CONFERENCE
PROGRAMME

BSODR
Annual General Meeting
14th-16th September 2015
Cardiff City Hall
The most recent in the professional range from LISTERINE® – a twice-daily mouthwash clinically proven to treat gum disease as an adjunct to mechanical cleaning.

Advanced Defence Gum Treatment is an alternative to chlorhexidine-based remedies. It’s formulated with unique LAE (Ethyl Lauroyl Arginate) technology that forms a physical coating on the pellicle to prevent bacteria attaching, and so interrupts biofilm formation.

When used after brushing it treats gum disease by reducing bleeding; 50.9% (p<0.001) in only 4 weeks.¹

In addition, Advanced Defence Gum Treatment is designed to not cause staining.²

To find out more visit www.listerineprofessional.co.uk

References:
1. Bleeding Index Reduction DOF 1 – 2013 (LAEBBA0001), 50.9% reduction in whole-mouth mean Bleeding Index at 4 weeks.
2. DOF 2 – 2013 (UNKPLT0006).
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WELCOME

A big warm Welsh welcome to this, the 63rd, Annual General Meeting of the BSODR in Cardiff. The BSODR has been meeting since 1953 with a major aim to facilitate the dissemination and application of research findings relating to oral health and the interactions between oral and systemic health. In addition the BSODR has a clear mission to encourage our junior researchers. This meeting is no exception with a focus on early career researchers (ECRs).

This year the BSODR provided membership to more than 30 ECRS throughout the UK to encourage them to become involved in the Societies activities and many are presenting at this meeting. We have also introduced a breakfast for ECRs to meet and network. ECRs also take centre stage for presentation of their research field at one of the scientific symposium.

As for previous years, our junior researchers are encouraged to enter the research prizes held during the conference. This year has seen record numbers apply for the Senior and Junior Colgate prizes, the Unilever poster prize and the Voco Dental Materials prize. This can only demonstrate true engagement of PhD and postdoctoral researchers. With this level of junior participation there are high hopes that the BSODR should continue to prosper in the future.

Local organising committee:
Rachel Waddington, David Williams, Mike Lewis (Cardiff University),
Victoria Hancock, Antonia Mitsis (In Any Event UK)
THE BSODR

The British Society for Oral and Dental Research

The BSODR was formed to advance research and increase knowledge for the improvement of oral health in the United Kingdom.

BSODR MANAGEMENT COMMITTEE
President: Professor Michael Lewis, Cardiff University
Secretary: Dr Marcello Riggio, University of Glasgow
Assistant Secretary: Professor Paul Anderson, Queen Mary University of London
Treasurer: Professor David Bartlett, King’s College London
Assistant Treasurer: Professor Alastair Sloan, Cardiff University
Editor: Professor Peter Robinson, University of Sheffield
Chair of the Awards Committee: Dr Simon Whawell, University of Sheffield

COUNCILLORS
Professor Paula Moynihan, Newcastle University
Professor Phil Stephens, Cardiff University
Professor Robert Allaker, Queen Mary University of London
Professor David Moles, Plymouth University
Professor Richard Lynch, GalaxoSmith Kline
Dr Christopher Nile, University of Glasgow (Early Career Researcher)
Chair of Local Organising Committee: Professor Rachel Waddington, Cardiff University

SCIENTIFIC GROUPS
Behavioural, Epidemiologic and Health Services Research (BEHSR): Blanaid Daly (blanaid.daly@kcl.ac.uk)
Dental Materials Group (DMG): Owen Addison (o.addison@bham.ac.uk)
Mineralised Tissue Research Group (MINTIG): Rachel Waddington (waddingtonrj@cardiff.ac.uk), Paul Anderson (p.anderson@qmul)
Oral Biology: Gordon Proctor (gordon.proctor@kcl.ac.uk)
Oral Medicine and Pathology Group (OMPG): Paula Farthing (P.Farthing@sheffield.ac.uk)
Oral Microbiology and Immunology Group (OMIG): Dave Spratt (d.spratt@ucl.ac.uk)
Periodontal Research Group (PRG): Francesco D’Auito (f.daiuto@eastman.ucl.ac.uk)

BUSINESS MEETINGS ARRANGED DURING THE CONFERENCE
Lunch provided during the conference is available to take to the business meetings.
BEHSR – Tuesday 15th September, 1-2pm Room A
ABSTD - Tuesday 15th September, 1-2pm Room B
OMPG Tuesday 15th September, 1-2pm Room C
OMIG – Wednesday 16th September, 1-2pm Room A
MINTIG - Wednesday 16th September, 1-2pm Room B
DMG - Wednesday 16th September, 1-2pm Room C
REGISTRATION

<table>
<thead>
<tr>
<th>TIME</th>
<th>DAY</th>
<th>LOCATION</th>
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<tbody>
<tr>
<td>1:00pm</td>
<td>Monday</td>
<td>Marble Hall</td>
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<tr>
<td>9:00am</td>
<td>Tuesday</td>
<td>Marble Hall</td>
</tr>
<tr>
<td>8:00am</td>
<td>Wednesday</td>
<td>Marble Hall</td>
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USEFUL INFORMATION

LOCATION
All meeting sessions will take place at Cardiff City Hall

PARKING
This is available in the Civic Centre avenues surrounding City Hall on a pay and display basis. Alternatively, parking is available in the NCP Car Parks situated around Cardiff (www.ncp.co.uk). The closest to City Hall and within walking distance are Cardiff Greyfriars NCP, CF10 3AD and Cardiff Dumfries Place NCP, CF10 3FN.

CONFERENCE BADGES
Please wear your conference name badge at all times to help conference organisation staff identify you as a delegate and promote networking.

ASSISTANCE
Use the registration desk for registration, replacement programme booklets or badges, restaurant information, invoicing, payment and conference dinner tickets. Student helpers wearing green polo shirts will be visible to help with directions, programme and poster questions, audio-visual, computer and room problems. Help will also be available in the speaker ready room for up-loading oral presentations.

SCIENTIFIC SESSION PROTOCOL
It is strictly forbidden to photograph, audio or video any of the information presented during oral sessions or on posters. This is because it is a requirement that all results data presented is unpublished.
INTERNET ACCESS
Wifi is available at the venue.

ORAL PRESENTATIONS
Please provide an electronic copy of your talk to a member of the AVA team in the slide preview room, syndicate room Gat least 60 minutes before the start of your session in which you are speaking. The slide ready room will be open from 8am to 5 pm throughout the meeting, and open from 10am on Monday 14th September for those participating in the prize presentations. The Talks will be subject to strict timing by light boxes: Green to 9 minutes, Orange to 11 minutes and red to 15 minutes. Plenary speakers only are permitted laptops.

POSTER PRESENTATIONS
Posters will be displayed throughout the conference meeting at the rear of the Assembly Hall. Presenters should mount their posters preferably by Monday afternoon, but at the latest by Tuesday 8am on their designated numbered board. Those participating in prize presentations must mount their posters by 12 noon on the Monday 14th September. Posters should be A0. Presenters are required to stand by their posters at the times indicated in the scientific programme.

CONFERENCE LUNCHES / REFRESHMENTS
To enhance networking opportunities tea and coffee and buffet lunches will be served as detailed in the programme overview in the Marble Hall on each day of the conference. Trade stands are also located here, which all are encouraged to visit as vital sponsors for this meeting.

MESSAGES
Messages will be posted on the board near the reception Desk. Messages to delegates can be left on telephone number 07779 989934. These message boards may also be used by delegates to advertise job vacancies and other conference meetings that may be of interest to conference delegates.
SOCIAL PROGRAMME

WELCOME RECEPTION
The Welcome Reception will take place in the Marble Hall from 6pm. Enjoy complimentary pies and beers to start the event.

CONFERENCE DINNER
“A Night at the Museum”
Starting with a drinks reception at 7pm in the picture galleries. Dinner will start at 7:45pm. In providing a flavour of Welsh music entertainment will be provided during the drinks reception and after the conference meal by Ben Creighton Griffith, a classical and contemporary jazz harpist. Dress code: lounge suits for men and smart female outfits.

EATING IN CARDIFF
City Hall is located next to Cardiff City Centre which is accessed via the subway or pedestrian crossing across Boulevard de Nantes. There are many restaurants on High Street, St Mary Street, Churchill Way, The Hayes and Mill Lane. More restaurants are located in Cardiff Bay 2 miles from the City Centre (train / bus and taxi connections).

SITES TO VISIT
If you have time Cardiff’s top attractions include the Castle and Cardiff Museum (free entry). On the outskirts of Cardiff is the St Fagan’s Welsh Folk Outdoor Museum (free entry) and in Cardiff Bay the Senedd (Welsh Assembly buildings), Millennium Centre, Norwegian Church with history, the Doctor Who Experience and wonderful views of the bay.

TAXIS
Cardiff City Centre is easily accessed from City Hall by foot. Cardiff Central Station is a 15 minute walk to Cardiff City Hall. If you need a taxi, local services include:
Dragon Taxis – 02920 333 333
Capital Cabs 02920 777 777
Premier Cabs 02920 555 555
HIGHLIGHT SESSIONS

**Plenary Lecture:** Professor Chris Lynch – “What does the Phase Down of Amalgam mean for UK Dental Education & Practice?”

**Graham Embery Lecture:** Professor Michael Curtis – “Periodontal Disease – who’s bad?”

**TC White Lecture:** Francesco D’Auito - “A Mouthful of Diseases”

**Early Career Researcher Breakfast:** This year we have organised a breakfast session for all ECRs who are members of the BSODR to attend. The session aims to provide a relaxed atmosphere for ECRs to network and a chance to learn more about the society and the benefits it can bring for career progression. The Breakfast will be held on Tuesday 15th September, 8am-9pm – Room I. Sponsored by Colgate

SPONSORED SYMPOSIUM

**OMIG;** Biofilm control, 11:30am - 1:00pm, Tuesday 15th September, Syndicate Room A

**MINTIG;** Emerging technologies for understanding and mimicking dental hard tissue growth: Early Career Researchers Focus, 11:30-1pm, Wednesday 16th September, the assembly rooms.

**Prize Sessions:** All oral prize presentation sessions are open for all to attend. Judging of posters prizes will be closed sessions, but posters will be presented again later in the scientific programme.

**Senior Colgate Prize:** Monday 14th September (oral session),
- Group A1, 2-3:30pm syndicate rooms B,
- Group A2, 4-5:30pm syndicate room B;
- Group B1 2-3:30pm syndicate rooms C
- Group B2 4-5:30pm syndicate room C.
Finals Tuesday 15th September, 11:30-12:30pm, Assembly Rooms

**Junior Colgate Prize:** Monday 14th September (oral session),
- Group 1, 2-3:30pm syndicate rooms A
- Group 2, 4-5:30pm, syndicate room A

**Unilever Poster Prize:** Monday 14th September, 2:00pm-4:00pm Assembly Room Poster Area

**BSODR Clinical Research Poster Prize:** Monday 14th September, 2:00pm-4:00pm Assembly Room Poster Area

**GSK-MINTIG travel Award:** Tuesday 15th September (oral session), 11:30-1pm, Syndicate Room C

**Voco Dental Materials Prize:** Tuesday 15th September (oral session), 11:30-1pm and 2-3pm, syndicate room D
# OVERVIEW TIMETABLE

## MONDAY 14TH SEPTEMBER

<table>
<thead>
<tr>
<th>TIME</th>
<th>TITLE</th>
<th>LOCATION</th>
<th>CHAIR</th>
</tr>
</thead>
<tbody>
<tr>
<td>02.00PM - 03.30PM</td>
<td>Senior Colgate Prize Group B1</td>
<td>Syndicate Room C</td>
<td>Bartlett, D.</td>
</tr>
<tr>
<td>02.00PM - 03.30PM</td>
<td>Junior Colgate Prize 1</td>
<td>Syndicate Room A</td>
<td>Riggio, M.</td>
</tr>
<tr>
<td>04.00PM - 05.30PM</td>
<td>Senior Colgate Prize Group A2</td>
<td>Syndicate Room B</td>
<td>Speight, P.</td>
</tr>
<tr>
<td>04.00PM - 05.30PM</td>
<td>Junior Colgate Prize 2</td>
<td>Syndicate Room A</td>
<td>Riggio, M.</td>
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## TUESDAY 15TH SEPTEMBER

<table>
<thead>
<tr>
<th>TIME</th>
<th>TITLE</th>
<th>LOCATION</th>
<th>CHAIR</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.30AM - 11.30AM</td>
<td>Tuesday Posters</td>
<td>Assembly Room</td>
<td>Whawell, S.</td>
</tr>
<tr>
<td>10.30AM - 11.30AM</td>
<td>Tuesday Posters</td>
<td>Poster Area</td>
<td>Whawell, S.</td>
</tr>
<tr>
<td>11.30AM - 12.30PM</td>
<td>Colgate Prize Finals</td>
<td>Assembly Room</td>
<td>Whawell, S.</td>
</tr>
<tr>
<td>11.30AM - 01.00PM</td>
<td>OMIG Symposium</td>
<td>Syndicate Room A</td>
<td>Spratt, D.</td>
</tr>
<tr>
<td>11.30AM - 01.00PM</td>
<td>MINTIG Prize Orals</td>
<td>Syndicate Room C</td>
<td>Waddington, R.</td>
</tr>
<tr>
<td>11.30AM - 01.00PM</td>
<td>Health Services Research Orals</td>
<td>Syndicate Room B</td>
<td>Chestnutt, I. G.</td>
</tr>
<tr>
<td>11.30AM - 01.00PM</td>
<td>VOCO Prize Session 1</td>
<td>Syndicate Room D</td>
<td>Addison, O.</td>
</tr>
<tr>
<td>02.00PM - 03.00PM</td>
<td>Voco Prize Session 2</td>
<td>Syndicate Room D</td>
<td>Addison, O.</td>
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</table>
**WEDNESDAY 16TH SEPTEMBER**

<table>
<thead>
<tr>
<th>TIME</th>
<th>TITLE</th>
<th>LOCATION</th>
<th>CHAIR</th>
</tr>
</thead>
<tbody>
<tr>
<td>02.00PM - 03.00PM</td>
<td><strong>Behavioural Science Orals</strong></td>
<td>Syndicate Room B</td>
<td>Robinson, P. G.</td>
</tr>
<tr>
<td>02.00PM - 03.15PM</td>
<td><strong>Cariology, Diagnosis and Prosthodontics</strong></td>
<td>Syndicate Room C</td>
<td>Bartlett, D.</td>
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<tr>
<td>09.00AM - 10.15AM</td>
<td><strong>Materials Science Orals</strong></td>
<td>Syndicate Room D</td>
<td>Walmsley, A. D.</td>
</tr>
<tr>
<td>09.00AM - 10.15AM</td>
<td><strong>Oral Cancer Orals</strong></td>
<td>Assembly Room</td>
<td>Khurram, A.</td>
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<tr>
<td>09.00AM - 10.30AM</td>
<td><strong>Epidemiology Orals</strong></td>
<td>Syndicate Room B</td>
<td>Newton, J.</td>
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<tr>
<td>09.00AM - 10.30AM</td>
<td><strong>Periodontology Orals</strong></td>
<td>Syndicate Room C</td>
<td>Taylor, J. J.</td>
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<tr>
<td>09.00AM - 10.30AM</td>
<td><strong>Pulp biology and Stem Cells</strong></td>
<td>Syndicate Room A</td>
<td>Sloan, A.</td>
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<tr>
<td>10.30AM - 12.00PM</td>
<td><strong>Wednesday Posters</strong></td>
<td>Assembly Room</td>
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<tr>
<td>11.30AM - 01.00PM</td>
<td><strong>Evaluation of interventions</strong></td>
<td>Syndicate Room D</td>
<td>Mason, S.</td>
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<tr>
<td>11.30AM - 01.00PM</td>
<td><strong>Microbiology Orals</strong></td>
<td>Syndicate Room B</td>
<td>Williams , D.</td>
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<tr>
<td>11.30AM - 01.00PM</td>
<td><strong>MINTIG Symposium</strong></td>
<td>Assembly Room</td>
<td>Al-Jawad, M.</td>
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<tr>
<td>11.30AM - 01.00PM</td>
<td><strong>Oral Health Promotion Orals</strong></td>
<td>Syndicate Room A</td>
<td>Kay, E. J.</td>
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BSODR Management Committee meeting Monday, 14/09/2015 12.30 PM-02:00 PM
Syndicate room H – Committee members only

Monday, 14/09/2015 02:00 PM-03:30 PM Syndicate Room B
Senior Colgate Prize Group A1 Chair: Speight, P.

1 Novel Bioactive Glass Cements For Bone Grafting: From An In-Vitro To An In-Vivo Study
D’Onofrio, A., Shahdad, S., Rawlinson, S., LIU, J., Kent, N., Hill, R. G.  Queen Mary University of London

2 Neutrophil directional chemotaxis in children with Papillon Lefèvre Syndrome
Roberts, H., White, P., Grant, M., Chapple, i.  University of Birmingham

3 Antibacterial Properties of Oral Progenitor Cells: The role of Soluble Factors
Board Davies, E., Moses, R., Sloan, A., Stephens, P., Davies, L.  1Cardiff University, 2Karolinska Institutet

4 Biomarker Study on Oral Cancer Exosomes
Qadir, F., Teh, M.  Queen Mary University of London

5 Trends Of Oral Cavity, Oropharyngeal, And Laryngeal Cancer Incidence In Scotland (2001-2012).
Purkayastha, M., McMahon, A., Gibson, J., Conway, D.  mUniversity of Glasgow

6 Salivary changes in mechanically ventilated patients are associated with respiratory pathogen colonization of dental plaque
Sands, K. M., Wilson, M., Wei, X., Xu, R., Brewis, I., Khanna, S., Lewis, M., Wise, M. P.  1School of Dentistry, 2Central Biotechnology Services, 3Adult Critical Care, University Hospital of Wales

Monday, 14/09/2015 02:00 PM-03:30 PM Syndicate Room C
Senior Colgate Prize Group B1 Chair: Bartlett, D.

7 Viral Status and Microenvironment In Oropharyngeal Cancer: A Therapeutic Target
Bolt, R., Lambert, D., Foran, B. H., Thomas, S., Murdoch, C., Hunter, K.  1School of Clinical Dentistry, 2School of Clinical Dentistry, 3Weston Park Hospital, 4Sheffield Cancer Research Centre, 5School of Clinical Dentistry

8 A Study of Biomarkers in Patients with Periodontal Disease
Gul, S. S., Douglas, I., Griffiths, G., Rawlinson, A.  The University of Sheffield
9 A parallel sided randomised controlled trial to determine if the process of warming composite resin restorative material prior to placement of a restoration leads to changes in postoperative sensitivity.  
Campbell, I. M. Leeds Dental Institute

10 A novel implant cleaning method - In vitro non-contact biofilm removal from SLA and polished titanium surfaces using cavitation from an ultrasonic scaler.  
Vyas, N., Sammons, R. L., Addison, O., Dehghani, H., Walmsley, A. D. University of Birmingham

11 A phenotypic re-evaluation of human γδ T cells in health and disease: Is Vδ2+ T cell depletion a risk predictor for BRONJ?  

12 The Role of the Outer Membrane Proteins of P. gingivalis in Host-Pathogen Interactions  
Naylor, K. L., Murdoch, C., Douglas, I., Stafford, G. P. The University of Sheffield

Monday, 14/09/2015 02:00 PM-03:30 PM Syndicate Room A  
Junior Colgate Prize 1 Chair: Riggio, M.

13 In vitro effects of a novel hydroxyapatite-fluoride agent (IWI2) upon enamel repair and remineralisation  
Holmes, W., Watson, T., Boyes, V., Verhaeghe, T. King's College London, Sylphar nv

14 Periodontal pathogens harvest sugar from human sialylated proteins, but which ones?  
Hussain, M. University of Sheffield

15 The relationship between periodontal disease and non-communicable diseases associated with endothelial dysfunction  
Johnson-Idan, J., Hon, J. M., Sabbah, W., Hughes, F. J. King's College London

16 Oral Cancer: Exploring the Stories in United Kingdom Newspaper Articles  
Kelly, C., Johnson, I. G., Morgan, M. Cardiff University

17 The association of calcium channel blockers with periodontal disease.  
Hon, J. M., Johnson-Idan, J., Linden, G. J., Winning, L., Sabbah, W., Hughes, F. J. King's College London, Queen's University Belfast

18 The Role of CALML3 in Oral Squamous Cell Carcinoma  
Hankinson, P. M., Lambert, D., Hunter, K. University of Sheffield

Monday, 14/09/2015 04:00 PM-05:30 PM Syndicate Room B  
Senior Colgate Prize Group A2 Chair: Speight, P.

19 Thermal Observation of Tooth Transfer Heat  
Lancaster, P., Brette, D., Carmichael, F., Clerehugh, V. University of Leeds, St James's University Hospital, Leeds Dental School
20 A Novel Orthodontic Adhesive Made From Bioactive Glass To Prevent Demineralisation.

21 Characterization of mouse incisor tooth mesenchymal stem cells
Walker, J. V., Zhuang, H., Tredwin, C., Hu, B. Plymouth University Peninsula Schools of Medicine & Dentistry, Peking University School and Hospital of Stomatology

Monday, 14/09/2015  04:00 PM-05:30 PM  Syndicate Room C
Senior Colgate Prize Group B2  Chair: Bartlett, D.

22 Recruitment of Tumour-Associated Neutrophils to Head and Neck Squamous Cell Carcinoma
NIAZ, H. A., Tazzyman, S., Muthana, M., Hunter, K., Murdoch, C. University of Sheffield, Department of Oncology, University of Sheffield

23 Signaling regulating active TG2 release from cells: implications for osteoarthritis.
Adamczyk, M., Griffiths, R. M., Seward, K., Knauper, V., Aeschlimann, D. Cardiff University

24 Bacterial products can directly activate trigeminal sensory neurons.
Kaewpitak, A., Bauer, C. S., Seward, E. P., Boissonade, F., Douglas, I. The University of Sheffield

Monday, 14/09/2015  04:00 PM-05:30 PM  Syndicate Room A
Junior Colgate Prize  Chair: Riggio, M.

25 Patients’ Valuation of Fluoride Varnish in Brazil: using Willingness to Pay<br />Adam, N., Walshaw, E., Lobato, M., Neves, M., Vernazza, C. R. Newcastle University, Newcastle University, Pontifícia Universidade Católica do Rio Grande do Sul

26 Isolation and Immortalisation of Tonsil Keratinocytes for Three-Dimensional Tissue Engineered Models
Grayson, A. K., Hearnden, V., Colley, H. E., Bolt, R., Jabreel, A., Murdoch, C. University of Sheffield, Sheffield Hallam University, Department of Materials Science and Engineering, Sheffield Teaching Hospitals NHS Foundation Trust

Monday, 14/09/2015, 2:00PM-4:00PM  Assembly Room Poster Area
BSODR Poster Prize Judging

BSODR Clinical Research Poster Prize
Unilever Poster Prize

Monday, 14/09/2015, 7:00PM-8:00PM  Welcome Reception
Tuesday, 15/09/2015  8AM -9AM Early Stage Researcher Breakfast (ECR eligible delegates only)

Tuesday, 15/09/2015  9.00AM – 9.30AM Opening Ceremony Assembly Rooms

Tuesday, 15/09/2015  9.30AM – 10.30AM Plenary Lecture, Professor Christopher Lynch
Assembly Rooms

Tuesday, 15/09/2015  10:30 AM-11:30 AM   Assembly Room Poster Area
Tuesday Posters

27 In vitro effect of amorphous calcium phosphate paste applied for extended periods of time on enamel remineralization  
Neves, J. G.¹, Danelon, M.¹, Stock, S.³, Cannon, M.³, Xiao, X.², De Carlo, F.², da Camara, D.¹, Delbem, A.¹ ¹Araçatuba Dental School , ²Argonne National Laboratory, ³Feinberg School of Medicine

28 Salivary antimicrobial proteins associate with <i>Streptococcus mutans</i>, age and dental disease status of children  
Malcolm, J.¹, Simon-Soro, A.², Sadique, S.³, Macpherson, L.³, Mira, A.², Culshaw, S.⁴, Sherriff, A.³ ¹University of Glasgow, ²FISABIO foundation, ³University of Glasgow Dental School, ⁴Oral Health Research Institute

29 In-situ Caries Evaluation of Experimental Dentifrices containing Functionalised β-TCP  
Churchley, D.¹, Bosma, M.¹, Hara, A.², Jain, R.¹, Kelly, S.², Lippert, F.², Martinez Mier, E.², Zero, D. T.² ¹GlaxoSmithKline Consumer Healthcare, ²Oral Health Research Institute

30 SEM imaging of cervical dentine treated with a desensitising toothpaste  
Morgan, E. R., Mnieinne, M., Gillam, D. G., Hill, R. G. Barts and the London School of Medicine and Dentistry, Queen Mary University of London

31 RCT of Experimental Cetylpyridinium Chloride Mouthwash Effects on Plaque Re-Growth  
Shaw, D.³, Gordon, J.¹, Maclure, R.², Bosma, M.¹ ¹GlaxoSmithKline Consumer Healthcare, ²Intertek CRS, ³inVentiv Health

32 Randomised Clinical Trial of Oral Tolerability of a NaHCO₃ Toothpaste  
Krishnan, N., Lomax, A., Ali, A., Jain, R., Bosma, M. Glaxo SmithKline

33 RCT of Experimental Stannous Fluoride Dentifrice in Dental Stain Removal  
Nehme, M.¹, Hall, C.¹, Siddiqi, M.², Hughes, N.³, Millenman, K.⁴, Millenman, J.³ ¹GSK Consumer Healthcare, ²Intertek, ³Intertek CRS

34 Antimicrobial activity of a stannous fluoride toothpaste in the PGRM  
Parkinson, C.¹, Maclure, R.¹, Payne, D.², Hall, P.¹, Jeffery, P.¹ ¹GSK Consumer Healthcare, ²Intertek, ³Intertek CRS
35 Three RCTs of Tolerability of Oral Healthcare Products in Children
Gordon, J.1, Newby, E.1, Millemans, K.2, Millemans, J.1, Payne, D.3, Maclure, R.4, Siddiqi, M.1, Bosma, M.1.1 GlaxoSmithKline Consumer Healthcare, 2Salus Research, 3Salus Research, 4Intertek CRS, 5inVentiv Health Clinical

36 Comparison of Different Periodontal Surgeries: A Longitudinal Meta-Analysis
Wu, Y., Lin, L., Tu, Y. Institute of Epidemiology and Preventive Medicine

37 Full-mouth Disinfection in the Treatment of Chronic Periodontitis: A Systematic Review and Bayesian Network Meta-analysis
Lu, Y., Chen, T., Wu, Y., Tu, Y. Institute of Epidemiology and Preventive Medicine

38 Association Between Periodontitis, Renal & Vascular-Function In Chronic Kidney Disease

Al-Qarakhli, A. M., Sloan, A., Moseley, R., Waddington, R. Dental School/ Cardiff University

40 Neutrophil cell glutathione changes in chemotaxis
Sham, N. B., Grant, M. University of Birmingham

41 Outcomes of periodontal therapy in Rheumatoid Arthritis: baseline data from a randomised controlled trial
Lopez-oliva, I.1, Stefan, S.1, de Pablo, P.2, Filer, A.2, Raza, K.2, Dietrich, T.1. 1Birmingham Dental Hospital, 2Queen Elisabeth Hospital, 3Sandwell and West Birmingham Hospitals NHS Trust

42 Effectiveness of Gingival Retraction Methods: A Systematic Review
Tabassum, S., Raza Khan, F. Aga Khan University Hospital

43 A qualitative study to explore the issues faced by patients when giving feedback on the communication of dental students
Coelho, C., Pooler, J., Lloyd, H. Plymouth University Peninsula Schools of Medicine and Dentistry

44 Teaching of posterior composites in UK and Ireland dental schools
Lynch, C. D.1, Blum, I. R.2, McConnell, R. J.3, Wilson, N. H.4. 1School of Dentistry, Cardiff University, 2Kings College London Dental Institute, 3University College Cork, 4Kings College London Dental Institute

45 A Mixed Methods Assessment Of An Orthodontic E-learning Resource
Mehta, S.1, Clarke, F.4, Fleming, P.3,2 1Cedars Dental Practice, 2Queen Mary University of London, 3Queen Mary University of London, 4Queen Mary University of London

46 New Graduates’ Professionalism And Communication Skills: Trainers’ Expectations and Experience
Jones, R. L., Gilmour, A. School of Dentistry, Cardiff University

47 Views of recent dental school graduates on teaching in prosthodontics.
Oliver, G.1, Lynch, C. D.2, Chadwick, B. L.3, Santini, A.4, Wilson, N. H.5. 1Oxford University Hospitals, 2School of Dentistry, Cardiff University, 3School of Dentistry, Cardiff University, 4The University of Medicine & Pharmacy, 5Kings College London Dental Institute
48 Are Thiel Cadavers a Better Model for Teaching Exodontia to Dental Undergraduates?
Macluskey, M., Hanson, C., Eisma, R.  University of Dundee

49 Peer Review of Teaching in UK Dental Schools
Cunningham, I. M., Lynch, C. D.  Cardiff University

50 In Vitro Real-Time Measurements of Rate of Enamel Remineralisation

51 Osteoarthritis biomarker discovery using a 3D human articular cartilage model
Heil, A., Dewitt, S., Waddington, R., Archer, C., Aeschlimann, D.  Cardiff University

52 Repair of Enamel Erosive Lesions with Adult and Children’s Dentifrices
Fowler, C., Brown, A., Lynch, R. J.  GlaxoSmithKline, Lucideon Ltd

53 Preparation of wild-type and mutant recombinant amelogenins for functionality studies of Al pathogenesis
Gabe, C., Brookes, S. J., Myers, S. L., Kirkham, J.  University of Leeds

54 Ordered Enamel Crystallite Formation Using Elastin-like Proteins
Shuturminska, K., Al-Jawad, M., Azevedo, H., Bushby, A., Mata, A., Anderson, P.  Queen Mary University

55 Pharmacological rescue of amelogenesis imperfecta and the NFkB pathway
Myers, S. L., Brookes, S. J., Feichtinger, G., Kirkham, J.  University of Leeds

56 Co-operativity of Statherin and Histatin in Demineralisation of Carious and Erosive HAp Model
Al Mandil, H. B., Al-Jawad, M., Williams, R., Anderson, P.  Queen Mary University of London

57 The Effect of Demineralisation on Enamel Porosity
Padidar, B., Burnett, G., Petroczi, A., Naughton, D.  Kingston University
London, GlaxoSmithKline Consumer Healthcare, Kingston University

58 Bacterial Toxicity Comparison Between Silver, Titanium Dioxide and Hydroxyapatite Nanoparticles
Besinis, A., Hadi, S., Le, H., Tredwin, C., Handy, R.  University of Plymouth

59 Comparison of Shaping Ability and Failure Incidence of WaveOne files and One Shape files.
Elashiry, M. M.  Faculty of Dentistry, Ain shams University

60 The Efficacy of Hydrogen Peroxide in Modulating Dentine Staining
Alraies, A., Waddington, R., Glassé, C., Rees, J., Mohan, V., Young, N., Sloan, A.  Tissue Engineering and Reparative Dentistry, Philips Research

61 Benchmarking the Chemical Solubility of Restorative Dental Ceramics
Hawsawi, R. A., Johnson, A., Stokes, C.  The University of Sheffield

62 Clinical Evaluation of Conventional Lithium Disilicate Single Crowns Issued in a Teaching Institution
Samer, M. S., Abdullah, H. B., Taiyeb Ali, T. B.  University of Malaya
63 Biocompatibility and biomineralization assessment of bioceramic, epoxy-resin based and calcium hydroxide root canal sealers. Dezan-Junior, E. UNESP

64 Development of Three-dimensional Models of Bone and Oral Mucosa Almela, T. K., Brook, I. M., Moharamzadeh, K. Sheffield University

65 The environmental fate of waste microplastics from resin-based dental composite Mulligan, S., Fairburn, A., Kakonyi, G., Thornton, S. F., Moharamzadeh, K., Martin, N. University of Sheffield

66 Biocompatibility of Surface-Modified Titanium Implants with Silver and Hydroxyapatite Nanoparticles Salaie, R. N., Besinis, A., Le, H., Tredwin, C., Handy, R. University of Plymouth

67 Comparison of Two Biomembranes in the Direct Pulp Capping klein-jr, c. a.1, Plepis, A. d.2 1lutheran university, 2USP

68 Development Of Bioactive Glass For Orthodontic Adhesive Removal And Remineralization TAHA, A., Patel, M., Fleming, P., Hill, R. G. Queen Mary University of London


70 Structural Integrity of Poly-Ether-Ketone-Ketone (PEKK) Based Bi-layered Molar Crowns Alsadon, O. A.1, Wood, D.1, Patrick, D.1, Copponnex, T.2, Pollington, S.1 1University of Sheffield, 2Cendres+Métaux SA


72 Layered Double Hydroxides in Experimental Composite Materials Abdulmohsen, B.1, Franks, M. A.2, Hine, C. E.2, Patel, M.2 1Newcastle University, 2Queen Mary University of London, Barts and The London School of Medicine and Dentistry

73 Differentiation Of Human Dental Pulp Stem Cells Into Neural Lineages Kyriakidou, E.1, Travers, P.3, Lopes, V.2 1University of Sheffield, 2Edinburgh Dental Institute, 3University of Edinburgh

74 The function of CD133 in tooth epithelium development Singer, D.1, Zhuang, H.2, Corbeil, D.3, Tredwin, C.4, Hu, B.5 1PUPSMD, 2Plymouth University, 3BIOTEC - Biotechnology Center TU Dresden, 4PUPSMD, 5Plymouth University

75 Role of Mesenchymal Cells in Salivary Gland Regeneration Davies, J.1, Hu, B. University of Plymouth

76 Peripheral Targeting Of Interleukin-1 Cytokine Signaling Pathway In Peripheral Nerve Injury SHEMBESH, H., Bird, E., Boissonade, F., Atkins, S. The University of Sheffield
77 Direct bacteria-neuron interaction via Toll-Like Receptors (TLRs): consequences for orofacial pain.
Helley, M., Abate, W., Jackson, S. K., Bennett, J. H., Thompson, S. W. Plymouth University

Tuesday, 15/09/2015  11:30 AM-12:30 PM  Assembly Room
Colgate Prize Finals  Chair: Whawell, S.

Tuesday, 16/09/2015  11:30 AM-01:00 PM  Syndicate Room A
OMIG Symposium  Chair: Spratt, D.
Sponsored by GlaxoSmithKline

Dr Andrew McBain (University of Manchester, UK)
Therapeutic and hygienic control of biofilms in the mouth and elsewhere

Prof Bart Keijser (TNO and ACTA, Netherlands)
Resilience: a new oral health concept beyond the absence of disease.

Prof Rob Allaker (QMUL, UK)
Use of nanotechnology in the control of oral biofilms

Prof Gordon Ramage (University of Glasgow, UK)
Polymicrobial biofilms on the denture surface

Tuesday, 15/09/2015  11:30 AM-01:00 PM  Syndicate Room C
MINTIG Prize Orals  Chair: Waddington, R.

78 Semi Real-Time Erosion of Human Dentine at the Nano-scale: An AFM Force-curve Based Imaging Study
Pattem, J. K. Newcastle University

79 Towards a 4-D spatial and temporal model of human incisor enamel biomineralisation using X-ray techniques.
Al-Mosawi, M., Davis, G. R., Al-Jawad, M. Queen Mary, University of London

80 Hydroxyapatite/Carbon Nanotubes as Composite Bone Implants - Biocompatibility vs Toxicity
Natesan, K., Le, H., Salih, V., Handy, R., Tredwin, C. Plymouth University

81 Radiographic assessment of regenerative endodontic treatment of traumatised non-vital immature teeth.
Nazzal, H., Monteiro, J., Webb, L., Duggal, M. S. University of Leeds

82 Characterization of White Spot Lesion Using Focused Ion Beam- Scanning Electron Microscopy
Moeinian, M., Shuturminska, K., Bushby, A., Hill, R., Wong, F. Institute of Dentistry, QMUL, QMUL, Queen Mary University
83 Referral pathways from general dental services to other primary dental care services in the UK: A systematic review and critical interpretive synthesis
Allen, Z. E., Nasser, M., Stenhouse, E., Richardson, J., Moles, D. R.  Plymouth University

84 The Future Dental Workforce In Malaysia: Drivers For Change
Che Musa, M., Bernabe, E., Gallagher, J. E.  King’s College London Dental Institute

85 Potential For Direct Access In Care Homes In Wales
Monaghan, N. P., Morgan, M. 1, 2  Cardiff University, 2Public Health Wales

86 Urgent Dental Care: Accessibility, Availability and Equity
Worsley, D., Marshman, Z., Robinson, P. G., Jones, K.  University of Sheffield

87 Fifteen-year Survival of Root Canal Treated Posterior Teeth.
Lessani, M., Lucarotti, S., Lumley, P., Burke, T.  University of Birmingham

88 Dental consultations and antibiotic use in UK General Medical Practice
Cope, A., Francis, N. A., Wood, F., Chestnutt, I. G.  Cardiff University

89 Mechanical Properties of Thermo-pressed Polyetheretherketone as a Denture Material
Muhsin, S. A. 1, Hatton, P. 1, Johnson, A. 1, Sereno, N. 2, Wood, D. 1 1University of Sheffield, 2JUVORA Ltd

90 Synthesis of glass-ceramics for dental applications
Alzahrani, A. S. 1, Cattell, M. 2, Karpukhina, N. 1 1Queen Mary University of London, 2Barts & The London School of Dentistry and Dentistry Institute of Dentistry Turner Street London

91 Water-Uptake and Mechanical Properties of Experimental Resin-Modified Glass-Ionomer Cements (RMGICs).
Agha, A. 1, Parker, S. 1, Fleming, G. 2, Patel, M. 1 1Barts and the London School of Medicine and Dentistry, 2Dublin Dental Hospital, Trinity College

92 Optimizing photo-initiator system of new fluoride-releasing acrylic orthodontic adhesive
Ismail, H. M., German, M. J., Rolland, S. L.  Newcastle University

93 Effect of Temperature and Substrate on Ordered Apatite Crystals for Coating Dental Implants.
Elsharkawy, S., Al-Jawad, M  Queen Mary University of London

94 Increased bioactivity in fluoride and high phosphate containing glasses and stimulation of VEGF production in MC3T3-E1 osteoblast-like cells
LIU, J., Rawlinson, S., Hill, R. G., Fortune, F.  Queen Mary University of London

Tuesday, 15/09/2015  1:00PM-02:00 PM  BERSH group lunch - Room A

Tuesday, 15/09/2015  1:00PM-02:00 PM  ABSTD group lunch - Room B
Tuesday, 15/09/2015  02:00 PM-02:00 PM  OMPG group lunch - Room C

Tuesday, 15/09/2015  02:00 -03:00 PM  TC White Lecture, Dr Francesco D’Auito  Assembly Rooms

Tuesday, 15/09/2015  02:00 -03:00 PM  Syndicate Room D
Voco Prize Session 2  Chair: Addison, O.

95 Odontogenic differentiation of human dental pulp stem cells (hDPSCs) under strontium treatment
HUANG, M., Hill, R., Rawlinson, S.  Queen Mary University of London

96 Modified Appen Model for Developing Restoratives with Enhanced Clinical Aesthetics
Duminis, T., Shahid, S., Frampton, P., Hill, R.  1Barts and the London School of Medicine and Dentistry, Queen Mary University of London, 2James Kent Group

97 2-Photon analysis of nanogel-infiltrated adhesive-dentine interfaces
Boyes, V., Stansbury, J., Festy, F., Thompson, V., Watson, T.  1King’s College London, 2Tissue Engineering and Biophotonics, 3University of Colorado Denver

Tuesday, 15/09/2015  02:00PM-03:00PM  Syndicate Room B
Behavioural Science Orals  Chair: Robinson, P. G.

98 Triggers for patients’ referral for NHS dental implant treatment and the subsequent decision making process; Patients’ encounters and Clinicians’ views
Kashbour, W. A., Ellis, J., Rousseau, N., Thomason, M.  Newcastle University

99 Tension field triad: Influences on development of professionalism in dental students
Ranauta, A., Davenport, E., Freeth, D.  1London School of Hygiene and Tropical Medicine, 2QMUL, Barts & The London School Of Medicine and Dentistry

100 Leadership in dentistry: an exploratory study of general dental professionals’ perceptions of leadership
Wardman, M. J.  University of Leeds

101 Exploring people’s online experiences of the teeth whitening industry using a web-specific mapping technique
Lala, R., Robinson, P. G., Gibson, B. J.  The University of Sheffield

Tuesday, 15/09/2015  02:00 PM-03:15 PM  Syndicate Room C
Cariology, Diagnosis and Prosthodontics  Chair: Bartlett, D.

102 Correlation between Optical Coherence Tomography and Synchrotron X-ray diffraction for the diagnosis of enamel defects
Bozec, L., Al-Jawad, M., Al-Azri, k., Cook, R. J., Festy, F., Parekh, S.  1University College London, 2Queen Mary University of London, 3King’s College London

103 Salivary proteins mediate greatest protection against dental erosion
Mutahar, M. A., Carpenter, G., Bartlett, D., Moazzaz, R.  King’s College London
104 Determination of Hydrogen-Ion Concentration Microenvironments within Biofilm by Two-Photon Excitation Fluorescence Lifetime Imaging Microscopy (2PE-FLIM)
Roulston, D. P., Pratten, J. R., Spratt, D. ¹UCL Eastman Dental Institute, ²GSK Consumer Healthcare

105 Comparing the Demineralisation Rates of Deciduous and Permanent Enamel.
Hassanali, I. ¹, Wong, F. ², Lynch, R. J., Anderson, P. ³ ¹GlaxoSmithKline, ²Institute of Dentistry, QMUL, ³Queen Mary University

106 Host Microbiome Interactions in the Oral Cavity of a Denture Wearer
O’Donnell, L. E., Robertson, D., Nile, C. J., Riggio, M., Bradshaw, D., Lambert, M., Crielaard, W., Zaura, E., Brandt, B., Ramage, G. ¹University of Glasgow, ²GSK, ³Academic Centre for Dentistry Amsterdam

Tuesday, 15/09/2015 03:30 PM-04:00 PM  NIHR funding, Professor Jimmy Steele, Assembly Rooms

Tuesday, 15/09/2015 04:00 PM-05:00 PM  Graham Embery Lecture, Professor Michael Curtis, Assembly Rooms

Tuesday, 15/09/2015 05:00 PM-06:00 PM  BSODR Annual Business meeting, Assembly Rooms (open to all members)

Tuesday, 15/09/2015 07:00 PM-10:00 PM  Conference Dinner “A Night at the Museum”
National Museum of Wales, Cathays Park (building next door to City Hall)

Wednesday, 16/09/2015

Wednesday, 16/09/2015 09:00-10:30AM Syndicate Room D
Materials Science Orals  Chair: Walmsley, A. D.

107 Debris accumulation following instrumentation with asymmetric file systems
OGLAH, F. S., Juneja, R., Robinson, J., Cooper, P., walmsley, A. D., Tomson, P.  University of Birmingham

108 In Vitro Characterisation of Teethmate: A Calcium-Phosphate Based Dentine Desensitiser
Duminis, T., Shahid, S., D’Onofrio, A., Hill, R., Gillam, D. G.  Barts and the London School of Medicine and Dentistry,

109 Antibacterial coating made of strongly adhered nanosilver to titania nanotubes for dental implants
Danookdharree, U. F., Le, H., Tredwin, C., Handy, R.  Plymouth University

110 The effect of sodium content on the bioactivity of glasses used in grit-blasting technique
Al-Khayvat, F., Hill, R. G., Rawlinson, S.  Queen Mary University of London
111 Multidisciplinary team perspectives on the quality of life of head and neck cancer patients at two years
Parhar, S.1, Rogers, S.2, Lowe, D.3 1University of Glasgow, 2University Hospital Aintree, 3Edge Hill University

112 Risk Modelling in Cancer Prediction: Are we falling behind in Head and Neck Cancer?
McCarthy, C. E.1, Marcus, M. W.1, Bonnett, L. J.2, Field, J.1 1Institute of Translational Medicine, 2Institute of Translational Medicine

113 The Scottish Audit of Head and Neck Cancer (SAHNC): Influence of socioeconomic deprivation status on 5- and 12-year survival.
Ingarfield, K.1, Savage, S.2, MacKenzie, K.3, Douglas, C.4, Conway, D. I.1, McMahon, A.1 1University of Glasgow, 2NHS Fife, 3Glasgow Royal Infirmary, 4Southern General Hospital

114 Cancer-associated fibroblasts contribute to macrophage recruitment in head and neck cancer.
Prajapati, P., Kabir, T., Melling, G., Hadley, L., Colley, H. E., Murdoch, C., Lambert, D. University of Sheffield

115 Modelling Ameloblastoma Behaviour With Bone-Like Co-Culture Scaffolds
Eriksson, T.1, Day, R.2, Fedele, S.3, Salih, V.4, 1University College London, 2University College London, 3University College London, 4Plymouth University

116 Body Mass Index and Dental Caries: A Systematic Review and Meta-analysis
Paisi, M.1, Kay, E. J.2, Bennett, C.3, Witton, R.1, Nelder, R.5, Lapthorne, D.4 1Plymouth University, 2Plymouth University, 3Coventry University, 4Public Health England, 5Plymouth City Council

117 Abdominal adiposity and periodontitis: a Mendelian randomisation study
Linden, G. J., Winning, L., Lundy, F., Kee, F., Patterson, C. Queen’s University Belfast

118 Impact of type-1 diabetes and periodontal status on life quality.
Desai, R.1, 2, Taylor, J. 1, 2, McCracken, G.2, Preshaw, P. 1, 2 1Institute of Cellular Medicine, School of Dental Sciences, Newcastle University, Newcastle upon Tyne, NE2 4BW, 2Centre for Oral Health Research, School of Dental Sciences, Newcastle University, Newcastle upon Tyne, NE2 4BW.

119 The association between periodontal pathogens and measures of adiposity
Winning, L., Patterson, C. C., Lundy, F. T., Kee, F., Young, I., Linden, G. J. Queen's University Belfast

120 Multilevel analysis of explanatory mechanisms in the relationship between income inequality and use of dental services
Bhandari, B., Newton, J., Bernabe, E. King’s College London dental institute
121 Global burden of oral and oropharyngeal diseases in 2010 as reflected in <i>Cochrane Database of Systematic Reviews</i>
Nasser, M., Karimkhani, C., Dellavalle, R.
Plymouth University Peninsula Schools of Medicine and Dentistry, Columbia University College of Physicians and Surgeons, Denver VA Medical Center

Wednesday, 16/09/2015 09:00 AM-10:30 AM Syndicate Room C
Periodontology Orals  Chair: Taylor, J. J.

122 A nano-biosensor for chair side monitoring of salivary MMP-8 in periodontitis
Taylor, J. J., Jaedicke, K., Williams, R. C., Bissett, S., Lansdowne, N., Stone, K., Pickering, K., Neeve, V., Lawson, V., Yatsuda, H., Kogai, T., Preshaw, P.
Newcastle University, Oj-Bio Ltd

123 Gingival Toll-like receptor and cytokine mRNA expression in equine periodontitis and oral health
Kennedy, R. S., Lappin, D. F., Bennett, D., Riggio, M.
University of Glasgow

124 Identification of novel salivary biomarkers for chronic periodontitis
Khudhir, A. S., Preshaw, P., Taylor, J. J.
Newcastle University

125 Zinc modulates IL-1b-stimulated chemokine secretion in human gingival fibroblasts
Michaud, S., Williams, R. C., Taylor, J. J., Valentine, R. A.
Newcastle University

126 Treatment of Periodontal Defects with Novel Bioactive Glass Containing Strontium
Hamed, N. A., Hill, R. G., Gillam, D. G., Karpukhina, N.
Queen Mary University of London, Queen Mary University of London

127 Ecology of volatile sulfur compound producing microbiota in health and chronic periodontitis
Queen Mary University of London, GlaxoSmithKline

Wednesday, 16/09/2015 09:00 AM-10:30 AM Syndicate Room A
Pulp biology and Stem Cells  Chair: Sloan, A.

Cardiff University

129 TNFα-induced p38 MAPK activation regulates TRPA1 and TRPV4 activity in human odontoblast-like cells
Queen’s University Belfast, Queen’s University Belfast, Aix University

130 Biodentine™ reduces TNF-α induced TRPA1 expression in odontoblasts
El Karim, I., About, I., Curtis, T., Linden, G. J., Lundy, F.
Queen’s University Belfast, Queen’s University Belfast, Marseille Université

131 Microfluidic Production of Stem-Cell Microcapsules for Spinal Cord Regeneration
Hidalgo, L.
Cardiff University
132 Exploring a role for biglycan in regulating the regenerative potential of mesenchymal stem cells in bone repair
Battersby, P., Sloan, A., Waddington, R.  Cardiff University

133 Development of 3D artificial niches for regenerative medicine
Ortega Asencio, I., Passley-Biggins, A. S., Thomson, O., Santocildes-Romero, M. E., Crawford, A., Hatton, P. The University of Sheffield

Wednesday, 16/09/2015  10:30 AM-12:00 PM  Assembly Room Poster Area
Wednesday Posters

134 Multilevel Principal Component Analysis (mPCA) for Shape Analysis: Initial Applications to Dental Research
Farnell, D. J., Popat, H., Richmond, S.  Cardiff University

135 Anatomical Variation At The Sites Of Autogenous, Mandibular Graft Harvest
Hamid, L., Adams, R., Binney, A., Claydon, N. C., Farnell, D. J., Thomas, D. W. 1  Cardiff University, 2Cardiff University, 3University of Wales

136 Feasibility of endoscope assisted surgery on oral and maxillofacial diseases with minimal incisions and invasion
Lai, Q., yuan, K., Luo, S., Yang, Z., Tang, X., Ci, J., zhang, z. 1  The second hospital of Shandong University, 2Peninsula Schools of Medicine and Dentistry, Plymouth University

137 Epoxy-Tiglianes Modulate Dermal Fibroblast-Myofibroblast Wound Healing Responses and Reduce Scarring
Dally, J., Moses, R., Midgley, A. C., Howard-Jones, R., Errington, R., Reddell, P., Steadman, R., Moseley, R. 1  Cardiff Institute of Tissue Engineering & Repair (CITER), School of Dentistry, 2QBiotics Ltd.

Maharjan, I. K. B.P koiral instutue of Health Science

139 Markers of disease activity and progression in Sjögren’s syndrome
Jazzar, A., Shirlaw, P., Carpenter , G., Proctor, G.  Kings College

140 The Perception Of Aesthetic Outcomes Of Dental Implant Restoration
Roopra, M., Claydon, N. C., Binney, A., Farnell, D. J., Thomas, D. W., Adams, R.  Cardiff University

141 Epoxy-tiglianes Stimulate Keratinocyte Proliferative and Migratory Responses, Enhancing Wound Re-epithelialisation.
Moses, R., Boyle, G., Reddell, P., Steadman, R., Moseley, R. 1  Cardiff University, 2QIMR Berghofer Medical Research Institute, 3QBiotics Ltd.

142 Hepatocyte Growth Factor Enhances Oral Mucosal Fibroblast Wound Healing Responses
Dally, J., Khan, J. S., Charalambous, C., John, H. L., Woods, E. L., Steadman, R., Midgley, A. C., Moseley, R. 1  Cardiff University, 2Institute of Nephrology, 3Institute of Nephrology

143 Randomised Clinical Trial of Biotène® Oral Rinse and Spray Efficacy
Akwagyiram, I., Gossweiller, A., Siddiqi, M., Bosma, M. 1  Oral Health Research Institute, Indiana School of Dentistry, 2InVentiv Health Clinical, 3GSK Consumer Healthcare
144 Extracellular vesicle mediated signalling in oral cancer progression
Ofield, M., Lambert, D., Hunt, S. University of Sheffield

145 Fibroblast ‘activation’ by media flow in cells of skin and oral origin.
Nithiananthan, S., Crawford, A., Lambert, D., Whawell, S. University of Sheffield

146 Activin A controls angiogenesis and influences oral squamous cell carcinoma development
Oliveira, C. E.1, Cervigne, N. K.1,2, Macedo, C. C.1, Leme, A. F.1, Graner, E.1, Lambert, D.4, Coletta, R. D.1
1State University of Campinas, 2Faculty of Medicine of Jundiai, 3Brazilian Biosciences National Laboratory, 4 University of Sheffield

147 The Role of Oral Cancer-associated Fibroblasts in Promoting Extracapsular Spread.
Pilborough, A., Lambert, D., Farthing, P., Khurram, A. University of Sheffield

148 Goblet cell mucin and the pathogenesis of salivary gland ductal stricture
Murtaza, U., McGurk, J., Brown, J., Hobbs, C., Proctor, G. King's College London

149 Use of 3D organotypic models of oral squamous cell carcinoma to examine the role of tumour-associated macrophages in tumour progression
Hadley, L., Colley, H. E., Murdoch, C. The University of Sheffield

150 The Chemokine Lymphotactin Increases Adhesion and Regulates Expression of Its Receptor In Oral Cancer Cells
Abdullah Zubir, A.1, Whawell, S.1, Wong, T.2, Khurram, A.1
1School of Clinical Dentistry, University of Sheffield, 2University of Sheffield

151 Functional expression of scavenger receptors by oral keratinocytes
Chasib, N. H., Colley, H. E., Murdoch, C. University of Sheffield

152 Preliminary results of a randomised controlled trial comparing regenerative endodontics versus root end closure with MTA in the management of non-vital, immature, permanent incisors
Gartshore, L., Jarad, F., Fox, K., Albadri, S. University of Liverpool

153 Marginal adaptation of CAD/CAM nanoceramic composite laminate veneers using two preparation designs
Zeitoun, R. M. Faculty of Dentistry Ain Shams University

154 Effects of 1% Micro-silicone Dioxide Filler on Soft Liner Mechanical Properties
Abood, A. Z. Middle Technology University, Foundation of Technical Education

155 Factors affecting OHRQoL with removable partial dentures: a retrospective cohort study
Ali, Z., Baker, S., Martin, N. University of Sheffield

156 Comparative Evaluation of Tricalcium Silicate, Mineral Trioxide Aggregate and Calcium Hydroxide as Dental Pulp Capping Materials: An In-Vivo Study
Agrawal, N. B.P. Koirala Institute of Health Sciences

157 A Randomised Clinical Study of the Measurement of Xerostomia Relief
JOSE, A.1, Skinner, J.2, Targett, D.1, Bosma, M.1
1GLAXOSMITHKLINE CONSUMER HEALTHCARE, 2Hill Top Research
158 Effects of ductal ligation/de-ligation on mTOR pathway and morphology of salispheres
Saleem, R. A., Proctor, G., Carpenter, G.
King's College London

159 Reasons for placement and replacement of restorations: an updated review
Eltahlah, D., Lynch, C. D., Chadwick, B. L., Blum, I. R., Wilson, N. H.
1Cardiff University, 2School of Dentistry, Cardiff University, 3School of Dentistry, Cardiff University, 4King's College, London Dental Institute

160 Exploratory Study Assessing Oral Health Knowledge Of Teachers In India.
PATIL, V., WESTON-PRICE, S. F., McNulty, C., HÖKSTRA, B., pine, c.
1QMUL Barts and the London School of Medicine and Dentistry, 2public Health England

161 Systematic review of the effectiveness of interventions using a component of habit formation theory to improve the uptake of preventive healthcare in adults
RAISON, M. H., Corcoran, R., Walley, S., Harris, R. University of Liverpool

162 Dental trauma in contact sports: Promoting prevention and emergency management
Dickie, J., Cross, L. 1University of Glasgow Dental School, 2Glasgow Dental Hospital and School

CHESTNUTT, I. G., Morgan, M., Monaghan, N. P., Collins, L., Sheppard, L. 1Cardiff University, 2Public Health Wales, 3NICE

164 The investigation of the effect of non-syndromic cleft lip and palate SNPs on lip morphological traits in an epidemiological study
WILSON-NAGRANI, C. E., Paternoster, L., Richmond, S. 1Cardiff University, 2Bristol University

165 The feasibility of alcohol misuse screening and treatment in a primary care general dental practice setting.
Roked, Z. Y., Moore, S., Shepherd, J. Cardiff School of Dentistry

166 Do outcomes in orthodontic research of cleft lip and palate patients reflect patient values?
Tsichlaki, A., Johal, A., Fleming, P. Queen Mary University of London

167 The relationship between adolescent's participation in community groups and dental caries in a deprived area in Brazil.
Rebelo, M. B., Silva, C. A., Rebelo Vieira, J. M., Vettore, M. V. 1Federal University of Amazonas, 2School of Clinical Dentistry, University of Sheffield, 3Municipal Health Secretariat of Manaus (SEMSA)

168 Surface Roughness Measurement of Carious Dentine Using Non-contact Optical Profilometry.
Ozel, B., Baysan, A., Anderson, P. 1Istanbul University, 2QMUL

169 Interim results investigating frequency of dietary acid intake and erosion.
O'Toole, S., Moazzez, R., Bartlett, D. King's College London

170 Antimicrobial Hydrogels For The Control Of Pulpal Disease
Everett, E. P., Waddington, R., Paul, A., Sloan, A. Cardiff University
171 Porphyromonas gingivalis preferentially invades oral keratinocytes in S phase of the cell cycle.
Al-Taweel, F. B., Douglas, I., Whawell, S. University of Sheffield

172 Porphyromonas gingivalis Lipopolysaccharide Isoforms Initiate Cytokine Tolerisation Responses in M1 and M2 Macrophages.
Strachan, A., Jackson, S. K., Harrington, Z., McIlwaine, C., Foey, A., Zarin, S. University of Plymouth

173 Novel Therapies Against Candida albicans Invasion And Biofilm Formation
Jack, A. 1, Pritchard, M. F. 1, Powell, L. C. 1, Onsøyen, E. 2, Rye, P. D. 2, Hill, K. E. 1, Thomas, D. W. 1
1Cardiff University, 2AlgiPharma AS

174 Unconventional protein secretion in innate immunity: The role of P2X7R in transglutaminase 2 export and activation
Griffiths, R. M., Adamczyk, M., Jones, A. T., Aeschlimann, D. Cardiff University

175 IL-34 suppresses Candida albicans-induced TNF-α production in M1 macrophages by down-regulating expression of Dectin-1 and TLR2
Xu, R., Wei, X., Song, B., Williams, D. W. Cardiff University

176 An in vitro model for evaluating denture cleaning toothpaste regimes
Marsh, L. L. 1, Williams, D. W. 2, Milward, P. 1, Wilson, M. 1, Lewis, M. 1, Rowe, W. 1, Bamford, S. 1, Bradshaw, D. 1, Roy, P. 2 1Cardiff University, 2GSK Consumer Healthcare

177 Lethal Photosensitisation of Prevotellaceae Under Anaerobic Conditions
Strother, M., Creber, H. K., Higham, S. M., Hope, C. K. University of Liverpool

178 Investigation Into The Rate of Bacterial Biofilm Removal by NaOCl Irrigant
Mohammed, S. A., Viana, M. E UCL- Eastman Dental Institute

179 Characterisation of a Temperate Phage Residing in the genome of the Anaerobic Bacteria Fusobacterium nucleatum polymorphum ATCC 10953

180 The Localisation Of Fluorescently Labelled OligoG In A Pseudomonal Biofilm<br />
Powell, L. C. 1, Pritchard, M. F. 1, Ferguson, E. 1, Onsøyen, E. 2, Rye, P. D. 2, Hill, K. E. 1, Thomas, D. W. 1 1Cardiff University, 2AlgiPharma AS

181 Trends in the antimicrobial susceptibility of anaerobic isolates from dentoalveolar abscesses
Pownall, M. R. 1, Thomas, O. D. 2, Lewis, M. 1, Williams, D. W. 1, Wilson, M. 1 1Cardiff University, 2University Dental Hospital. Cardiff and Vale University Local Health Board

182 The Candida albicans protein Ece1p promotes activation of human microvascular endothelial cells in vitro and drives systemic candidiasis in a zebrafish model.
Fantom, A. 1, Khalaji, M. 1, Tazzyman, S. 1, Moyes, D. L. 2, Wilson, D. 2, Hube, B. 2, Naglik, J. 2, Murdoch, C. 2 1University of Sheffield, 2Sheffield Hallam University, 3Dept Oncology, University of Sheffield, 4King's College London, 5Hans Knöll Institute, 6University of Aberdeen

183 Fungal bacterial interactions: from opportunistic pathogen to mutualistic biofilm communities
Rajendran, R. 1, Haggarty, J. 1, Kean, R. 3, O'Donnell, L. E. 1, Williams, C. 2, Burgess, K. 1, Ramage, G. 1 1University of Glasgow, 2University of the West of Scotland
184 Development of a sampling matrix and topic guide for qualitative in-depth interviews exploring the landscape of child protection in dentistry
Park, C. M.¹, Welbury, R.¹, Anderson, P.² ¹University of Glasgow, ²Glasgow School of Art

185 Can Dental Service Utilization Decrease The Occurrence Of Dental Pain?
Constante, H.¹, Peres, M.², Schroeder, F.¹, Bastos, J.¹ ¹Federal University of Santa Catarina, ²The University of Adelaide

Wednesday, 16/09/2015 11:30 AM-01:00PM Syndicate Room D
Evaluation of interventions  Chair: Mason, S.

186 A New Fluoride Containing Bioactive Glass Additive For Toothpastes
Hill, R. G.³, Gillam, D. G.¹, D’Onofrio, A.², Mneimme, M.¹, Shah, P.², Karpukhina, N.³ ¹Barts & The London, ²Queen Mary University of London, ³BioMin Technologies, ⁴QMUL, ⁵QMUL

187 Dentifrice Delivery into Dental Plaque Biofilms by High-velocity Microsprays
Fabbri, S.⁶, Johnston, D. A.¹, Rmaile, A.², Aspiras, M. B.³, de Jager, M.², Starke, M.², Ward, M.², Stoodley, P.⁵,⁶ ¹Biomedical Imaging Unit, ²Oral Healthcare Research, ³Wm. WRIGLEY Jr. Company, ⁴Philips Oral Healthcare Inc. (POH), ⁵The Ohio State University, ⁶University of Southampton

188 Stain Control Efficacy and Tolerability of an Oral Hygiene Regimen
Creeth, J. E.¹, Milleman, K.², Milleman, J.², Gordon, J.³, Jain, R.¹, Butler, A.¹ ¹GlaxoSmithKline Consumer HealthCare, ²Salus Research

189 A novel intervention for oral malodour reduction: A randomized clinical trial on the effect of Philips Sonicare TongueCare+ and BreathRx
Gomez-Pereira, P. R.¹, Saad, S.², Hewett, K.², Horstman, P.¹, Patel, J.¹, Greenman, J.² ¹Philips Research, ²University of West of England,

190 The effect of toothbrush abrasion force on dentine hypersensitivity <i>in-vitro</i>
Sehmi, H., Bartlett, D., Olley, R.  King’s College London

191 Polymer Therapeutics for Sustained, Controllable Release of Growth Factors to Promote Cranial Nerve Repair and Regeneration by Stem Cells.
Ferguson, E.¹, Naseer, S.¹, Powell, L. C.², Zhu, B.¹, Liu, Q.¹, Song, B.¹, Thomas, D. W.⁴ ¹Cardiff University, ²Cardiff University, ³Cardiff University, ⁴Cardiff University

Wednesday, 16/09/2015 11:30 AM-01:00PM Syndicate Room B
Microbiology Orals  Chair: Williams, D.

192 Influence Of Nanopatterning On Oral Bacteria Adhesion onto Surfaces<br />
Aguayo, S.¹, Dalby, M.³, Gadegaard, N.³, Donos, N.², Spratt, D.², Bozec, L.³ ¹UCL Eastman Dental Institute, ²UCL Eastman Dental Institute, ³University of Glasgow, ⁴UCL Eastman Dental Institute

193 Dentures: a pathogenic reservoir for respiratory pathogens
Hannah, V. E.¹, O’Donnell, L. E.¹, Smith, K.²,¹, Nile, C. J.¹, Lappin, D. F.¹, Dickie, J.¹, Calvert, G.¹, Robertson, D.¹, Bagg, J.¹, Ramage, G.¹ ¹University of Glasgow, ²University of West Scotland
194 Development of an In-Vitro Tissue Model for Biofilm Infection
Morse, D. J., Williams, D. 2, Wei, X. 4, Wilson, M. 3, Lewis, M. 6, Bradshaw, D. 5
1Cardiff University, 2Cardiff University, 3Cardiff University, 4Cardiff University, 5GlaxoSmithKline, 6Cardiff University

195 Interleukin-24 Is Expressed In Gingival Fibroblasts And Promotes Keratinocyte
Interleukin-17
Williams, R. C., Serrage, H., Rowan, A. D., Preshaw, P., Taylor, J. J. Newcastle University

196 Cholinergic modulation of Candida albicans pathogenic potential
Rajendran, R. 1, O’Donnell, L. E. 2, Lappin, D. F. 3, Ramage, G. 4, Nile, C. 1
1University of Glasgow, 2University of Glasgow, 3Dental School, 4Dental School

197 Periodontal Pathogens and their Sialidases
Frey, A. M. 1, Phansopa, C. 1, Parker, J. 1, Pratten, J. R. 2, Middleton, A. 2, Patel, N. 2, Douglas, I. 3, Murdoch, C. 3, Stafford, G. P. 1
1The University of Sheffield, 2GlaxoSmithKline

Wednesday, 16/09/2015 11:30 AM-01:00 PM Assembly Room
MINTIG Symposium Chair: Al-Jawad, M.
Sponsored by Colgate Oral Health Network

Emerging technologies for understanding and mimicking dental hard tissue growth: Early Career Researchers Focus

Dr Wayne Nishio Ayre (Cardiff University)
Delivery of osteogenic molecules to encourage regenerative bone repair around polymeric biomaterials

Dr Yvonne Pang (Kings College London)
A stem cell niche at the tip of mouse incisors regulates homeostatic tissue repair

Kseniya Shuturminska (Queen Mary University of London)
Use of elastin-like proteins in understanding and controlling apatite mineralisation

Dr Colin Freeman (University of Sheffield)
Understanding the early stages of Apatite Growth

Wednesday, 16/09/2015 11:30 AM-01:00 PM Syndicate Room A
Oral Health Promotion Orals Chair: Kay, E. J.

198 A Review of Approaches for Dental Practice Teams for Promoting Oral Health
Kay, E. J. 2, Vascott, D. 1, Hocking, A. 1, Helen, N. 2, Charles, D. 1, Scales, H. 1
1Plymouth University, 2Plymouth university, 3British Dental Association

199 Economic Evaluation of public health interventions to reduce caries in children at high risk
1Plymouth university, 2National Institute for Health and Care Excellence, 3York Health Economic Consortium

200 Feasibility of screening for diabetes in General Dental Practice
Bould, K. J., Dunne, S., Scott, S., Asimakopoulou, K. King’s College London
201 Urinary fluoride excretion in children aged 4-5 years living at low and high altitude in Nepal
Sah, O. P.¹, Atkinson, G.², Maguire, A.³, Zohoori, V.⁴ ¹Health and Social Care Institute, Teesside University, ²Health and Social Care Institute, Teesside University, ³Centre for Oral Health Research, School of Dental Sciences, Newcastle University, ⁴Health and Social Care Institute, Teesside University

202 Head Injuries in Early Childhood: Is There a Social Gradient?
Letelier, A. University College London

203 The impact of oral health risk indicator labels on prevention uptake
Sharma, S.¹, Vernazza, ², Steele, J.²,³, Finch, T.⁴ ¹NEWCASTLE UNIVERSITY, ²Newcastle university, ³Oral Health Services Research, ⁴Institute of Health and Society

Wednesday, 16/09/2015  1:00PM-02:00 PM  OMIG group lunch - Room A
Wednesday, 16/09/2015  1:00PM-02:00 PM  MINTIG group lunch - Room B
Wednesday, 16/09/2015  1:00PM-02:00 PM  DMG group lunch - Room C
ABSTRACTS

Novel Bioactive Glass Cements For Bone Grafting: From An In-Vitro To An In-Vivo Study
D’Onofrio, A., Shahdad, S., Rawlinson, S., Liu, J., Kent, N., Hill, R. G.

Objectives An ovine study has previously been conducted to assess the performance of novel bioactive glass based calcium-phosphate cements and indicated that are osteoconductive and able to osseointegrate with host bone (bone to graft contact: 90%). In this study we developed new strontium (Sr) containing compositions. Cement properties, bone cells activity were investigated. The addition of Sr is hypothesised to enhance bone remodelling in-vivo.

Methods Glasses were synthesised by progressively substituting Sr\^{2+} for Ca\^{2+} on a molar basis. Cements were prepared by mixing the glass powders with Ca(\(\text{H}_2\text{PO}_4\))\(_2\) and a 2.5% Na\(_2\)HPO\(_4\) solution. XRD, compressive strength and ions release were measured after 1h, 1d, 7d and 28d immersion in TRIS buffer solution. Setting times and Radiopacity were tested. Cell culture was performed using MC3T3-E1 osteoblast cell line. A pilot minipig study was conducted to compare performances of Sr free against 25%-Sr containing compositions for socket preservation and guided bone regeneration around dental implants. Specimens were retrieved after 3 and 6 months and analysis using XMT, BSSEM and Histology has been carried out.

Results Sr release and radiopacity increased proportionally with Sr content in the glass. Compressive strength showed a maximum value of 12.5 MPa. XRD showed that octacalciumphosphate was the main phase present after 1h and 1d and after 28d was completely transformed to Sr-containing HA (SrHA). In general, fewer cells grew on the surface of 0%-Sr discs when compared to those with higher content. Preliminary in vivo results suggests high osseointegration rates (90%) and high remodelling rates for the Sr-containing cement after 6 months of implantation (>50%).

Conclusions A novel method to develop a bone substitute forming in vitro SrHA as a final product by using a bioactive glass as a precursor was shown. A correlation cements properties and Sr content was witnessed. The addition of Sr might promote a faster bone remodelling in-vivo.

Notes
Neutrophil directional chemotaxis in children with Papillon Lefèvre Syndrome
Roberts, H., White, P., Grant, M., Chapple, i.
University of Birmingham

Objectives Aim: To functionally characterise neutrophil behaviour in PLS patients compared with healthy gender and age-matched controls.

Papillon-Lefèvre Syndrome (PLS) is an extremely rare inherited autosomal recessive disease apparent in children aged between 1-4 years and a prevalence of 1-4 people per million. PLS is characterised by palmoplantar keratosis and severe periodontal destruction leading to premature and permanent loss of teeth, 20-25% of PLS cases suffer from an increased susceptibility to other infections.

PLS is caused by a mutation in the cathepsin C gene (CTSC), resulting in a complete loss of activity and subsequent failure to activate immune response proteins. The underlying cause of periodontal disease in PLS patients is thought to arise from a consequent defect in neutrophils, which are vital for defence against pathogens and in the progression of periodontal inflammation.

Methods
Neutrophils were isolated from the peripheral blood of 5 genotyped PLS patients alongside healthy age and gender-matched controls. Chemotaxis was analysed by real-time video-microscopy. Other neutrophil immune functions including Reactive Oxygen Species (ROS) generation and Neutrophil Extracellular Trap (NET) formation were also assayed using periodontally relevant bacteria/other stimuli.

Results No significant differences were observed for neutrophil speed or velocity towards host and bacteria-derived chemoattractants, however PLS neutrophils exhibited reduced chemotactic accuracy. PLS neutrophils generated significantly more ROS and significantly lower NETs when stimulated.

Conclusions These results enhance our understanding of the underlying neutrophil defect in PLS patients.

Notes
**Antibacterial Properties of Oral Progenitor Cells: The role of Soluble Factors**

**Board Davies, E.**, Moses, R., Sloan, A., Stephens, P., Davies, L.  

*Cardiff University, *Cardiff University, *Karolinska Institutet*

**Objectives** Oral mucosal lamina propria-progenitor cells (OMLP-PCs) are a multipotent PC population with known immunosuppressive properties. Many immunomodulatory soluble factors are also documented to be antimicrobial; leading to the hypothesis that OMLP-PCs, in addition to their immunoregulatory actions, may possess antibacterial properties. We have previously presented data demonstrating the broad spectral antibacterial properties of OMLP-PCs through a constitutive and contact-independent mechanism. The current aims of this study are to elucidate the soluble factors involved in mediating this effect.

**Methods** OMLP-PCs (± pre-treatment with interferon (IFN)-γ) were incubated with Gram positive or Gram negative bacteria for 7-14hrs (midlog of each bacterium). Genomic and protein levels of potential soluble antibacterial factors were examined by QPCR, ELISA and/or Western Blotting. Retained supernatants were filter-sterilised and cultured with live bacteria ± blocking antibodies to identified factors. The antibacterial effects of the identified factors were also confirmed by co-culture of the pure peptide form with each bacterium.

**Results** Indoleamine 2,3 dioxygenase (IDO) expression and activity directly correlated with IFN-γ exposure and did not contribute to OMLP-PC antibacterial activity. LL37 expression was not detected, irrespective of IFN-γ and/or bacterial exposure. Haptoglobin was detected in the supernatant samples and found to be antibacterial against Gram negative bacteria at low levels (50μg/mL), with blocking restoring bacterial growth (P<0.01). OPG expression and secretion was constitutive and found to be antibacterial against Gram positive bacteria, with blocking significantly restoring bacterial growth (P<0.05).

**Conclusions** OMLP-PCs secrete a range of soluble factors capable of mediating their antibacterial effects via a complex interplay of direct bacterial interactions and also potential signalling to innate immune cells. Unlike bone marrow mesenchymal stromal cells, LL37 and IDO do not play a role in mediating OMLP-PC antibacterial effects, confirming the role the *in vivo* microenvironment plays in modulating the phenotype of PCs.

**Notes**
Biomarker Study on Oral Cancer Exosomes
Qadir, F., Teh, M.
Queen Mary University of London

Objectives Extracellular vesicles (EVs) are released by almost all cell types. They are involved in intracellular communications as vehicles for transfer of functional membranes, cytosolic proteins, lipids, RNAs and DNA. EVs are classified according to their mechanism of origin, composition, size and density. Exosomes are nano-size particles, measuring 40-100 nm. Depending on their origin they are capable of altering the fate of recipient cells through the transferred information. In cancer biology exosomes offer both diagnostic and therapeutic advantage. Their involvement in cell-cell communication indicates their influence in tumour development, progression, metastasis and therapeutic efficacy. Exosomes released by cancerous cells carry numerous biomarkers, which are passed on to healthy cells. In this study we characterised and investigated the functional significance of exosomes derived from SVpgC2a (oral epithelial pre-cancerous) and SVFN10 (transformed) cell lines.

Methods The characteristics of exosomes were first confirmed by Scanning Electron Microscopy, Transmission Electron Microscopy, Zetasizer and Nanosight Tracking Analysis, along with western blot for specific exosomal membrane proteins. Further we validated the presence of RNA within exosomes that remained stable after treatment with proteinase K and RNase. The quality and size distribution of RNA extracted from exosomes were analysed on Agilent BioAnalyser.

Results We found that, similar to parental cell line SVFN10, exosomes derived from SVFN10, but not SVpgC2a, were abundant in FOXM1 mRNA, an oncogene involved in cancer initiation and progression. Exosomes derived from SVFN10 were found to be 20-50% larger than exosomes derived from SVpgC2a cells. Exposure of SVpgC2a cells with exosomes derived from SVFN10 induced a distinct morphological change in the SVpgC2a cells within 24 hours. Exposure of SVpgC2a cells with its own exosomes or exosome-free supernatant did not induce significant morphological change.

Conclusions This is the first evidence showing the presence of oncogene mRNA within exosomes secreted by a transformed malignant oral keratinocyte cell line. This finding has tremendous potential for clinical translation into a non-invasive oral cancer diagnostic tool.

Notes

Purkayastha, M., McMahon, A., Gibson, J., Conway, D.
University of Glasgow

Objectives To assess the current incidence burden of oropharyngeal (OPC), oral cavity (OCC), and laryngeal cancer in Scotland, and examine the factors influencing trends between 2001 and 2012.

Methods Our study included all diagnosed cases of OCC (C00.3-C00.9, C02-C06 excluding C2.4), OPC (C01, C2.4, C09-C10, C14), and larynx (C32), registered at the Scottish Cancer Registry between 2000 and 2012. We collated annual midterm population estimates by age, sex, Scottish Index of Multiple Deprivation (SIMD), and region. Age-standardized rates and fully adjusted Poisson regression rate-ratios (RR) were used to compare all subsites, by age, sex, region, SIMD, and year of diagnosis. All statistical analyses were performed using SAS 9.3, and level of significance was fixed at 0.05.

Results The fully adjusted Poisson regression model showed that males exhibited significantly higher rates of OPC (RR 3.31; 95% Confidence Interval (CI) 3.02-3.62) and OCC (RR 1.82; 95% CI 1.71-1.94) compared to females. The peak age of incidence of OPC was slightly lower (61-65 years) than the other sub-sites (71-75 years). Between 2001 and 2012, there was a two-fold increase in rates of OPC (RR 1.85; 95% CI 1.53-2.25), rates of OCC were seen to plateau, and rates of laryngeal cancer decreased (RR 0.77; 95% CI 0.65-0.90). The most socioeconomically deprived areas had the highest rates of OPC (RR 3.33, 95% CI 2.72-4.07), OCC (RR 2.69, 95% CI 2.31-3.13), and laryngeal cancer (RR 4.98, 95% CI 4.15-5.97), and an almost dose-like response was observed with increasing deprivation increasing cancer risk.

Conclusions In Scotland in the decade to 2012, the incidence rates of OPC rose, while OCC were stable and laryngeal cancer decreased. The highest rates of cancer were seen in males in the 60-75 year age group and those from the most deprived areas.

Notes
Salivary changes in mechanically ventilated patients are associated with respiratory pathogen colonization of dental plaque


School of Dentistry, Central Biotechnology Services, Adult Critical Care, University Hospital of Wales,

**Objectives** Ventilator associated pneumonia (VAP) is an infection of mechanically ventilated (MV) patients with high morbidity and mortality. Importantly, VAP has been linked to changes in the oral microbiome where dental plaque becomes colonized with respiratory pathogens during mechanical ventilation. The cause(s) of this microbial change has not been determined, and this is important since the prevention of dental plaque providing a reservoir of VAP pathogens would be a key management tool. The objectives of this study were to analyse salivary parameters in MV patients and relate any changes to the dynamics of plaque microbial composition.

**Methods** Dental plaque and saliva samples were obtained from 107 MV patients during mechanical ventilation and post extubation, using paper points and Salivette devices, respectively. The microbial composition of plaque was established by culture and next generation sequencing, whilst volume, pH and total protein concentration of saliva was also measured. Protein composition of saliva was also determined using gel-based and LC-MALDI methodology. Inflammatory cytokines in saliva, including TNFα, IL-1β, IL-6, IL-8 and IL-1β were also determined using cytometric beads array (CBA).

**Results** A ‘microbial shift’ in the dental plaque of MV patients was established with colonization by respiratory pathogens occurring during MV. Importantly, a reversion of this change to a dental plaque associated with health was encountered following extubation. During MV associated changes in saliva included a reduced volume and pH, together with changes in the salivary proteome. Significant increases in the pro-inflammatory cytokines IL-6, IL-1β and IL-8 were also evident during MV, and these reduced to levels observed in healthy volunteers post extubation.

**Conclusions** Observed changes in salivary factors in MV patients may be lead to an oral environment conducive to the colonization of respiratory pathogens in dental plaque, recognized as a risk factor for VAP. These results can be exploited for the management of MV patients. Administration of an appropriate artificial saliva of protein content and pH could limit respiratory pathogen colonization of plaque, whilst monitoring salivary cytokine profiles may serve as a non-invasive diagnostic/prognostic marker for VAP in MV patients.

**Notes**
Viral Status and Microenvironment In Oropharyngeal Cancer: A Therapeutic Target
Bolt, R., Lambert, D., Foran, B. H., Thomas, S., Murdoch, C., Hunter, K.

1School of Clinical Dentistry, 2School of Clinical Dentistry, 3Weston Park Hospital, 4Sheffield Cancer Research Centre, 5School of Clinical Dentistry

Objectives
1. Determine whether there is a difference between microenvironmental interactions in HPV-positive and -negative oropharyngeal carcinoma.
2. Establish whether these differences influence tumour behaviour
3. Define the underlying secretome responsible for altering tumour behaviour
4. Establish whether targeted therapeutics can limit microenvironmental support for tumour invasion in vitro, as evidence for therapeutic potential in-vivo

Methods
Conditioned media was collected from HPV-positive and HPV-negative oropharyngeal carcinoma lines and used to stimulate normal fibroblasts. Further conditioned media was then collected from the stimulated fibroblasts, and used in migration/proliferation experiments. A multitude of techniques were used to determine candidate molecules responsible for observed differences between HPV-positive and -negative cell line interactions. Clinically relevant inhibitors were then used to confirm whether interactions could be disrupted in-vitro and also to validate candidate molecules. Finally, a range of functional and analytical toxicity assays were undertaken to confirm that the efficacy of these inhibitors was attributable to receptor targeting.

Results
HPV-negative lines consistently demonstrated increased migration in response to stimulated fibroblast media, whereas HPV-positive lines did not. HGF and IL-6 were identified as key factors promoting cell migrations. Co-incubation of Foretenib or INCB28060 led to near-total abrogation of the additional migrations; all toxicity assays confirmed this effect was due to receptor targeting.

Conclusions
HPV-negative lines induced a characteristic stromal reaction that supported migration. HGF has a central role in the modelled interactions, although supplementation of intracellular STAT activity via IL-6 and/or constitutive STAT activation appears to have compounded HGF’s effect; these additional derangements noted in HPV-negative cell lines may be the consequence of greater tumour evolution in HPV-negative disease. Clinically-relevant cMet inhibitors show promise in therapeutically targeting Oropharyngeal Carcinoma, and may be employed on the basis of Virus status in combination with biomarker analysis.

Notes
A Study of Biomarkers in Patients with Periodontal Disease

Gul, S. S., Douglas, I., Griffiths, G., Rawlinson, A.

1The University of Sheffield, 2The University of Sheffield

Objectives Individual biomarkers have failed to provide useful predictive information for the response of sites to periodontal treatment. Therefore, the aim of this study is to determine whether combinations of enzymes (MMP-8, Elastase, Sialidase) in gingival crevicular fluid (GCF) along with levels of Porphyromonas gingivalis and Tannerella forsythia in subgingival plaque can be used as an improved prognostic “finger print” for the outcome of periodontal treatment.

Methods 89 subjects participated in a 6-month longitudinal study. Full mouth clinical parameters (probing pocket depth, clinical attachment loss, plaque index and bleeding index) plus GCF and plaque samples were collected from 3 representative sites: healthy (≤ 3mm), deep non-bleeding (DNB) (≥ 6mm) and deep bleeding (DB) (≥ 6mm) at baseline, 3 and 6 months. Patients received standard nonsurgical periodontal treatment. GCF was assayed for each enzyme and plaque for bacteria using colourimetric substrates and qPCR, respectively. Biomarker profiles were analysed by logistic regression on a site-by-site basis for prediction of a 2mm improvement in probing pocket depth.

Results 50 subjects have completed the 6-month interval to date. Clinical treatment resulted in reduction of mean probing pocket depths (≥6mm) by 2.1mm. However, 33% of DNB and 35% of DB sites did not improve by 2mm in pocket depth. All biomarkers were significantly higher in diseased sites than healthy sites and low levels of these biomarkers at baseline were associated with better treatment outcome. Logistic regression showed that a combination of enzymes at base-line provided accurate predictions of treatment outcome for DNB sites (84.8%) and DB sites (82%). When combined with the levels of bacteria, prediction values were 91% (DNB) and 92% (DB). Each biomarker alone failed to predict >61% of improvements.

Conclusions Combined profiles of the above biomarkers offer a significantly improved indication of a site’s response to non-surgical periodontal treatment than they do as single biomarkers.

Notes
A parallel sided randomised controlled trial to determine if the process of warming composite resin restorative material prior to placement of a restoration leads to changes in postoperative sensitivity.

Campbell, I. M.
Leeds Dental Institute

Objectives To determine if preheating a composite resin restorative material leads to an increase in postoperative sensitivity after 24 hrs.

Methods 120 patients (aged between 18 and 70) from a private primary care dental practice who required a one or two surface restoration were randomly divided into two groups of 60. One group had a microhybrid composite placed at room temperature the other group had the composite warmed to 39°C before placement. The patients were randomly allocated to each group by the Dental Translational Research Unit, University of Leeds. All recruitment and procedures were carried out by a single operator. VAS sheets were used to record sensitivity scores before restoration placement and to measure the primary outcome after 24 hours. Additional sheets were used to record secondary outcomes at 1 week, 2 weeks and 1 month. The patients were telephoned to remind them to fill in the sheets. The operator and patients were blinded to the type of intervention throughout the study.

Results 120 patients were recruited and randomised. Data from 57 patients in the heated group and 58 in the room temperature group were analysed. A Shapiro-Wilk test indicated a non-parametric analysis would be required ($p < 0.05$). A Mann-Whitney test of postoperative sensitivity showed no detectable difference between the 2 sides of the trial. The null hypothesis was retained that there is no difference in postoperative sensitivity between composites placed at room temperature and preheated to 39°C ($p$-value of 0.162). No adverse effects were reported throughout the trial.

Conclusions There was no difference in postoperative sensitivity between the two groups. This study was part of a Masters research project. Trial register/number: ISRCTN76727312

Notes
A novel implant cleaning method - *In vitro* non-contact biofilm removal from SLA and polished titanium surfaces using cavitation from an ultrasonic scaler

**Vyas, N., Sammons, R. L., Addison, O., Dehghani, H., Walmsley, A. D.**

University of Birmingham

**Objectives** Removal of biofilm from dental implants is difficult and many methods damage the surface coating. The aim of this study was to investigate the ability of cavitation from ultrasonic scalers to remove biofilm grown on polished and sandblasted, large-grit acid-etched (SLA) titanium surfaces via high speed imaging and scanning electron microscopy (SEM).

**Methods** 24 CpTi Grade II discs were either polished to mimic a dental implant collar or sandblasted and acid etched to produce a surface mimicking the Straumann SLA surface. *Streptococcus mutans* biofilm was grown on the discs in a bioreactor. A Satelec P5 Newtron ultrasonic generator with tip 10P was operated at medium power at either 0.5 mm or 1 mm away from each disc for 30s. High speed video was recorded during the removal process at 128,000 frames per second to visualise cavitation. SEM images of the discs before and after removal were used to evaluate the effectiveness of cleaning, using SEM finder grids and image registration to show the exact areas the biofilm had been removed from. The results were compared to a control experiment performed at low power where cavitation did not occur around the scaler.

**Results** SEM showed biofilm was removed from the discs at distances of both 0.5 mm and 1 mm away when the ultrasonic scaler was operated at medium power, but not at low power. High speed videos showed cavitation clouds from the scaler tip impacting on the disc at 0.5 mm separation. This did not occur at 1 mm separation, but cavitation bubbles were still observed to be growing and collapsing on the surface of the disc.

**Conclusions** Cavitation does remove biofilm from roughened dental implant surface without causing damage. Ultrasonic scalers may be used in a non-contact mode as a new method of cleaning dental implants.

**Notes**
A phenotypic re-evaluation of human γδT cells in health and disease: Is Vδ2(+) T cell depletion a risk predictor for BRONJ?
1Barts and The London Medical School, Queen Mary University of London, 2Institute of Dentistry, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, 3Institute of Dentistry, Queen Mary University of London, 4Eastman Dental Institute, University College London, UCL, 5Royal London Hospital, 6Barts Health NHS Trust

Objectives Bisphosphonate Related Osteonecrosis of the Jaw (BRONJ) is a chronic jawbone necrosis occurring in ≈ 0.1-7% of patients receiving bisphosphonate medication for osteoporosis and certain cancers. Bisphosphonates potently activate human γδ T cells and specifically the Vδ2(+) subtype. The pathogenesis of BRONJ is still uncertain but to date an immune-mediated pathology has largely been ignored. The objectives of the study were to firstly re-evaluate the most appropriate Vδ2(+) phenotypic markers to describe Vδ2(+) subsets, secondly to determine the functional potential of these subsets, and finally to assess whether Vδ2(+) T cells are implicated in BRONJ pathogenesis.

Methods Peripheral blood mononuclear cells were isolated by density gradient separation from 63 healthy individuals. Flow cytometry was used to determine extracellular marker expression on Vδ2(+) T cells with further characterisation using intracellular cytokine staining and proliferation assays. A gene microarray was subsequently performed to further characterise sorted Vδ2(+) subsets. Finally, the healthy cohort was compared to 8 BRONJ and 10 long-term bisphosphonate patients without BRONJ to determine peripheral blood Vδ2(+) levels and phenotype.

Results Re-evaluation of conventional Vδ2(+) T cell surface markers in healthy individuals showed that CD45RA is an unreliable marker for accurate identification of functionally-relevant subsets. An alternative marker set (CD28, CD27, and CD16) unambiguously identifies four Vδ2(+) T cell subsets which can be used to subdivide human individuals into six stable “Vδ2-profiles”. Analysis of these profiles demonstrates a surprisingly wide spectrum of Vδ2(+) phenotypes. Patients on long-term bisphosphonates including BRONJ patients had significantly depleted peripheral blood Vδ2(+) T cells with no apparent characteristic disease signature phenotype (p < 0.01).

Conclusions Depletion of peripheral blood Vδ2(+) T cells is characteristic of BRONJ and patients on long-term bisphosphonates and may represent a useful risk predictor for BRONJ.

Notes
The Role of the Outer Membrane Proteins of *P. gingivalis* in Host-Pathogen Interactions

Naylor, K. L.¹, Murdoch, C.², Douglas, I.³, Stafford, G. P.⁴

¹The University of Sheffield, ²University of Sheffield, ³The University of Sheffield, ⁴The University of Sheffield

**Objectives** *Porphyromonas gingivalis* is considered a keystone pathogen in periodontal disease. As part of its pathogenic features its ability to interact with host epithelial cells is central. During the invasion process, surface associated proteins, e.g. fimbriae, are crucial. Work in Sheffield identified a signature set of differentially regulated genes identified in an invasive subtype of the natural *P. gingivalis* population. These included the outer membrane protein encoding *ompA* and associated *ompH* genes. The aim of this study is to investigate the role of *ompA* and other signature set genes in interaction with oral epithelial cells and biofilm formation.

**Methods** Using knockout mutagenesis, strains lacking the *ompA*₁-₂ operon and individual *ompA* genes alongside an *ompH*₁-₂ mutant were created. The effects on invasion and biofilm formation were investigated using antibiotic protection assays and biofilm staining respectively. For protein-protein interaction studies, binding of purified recombinant truncated OmpA protein and biotinylated OmpA peptides with oral cells is being investigated using fluorescence microscopy, cross-linking, and affinity chromatography to isolate interacting partners that will be identified by Mass Spectroscopy.

**Results** *ompA*₁-₂ and *ompA*₂ gene knockouts display reduced attachment and invasion of epithelial cells, while *ompA*₁ does not. Biofilm formation by the mutants mirrors these data with a 20-fold reduction in the *ompA*₁-₂ mutant and two-fold reduction for *ompA*₂. Meanwhile, a ∆*ompH* mutant displays altered cellular protein profile (SDS-PAGE) and is being investigated similarly. To investigate direct OmpA-human interactions we are using fluorescent antibodies with a recombinant soluble form of OmpA2 and biotinylated predicted OmpA2 surface loop peptides. In addition, these peptides reduce interaction of *P. gingivalis* with oral cells in vitro.

**Conclusions** These results illustrate a role for OmpA2 and its predicted surface loops in cellular interactions and biofilm formation and highlight a role for *ompH* in assembly of OmpA and other surface proteins.

**Notes**
In vitro effects of a novel hydroxyapatite-fluoride agent (IWI2) upon enamel repair and remineralisation

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Objectives
1. To trial air abrasion to generate enamel surface defects of controlled extent matched to a control smooth enamel surface
2. To evaluate the repair and remineralisation of enamel surface defects and artificial white spot lesions (WSLs) using two variations of a novel remineralisation agent, IWI2 (Sylphar Ltd), against positive and negative controls

Methods
Polished human enamel specimens were allocated to six treatment groups: two IWI2 variations, casein phosphopeptide amorphous calcium phosphate (CPP-ACP) (GC Corp.), 1500ppm fluoride gel, a placebo gel and a negative control. Alumina powder air abrasion was used to generate enamel surface defects.

Repair was evaluated using SEM, confocal profilometry, and Raman spectroscopy. Optical coherence tomography was used to evaluate the remineralisation of a separate artificial white spot lesion model.

Specimens were maintained in artificial saliva at 37.5°C and material applied to samples for 20 minutes for 5 consecutive days. They were assessed at 0, 1, 2, 5 and 30 days.

Data were analysed using one-way analysis of variance tests (p = 0.05).

Results
SEM and confocal profilometry showed significantly more infill of enamel surface defects with application of IWI2 than the negative controls. Similar results were achieved with CPP-ACP. One of the IWI2 formulations, IW37, resulted in significantly more mineralisation of enamel surface defects. Fluoride performed similarly well, but no other treatment group significantly differed from the negative control.

Furthermore, IW37 and CPP-ACP resulted in a significant reduction in OCT subsurface light scattering of WSLs relative to other treatment groups. No other treatment group was better than the negative control.

Conclusions
Air abrasion is a suitable method for creating enamel surface defects. Both IWI2 formulations enhanced enamel surface repair; and IW37 contributed to the mineralisation of both models.

Notes
Periodontal pathogens harvest sugar from human sialylated proteins, but which ones?

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Objectives Sialic acids are a group of carbohydrates, found extensively in eukaryotic glycoproteins. Periodontal pathogens possess sialidase enzymes which grant the ability to cleave sialic acid from human sialoglycoproteins, serving a variety of functions for pathogens including nutrient acquisition, biofilm formation, immunoregulation, and host cell association. This work aims to identify the sialoglycoproteins targeted by the important red complex pathogen *Tannerella forsythia*, and the potential use of novel sialidase inhibitors to prevent loss of sialic acid from sialoglycoproteins.

Methods Labelling cell surface sialoglycoproteins of oral epithelial cell lines was achieved by aniline oxidation of surface sialic acids followed by biotinylation and visualisation by Streptavidin-Texas-Red staining by fluorescent microscopy. Purification of biotinylated sialoglycoproteins with streptavidin agarose beads allowed analysis of the sialoglycoproteome by SDS-PAGE. The effect on the sialoglycoproteome to sialidase from *Tannerella forsythia* in the presence or absence of sialidase inhibitors was observed. A fluorescence based assay was used to assess the efficacy of potential inhibitors for pathogen sialidases. Sialidase activity was quantified by release of fluorescent methylumbelliferone from a methyumbelliferyl-sialic acid conjugate by *T. forsythia* sialidase, in the presence and absence of inhibitors.

Results Fluorescence microscopy revealed that sialic acids were abundant on surfaces of oral epithelial cells, and treatment with *T. forsythia* sialidase drastically reduced the level of sialic acid. Extraction of these membrane sialoglycoproteins is ongoing. Novel inhibitors were capable of significantly reducing *T. forsythia’s* sialidase activity, with some decreasing activity by ~80% at 1mM.

Conclusions Analysis of the sialoglycoproteome of oral cell lines is ongoing. A range of inhibitors tested proved successful in reducing *T. forsythia’s* sialidase activity. These results highlight the potential for development of sialidase inhibitors for periodontitis therapy.

Notes
The relationship between periodontal disease and non-communicable diseases associated with endothelial dysfunction

Johnson-Idan, J., Hon, J. M., Sabbah, W., Hughes, F. J.
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Objectives Periodontal diseases (PD) have been associated with risk of a number of non-communicable diseases and in particular those associated with endothelial dysfunction such as cardiovascular disease, chronic kidney disease (CKD) and cerebrovascular disease. Given our recent finding of the association of Calcium Channel Blocker drugs (CCBs) with Periodontal Disease, the aims of this study were to investigate the association of periodontal disease with these conditions in the NHANES 2011-12 dataset and secondly to determine if CCBs might act as a confounder for this association.

Methods We performed a retrospective analysis of the 2011-2012 NHANES (National Health and Nutritional Examination Survey) dataset. We tested the relationship of moderate to severe periodontitis (Page and Eke (2012) against CKD (stages 3-5), Ischaemic heart disease (IHD) and cerebrovascular disease (CVD).

Results 3313 participants of ages 30 – 80 (mean 52 ± 14.2) were identified with full periodontal charts. 1625 of these had moderate – severe periodontitis. 200 had CKD, 103 had IHD and 93 had CVD. In univariate analyses (Fishers Exact test) periodontitis was associated with CKD (P <0.001, OR: 1.83, 1.36 – 2.46 95% CI); IHD (P<0.001, OR 2.574, 1.69 – 3.9) and CVD (P < 0.001, OR 3.073 1.92 – 4.91). When subjects taking CCBs were excluded from the analyses, the associations seen persisted although the odds ratios were slightly reduced (CKD 1.53; IHD 2.38; CVD 2.8).

Conclusions The results support the findings of studies of other datasets of the association of moderate to severe PD with presence of CKD, IHD and CVD. Further multivariate analyses are required to investigate the effects of the many potential confounders in this data, and particularly to investigate more deeply if CCBs may act as a confounding factor.

Notes
Oral Cancer: Exploring the Stories in United Kingdom Newspaper Articles
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**Objectives** Reports suggest that many patients with oral cancer delay seeing help because they are unaware of the symptoms. Studies indicate that 90% of adults engage with news reports and 4 out of 10 people read newspapers. Newspaper stories on oral cancer may influence awareness and health seeking behaviour. This study aimed to explore how oral cancer is portrayed in UK newspaper print media.

**Methods** News articles were retrieved from the 10 UK newspapers with the greatest print circulation. A newspaper archive database was used to retrieve all “mouth cancer” and “oral cancer” articles between 1 August 2011 and 31 October 2014. Duplicates, non-cancer and non-human articles were excluded. Content analysis was undertaken assisted by NVivo 10 software and themes were identified.

**Results** 241 Articles were identified. Themes identified included: “recent research”, “survivor stories”, “celebrity linkage” and “health information”. Predominately articles had focussed headlines linked to risk and content was written to elicit emotions. For example survivor stories included dramatic tragedy or hope. When present, signs and symptoms in oral cancer articles were used to draw attention, elicit emotion and often generate concern. Many articles omitted to convey accurate or complete health information about signs, symptoms or signposting. Even where information was present, it was placed at the bottom of the article where it was least likely to be read.

**Conclusions** Opportunities to increase awareness, early detection and early treatment for oral cancer through UK newspaper media are being missed. Further work to improve the quality of information and health reporting in newspapers and enhance social responsibility are indicated.

**Notes**
The association of calcium channel blockers with periodontal disease.
Hon, J. M., Johnson-Idan, J., Linden, G. J., Winning, L., Sabbah, W., Hughes, F. J.

1King’s College London, 2Queen’s University Belfast

Objectives Calcium channel blockers (CCBs) are widely used as anti-hypertensives and are known to cause gingival overgrowth. The objective of this study was to determine if there is an association between CCB use and periodontal disease.

Methods Two existing datasets with periodontal phenotyping were analysed: the PRIME database from Belfast, which was collected to study risk factors for coronary heart disease, and the NHANES 2011–2012 database from the USA. For both databases, we analysed the demographics, medical and drug history and smoking status of all those who had 6-point pocket chart measurements, which were converted into Page & Eke case definitions of periodontitis. Univariate analyses and multivariate modelling were performed for each dataset.

Results 9.8% of 1397 PRIME subjects and 9.5% of 3313 NHANES subjects were prescribed CCBs. In the PRIME dataset, CCBs were significantly associated with moderate/severe periodontitis (p = 0.010), with 52.6% of CCB users having this level of disease compared with 41.1% of non-CCB users. Although hypertension was also associated with moderate/severe disease (p = 0.023), this was not significant when those on CCBs were excluded from the analysis. In the NHANES dataset, CCBs were also significantly associated with moderate/severe disease (p < 0.001), with 67.9% of CCB users having this level of disease compared with 47.1% of non-CCB users. This association remained after adjusting for age, gender and smoking status.

Conclusions These data indicate that CCB use may be an independent risk factor for developing moderate-severe periodontitis. Further research is required to investigate this preliminary finding, but an increased side effect profile of CCBs may have some influence on existing prescribing practice.

Notes
The Role of CALML3 in Oral Squamous Cell Carcinoma
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University of Sheffield

Objectives The aims of the project were to determine the expression of CALML3 in a cohort of normal, dysplastic and oral squamous cell carcinoma cell lines and ex vivo, in normal oral mucosa, oral potentially malignant lesions (OPML) and oral squamous cell carcinoma (OSCC). We also aimed to determine the phenotypic changes resulting from overexpression of CALML3 in OSCC cells.

Methods The mRNA and protein expression of CALML3 were measured by qPCR and western blot in cell lines and in normal oral mucosa, OPML and OSCC by immunohistochemistry. The relationship of CALML3 expression to that of markers of differentiation and expression of myosin-X (a protein related to CALML3 activity) was also determined by qPCR and western blot respectively. The effects of re-expressing CALML3 in OPML and OSCC cell lines were investigated by means of transient transfection of CALML3. The effects of expression of CALM3 on migration and proliferation of the cells were assessed as well as on expression of differentiation markers and myosin-X.

Results CALML3 was down-regulated in OSCC with a pattern of localisation in tissues similar to previous studies. It was found that CALML3 expression changes are unlikely to merely be a consequence of immortalisation. The expression of CALML3 mRNA correlates with involucrin mRNA expression. CALML3 re-expression has no effect on the migration of OSCC cells and this may be due to the increased expression of myosin-X in OSCC. Finally, ectopic expression of CALML3 had no effect on proliferation or expression of involucrin.

Conclusions This study has supported previous findings regarding loss of CALML3 expression in OSCC. However, it has shown that CALML3 is not important in the migration of OSCC as it is in other cancers. The phenotypic changes resulting from this down-regulation in OSCC remain unclear.

Notes
Thermal Observation of Tooth Transfer Heat

Lancaster, P.¹, Brettle, D.², Carmichael, F.³, Clerehugh, V.¹

¹University of Leeds, ²St James’s University Hospital, ³Leeds Dental School

Objectives All tissue with a temperature above absolute zero emits infrared radiation - the wavelength and quantity of which depends on tissue characteristics and environmental conditions.

The primary objective was to visually characterise the internal surfaces of human enamel and dentine tooth sections, following the transfer of heat with a thermal camera. The secondary objective was to assess the diagnostic potential of heat-transfer for demineralisation and future vitality-testing of teeth.

Methods Two ethically-sourced human third-molars, one sound and one demineralised, were sliced bucco-lingually into 1 mm-thick sections (Accutom-5), polished, measured with a digital micrometer and stored in distilled water. Pre- and post-slice photographs and radiographs were taken. A thermally-stable environment (22°C±0.1°C) was achieved with macro- and micro-controlled thermal sensors. Tooth-slices were placed on a copper baseplate (0.5mm) with a thermal pad, and cooled on an ice-block. Activation of the thermal camera (FLIR SC305) recorded a heat transfer sequence at nine frames per second with ThermaCAM Researcher Professional 2.10 Software when moved to a hotplate (37°C), to reach thermal equilibrium. Heat-transfer data was processed in Microsoft Excel and a unique MatLab Programme, to produce original images according to frame-number and characteristic time of the heating curve.

Results Thermal equilibrium was reached within three minutes for all tooth-slices. Heat-transfer images characterise enamel and dentine within 1 frame, with the best contrast and resolution seen at 9 frames (1 second).

Demineralised enamel and dentine was differentiated within the same time-line. The characteristic time-curve successfully distinguished tissue-type and demineralisation.

Conclusions This innovative imagery of heat-transfer data has enabled visualisation of enamel and dentine of the tooth-slices and shows diagnostic capability for demineralisation. This methodology will be translated for tooth vitality in-vivo.

Notes
A Novel Orthodontic Adhesive Made From Bioactive Glass To Prevent Demineralisation. 
Al-eesa, N. A. 1, 2, Wong, F. 2, Hill, R. G. 1, Johal, A. 2
1Queen Mary university of London, 2Barts and the London Dental institute

Objectives To design a novel orthodontic adhesive from bioactive glass and resin capable of preventing demineralisation around orthodontic brackets by releasing Ca\(^{2+}\), PO\(_4^{3-}\) and F\(^-\) ions.

Methods Three bioactive glasses of high fluoride and low sodium content were synthesized via the melt-quench route. The refractive index was calculated to match that of the resin, and a light curable paste was made from the glass powder and a resin made from BisGMA and TEGMA. 20 Human teeth imbedded in acrylic discs were bonded with incisor brackets and tested for shear bond strength in comparison with (Transbond XT). The glass powders, and the adhesive in the form of discs, were characterized for their bioactivity through FTIR and XRD studies both before and after immersion in tris buffer solution which were then analysed for fluoride and calcium release and pH change by ion selective electrode and pH meter respectively. Adhesive discs were examined by scanning electron microscopy (SEM) for confirmation of apatite formation.

Results FTIR and XRD data clearly revealed the formation of apatite. SEM images showed an apatite layer around glass particles. Around 30 ppm F ions were released after 24 hours which then reduces to 20ppm after 1 week while Ca\(^{2+}\) ions released within 24 hours were 127 ppm. pH values of the tris were elevated to 8.8 from the original 7.34 after immersion. Comparable values of shear bond strength were found for the new adhesive and Transbond XT (8 and 10 MPa respectively).

Conclusions This formula of bioactive glass with the resin is successful in producing an applicable orthodontic adhesive that release a considerable amount of F and Ca\(^{2+}\) ions with apatite formation which are important elements for prevention of decalcification.

Notes
Characterization of mouse incisor tooth mesenchymal stem cells
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Objectives The continuously growing mouse incisor provides an excellent model for studying molecular mechanisms of stem cell fate determination. This work aims to: (1) Define the molecular signatures of a mesenchymal stem cell (MSC) region within the mouse incisor; (2) Develop a methodology to culture MSCs in vitro.

Methods Based on the Notch signalling pathway activation levels in the cells, we used Laser Capture Microdissection to capture different regions of interest within the mouse incisor dental mesenchyme. A real time PCR screening of the cDNA from the cells was performed with specific probes targeting a group of known mesenchymal quiescent stem cell markers. The corresponding protein expression levels were evaluated using immunofluorescent microscopy. Furthermore, cell culture of dissected MSC containing incisor mesenchyme was performed and stem cell status manipulation has been attempted using an in vitro synchronisation assay.

Results We have found that mouse incisor MSCs express many similar markers to MSCs contained in other organs, such as muscle and the hematopoietic system. These cells could be cultured in vitro. Cellular quiescence and activation can be simulated using a synchronization assay. Notch signalling pathway members express differently in the MSCs and transit amplifying (activated) cells.

Conclusions Our study showed that mouse incisor MSCs are located in a distinct region and express specific markers even when cultured in vitro. Notch signalling may play a key role in incisor MSCs maintenance and differentiation. It is expected that the molecular mechanisms driving dental stem cell behaviour, can be applied to direct differentiation of stem cells into dental tissues and possibly even utilised to grow an entire adult tooth.

Notes
Recruitment of Tumour-Associated Neutrophils to Head and Neck Squamous Cell Carcinoma

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Objectives
Objectives: The presence of tumour-associated neutrophils (TAN) has been associated with poor prognosis in patients with head and neck squamous cell carcinoma (HNSCC). The aim of this study was to identify factors that are responsible for neutrophil recruitment into HNSCC.

Methods
Methods: TAN numbers within human HNSCC was evaluated by immunohistochemical staining for neutrophil myeloperoxidase (MPO). Factors secreted by FaDu HNSCC multi-cellular tumour spheroids (MCTS) were analysed by cytokine array and ELISA. Peripheral blood neutrophils from healthy volunteers were used to measure neutrophil migration to factors identified from the array. The recruitment of neutrophils to MCTS was assessed over time by flow cytometry in the absence and presence of small molecule inhibitors. FaDu xenograft mouse models were used to confirm the effect of these inhibitors on neutrophil recruitment in vivo.

Results
Results: MPO staining confirmed the presence of marked numbers of TAN in HNSCC compared to normal oral epithelium. TAN were most abundant in the tumour invading front and necrotic areas. HNSCC MCTS resemble in vivo tumours, displaying areas of hypoxia, necrosis and cell proliferation. Neutrophils migrated in a dose-dependent manner to recombinant CXCL8, CXCL1 and MIF and these chemoattractants were found in abundance in the conditioned medium of FaDu MCTS. The recruitment of neutrophils to FaDu MCTS was significantly inhibited when neutrophils were pre-treated with antagonists for CXCR2 and CXCR4, the receptors for CXCL8, CXCL1 and MIF respectively. Moreover, use of the specific MIF inhibitor, ISO-1 caused a dramatic reduction in the number of neutrophils recruited into FaDu MCTS. In addition, in vivo, ISO-1 significantly reduced the number of TAN in xenograft FaDu tumours.

Conclusions
Conclusions: Collectively, these data suggest that CXCL8, CXCL1 and MIF in particular are important in the recruitment of TAN into HNSCC. Inhibition of these factors represent a potential novel anti-cancer target.

Notes
Signaling regulating active TG2 release from cells: implications for osteoarthritis.
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Objectives Although altered joint mechanics is considered the main risk for osteoarthritis (OA), disease development is linked to inflammatory processes as well. Importantly, OA can affect the temporomandibular joint, causing facial pain, headaches and limiting mouth mobility. Transglutaminase 2 (TG2) is potentially an important player in OA pathogenesis, as its extracellular actions promote aberrant chondrocyte hypertrophy and cartilage calcification. However, TG2 release from cells occurs through a non-conventional and currently unknown process. We hypothesize that ATP secretion from immune cells, and damaged or mechanically stimulated chondrocytes drives TG2 release in OA via P2X7 receptor (P2X7R) activation. The aim of this study was to investigate whether TG2 externalization is mediated by activation of P2X7R.

Methods Cell models were differentiated THP-1 monocytes and HEK293 cells expressing P2X7R. TG2 secretion was quantified using Western blotting. Release of vesicles was analyzed by nanoparticle tracking. Pharmacological agents were used to determine the role of Ca\(^{2+}\)signaling and P2X7R-dependent pore formation in TG2 secretion. Finally, TG2 externalization was monitored during IL-1ß/oncostatin-M treatment of 3D cartilage constructs.

Results In macrophage-like cells TG2 release was dependent on P2X7R. In HEK293 cells P2X7R activation induced time-dependent release of TG2 but not other cytoplasmic proteins. TG2 release was independent of inflammasome formation, occurred through a mechanism distinct from microvesicle shedding and was not due to apoptosis. Significantly, P2X7R-dependent membrane pore formation but not the initial ion flux or membrane depolarization led to TG2 secretion. IL-1ß/oncostatin-M treated cartilage displayed enhanced TG2 expression and activity, thus demonstrating the importance of inflammatory signaling in regulation of TG2 function.

Conclusions We demonstrate that P2X7R-dependent membrane pore formation is regulating TG2 release from cells. Our data indicates that P2X7R has a central role in controlling TG2 externalization and downstream events, especially in the context of inflammation. This identifies a new avenue for therapeutic intervention.

Notes
Bacterial products can directly activate trigeminal sensory neurons.

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Objectives Pulpal infection often results in acute and chronic dental pain. Evidence suggests that sensory neuronal activation can be initiated by the action of inflammatory mediators as well by the bacteria causing the infection, however, the mechanisms involved in the latter are unclear. The aims of this study were to determine whether trigeminal neurons are directly activated by bacterial cellular products and whether these lead to increased expression of calcium gene related peptide (CGRP).

Methods LPS and arginine-gingipain from Porphyromonas gingivalis were used to stimulate mouse trigeminal ganglia cells that had been cultured in vitro for 2-3 days. Neuronal response was assessed by calcium-imaging using Cal-520 AM and intracellular levels of CGRP were assessed by immunocytochemistry. Neurons were differentiated from non-neuronal cells, such as satellite glial cells and Schwann cells, by response to 60 mM KCl. Responses were compared with that achieved with Escherichia coli LPS and with agonists for the PAR2, TRPA1, and TRPV1 receptors.

Results P. gingivalis LPS stimulation resulted in an activation response in more than 20% (24/90) of trigeminal neurons and 40% (18/57) of non-neuronal cells using calcium imaging and there was increased neuronal expression of CGRP. Similar results were obtained with E. coli LPS. Arg-gingipain mediated a response from 46% (11/24) of neurons and almost all of the non-neuronal cells present. However, the bacterial-responsive neurons were not always the same as those that responded to the TRPV1 agonist, capsaicin, or the TRPA1 agonist, cinnamaldehyde, suggesting differences between neuronal subpopulations.

Conclusions These data suggest that direct activation of neurons by P. gingivalis LPS and arg-gingipain may contribute to pain processing. This is likely to involve alteration of CGRP expression as well as TLRs, TRPs, and PAR2. It is possible that non-neuronal cells are also involved in mediating neuronal responses to these bacterial agonists.

Notes
Patients’ Valuation of Fluoride Varnish in Brazil: using Willingness to Pay

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Objectives Decisions regarding resource allocation within a health care system, such as the Sistema Único de Saúde (SUS), can be aided by understanding patient preferences. Where patient preferences are used to inform decision making, the result can be a more patient-centred system. Health economics has measures to allow systematic elicitation of preferences which can then be incorporated in economic evaluations to inform decision making so resources are used more efficiently. One such measure is Willingness to Pay (WTP) which values preferences in monetary terms. The aim of this study was therefore to ascertain the value of preventative dental care using fluoride varnish as an exemplar amongst citizens in Porto Alegre, Brazil using WTP.

Methods A questionnaire eliciting willingness to pay for fluoride varnish alongside demographic and dental attitudinal data was administered to a consecutive sample of 100 patients aged 18 and over, using the SUS for their dental treatment. The setting was dental clinics at the Pontifical Catholic University of Rio Grande do Sul, Brazil

Results Dental caries prevention, in the form of fluoride varnish, was valued by users of the SUS at a mean level of R$60.37 (=£15.17). There was significant variation within the sample with standard deviation of 63.44. Regression modelling revealed that higher income and infrequent visits to the dentist were significant positive influences on WTP. However, participant age, previous dental treatment and perceived need for future dental treatment did not appear to influence WTP.

Conclusions WTP for fluoride varnish application in Brazil is highly variable, with significant influence from income and dental visit frequencies. This is an important finding for policy makers who may be able to use these findings to target caries preventive programmes better.
Isolation and Immortalisation of Tonsil Keratinocytes for Three-Dimensional Tissue Engineered Models
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Objectives The ability to isolate and culture primary cells is important for the in vitro investigation of specific tissues and diseases as these are more representative than cancer-derived cell lines. This study aimed to isolate primary keratinocytes from tonsils removed during routine tonsillectomies (ethics ref 09/H1308/66) using enzymatic digestion and use these to create functional tissue-engineered tonsillar tissue.

Methods The efficiency of cell isolation using two different enzymes (trypsin and dispase) was compared. The growth of primary tonsil keratinocytes was measured and compared to cells cultured in the presence of the Rho kinase inhibitor Y27632. Keratinocytes were used to develop a tissue-engineered model of tonsil epithelium using primary tonsil fibroblasts and de-epithelialized dermis and these models were then incubated with Streptococcus pyogenes to model tonsillitis.

Results Enzymatic digestion of tonsillar tissue with trypsin resulted in the isolation of significantly more keratinocytes compared to dispase isolation. Keratinocytes cultured without the Rho kinase inhibitor Y27632 survived in culture for less than 10 population doublings whereas cells cultured in the presence of this inhibitor grew for over 30 population doublings without changing their phenotype. Tonsil keratinocytes and fibroblasts cultured in three dimensions produced a multi-layered differentiated epithelium that histologically resembled the surface epithelium of normal tonsils and responded to S. pyogenes by increasing expression of pro-inflammatory cytokines.

Conclusions Tonsil keratinocytes can be successfully isolated and cultured in vitro. Y27632 was able to markedly prolong the life span of keratinocytes without any deleterious consequences to the cell phenotype making these cells useful for a number of applications that require longer term culture. A functional tissue engineered model of tonsil epithelium was generated which will provide a useful tool for studying cells in a more physiologically relevant way.

Notes
In vitro effect of amorphous calcium phosphate paste applied for extended periods of time on enamel remineralization

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¹ Araçatuba Dental School, ² Argonne National Laboratory, ³ Feinberg School of Medicine

Objectives The objective of this study was to determine the ability of topically applied ACP (amorphous calcium phosphate casein phosphopeptide) to re-mineralize subsurface lesions when applied for extended periods of time (3 h and 8 h).

Methods Artificially induced carious lesions were produced in 50 enamel blocks previously selected by surface hardness. After treatments (Placebo, neutral fluoride gel 1 min, ACP 3 min, ACP 3 h and ACP 8 h), the enamel blocks were submitted to the re-mineralization pH-cycling. Surface hardness and synchrotron micro-tomography were used to determine the percentage of surface hardness recovery (%SHR) and to calculate integrated loss of mineral (g HAp × cm⁻³) and lesion depth (µm), respectively. The data were submitted to ANOVA followed by the Student-Newman-Keuls test (p < 0.05). Fluoride gel presented higher %SHR followed by ACP 3 min (p < 0.001).

Results No difference (p = 0.148) was found for Placebo, ACP 3h and ACP 8h groups for %SHR. Fluoride gel showed greater mineral concentration (p < 0.001) when compared to the other groups. ACP 3 min demonstrated a significant difference (p < 0.001) from ACP 3 h and ACP 8 h. The ACP 3 h and 8 h presented a subsurface lesion with development of laminations in all blocks.

Conclusions It was concluded the use of ACP Paste for extended periods of time did not produce an additive effect in the remineralization process. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. DE-AC02-06CH11357.

Notes
Salivary antimicrobial proteins associate with Streptococcus mutans, age and dental disease status of children
Malcolm, J.\(^1\), Simon-Soro, A.\(^2\), Sadique, S.\(^3\), Macpherson, L.\(^3\), Mira, A.\(^2\), Culshaw, S.\(^4\), Sherriff, A.\(^3\)
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**Objectives** We aimed to investigate the association of antimicrobial proteins (AMPs) with \textit{S. mutans} colonisation, relative to changes in dental disease status overtime. Specifically, we sought to determine whether appropriate AMP production could modulate caries risk in individuals colonised by \textit{S. mutans}. We also sought to investigate whether AMP responses associated with changes in the bacterial diversity of saliva in relation to disease status.

**Methods** Oral examinations were carried out on 132 children, aged 4-years, followed-up at age 5-years. Whole, unstimulated saliva was collected for immunoassays of lactoferrin, calprotectin, the human neutrophil peptides 1-3 (HNPs-1-3) and LL37, detection of \textit{S. mutans} and assessment of bacterial diversity.

**Results** Our data confirm that \textit{S. mutans} associates with caries experience, but not in all children, 34\% of children with detectable \textit{S. mutans} at baseline remained caries-free (CF) at follow-up. Analysis of the shared co-variance among AMPs indicated that \textit{S. mutans} colonisation (in the absence of dental disease) was sufficient to increase AMP responses. However, there was no evidence to suggest that AMPs protected against caries development in children colonised by \textit{S. mutans}. Overtime, AMP concentrations tended to increase with age. The most dramatic increases in AMP responses occurred in children who developed caries during the study compared with those who remained caries free or those who had established caries at the onset of the study. These increases reflected changes in \textit{S. mutans} numbers, which increased in the saliva of all children overtime but increased to a greater degree in the children who developed caries. We did not detect changes in the bacterial diversity of saliva according to age or disease status and we found no association between measures of bacterial diversity and AMP responses.

**Conclusions** These data indicate that acquisition of \textit{S. mutans} remains a strong risk factor for caries development in children of this age. AMP responses associate with \textit{S. mutans} colonisation and likely only modestly contribute to caries susceptibility.

**Notes**
In-situ Caries Evaluation of Experimental Dentifrices containing Functionalised ß-TCP

Churchley, D.1, Bosma, M.1, Hara, A.2, Jain, R.1, Kelly, S.2, Lippert, F.2, Martinez Mier, E.2, Zero, D. T.2

1GlaxoSmithKline Consumer Healthcare, 2Oral Health Research Institute

Objectives The objective of this study was to use an in situ caries model to compare the anti-caries potential and fluoride uptake from two experimental dentifrices containing functionalized ß-tricalcium phosphate (ß-TCP) and sodium fluoride (NaF) with a NaF reference dentifrice.

Methods Human enamel specimens were demineralised for 7 days in a 4% methylcellulose gel covered with an equal mass of 0.1M lactic acid, pH 4.6. Two gauze-covered, enamel lesions were placed in the buccal flange area of subjects’ mandibular partial dentures. Forty-eight subjects completed a double-blinded (to examiner and specimen analyst) cross-over design with five randomly assigned dentifrice treatments: 1) Dentifrice containing 100ppm ß-TCP/1100ppm F (EXP-100); 2) Dentifrice containing 200ppm ß-TCP/1100ppm F (EXP-200); 3) Dentifrice containing 1100ppm F (D-1100); 4) Dentifrice containing 250ppm F from NaF; 5) Placebo dentifrice (0ppm F) (Note: all dentifrices used silica as the abrasive).

Subjects brushed twice daily for 28 days with the test dentifrices. The enamel specimens were assessed by Transverse Microradiography (TMR) for changes in mineralization (∆M), and lesion depth (∆L); Cross-sectional microhardness (CSMH) for integrated mineral loss (∆Z) (post-treatment only) and for enamel fluoride uptake (EFU). Statistical analyses involved ANCOVA (∆M/∆L/∆Z) / ANOVA (EFU) and pairwise comparisons between treatments (α=0.05).

Results The model demonstrated a fluoride dose-response for ∆M, ∆L, ∆Z and EFU. There were no significant differences in ∆M and ∆L measured by TMR and ∆Z measured by CSMH between EXP-100, EXP-200 and D-1100 dentifrices. Whilst EXP-100 and EXP-200 promoted greater EFU vs. D-1100, this was only significant for EXP-100 (P_{EXP-100}=0.0002 / P_{EXP-200}=0.0667).

Conclusions In conclusion, in this model the experimental dentifrices containing functionalized ß-TCP did not deliver an enhancement in remineralization compared with the NaF reference dentifrice despite an increase in EFU (for EXP-100 only).

Notes
SEM imaging of cervical dentine treated with a desensitising toothpaste

Morgan, E. R., Mneimne, M., Gillam, D. G., Hill, R. G.

1Barts & The London, 2Barts and the London School of Medicine and Dentistry, 3Barts and the London School of Medicine and Dentistry, Queen Mary University of London

Objectives

The effect of a calcium sodium phosphosilicate (NovaMin®) desensitising toothpaste on cervical dentine was evaluated by scanning electron microscopy (SEM). Cervical dentine was chosen rather than mid coronal dentine as Dentine Hypersensitivity (DH) generally occurs on the exposed dentine of the cervical region of a tooth, particularly in patients with gingival recession.

Methods

Three root sections were cut and etched with 5% citric acid for 5 minutes. These sections were then brushed with 2mg of Sensodyne Repair and Protect (NovaMin®) toothpaste for 2 minutes with a powered toothbrush and left in artificial saliva (AS) for an hour at 37 degrees celsius. One group, a control, was brushed only with distilled water and a third group was exposed to a one-minute acid challenge with 5% citric acid. The cervical regions of the samples were then analysed by SEM.

Results

The dentine tubules identified on the cervical dentine sections were narrower and sparser compared with a typical mid-coronal section. SEM observation of the treated cervical dentine indicated that all tubules were occluded by the NovaMin® toothpaste. After a subsequent acid challenge, one representative SEM image showed that approximately 6% of tubules were not occluded and 42% were only partially occluded with less than 50% of the diameter of the tubule occluded.

Conclusions

The results from this pilot study would suggest that a NovaMin® desensitising toothpaste has the potential as a tubule occludent for the treatment of patients with gingival recession and associated DH. Furthermore, the application of the toothpaste may also provide effective treatment from DH following an acidic challenge. These findings are more clinically relevant than studies showing occlusion of mid-coronal dentine as it demonstrates that the material may be useful for the treatment of hypersensitivity resulting from gingival recession.

Notes
RCT of Experimental Cetylpyridinium Chloride Mouthwash Effects on Plaque Re-Growth
Shaw, D. 1, Gordon, J. 1, Maclure, R. 2, Bosma, M. 1
1GlaxoSmithKline Consumer Healthcare, 2Intertek CRS, 3inVentiv Health

Objectives To evaluate the effects on plaque re-growth over 24 hours and the tolerability of an experimental mouthwash containing 0.05% w/w cetylpyridinium chloride and 100 ppm fluoride (as sodium fluoride) compared with sterile water.

Methods This was a randomised, controlled, examiner blind, two treatment, replicate, four period crossover, single centre study. Eligible subjects aged ≥18 years with 40 gradable teeth surfaces needed a whole mouth Turesky Plaque Index (TPI) score of ≥2.00 after receiving a prophylaxis to remove all plaque, using a standard fluoride dentifrice for 3–7 days then abstaining from oral hygiene for 24 hours. At least 7 days after screening, and following a second prophylaxis, subjects rinsed their mouths with 10 mL of their allocated study mouth rinse for 60 timed seconds, repeated 4–12 hours later. Subjects then abstained from oral hygiene for 24 hours (±1 hour), after which plaque re-growth assessment was made using the TPI and the Gingival Margin Plaque Index (GMI). This was repeated with the alternative mouth rinse after 3–7 days washout. Following another 5 weeks washout, the study protocol was repeated with each mouth rinse, either in the same or a reversed sequence, depending on randomisation assignment.

Results Of 83 screened subjects, 46 were randomised to treatment and completed the study. There were no significant differences between the mouth rinses in plaque re-growth 24 hours after treatment (Table 1). Of the 28 treatment-emergent adverse events (Experimental = 12, Sterile Water = 16), none were considered treatment-related or serious.

Conclusions There were no significant differences between the experimental mouth rinse and sterile water for measures of plaque re-growth after 24 hours. Both treatments were well tolerated. This study was funded by GSK Consumer Healthcare.

Notes
Randomised Clinical Trial of Oral Tolerance of a NaHCO$_3$ Toothpaste

Krishnan, N., Lomax, A., Ali, A., Jain, R., Bosma, M.
Glaxo SmithKline

Objectives To evaluate the oral tolerance of twice daily brushing with an experimental toothpaste containing 67% sodium bicarbonate (NaHCO$_3$) as an anti-plaque and whitening ingredient compared to a reference product with 0% NaHCO$_3$.

Methods This was a 14 day, single centre, examiner blind, two arm, parallel group, randomised clinical study in healthy adult volunteers. Oral soft tissue (OST) examinations were performed at screening, followed by Visit 2 (Day 7±1) and Visit 3 (Day 14±3). Participants were randomised to receive a 67% NaHCO$_3$ containing toothpaste (Experimental group) or a toothpaste containing 0% NaHCO$_3$ (Reference group). Participants brushed their teeth twice daily for one timed minute with a ribbon of toothpaste to cover the head of the provided toothbrush. OST examinations were performed on Day 7(±1) and Day 14(±3). Adverse Events (AEs) and Serious AEs (SAEs) were reported throughout the study.

Results A total of 150 subjects (75 in each group) of 160 screened subjects were included in the Safety population. At Day 14, there were 14 treatment-emergent AEs (TEAEs) reported in total. There were eight oral TEAEs, of which five were reported in the Experimental group by four subjects (5.3%), three in the Reference group by two subjects (2.7%). Of these oral TEAEs, four were considered treatment-related, two in each group (tongue ulceration occurring twice in one subject in the Experimental group and once in one Reference group subject; gingival ulceration in one Reference group subject). The majority of TEAEs were mild in intensity except one moderate report of ‘pain in jaw’ in the Experimental group; all resolved before the end of the study. No SAEs were reported.

Conclusions The results of this study indicate that the experimental toothpaste containing 67% NaHCO$_3$ was well tolerated over the 14 day treatment period relative to the reference toothpaste. This study was funded by GSK Consumer Healthcare.

Notes
RCT of Experimental Stannous Fluoride Dentifrice in Dental Stain Removal
Nehme, M.1, Hall, C.1, Siddiqi, M.2, Hughes, N.3, Milleman, K.4, Milleman, J.4
1GSK Consumer Healthcare, 2InVentiv Health Clinical, 3Salus Research, 4Salus Research

Objectives To evaluate and compare extrinsic dental stain removal efficacy of an experimental 0.454% stannous fluoride and 5% sodium tripolyphosphate (STP) dentifrice (‘Test’), with that of a daily use dentifrice indicated for whitening (Colgate Total Whitening®, Colgate Palmolive, USA) (‘Comparator’).

Methods This was a randomised, controlled, examiner-blind, parallel group study. At baseline, subjects with good oral health, ≥ 16 natural teeth with the 12 anterior teeth gradable for Lobene Stain Index (LSI), and a LSI Score ≥ 25, were stratified (by baseline LSI score and smoking status) and randomised to study treatment. Subjects returned after 4 and 8 weeks twice daily brushing with their assigned dentifrice for assessment of dental stain (LSI: Area and Intensity; facial surfaces of the 12 anterior teeth; lingual surfaces of six anterior teeth).

Results Of 126 randomised subjects, 121 completed the study (Test=61, Comparator=60). Both treatment groups showed statistically significant decreases in mean LSI Area x Intensity from baseline after 4 and 8 weeks treatment (all p<0.0001). With the exception of mean LSI Area at 8 weeks, which favoured the Comparator dentifrice (p=0.0289), there were no other significant between treatment differences for any measure at any time point. Three treatment-emergent adverse events were reported for two subjects (Comparator group). None were considered treatment related. No serious adverse events were reported.

Conclusions The experimental 0.454% stannous fluoride/5% STP dentifrice performed similarly to a marketed whitening dentifrice in significantly reducing established natural stain when used twice daily over the 8-week treatment period (with the exception of mean LSI Area at 8 weeks). Inclusion of 5% STP in the experimental formulation is postulated to have mitigated against the tooth stain issues historically associated with stannous fluoride-containing toothpastes. Both study products were well tolerated. This study was funded by GSK Consumer Healthcare.

Notes
**Antimicrobial activity of a stannous fluoride toothpaste in the PGRM**

**Parkinson, C.**¹, Maclure, R.³, Payne, D.², Hall, P.³, Jeffery, P.¹

¹GSK Consumer Healthcare, ²Intertek, ³Intertek CRS

**Objectives** To assess *ex-vivo* antimicrobial activity of a non-aqueous toothpaste containing 0.454% stannous fluoride (SnF₂) on *de-novo* plaque in two plaque glycolysis and regrowth models.

**Methods** Two single-centre, analyst/examiner blind, randomised, three-treatment studies were conducted in healthy adults with plaque acidity pH 5.0–5.7. Test (0.454% SnF₂) and Positive Control (Crest® ProHealth: 0.454% SnF₂ and zinc citrate) toothpastes were compared to a Negative Control (regular) toothpaste. Baseline plaque samples were collected from maxillary dentition. Subjects then either brushed maxillary dentition with their assigned toothpaste for 30 seconds and swilled the resulting slurry for 30 seconds (Study 1) or rinsed the whole mouth with a pre-prepared toothpaste slurry for 60 seconds (Study 2). Plaque samples (mandibular, except right maxillary for Study 2, 15 minutes) were collected at 15, 45 (both studies) and 90 minutes (Study 2) and analysed for acidogenicity and regrowth activity. Area-under-the-curve (AUC) for regrowth and glycolysis were calculated over 45 (both studies) and 90 minutes (Study 2).

**Results** Study 1: Differences in mean AUC<sub>glycolysis</sub> and AUC<sub>regrowth</sub> between Positive and Negative Controls weren’t significant, study validity was not achieved (Table 1). Study 2: Significant differences were shown in mean AUC<sub>glycolysis</sub> and AUC<sub>regrowth</sub> between Positive and Negative Controls and between Test and Negative Control at both timepoints, favouring the first named treatments. Mean difference in AUC<sub>regrowth(0-90)</sub> between Test and Positive Control was statistically significant, favouring the latter, with no significant difference for AUC<sub>glycolysis(0-90)</sub> or for either measure at 45 minutes (Table 2). There were no treatment-related or serious adverse events.

**Conclusions** While Study 1 was not validated, Study 2 was. In Study 2, antimicrobial biological activity was demonstrated for the 0.454% SnF₂ non-aqueous toothpaste. The difference in AUC<sub>glycolysis(0-90)</sub> observed for the Positive Control compared to the Test is attributed to the presence of zinc. All study products were well tolerated. Study funded by GSK Consumer Healthcare.

**Notes**
Three RCTs of Tolerability of Oral Healthcare Products in Children

Gordon, J.1, Newby, E.1, Milleman, K.2, Milleman, J.3, Payne, D.4, Maclure, R.4, Siddiqi, M.5, Bosma, M.1

1GlaxoSmithKline Consumer Healthcare, 2Salus Research, 3Salus Research, 4Intertek CRS, 5inVentiv Health Clinical

Objectives To investigate oral tolerability in children of four experimental fluoride toothpastes (distinguished by flavour), an experimental toothbrush and a fluoride mouthrinse (220 ppm fluoride), versus commercially available controls over 14 (toothpaste and mouthrinse) or 28 days (toothbrush).

Methods All products were investigated in randomised, examiner blind, multiple arm, parallel group studies in children aged 6-10 years (toothpaste), 2 years (toothbrush) or 7-10 years (mouthrinse). Key tolerability outcome measures were overall incidence, severity and frequency of oral treatment-emergent adverse events (OTEAEs) and OST abnormalities.

Results In the toothpaste study (Toothpaste1 n=71; Toothpaste2 n=72; Toothpaste3 n=73; Toothpaste4 n=72; Control n=72 subjects), two OTEAEs at Day 7 (one with Toothpaste2, one with Control) and seven at Day 14 (five with Toothpaste2, two with Control) were reported (all mouth ulceration) (Table 1) but not considered treatment-related. By Day 28 of toothbrush use (Experimental n=71; Control n=72 subjects), only one, non-treatment related, OTEAE was reported (Control group) (Table 2). In the mouthrinse study (n=83 for both groups), a greater number of oral TEAEs were reported for the Experimental mouthrinse (53 by 25 subjects) than the Control mouthrinse (22 by 20 subjects). Of these, 22 in the Experimental group and three in the Control group were considered treatment-related, reported by three and two subjects respectively in Centre 1 (Table 3). Two in the Experimental group withdrew due to these OTEAEs. No studies reported serious OTEAEs.

Conclusions These studies suggest that the experimental toothpastes and toothbrush have similar tolerability to currently marketed control products. However, in the mouthrinse study, more OTEAES were reported for the Experimental mouthrinse compared to control. This was postulated to be due to subject inexperience with mouthrinse use. The Experimental mouthrinse was not progressed to market due to the unexpectedly high number of OTEAEs. Studies were funded by GSK Consumer Healthcare.

Notes
Comparison of Different Periodontal Surgeries: A Longitudinal Meta-Analysis

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Institute of Epidemiology and Preventive Medicine

Objectives Guided tissue regeneration (GTR) and enamel matrix derivatives (EMD) are the two most commonly used surgical techniques for periodontal regeneration. Several meta-analyses showed that GTR and EMD achieved better treatment outcomes such as pocket depth reduction and clinical attachment level gain in one year compared to periodontal flap operation. However, their long-term benefits remain unclear. The aim of this study is therefore to conduct a longitudinal meta-analysis of for the long-term differences in treatment outcomes between periodontal regeneration and flap operation.

Methods A systematic literature search was conducted using the Medline, EMBASE, Pubmed and CENTRAL databases up to Dec 2014. Treatment outcomes were changes in probing pocket depth (PPD) and clinical attachment level (CAL). We extracted data reported at different time points after periodontal surgery and incorporated all data into the same model to estimate the trend in the treatment outcomes. The restricted cubic spline model was used to evaluate the nonlinear trend, and as some studies reported outcomes at multiple time points. Various correlation structures for data reported by the same study were considered.

Results A total of 40 randomized Controlled Trials were included. There are 10 time points for PPD and CAL, and the follow-up length ranged from 0.5 year to 8 years. The results are similar under the different correlation structures. GTR and EMD achieve greater PPD reduction and CAL gain than FO in both short-term and long-term follow-up, but the uncertainty in their long-term benefits was large due to few studies reporting results after one year.

Conclusions Compared with traditional flap operation, periodontal regeneration surgeries achieved greater PPD reduction and gain in CAL after one year, and its effects may last for 5 to 8 years.

Notes
Full-mouth Disinfection in the Treatment of Chronic Periodontitis: A Systematic Review and Bayesian Network Meta-analysis
Lu, Y., Chen, T., Wu, Y., Tu, Y.
Institute of Epidemiology and Preventive Medicine

Objectives Quadrant scaling (Q-SRP) in consecutive appointments and one-appointment full-mouth scaling (FM-SRP) are two common non-surgical treatment strategies for chronic periodontitis. Both have also been used in conjunction with other supplemented materials. Several meta-analyses have been conducted but they only made pairwise comparisons. The aim of this study is to conduct a Bayesian network meta-analysis for comparing the treatment effects of Q-SRP, FM-SRP and their combination therapies.

Methods A systematic literature search was conducted using Medline, Pubmed and CENTRAL databases up to May 2015. All randomized control trials (RCTs) were included. Treatment outcomes were changes in probing pocket depth (PPD) and clinical attachment level (CAL). We extracted data reported at 3-month and 6-month after periodontal surgery. Bayesian network meta-analysis was then used to compare different treatment strategies and their ranking in performance.

Results A total of 18 RCTs were included in this review. Seven treatment strategies including Q-SRP, FM-SRP, FM-SRP plus chlorhexidine (FM-SRP+CHX), FM-SRP plus iodine (FM-SRP+I), FM-SRP plus fluoride (FM-SRP+F), FM-SRP plus chlorhexidine and fluoride (FM-SRP+CHX+F), and FM-SRP plus chlorhexidine varnish (FM-SRP+CHX Vanish) were identified. No statistically significant difference in either PPD reduction or CAL gain was found amongst various quadrant SRP or FM-SRP treatment strategies. FM-SRP+CHX+F had the best performance on PPD reduction both in 3-month and 6-month; FM-SRP+CHX Varnish had the best performance on CAL reduction in 3-month and FM-SRP+CHX had the best performance on CAL reduction in 6-month.

Conclusions This study did not find substantial differences in benefits of different non-surgical treatment strategies for chronic periodontitis, although FM-SRP+CHX+F have the best performance on PPD; FM-SRP+CHX Varnish and FM-SRP+CHX have the best performance on CAL.

Notes
Association Between Periodontitis, Renal & Vascular-Function In Chronic Kidney Disease

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Objectives Patients with chronic kidney disease (CKD) experience a higher rate of mortality, mainly through cardiovascular events. These events are associated with declining renal function and increased vascular stiffness, in patients with CKD. Declining renal function is also associated with increased morbidity as in end-stage-renal-disease, patients may need dialysis or kidney transplants. Periodontitis may contribute to the systemic inflammatory or oxidative stress burden in patients with CKD and hence act as a co-morbid factor in the morbidity and mortality associated with CKD. We aim to investigate the association between measures of periodontitis and estimated glomerular filtration rate (eGFR) and pulse wave velocity (PWV), a measure of vascular stiffness.

Methods Results are presented from an on-going longitudinal observational cohort study of patients with high-risk CKD. Over 700 patients underwent a detailed medical and dental examination including a periodontal examination (interproximal sites of all teeth). eGFR was calculated using the 4v-MDRD equation. The carotid-femoral PWV was assessed as a measure of vascular stiffness (surrogate marker of cardiovascular risk) using the Vicorder system. Multiple linear regression models were used to determine the association between various periodontal measures and eGFR and PWV, accounting for age, sex, ethnicity, diabetic and smoking status, albumin-creatinine ratio, BMI, blood pressure and socio-economic status.

Results The mean age of this cohort (N=678) was 64 (S.D.16; Range 19-92) with 60% males, 13% current smokers and 36% diabetic. 16% were edentulous, 42% had severe periodontitis and only 4% were periodontally healthy (CDC/AAP classification, 2007). Measures of active periodontal disease (mean probing depth, bleeding on probing and periodontal inflamed surface area) were significantly associated with declining renal function and increasing PWV in a dose-dependent fashion. This association was not significant in patients with severe periodontist compared to whose with healthy/moderate periodontitis.

Conclusions Measures of active periodontal disease correlate with declining eGFR and increasing PWV in CKD patients. It remains to be seen if periodontal health of patients at baseline has an impact on CKD progression and increased.

Notes
The Influence of Supra-physiological Glucose Levels on Mesenchymal Stem Cells. Consequences for Diabetic Bone Repair.
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Dental School/ Cardiff University

Objectives To gain a greater understanding of the effect of high glucose levels on MSC populations.

Methods MSCs were isolated from rat femurs. Bone marrow was removed and bone-associated cells were detached with collagenase. Cells were cultured in normal (5.5mM) and high glucose culture levels (25mM) for over 250 days, during which population doublings (PD) were calculated. At PD 15, 50, 100 and 200, regenerative capacity and expression of age-related markers for MSCs were assessed by measuring cell morphology and size; colony forming efficiency (CFEs); cell cycle markers, p16\textsuperscript{INK4a}, p53 and p21\textsuperscript{WAF1} and senescent markers β-galactosidase staining, telomerase expression and telomere length.

Results During culture expansion in normal and high glucose, cells failed to attain senescence at any time point up to PD 200. For normal glucose media, a progressive decrease in cell size and increase in CFE between PD15 and PD100 was observed. However, CFE decrease at PD200. In high glucose, CFEs were lower; cell size decreased and exhibited a higher expression for senescent genes markers compared to normal indicated a different size distribution compared to normal cells. Compared to cells grown in normal media, cells in high glucose exhibited a higher expression for senescent genes markers, p16\textsuperscript{INK4a}, p53 and p21\textsuperscript{WAF1}.

Conclusions These results suggest that as cells expand in culture, there is a change in the heterogeneous cell population, with the possible loss of mature lineage restricted cells around PD50. In high glucose conditions, results could suggest a reduced regenerative capacity of remaining cells.

Notes
Neutrophil cell glutathione changes in chemotaxis

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Objectives Chronic periodontitis is an inflammatory disorder affecting the teeth, connective tissues and alveolar bone within the oral cavity. Neutrophils from periodontitis patients have decreased intracellular glutathione.

Intracellular glutathione is essential for a number of homeostatic and cellular processes, including chemotaxis and is the main intracellular redox buffer.

The objective was to analyse the effect of alteration in glutathione levels on chemotactic behaviour of neutrophils.

Methods Neutrophils were isolated from the peripheral blood of healthy volunteers. Real Time Glo was used as a viability assay to determine time course for neutrophil cells experiment. A Boyden type chamber was used to assess neutrophil chemotaxis with and without the pre-incubation of glutathione depletion reagents: BSO, CDNB, BCNU. Chemoattractants used were FMLP and IL8.

Results CDNB, a deplete of glutathione through thioester conjugation, and BCNU, an inhibitor of glutathione reductase both altered neutrophil chemotaxis relative to control treated cells responding to both FMLP and IL8. However BSO, an inhibitor of rate limiting gamma-glutathione cysteine synthetase, did not appear to have an effect.

Conclusions Alterations in chemotaxis and intracellular glutathione in periodontitis patients are already known separately, this study may begin to help in our understanding the mechanism by which these two factors may be linked.

Notes
Outcomes of periodontal therapy in Rheumatoid Arthritis: baseline data from a randomised controlled trial

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Objectives OPERA is a feasibility randomized controlled clinical trial on the effect of non-surgical periodontal therapy on clinical, immunological, microbiological and inflammatory parameters in rheumatoid arthritis (RA). The objective of this paper is to present baseline characteristics of the study population.

Methods Patients with RA were approached and screened for the presence of chronic periodontitis. Major inclusion criteria were DAS 28>3.2, cumulative pocket depth (CPD) of 40 mm and being in stable medication for more than 2 months. At baseline, a range of demographic, periodontal and rheumatologic clinical and laboratory parameters were collected. In addition, saliva, blood, GCF and plaque samples were taken for biobanking. Eligible patients were randomized to either immediate or delayed (6 months) non-surgical periodontal therapy. Follow-up appointments were carried out after 3 and 6 months post baseline.

Results A total of 296 patients with Rheumatoid Arthritis were approached and consented in 3 different hospitals in Birmingham. 196 patients were screened (mean age 58 years old, 80% female), 46% of screened patients had moderate to severe periodontitis, and 26% met all the inclusion criteria for the study. The mean DAS 28 score of our population is 3.79 (ED=0.13), median cumulative pocket depth 34.0 (IQR=36-49), mean ESR 17.71 (N=21). Therefore, a total of 60 patients were randomized for immediate or delayed non-surgical periodontal therapy. This group presented a higher DAS 28 (mean 4.6) compared to the non-eligible group (mean 3.5).

Conclusions Out of all the patients consented, 33.7% patients did not attend the screening appointment. Feasibility data from our study will support future definitive studies to investigate the outcomes of periodontal therapy in rheumatoid arthritis and the analysis of the biological samples will help to understand the link between the two conditions.

Notes
Effectiveness of Gingival Retraction Methods: A Systematic Review
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Aga Khan University Hospital

**Objectives** The aim of this systematic review was to assess the effectiveness of different gingival retraction methods in terms of the amount of gingival retraction achieved and changes observed in various clinical parameters e.g. Gingival Index (GI), Plaque Index (PI), Probing depth (PD) and Clinical attachment level (CAL).

**Methods** Data sources included three major databases i.e. PubMed, CINAHL plus(Ebsco), COCHRANE along with hand search. Search was made using the key terms in different permutations of gingival retraction* AND displacement method* OR technique* OR agents OR material* OR medicament*. The initial search results yielded 142 articles which were narrowed down to final 10 articles after a strict eligibility of including clinical trials or experimental studies on gingival retraction methods with the amount of tooth structure gained and assessment of clinical parameters as the outcomes conducted on human permanent teeth only.

**Results** The total number of teeth assessed in the 10 selected studies were 400. Gingival retraction was measured in 6/10 studies whereas the clinical parameters were assessed in 5/10 studies. The results were highly heterogeneous with regards to the outcome variables.

**Conclusions** No method seemed to be significantly superior to the other in terms of gingival retraction achieved. Clinical parameters such as Plaque Index (PI), Probing Depth (PD), Clinical Attachment Level (CAL), Bleeding on Probing (BOP) etc. were not significantly affected with gingival retraction. Except for Gingival Index (Gingival Index), this was significantly altered in some studies.

**Notes**
A qualitative study to explore the issues faced by patients when giving feedback on the communication of dental students

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Objectives Whilst the DoH and NIHR are committed to involving patients in improving clinical education, research and service delivery, there is a limited body of evidence on the perceptions of patients when asked to be involved in this way, and specifically when asked to feedback on the communication skills of dental students. This study seeks to address this gap and heighten the understanding of the issues faced by patients when asked to be involved in clinical education.

Methods Qualitative research methodology was used in the form of focus groups to gather data from patients being treated at the Peninsula Dental School on the thoughts, feelings and beliefs of the target population when asked to feedback hypothetically on the communication skills of dental students. Data analysis involved inductive thematic analysis of the transcribed audio recordings. The rigor of the research was tested by participant validation of emergent themes, prior to final analysis.

Results The data revealed that patients want to be involved in this way and perceive themselves as co-educators who can enhance the students teaching and learning experiences. Issues of anonymity, confidentiality and ownership of the feedback process were worrisome. Careful support of the students was highlighted if feedback was negative, and the positioning patient feedback in the programme was seen as critical, with higher year students being perceived as more able to cope than younger students. Potential patient vulnerability was observed with the power balance between patient and student being important.

Conclusions Patients have a valuable contribution to make to the development of the communication skills of dental students and are willing be involved in the feedback loop. As co-educators, they can mutually work together in the supportive environment of dental schools to enhance these skills.

Notes
Teaching of posterior composites in UK and Ireland dental schools
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Objectives Despite many advances in prevention, the restoration of teeth, affected by caries or trauma or in need of a replacement restoration, is still one of the most commonly performed procedures in dental practice today. The aim of this study was to investigate the teaching of posterior composites in UK and Ireland dental schools in 2015.

Methods Following ethical approval, an internet-based questionnaire was distributed by email to the person identified as being responsible for leading teaching in Operative Dentistry within each of the 18 UK and Ireland dental school with undergraduate programmes.

Results Responses were received from 17 of the 18 schools surveyed (94%). All schools included clinical teaching of posterior composite placement in occlusal cavities. However, 2 schools did not include teaching of 3-surface posterior composites in premolars or molars. The ratio of posterior composites: amalgams placed by students, on average, was 2:1 (67% posterior composite: 33% amalgam). 4 schools (24%) taught preparation of enamel bevels on the occlusal and proximal cavosurface margins prior to placement of posterior composites. For management of “moderate cavities” (involving middle third of dentine), 11 schools taught (65%) taught a ‘total etch’ (no base) approach. Six schools (35%) included teaching of newer bulk filling composites. Following the signing of the Minamata Treaty, a majority of schools (n=10) felt that the teaching of amalgam should be “phased down” over the next 7 years.

Conclusions This survey has seen a further increase in the placement of posterior composites since the time of the previous surveys in 2004 and 2009. Further investigations are indicated into understanding barriers to further increases of teaching of posterior composites, particularly given the desire to practice minimally invasive dentistry, as well facilitating a ‘phase-down’ in the use of dental amalgam as recommended by the UN Minamata Treaty.

Notes
A Mixed Methods Assessment Of An Orthodontic E-learning Resource

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1Cedars Dental Practice , 2Queen Mary University of London, 3Queen Mary University of London, 4Queen Mary University of London

Objectives Traditional forms of undergraduate orthodontic teaching include lectures, seminars and clinical teaching. More recently e-learning has become imbedded within undergraduate curricula. The aims of this study were to describe the development of a novel e-learning resource and to assess the impact of the resource on student learning experiences and orthodontic knowledge.

Methods Thirty-two 4th year undergraduate students at Queen Mary University of London were randomly allocated to receive electronic access to e-learning material comprising of fundamentals of orthodontics and malocclusion types over a 6-week period. Thirty-one student controls were not given access during the study period. All students were asked to complete electronic quizzes both before (T0) and after (T1) the study period and a general questionnaire concerning familiarity with e-learning. The test group, also completed user satisfaction questionnaires at T1. Two focus groups (5-7 participants) were also undertaken to explore learners’ experiences and suggestions in relation to the resource.

Results The mean quiz result improved by 3.9% and 4.5% in the control and test groups, respectively. An independent t-test failed to demonstrate a statistically significant difference in knowledge gain between control and test groups, however (P= 0.941). The qualitative feedback indicated that students believed that use of the resource enhanced knowledge and basic understanding with students expressing a wish to ingrain similar resources in other areas of undergraduate teaching.

Conclusions Use of the novel orthodontic e-resource over a short period did not result in a significant change in subject knowledge. However, the e-learning has proven popular among undergraduate resources and will continue to be refined.

Notes
New Graduates’ Professionalism And Communication Skills: Trainers’ Expectations and Experience
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Objectives A one-year period of Dental Foundation training is mandatory to work in the NHS in the UK. Anecdotal feedback from trainers suggests concern relating to the non-clinical skills of the new graduate. The objective was to investigate foundation trainers’ expectations and experience of new graduates’ professional values and communication skills.

Methods Data were collected from an online questionnaire distributed to all Dental Foundation trainers in the Wales Deanery (N=69). The questionnaire investigated trainers’ expectations and experiences of new graduates’ skills. General expectations of graduates’ communication and professional values were explored using eleven direct statements. For comparison, identical statements were used to gauge each trainer’s experience of their current trainee. Trainers were also asked to indicate if they had experienced specific difficulties with their current trainee. Respondents were invited to comment further.

Results 60 complete questionnaires were returned (87%). Trainers’ expectations outstripped experience for the eleven direct skill statements. Experience was most adrift from expectations for the ability to: keep accurate patient records, listen to a patient effectively and respond appropriately; and put patient’s interest first, through adopting a professional approach to their work. Trainers’ expectations were highest amongst these skills. In response to the difficulties questions, 72% (n=43) of trainers had experienced none of the listed difficulties. However, 20% (n=12) felt that their current trainee had poor time management skills; 17 % (n=10) reported that their current trainee did not know when to seek help and 15% said they did not integrate well with team.

Conclusions Open comments were illuminating and reinforced the findings. In conclusion the study highlights a number of skills where trainers’ expectations of trainees are higher than their current experience and areas where the new graduate experiences difficulties.

Notes
Views of recent dental school graduates on teaching in prosthodontics.
Oliver, G.1, Lynch, C. D.2, Chadwick, B. L.3, Santini, A.4, Wilson, N. H.5
1Oxford University Hospitals, 2School of Dentistry, Cardiff University, 3School of Dentistry, Cardiff University, 4The University of Medicine & Pharmacy, 5Kings College London Dental Institute

Objectives The aim of this study was to investigate the views of recently graduated dentists on their undergraduate training in fixed and removable prosthodontics and as well as their views on possible areas for improvement.

Methods An electronic questionnaire was distributed by email to Fellows and Members of the Faculty of General Dental Practice (FGDP, UK) via email containing questions asking about respondents’ dental education history, opinion on educational experience at dental school and how well the undergraduate programme prepared them for life post-graduation in relation to prosthodontics.

Results 194 responses were received from graduates qualifying between 2004-2013. A majority of respondents agreed they had learned ‘a lot’ or ‘enough’ in relation to crowns (69%), acrylic removable partial dentures (65%), resin-bonded bridges (62%), complete dentures (60%), and metal-based removable partial dentures (54%). However a majority reported they wished they had ‘learned more’ or felt their ‘training was deficient’ in relation to implants (77%), porcelain veneers (64%), conventional bridges (53%), and copy dentures (52%). Comments received criticised a lack of clinical time and experience while at dental school. However most appeared to appreciate the constraints of the undergraduate curriculum as well as the need for life-long learning.

Conclusions The findings of this survey will be of interest to those working with dental students, Dental Foundation Trainees and young dental practitioners in helping to produce a ‘safe beginner’. These results demonstrate a demand for further training in implants, porcelain veneers, conventional bridges and copy dentures although this may be improved with further clinical experience. However those involved in dental education must ensure that undergraduate education is fit for purpose and meets the expectations of the General Dental Council.

Acknowledgement The assistance of the FGDP(UK) Secretariat in conducting this study is greatly appreciated.

Notes
Are Thiel Cadavers a Better Model for Teaching Exodontia to Dental Undergraduates?

Macluskey, M., Hanson, C., Eisma, R.

University of Dundee

Objectives The objective was to determine whether the Thiel embalmed cadavers were perceived to be an improved model for the teaching of exodontia by the undergraduates.

Methods From 2011 to 2013 second year undergraduates were randomly assigned into two groups, those taught traditionally using mannequins followed by observation of patient treatments and those who additionally attended cadaveric teaching at the Centre for Anatomy and Human Identification (CAHID).

Results In total 128 students of the 219 attended CAHID. Feedback was collected from 69 students (54%) and 100% had no moral or cultural objection to using cadavers. The majority (98%) thought using the cadavers was advantageous, gave a realistic feel for soft tissue management (89%) and felt it was similar to managing a patient (81%). Compared to a mannequin they felt that the cadaver offered a greater challenge (78%) but was not as challenging as treating a real patient (95%). No difference was found in self-reported confidence at exodontia between those that attended CAHID and those that did not. No one who attended CAHID failed their clinical exam and the failure rate was 3%, 7% and 6% in 2011, 2012 and 2013.

Conclusions The use of Thiel cadavers was well received by the students who found it a more realistic model for exodontia. Future work on these cadavers may be expanded to include surgical procedures thus better preparing our undergraduates.

Notes
Peer Review of Teaching in UK Dental Schools

Cunningham, I. M., Lynch, C. D.
Cardiff University

Objectives The aim of the project was to investigate the use of Peer Review of Teaching (PRT) in UK Dental Schools.

Methods A structured questionnaire consisting of 12 open and closed questions was emailed to the Deans of all 16 UK Dental Schools. Deans were invited to forward the questionnaire to the person most knowledgeable about PRT within their school. Follow-up emails were sent at 4 and 6 weeks. Data was made anonymous prior to analysis.

Results Responses were received from 16 schools (100%). The majority of responders (10) were leads for learning and teaching, with 3 being PRT leads and 3 school Deans. 14 schools reported having a PRT scheme. 11 schools define a minimum level of staff engagement, most frequently that all teaching staff engage annually. Although most schools stated that their schemes were fully operational (9), only 4 considered their staff to be fully engaged. Reasons for sub-optimal engagement included: newly introduced or changing schemes, problems with compliance for off-campus staff, cynicism and loss of momentum. 9 schools felt that changes to their scheme were required, including: a larger reviewer pool, pre-allocation of partners (rather than self-selection), reviews by more experienced teachers, and expansion to include clinical teachers.

Conclusions This survey reveals that PRT is operating within the majority of UK dental schools, although there is a variation in scheme maturity, staff engagement and format. The survey generated ideas for improving schemes further which should be explored in future research.

Notes
In Vitro Real-Time Measurements of Rate of Enamel Remineralisation

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Objectives Fluoride can significantly improve the enamel remineralisation capability of enamel dentifrices. The primary objective of this in vitro study was to quantify the amount of mineral deposition following the application of fluoride containing dentifrices in real-time using scanning microradiography (SMR).

Methods Enamel sections were cut and polished to a thickness of 1mm to remove the underlying dentine. Sections were then placed into a flow cell, which had solutions circulating through at a rate of 0.8ml/min. The mineral mass was simultaneously measured throughout the experiments. Initially, enamel sections were demineralised by 0.1M acetic acid (pH 4) for 24 hours. Samples were then remineralised by one of four treatments; artificial saliva control, a fluoride-containing (100ppm F) artificial saliva, artificial saliva with 2 daily applications of a nano-hydroxyapatite and fluoride containing toothpaste (Ultradex Recalciﬁying and Whitening Toothpaste diluted 1 in 3 with water to give 333ppm F) and ﬁnally artificial saliva with 2 daily applications of the aforementioned toothpaste and 2 applications of a nano-hydroxyapatite and fluoride-containing oral rinse (Ultradex Oral Rinse also diluted 1 in 3 with water to give in total 533ppm F).

Results Enamel was remineralised by the control at a constant rate of $2.2 \times 10^{-5}$ gcm$^{-2}$ hr$^{-1}$ for the duration of the experiment. The fluoride-containing artificial saliva signiﬁcantly increased the rate of remineralisation of enamel for up to 30 hours before the remineralisation rate plateaued. The remineralisation rate of enamel with the toothpaste was also greater than the control. Finally, the combined use of the toothpaste and oral rinse appeared to signiﬁcantly enhance the rate of enamel remineralisation and surpassed both the fluoride-containing artificial saliva and individual toothpaste treatments.

Conclusions SMR can be successfully used to observe the real-time changes in the increase in mineral gain during enamel remineralisation by various remineralising agents. The rate of remineralisation of enamel is signiﬁcantly increased by the presence of fluoride in a toothpaste and oral rinse.

Notes
Osteoarthritis biomarker discovery using a 3D human articular cartilage model
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*Cardiff University, †Cardiff University, ‡Swansea University

Objectives Osteoarthritis (OA) is the most common form of joint disease, yet no reliable diagnostic or prognostic biochemical markers are available. Diagnosis is confirmed by x-ray (or MRI), which detects late-occurring gross structural changes to joints. Here, we report an in vitro model of human articular cartilage that enables identification of diagnostic indicators for early (molecular) disease stages.

Methods Chondrocyte progenitor cells isolated from human articular cartilage were used to generate cartilage-like tissue in vitro. The 3D cartilage model was characterized and optimized for biomarker identification, employing qPCR, immunohistochemistry, quantitative biochemical methods and compression testing. To investigate OA-related changes in expression of glycosylation-related genes, constructs were subjected to inflammatory regimens known to be present in OA. Treated and untreated constructs were analyzed using GLYCO v4 gene chips which specifically detect genes involved in glycosylation and related post-translational modifications.

Results Cartilage constructs were 6mm by 1mm discs, with full-depth cellular differentiation and tissue stratification, matrix synthesis, and biomechanical properties comparable to articular cartilage. Testing of constructs with cyclical loading or inflammatory cytokines induced mechanoresponsive genes and MMP-13/ADAMTS-4 mediated cartilage breakdown, respectively. Microarray analysis of the glycome following inflammatory stimulation identified changes in protein modifications associated with OA and revealed epitopes that could be biomarkers for OA. Using our cartilage model, we have verified the upregulation of a selected candidate biomarker (qPCR, immunohistochemistry), and confirmed a similar pattern of expression of this epitope in patient derived OA cartilage.

Conclusions De novo formation of artificial human cartilage tissue in vitro from chondrocyte progenitor cells provides a new tool for analyzing cartilage responses to external stimuli (loading, inflammation, drugs). We have successfully used this model to identify previously unknown qualitative changes occurring in protein glycosylation during development of human OA. The potential use of these epitopes as diagnostic OA biomarkers is now under investigation.

Notes
Repair of Enamel Erosive Lesions with Adult and Children's Dentifrices

fowler, c.1, Brown, A.2, Lynch, R. J.1
1GlaxoSmithKline, 2Lucideon Ltd

Objectives To compare the ability of two commercial adult’s dentifrices and an experimental children’s dentifrice to reharden artificial erosive lesions in vitro. Fluoride uptake into erosive lesions after treatment with some of the dentifrices was also determined using Dynamic Secondary Ion Mass Spectrometry (DSIMS).

Methods Rehardening and fluoride uptake (DSIMS) experiments were conducted. Permanent and deciduous human enamel specimens were polished and immersed in 1.0% citric acid, pH 3.8, for 5min during the fluoride uptake experiment and 30min during the rehardening experiment to create artificial erosive lesions. Specimens were then divided into four treatment groups: Adult erosion dentifrice - Sensodyne Pronamel (AED), Adult dentifrice - Colgate Cavity Protection (MAD), Fluoride-free control, Experimental children's dentifrice (ECD). Dentifrices were prepared as 1:3w/w slurries with deionised water, and specimens incubated for 2min in one of the slurries (AED not included in fluoride uptake experiment). Specimens for rehardening were subsequently immersed in artificial saliva for 48h. Microhardness measurements were made after 24h and 48h. For fluoride uptake, DSIMS cross-sectional imaging was used to generate linescans of fluoride depth distributions after treatment.

Results All lesions rehardened after 24 and 48h incubation in artificial saliva. Lesions treated with fluoride-containing dentifrices exhibited superior rehardening to the placebo dentifrice. Specifically, AED and ECD treatments were superior to MAD. DSIMS showed that for specimens treated with ECD, fluoride penetration was substantially greater than for MAD, despite the formulations containing equal quantities of sodium fluoride. There were no significant differences between deciduous and permanent enamel exposed to the same treatments in either experiment.

Conclusions This in vitro investigation has shown that rehardening and fluoride uptake were higher for the experimental children's dentifrice than for specimens treated with a leading marketed adult's dentifrice, for both permanent and deciduous enamel.

Notes
Preparation of wild-type and mutant recombinant amelogenins for functionality studies of Al pathogenesis
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1University of Leeds, 2University of Leeds

Objectives Amelogenesis imperfecta (AI) describes a group of inherited biomineralisation defects of enamel. Mutations in AMEL (encoding amelogenin, the principle enamel matrix protein), underlie AI in man. We showed that a Y64H point mutation in murine amelogenin is associated with abnormal intracellular amelogenin accumulation, endoplasmic reticulum (ER) stress and an unfolded protein response (UPR), ultimately leading to ameloblast apoptosis. Our present aim was to identify an efficient and rapid methodology for production of wild-type (WT) and Y64H mutant recombinant amelogenins for functionality studies.

Methods Recombinant WT and Y64H His-tagged amelogenins were expressed in E. coli transfected with pET28/AMELXWT or pET28/AMELX MUT vectors. Cells were harvested, washed in 150mM NaCl and centrifuged. Cell pellets were resuspended in 3% v/v acetic acid and heated at 65°C for 20 minutes before centrifugation. Supernatants were freeze-dried, dissolved in tris-buffered 10mM imidazole, 4M urea (pH7.4) and loaded onto a nickel column. His-tagged amelogenin was eluted by increasing the imidazole concentration in the buffer to 200mM. Purification was monitored using a Nanodrop-2000, SDS-PAGE and Western blotting using rabbit anti-amelogenin antibodies (1:10,000) and goat anti-rabbit secondary antibodies conjugated to horse-radish peroxidase (1:3,000) with a 3, 3'-diaminobenzidine chromogenic substrate.

Results SDS-PAGE and Western blotting showed that selective acid extraction as a first step removed almost all contaminating E. coli proteins from the crude extracts. The recombinant proteins were further purified using affinity column chromatography. The methodology proved scalable, producing mg amounts of WT and Y64H amelogenins.

Conclusions Selective acid extraction of WT amelogenin was described previously but these data show that Y64H mutant amelogenin, believed to be more highly aggregative than WT, can also be selectively purified using this process. This simple, rapid, large scale methodology provides purified Y64H amelogenin for use in functionality studies that will help elucidate the underlying mechanisms of ER stress and its pharmacomodulation in AI.

Notes
Ordered Enamel Crystallite Formation Using Elastin-like Proteins


1Queen Mary University, 2Queen Mary University, 3Queen Mary University

Objectives Enamel is a mineralised tissue consisting of highly ordered hydroxyapatite crystallites, essential for its mechanical properties. Novel remineralising products exploit the use of self-assembled peptides as a minimally invasive option to treat carious enamel. Although new mineral is deposited within the lesion, there is little evidence of ordered enamel-like structures forming. The overall aim is to understand the role of bio-organic molecules in guiding mineral growth. Consequently therapeutic remineralising systems can be developed which promote the formation of ordered enamel-like structures.

Methods Recombinantely synthesized elastin-like proteins (ELPs) with and without bioactive domains were obtained. Five different ELPs were used for comparison: Stn15-with 15 amino acid statherin N-terminal sequence; RGD- with arginine-glycine-aspartic acid (RGD) amino acid sequence; Stn15-RGD- with statherin and RGD sequence; control-no bioactive segment; acidic di-block containing acidic domains but no basic domains. The ELPs were coated on glass slides and incubated for 8 days in mineralising solutions of dissolved hydroxyapatite (2 mM) and sodium fluoride (2 mM), adjusted to pH of 6.0.

Results Scanning electron microscopy (SEM) showed that, with ELPs on glass, crystallites grew with a preferential direction. However, when ELPs were added to the solution, no platelet structures were present. Energy dispersive X-ray spectroscopy (EDX) showed that mineral formed in the presence of ELPs is apatite-like and had organic matter incorporated into it. During incubation the mineral underwent morphological changes which may be attributed to pH dropping to 3.8. The crystallites changed from single platelets to multi-layered platelets. Polarised light microscopy (PLM) showed that crystallites are mostly isotropic at early stages, compared to minerals present after 1 day of incubation.

Conclusions ELPs affect the morphology of mineral formed in a supersaturated solution. However, only when ELPs were coated on glass was there a preferential direction of growth for the minerals. This demonstrates that control of mineral growth is dependent on the presence of proteins and how they are introduced into the solution. This studentship is funded by QMUL as part of the Life Sciences Initiative.

Notes
Pharmacological rescue of amelogenesis imperfecta and the NFκB pathway

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Objectives Amelogenesis imperfecta (AI) is an inherited disease affecting enamel biomineralisation. We showed that a Y64H point mutation in mouse amelogenin phenocopying human AI caused ER stress in ameloblasts with subsequent apoptosis. COS7 cells transiently transfected with Y64H amelogenin also underwent ER stress driven apoptosis. Pharmacological intervention with the FDA-approved drug phenylbutyrate (PB) ameliorated ER stress and rescued the phenotype in both mice and COS7 cells. To determine the mechanism of PB action, our aim was to investigate whether PB affects NFκB expression, which, depending on cell context, favours either cell survival or apoptosis.

Methods COS7 cells were stably transfected with a secreted-luciferase gene driven by a NFκB enhancer element (pNFκB-MetLuc2- (secreted-luciferase reporter vector); Clontech). Cells were cultured in DMEM containing 10% foetal bovine serum. Sodium phenylbutyrate (PB) was added to the cultures at concentrations ranging from 12.5 to 1000µM for 48h. Control cultures contained no PB. Secreted luciferase was quantified using a luciferase assay (Ready-To-Glow™ Secreted Luciferase Reporter System; Clontech) using a 96 well microplate reader.

Results PB at concentrations 12.5 - 500µM significantly increased luciferase expression relative to controls in a dose dependent manner. PB at 12.5µM induced a 6.1±1.3 fold increase in expression (p<0.001) increasing to 9.4±1.9 fold at 500µM (p<0.05). With 1000µM PB, expression levels fell to levels similar to those obtained with 12.5µM PB.

Conclusions PB increases the efficiency of the NFκB enhancer element in COS7 cells. We propose that PB rescues the Y64H phenotype by promoting cell survival via NFκB pathways. Ongoing studies are examining the effect of PB on NFκB expression and its up-regulation of downstream pro-survival targets (e.g. Bcl-2, BcL-XL and Bfl-1/A1) in COS7 cells transfected with Y64H amelogenin. Elucidation of the molecular mechanisms whereby PB promotes cell survival will aid in the development of pharmacological based treatments for AI.

Notes
Co-operativity of Statherin and Histatin in Demineralisation of Carious and Erosive HAp Model
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Objectives One aim of preventive and minimal invasive dentistry is to alter the oral environment to a less demineralisation setting which may allow early caries and erosive lesions to be reversed. Salivary proteins such as statherin (StN43) are known to be involved in de-mineralisation. The active N-terminal is involved in inhibition of precipitation and responsible for binding with Ca$^{2+}$. StN21 is an analogue peptide that contains the N-terminal 21 residues similar to StN43. Histatin-1 (HtN38), similar to salivary proline-rich proteins, is also understood to inhibit crystal growth of calcium phosphate salts, but does not inhibit spontaneous precipitation. The objective of this study was to measure the effect of these test peptides (StN21 and HtN38 individually and in combination) on the demineralisation rate of previously demineralised hydroxyapatite discs (HAp) using scanning microradiographic (SMR).

Methods HAp discs were mounted within SMR cells. Three scanning positions were located on each disc for the measurement of the rate of mineral loss (RD$_{\text{HAp}}$). The discs were exposed to de-mineralising solutions (pH 4.0, 0.1M acetic acid) for 3 days. After this preliminary acidic exposure, the HAp discs were rinsed thoroughly with distilled water and then exposed to 2.0ml of the test solution containing the peptides dissolved in phosphate-buffered saline (PBS) at a concentration of 0.2mM. After 24hrs the HAp discs were then exposed to the acidic demineralisation solution for a further 3 days. A HAp disc exposed to PBS was used as a negative control. RD$_{\text{HAp}}$ was measured continuously using real-time SMR.

Results StN21 and HtN38 reduced the RD$_{\text{HAp}}$ by 40.0±3.2% and 39.2±2.9% respectively compared to the control. The peptides in combination reduce the RD$_{\text{HAp}}$ by 52.6±2.2%.

Conclusions This study has shown that StN21 and HtN38 have considerable effect in reducing HAp demineralisation under caries/erosive simulation conditions. Their increase in efficacy in combination suggests a cooperative action. This suggests that StN21, HtN38, and a combination of both can be used as a therapeutic agent for preventive treatment of enamel demineralisation.

Notes
The Effect of Demineralisation on Enamel Porosity

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Objectives The intrinsic porosity of enamel is an important consideration in erosion studies since diffusion pathways for acids and minerals may affect the dissolution of enamel. In this study, the effects of enamel acid erosion on the depth and distribution of enamel pores of erosive lesions were studied. Scanning electron microscopy (SEM) for topographic investigation and confocal laser scanning microscope (CLSM) in fluorescent mode for obtaining non-destructive microscope tomographies (3D imaging) of enamel pores were utilised to visualise the dissolution process.

Methods 35 polished bovine enamel samples were randomised into 7 groups and were immersed in acetic acid solution (0.1M, pH4.0) for 0, 5, 15, 30, 60, 90 or 120 minutes (i.e. 5 specimens per time group). The enamel surfaces were investigated with SEM backscattered imaging, then the specimens were soaked in fluorophore (Rhodamine B, 0.1mM) for 24 hours, rinsed with water and imaged by CLSM.

Results CLSM cross-section images showed the depth of the penetration of fluorophore into the enamel pores of the control and erosive lesions. The acid dissolution process created a demineralised and more porous surface layer, with deeper dye penetration observed with longer demineralisation times (6.10, 17.0, 8.90, 13.5, 19.8, 18.2 and 34.0 µm for 0, 5, 15, 30 60, 90 and 120 minutes erosive lesions, respectively). Acid-dependent etching patterns that progressed with exposure duration, were observed from top-view SEM images.

Conclusions CLSM was found to be a useful tool for studying enamel demineralisation, 3D images of enamel pores were observed with increased pore distribution and depth observed with increasing acid exposure time. SEM images showed rougher surface as the erosive lesions progressed.

Notes
Bacterial Toxicity Comparison Between Silver, Titanium Dioxide and Hydroxyapatite Nanoparticles

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Objectives This study aimed to determine the toxicity to *Streptococcus sanguinis* of a range of engineered nanomaterials (ENMs) compared to their equivalent bulk materials or metal salts, and against a positive control of chlorhexidine.

Methods Bacterial suspensions of *S. sanguinis* (10⁷ CFU ml⁻¹) were exposed to dilutions series (400-3.125 mg l⁻¹) of each material using the minimum inhibitory concentration assay (MIC). The lactate production by *S. sanguinis* was also measured. The materials tested included Ag, TiO₂ and hydroxyapatite (HA) nanoparticles (NPs). The antibacterial activity of these ENMs was compared to that of AgNO₃, TiO₂ bulk and HA microparticles respectively. Chlorhexidine was used as positive control. All test solutions were prepared in physiological saline (modified Krebs solution) and normal saline (0.85 % NaCl). The materials under investigation were characterised in solution and the aggregate size was measured using nanoparticle tracking analysis (NTA).

Results Additions of the ENMs to physiological saline caused some aggregation. Silver nitrate was effective at growth inhibition at all the dilutions tested compared to unexposed controls. Ag NPs caused growth inhibition at 100 mg l⁻¹ or less. The MIC for complete growth inhibition for TiO₂ NPs was 50 mg l⁻¹, with the nano form being more toxic than bulk TiO₂. HA particles (nano or micro) were less toxic. Apparent lactate production by *S. sanguinis* was abolished by all concentrations of AgNO₃, but only at 400 mg l⁻¹ for Ag NPs. The different forms of neither TiO₂ nor HA had any effect on lactate production.

Conclusions Data showed that Ag NPs were a better antibacterial than TiO₂, and that HA particles were not overly toxic to *S. sanguinis* in the conditions used here. All ENMs that demonstrated antibacterial action were found to be more effective in saline compared to physiological saline.

Notes
Comparison of Shaping Ability and Failure Incidence of WaveOne files and One Shape files.

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Objectives The aim of this study was to compare and evaluate the efficacy of different single file techniques in terms of shaping ability of the canal according to canal centering and the failure incidence of the instruments.

Methods The study was divided into two parts. Forty mesiobuccal canals of human mandibular molars were utilized in part I of the study and divided in two groups 20 mesiobuccal canals each. Group A prepared with WaveOne files and group B prepared with One Shape files. Gutta percha pieces were attached to the mesial surface of the mesial roots at 3mm intervals from the apex guided by a digital caliber, these gutta percha pieces serve as a reference for standardization. The teeth were fixed in position inside two blocks of acrylic resin during the whole study for each group and then the teeth were scanned using Cone Beam Computed Tomography before and after preparation. Fusion of the pre-instrumentation and post-instrumentation records was achieved using special software that allows manual and then automatic registration of the pre and post-instrumentation records. Part II of the study was divided into group A and group B as in part I where the instruments were used to prepare the mesiobuccal canals of mandibular molars until instrument fracture and the number of canals prepared until fracture was recorded. The time that was taken by each file to reach the full working length of the canal was recorded. The data was tabulated and statistically analysed.

Results The results showed that in part I of the study there was no significant difference between the two groups regarding canal centering where in part II of the study there was a significant difference between the two groups regarding the fracture incidence with group A showing higher number of canals prepared till fracture. However, group B was significantly faster in reaching the full working length of the mesiobuccal canals than group A.

Conclusions From the results of the current work, it could be concluded that: - Reciprocating motion utilized by WaveOne files is promising in providing a well centered preparation, with less risk of instrument fracture and preservation of the canal curvature. - However it may require more time to reach the full working length than the One Shape files utilizing rotation motion.

Notes
The Efficacy of Hydrogen Peroxide in Modulating Dentine Staining
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Objectives Hydrogen peroxide (H₂O₂) is described as an effective and economical method for tooth bleaching treatment. Despite the ability of H₂O₂ to disintegrate staining molecules, the long-term effect on tooth structure has not been elucidated. Our previous work has demonstrated that dentine matrix proteins, predominantly collagen, facilitate binding of stains; the mineral crystals play little role in the process. The aim of this study is to investigate the efficacy of H₂O₂ in preventing staining of dentine matrix and its potential effect on collagenous structures within the tooth.

Methods Human teeth (15) were obtained from the Cardiff School of Dentistry Tooth bank. Enamel, pulp, and any caries were removed. Teeth were sectioned into 100µm slices and treated with 0.5M EDTA pH8 for 3 days, for demineralisation. Teeth were stained with Orange II Sodium salts for 7 days. After staining, teeth were treated with 10% or 30% H₂O₂ and colour loss was monitored over 1-168h. Teeth were re-stained with Orange II and re-incorporation of the stain was similarly monitored over 168h. Additionally, the direct effect of 25%, 12.5%, 6% and 3% H₂O₂ on purified collagen type I (from human placenta, Sigma-Aldrich) was analysed with degradation visualised by SDS-polyacrylamide gel electrophoresis.

Results After 7 days treatment with H₂O₂ (10% and 30%), teeth were completely de-stained. Re-stained teeth showed less intensity compared with teeth stained before H₂O₂ treatment. Following 24h H₂O₂ treatment of collagen type 1, progressive degradation of the alpha chains and cross links; little effect was apparent after 1h.

Conclusions H₂O₂ can result in tooth whitening. The mechanism is unclear but may in part be due to degradation of collagen, which we previously reported as a major protein to attract staining molecules. Additionally, reduced re-staining of sections may be brought about by the persistence of decolourised stain molecules blocking further matrix-stain interaction within the dentine tissue.

Notes
Benchmarking the Chemical Solubility of Restorative Dental Ceramics
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The University of Sheffield

Objectives The current ISO 6872:2008 specifies a standardized testing method to measure the chemical solubility of dental ceramics. Unfortunately, few published studies of chemical solubility have adhered to the ISO test. Therefore, the aim of this study was to assess the current ISO solubility test for benchmarking the chemical solubility of restorative dental ceramics and to investigate the impact of the flexibility in sample size afforded by the new standard.

Methods The chemical solubility analysis was performed to the study samples according to the (EN ISO 6872:2008). Two types of dental ceramics were involved (Vitablocs Mark II and Vita In-Ceram Alumina). Three different size groups (cubes) were prepared of both materials in order to investigate the chemical solubility of the varied geometrical specimens. The surface microstructure of test specimens was analyzed by scanning electron microscope (SEM, CamScan) before and after the solubility test.

Results It was found that increasing the individual surface area of the specimens across the range permitted by ISO 6872:2008 resulted in a decreasing chemical solubility rate (Table 1). ANOVA indicated a significant difference between groups 1 and 2, and 1 and 3. Particularly, the present outcomes possibly indicated as the overall sample edge length increases, the chemical solubility also increases. The SEM images indicated that the edges of the specimens were more vulnerable to chemical dissolution than other areas of a specimen.

Conclusions Although the results of the current ISO chemical solubility test appear with low variability, these results can be manipulated by modifying the specimens’ dimensions or morphology. Maintaining a standard surface area, the chemical solubility decreases as the sample size increased for both ‘enamel’ and ‘core’ classes of dental ceramics. The test did not succeed to improve the reproducibility of solubility measurement. It is recommended that the test be amended to stipulate sample morphology and geometry.

Notes
Clinical Evaluation of Conventional Lithium Disilicate Single Crowns Issued in a Teaching Institution

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Objectives There are various developments which have been made in relation to the use of ceramic materials in dentistry, especially in the construction of all porcelain crowns. To investigate the clinical performance and clinical survival rate of lithium disilicate (IPS e.max Press) material utilized in single crowns as well as assessment of the periodontal health status of these crowns.

Methods A cross-sectional design on the clinical performance of IPS e.max Press single crown was utilized. A total of 47 suitable patients with 88 crowns issued by students at the Dental Centre of University of Malaya, were recruited. Modified United States Public Health Service evaluation criteria (USPHS) was used. For periodontal parameters, Plaque Index (PI), gingival recession, Modified Papillary Bleeding Index (MPBI) and probing pocket depth (PPD) were used for comparison between the crowns and contralateral natural sound teeth (control)

Results The distribution of the crowns according to the tooth types was 8 premolars, 1 molar, 76 incisors, and 3 canines. 96.6% of IPS e.max Press crowns were rated satisfactory. The rate of survival at mean evaluation time of 3 years was 95.1% and at two years was 100%. There were no differences in mean gingival recession (p=0.182) and mean plaque scores (p=0.102) between crowns and contralateral natural teeth (control). The crowns had higher mean Modified Papillary Bleeding Index (MPBI) p=0.000 and Probing Pocket Depth (PPD) p=0.051 as compared to contralateral sound teeth.

Conclusions IPS e.max Press crowns can achieve satisfactory clinical performance. These crowns have enhanced survival rate along with reduced risk of fractures and minimal dentinal sensitivity. Plaque retention and gingival recession were almost similar to the contralateral natural sound teeth.

Notes
Biocompatibility and biomineralization assessment of bioceramic, epoxy-resin based and calcium hydroxide root canal sealers.

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Objectives To evaluate in vivo biological response and biomineralization capacity of the endodontic sealers Smartpaste Bio, Sealapex and Acroseal.

Methods Subcutaneous implants in 40 Wistar rats were performed. Analysis was at 7, 15, 30 and 60 days experimental periods (10 animals for each time period). Each animal received four implants, three polyethylene tubes with the sealers in test and one empty tube as control. After each post-operative period animals were euthanized and the polyethylene tubes, along with surrounding tissue were removed and fixed. In order to histologically analysis fibrous capsule thickness, inflammatory infiltrate and mineralization, the pieces were included in historesin and stained in HE, Von Kossa or remained without staining for observation under polarized light. The results were statistically analyzed by Kruskal-Wallis and Dunn’s test (p<0,05).

Results All sealers promoted moderate inflammatory reaction at initial periods. Smartpaste Bio presented the lowest inflammatory reaction at 15 days period (p<0.05). Sealapex induced higher mineralization, followed by Smartpaste Bio. Acroseal showed no mineralization areas.

Conclusions At the end of the experiment, all tested sealers Smartpaste Bio, Sealapex and Acroseal presented biocompatibility. Smartpaste Bio and Sealapex induced biomineralization.

Notes
Development of Three-dimensional Models of Bone and Oral Mucosa
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Objectives To develop and characterise three-dimensional tissue-engineered models of bone and oral mucosa in order to construct a full-thickness composite osteo-mucosal model.

Methods Bone cells were expanded in vitro, seeded onto porous hydroxyapatite/tricalcium phosphate (HA/TCP) scaffold discs and cultured in a spinner bioreactor to enhance the nutrient transport. Two different types of bone cells were investigated including primary human osteoblasts and rat osteosarcoma cells. Regular assessments of the bone reconstructs were carried out throughout the study both quantitatively by using PrestoBlue cell viability assay and qualitatively by Scanning Electron Microscopy (SEM). The oral mucosa model was constructed separately by air-liquid interface culture of oral keratinocytes on fibroblast-populated collagen gels. The engineered mucosa was then transferred and laminated onto the engineered bone surface at the endpoint of its culture to form a full-thickness osteo-mucosal model which was fixed and processed for histological examinations.

Results PrestoBlue fluorescence spectroscopy showed that the engineered bone remained vital throughout the study if cultured within the bioreactor in a dynamic condition. SEM images showed that bone cells were evenly distributed across the scaffold and progressively expanded in numbers with osteoid-like new bone formation inside the interconnected macro pores. The histological examination of the oral mucosal model showed presence of a continuous multilayer epithelium on top of a connective tissue layer densely populated with viable fibroblasts.

Conclusions Tissue-engineered models developed in this study resembled the natural bone and oral mucosa and have the potential to be used as relevant models to include both bone and oral mucosa for various in vitro applications including biological evaluation of biomaterials and disease modelling.

Notes
The environmental fate of waste microplastics from resin-based dental composite

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Objectives
Resin-based composite restorations have become the ubiquitous alternative to dental amalgam. A trend that is set to continue following the Minamata Convention (signed in 2013), that highlighted the environmental impact of Hg. The fate of the microplastics and the eluted chemicals from resin-based dental composites following clinical finishing regimes is unknown. These are released directly into the urban water sewage system.

Aim: To quantify the potential environmental pollutant effect from the release of microplastics and partly polymerised components of dental composite following clinical finishing regimes.

Methods
Two dental composites (one representative of a current commercially available material and one custom-made calibration composite) containing HEMA, TEGDMA, UDMA and BisGMA, were tested. These composites were polymerised and ground to simulate standard clinical finishing regimes. The microplastic particulates were stored in tap water and sampled at regular intervals. We measured: (i) the particulate size in solution via laser diffraction analysis; (ii) the concentration and release profiles of the eluted monomers with a combined HPLC and SPME method at trace level concentrations below 20µg/L.

Results
Particulate size was found to be in the range of 1-500µm (mean 289µm). Over 6 months the concentration/elution patterns of the monomers varied. The concentration of all monomers (including residual BPA) spiked in the first 48 hours followed by increased leaching of all monomers into solution over 24 hours to 3 days. A decrease in the leaching of all monomers from 72 hours to 14 days was observed, followed by a relatively large increase at 14 days to 1 month (excluding TEGDMA). This consistent leaching remained for 4 months. BPA is detected throughout the 6 month period. Concentrations of eluates were: HEMA 50-450 ppb, BPA 0-275 ppb, TEGDMA 100-1800 ppb, UDMA 0-3000 ppb, BisGMA 0-150 ppb.

Conclusions
Monomers leached out from particulates at quantifiable levels over a prolonged period of time. Molecular weight, hydrophilicity and diffusion rates of the constituent monomers and high surface area of the particulates contribute to the release patterns observed. This study highlights the need to consider responsible waste management strategies for resin-based dental composite.

Notes
Biocompatibility of Surface-Modified Titanium Implants with Silver and Hydroxyapatite Nanoparticles
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Objectives To examine the stability of silver and hydroxyapatite nanocoatings applied to the surface of titanium dental implants in cell culture media. Also, to investigate the biocompatibility of these surface-modified dental implants.

Methods Ti6Al4V discs were coated with silver nanoparticles (Ag NPs), silver and hydroxyapatite nanoparticles (Ag+nHA) or microparticles (Ag+mHA). The silver and HA coatings were applied using the electroplating and sintering techniques respectively. The stability of the silver-hydroxyapatite nanocoatings was tested in different cell culture media (physiological saline, DMEM, human osteoblast growth medium; HOB) using inductively coupled plasma mass spectrometry (ICP-MS). The biocompatibility of the coatings was tested with primary human osteoblasts in 24-well microplates (n = 9 discs/treatment; 20,000 cells/well). The cell viability was assessed by measuring the LDH and ALP activities as well as the cell protein content over 72h. The cell morphology was investigated using scanning electron microscopy (SEM). Ag and cell electrolyte (Na+, K+) concentrations in the media were measured using (ICP-MS).

Results SEM and energy dispersive X-ray spectroscopy (EDS) confirmed that Ti6Al4V discs were successfully coated. Electron microscopy confirmed that the primary particle size of Ag, nHA and mHA was 111.58±14.99 nm, 23.90±1.49 nm and 4.72±0.38 µm respectively. Metal analysis showed that silver coatings remain stable in all media tested (<0.57%), but dissolution of Ag NPs in DMEM was 4-fold higher compared to HOB media. The presence of FBS in the media was found to increase the silver release from the nanocoatings significantly. The findings of the LDH, ALP and protein assays agreed that viability of the human osteoblasts cells adherent to the implants was lower for the Ag+mHA coated specimens compared to Ag+nHA.

Conclusions Ag and HA NPs formed stable coatings on titanium implants. Implants coated with Ag+HA NPs maintained a higher degree of biocompatibility compared to those coated with Ag+mHA suggesting a benefit for clinical use.

Notes
COMPARISON OF TWO BIOMEMBRANES IN THE DIRECT PULP CAPPING
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Objectives The aim of this study was evaluate the response of direct pulp capping in rat molars from the use of bacterial cellulose and bovine pericardium biomembrane.

Methods For bovine pericardium biomembrane preparation, small pieces of bovine pericardium (PB) were treated with an alkaline solution of sulfates and hydroxides. A solution of 1% chitosan was allocated in Teflon molds, freeze-dried in N₂ and covered with PB hydrated sample and lyophilized. After that, at chitosan side it was added a mixture of Ca(OH)₂ and gelatin, dried at air flow and ambient temperature. Bacterial cellulose was purchased from Bionext®. Both materials were sterilized by ethylene oxide. The cavities were prepared on the occlusal surface of lower first molar in Wistar rats. The animals were randomly divided into 3 groups: G₁: control group: Cortisporin (CO). + Ca(OH)₂ + glass ionomer (GI) + adhesive system (AS) + composite resin (CR); G₂: CO + bacterial cellulose biomembrane + AS + CR; G₃: CO, + biomembrane bovine pericardium + AS + CR. The ages of analyzes were 7, 14 and 30 days.

Results This study utilized the Kruskal-Wallis test and Student-Newman-Keuls (P ≤ 0.05). In group 1 and 3 predominated gentle desorganization of odontoblastic cell layer, but with normal appearance of the pulp and normal cells too, both as 7, 14 and 30 days. In the same period, in group 2, predominated the general loss of cellular morphology pulp necrosis and periapical abscess in some teeth.

Conclusions It is possible to conclude that bacterial cellulose did not to use the scaffold to induce the pulp protection, but calcium hydroxide is still the material most suitable for pulp protection, and when associated with pericardium biomembrane, induced to favorable results in cases of pulp exposure.

Notes
Development Of Bioactive Glass For Orthodontic Adhesive Removal And Remineralization
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Objectives To prepare a bioactive glass powder propelled via an air-abrasion handpiece to safely remove orthodontic bonding resin after orthodontic treatment while promoting re-mineralization of white spot lesions (WSLs).

Methods Experimental bioactive glasses were prepared using the melt quench route with a similar composition to Bioglass 45S5, but with high sodium and phosphorus contents and a constant ratio of fluoride. The glass transition temperature (Tg) of each glass was measured using Differential Scanning Calorimetry (DSC) and the hardness of each glass was calculated. Bioactivity of each glass was tested before and after immersion in Tris buffer solution, using FTIR, and XRD.

Results Based on the DSC data, increasing Na2O content in the glass resulted in a decrease in Tg from 524 to 465 for Na2O concentration of 20% and 30% respectively. There was a linear relationship between hardness and Tg. Hence, hardness decreased with increasing Na2O content. Based on representative enamel hardness of ~3.5 GP, a glass with hardness comparable to that of enamel (3.64 GP) has been achieved. The results of XRD and FTIR revealed that these glasses are amorphous and can form apatite earlier than 45S5.

Conclusions A novel bioactive glass with appropriate hardness and Tg with promise for removal of orthodontic cement has been developed. Further testing in terms of safety and efficiency of removal will be undertaken on healthy extracted premolars and with artificially-induced WSLs.

Notes
A New Photoelastic Dental Model for 3D Photoelasticity Applications.

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**Objectives** The aim of this study is to construct a resin dental model of the teeth and supporting bone produced from two materials that have moduli of elasticity of the same ration as that of tooth to bone in order to carry out photoelasticity experiments. Photoelasticity is a useful technique to evaluate the stress distribution in dental applications. However most of the studies highlighted the use of intergrated 3D photoelasticity at room temperature with a little can be concluded regarding the state of stress at any point. This study aims to use a stress freezing technique to allow accurate analysis of interior stresses at specific points and locations.

**Methods** To estimate and mimic the tooth-bone modulus of elasticity ratio, four materials have been tested in this study: epoxy resins PL1, and PL2 (Vishay Precision Group, USA); epoxy resin Araldite 2020 (Huntsman Advanced Materials, Swiss) and PMMA (Candulor AG, Germany).

Sample were prepared to ISO Standardization (1567) for 3-point bend testing at a dimension of (65x10x2.5mm). The specimens tested using Dynamic Mechanical Analyser machine (DMA) with supporting span of 30mm (Perkin Elmer DMA 8000). The temperature monitoring from low to high was performed with a heating rate of 2°C/min at an oscillation frequency of 0.05mm. In oder to select the appropriate material for model construction the material ratio was determined as: $E_{(tooth substitute material)}$ at $T_g(bone substitute material)$ /$E_{(bone substitute material)}$ at $T_g = E_{(dentine)} /E_{(bone)}$.

**Results** The elastic modulus of each material at $T_g$ is shown in Table (1). The elastic modulus of each material at the $T_g$ of the other materials tested is also shown where applicable.

**Conclusions** This study concluded that the Araldite 2020 with PL1 fabricated by using (DMA) could mimic the tooth-bone modulus of elasticity ratio. This ratio suggests the technique may give a promise to be used as a dental model to evaluate the stress distribution over supporting stractures.

**Notes**
Structural Integrity of Poly-Ether-Ketone-Ketone (PEKK) Based Bi-layered Molar Crowns

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Objectives

To evaluate the occlusal fracture resistance of mandibular molar crowns made from a Poly-Ether-Ketone-Ketone (PEKK) based polymer (Pekkton®ivory). Composite veneered Pekkton®ivory crowns will be tested and compared with equivalent zirconia-composite and metal-composite bi-layered crowns.

Methods

A mandibular first molar was prepared (1.5mm reduction) and duplicated. Abutments were produced from polyurethane (AlphaDie MF, Schutz Dental Group, Germany) and mounted in a base of the same material. Light bodied impression material (President, Coltene/Whaledent, Switzerland) was used to simulate the periodontal ligament. Three groups of crowns (n=20) were fabricated with different substructure materials: polymer (Pekkton®ivory, Cendres+Métaux SA), zirconia (In-Ceram® YZ, Vita Zahnfabrik, H. Rauter GmbH & Co. KG) and metal (Talladium Tilite V, Talladium, Inc, USA). All groups were veneered with the same light-cured composite material (VM LC, Vita Zahnfabrik, H. Rauter GmbH & Co. KG) and were cemented using resin cement (Multilink Automix, Ivoclar Vivadent AG, Liechtenstein). Each sample set was divided into two groups (central fossa or buccal cusp) to allow the effect of different occlusal loading to be compared. A Lloyd LRX universal testing machine was used to apply a static load through a 4mm diameter ball steel indenter at a crosshead speed of 1mm/min. The force was measured in Newtons (N) and the mode of fracture was also recorded and categorized.

Results

The fracture strength results of all groups are shown in the attached table. IBM SPSS Statistics 22 software was used to compare them using one-way ANOVA and Games-Howell tests to determine any significant difference between groups. Groups with different superscript letters indicate significant differences (P<0.05) and groups with same superscript letters indicate no significant difference (P>0.05).

Conclusions

The occlusal fracture resistance of PEKK based crowns showed significantly higher strength than zirconia based crowns, and comparable results to metal-based crowns with no significant difference.

Notes
Stain on denture base materials – Development of an in vitro model for assessing stain removal by brushing
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Objectives This study investigated different staining methods for their suitability to assess stain removal on denture base materials by brushing with experimental denture paste formulations and commercial toothpastes.

Methods Specimens of various denture base materials were prepared by mechanically grinding to obtain an appropriate surface roughness. Pre-tests were conducted for following different staining models based on (1) liquorice staining, (2) chlorhexidine-tea staining with human saliva, (3) chlorhexidine-tea staining with artificial saliva, (4) occlusion spray, (5) combined method of (3)+(4), (6) seven day tea-staining method. Main investigations were performed with staining model (3). Colorimetric measurements were performed at initial stage, after staining and after stain removal. ∆E was determined to assess the stain removal properties. The samples were brushed in a brushing simulator for different brushing times. The influence of paste dilution degree with water was also investigated. Three experimental formulations of denture pastes, three toothpastes ((I) Crest Cavity Protection, (II) Oral-B Pro Health, (III) Colgate Cavity Protection) and water only treatments were investigated.

Results A brushing time of 20sec and paste dilution of 1:1 was identified as most practical. Brushing with all pastes caused a significantly higher stain removal compared to water. For two experimental denture paste formulations, a better or equal behaviour of stain removal was observed in comparison to the commercial toothpastes. One experimental denture paste formulation showed significantly lower cleaning performance compared to other pastes.

Conclusions This study shows it is possible to differentiate stain removal ability of different pastes on denture materials hence this can be used as a suitable model for evaluating the stain removal efficacy of denture cleaning formulations which have to applied with brushing. Future research, it must be considered to investigate the material compatibility of brushing with pastes to understand the impact of this cleaning regime on denture material surfaces.

Notes
Layered Double Hydroxides in Experimental Composite Materials
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Objectives To investigate the effects on water absorption/desorption properties, of incorporating Layered Double Hydroxides (LDH), into experimental fissure sealants based on composite resins. These systems have been developed for use as fluoride batteries for dental applications.

Methods LDH was incorporated at ratios (7%, 20% and 33%) into experimental BisGMA/UDMA/TEGMA (ratio 35%/35%/30% respectively) composite (light cured) prior to charging these systems with fluoride. Twenty discs (15 ×1mm²) were prepared (n=5 each group) and weighed. Each specimen was immersed in 15ml deionised water (DW), weighed at regular intervals until equilibrated, and then removed from DW, placed at 23°C and weighed at regular intervals until equilibrated. The percentage equilibrium water uptake/loss, %solubility and diffusion coefficients (D) for uptake/loss processes were calculated. A nonparametric statistical test was used at a significant level of 0.05.

Results The addition of LDH at all ratios showed a significant increase in all water absorption/desorption parameters, apart from the 33% LDH system with respect to % solubility and diffusion coefficient during absorption, which showed no significant difference to the unloaded system (Table 1).

Conclusions Incorporation of LDH (at all ratios) to the system enhanced its water absorption/desorption characteristics; this increase was dose dependent. This enhanced uptake will potentially aid the release of fluoride ions (cariostatic action) once the fissure sealant systems have been charged with fluoride. Systems with 33% LDH showed no significant increase in % solubility and diffusion coefficient compared with the control.

Notes
Differentiation Of Human Dental Pulp Stem Cells Into Neural Lineages

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Objectives The present study characterised and compared a population of adult stem cells derived from human dental pulp (hDPSCs).

Objectives:
1. Investigation of self-renewal capacity of the cell population
2. Induction of neural differentiation
3. MSC characterisation of cells via immunocytochemistry using CD105 and CD73 antibodies and respectively via qPCR using CD105, CD90, SOX2 and COL15A1 at three different time intervals: Day 0 (undifferentiated cells), Day 8, and Day 24 in neural differentiation media
4. Analysis at protein and mRNA levels of neural differentiated cells at three time intervals using TUJ1, S100, SOX10, GFAP, NCAM, MAPT

Methods Dental pulp was extracted from wisdom teeth of ten patients and subsequently cultured as explants in growth media and in neurobasal media to assess for neuronal and glial markers. Analysis was carried out at mRNA and protein level using qPCR and immunocytochemistry. Furthermore undifferentiated hDPSCs were analysed at mRNA and protein level to evaluate the expression of MSCs and neural markers.

Results We successfully isolated a population of MSCs from the dental pulp, as confirmed by positive CD105 and CD73 staining and qPCR analysis. Undifferentiated DPSCs spontaneously expressed glial marker S100 independent of passage number. Neural differentiation was confirmed by positive immunocytochemical analysis of TUJ1, GFAP and S100. Quantitative qPCR analysis showed great expression of NCAM and moderately to low expressions of MAPT and S100 when compared to controls.

Conclusions The results obtained from the current study indicate that dental pulp is a promising source of MSCs. Induced hDPSCs showed a great expression of NCAM, which plays a role in myelination and remyelination as well as analgesic effect of glial cell–derived neurotrophic factor in neuropathic pain. Spontaneous expression of glial marker S100 was evident in the undifferentiated dental pulp stem cells, thus protein expression as the only evidence of MSC differentiation towards a neuronal phenotype should be prohibited.

Notes
The function of CD133 in tooth epithelium development

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Objectives CD133 is widely used as a stem cell marker in a broad organ spectrum. The role of this molecule in tooth epithelium development has not yet been fully elucidated. The aim of this study is to test the expression pattern of CD133 in developing mouse incisors and to evaluate the function of CD133 in controlling incisor tooth epithelial stem cell fate determination.

Methods The expression of CD133 in different epithelial compartments of the mouse lower incisor was evaluated using postnatal day 7 ICR/CD1 mice. Laser capture microdissection was applied to isolate cells from the stem cell (SCs), transit amplifying (TACs), inner dental epithelium (IDE) and ameloblast (AM) regions. Real time RT-PCR was performed and the results were further validated using immunofluorescent analysis by applying different anti-CD133 antibodies that target different domains of CD133. The ability of incisor tooth epithelial cells growing in vitro was tested and their expression of CD133 expression levels were assessed. Finally the incisor tooth phenotypes of the CD133 knockout mice were analysed using transmission microscopy as well as immunostaining for ameloblastin and amelogenin.

Results Molecular profiling revealed the expression of CD133 in the incisor tooth cervical loop epithelium. The intracellular C-terminal domain is broadly expressed in the cervical loop whereas the expression of CD133’s extracellular loops is restricted to TACs. CD133 knockout mice exhibited defects in enamel maturation as well as reduced ameloblastin and amelogenin expression.

Conclusions We have found novel evidence that CD133 is a marker of mouse incisor tooth epithelial stem cells and that its extracellular loops might be a potential marker for tooth epithelial TACs. CD133 deficiency could induce tooth enamel development defects. Currently we are conducting research on the functions of CD133 in tooth epithelial stem cell maintenance, lineage differentiation and crosstalk with other signalling pathways.

Notes
Role of Mesenchymal Cells in Salivary Gland Regeneration

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**Objectives** To test the function of adult salivary gland mesenchymal cells (MCs) in tissue regeneration.

**Methods** In order to identify the presence and location of MCs in adult salivary glands, we first tested a panel of general mesenchymal cell markers such as Vimentin and PDGFRb on one-month-old ICR/CD1 mice using immunofluorescent analysis. We then could establish a novel protocol for isolating and culturing MCs from the glands, based on their growing capabilities on defined culture surfaces and in different media. The cells were passaged 2-3 times before reaching a homogenous MCs morphology. The expression of MCs markers were checked using real time RT-PCR and immunofluorescence. The ability of the MCs in salivary regeneration was then tested in a two-stage 3D organogenesis system by mixing MCs with salivary gland epithelial cells and seeding into different extracellular matrix. Morphology, branching and differentiation of the derived organoid spheres’ were assessed using stereo and confocal microscopies as well as 3D reconstruction, by applying specific antibodies for cell type and function analysis.

**Results** We have identified that Vimentin and PDGFR-beta were the two most persistent MCs markers for adult salivary gland MCs that could continuously express in the cultured MCs. While salivary gland epithelial cells alone were able to form organoid spheres in vitro, MCs could significantly improve the sphere number, size and branching. In addition, the formed tissues could further develop; with duct and acinar like structures forming.

**Conclusions** Salivary glands develop through active epithelial-mesenchymal interactions. Adult glands have little regenerative capability after damage from radiotherapy and Sjorgrens’ syndrome, leaving regeneration of the organ a major challenge. We have successfully established a system to purify and culture salivary gland MCs and found they are important to accelerate tissue regeneration. An intriguing future research direction would be the translation of this research into the human case.

**Notes**
Peripheral Targeting Of Interleukin-1 Cytokine Signaling Pathway In Peripheral Nerve Injury

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Objectives The aim of this study was to investigate the effects of peripherally applied cytokines and/or their modulators on the inflammatory reaction at the site of peripheral nerve injury. In order to establish their possible contribution to the reduction or prevention of neuropathic pain, and determine their potential effects on nerve regeneration.

Methods In four groups of 6 animals (Sprague-Dawley rats) under general anaesthesia the left sciatic nerve was transected and microsurgically repaired with four epineurial sutures. At time of repair 3 groups received either Interleukin-1 antagonist, Interleukin-10, or sterile water. A further 6 animals acted as control shams. The investigators were blinded. Following 7 days recovery period, the animals were perfusion fixed and the repaired nerves and spinal cord harvested. The tissue was processed for immunohistochemistry, and image analysis used to quantify the expression of macrophages (CD68) at the site of nerve repair.

Results Qualitative analysis revealed immunoreactivity to CD68 was present in all three groups of injured and repaired nerves, with a more concentrated pattern of labelling at the site of nerve repair and around the repair sutures, compared to the proximal and distal end of the repaired nerves. No immunoreactivity to CD68 was observed in the control nerves. Groups injected with water and Interleukin-10 had a significant increase in macrophage expression at the injury site at seven days (p=0.003, p=0.038, respectively Kruskal-Wallis). However, following administration of an antagonist to Interleukin-1, macrophage immunoreactivity was not significantly different to sham controls (p>0.05, Kruskal-Wallis).

Conclusions These findings indicate that targeting cytokine signalling by administration of an antagonist to Interleukin-1 at a time of peripheral nerve repair shows a reduction in macrophage influx and suggest a local decrease in inflammatory response. This may have a positive influence on neural regeneration and potentially reduce signalling regulatory molecules that are responsible for development of neuropathic pain.

Notes
Direct bacteria-neuron interaction via Toll-Like Receptors (TLRs): consequences for orofacial pain.
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Objectives Expression of TLRs on high-threshold sensory neurons (nociceptors) permits direct interaction between neurons and pathogens. Previously we have shown that TLR4 is preferentially expressed by nociceptors within the trigeminal ganglion (TG). Here we test the hypothesis that sensory neurons directly detect and respond to challenge by the TLR4 agonists *E. coli* and *P. gingivalis*-derived lipopolysaccharide (LPS) and the TLR2 agonist Pam3CSK4.

Methods Indirect, dual-labelled immunohistochemistry was performed on fixed, frozen rat TG to detail the expression of TLR2 within specific neurochemically-identified sensory neuron sub-populations. Primary TG neuron cell cultures were prepared from mechanically and enzymatically dissociated TG. Cultures were maintained for 48 hours prior to exposure to either *E. coli* LPS (O111:B4, 1µg/mL), *P. gingivalis* LPS (1690/1435 isoforms, 1µg/mL) or Pam3CSK4 (500ng/mL) for 2 hours. Induction of TNFα, IL-1β, IL-6 and IFNβ gene expression was measured by qPCR using GAPDH, ß-actin and 18s as endogenous controls. TLR2 (CU CPT 22, 8µM) and TLR4 (CLI-095, 3µM) specific inhibitors were used to demonstrate receptor specificity of the various ligands.

Results TLR2 is expressed by 27.4±3.1% of total neurons, 62.7±7.9% of TRPV1-positive and 61.5±6.8% of P2X3-positive neurons within the TG (n=3). Following exposure to *E. coli* LPS both TNFα (8.1±0.5 fold, p<0.0001) and IL-1β (4.1±0.5 fold, p<0.001) gene expression are significantly induced relative to endogenous controls. Pam3CSK4 exposure significantly induced TNFα (18.2±1.5 fold, p<0.001) and IL-1β (4.6±0.3 fold, p<0.001) gene expression. Investigations into the response to *P. gingivalis* LPS isoforms are currently underway.

Conclusions Expression of TLR4 and TLR2 within TG sensory neurons is nociceptor specific. Acute activation of these TLRs induces TNFα and IL-1β gene expression, both of which contribute toward the onset of inflammatory pain. Thus, TG nociceptors may directly respond to pathogenic challenge by synthesis of pro-inflammatory cytokines impacting on acute nociception and the local inflammatory response.

Notes
Semi Real-Time Erosion of Human Dentine at the Nano-scale: An AFM Force-curve Based Imaging Study

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Objectives Dietary-acid mediated damage to tooth enamel results in softening and dissolution of mineral on the exposed surface. Once the enamel has been lost, dentine is exposed. Our knowledge of the role dietary acid concentration plays when pH is dietary relevant upon dentine erosion has yet to be established. We therefore investigated the effect of dietary acid pH and concentration when pH is dietary relevant, upon the morphological and mechanical property changes of short-term exposed human coronal dentine.

Methods Human coronal dentine specimens (n=25) were obtained from 20 erupted 3rd molars and monitored using atomic force microscopy (AFM), operating in quantitative imaging™ mode (Force-curve based imaging). In vitro early stage erosion of human dentine was studied in terms of dietary relevant citric acid concentration and pH, monitoring changes in average roughness ($R_a$), load bearing area ($\delta$) and stiffness ($S$) in a liquid environment (PBS, pH 7.2), over the same 50x50µm area, up to 180s, at 30s intervals.

Results The severity of human dentine’s $R_a$ (nm), and $S$ (N/m) changes post treatment were found to be significantly influenced by acid concentration only (p<0.001), while the $\delta$ (nm) changes were influenced by both pH and concentration (p<0.001). Within the limitations of the study, human dentine erosion was found to be dominated by acid concentration, regardless of pH.

Conclusions Of the acid characteristics analysed, dietary-acid concentration was found to be the dominant characteristic in the erosive severity of human dentine. Concentration-dependent changes in morphology and stiffness were obtained and were independent of acid pH. These findings could support the possibility to manufacture dentine-friendly drinks with an aim to lower acid concentration and or, focus on acid concentration in dentine erosion studies as the main driver of erosive severity, in both clinical applications and public health policy.

Notes
Towards a 4-D spatial and temporal model of human incisor enamel biomineralisation using X-ray techniques.
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**Objectives** Precise timings and spatial progression of human enamel biomineralisation are still largely unknown due to scarcity of developing human enamel specimens for investigation. However, this information is crucial for optimising emerging biomimetic regenerative/reparative dentistry routes. The aim was to characterise and compare the crystallography, microstructure and mineral density (MD) of immature incisal enamel at various development stages.

**Methods** Five developing primary upper incisors were obtained from an archaeological source. Two type-matched mature contemporary teeth were used for comparison. X-ray microtomography (XMT) with 15µm³ resolution at 90kV was used to map MD distribution. Linear attenuation coefficient was used to calculate MD (gcm⁻³). Specimens were then sectioned into 0.3mm slices to carry out synchrotron X-ray diffraction (S-XRD) on XMaS (BM28) at the European Synchrotron Radiation Facility and B16 at Diamond Light Source in order to map crystallographic structural parameters. 2D diffraction images were collected using 15keV or 18keV X-ray energy, 50µm and 43µm beam size, and a 2048x2048 pixel and 3056x3056 pixel CCD detector on XMaS and B16 respectively.

**Results** XMT revealed the average MD of least developed enamel is lower (2.34±0.3gcm⁻³) than that of mid (2.63±0.2gcm⁻³) and fully developed (2.75±0.1gcm⁻³). In fully developed enamel, the MD increased when moving from the enamel-dentine junction (EDJ) to the outermost surface enamel. In contrast, developing enamel MD was relatively higher near the EDJ compared to the surface. The least developed enamel had higher distribution of MD values. S-XRD showed the texture distribution to be more spatially uniform and lower in early developed enamel, becoming more spatially heterogeneous and on average higher in full development. Hydroxyapatite crystallite orientation was perpendicular to EDJ regardless of development stage, indicating initial preferred directions of crystallites persist from early through to full maturation.

**Conclusions** These results provide new insight into the fundamental understanding of natural growth and formation of human incisal enamel.

**Notes**
**Hydroxyapatite/Carbon Nanotubes as Composite Bone Implants - Biocompatibility vs Toxicity**

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**Objectives** Poor wear resistance and low fracture toughness are the main disadvantages of using hydroxyapatite (HA) for orthopaedic implants. This can be overcome by the use of Carbon nanotubes (CNTs) as reinforcements due to their versatile properties e.g. high stiffness and mechanical strength. The main aim of this study is to develop HA composite reinforced with CNTs and to investigate their biocompatibility.

**Methods** HA in the presence of CNTs was synthesised following a sol-gel technique. Six different types of powders were produced by altering two variables – functionalization and presence of surfactants. The composites were produced by mixing Hydroxyapatite/carbon nanotube powder with Polyvinyl alcohol (PVA) in equal proportions. Primary Human Osteoblast cells were used for the biocompatibility study. LDH, ALP, pH and Ion content analyses were performed on external media every 24 h for 3 days and at the end of the study LDH, ALP and protein assays were performed using cell homogenate to measure various cell activities. SEM analysis was also performed.

**Results** A drop in pH was observed after 24 h which recovered to neutral pH by the end of day 3. Total protein content was confirmed on all materials. Cell survival was analysed by performing LDH assay on cell homogenate at the end of day 3. ALP assay was performed to determine the mineralization activity of the cells. Finally, the material was qualitatively analysed under SEM and the presence of cell material was observed.

**Conclusions** CNTs possess properties that are highly desirable in the development of biomaterials. However, there has been controversy regarding their biocompatibility and cytotoxicity. This study explores the biocompatibility of HA/CNTs composite as bone implants. The results show that CNTs are biocompatible and can be employed in the development of bone implants.

**Notes**
Radiographic assessment of regenerative endodontic treatment of traumatised non-vital immature teeth.

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Objectives To evaluate the radiographic outcomes of the regenerative endodontic technique (RET) in managing traumatised non-vital immature teeth. To compare two methods of radiographic assessment of continued root development; expert visual assessment and objective measurements of radiographic parameters.

Methods Fifteen healthy children (age range=7-11 years) with traumatised non-vital immature upper incisors were included. RET using two antibiotics (ciprofloxacin and metronidazole), was used for treatment with an average follow up of 9.9 months. One operator undertook all treatments, reviews and standardised radiographs. Radiographic assessment of root length, dentinal wall thickness and apical foramen width at baseline and follow-up were assessed visually and measured using Infinitt digital radiographic assessment software, Seoul, Korea, by 2 experienced clinicians.

Results When the radiographs were evaluated visually, it was agreed that only 2, 4 and 6 out of the 13 teeth showed any evidence of increased root length, increased dentinal thickness, and reduced apical foramen width (Kappa=0.278, 0.270, 0.870), respectively. However, when the radiographic parameters were objectively measured, inter-operator measurement reliability was excellent (overall intra-class correlation= 0.974 (95% CI 0.955-0.986)). No statistical significant differences were found in any of the measured parameters between pre and post-operative radiographs. Clinically 100% resolution of clinical signs and symptoms was found.

Conclusions Our data showed that visual evaluation of radiographs was not as reliable as objective measurements with both methods showing no significant increase in root length and dentinal wall thickness. Most teeth treated with RET did not demonstrate any continuation of root development, but complete clinical healing was evident in all non-vital immature traumatised incisors.

Notes
Characterization of White Spot Lesion Using Focused Ion Beam-Scanning Electron Microscopy

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Objectives A white spot lesion (WSL) is defined as subsurface enamel porosity from carious demineralisation on the smooth surfaces of the tooth. It appears as a milky white opacity. Lesions shown an apparently intact surface layer, followed underneath by the more porous lesion body. The small pores within the body of the lesion act as diffusion pathway for both acids and minerals, so allowing the demineralisation of enamel to occur at the advancing front of the lesion. The objective is to map the porosity and its size on WSL with Focused Ion Beam-Scanning Electron Microscopy (FIB-SEM).

Methods The basic method used for FIB-SEM consisted of depositing a one micron thick layer of platinum over 25µmx 25µm of the interest region of enamel. Then, making a rough cut (25µmx 5µmx 20µm) with 3nA current and 30Kv was applied with the help of drift suppression (DS), using a standard “cross-sectional” cutting pattern, which ended at the front of the deposited platinum layer. Two adjacent areas (25µmx 5µmx 20µm) on the both sides of the platinum layer were milled under the same conditions. Subsequent, cleaning cross-sections were applied to polish the sub-surface edge of interest running perpendicular to the surface. The “slice and view” was carried out overnight for milling almost 700 slices with 2Kv and 4nA and taking backscattered (BS) images. Then, images were imported into imageJ and analysed.

Results The prism structure is clearly apparent on FIB-SEM slices of WSL with dissolution of prism boundaries as well as internal porosity within the prism itself. Porosity scales roughly 100-400nm which is comparable to the light wavelength (500nm).

Conclusions FIB-SEM is useful to characterize the porosity of WSL and it clearly shows the difference between WSL and normal enamel.

Notes
Referral pathways from general dental services to other primary dental care services in the UK: A systematic review and critical interpretive synthesis

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Objectives To define the characteristics of referrals from general dental services (GDS) to other primary dental care services (PDCS) and to identify factors which influence the decisions leading to these referrals.

Methods The review has a configurative approach. A systematic literature search was conducted in Medline, CINAHL and Embase. All articles meeting the inclusion criteria were analysed using critical interpretive synthesis, extracting first and second order constructs and generating synthetic constructs.

Results The search identified 16,109 articles, of which 73 were included in the review. Key constructs influencing patients’ journeys and care outcomes were: policy change, contractual systems, geography, communication barriers and dentists’ perceptions about the quality and accessibility of referral services. Some authors described referrals as ‘inappropriate’ if they included poor documentation, unsuitable treatment planning or patient selection. This term was usually applied by dentists receiving referrals into services managing a high demand with limited resources. The literature described two principal referral pathways originating in the GDS. Referrals were made to specialists for their technical skills and knowledge and represented a temporary shared-care arrangement. Referrals were made to Community Dental Services for patient management reasons and represented a semi-permanent transfer of responsibility for patients whose care was considered by referring dentists to be outside their GDS role.

Conclusions Diverse non-clinical factors are involved in decisions about making and accepting referrals from the GDS to other PDCS. These factors have not been adequately considered in designing and implementing interventions to optimise the management of referral processes. There is limited information in the literature exploring patients’ and policymakers’ perceptions of referral within primary dental care and this merits further research.

Notes
The Future Dental Workforce In Malaysia: Drivers For Change
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Objectives To explore key stakeholders views on recruitment, retention, training and team working of the dental workforce in Malaysia and drivers for change.

Methods Elite players from key dental organisations and professions in Malaysia were invited to participate in an audio-taped semi-structured interview. The one-to-one interviews were conducted in English, which is the medium for dental education nationally, and used a pre-tested topic guide to test a framework influenced by the health workforce literature and previous research amongst Malaysian dental students. The qualitative data were transcribed and analysed using a Framework Analysis.

Results Elites from top level management representing government and non-government dental organisations; and representatives from dentistry and dental therapy agreed to participate (n=20). Thematic analysis suggests the main drivers influencing the future dental workforce in Malaysia include policy (growth in dental education; moratorium on dental schools) and politics (healthcare governance; trade liberalisation), social (neo-liberalisation including globalisation of dental education; value in academic success), economic (country wealth), demography (growing and ageing population, inequalities in oral health) and disease (caries levels, chronic diseases) as well as developments in science and technology (dental implants; cosmetic dentistry). The pace of change and possible interplay between drivers was a source of concern.

Conclusions Stakeholders’ views on drivers for change broadly mirror other studies in high income countries; however, specific challenges for Malaysia relate to the rapid expansion of dental education and a young workforce with significant aspirations. A broad and flexible strategy is required to overcome an issue of training and retention of the dental workforce; and the influence of globalisation for the benefit of healthcare systems and the population.

Notes
Potential For Direct Access In Care Homes In Wales
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Objectives Many care home residents require simple dental treatment, complicated by the need for extra time to deliver dental care. The proportion of their care which could be delivered wholly by hygienists or therapists is unknown. This re-analysis of recently collected data aims to estimate the proportion of care home resident’s dental treatment needs which could be delivered wholly by hygienists or therapists.

Methods 2010 Welsh dental care home survey data on clinical opinion of treatment need and special care skill level required for 655 residents was cross referenced with the General Dental Council Scope of Practice document for dental therapists, dental hygienists, clinical dental technicians and extended duties dental nurses.

Results 22% and up to 27% of care home residents had treatment needs which could be wholly addressed by a generalist dental hygienist or therapist respectively. The percentages were 43% and up to 53% for hygienists or therapists with special care dental experience. Equivalent findings for clinical dental technicians were 6% for generalists and 12% for those with experience of special care. Extended duties dental nurses could not provide any residents with care needs. A large proportion of need in care homes could be wholly provided by hygienists or therapists, especially those with special care experience. The potential efficiency gain of direct access arises from individuals who do not need to see a dentist for any aspects of their care. This should be further explored.

Conclusions Hygienists and therapists could make a large contribution to addressing dental treatment needs of care home residents. Direct access could be an efficient model of care for this setting. Direct access to hygienists/therapists for dental care of care home residents should be piloted and evaluated.

Notes
Objectives To evaluate an emergency dental service available in-hours and out-of-hours to a population of half a million people in Sheffield, England. Treatment at a dental clinical provider (CP) was accessed via a 24 hour telephone triage provider (TTP).

Methods Analysis of retrospective activity data from the TTP and CP over one year and data analysis of a patient experience questionnaire. The demographic profile of the patients and local population were compared.

Results During the evaluation period 17,559 people contacted the TTP, 6,367 were referred to the CP, of whom 5,365 attended. Most calls to the TTP were answered within 60 seconds (96.6%) and sufficient appointments were provided at the CP. Most referrals to the CP were appropriate (93.7%), the main reasons for attendance being pain (70.4%) or swelling (18.8%). Most treatments involved surgical interventions (77.9%), whereas 18.1% were prescription only. There was high satisfaction with the CP. Cost per person calling the TTP was £5.04 and cost per person utilising the CP was £65.75. There was proportionally higher use of the TTP and CP by people living in more deprived areas and proportionally higher use by 20 to 44 year olds. Almost 50% of CP attendees were recorded as not having a dentist.

Conclusions An equitable high quality service provided timely advice and treatment for people with emergency dental conditions. The supply of clinical care met demand. Comparison with other service models would inform on the relative efficiency of the service.

Notes
Fifteen-year Survival of Root Canal Treated Posterior Teeth.
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Objectives To measure the fifteen year survival curve for root filled posterior teeth in adult patients treated within the General Dental Services in England and Wales (GDS).

Methods The data source was adult GDS patients and the treatments they received from 1/10/1990 to 31/3/2006. Re-intervention was either re-root canal treatment, apicectomy or extraction after root canal treatment. Analysis by modified Kaplan-Meier methodology was utilized to establish survival curves extending to fifteen years. The period of 4 year follow up in line with European Society of Endodontology (ESE) guidelines were noted. Associations between the survival curve and tooth notation and presence of a cuspal coverage restoration as a crown were also investigated.

Results The full database contains over 25 million courses of treatments on 2.7 million adult patients and 894,036 teeth were root canal treated. The 4 year follow up showed that 93.5% and at 15 years, 86% of root canal treated teeth had not received re-intervention. After 15 years there was variation in survival by tooth position. Root canal treated teeth with a crown survived 96% at 4 years compared to 93% for those without and at 15 years 88% versus 85%.

In the first year 24,969 teeth required re-intervention., 68% were extracted, 30% re-root canal treated and 2% apicected. At 10 years figures were: 85%, 15% and less than 1%.

Conclusions It was found that the 86% of teeth that had been root treated survived for 15 years without requiring re-root canal treatment, apicectomy or extraction. The teeth that had the best prognosis were lower premolars and lower third molars at 88%. The posterior teeth that had been crowned survived better than without a crown by around 3 percentage points.

Notes
Dental consultations and antibiotic use in UK General Medical Practice
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Objectives This study aimed to: characterise attendances for dental problems in UK general medical practice 2004-2013; describe the use of antibiotics in such consultations, and identify patient, practice, and appointment characteristics predictive of antibiotic prescription in dental consultations.

Methods A retrospective cohort study utilising routinely collected primary care data held by the UK Clinical Practice Research Datalink (CPRD). An inclusive sample of all dental consultations between 2004 and 2013 were extracted and cleaned according to CPRD-recommended quality assurance protocols. Multilevel logistic regression analysis was conducted to examine the relationship between patient, practice, and consultation characteristics, and antibiotic prescription.

Results Between 1st January 2004 and 31st December 2013 there were 288,169 consultations for dental problems at 655 general practices. During the study period median annual consultation rates varied between 3.37 and 6.32 consultations per 1000 patient-years. Rates of dental consultations remained relatively static between 2004 and 2008, but decreased between 2008 and 2013. Consultations for dental problems were highest amongst females and adults 20-29 years and an antibiotic was prescribed in 55.0% of consultations. Factors significantly (p<0.05) associated with increased likelihood of antibiotic prescription included: patient middle-age; patients with diabetes mellitus (OR 1.06); previous consultations for tooth-related problems (OR 1.20); consultations in December (OR 1.19), and consultations on a Monday (OR 1.11) or a Friday (OR 1.15). Females (OR 0.93), patients consulting in Scotland (OR 0.75), patients consulting on a weekend (OR 0.11), and patients consulting in January (OR 0.93) all had a significantly (p<0.05) reduced likelihood of being prescribed an antibiotic.

Conclusions Although rates of attendance for dental problems in general practice were relatively low, consultations commonly result in antibiotic provision. This may contribute to patient morbidity from untreated dental disease, and antimicrobial resistance. Interventions are therefore required to support patients in accessing the most appropriate care when experiencing dental problems.

Notes
Mechanical Properties of Thermo-pressed Polyetheretherketone as a Denture Material
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Objectives Since the injection moulding technique was adopted to fabricate thermoplastic products, the injection moulding condition might influence the processing parameters. Polyetheretherketone (PEEK) has been highlighted as having the potential for dental application despite the wide choice of polymer materials that are already broadly acceptable. However, no systematic studies have reported PEEK mechanical properties as a denture material. Therefore, the present study will investigate the mechanical properties of thermo-pressed PEEK using injection moulding technique. Assess the impact strength, tensile strength, and flexural properties (4-point bend test) of PEEK after processing at two different mould temperatures compared to PMMA.

Methods Samples (n=10) were prepared for Izod test according to (ASTM-D-256/ISO-180), tensile test (ISO 527), and 4-point bend test (ISO 1567). They were produced using thermopress 400 unit (Bredent, Germany). PEEK-Optima®NI1 (Invibio Ltd.) of 143°C (Tg) and 380°C (Tm) was injected at 175 and 200(±3)°C mould temperatures, while Polymethylmethacrylate (PMMA/control) of 280°C (Tm) processed at 40(±3)°C mould temperature (Bre.Crystal-HP,Bredent). Impact specimens were notched with V-shape cutting depth 2(±0.1)mm, base-radius 0.25(±0.05)mm, and testing was carried out using impact tester machine (Tinius Olsen, IT-503). A Lloyd testing machine (2000, England) was used for both tensile and 4-point bend tests. Tensile specimens tested at 50mm grip-to-grip distance and crosshead speed of 30mm/min. Whilst 4-point specimens tested at a crosshead speed of 5(±1)mm/min with 50(±0.1)mm distance between the supports centres. The force of the loading plungers for bend test was increased uniformly from 5N up to 100N. All study specimens were stored in water before the tests [37°C, 50(±2)h] and then statistically analyzed (ANOVA, P<0.05).

Results There was statistical significant difference between the mechanical properties of injected PEEK compared to PMMA (P<0.05), as shown in table (1).

Conclusions PEEK processed at 175 and 200°C mould temperatures showed the most promising mechanical properties as a denture material compared to conventional PMMA.

Notes
Synthesis of glass-ceramics for dental applications
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Objectives Glass-ceramics used in dentistry must possess superior chemical, physical and mechanical properties. Outside dentistry it has been reported that glass-ceramics based on the feldspathoid minerals in the system SiO₂-Al₂O₃-Na₂O-CaO-K₂O led to the development of crystalline phases such as Leucite, Nepheline, Kalsilite, Albite and/or Anorthite. Glass-ceramics in this phase field are reported to have good mechanical properties which are suitable to be used for dental applications. The aims of the study were to explore the potential of synthesizing dental glass-ceramics based on the chemical composition of nepheline (NaAlSiO₄) and kalsilite (KAlSiO₄).

Methods Novel glasses based on multicomponent sodium potassium calcium aluminosilicate system were synthesized using melt-quench methods. Glasses were characterized using differential scanning calorimetry and dilatometry. Experimental glass powders were heat treated to produce glass-ceramics. The crystalline phases and microstructures of the glass-ceramics were analysed using X-ray diffraction, solid state nuclear magnetic resonance and scanning electron microscopy. The mechanical properties of the glass-ceramics were evaluated using the biaxial flexural strength test.

Results Preliminary results showed glass-ceramics containing fine crystalline structures with desirable mechanical properties based on this multicomponent system, which has the potential to be used in dentistry.

Conclusion The final glass-ceramic obtained from the new glass system designed in this study may have the potential to be developed and used as a glass-ceramics material for dental application.

Notes
Water-Uptake and Mechanical Properties of Experimental Resin-Modified Glass-Ionomer Cements (RMGICs).

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Objectives To investigate water-uptake, compressive fracture strength (CFS), compressive modulus (CM), three-point flexure strength (TFS) and flexural modulus (TFM) of four experimental RMGIC liquid compositions compared to two commercial and two in-house RMGICs based on the commercial materials used.

Methods Two commercial RMGICs included in this study were Fuji Plus (GC, Japan) and RelyX Luting (3M ESPE, USA). Two additional in-house liquids were prepared based on the commercial materials published composition data. Four experimental liquid compositions (F1 and F2 based on Fuji Plus, R1 and R2 based on RelyX Luting) were prepared replacing either 50% of hydroxyethyl-methacrylate (HEMA) with tetrahydrofururyl-methacrylate (THFM) in F1 and R1 or 30% in F2 and R2 compared to the in-house liquids. Six discs for water-uptake (16mm-diameter, 1mm-thickness), twenty cylinders (6mm-height, 4mm-diameter) for CFS and CM and twenty bars (25mm-length, 2mm-width, 2mm-thickness) for TFS and TFM, respectively, were prepared at the manufacturers recommended powder:liquid mixing ratio. The powder used was the same corresponding commercial powder. Data were analysed using one-way ANOVA followed by post-hoc Tukey test at a significance level of p=0.05.

Results All experimental compositions showed significantly lower weight change compared to the commercial materials (p<0.001). All experimental materials showed improved mechanical properties (CFS and TFS) compared to their in-house counterparts (p≤0.013), except CFS of R1 (p=0.734) and TFS of R2 (p=0.781). Two compositions (F1 and F2) showed significantly higher CFS compared to RelyX Luting and the two in-house materials (p<0.001). F1 moreover presented significantly higher CM (p≤0.04) and TFM (p<0.001) values compared to RelyX Luting and the two home materials.

Conclusions Replacement of HEMA with THFM reduced the water-uptake, a common problem associated with RMGICs. Two experimental compositions (F1 and F2) showed improved mechanical properties compared to RelyX Luting but further modifications of the remaining experimental compositions is required to improve their strength.

Notes
Optimizing photo-initiator system of new fluoride-releasing acrylic orthodontic adhesive
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**Objectives** White spot lesions are a common complication with orthodontic treatment. We are developing a photo-polymerizing fluoride-releasing orthodontic adhesive. This study aims to investigate the effect of different initiator systems on degree of conversion (DoC) of the developed material.

**Methods** Sodium fluoride at 10wt% was added to 90wt% polymethylmethacrylate powder and mixed at a powder:liquid ratio of 2:1 with liquid 2-hydroxyethylmethacrylate and methylmethacrylate (40wt%:60wt%), alongside two concentrations of acetone (A-0wt% and 10wt%). Four groups of resin were prepared with varied photo-initiator systems: (1) 1wt% camphorquinone and 1wt% N,N-dimethylaminoethylmethacrylate (DMAEMA); (2) 1wt% camphorquinone and 1wt% Ethyl 4-(dimethyl-amino) benzoate (EDAB) activator; (3) 1wt% diphenyl (2, 4, 6-trimethylbenzoyl) phosphine oxide (Lucirin TPO) and (4) 1.5wt% Lucirin TPO (table 1). FTIR was used to measure DoC for all the materials at 10, 20, 30, 40 and 80s of light curing using Bluephase®20i at 1130mW/cm².

**Results** The DoC of group CQ/DMAEMA at 10%Acetone are higher than CQ/EDAB at 30, 40 and 80s (p<0.05). The DC of all groups with Lucirin TPO was higher than groups with CQ initiator system (p<0.05, one-way ANOVA).

**Conclusions** Lucirin TPO is an effective photo-initiator system which results in higher and faster polymerisation than the CQ system.

**Notes**
Effect of Temperature and Substrate on Ordered Apatite Crystals for Coating Dental Implants.

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Objectives Coating dental implants with apatite is well-known to improve the osseointegration and subsequently the healing time. Recently, wet chemical routes to synthesise apatite crystals has gained a lot of interest due to their ability to produce highly-ordered apatite crystals and cost-effectiveness. On the other hand, controlling organised crystal growth is dependent on many parameters such as temperature, substrate, pH and ionic concentration. Therefore, better understanding is needed to investigate the effect of temperature and substrate on the chemistry and organisation of the synthesized apatite crystals.

Methods Mineralisation experiments were undertaken at different temperatures (37, 54, 70 and 90 °C) on different substrates including; titanium, silicon, sapphire, and hydroxyapatite disks. The grown crystals were investigated chemically using FTIR, ¹⁹F MAS-NMR, Synchrotron XRD and morphologically using SEM and TEM.

Results Self-assembled nanocrystals (dandelion-like structures) were observed to develop from the less-ordered spherical structures. Interestingly, apatite growth on hydroxyapatite substrates shows highly-ordered bundles of crystals with a close likeness to those found in biological hard tissues. Synchrotron XRD data show that at all temperatures, diffraction peaks arose from fluorapatite, however the relative intensity between (012) and (120) reflections varied at different temperatures. This is likely caused by the presence of fluorite (CaF₂), which has Bragg peaks which overlap the (012) and (222) reflections of the apatite phase. Similarly MAS-NMR measurements support the presence of the two phases and confirm that the relative percentage of fluorapatite increases with increasing temperature. FTIR spectra show typical apatite peaks at all temperatures.

Conclusions Fluorapatite ordered crystals were successfully synthesised at all temperatures. However, there is an optimum temperature to promote fluorapatite formation between 37 and 54 °C. Hydroxyapatite surfaces were observed to guide the growth of crystals into highly-ordered crystals that could be investigated further in order to mimic biological hard tissues.

Notes
Increased bioactivity in fluoride and high phosphate containing glasses and stimulation of VEGF production in MC3T3-E1 osteoblast-like cells

LIU, J., Rawlinson, S., Hill, R. G., Fortune, F.
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Objectives
Optimal bone regeneration is dependent on sufficient blood supply; bioactive glasses (BG) could act as a delivery system to promote local vasculogenesis. Phosphate plays an important role in BG apatite formation and fluoride (F) stimulates osteoblast activity in vitro. Vascular endothelial growth factor (VEGF) is important for the regulation of vasculogenesis and angiogenesis. The aim of this study was to investigate bioactivity and VEGF producing potential in osteoblast-like cells of high P₂O₅ containing bioactive glasses with low F levels.

Methods
BG (0-7% F content, and 6.33% P₂O₅ in mole%) were produced by a melt-quench method and then ground and sieved to <38μm. Glass powder was immersed in Tris Buffer solution (1.5g/L) for 2h, 8h, 24h, 72h and 168h to determine apatite formation through X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). Ion (Ca, P, Si and F) release by dissolution was determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) and fluoride-selective electrode. RT-PCR and Western Blot were used to quantitate the effects of 72 hour glass-conditioned medium on VEGF gene and protein expression in MC3T3-E1 cells.

Results
XRD and FTIR demonstrate an amorphous glass structure in all groups (0-7% F). Apatite formation was accelerated in the F-added group (even 1% content) after only 2-8h immersion in Tris buffer. Apatite was not found in the F-free group until 72h immersion. Ca, P and F release reached the peak after 2h immersion and then decreased. VEGF gene expression was increased, and protein expression dose-dependently promoted with media conditioned by F-containing glasses from 1 to 21 days in culture.

Conclusions
The combination of a low F content with high phosphate significantly accelerated apatite formation in Tris Buffer. F-containing glass conditioned medium significantly promoted VEGF production in MC3T3-E1 cells. F-containing BGs might accelerate optimal bone regeneration.

Notes
Odontogenic differentiation of human dental pulp stem cells (hDPSCs) under strontium treatment
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Objectives As a trace element similar to calcium, strontium (Sr) is commonly used in dental applications. For example it has been doped in toothpaste to treat dentine hypersensitivity, or in glass ionomer cement in dental restorative materials. However, the biological effects of Sr on the dentine-pulp complex require further elucidation. The use of human dental pulp stem cells (hDPSCs) in tooth regeneration is favoured as they have the ability to generate the components of dentine-pulp complex. The aims of this study are to investigate the influences of strontium on the behaviour of human dental pulp stem cells (hDPSCs).

Methods hDPSCs were treated with or without Sr, then cell proliferation and alkaline phosphatise activity (ALP) was quantified by DNA fluorometric assay and ALP activity assay respectively. Odontogenic differentiation related gene expression: DSPP, DMP-1, ALP and type I collagen (COL-1) was assessed by quantitative polymerase chain reaction (qPCR). Western-blot and immunocytochemical staining were used to determine the level and location of DSPP and DMP-1. Alizarin Red S staining was used to quantify the degree of Sr mediated mineralization.

Results Low doses of Sr (≤ 2.5mM) significantly influence proliferation ability and ALP activity of DPSCs, while 5mM Sr treatment induces cell proliferation only. The Sr exposure groups displayed significant increase of DSPP and DMP-1 expression compared with negative control group at mRNA and protein level. Sr also stimulated mineralization. DMP-1 appeared to be ubiquitous in its staining pattern both in cytosol and nucleus, while DSPP is observed to be localised in perinuclear structure.

Conclusions This study provides the first evidence that Sr at specific doses significantly influences proliferation, odontogenic differentiation and mineralization of hDPSCs. Therefore Sr is a promising candidate for dental hard tissue regeneration. It is hoped this information could pave the way for using of strontium in dental applications for dental tissue regeneration.

Notes
Objectives Acid-degradable and inert glasses containing CaF\(_2\) and SrF\(_2\) are widely used as fillers in aesthetic dental restorative materials. Aesthetically superior polyalkenoate cements and dental composites are produced by matching the refractive index (RI) of the glass to the resin or the polysalt matrix to avoid light scattering at the interfaces. RIs of glasses can be calculated by the means of Appen factors. However, there were no Appen factors for amorphous metal fluorides published to date. Hence, the objective of this study was to empirically derive Appen factors for amorphous Calcium and Strontium fluorides.

Methods Several series of acid-degradable fluoroaluminosilicate glasses of varying metal fluoride content were selected for this study. The Appen equation was used to estimate RIs of glasses by using previously published RIs for the crystalline metal fluorides, in addition to Appen factors for the glass oxides. RIs of glasses were measured by the Becke line technique. Fragments of coarse glass samples were dispersed in mineral oils of varying RI (1.45-1.55) and observed under a phase contrast microscope until RI match was found. Subsequently, once experimental RIs of glasses became available, Appen factors for amorphous CaF\(_2\) and SrF\(_2\) were derived.

Results Empirical Appen factors for the amorphous metal fluorides were found to be higher than those for the crystalline metal fluorides. It was also found that the RIs of glasses reduced linearly (R\(^2\)=0.98) with increasing fluorine content.

Conclusions Empirical Appen factors have been successfully derived for the amorphous metal fluorides that enable accurate RI estimation of fluoride-containing glasses for the development of dental restoratives with enhanced clinical aesthetics.
2-Photon analysis of nanogel-infiltrated adhesive-dentine interfaces

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**Objectives** A new class of dental adhesive based on reactive nanogel additives controls the hydrophobic character of the adhesive resin whilst reducing shrinkage stress and improving mechanical properties. Nanogels are globular methacrylate-based cross-linked particles 10-90 nm in diameter, and can be swollen by and dispersed in resin monomers. Nanogels migratory/ swelling behaviours within such a system are unclear. 2-photon scanning laser microscopy allows high-resolution images of the fluorescently tagged nanogel adhesive interface, providing detailed morphological information and details of nanogels’ migration. Such an understanding may lead to further optimisation of these materials.

**Methods** Model BisGMA/HEMA 60:40 w/w solvated adhesives were prepared. Nanogels were a 70:30 molar ratio of isobornyl methacrylate (IBMA) and urethane dimethacrylate (UDMA), labelled with a Rhodemine Red fluorescent probe. Labelled nanogels were added to the resin at 20 wt%. Six human third molars each had mesial and distal class V cavity preparations and were restored at 1h, 3h, 24h, 48h, 7d and 14d after creation of the nanogel adhesive. Adhesive interfaces were imaged using a tunable two-photon laser using appropriate excitation-emission profiles for dentinal collagen and labelled nanogels.

**Results** The 2-Photon images show nanogel distribution and microstructural interface features across all hybrid interfaces studied. Nanogels were shown to homogeneously disperse within the resin matrix and infiltrate finer tubular structures (<1µm).

**Conclusions** The 2 photon imaging modality provides detailing of interface structure, using both intrinsic fluorescence of tooth tissue and with the selection of suitable fluorescent probes. This technique could assist in further studies surrounding adhesive placement protocol and interaction between adhesives and tooth tissue.


**Notes**
Triggers for patients’ referral for NHS dental implant treatment and the subsequent decision making process; Patients’ encounters and Clinicians’ views

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Objectives Within the NHS, implant provision is restricted in order to manage a limited resource and prioritise those with greatest need. This study aims to explore 1) the main triggers for referral of patients for implant treatment via NHS secondary care, and, 2) patients’ and clinicians’ thoughts of, and their roles in, the decision making process in the context of ‘restricted’ NHS implant provision

Methods This qualitative study used audio-recorded semi-structured interviews with a purposive sample of patients and clinicians at a NHS secondary care clinic. Thematic content analysis was undertaken to reveal emerging themes. Sample : Patients (, 35 interviews with 31 individuals, 50% edentulous, 12 women and 19 men, age range 19-76 with 50% under age of 40) and clinicians (n=7)

Results Three core themes in relation to patients’ experiences of the decision stage were identified;
(1) The ambiguity of patient selection criteria (2) The length of time for the decision process to be undertaken (3) The “risk” of ineligibility for implants restorations within the NHS and the subsequent impact on their quality of life and tolerance of other treatment options.
In contrast the two themes emerging from the clinician interviews were;
(1) The status of current guidelines (RCS, 2012)[1] which are open to personal interpretation (2) The decisions on implant provision were dependant on local conditions (i.e. the implant team and local resource allocation)
In addition, patients’ referrals from primary care to NHS clinic were mainly steered by RCS implant guidelines. [1] Royal college of Surgeons, Faculty of Dental Surgery: Guidelines for Selecting Appropriate Patients to Receive Treatment with Dental Implants: Priorities for the NHS

Conclusions More robust national NHS implant selection criteria may be required to facilitate shared decision making within primary and secondary care. Patients need clearer information about the planning stages for implants and how this can affect selection and treatment progression. Duration of treatment stages and the need for implant maintenance should be highlighted & discussed early during the decision making process as these factors influence patient’s decision to proceed with implants if offered.

Notes
Tension field triad: Influences on development of professionalism in dental students
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Objectives To explore dental students’ views on what influences development of professionalism

Methods This was a cross sectional qualitative study designed to explore the influences on development of professionalism amongst dental students. Five focus groups, one for each year of undergraduate study with 6-8 participants were selected using a sample matrix. A topic guide was formulated based on pilot study findings to facilitate discussion which was audio taped and transcribed. Thematic analysis was carried out using the software QSR NVivo 9. Ethics approval was granted (QMREC2011/93).

Results The students were able to pinpoint particular episodes and interactions which influenced their development of professionalism. Those identified by the students included explicit teaching, care of patients, clinical teachers as role models and significant critical incidents. An unanticipated finding was the role of patients as mentors in the development of professionalism. Patients were supportive, interested and keen to encourage the individuals who cared for them to be successful in completing their course. This extended to patients mentoring students and giving informal feedback to boost confidence. A constant state of tension was detected in the student’s professional development. This is represented by the influences of three domains interacting, the curriculum content, the emotions of the students and the environment.

Conclusions This tension field created by the interactions between the three domains is similar to Illeris model of learning (2004). There is a psychological interaction between learning experiences and the emotions evoked by this within the learner as well as a social interaction of the learner with the learning environment. The students’ engagement in these multiple interactions creates the tension field which stimulates the learning of professionalism.

Notes
Leadership in dentistry: an exploratory study of general dental professionals’ perceptions of leadership
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University of Leeds

Objectives This study aims to explore the following questions:
What does leadership mean in the context of the dental profession and how does it manifest?
What are the leadership and management skills and personal attribute requirements of individuals working in the dental profession?
Do members of the profession feel adequately prepared, through their own educational experience, for these requirements?
What is their experience of leadership and management in practice?

Methods A qualitative methodology was applied, using an interpretive, constructionist approach. One-to-one exploratory semi-structured interviews with ten dental professionals (dentists, dental hygiene-therapists, dental nurses and practice managers/receptionists) working in general dental practice were held. Participants were asked a series of pre-prepared questions but the conversations were open in structure to allow elaboration and accommodation of topics not previously identified. Interviews were audio recorded and transcribed. Analysis was initially thematic in approach but a reflexive reaction to the findings was to examine narratives that emerged from the data.

Results Participants and the practices in which they worked were representative of general dentistry in the UK. Identified themes were: ‘multiple meanings of leadership’, ‘personal experience’ ‘embodiment’, ‘challenges’ and ‘leadership learning’. Deeper and emerging findings were ‘narratives of practice’ which illustrate common leadership issues/stories happening in many general dental practices: a recent corporate buy-out with a change in leadership culture; the responsibility and pressure of a single owner practice.

Conclusions This study explored the concept of leadership in general dental practice by identifying themes and narratives. This work has resonance for practitioners throughout the dental team and tells the ‘real-life’ experience of leadership in practice. The outcome of this work has informed a planned further in-depth ethnographic study of leadership in general dental practice.

Notes
Exploring people’s online experiences of the teeth whitening industry using a web-specific mapping technique
Lala, R., Robinson, P. G., Gibson, B. J.
University of Sheffield

Objectives To explore the utility of web epistemology in dental research, illustrated using ‘issuecrawler’ to unravel the actors in the teeth whitening industry.

Background Online interactions supplement, or create more ‘real’ interactions. Dentists, dental regulatory and union organisations, industrial partners, the public sector, the media employ web practices such as social media and websites to reach people and promote teeth whitening online. However, search engines are epistemological devices that algorithmically index, cache and order data. Hyperlink patterns form part of the algorithms for ranking search results. Organisations are cognizant of these algorithms and use search engine optimisation (SEO) techniques to elevate their search engine rankings to influence people’s behaviours. To capture these distinct web cultural dynamics, it is proposed that web-based research considers medium-specific techniques such as ‘issuecrawler’ that will reflect people’s web searching experiences.

Methods Preliminary analysis using Issuecrawler, a web-specific tool that performs hyperlink analysis was used to explore the relevant actors in the teeth whitening industry. The inductively identified actors’ websites including the GDC, BDA and beauty magazines were crawled for hyperlinks to observe if actors interact with one another using ‘social network analysis’ and ‘co-link analysis’. The technique created a graphical network revealing the relevant actors in the teeth whitening network.

Results The dominant actors included in the Issuecrawler teeth-whitening network were women’s magazines, fashion magazines, sports magazines as well as the dental institutions. The BDA, GDC, DoH, BACD were also included in the network. The online teeth whitening network was larger than anticipated and also included unpredicted actors such home magazines.

Conclusions Dental research can ground claims about culture within online dynamics. Due to the web’s distinct nature, the medium-specific techniques used in this study revealed unexpected interactions between the beauty, dental and domestic fields in relation to tooth whitening. Web-specific approaches may therefore allow us to explore the dynamics operating behind people’s online experiences.

Notes
Correlation between Optical Coherence Tomography and Synchrotron X-ray diffraction for the diagnosis of enamel defects

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Objectives Currently, dental enamel defects (e.g. Molar Incisor Hypomineralisation, MIH) are diagnosed clinically by inspection (and radiographs), which have limitations: visual examination is subjective; being dependent on the dentist, lighting, and dryness of the tooth. Radiographs have poor spatial resolution, expose the patient to ionizing radiation and under-represent superimposed buccal and lingual features. We propose Optical Coherence Tomography (OCT) as a new diagnostic tool for assessing the prognosis of MIH, which we are currently developing for clinical use. Since enamel is a hierarchically ordered material with organisation at the crystallographic, nano- and micro-scale, a crucial aspect of the successful translation of OCT into clinical use, is the precise and reliable interpretation of how the OCT image data, reports the underlying enamel crystallography, nano- and micro- structures.

Methods In this study, we characterise the crystallographic texture, crystal lattice parameters, and particle sizes in healthy and affected enamel specimens by X-ray Micro-tomography (XMT) and Synchrotron X-ray Diffraction (SXRD) before relating these parameters to the OCT profiles in the same regions of the same specimens.

Results We demonstrated how OCT using en-face imaging mode can be used as a simple and convenient diagnostic technology, to evaluate the extent of the MIH lesion 3-dimensionally, including subsurface lesions. In a recent experiment on XMaS (ESRF Grenoble), we found correlative evidence between OCT image contrast (localised photons scattering) and crystallite organization (azimuthal angles extracted from sequential diffraction patterns) in enamel affected by MIH.

Conclusions The use of OCT for the clinical diagnostics of enamel defects is promising and may pave the way for this approach to be adopted to advance the diagnostics of more common dental conditions, such as tooth decay or erosion.

Notes
Salivary proteins mediate greatest protection against dental erosion
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Objectives The aim is to investigate the effect of salivary ions and proteins on eroded enamel in a laboratory investigation.

Methods 40 polished enamel specimens were prepared from extracted human teeth (Research ethics approval, Northampton REC, 14/EM/0183) and randomly assigned to 4 subgroups. 10 enamel samples per group were allocated to parotid, whole mouth, artificial saliva and water and immersed in the corresponding solution for 24 hours followed by a further 30 minutes prior to exposure to a 10-min erosion cycle in 80 ml of 0.3% pH 3.2, citric acid, agitated at room temperature, followed by 2-min water rinse. The 30 min immersion in the corresponding solution followed by the acid was repeated 5 times for all samples. Mean step height change from 5 randomly assigned points was measured using a non-contacting profilometer and Knoop microhardness measured at baseline (KHNb) and on the eroded surface of each sample (KHNe) and SMH change = (KHNb – KHNe) was calculated. Linear Regression model and Stata12.0 were used for the statistical analysis.

Results Whole and parotid saliva produced significantly less step height (4.16±0.57 µm, 6.41±0.71 µm respectively) than artificial saliva (7.47±0.98µm) and these differences were statistically significant compared to water (10.89±0.98µm and p< 0.0001). Microhardness change, for whole mouth (224.11 ±29.29 KHN p<0.0001), parotid (208.16 ±50.20 KHN p<0.0001) and artificial saliva (194.0±19.75KHN p<0.002) was significantly greater than water (155.34±18.4 KHN). Whole mouth saliva had significantly greater microhardness change than artificial (p<0.012).

Conclusions Saliva, containing proteins, appears to offer greater protection against dental erosion than artificial salvia and water. Whole mouth saliva provided less step height and greater hardness change than parotid saliva.

Notes
Determination of Hydrogen-Ion Concentration Microenvironments within Biofilm by Two-Photon Excitation Fluorescence Lifetime Imaging Microscopy (2PE-FLIM)

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Objectives Dental caries is caused by organic acids produced by bacterial biofilm on the tooth surface. These acids are produced when this biofilm (plaque) comes in contact with fermentable carbohydrates. Acid production lowers the pH, promotes mineralisation loss and encourages the growth of aciduric, acidogenic microorganisms. An important feature of biofilm is the formation of multi-dimensional physicochemical gradients. Currently employed methods for the determination of pH in biofilms are not without drawbacks. Therefore, novel methods are required in order to visualise these microgradients and determine their effect upon the microbial ecology. Here, we discuss a method for the measurement of multi-dimensional pH in a natural state, independent of probe concentration or excitation intensity.

Methods The pH-sensitive benzo[c]xanthene dye, seminaphthorhodafluor-4F 5-(and-6)-carboxylic acid (SNARF-4F), was selected due to its low pKₐ allowing measurements in the optimum range. The two-photon excitation (2PE) wavelength was determined by total photon counts, scanning between 760 and 900 nm. The dye was calibrated in buffers and utilised for fluorescence lifetime measurements of both planktonic bacteria and biofilm. Images were collected using BH SCPM software and the fluorescence lifetimes analysed by time-correlated single photon counting using BH SPCImage software.

Results An optimum λₑ of 2PE of SNARF-4F was determined to be 840 nm. Calibration between pH 3.0 and 7.6 exhibited a linear relationship between lifetime and pH. This allows the calculation of pH from the fluorescence lifetime. Bacterial suspensions supplied with glucose demonstrate a decrease in fluorescence lifetime over time. The images obtained demonstrate a lifetime/pH change with significant visible heterogeneity.

Conclusions Here, we describe the use of a pH indicator, SNARF-4F, and 2PE-FLIM for the determination of extracellular pH. By obtaining fluorescence lifetime of the fluorophore in buffers we were able to visualise a linear relationship between fluorescence lifetime and pH. Although the use of fluorescence emission intensity is limited by the pKₐ, we have demonstrated that fluorescence lifetime imaging is reliable and reproducible for determination to below pH 4 making this a useful method for acidic environments, such as dental biofilm.

Notes
Comparing the Demineralisation Rates of Deciduous and Permanent Enamel.
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**Objectives** GA dental extractions for children in the UK are increasing. The causes are many-fold. Although there are many studies on the physical chemistry underlying the aetiology of demineralisation in human permanent enamel (PE), data relating specifically to deciduous enamel (DE) are relatively scarce. The overall aim is to understand the differentiating demineralisation characteristics of PE and DE. The objectives of this study were to use scanning microradiography (SMR) to; establish DE baseline demineralisation rates; measure the effect of increasing the calcium and phosphate ion concentration in the demineralising solution on DE rates; and to establish whether a statistically significant difference exists between the demineralisation rates of DE and PE.

**Methods** Pairs of PE and DE 2mm slabs with natural surfaces exposed were mounted into 3 SMR cells. 18 points on DE and 13 points on PE slabs were analysed using SMR. Samples were initially immersed in deionised water for 48h; then demineralising solution (acetic acid pH 4.0) was circulated for 48h. Real-time SMR was used to measure the rate of mineral loss at each point. Then, 0.5mmol l\(^{-1}\) calcium ions and 0.3mmol l\(^{-1}\) phosphate ions were added to the demineralising solutions, and mineral loss rates re-measured. Thereinafter; 1mmol l\(^{-1}\) calcium ions and 0.6mmol l\(^{-1}\) phosphate ions were sequentially added to the demineralising solution every 48h and the rates of mineral loss measured continuously using SMR.

**Results** The mean baseline demineralisation rates were not significantly different (p<0.05\%) at 3.96 x 10\(^{-4}\) g cm\(^{-2}\) hr\(^{-1}\) (DE) and 4.54 x 10\(^{-4}\) g cm\(^{-2}\) hr\(^{-1}\) (PE). The demineralisation rate in DE and PE decreased as the calcium ion concentration was increased, and both followed similar trends.

**Conclusions** These results show that DE demineralises slower than PE but this was not significant (p<0.05\%). Increasing the calcium and phosphate ion concentrations in the demineralising solution reduces the PE and DE demineralisation rates, and both follow similar trends.

**Notes**
Host Microbiome Interactions in the Oral Cavity of a Denture Wearer
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Objectives Currently there is limited understanding of how the composition of denture plaque directly influences denture related stomatitis (DS), and in turn the role of the host in this disease. Therefore, the aims of this study were to understand and compare the composition of plaque taken from different oral sites, including the denture, palatal mucosa and dental plaque from the mouths of denture wearers. Furthermore, to assess changes in the diversity and composition of the microbiome against increasing Candida CFU, as well as understanding the relationship between host antimicrobial peptides and denture plaque composition.

Methods Samples were obtained from 131 denture wearers, all of which wore full or partially removable upper dentures. Swabs were taken of the palatal mucosa and the denture. If the patients had any natural teeth remaining, a sample of dental plaque was taken. Whole unstimulated saliva was obtained by expectorating into a collection tube. DNA was extracted from the denture plaque and amplicons were prepared and purified for MiSeq sequencing of the V4 region of 16S rRNA. Antimicrobial peptide (AMP) levels in patient’s saliva were assessed by ELISA.

Results Over 550 OTU’s were identified across the three samples. Principal component analysis (PCA) demonstrated clear cluster differentiation between dental plaque and denture plaque. No significant differences in denture plaque composition was shown between health and disease, although on the mucosa significant differences were observed in terms of diversity. Higher levels of Candida spp. appear to induce a compositional switch to a more aciduric environment, where by Lactobacillus levels increased alongside Candida.

Conclusions This is the first study to give a detailed insight into the microbiome of denture plaque in health and disease. Furthermore it has provided evidence that DS development is not solely a result of fungal infection, but somewhat of an interaction between the two kingdoms.

Notes
Debris accumulation following instrumentation with asymmetric file systems

OGLAH, F. S., Juneja, R., Robinson, J., Cooper, P., walmsley, A. D., Tomson, P.
University of Birmingham

Objectives To determine the amount of debris which accumulates in root canal spaces after instrumentation with asymmetric rotary NiTi file systems called Revo-s (Micro-Mega, France) and Protaper Next (Dentsply Switzerland) using micro-computer tomography (µCT) analysis.

Methods Mandibular molars with consistent continuous isthmi between the mesial canals were chosen using low resolution µCT (Skyscan 1172, Bruker Micro CT, Belgium) from the Birmingham Dental School Tooth Bank (REC Ref: 14/EM/1128). Teeth were accessed using 501 diamond bur and following identification of the mesial canals a glide path was created up to size 15 in each canal. Both canals of mesial roots were instrumented by either Revo-S (n=20), Protaper Next (n=20) or Protaper Universal (Control n=19) according to the manufacturer instructions. Strict irrigation protocol was adhered to using 1ml of 5.25% aqueous sodium hypochlorite after each file. Teeth were scanned before and after instrumentation using µCT at resolution of 13.5901 µm. The acquired images were co-registered with 3D Slicer software to ensure correct alignment and processed with the ImageJ software in order to isolate debris accumulation following instrumentation. 2-sample t test was used to determine statistical differences of debris accumulation between file systems.

Results Roots prepared with the Revo-S file system showed the least debris accumulation (5.1%) of all file systems tested. Both asymmetric rotary file systems showed significantly less debris accumulation compared to Protaper Universal (p< 0.05).

Conclusions Based on this assessment, asymmetric NiTi file systems produce less debris accumulation in root canal isthmi compared to Protaper Universal.

Notes
In Vitro Characterisation of Teethmate: A Calcium-Phosphate Based Dentine Desensitiser

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¹Barts and the London School of Medicine and Dentistry, ²Barts and the London School of Medicine and Dentistry

Objectives To characterise apatite formation and to assess effectiveness of Teethmate (TM) in treating dentine hypersensitivity.

Methods Healthy molars were sectioned mid-coronally to produce dentine discs. Pashley type hydraulic conductance was performed on the discs to assess tubular occlusion before and after treatment with TM. Thereafter, the discs were desiccated, fractured and observed under SEM. To assess rate of apatite formation, 4mm (D) X 6mm (L) cylinders of TM were produced and immersed in TRIS-buffer and artificial saliva for different time periods. This was followed by characterisation of the cements using FTIR, XRD and ³¹P MAS-NMR.

Results TM treated discs showed a reduction in hydraulic conductance compared to non-treated samples. SEM analysis also confirmed tubular occlusion through formation of plate-like crystals. Characterisation studies indicated that TM cement samples immersed in TRIS-buffer formed an apatite phase, most likely calcium-deficient apatite, within 6 hours of immersion. This change was more rapid for samples stored in artificial saliva.

Conclusions This study suggests that TM converts to apatite like phases and provides an effective option for the treatment of dentine hypersensitivity.

Notes
Antibacterial coating made of strongly adhered nanosilver to titania nanotubes for dental implants
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1Plymouth University, 2Plymouth University, 3Plymouth University

Objectives
An antibacterial and biocompatible coating on Ti-6Al-4V alloy was synthesised. A method of making the antibacterial effect long term, without any acute release of silver from a dental implant, on insertion was devised.

Methods
Titania nanotubes were grown on Ti-6Al-4V alloy using anodisation in the presence of phosphate and fluoride ions. Following alkali treatment of the latter surface, silver nanoparticles were chemically reduced on the nanotubes. The latter surface was then characterised using high resolution electron microscopy (SEM) in association with energy dispersive X-Ray Spectroscopy (EDS) and Raman Spectroscopy. A silver release test was performed. Following a toxicity test in the presence of Human Osteoblast Cells, the biocompatibility of the coating would be assessed.

Results
Initially, titania nanotubes with diameter of c. 100 nm were formed following the deposition of silver nanoparticles (10-25nm) on the outer and inner walls. The chemical structure of the nanoparticles was confirmed by Raman Spectroscopy and EDS. The results also highlighted the different bonding attaching the nanosilver to the walls. Subsequently a very low amount of silver was released from the coating during the beginning of the silver release test confirming a good adherence between the nanosilver and the nanotubes. The low leaching was expected to reduce the toxicity of the implant in general and it was confirmed by growth of human osteoblast cells on the coating.

Conclusions
The strong adhesion of silver nanoparticles validated the fact that the coating on titanium alloy can prevent an acute release of silver as such having the possibility of having a long term antibacterial effects. This work gave rise to a novel method of synthesising an antibacterial coating for dental implants.

Notes
The effect of sodium content on the bioactivity of glasses used in grit-blasting technique
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1Queen Mary University of London, 2Queen Mary University of London

Objectives Bioactive glasses are widely used as implant coatings due to their ability to form a chemical bond with bone. Glass composition has an important role in determining the glass bioactivity and osseointegration. Coating the titanium implants with bioactive glasses using grit blast technique depends on their ability to embed physically in the surface. As there is a direct relationship between the sodium concentration in the glasses and their hardness, glasses with variable sodium content were designed to investigate their ability to embed in the titanium surface. The aim of this study is to investigate the effect of increasing sodium content on the bioactivity of the glasses.

Methods Three glasses in the system of SiO$_2$-CaO-Na$_2$O-P$_2$O$_5$-CaF$_2$ were prepared by the melt quench technique where Na$_2$O is substituted for CaO on molar basis. Each glass powder with particle size less than 38µm was immersed in tris buffer solution and simulated body fluid (SBF) at 37ºC for 3, 6, 9, 24, 72 and 168 hours. The pH changes and apatite formation for all glasses after immersion were investigated. The crystalline phases of the glasses were examined by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and $^{19}$F Nuclear Magnetic Resonance (NMR).

Results Increasing sodium content in the glasses showed a pronounced rise in the pH level of the two solutions with a slight drop at the 6 hours time point for the SBF. The apatite-like phase started to form within 3 hours of immersion for all glasses in tris buffer solution while in SBF, the formation of apatite clearly occurred after 24 hours immersion. $^{19}$F NMR showed that the glasses form fluorapatite when immersed in these solutions.

Conclusions This study shows that the variation of sodium content in soda-lime phosphosilicate glasses has no adverse effect on the reactivity and apatite formation of these glasses.

Notes
Multidisciplinary team perspectives on the quality of life of head and neck cancer patients at two years
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University of Glasgow, University Hospital Aintree, Edge Hill University

Objectives The aim of this study was to assess the extent to which core members of the head and neck (H&N) multidisciplinary team (MDT) used health-related quality of life (HRQoL) data and to assess their familiarity with specific HRQoL outcomes for different groups of H&N cancer patients

Methods A survey was undertaken of members of the H&N MDT in the Merseyside Regional Head and Neck Cancer Centre, including consultants, clinical nurse specialists and allied health professionals, as to their views on patient-reported outcomes for 8 common clinical scenarios following treatment for H&N cancer

Results The response rate was 63% (17/27), of which 71% were using HRQoL data. There was a wide scatter of estimates of patient-reported outcome from the participants within each scenario for each domain. There were no notable differences between consultants and others. In regard to speech, saliva and swallowing there was a tendency for participants to estimate worse outcome for patients than reported by patients themselves

Conclusions Although most clinicians use HRQoL data, this is for research purposes, rather than as part of patient information. HRQoL data can be used to allow the clinicians to include more accurate information in discussions with patients and carers, but it is important to remember that individual HRQoL outcomes can differ. There is scope for further study to explore the decision-making process for treatment modalities with equivalent survival, both from the MDT perspective and the patient perspective.

Notes
Risk Modelling in Cancer Prediction: Are we falling behind in Head and Neck Cancer?

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Objectives Risk prediction models incorporating multiple risk factors have been recognised as a method of identifying individuals at high risk of developing cancer. Our objectives are to:

◊ Discuss the concept of risk models and their use in healthcare
◊ Provide examples of risk models currently in use in medical practice, and specifically cancer.
◊ Propose the need for these models within the field of Head and Neck Cancer (HNC)

Methods Two of the authors (CEM and MWM) will independently conduct a systematic search of PubMed and Embase in accordance with the MOOSE consensus statement to identify risk models developed within the field of cancer in the last twenty years. Retrieved studies from both Pubmed and Embase will be imported into Endnote where duplicate studies will be excluded. Titles and abstracts of the residual studies will be interrogated by the two aforementioned authors. The extracted studies will be compared, and inconsistencies will be resolved by consensus. The full texts of the remaining studies will then be read to determine whether they met our inclusion criteria. In addition, the reference lists from all identified studies will be examined.

Results Risk prediction models are statistical algorithms used to predict population or individual risk of a particular disease, within a specified time frame. Models have been developed for lung, breast, ovarian and colorectal cancer, as well as cardiovascular disease. To date, there are no risk prediction models in use within the field of HNC. If developed, these models would hold promise for guiding selection of individuals at the population level, for screening. The challenge lies in identifying this high risk cohort of patients, who are most at risk of HNC and for whom screening programmes would be most cost-effective.

Conclusions Risk prediction models have been developed in many areas of healthcare but they are lacking in oral and dental research. Head and Neck Cancer prediction models have the potential to inform screening programmes and facilitate early detection. Because of the public health significance, the National Cancer Institute have recognised risk modelling as an area of extraordinary opportunity. A cost-effective robust risk prediction algorithm is required to identify the cohort of patients at highest risk of developing HNC.

Notes
The Scottish Audit of Head and Neck Cancer (SAHNC): Influence of socioeconomic deprivation status on 5- and 12-year survival.
Ingarfield, K.\(^1\), Savage, S.\(^2\), MacKenzie, K.\(^3\), Douglas, C.\(^4\), Conway, D. I.\(^1\), McMahon, A.\(^1\)

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**Objectives** Socioeconomic factors have been implicated in affecting the survival of head and neck cancer patients, but the association with long-term survival is poorly understood. The aim of this project is to assess how socioeconomic deprivation status impacts on the long-term survival of head and neck cancer patients, and to investigate the impact on long-term survival associated with patient, tumour and treatment factors.

**Methods** SAHNC is a large clinical head and neck cancer cohort recruited between 1999 and 2001 (n = 1910). Several factors are considered associated with inequalities in survival between varying deprivation categories including patient (age, sex, lifestyle, WHO status), tumour (site, stage), and treatment (modality, geographic region). Deprivation category is determined using the Carstairs and Morris index of deprivation. 5- and 12-year Kaplan-Meier Survival Analysis has been completed and differences in survival determined by the log-rank statistic.

**Results** The overall 5- and 12-year survival by socioeconomic deprivation status are displayed below:

**5-year Survival**
- Overall 44.7% (95% CI 42.4% 46.9%)
- Affluent 47.4% (95% CI 41.1% 53.4%)
- Deprived 38.5% (95% CI 32.1% 44.9%)

**12-year Survival**
- Overall 25.1% (95% CI 23.2% 27.1%)
- Affluent 25.7% (95% CI 20.5% 31.3%)
- Deprived 22.0% (95% CI 16.8% 27.7%)

The difference in survival between the most affluent group and the most deprived group reduced from 8.9% at 5 years to 3.7% at 12 years.

**Conclusions** This is a thorough analysis of the long-term survival of a national clinical cohort of head and neck cancer patients over an extended follow-up period. We have seen a reduction in the influence of deprivation on long-term survival in comparison to 5-year survival. The potential influences of patient, tumour and treatment factors on long-term survival are explored in further models.

**Notes**
Cancer-associated fibroblasts contribute to macrophage recruitment in head and neck cancer.

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**Objectives** To examine and compare the ability of factors secreted from cancer-associated fibroblasts (CAFs) to promote recruitment of monocytes/macrophages, the presence of which is known to be associated with poor disease outcome in a number of solid tumours including head and neck cancers.

**Methods** Senescence (a feature of some CAF) was induced in primary human oral fibroblasts using cisplatin, and monitored using senescence associated ß-galactosidase assay and by measuring markers of senescence by qPCR. Oral fibroblasts were also transdifferentiated into myofibroblasts (another CAF phenotype) by treating with TGF-ß, and the activated phenotype confirmed by qPCR and Western blot of myofibroblast markers. Chemotaxis assays were used to examine the ability of fibroblast secreted factors to recruit monocytic THP1 cells and monocytes isolated from peripheral blood.

**Results** Cisplatin treatment induced senescence of oral fibroblasts, while TGF-ß transdifferentiated normal oral fibroblasts to myofibroblasts with highest activation seen at 96 h exposure. IL-6 secretion was reduced with increasing TGF-ß exposure in myofibroblasts, while secretion of this cytokine was increased 22-fold in cisplatin-induced senescent fibroblasts compared to myofibroblasts. Similarly, higher level of CCL2 (8-fold) was secreted by senescent fibroblast in comparison to myofibroblasts. THP-1 migration was reduced with increasing duration of TGF-ß treatment while senescent fibroblasts induced significantly increased migration (~5-fold) compared to conditioned medium from myofibroblasts. Monocytes from peripheral blood migrated more towards both myofibroblasts and senescent fibroblasts with 33-fold and 31.5-fold increases, respectively, compared to untreated controls.

**Conclusions** It is likely that different sub-populations of CAFs play different roles in the tumour microenvironment. These data suggest that senescent fibroblasts may promote recruitment of macrophages more than myofibroblasts by secreting a discrete repertoire of factors, including CCL2.

**Notes**
Modelling Ameloblastoma Behaviour With Bone-Like Co-Culture Scaffolds

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Objectives Ameloblastoma (AM) is a benign yet destructive tumour of the jawbones and surrounding soft tissues with a high recurrence potential. Therapy of AM is typically surgical, involving large resection of the jawbone and surrounding soft tissues. Disease recurrence is caused by the presence of cell islands surrounding the main tumour, which can result in incomplete tumour removal.

Little is known about the cellular mechanisms of tissue invasion of this tumour. We have developed an in vitro bone-like co-culture scaffold to model the initial AM tumour growth, invasion and examine changes in gene expression.

Methods The co-culture models were assembled from two parts: first, a cellularised ‘hard’ tissue-like construct was made using plastic compressed collagen and bone cells. These constructs were placed next to collagen gels with cells from the AM-1 cell line to create co-culture models. Confocal microscopy, quantitative real-time PCR, scanning electron microscopy and cell proliferation assays were used to characterise the behaviour of the cells in the scaffolds.

Results The in vitro model described here was shown to closely mimic the native ameloblastoma growth environment. AM cells were shown to interact with the bone cells, upregulate bone resorptive genes, and express fibroblast-like markers with increasing culture time. This model enables close characterisation of AM cells in the model using fluorescence microscopy, as well as gene and protein expression assays. Furthermore, using this model we have been able to easily assess the effects of potential therapeutic agents on AM growth in the model prior to clinical studies.

Conclusions Our novel model can be used to determine the underlying molecular events in AM growth and to efficiently test drugs in an in vitro organotypic setting. Furthermore, with minor refinement, this scaffold may be used to model other oral tumours.

Notes
Body Mass Index and Dental Caries: A Systematic Review and Meta-analysis
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Objectives To investigate the relationship between Body Mass Index (BMI) and dental caries in children and adolescents aged up to 18 years, by conducting a systematic review and meta-analysis of the published literature.

Methods A search of the literature published between 1980 to June 2014, using electronic bibliographic databases and manual searching of bibliography lists from relevant publications was conducted. Assessment of study quality (internal and external validity) was based on the validated Methodological Evaluation of Observational Research checklist (MEVORECH) against predetermined criteria to identify research specific flaws. Reporting quality was also assessed using the same checklist. Two independent reviewers conducted the appraisal and any disagreements were resolved by consensus.

Results The initial search identified 4208 potential studies. After de-duplication and exclusion by title/abstract and full text, 67 papers met the inclusion criteria and were included in the review. Three main types of association between BMI and dental caries were found, with some of the studies showing more than one pattern of association: 23 studies found a positive relationship between BMI and caries, 14 showed an inverse association, and 33 found no association between the variables of interest. Confounder assessment was the domain most commonly found to be flawed, followed by sampling and research specific bias domains. Among the seven studies which were found to be of better methodological quality, four found no association between BMI and dental caries and three showed a positive association between the two variables; that is, children with higher BMI had a higher burden of dental caries.

Conclusions The evidence for a positive association between BMI and dental caries was inconsistent even in those studies found to be of better quality. A quantitative analysis of the findings (meta-analysis) will be presented.

Notes
Abdominal adiposity and periodontitis: a Mendelian randomisation study

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1Queen’s University, 2Queen’s University, 3Queen’s University

Objectives Mendelian randomization (MR) uses a genetic variant which is associated with an exposure as an instrumental variable to determine whether the exposure is causally related to an outcome. MR exploits the random assignment of genes as a means of reducing confounding in examining exposure-outcome associations. The aim was to investigate whether abdominal adiposity (waist circumference) is causally related to periodontitis in a group of 60-70 year old men.

Methods The study was based on 1271 dentate men in Northern Ireland who had a comprehensive periodontal examination and provided a DNA sample. The adiposity-associated variant rs17782313 in MC4R (melanocortin-4 receptor) was used as an instrumental variable for waist circumference in a MR design. Periodontitis was classified by mean clinical attachment level (CAL). Age and smoking adjusted regression analysis was used to test for associations.

Results There was an association between variants in rs17782313 and waist circumference ($P_{\text{trend}}=0.02$). After adjustment for age and smoking each extra C allele was associated with a 1.2 (95% confidence interval 0.3 to 2.1) cm increase in waist circumference, $P=0.01$. Abdominal adiposity was significantly associated with CAL, $P=0.007$: each 1 cm increase in WC was associated with 0.01 (95% CI 0.00 to 0.02) mm increase in CAL. However, when the instrumental variable was applied to investigate the effect of WC on CAL the association was not significant, $P=0.44$: each 1 cm increase in WC mediated through genotype was associated with -0.04 (95% CI -0.14 to 0.06) mm increase in CAL.

Conclusions Mendelian randomisation did not support a causal relationship between abdominal adiposity and periodontitis via the instrumental variable method applied.

Notes
Impact of type-1 diabetes and periodontal status on life quality.
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Objectives To determine the impact of periodontal status on quality of life (QoL) in patients with type-1 diabetes (T1DM) pre- and post-periodontal treatment.

Methods 57 T1DM and 43 non-diabetic patients matched for gender and periodontal status (health, gingivitis, periodontitis) were recruited. The Well-being Questionnaire 12 (W-BQ12) and Audit of Diabetes Dependent Quality of Life-19 (ADDQoL-19) were self-completed by the T1DM patients at baseline and those with periodontitis at 3 months and 6 months after non-surgical periodontal therapy.

Results There were no significant differences in the overall general W-BQ12 score between patients with healthy periodontal tissues (mean±SD; 24.6±2.19), gingivitis (24.1±5.14) or periodontitis (23.0±5.19) (p>0.05). A significantly higher general W-BQ12 score (indicating better QoL) was observed at month 3 (25.7±5.85) compared to pre-treatment baseline score (22.1±5.11) (p<0.05), suggesting an improvement in QoL post-periodontal treatment. The range of the ADDQoL-19 scores was -6.68 to 0.00, with a median score of -1.37 and lower quartile cut-off of -2.59. 42 (75%) T1DM patients reported an ADDQoL score of -2.59 or more, and 14 (25%) patients reported an ADDQoL score less than -2.59 (indicating lower QoL). Based on periodontal diagnosis, 73.7% of patients with periodontitis reported a lower QoL (using lower quartile cut-off) compared to only 22.2% of healthy periodontal tissues and 25% of gingivitis patients at baseline. Diabetes had the greatest impact on the “freedom to eat” domain (-1.83±1.03) with the greatest importance placed on the “family life” domain (2.76±0.47) of the ADDQoL-19 for all T1DM patients. No significant changes in the ADDQoL-19 scores were shown following periodontal treatment in T1DM patients (p>0.05).

Conclusions T1DM did impact on certain life aspects in this group of patients, however T1DM had no significant influence on QoL based upon their periodontal condition, as measured by the W-BQ12 and the ADDQoL-19 questionnaires, which may be inappropriate tools for assessing oral health-related QoL.

Notes
The association between periodontal pathogens and measures of adiposity

Winning, L., Patterson, C. C., Lundy, F. T., Kee, F., Young, I., Linden, G. J.
Queen’s University Belfast

Objectives Whilst an association between periodontitis and obesity has been described, a possible association between subgingival periodontal pathogens and obesity is less clear. This study investigated associations between the presence of periodontal disease pathogens and various measures of adiposity in a group of 60-70 year old men.

Methods A cross-sectional analysis was performed on 642 dentate men enrolled on a longitudinal study of cardiovascular disease in Northern Ireland. A comprehensive periodontal examination, including subgingival plaque sampling, was performed. Body mass index, waist circumference, waist-to-height ratio and waist-to-hip ratio were analysed as dependent outcome variables in multiple linear regression models. The detectable presence of four subgingival periodontal pathogens (Aggregatibacter Actinomycetemcomitans, Porphyromonas gingivalis, Treponema denticola, and Tannerella Forsythia) at different detection thresholds were entered as predictive variables with adjustment for the various confounders.

Results The mean age of the men studied was 63.7 (SD 3.0) years. Of the 642 men, 347 (54%) were classified as being overweight (BMI 25-29.9 kg/m²), and 144 (22.4%) as obese (BMI ≥30 kg/m²). At a detection threshold of 1x10³ bacteria, the mean BMI of the 178 men that had Porphyromonas gingivalis was 28.0 (SD 3.6) kg/m² compared to 27.3 (SD 3.6) kg/m² for men that did not, p=0.03. Multiple linear regression analysis showed the detectable presence of Porphyromonas gingivalis was significantly associated with an increased BMI p=0.03, waist circumference p=0.04, and waist-to-height ratio p=0.02, after adjustment for age, CRP, smoking, socio-economic status, cholesterol and diabetes. There were no associations between the presence of the other bacteria and measures of adiposity.

Conclusions In these 60-70 year old dentate men, the presence of Porphyromonas gingivalis in subgingival plaque was significantly associated with an increased BMI, waist circumference, and waist-to-height ratio after adjustment for known confounders.

Notes
Multilevel analysis of explanatory mechanisms in the relationship between income inequality and use of dental services
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Objectives To determine the role of disinvestment in public services, social capital and psychosocial stress in explaining the association between income inequality and use of dental services.

Methods This study pooled individual-level data from 42 of the 70 countries participating in the World Health Organization’s World Health Survey (WHO/WHS, 2002-2004) and country-level data from World Bank and WHO statistics. Individual-level data included participants’ socio-demographic characteristics (age, sex, education and household wealth index), edentulous status, and use of dental services. Use of dental services was defined as having had dental problems in the last 12 months for which treatment was received. Country-level data included income inequality (Gini coefficient for years 1994-2005) and average national income (Gross Domestic Product in US$ for year 2000) respectively. Potential intermediate variables were disinvestment in public services (total health expenditure and government share as a proportion of GDP, dental health systems and responsiveness of health systems), social capital (trust, safety, participation) and psychosocial stress (control and coping). A two-level binomial logit model with individuals nested within countries was used to test the association between Gini coefficient and use of dental services.

Results Data from 137121 adults aged 18 years and older in 42 countries, covering the 6 WHO regions (16 from Africa, 5 from the Americas, 3 from South-East Asia, 12 from Europe, 2 from the Eastern Mediterranean and 4 from the Western Pacific) were analysed. Gini coefficient was negatively associated with use of dental services. This association was attenuated but remained significant after adjustments for indicators of social capital (OR: 0.82; 95% CI: 0.69-0.87) and psychosocial stress (OR: 0.83; 95% CI: 0.70-0.98), but not for disinvestment in public services (OR: 0.94; 95% CI: 0.79-1.10).

Conclusions Disinvestment in public services provided the strongest explanation in the association between income inequality and utilisation of dental services.

Notes
Global burden of oral and oropharyngeal diseases in 2010 as reflected in Cochrane Database of Systematic Reviews
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Objectives The purpose of this study is to analyze the Cochrane Database of Systematic Reviews (CDSR) and determine whether oral disease related systematic reviews and protocols represent disease burden, as measured by disability-adjusted life years (DALYs) from the Global Burden of Disease (GBD) 2010 Project.

Methods Eight conditions have been identified in the GBD 2010 project as relevant to oral and oropharyngeal disease. The conditions were ranked based on % of total DALYs (disability-adjusted life years). Upon reviewing search results within CDSR, systematic reviews and protocols were matched to oral diseases based on subject content and study objectives. In order to be included, a review/protocol must have included the oral disease as a predominant focus of the subject content.

Results The condition were ranked in order of decreasing (esophageal cancer, edentulism, dental caries, periodontal disease, mouth cancer, Cancer of the other part of pharynx and oropharynx, Nasopharynx cancer, Cleft Lip and Cleft Palate). All conditions are represented with at least one systematic review and one protocol in the library. Edentulism had higher % total 2010 Daly compared to periodontal diseases but has been less represented in the Cochrane library (11 versus 13) and clinical trials included in the reviews (87 versus 101 trials).

Conclusions Edentulism was proportionally under-represented in the CDSR. This also reflected in the number of clinical trials included in each reviews. We will be also looking at mapping CDSR against 2010 DALY rank and median percentage change in DALY from 1990-201. These results provide high-quality and transparent data that may guide future prioritization decisions. The project is part of a larger project mapping the CDSR again Global Burden of Disease 2010.

Notes
A nano-biosensor for chair side monitoring of salivary MMP-8 in periodontitis

Taylor, J. J.¹, Jaedicke, K.¹, Williams, R. C.¹, Bissett, S.¹, Lansdowne, N.¹, Stone, K.¹, Pickering, K.¹, Neeve, V.², Lawson, V.², Yatsuda, H.², Kogai, T.², Preshaw, P.¹

¹Newcastle University, ²Oj-Bio Ltd

Objectives Chair-side monitoring of inflammatory mediators of periodontitis can provide immediate information about disease activity, which can inform patient management. For this purpose, we aimed to develop a novel prototype device, which can effectively measure salivary MMP-8, an established biomarker of periodontitis.

Methods Surface acoustic wave (SAW) biosensor chips were coated with MMP-8-specific capture antibody. The detection of soluble MMP8 in saliva samples was carried out by application of the sample to the biosensor chips followed by signal enhancement with a second MMP-8 specific antibody. The analytical performance of the SAW biosensor was compared to standard ELISA (Quantikine, R&D Systems) using unstimulated saliva samples obtained from patients with untreated periodontitis (N=58), gingivitis (N=54) and periodontally healthy volunteers (N=65).

Results In agreement with the published literature, levels of salivary MMP-8 detected using both analytical approaches were significantly higher in periodontitis as compared to gingivitis (p< 0.001) and healthy controls (p< 0.001). Receiver operator characteristic analysis for distinguishing periodontitis from health revealed an almost identical performance (area under curve values: ELISA 0.93; SAW 0.89). The assay time for our prototype device is ~20 minutes.

Conclusions The prototype SAW biosensor represents a promising development towards effective chair side monitoring of periodontitis. Further developments will focus on the refinement and clinical efficacy of the novel biosensors.

Notes
Gingival Toll-like receptor and cytokine mRNA expression in equine periodontitis and oral health

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Objectives Equine periodontitis is a common and painful condition, causing severe pain and eventual tooth loss. Despite this, the disease often goes unnoticed by owners and is thus a major welfare concern. The aetiology pathogenesis of the condition remains relatively poorly understood with few recent studies performed. The innate immune system is known to play an important role in human periodontitis, however its role in equine periodontitis has not been examined. The aim of this study was to quantify the expression levels of Toll-like receptor (TLR) and cytokine mRNAs in gingival tissue from orally healthy horses and those affected by periodontitis.

Methods Gingival tissue samples were taken post-mortem from seven orally healthy horses and thirteen horses with periodontitis. mRNA expression of TLR2, TLR4 and TLR9 and cytokines IL-1β, TNFα, IL-4, IL-6, IL-10, IL-12, IL-17 and IFN-γ was determined using qPCR. Statistical significance of results was assessed using f-tests and paired t-tests.

Results All TLRs and cytokines showed up-regulation of mRNA expression in disease. However, statistically significant increases were noted in expression of mRNAs for TLR2 (p=0.005), TLR4 (p=0.03) and TLR9 (p=0.0001) and cytokines IL-1β (p=0.02), IL-4 (p=0.03), IL-6 (p=0.005), IL-10 (p=0.005), IL-12 (p=0.02), and IFN-γ (p=0.003). The fold change between oral health and periodontitis was largest for IFN-γ, which increased 53-fold in disease.

Conclusions This study has provided an initial insight into the involvement of the innate immune system in equine periodontitis. Increased expression of TLR2 and TLR9 mRNAs indicates increased need for recognition of and response to microbial products and bacterial DNA in disease. Increased expression of mRNAs for pro-inflammatory, anti-inflammatory and Th1/Th2/Th17 cytokines indicates a complex immune response to periodontal pathogens. Further studies are required to fully characterise the role of the innate immune system in equine periodontitis.

Notes
Identification of novel salivary biomarkers for chronic periodontitis
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Newcastle university

Objectives This study aimed to compare selected protein profiles of whole saliva from patients with chronic periodontitis and healthy subjects, to identify and characterize novel salivary biomarkers for chronic periodontitis.

Methods Salivary levels of 102 cytokines and 34 proteases were measured using Proteome Profiler Arrays (PPA, R&D Systems). PPA assays were performed on saliva from 12 patients with untreated chronic periodontitis and 12 healthy volunteers. Protein expression on PPA membranes was quantified using image analyser software. PPA data was confirmed by enzyme-linked immunoassay (ELISA).

Results Overall analysis of salivary PPA revealed that; 75 of 102 cytokines were positively expressed in both groups (63 were higher in the patients, compared to 12 highly expressed cytokines in the healthy volunteers). Similarly, 24 proteases were higher in the patient group and 10 higher in the healthy volunteers. Interleukins and matrix metalloproteases such as IL-1ß, MMP-8 and MMP-9, which are previously identified biomarkers associated with periodontal diseases, were detected along with several novel proteins. Urokinase plasminogen activator (uPA) which is a serine protease enzyme involved in MMP activation, extracellular matrix proteins degradation, inflammation and tissue repair, was identified as a candidate salivary biomarker. ELISA results revealed that salivary uPA levels in the patients ranged between 360.03-4196.88 pg/ml, which were significantly higher than in saliva from the healthy subjects (31.65-632.48 pg/ml, p < 0.001).

Conclusions PPA assays can be used to identify salivary biomarkers, an approach that may contribute positively to the diagnosis of periodontal diseases. Salivary uPA is a candidate biomarker for chronic periodontitis.

Notes
Zinc modulates IL-1b-stimulated chemokine secretion in human gingival fibroblasts.
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Objectives Zinc (Zn) is an essential micronutrient involved in a vast array of cellular functions. There is well established evidence that sub-clinical Zn deficiency is important with respect to immune function. Despite the evident link between immune response and dietary intake of Zn, the precise mechanisms by which cellular Zn influences immune function are unclear. The effect of Zn deficiency on the pathogenesis of periodontal disease has not been examined; however preliminary experiments in rat models indicate Zn deficiency as a risk factor for periodontal disease. Objective: to investigate whether Zn availability could modulate chemokine expression by human gingival fibroblasts (HGFs).

Methods HGFs were cultured in serum free media (depleted) or media containing 20 µM (physiological) or 100 µM (supra-physiological) extracellular ZnCl₂ for 2 h prior to stimulation with 1 ng/ml IL-1β for a further 24h. Cytokine levels were measured using a cytokine protein array (Proteome Profiler, R&D Systems). Confirmation of results was carried out for IL-8 using a Human CXCL8/IL-8 DuoSet (R&D systems) ELISA kit.

Results Stimulation of HGFs with IL-1β alone (Zn-depleted medium) resulted in a 5-fold increase in expression of CXCL1, IL-8 and MCP-1 compared with IL-1b stimulated HGF cells under physiological Zn concentrations (20 µM). However, in the presence of supra-physiological Zn concentrations (100 µM), IL-1b stimulated CXCL1, IL-8 and MCP-1 were at levels similar to those manifested in Zn depleted cells and these cultures exhibited a wider range of cytokine expression (IL-6, CXCL5, CXCL10, and CCL20) compared with other conditions. Quantitation of IL-8 demonstrated a significant increase in IL-1b stimulated cells in Zn-depleted and 100 µM Zn-supplemented media compared with cells exposed to 20 µM Zn (Table 1).

Conclusions This preliminary data shows that Zn has a biphasic effect on chemokine secretion on HGF cells suggestive of a molecular role for Zn in periodontal disease. Further research investigating the mechanisms through which Zn is acting is necessary.

Table 1: IL-8 expression in HGFs cells exposed to Zinc

<table>
<thead>
<tr>
<th>Chemokine expression (pg/mg protein)</th>
<th>Zinc treatment</th>
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<tbody>
<tr>
<td></td>
<td>Untreated</td>
</tr>
<tr>
<td>IL-8</td>
<td>Median</td>
</tr>
<tr>
<td></td>
<td>0.96**</td>
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Median values are stated, n = 3 for each treatment, measured in duplicate. ** P < 0.01, by Kruskal Wallis one way ANOVA compared with 20 µM zinc treatment.

Notes
Treatment of Periodontal Defects with Novel Bioactive Glass Containing Strontium

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1 Queen Mary University of London, 2 Queen Mary University of London

Objectives To investigate the role of phosphate and strontium content on the bioactivity in order to develop improved bioactive glasses for the treatment of periodontal bony defects. Strontium is known to up-regulate osteoblast and down-regulate osteoclast.

Methods The glasses in the system of (SiO$_2$-P$_2$O$_5$-CaO-Na$_2$O-SrO) with varying phosphate content and 10% of strontium substituted for calcium on a molar basis were synthesized via the melt-quench route. The glass was ground and sieved to obtain particles size between 100 and 400 µm. The glass powder was analysed by (Fourier transform-infrared spectroscopy) FTIR and (X-ray diffraction) XRD both before and after immersion in Tris buffer solution for the purpose of investigating glass bioactivity and its amorphous structure. Glass particles size was determined by Scanning Electron Microscopy (SEM). The pH change after immersion in Tris buffer solution was measured at specific time points (4, 6, 8, 24 and 72 hours). Quantitative analysis of the Ca, P, Na, Si and Sr ions were detected by (Inductively couple plasma-optical emission spectroscopy) ICP-OES technique.

Results XRD patterns and FTIR spectra of untreated glasses indicated that all glasses were amorphous. FTIR and XRD revealed the formation of apatite upon immersion. The pH values after immersion increased slightly during the experiment. The speed of apatite formation and the amount of apatite formed increased with phosphate content of the glass.

Conclusions The present study showed that the phosphate content was overriding factor in terms of increasing the glass bioactivity as well as reducing the rapid pH rise, whereas strontium content illustrated a little influence on the glass bioactivity. However, further cell culture studies may demonstrate the biological benefit of strontium.

Notes
Ecology of volatile sulfur compound producing microbiota in health and chronic periodontitis

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1Queen Mary University of London, 2Queen Mary University of London, 3GlaxoSmithKline, 4GlaxoSmithKline, 5Queen Mary University of London

Objectives Sulfur metabolism of the oral microbiota confers selective advantages during an inflammatory challenge, and is consistent with increased volatile sulfur compound (VSC) concentrations in the breath of periodontitis patients. The aim of this study was to elucidate the VSC producing bacterial taxa in the oral cavity of subjects with chronic periodontitis, gingivitis and periodontal health, and study the ecology between these disease states.

Methods Tongue scrapings, subgingival and interdental plaque were collected from individuals with gingivitis (n=19), chronic periodontitis (n=14) and periodontal health (n=18). DNA extracted from the samples was analysed by HOMINGS (Human Oral Microbe identification by Next Generation Sequencing), to determine the percentage abundance of 667 oral taxa (538 species and 129 genera). VSC producing species were established by database searches for taxons carrying genes involved in VSC production (cysteine desulphhydrases and methionine gamma lyases). Principal Component and Partial Least Squares multivariate analyses was used to determine the taxons that influenced the community structure of samples.

Results Interdental plaque was significantly more rich compared to tongue and subgingival plaque across all cohorts. Subgingival plaque and tongue communities were more alike than interdental plaque in health, however, a transition to less rich and overlapped communities between all niches in gingivitis and chronic periodontitis was observed. VSC producing species were the major drivers of the ecological shifts occurring in the three niches between health, gingivitis and chronic periodontitis. Species with a higher rate of VSC production in vitro such as Treponema spp, Fusobacterium spp. and Porphyromonas endodontalis were strong determinants of the community structure of interdental and subgingival niches in chronic periodontitis and gingivitis.

Conclusions Overlap of tongue communities within interdental and subgingival niches in chronic periodontitis highlights the role of the tongue microbiota. VSC producing species driving the differentiation of subgingival and interdental niches in chronic periodontitis from health suggests VSC production in these niches could be important in the aetiopathogenesis of chronic periodontitis.

Notes

Objectives Previously, we have developed an ex vivo dental pulp infection model, however it was not fully validated for therapeutic intervention. Therefore the aim of this study was to (I) validate this model and (II) evaluate its histological and inflammatory response to an antimicrobial agent, triclosan.

Methods Transverse tooth slices (2mm) were prepared from rat incisors and cultured in Dulbecco’s Eagles Media (DMEM) for 24h. Then the tooth sections ± triclosan (12µg/mL) treatment were inoculated with fluorescein diacetate stained Streptococcus anginosus Group (SAG) bacteria in DMEM + 10% Brain Heart Infusion media and incubated for 24h. Furthermore, triclosan pre-treated pulp tissues were included to find its impact on area of colonisation. The samples were harvested at 0, 6, 12 or 24h for further analysis. Tissue damage was assessed by histological analysis. Expression of cytokines was assessed by qRT-PCR technique.

Results A significant (p<0.001 & p<0.01) reduction in dental pulp cells and odontoblast count was found with infected tooth slices from 12h and 24h respectively. The percentage area of SAG colonisation increased between 6-24h (p<0.01). The pro-inflammatory markers IL-1β, TNF-α, IL-18 were significantly (p<0.01) upregulated by SAG infection. Conversely, anti-inflammatory marker IL-10 was found to be significantly down regulated. Triclosan treatment significantly reduced the SAG area of colonisation in a time depended manner. Triclosan reduced the IL-1β, TNF-α, IL-18 expression and maintained IL-10 levels.

Conclusions The ex vivo model showed similar inflammatory responses to human pulpal infections as described in the literature. Triclosan offers the potential as an antimicrobial and anti-inflammatory agent for endodontic treatment.

Notes
TNFα-induced p38 MAPK activation regulates TRPA1 and TRPV4 activity in human odontoblast-like cells

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1Queen’s University Belfast, 2Queen’s University Belfast, 3Aix University

Objectives Odontoblasts express multiple mechano-sensitive ion channels, including members of the transient receptor potential (TRP) channel family. TRPA1 and TRPV4 are candidate mechano-sensitive TRP channels that play pivotal roles in inflammatory pain and mechanical hyperalgesia, however the effects of inflammation on the activity of these channels is not known. The objective of the current work was to determine whether TNFα directly sensitises TRPA1 and TRPV4 in odontoblasts.

Methods Dental pulp cells were derived by explant culture and differentiated towards an odontoblast phenotype in the presence of β-glycerophosphate. The odontoblast phenotype of the cells was confirmed by the expression of dentin sialophosphoprotein (DSP). Cells were treated with tumour necrosis factor alpha (TNFα) and the TRPA1 and TRPV4 gene and protein expression was determined by q-RT-PCR and immunohistochemistry. Functional studies of channel activity employed calcium imaging. Western blotting was used to study the signalling pathway involved in TNFα modulation of TRP channels.

Results Short treatment of odontoblast-like cells with TNFα significantly enhanced TRPA1 and TRPV4 responses to their specific chemical agonists and to membrane stretch. This enhanced channel activity was accompanied by expression of phospho-p38MAPK. Furthermore, treatment of cells with the p38 inhibitor SB202190, significantly reduced TNFα effects, suggesting signalling via the p38MAPK pathway. When cells were treated with TNFα for 24hrs there was an up regulation inTRPA1 expression but down regulation of TRPV4. The induced TRPA1 expression was significantly reduced by treatment with SB202190.

Conclusions Our findings demonstrated that TNFα is capable of enhancing TRPA1 and TRPV4 responses via the p38MAPK pathway and inhibitors of p38MAPK might therefore constitute a novel drug target for pain and inflammation mediated by non-neuronal TRP expressing cells.

Notes
**Biodentine™ reduces TNFα - induced TRPA1 expression in odontoblasts**

El Karim, I., About, I., Curtis, T., Linden, G. J., Lundy, F.

1Queen’s University Belfast, 2Queen’s University Belfast, 3Marseille Université,

**Objectives** Members of the transient receptor potential (TRP) family of ion channels have emerged as important cellular sensors. Amongst the TRP channels, TRPA1 plays a central role in nociception, and is considered the gate keeper of inflammation. TRPA1 has previously been identified in human odontoblasts and has been suggested to mediate odontoblast sensory function. Biodentine™ is a calcium silicate cement with promising regenerative potential and is proposed for use in pulp capping. Emerging clinical observations after Biodentine™ application as a restorative material have shown absence of post-operative sensitivity and pain relief effect in cases of symptomatic pulpitis. However the mechanism by which Biodentine™ induces this pain relief effect is not known.

**Objectives:** To investigate the effect of Biodentine™ on cytokine-induced TRPA1 expression and function in odontoblasts.

**Methods** Odontoblasts were differentiated from dental pulp stem cells grown in osteogenic media. TRPA1 expression was determined with immunohistochemistry, qPCR and Western blotting in dental pulp and odontoblasts. The functionality of the channel was confirmed by ratiometric calcium imaging.

**Results** Immunohistochemistry showed TRPA1 is expressed in odontoblasts and staining intensity was increased in the odontoblast layer of carious compared to intact teeth. *In vitro* simulation of caries induced inflammation, by treatment of odontoblasts with TNFα, confirmed TRPA1 up regulation at gene and protein level. Calcium imaging confirmed increased channel activity following TNFα treatment. On the other hand treatment with Biodentine™ significantly reduced TNFα-induced TRPA1 expression while Biodentine™ was shown to have no effect on basal expression or activity of the channel.

**Conclusions** Modulation of TRPA1 expression and activity in odontoblasts may provide an explanation for the anti-inflammatory and pain relief effects of Biodentine™ observed in the clinic.

**Notes**
Microfluidic Production of Stem-Cell Microcapsules for Spinal Cord Regeneration

Hidalgo, L.
Cardiff University

Objectives Cell transplantation is a promising technique to replace damaged tissue however, it is still difficult to guide cell fate during this process. Cell encapsulation permits a better control of cell responses and also protects the cells from any adverse environmental conditions post grafting. Microfluidics is a technique for generating monodisperse microcapsules in which the size of the capsules can be easily controlled.

Methods Alginate microcapsules were formed on a customised Polytetrafluoroethylene microfluidic chip. Actin-driven GFP expressing Dental Pulp Stem Cells (DPSCs) were encapsulated (figure 1,2) and incubated in a humidified 37°C, 5% CO2 environment. Medium was change every 2-3 days. Cell viability was measured using a Trypan Blue exclusion assay and a Live/Dead Viability Kit at different time points. In addition, the capacity of DPSCs to differentiate into neuronal-like cells in 2D culture was studied as a pre-requisite for 3D studies. Neural marker expression was analysed using q-PCR.

Results Flow rate alteration produced alginate beads of between 270-380µm diameter with a consistent shape. An increase in the flow rate of the continuous phase gave rise to smaller beads with the result that the distance between droplets was increased (thereby preventing bead fusion). Experiments with varying cell numbers demonstrated that DPSCs encapsulated at a density of 1x10⁶ cells/mL (in 350µm microcapsules) produced the best cell-laden beads (increased viability, notable GFP-actin expression). With respect to DPSC differentiation, the cells demonstrated a good, but somewhat variable, tendency to differentiate into neuronal-like stem cells.

Conclusions Alginate microcapsules containing viable stem cells were obtained using a microfluidics-based approach. Droplet diameter was controlled by flow rate modification. The next stage of this project is to investigate the potential for controlled neuronal differentiation of the stem cells within the microcapsules as part of the long-term aim to develop a stem cell-based therapy for neuronal regeneration in oral tissues.

Notes
Exploring a role for biglycan in regulating the regenerative potential of mesenchymal stem cells in bone repair

Battersby, P., Sloan, A., Waddington, R.
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Objectives To investigate how the BGN expression correlates to the regenerative capacity of the MSC population.

Methods Heterogeneous MSC populations, isolated from femoral compact bone of 28 day old male Wistar rats, were culture expanded monitoring expression of MSC and cell senescent markers. At PD15 and 50 temporal expression of cell proliferation marker, Ki-67, matrix proteins, BGN and Col1a1 and BGN cell internalisation receptor, HARE were quantified by qPCR and Western blot analysis during osteogenic differentiation. Mineral deposition was identified by alizarin red staining.

Results Extensive characterisation identified MSC populations with differing heterogeneous profile. PD15 cells likely contained predominately lineage-restricted cells with low proliferative capacity and high osteogenic potential. At PD50 these cells appear to be lost to leave immature transit amplifying MSCs of higher proliferative capacity and take longer to synthesise a mineralised matrix. During osteogenic stimulation, cell populations responded differently. Compared with PD15 cells, expression of ki-67 in PD50 cells was initially reduced in osteogenic media, which correlated with a delay in mineral deposition. No difference in BGN or Col1a gene expression was observed between the MSC populations, however, HARE was initially reduced in PD50 populations, possibly correlating with the prolonged presence of BGN protein within the extracellular matrix.

Conclusions The results of this study support previous purported evidence suggesting a role for BGN in stimulating early osteogenic differentiation, which may be of value in enhancing bone tissue engineering approaches in dentistry.

Notes
Development of 3D artificial niches for regenerative medicine

Ortega Asencio, I., Passley-Biggins, A. S., Thomson, O., Santocildes-Romero, M. E., Crawford, A., Hatton, P.

1The University of Sheffield, 2The University of Sheffield

Objectives The use of stem cells in tissue regeneration strategies offers great possibilities but is still an emerging field and few cell-based therapies have reached the clinic. Human embryonic stem cells (hESC) show great therapeutic potential and they are an invaluable research tool for the development of models in which to study disease and tissue regeneration mechanisms. The objective of this research is to create a platform technology for the development of niche-equipped smart microfabricated constructs for controlling and directing embryonic stem cell behaviour for musculoskeletal tissue regeneration applications.

Methods The microfabricated membranes were computer-designed and manufactured via a combination of novel 3D-printing techniques and conventional electrospinning. The additive manufacturing part of the fabrication allows the creation of intricate structures which will simulate to a certain extent the stem cell niche; on the other hand, the use of electrospinning techniques allows the creation of biodegradable membranes and the use of FDA approved polymers (polycaprolactone has been used in this work). In terms of cell culture work, a range of differentiation factors for directing hESCs towards a musculoskeletal pathway will be used, these factors including sonic hedgehog, Wnt1 and MyoD; furthermore, aspects such as cell morphology and cell differentiation will be studied using markers such as SSEA3, Oct4 and nanog.

Results We have optimised the design and fabrication of a broad range of biodegradable constructs equipped with artificial niches (Fig.1) and we have used these constructs for the study of embryonic stem cell behaviour. Initial studies have shown that the shape and morphology of the underlying 3D patterns seem to affect cell responses.

Conclusions The development of this platform technology sets the basis for the creation of artificial 3D microfabricated membranes for controlling stem cell fate which could be ultimately translated to a biomaterial device with potential use in the regeneration of musculoskeletal tissue.

Notes
Multilevel Principal Component Analysis (mPCA) for Shape Analysis: Initial Applications to Dental Research
Farnell, D. J., Popat, H., Richmond, S.
Cardiff University

Objectives To investigate the interplay between- and within-subject variation in statistical models of shape using mPCA. To determine how mPCA methods compare to standard PCA analyses of shape in dental applications.

Methods The major modes of variation can be found for a set of related shapes by using standard PCA. Broadly, a specific “new” shape is modelled via: \[ \text{Shape} = \text{Mean Shape} + \text{Modes due to Between-Subject Variation} \]
Multilevel methods allow for variation that is due to between- and within-subject effects.

In this case: \[ \text{Shape} = \text{Mean Shape} + \text{Modes due to Between-Subject Variation} + \text{Modes due to Within-Subject Variation} \]

Data Sets: Monte Carlo data: simulated mouth shapes using seven “landmark” points, shown in Figure 1.
OSTEODENT data: from two experts outlining the cortical bone in the mandible from dental panoramic radiographs using the graphical user interface shown in Figure 2.

Results Simulated Data Set: mPCA correctly partitions the between-subjects variation that correspond to scale changes (size of mouth) and within-subjects variation that correspond to shape changes (smiling, neutral, and sad). mPCA is much better at fitting to more extreme (yet still possible) cases.

OSTEODENT Data Set: mPCA indicates correctly that between-subject variation (shape changes of the mandible) are more important than within-subjects variation (variability due to different experts) by comparison of eigenvalues (left-hand side, Figure 3). mPCA provides adequate fits using “miss-one-out” testing (right-hand side, Figure 3).

Conclusions Between- and within-subject variations are modelled in a statistically correct manner using mPCA.
mPCA has more flexibility, control, and accuracy than standard PCA.
mPCA provides the correct method of combining sets of landmark points provided by different experts for the same images.

Notes
Anatomical Variation At The Sites Of Autogenous, Mandibular Graft Harvest
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Objectives Intra-oral bone grafting is an important component of implant treatment. The relationships between the bone surface and the underlying nerves and vessels at the two most commonly-employed intra-oral (retro-molar and symphyseal) sites for bone-harvesting were characterised.

Methods Data was collected by direct measurement from patients (n=103) undergoing CT scanning prior to implant treatment (50 retro-molar: 53 symphyseal). Measurement included the distance between the bone-surface and the underlying ID/incisive canal, and the thickness of the bone available for harvesting (incisive and retro-mandibular regions). Linear regression was performed to study the relationship of these anatomical variables to patient demographics.

Results The retro-molar analysis comprised 33 female and 17 male patients. Data recorded included: maximum thickness of the mandible across the inferior dental canal (IDC) (mean 13.79mm; range 10.2–18.2mm), distance from the IDC to the outer buccal cortex (mean 3.2mm; range 1.0–7.9mm); distance from the most buccal point of the lower second-molar root to the outer buccal cortex (mean 3.3mm; range 1.0-6.5mm). Symphyseal analysis included 26 female and 27 male patients. Data recorded included: maximal width of mandible between the lower central incisor root tips (mean 13.4mm; range 8.91-16.39mm); tip of canine to the mandibular incisive nerve (mean 4.30mm; range 1.2–8.9mm), canine root tip to the outer labial cortex (mean 3.96mm; range 1.85–7.30mm), mid-line distance from the incisive nerve to the outer labial cortex (mean 2.97mm; range 1.02–6.86mm). Linear regression indicates that mandibular thickness decreases with age in women (p=0.032). Whilst the thickness of bone buccally to the IDC remains relatively constant in females (p=0.43), it decreases with age in men (p=0.075).

Conclusions This study demonstrated that, although bone grafts are common, the proximity of underlying vital structures may be overlooked in routine examination of implant patients and may be related to patient characteristics.

Notes
Feasibility of endoscope assisted surgery on oral and maxillofacial diseases with minimal incisions and invasion

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Objectives To investigate the feasibility of endoscope assisted surgery on some oral and maxillofacial diseases with minimal incisions and invasion.

Methods We choose four patients suffering from oral and maxillofacial diseases from 2012 to 2013 in the Second Hospital of Shandong University. Two patients were diagnosed as submandibular dermoid or epidermoid cyst and two patients suffered from maxilla osteomyelitis after maxillary fracture operations with titanium plates. Each patient was given a careful design of dermatoglyph incision preoperatively. The endoscope assisted surgeries were performed on all of the patients.

The details of the surgery procedure were as follows. A 1.5 cm dermatoglyph incision was performed on the submandibular area. The facial artery, submandibular gland branch of submental artery, branches of anterior facial vein were identified and treated with ultrasound scalpel. The outer, inside, former and rear border of the cyst were separated and the cysts were resected en bloc. (Fig1 and Fig.2). The two patients suffering from maxilla osteomyelitis due to titanium plates just received extraction surgeries of titanium plates. A minimal incision with 0.5 cm long on the infraorbital area was performed. The titanium plates and screws were all taken out.(Fig.3)

Results The incision lengths on the submandibular area varied from 1.5-2.0 cm for the submandibular cyst patients. Neither facial paralysis nor infection occurred post-operatively. No relapse was detected in the long-term follow-up (two years). As for the two patients suffering from maxilla osteomyelitis after maxillary bone fracture operation with titanium plates. The incision lengths on the infraorbital area varied from 0.5-1.0 cm. Again there was no damage of infraorbital nerve, no ectropion and no inflammation.

Conclusions Endoscope assisted resection of submandibular cysts and extraction surgeries of titanium materials through dermatoglyph incision will decrease the damage to surrounding tissue and acquired good cosmetic results.

Notes
Epoxy-Tiglianes Modulate Dermal Fibroblast-Myofibroblast Wound Healing Responses and Reduce Scarring

Dally, J., Moses, R., Midgley, A. C., Howard-Jones, R., Errington, R., Reddell, P., Steadman, R., Moseley, R.

1Cardiff Institute of Tissue Engineering & Repair (CITER), School of Dentistry, 2Cardiff Institute of Tissue Engineering & Repair (CITER), School of Medicine, 3Cardiff Institute of Tissue Engineering & Repair (CITER), School of Dentistry, 4Cardiff Institute of Tissue Engineering & Repair (CITER), School of Medicine, 5QBiotics Ltd.

Objectives Unlike oral mucosal wounds, dermal wounds are characterised by prominent scar formation. Excessive scarring (fibrosis) occurs due to aberrant wound healing or insult, during situations such as keloid/hypertrophic scarring, burns or trauma. Such situations pose significant challenges to Healthcare Services; confounded by acceptance that existing therapies are clinically unsatisfactory. The epoxy-tiglianes, EBC-46 and EBC-211, occur within seeds of the Fontain’s Blushwood Tree, indigenous to Queensland’s tropical rainforest. EBC-46 is currently being developed by QBiotics, as an anti-cancer drug. In clinical studies, EBC-46 stimulates exceptional dermal wound healing responses following tumour destruction, including minimal scarring. As TGF-β-driven, fibroblast-myofibroblast differentiation is pivotal to dermal scarring, fibroblasts and myofibroblasts represent viable targets for the anti-scarring properties of epoxy-tiglianes. Therefore, this study examined epoxy-tigliane effects on dermal fibroblast proliferation, migration and TGF-β-driven, myofibroblast differentiation.

Methods Human dermal fibroblasts were cultured with EBC-46 or EBC-211 (0-100μg/ml). Fibroblast proliferation and cell cycle analysis were analysed by MTT assay and Draq5/FACS. Migration was assessed by in vitro scratch wounds and Time-Lapse Microscopy. TGF-β-driven, fibroblast-myofibroblast differentiation was examined by the detection of α-smooth muscle actin (α-SMA) expression and stress fibres, by ICC and QRT-PCR.

Results Both EBC-46 and EBC-211 induced significant fibroblast cytotoxicity at 100μg/ml and retarded proliferation at 0.001-10μg/ml, but no significant effects on fibroblast migration were evident. Although EBC-46 had no effects on α-SMA expression, stress fibres and myofibroblast formation at 0.001-0.01μg/ml or 1-10μg/ml, EBC-46 significantly inhibited α-SMA expression and fibre formation at 0.1μg/ml, with cells retaining normal fibroblastic morphologies. EBC-211 induced similar inhibitory effects at 10μg/ml.

Conclusions These findings suggest that epoxy-tiglianes attenuate fibroblast proliferation and TGF-β-driven myofibroblast differentiation, explaining their enhanced anti-scarring responses in treated skin. Such findings highlight the potential of epoxy-tiglianes as novel therapeutics for excessive dermal scarring.

Notes
EFFECTIVENESS OF DEXAMETHASONE IN TREATMENT OF ORAL MUCOCELE: A CASE SERIES.
Maharjan, I. K.
B.P koiral instutue of Health Science

Objectives We evaluated the effectiveness of dexamethasone injections in treatment of oral mucocele.

Methods Nine patients with mucocele and one with ranula were treated with two having reoccurrence after surgery in our department. Diagnosing was made by on and off lesion, non progressing, history of trauma, dome-shaped, smooth surface, soft to firm consistency, non reducible and non pulsatile with mucus fluid on aspiration and sent for cytopathological examination. Eight were treated with intralesional and ranula was treated with combination of micromarsupialization and intralesional injection.

Results Intralional dexamethasone 4mg/ml 0.2 to 1 ml on weekly under topical anesthesia. We injected 2 to 4 sessions over 14 or 28 days, depending on the clinical response. Complete healing was seen in seven patients, without recurrences for a mean time of 8 months (range, 4-12 months) one patient choosed to under go surgery and one was lost to follow up after 1 month.

Conclusions Intralional dexamethasone can be used as an alternative to surgery in patients who are anxious about surgery procedure and in case of reccurence after surgery. The patient compliance is good, as the treatment is done under topical anesthesia specially in pediatric patient. The multiple follow up is one of the disadvantage.

Notes
Markers of disease activity and progression in Sjögren's syndrome

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Kings College

Objectives Sjögren’s syndrome (SS) is still far from being completely understood. The absence of early diagnostic markers contributes to delays in its diagnosis. Furthermore it is still difficult to identify who among them will develop lymphoma. The aim of the study is to identify salivary markers of disease activity and progression in Sjögren’s syndrome.

Methods Seventy six parotid saliva samples were collected from patients attending Oral Medicine clinics and were grouped as follows: SS (n=20), SS at risk of developing lymphoma (mucosa associated lymphoid tissue lymphoma) (MALT-L) (n=20), SS with confirmed MALT-L (n=18). Age, sex- matched healthy control samples were also collected (n=18). Twelve saliva samples (3 healthy controls, 3 SS, 3 SS at risk of MALT-L and 3 SS with MALT-L) were analysed using the proteome profiler-Human XL Cytokine Array (R&D Systems; Minneapolis MN).

Six of them (2 healthy controls, 2 SS and 2 SS at risk of MALT-L) were sent for proteomic analysis using SDSPAGE, trypsin digestion and LC-MS/MS.

Results Six proteins/cytokines (ST2, G-CSF, MIP-3α, TFF3, IL-24 and Vitamin D BP) were significantly different in the MALT-L risk group compared to healthy controls (independent t-test, df=4, p ≤0.05) with a fold change of at least 1.5 in 4/6. Both SS and SS with MALT-L did not show significant differences but some samples showed increased fold change especially the SS with confirmed MALT-L. Proteomic analysis of saliva revealed that a number of proteins (e.g. Annexin A1, Ig gamma-1 chain C region and Protein S100-A8) were overexpressed in SS compared to control and in SS at risk of MALT-L compared to SS and control.

Conclusions It is concluded that a number of potential protein biomarkers of SS and SS at risk of lymphoma are present in parotid saliva. These will be further characterized, analysed and validated on the larger groups of patients.

Notes
The Perception Of Aesthetic Outcomes Of Dental Implant Restoration
Roopra, M., Claydon, N. C., Binney, A., Farnell, D. J., Thomas, D. W., Adams, R.
Cardiff University

Objectives Dental implants present an increasing challenge in terms of matching outcome to patient expectations when undertaking implant treatment in the aesthetic zone. The aim of the project was to compare aesthetic perception between lay people and the dental profession.

Methods 31 individuals participated in the study (16 dental practitioners and 15 lay people). The 16 dental professionals were either hospital-based specialists (n=8) or family dental practitioners not practicing implant dentistry (n=8). Participants were asked to assess the cosmetic photographs of 20, completed single-tooth implant cases in the maxillary aesthetic zone. The cases were randomly sequenced and presented to each participant. Lay people were asked to judge overall cosmetic appearance (tooth shape, colour and “gum” appearance using a previously-established visual analogue score (VAS). Dentists additionally undertook a Pink Aesthetic Score (PES) and White Aesthetic Score (WES) for each case.

Results The aesthetic ratings of implant restorations differed significantly between lay people and dental professionals. Dental professionals were more critical than lay people for general appearance (p=0.0001), gum appearance (p=0.0228) and tooth shape (p=0.0057). There was no difference between lay people and dental professionals when assessing tooth colour (p=0.2471). Family dental practitioners were significantly more critical than hospital-based specialists in all assessed variables (p<0.01) except tooth colour (p=0.1259). Spearman’s correlation coefficient showed a high positive correlation between VAS and PES/WES for dental professionals.

Conclusions This study demonstrated that lay people are less critical of aesthetic outcome than dental professionals for all parameters except tooth colour. The correlation between VAS and PES/WES as judged by dental professionals confirms the utility of these scores in practice.

Notes
Epoxy-tiglianes Stimulate Keratinocyte Proliferative and Migratory Responses, Enhancing Wound Re-epithelialisation.

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¹Cardiff University, ²QIMR Berghofer Medical Research Institute, ³QBiotics Ltd.

Objectives The novel epoxy-tiglianes, EBC-46 and EBC-211, occur within seeds of the Fontain's Blushwood Tree (Fontainea picrosperma), indigenous to Queensland's tropical rainforest. EBC-46 is being developed by biotechnology company, QBiotics (www.qbiotics.com/), as an anti-cancer pharmaceutical. In clinical studies, EBC-46 stimulates exceptional dermal wound healing responses following tumour destruction, manifested as accelerated wound re-epithelialisation and closure; reminiscent of preferential healing in oral mucosal wounds. As little is known on how epoxy-tiglianes induce this response, this study examined their effects on epidermal keratinocyte wound healing responses and the underlying mechanisms of action.

Methods Immortalized human epidermal keratinocytes (HACATs) were cultured with EBC-46 or EBC-211 (0-100µg/ml). HACAT proliferation and cell cycle analysis were assessed by MTT assay and Draq5/FACS. Migration was assessed using in vitro scratch wounds and Time-Lapse Microscopy. Global gene expression analyses were performed by Microarrays (Human HT-12 v4 Expression BeadChips), with significant differences identified using GeneSpring, and Ingenuity Pathway Analysis elucidating the key pathways involved. Differentially expressed genes were confirmed by protein level analysis (Western blotting, ELISAs, activity assays).

Results Both EBC-46 and EBC-211 induced significant HACAT cytotoxicity at 100µg/ml, but stimulated significant HACAT proliferation at 0.001-10µg/ml. These epoxy-tiglianes also induced significant HACAT scratch wound closure at 0.001-0.1µg/ml (EBC-46) and 0.001-10µg/ml (EBC-211). Microarray analyses identified key genes differentially expressed in EBC-46 and EBC-211-treated HACATs, which contribute to the stimulatory effects on keratinocyte proliferation and migration. Up-regulated genes included certain keratins (KRT9, KRT13, KRT15, KRT81), positive cell cycle/proliferation regulatory genes (CCNB2, CDKN3, CDCA7, GINS2, KIAA0101); and proteinases (MMP-1, MMP-7, MMP-10). Other keratins were down-regulated (KRT6B, KRT16, KRT17) and many cytokine/growth factor/chemokine-related genes.

Conclusions These findings provide evidence to explain the enhanced re-epithelialisation responses in epoxy-tigliane-treated skin; and their potential as novel therapeutics for impaired dermal wound healing situations.

Notes
Hepatocyte Growth Factor Enhances Oral Mucosal Fibroblast Wound Healing Responses

Dally, J.1, Khan, J. S.1, Charalambous, C.1, John, H. L.1, Woods, E. L.2, Steadman, R.3, Midgley, A. C.4, Moseley, R.1

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Objectives Unlike dermal wounds, oral mucosal wounds are characterized by minimal inflammation, rapid healing/remodelling and reduced scarring. These responses are partly attributable to the ‘enhanced’ wound healing phenotype of oral mucosal fibroblasts (OMFs), characterised by increased migratory and proliferative capabilities; and resistance to transforming growth factor-β1 (TGF-β1)-driven, myofibroblast differentiation. Hepatocyte growth factor (HGF) is a pleiotropic growth factor, with key roles in wound healing and fibrosis. OMFs display increased HGF expression, compared to dermal fibroblasts, suggesting HGF involvement in mediating preferential OMF wound healing responses. Therefore, this study investigated the role of enhanced HGF expression in promoting these wound healing responses in OMFs; and the influence of pro-fibrotic growth factor, TGF-β1, on these responses.

Methods Human OMFs were isolated from adult buccal mucosa. HGF production by OMFs was analysed with/without TGF-β1 (10ng/ml) supplementation within in vitro scratch wounds, by RT-QPCR and ELISA. OMF migration and proliferation were assessed by time-lapse microscopy and Alamar blue, respectively. OMFs were transfected with short-interfering RNA-targeting HGF (siHGF) to knockdown HGF expression, confirmed by RT-QPCR and ELISA. siHGF effects on OMF migration and proliferation were assessed by time-lapse microscopy and MTT assay. siHGF influence on OMF resistance to TGF-β1-dependent differentiation was assessed via α-smooth muscle actin (α-SMA) expression and stress fibre formation, using RT-QPCR and immunocytochemistry, respectively.

Results TGF-β1 treatment significantly inhibited OMF migratory and proliferative capabilities, concomitant with significantly down-regulated HGF expression/protein levels. Stable knockdown of HGF expression by siHGF, was confirmed by RT-QPCR and ELISA. siHGF transfection also significantly inhibited OMF migratory and proliferative capabilities; and further impaired OMF resistance to TGF-β1-driven, myofibroblast differentiation, evident by increased detection of α-SMA expression and stress fibre formation, versus controls.

Conclusions This study demonstrates the key role HGF possesses in mediating the ‘enhanced’ wound healing properties of OMFs; and the interplay between TGF-β1 and HGF in regulating these responses.

Notes
Randomised Clinical Trial of Biotène® Oral Rinse and Spray Efficacy

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Objectives To compare efficacy of an oral rinse/mouth spray combination versus water using the Product Performance Attributes Questionnaire (PPAQ) after 7 and 28 days, examine quality of life (QoL) measures and monitor oral adverse events (AEs).

Methods This was a single centre, examiner blind, stratified, randomised, parallel group study in healthy adults with dry mouth. Subjects received biotène® Dry Mouth Oral Rinse plus biotène® Moisturising Mouth Spray ('Test') or water. Test group rinsed with 15 mL oral rinse for 30 seconds after brushing and up to a further 3x/day and used the spray 2–5x/day. Water group sipped as required. Subjects rated their product for each PPAQ question from 1–5 (Poor–Excellent) and QoL questions from 0–4 (not at all–very much).

Results Of 172 randomised subjects, 165 completed the study (Test = 81; Water = 84). Primary efficacy, response to PPAQ Question 1 (“Relieving the discomfort of dry mouth”), significantly favoured the Test group (p<0.0001) at Days 7 (mean rating scores 3.2 for Test, 2.4 for Water) and 28 (3.4, 2.5 respectively). At Day 28, 78.3% of Test and 35.7% of Water subjects rated the products ‘Good’–’Excellent’ for Q1. All 17 other questions showed significance in favour of the Test group at both timepoints (p<0.05). QoL significantly improved from baseline for both groups on all questions (p<0.05); Test group showed significant improvements over Water for Q1 “Has your dry mouth caused discomfort?”.

Of 31 treatment-emergent oral AEs (Test = 11, Water = 20), four were treatment-related, three in the Test group, one in the Water group; none discontinued treatment. The Test group had one fatal serious AE and one non-fatal AE, both non-oral, non-treatment-related.

Conclusions Mouthrinse plus mouth spray produced significantly better relief than water for all PPAQ questions after 7 and 28 days. Study funded by GSK Consumer Healthcare.

Notes
Extracellular vesicle mediated signalling in oral cancer progression
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University of Sheffield

Objectives Oral cancer includes cancers of the throat, tongue and mouth with 6767 new cases and 2119 deaths per year; worryingly mortality rates are slowly on the rise. Efforts to improve survival are hampered by a limited understanding of the underlying molecular complexity of the disease. The objectives of this study are to identify the role extracellular vesicle (EVs) mediated signalling plays in oral cancer development. EVs are nanometre sized vesicles with a range of bioactive contents released by cells, but released in higher numbers by cancer cells. Since the early 2000's these vesicles have been thought of as a new signalling system with a vital role in driving cancer progression. Developing tumours exist as a complex milieu comprising multiple cell types, which require complex cross talk as the tumour develops. Using fluorescent staining techniques this study will identify potential vesicle mediated interactions between the different cell types of the oral cancer microenvironment.

Methods EVs were extracted from the culture media of oral cancer cell lines using ultracentrifugation and then characterized using transmission electron microscopy, western blotting and tuneable resistive pulse sensing. EVs were labelled with fluorescent markers and transferred to cells of a different line to visualise transfer of RNA.

Results We have successfully isolated and characterised EVs from a panel of cell lines representative of the stages of oral cancer development and confirmed their presence by western blot and transmission electron microscopy. Using fluorescently labelled EVs the horizontal transfer of RNA between oral cancer cells and stromal cells that would be present in the tumour microenvironment has been visualized.

Conclusions Using a combination of techniques the beginnings of an EV mediated signalling network in the oral cancer microenvironment has been revealed. Future work will expand on this by focusing on the RNA and protein cargo of the isolated EVs in order to identify their roles in oral cancer progression.

Notes
Fibroblast ‘activation’ by media flow in cells of skin and oral origin.
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University of Sheffield

Objectives Fibroblasts can trans-differentiate into a specific type of ‘activated’ cell in wound healing and tumour stroma resulting in enhanced expression of markers such as alpha smooth muscle actin (αSMA) and collagen I (COL1A1). Transforming growth factor-β1 (TGF-β1) stimulates this process, although other physical signals may also play a role. The aim of this study was to investigate effect of medium movement in the Quasi-vivo® bioreactor (Kirkstall Ltd.) on the activation fibroblasts of oral and dermal origin.

Methods Normal oral (NOF) and dermal (NHDF) fibroblasts were plated on collagen coated Thermonox cover slips, transferred into the bioreactors and subjected to flow (150 μl/min) for 24 h. Expression of α-SMA and COL1A1 was assessed by qPCR and microarray gene expression analysis was performed using RNA harvested from the cells. α-SMA and phospho-SMAD3 protein expression was studied using immunofluorescence.

Results Media flow altered gene expression in oral and skin fibroblasts with increased expression of α-SMA by 5.2 fold (NHDF’s) and 2.6 fold (NOF’s) and COL1A1 by 2.9 fold (NHDF’s) and 3.2 fold (NOF’s) when compared to cells in static culture. TGF-β1 stimulation in the presence of flow decreased expression of these markers. In dermal fibroblasts, flow stimulated α-SMA protein expression and translocation of phosphorylated SMAD3 to the cell nucleus. Gene array analysis revealed significant variations in the expression of 54,700 genes functionally associated with blood vessel development, angiogenesis and activation of the TGF-β1 pathway upon exposure to flow.

Conclusions Media flow ‘activates’ both oral and skin fibroblasts to a similar extent as TGF-β1; treatment with both flow and TGF-β1 reduces expression of activation markers. It is possible that TGF-β1 stimulation of fibroblasts under flow leads to receptor internalization or competition of SMAD intracellular signaling pathways resulting in decreased expression of activation markers. Even the modest physical forces associated with the medium flow rates used in these experiments has a dramatic effect on fibroblast phenotype and genotype.

Notes
Activin A controls angiogenesis and influences oral squamous cell carcinoma development
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Objectives Angiogenesis is essential for tumor development, as solid tumors must develop ways to induce angiogenesis in order to continue progression and expansion. Previous studies have shown that activin A, a member of the TGF-ß superfamily, controls important events related to development and progression of oral squamous cell carcinomas (OSCC), but its effects on angiogenesis are unknown. Here we examine the role of activin A in oral tumor angiogenesis.

Methods The effect of activin A on human umbilical vein endothelial cells (HUVECs) tubulogenesis was investigated using matrigel assays, cellular proliferation was assessed by BrdU incorporation index, cellular apoptosis was analysed by flow cytometry and cellular migration was investigated using transwell assays. The influence of endogenous activin A was evaluated in HUVEC cells stably expressing shRNA targeting activin A (shINHBA). The effect of activin A released from OSCC cells on HUVECs was assessed using conditioned medium collected from OSCC shINHBA cells. We also investigated the effects of activin A on oral tumor growth in vivo using a mouse xenograft model.

Results We demonstrate increased tubulogenesis activity concomitantly with high proliferation in activin A-treated HUVECs; however, it did not alter the rates of apoptosis or cell migration. Conversely, follistatin, an activin A antagonist, and activin A knock down in HUVECs significantly inhibited proliferation, induced apoptosis, cell migration and decreases tube formation. Similarly, conditioned media harvested from control shScramble OSCC cells increased the proliferative rate and the tubulogenic activity, whereas the ability of proliferation and tube formation of HUVECs was significantly decreased in tumor conditioned media of shINHBA OSCC cells. In vivo, tumors incorporating shINHBA HUVECs were significantly smaller than tumors incorporating shScramble HUVECs.

Conclusions These results presented here highlight a role for activin A in oral tumor angiogenesis, suggesting that activin A signaling could be an important target for tumor vascular disruption in oral carcinomas. Financial support: FAPESP.

Notes
The Role of Oral Cancer-associated Fibroblasts in Promoting Extracapsular Spread.
Pilborough, A., Lambert, D., Farthing, P., Khurram, A.
University of Sheffield

Objectives Oral squamous cell carcinomas (OSCC) are a significant cause of morbidity and mortality worldwide, and account for the majority of head and neck cancers. The prognosis of patients with extracapsular spread (ECS) of metastatic tumour from the lymph nodes into the neck, is particularly poor. However the factors affecting this process are poorly understood and detection is difficult pre-surgery. Work on the primary tumour has highlighted the importance of cancer-associated fibroblasts in promoting tumour growth, invasion and metastasis. However, relatively little is known about their role in extracapsular spread. The objective of this work is to investigate the role of fibroblasts in altering cancer cell behaviour in the lymph node and to determine if this influences ECS.

Methods ECS-positive and -negative tumour sections were examined for markers of epithelial-mesenchymal transition (EMT), microvessel density and proliferation (Ki67) using immunohistochemistry. In addition, indirect co-culture experiments were carried out with oral fibroblasts and a range of oral cancer cell lines derived from OSCC and lymph node metastasis. The effects on cancer cell behaviour were analysed using western blotting and qRT-PCR for expression of EMT markers (Slug, Snail, Twist, ZEB).

Results ECS-positive tumour sections showed significantly higher microvessel density, Ki67 staining (p<0.05) and altered EMT marker expression. Oral fibroblast conditioned media was found to affect the expression of EMT markers in a range of oral cancer cell lines (fold increase compared to control by qRT-PCR: 0.96-6.3 (Slug); 1.4-3.1 (Snail); 0.6-1.5 (Twist); 0.6-1.7 (ZEB)).

Conclusions This work begins to elucidate the importance of fibroblasts in ECS. With further research it is hoped that this may lead to improved detection and treatment strategies.

Notes
Goblet cell mucin and the pathogenesis of salivary gland ductal stricture


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Objectives The aim of the study was to investigate whether ductal cells in the affected gland undergo transdifferentiation into metaplastic and hyperplastic cells which may eventually lead to stricture formation.

Methods Mucoid plugs were collected from the Stenson’s duct of the affected gland of patients and analyzed for the presence of mucins. An in-vivo experiment was conducted which involved the introduction of the bacterial mimic lipopolysaccharide (LPS) into the parotid duct followed by assessment of metaplastic and hyperplastic changes by the ductal cells. LPS induced signaling was examined in a human parotid duct (HSY) cell line. Real time PCR and western blots were performed to demonstrate if there is an increased expression of TLR4 on ductal epithelial cells in response to bacteria (LPS).

Results The parotid gland does not secrete mucin yet stricture formation is associated with the presence of ‘mucoid plugs’. We collected mucoids plugs from patients with stricture and identified the presence of mucins (MUC5AC, MUC5B, MUC2, and MUC1) using SDS PAGE/ proteomics analysis and western blotting. Thus, under pathological conditions the parotid gland expresses aggregates (mucoid plugs) containing mucin and proteins associated with defense and inflammation.

An in-vivo experimental model was developed in which lipopolysaccharide (LPS) was instilled into the parotid ducts of rats. After 7 days tissue was removed, fixed and examined morphologically. Inflammatory cell infiltrates in the main parotid duct were associated with increased expression of TLR4 and the novel appearance of MUC5AC containing goblet cells in the ductal epithelium.

LPS induced signaling was examined in a human parotid duct (HSY) cell line. Real time PCR and western blots results demonstrated an increased expression of TLR4 on ductal epithelial cells in response to bacteria (LPS).

Conclusions In conclusion, it appears that goblet cell metaplasia and aberrant mucin production occurs in the main duct of the parotid gland in response to infection. The formation of plugs of mucous in the duct and persistence of infection may be a causative factor in stricture and obstructive disease.

Notes
Use of 3D organotypic models of oral squamous cell carcinoma to examine the role of tumour-associated macrophages in tumour progression
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¹The University of Sheffield, ²The University of Sheffield, ³The University of Sheffield

Objectives Tumour-associated macrophages (TAM) represent a prominent component of the leukocytic infiltrate of human tumours and their accumulation in oral squamous cell carcinoma (OSCC) has been shown to be a predictor of poor prognosis. Recent evidence suggests that the tumour microenvironment drives TAM into a pro-tumour phenotype that exacerbates tumour growth and metastasis but evidence for this in OSCC is lacking. In this study we further develop a previously generated model of OSCC to incorporate human macrophages in order to examine their phenotype and the molecular mechanisms of TAM in oral cancer progression.

Methods Human monocyte-derived macrophages with induced M1 and M2 phenotypes were cultured with normal and cancer-associated oral fibroblasts within a type 1 collagen hydrogel. OSCC cell lines were seeded on top and cultured at an air-to-liquid interface to form a multi-layered epithelium. After 14 days, macrophages were retrieved from the connective tissue by collagenase digestion, and their viability and the expression of a number of macrophage differentiation and polarisation markers were assessed using polychromatic flow cytometry and quantitative PCR. The effects of macrophages on OSCC invasion into the connective tissue was analysed using immunohistochemistry.

Results Macrophages were viable after prolonged culture within OSCC models and showed differences in the expression of differentiation and polarisation markers, and changes in immunohistochemical staining in an induced tumour microenvironment, compared to a malignancy-free environment.

Conclusions 3D in vitro models of OSCC show considerable promise for delineating the molecular mechanisms of TAM-induced tumour progression in OSCC.

Notes
The Chemokine Lymphotactin Increases Adhesion and Regulates Expression of Its Receptor In Oral Cancer Cells
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Objectives To determine if Ltn acts on OSCC cells in an autocrine manner and whether adhesion to extracellular matrix (ECM) components collagen I and collagen IV is increased after Ltn exposure. Oral squamous cell carcinoma (OSCC) has a poor prognosis which further worsens after metastasis to cervical lymph nodes. Recent studies show a role for chemokines and their receptor in cancer growth and metastasis. Expression of the chemokine lymphotactin (Ltn) and its receptor XCR1 has been shown in OSCC facilitating cell migration, proliferation and invasion with a potential role in metastasis. However the precise role and mechanism of Ltn/XCR1 interaction in the context of OSCC remains poorly understood.

Methods Oral cancer cell lines (OCCL) and a dysplastic cell line were incubated with 100 ng/mL of Ltn for 24 hours. XCR1 expression was determined using qRT-PCR and flow cytometry. Adhesion to collagen I and IV with and without Ltn stimulation was also studied using a range of concentrations (1-10 µg/mL).

Results Up-regulation of XCR1 expression at both mRNA (~75 fold) and protein level was seen in H357 OCCL after Ltn exposure. FNB6 (dysplastic) showed little increase in mRNA (~3 fold) and protein level. OCCL showed significantly higher attachment to collagen I and IV compared to controls. This attachment was further increased after Ltn stimulation in both H357 (p<0.05) and SCC4 (p<0.001). Attachment of SCC4 (p<0.0001) to collagen I was significantly higher than H357 (p<0.001). The difference between collagen I and IV adhesion was significant for SCC4 (p<0.001) and H357 (p<0.001).

Conclusions The results show that exposure to Ltn can increase XCR1 expression in cancerous but not dysplastic cells suggesting that it can act in an autocrine manner in OSCC. Increased adhesion to collagen I and IV suggests a potential role for Ltn in migration and invasion in OSCC.

Notes
Functional expression of scavenger receptors by oral keratinocytes
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Objectives Objective: Scavenger receptors (SR) constitute a large family of receptors that primarily bind lipid-containing molecules. Until recently their expression has been observed on macrophages and endothelial cells where their main role is binding bacterial molecules or lipoproteins. The aim of this study was to determine if normal oral keratinocytes (NOK), dysplastic or oral cancer cell lines express SR and if activation of these affects keratinocyte cell biology.

Methods Methods: A panel of NOK, dysplastic, metastatic and cancerous oral keratinocytes from various oral sites were tested for the mRNA and protein expression of scavenger receptor family; SR-A1, SR-A6, SR-B1, SR-B1.1 and SR-I1 by qPCR, immunoblotting, flow cytometry and immunofluorescence staining. Keratinocytes expressing SR were examined for their ability to internalize oxidized (ox) and acylated (ac) low-density lipoproteins (LDL) and their functional response to these ligands measured.

Results Results: SR-B1 and its sliced variant SR-B1.1 were expressed at the mRNA and protein level by all oral keratinocytes cells tested. The expression of other SR members was differentially expressed by keratinocytes with the oral cancer cell line SCC4 overexpressing many SR family members. Some SR family members were expressed at higher levels by oral cancer cells than normal oral keratinocytes. None of the keratinocytes tested expressed SR-A1 and this receptor is macrophage-specific. SR-positive keratinocytes bound and internalized ox-LDL and ac-LDL in a time and dose-dependent manner initiating intracellular signaling mechanisms.

Conclusions Conclusion: Oral keratinocytes express functional scavenger receptors indicating that these receptors may play an important role in oral keratinocyte biology.

Notes
Preliminary results of a randomised controlled trial comparing regenerative endodontics versus root end closure with MTA in the management of non-vital, immature, permanent incisors

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Objectives Traditionally, clinicians carried out apexification with calcium hydroxide. More recently, mineral trioxide aggregate (MTA) has established a recognised role in the management of the open apex. Reports of the possible benefits of a more novel, conservative approach of regenerative endodontics has emerged. Each of these interventions may have a place in the management of non-vital immature incisors, yet each also has its limitations. This trial seeks to compare two interventions and help guide practice. The objective is to compare the outcomes of regenerative endodontics versus root end closure with MTA in the management of non-vital, immature, permanent incisors.

Methods Ethical approval was granted by the local Research Ethics Committee. 30 participants aged between 7 and 19 years, who had at least one traumatised non-vital maxillary central incisor with incomplete root development, were recruited to the study at a single centre, following referral to the Paediatric Dentistry Department of a UK teaching hospital. Participants were randomly assigned into intervention groups for either regenerative endodontics or MTA root end closure via computerised sequence generation and allocation concealment with opaque envelopes. Follow-up took place at 3, 6, 9 and 12 months. This abstract presents the clinical outcome data for 21 patients who have completed a minimum of 6 months follow-up to date.

Results The mean age of participants was 10 years and 70% were male. At presentation, 48% complained of pain/tenderness to percussion, 38% exhibited infection and 14% were asymptomatic. 24% of children complained of tooth discoloration prior to treatment. 11 teeth have been treated via regenerative endodontics and 10 teeth via root end closure with MTA. Following treatment, 100% of patients were asymptomatic with no clinical signs of infection / failure. Two teeth (18%) managed via regenerative endodontics produced a positive response to sensibility testing. Deterioration in tooth shade (recorded with standardised clinical photography and a digital shade matching system) was recorded for all teeth in both groups.

Conclusions There was no difference in clinical success between the intervention groups. Both interventions were largely acceptable to participants despite their limited dental experience.

Notes
Marginal adaptation of CAD/CAM nanoceramic composite laminate veneers using two preparation designs
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Objectives The study was performed to assess the marginal adaptation of CAD/CAM laminate veneers constructed from:
- Nanoceramic composite
- Leucite reinforced ceramic
Using two proximal preparation designs:
- Incisal butt joint with slice preparation
- Incisal butt joint with no slice preparation

Methods Twenty natural central incisors were selected to receive a standard veneer preparation with difference only proximally. Ten were constructed to receive nanoceramic composite material (Lava Ultimate 3M) and the other ten received the conventional leucite reinforced ceramic (IPS Empress CAD). Within each group five were prepared with a standard preparation where the preparation was confined to the labial surface with no proximal extension. The other five had their proximal contact sliced so that veneer preparation ended just lingual to the contact. Twenty CAD/CAM milled veneers were constructed to fit on the corresponding scanned teeth for each proximal preparation, using inLab 3D software (V3.8). Using a stereomicroscope with a digital camera, the vertical marginal gaps were measured for the four different margins of each veneer. The internal surfaces of the veneers were sandblasted, and the internal surfaces of the leucite reinforced veneers were etched using hydrofluoric acid gel and then were silanized by a primer before application of resin cement. However, no etching was done for the nanoceramic composite group. The veneers were seated, during cementation, utilizing static finger pressure. The mean vertical gap (in microns) for each specimen was then calculated and saved in an Excel sheet for statistical analysis.

Results The results showed significantly better marginal gap values for the Lava Ultimate group than the IPS Empress CAD group, with no significant effect of preparation design on the marginal adaptation.

Conclusions Within the limitations of this study, the following conclusions could be drawn:
1- Nanoceramic reinforced composite showed improved marginal adaptation when compared to the conventional leucite reinforced ceramic.
2- The proximal slice preparation did not affect the marginal adaptation of either materials
3- For the two tested materials, the marginal vertical gap readings recorded in this study were within the limits of clinically acceptable standards (50-120um)

Notes
Effects of 1% Micro-silicone Dioxide Filler on Soft Liner Mechanical Properties

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Objectives Denture lining material has a key role in modern prosthodontics due to restoring health of inflamed and distorted mucosa. The soft lining ability to resist plastic deformation under load is of practical importance. Therefore, this study aims to investigate the effect of micro-silicone dioxide filler on the surface hardness and transverse strength of soft denture liner material.

Methods Samples (n=10) were prepared for surface hardness test according to ANSI/ADA Specification (No.12); and transverse strength test ADA (No.57&12). Specimens prepared using old denture mould technique. The respectable thickness of old hard resin denture was 2mm, while the relining layer was 1mm in thickness (Vertex-Germany). 1% micro-silicone dioxide particles (USA) of 0.01-0.04µm by weight were added to the reline powder and settled homogenously by mixing together for 1min. Then, the special liquid added according to the manufacturer and mix for 30sec. The curing process was carried out using water-bath technique starting at room temperature to set up at 70˚C for 90min, and then at 100˚C for 30min. Sample left to cool at room temperature, finished, polished and stored in distilled water (48h). Surface hardness specimens tested using (Shore A, Italy) tester device with indenter cone diameter of 0.79mm. While the transverse specimens tested using Universal Instron machine (Germany) with cross-head speed of 1mm/min.

Results There was statistical significant difference in the mechanical properties of the soft denture liner with micro-silicone dioxide particles (P<0.05). Denture soft liner with 1% micro-silicone dioxide particles had surface hardness of 79(±2) Shore A, while that liner without filler had 64(±1) Shore A. The transverse strength of lining resin has been improved from 43(±5)N/mm$^2$ without filler to 75(±3)N/mm$^2$ with 1% micro-silicone dioxide particles.

Conclusions 1% of micro-silicone dioxide particles as reline filler could improve the mechanical properties of soft denture liner material giving a promise to superior resist to plastic deformation under load.

Notes
Factors affecting OHRQoL with removable partial dentures: a retrospective cohort study

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Objectives

Introduction Removable partial dentures (RPDs) are used for restoration of missing teeth in partially dentate patients. Previous studies have investigated patient satisfaction and continued use of RPDs as an outcome for success. These studies have highlighted that the replacement of anterior teeth and the number of teeth being replaced are associated with greater success. A detailed search and review of the literature has not revealed any studies that have investigated the impact of particular independent variables such as framework material OHRQoL measures.

Aim Investigate whether framework material, Kennedy classification, number of missing teeth and patient age are important to success with RPDs as measured by a validated OHRQoL outcome.

Methods Power calculation based on minimally important difference for OHIP-20 indicated a sample size of 64 patients.

Questionnaires, which included the OHIP-20, were posted to 95 patients who received RPDs at the Charles Clifford Dental Hospital-Sheffield during a prior 8-month period. Comparison of OHIP scores between groups was done using non-parametric tests as OHIP-20 results were non-normally distributed. Kruskal Wallis ANOVA and Mann-Whitney u tests were used to evaluate between group differences.

Correlation was explored with Spearman’s rank (Age/Missing teeth vs OHIP-20).

Results 57% (n=60) questionnaires returned, accounting for 88 RPDs: 64% (n=56) combined upper and lower, 24% (n=21) upper only, 12% (n=11) lower only. 89% (n=78) were still being worn. Median OHIP-20 was lower for acrylic RPDs ($M$ = 17) than for chrome dentures ($M$ = 24), and lower still in patients wearing chrome and an acrylic RPD ($M$ = 9). Differences were not statistically significant (p=0.428). Outcomes based on Kennedy classification, and correlations of OHIP-20 with number of teeth missing and age are presented.

Conclusions Results indicate that it is too simplistic to conclude continued use of RPDs constitutes success. Further research is required to explore the construct of OHRQoL with patients wearing RPDs.

Notes
Comparative Evaluation of Tricalcium Silicate, Mineral Trioxide Aggregate and Calcium Hydroxide as Dental Pulp Capping Materials: An In-Vivo Study
Agrawal, N.
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Objectives □ Clinical and radiographic assessment of reparative dentin formation using Tricalcium Silicate (Biodentine), MTA and Ca(OH)₂ as Pulp Capping Agents.

Methods 114 teeth with deep dentinal caries involving inner third of the dentine with no symptoms of irreversible pulpitis were treated randomly with one of the pulp capping agents and intermediate restorative material Fuji IX GIC. They were assessed in 2nd and 6th month, if successfully treated underwent final restoration with composite/amalgam.

Radiographic success criteria: (1) The contours width and structure of the periodontal margin within normal limits; (2) No signs of pathological tooth resorption.

Clinical success criteria : No history of spontaneous, persistent pain or sensitivity to palpation/percussion and/or presence of clinical signs (abscess, sinus tract, and abnormal mobility).

Results Three Biodentine pulp-capped teeth and 1 MTA pulp-capped teeth were recommended for either extraction or root canal therapy; Ca(OH)₂ pulp-capped teeth showed no failure. The overall analysis indicated few failure in the Biodentine group (7.9%; n=3) and MTA group (2.6%; n=1) than in the Ca(OH)₂ group (0%; n=0). The failure was seen in the 1st week itself due to the poor choice of cases and an inch forward taken to treat cases with features of irreversible pulpitis. The results were unfavourable for cases with feature of irreversible pulpitis. The 4 failed cases underwent root canal treatment and were included in the analysis as failure. However, on comparison between Biodentine and MTA using Chi Square Fisher’s Exact Test (=0.615), it revealed no significant difference between the group proving either of the material can be safely used for indirect pulp-capping procedure.

Conclusions This study conducted for up to 6 months provided confirmatory evidence for a performance with Biodentine as an indirect pulp-capping agent as compared with MTA and Ca(OH)₂. However, long term study is needed in future.

Notes
A Randomised Clinical Study of the Measurement of Xerostomia Relief
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¹GLAXOSMITHKLINE CONSUMER HEALTHCARE, ²Hill Top Research

Objectives To assess test-retest reliability and construct validity of an 11-point Visual Rating Scale (VRS); to determine test product efficacy versus water using the VRS on questions regarding moisturisation, lubrication and dry mouth relief; to compare efficacy using a 5-point Product Performance and Attributes Questionnaire (PPAQ) and the VRS.

Methods This was a single centre, examiner-blind, four treatment arm, stratified, randomised, parallel group study. Subjects (35–84 years) with self-reported dry mouth used their assigned treatment (experimental OralBalance gel ['Gel'], biotène® Dry Mouth Oral Rinse ['Rinse'], biotène® Moisturising Mouth Spray ['Spray'] or tap water ['Water'] in a 2:2:2:1 randomisation) then completed the VRS immediately and at 30, 60 (plus modified PPAQ), 90 and 120 minutes on Visit 1 and again on Visit 2, 3 days later.

Results Of 175 subjects randomised, 170 completed the study. Intra-class correlation values (Visits 1 and 2 test-retest reliability) for moisturisation, lubrication and dry mouth relief questions were 0.695, 0.700 and 0.677. Responses to the VRS-rated questions were not wholly consistent across visits but with occasional significant differences (p<0.05) noted for each of the dry mouth products compared to Water. Overall, Gel scores were significantly higher than Water at more timepoints than the other treatments, notably for moisture and lubrication questions. Modified PPAQ analysis demonstrated lower sensitivity to detect treatment differences but less inconsistency between days than VRS. Construct validity of the VRS-rated questions assessed against the modified PPAQ question at 60 minutes demonstrated correlation values between 0.713 and 0.792. Two subjects (both Rinse) reported three treatment-related oral adverse events.

Conclusions While OralBalance gel performed best overall, these data should be interpreted cautiously as test-retest reliability of the VRS was borderline of what is considered a consistent and reliable measurement scale. Construct validity of the VRS scale (compared to PPAQ) was acceptable. Study was funded by GSK Consumer Healthcare.

Notes
Effects of ductal ligation/de-ligation on mTOR pathway and morphology of salispheres

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Objectives Cultured salivary gland stem/progenitor cells form spherical, floating clusters in vitro named as salispheres. Salispheres usually develop around day two to day three of culture and express different stem cell markers such as; c-kit. Ductal ligation of submandibular gland (SMG) induces atrophy where by cells undergo apoptosis and fibrosis while de-ligation results in the recovery of the gland. The mammalian target of rapamycin (mTOR) is one of the signaling pathways that plays a role in cell growth and proliferation. It consists of two main complexes; mTORC1 and mTORC2. The activation of mTORC1 leads to the phosphorylation of the two main substrates; 4e-bp1 and S6K. Since mTOR is active during healthy salispheres development, the aim of this study was to measure mTOR activity in ligated/de-ligated models and evaluate the morphological changes in salispheres.

Methods The SMG of female, ICR mice were used in this study. Duct ligation of SMG was performed for 7 days whereas de-ligation was processed for additional 7 days. All glands were collected and digested with collagenase II and hyaluronidase, washed, filtered and plated in 12-wells dish. Salispheres were then imaged by the phase-contrast microscopy and collected for analysis using western blot and immunofluorescence.

Results Results showed that number of salispheres was significantly lower in ligated glands when compared to un-operated glands and to de-ligated glands. In addition, salispheres from ligated glands showed mTOR activity and both ligation and de-ligation cause morphological changes to salispheres. These results suggest that the morphological changes caused by ligation/de-ligation are not mTOR related but instead Rho-associated protein kinase (ROCK) maybe a reason for these morphological changes.

Conclusions In conclusion, stem/progenitor cells from atrophic/regenerated glands are in a different state compared to stem/progenitor cells from normal tissues.

Notes
Reasons for placement and replacement of restorations: an updated review
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Objectives To investigate the reasons for placement and replacement of restorations from published literature.

Methods This study is an update on the previous review carried out by Deligeorgi et al. (2001). The literature was reviewed to identify published studies based on the protocol of Mjor (1981) which investigated the reasons for placement and replacement of restorations. These studies were identified using searches of electronic databases, as well as hand-searching of the literature and reference lists.

Results The 2001 review included ten papers, detailing the placement and replacement of 32,777 restorations, of which 56% (18,229) were replacements and 44% (14,548) were initial placements. Twelve additional papers were identified as being published since the time of the 2001 review. The new studies considered 54,023 further restorations. Of these, 42% (22,625) were initial placements and 58% (31,398) replacements. Within the newer papers, primary caries was the most common reason for initial restoration placement (86%), and secondary caries was the most common reason for the replacement of restorations (ranging from 29% to 59% for resin composite and 29% to 57% for amalgam). The second most common reason for the replacement of resin composite was bulk/marginal discoloration (12-59%) followed by bulk/marginal fracture (9-37%). For amalgam restorations, this was bulk/marginal fracture (12-29%) followed by tooth fracture (8-24%). Other reasons included pain, sensitivity, lost restoration, poor anatomic form, aesthetics and root canal treatment. The use of amalgam as a restorative material has decreased from 57% (18,552/32,697) in the initial review to 31% (16,815/54,023) in the additional papers. On the other hand, the use of resin composite increased from 37% (11,983/32,697) to 48% (26,102/54,023).

Conclusions In the years subsequent to the initial review, replacement of restorations still accounts for more than half of restorations placed by dentists, and the proportion of replacement restorations continues to increase. Trends towards the increased use of resin composites was noted in recent years. Further research is required in this area to investigate changes in the approaches to the restoration of teeth, especially with increased understanding of the concept of restoration repair as an alternate to replacement.

Notes
Exploratory Study Assessing Oral Health Knowledge Of Teachers In India.
PATIL, V.1, WESTON-PRICE, S. F.1, McNulty, C.2, HOEKSTRA, B.2, pine, C.1
1QMUL BARTS AND THE LONDON SCHOOL OF MEDICINE AND DENTISTRY, 2PUBLIC HEALTH ENGLAND

Objectives This study aimed to explore baseline oral health knowledge of primary school teachers in an urban area of Maharashtra state, India. The objective was to obtain initial data examining suitability of a pre-existing oral health education resource for primary school teachers, created for the Public Health England website www.e-bug.eu.

Methods Science teachers to 7-11 year olds, from a convenience sample of four schools in Nagpur district, Maharashtra were invited to complete a validated oral health knowledge questionnaire.

Results All 32 teachers who met the inclusion criteria agreed to participate. Of those who completed questionnaires 81% were aware that bacteria and sugar caused tooth decay yet 72% did not think avoiding sugar would prevent decay, with 75% favouring tooth cleaning and rinsing with water as methods of preventing decay. 84% identified plaque bacteria as a cause for bleeding gums but only half highlighted tooth brushing as prevention. 97% rated the teeth of children in their class as fair or above with 91% judging their pupils required some dental treatment. All stated schoolteachers should teach children how to take care of their teeth, with 94% considering they have sufficient knowledge to do this.

Conclusions There was a good awareness of the aetiological causes of dental disease yet correct preventive behaviors were not elucidated, indicating a deficit in oral health knowledge. Due to the limited sample these results should be viewed with caution they do however support data from Petersen et al, (WHO). Using the same questionnaire format in an urban area of central China they found only half of teachers had a high knowledge of oral health rising to 94% following oral health education workshops. Recommendation: From this study, it appears advisable that a short fact-based module should be provided for teachers before they go on to teach oral health care to primary school children.

Notes
Systematic review of the effectiveness of interventions using a component of habit formation theory to improve the uptake of preventive healthcare in adults

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Objectives Low socio-economic status (SES) groups are less likely to attend for dental check-ups and this is recognised as contributing to inequalities in health. Habit formation theory suggests that behaviour can become automatic (i.e. habitual), requiring little or no cognitive processing, if it is repeated in a stable environment and initiated by an environmental cue. Initiating habit may be a mechanism particularly appropriate for increasing the preventive use of services for low SES groups. We aimed to undertake a systematic review to assess the effectiveness of interventions informed by habit formation theory at improving the uptake of adult preventive healthcare.

Methods An electronic search was developed using key papers. Interventions which contained a component of habit formation theory, designed to influence the utilisation of adult preventive healthcare were included. Studies were limited to RCTs, quasi-RCTs, pilot studies, feasibility studies, controlled randomised trials, cluster randomised trials and English language.

Results Electronic searching alongside backward and forward citations identified 11888 titles and abstracts. 23 full papers were screened, six of which met full inclusion criteria. Four included papers involved cancer screening, the remaining two targeted vaccinations. All six interventions incorporated an implementation-intention component to the intervention, with five showing a significantly positive increase in preventive health service usage whilst one did not. Of particular note, an intervention delivered to low SES participants showed a positive increase in uptake of colorectal cancer screening compared to an educational intervention.

Conclusions Interventions which incorporate a component of habit formation theory are rare and limited to one methodology. However, those that do exist appear promising in relation to increasing preventive adult healthcare utilisation. More work is required to explore the effect of habit formation interventions on individuals within a low SES, and how this type of intervention could be translated into increasing attendance for dental check-ups.

Notes
Dental trauma in contact sports: Promoting prevention and emergency management
Dickie, J.¹, Cross, L.²

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Objectives
Promote wear of mouthguards amongst players of contact sports. Raise awareness of advantages and disadvantages of different mouthguard styles. Raise awareness of risks of not wearing mouthguards whilst participating in sport. Promote a Smart Phone App providing guidance on initial management of dental injury.

Methods
Online questionnaires relating to dental trauma experience, mouthguard wearing habits and their perceived benefits were distributed to players at professional, amateur and school contact sport clubs throughout central Scotland. An additional questionnaire was sent to referees enquiring about policies of mouthguard wear, dental trauma experience and its management. Questionnaires distributed to players and referees also gauged interest in the use of Smart Phone Application (App) detailing the immediate treatment of mouth injuries. A final questionnaire was supplied to GDPs investigating their experience in managing dental trauma as well as their promotion and provision of mouthguards.

Results
The pilot study identified questionnaire design flaws and the need for wider data collection. Consequently the questionnaires for the definitive study have been amended accordingly and distributed to a greater number of sports clubs. At the time of writing this abstract, data collection is ongoing. Pilot study data revealed that players were more likely to wear mouthguards when playing competitive matches than when training. 39% of respondents had previous experience of dental trauma and 22% had required dental treatment following injury. A majority of respondents stated that they did not know how to immediately manage dental injuries, with 72% stating that a Smart Phone App providing guidance would be beneficial.

Conclusions
It is concluded that this study will help to increase awareness amongst sports club members of the risk of dental trauma occurring during contact sport participation and raise awareness of the preventive benefits of wearing a mouthguard. These factors combined with access through the ‘app’ to guidance on effective initial management of dental trauma will help reduce the incidence of sports-related dental trauma or improve the chance of survival of traumatised teeth through effective immediate trauma management.

Notes

Chestnutt, I. G., Morgan, M., Monaghan, N. P., Collins, L., Sheppard, L.

1Cardiff University, 2Public Health Wales, 3NICE

Objectives Assessing the oral health needs of a population is key to effective planning and commissioning of dental services, particularly for vulnerable and “at-risk” peoples. This work reviewed the available literature on conducting an oral health needs assessment covering vulnerable groups.

Methods The following electronic databases; MEDLINE, EMBASE, CINAHL and the Cochrane Library were searched from inception to June 2013, without language restrictions. Papers which described the conduct of oral health needs assessment (OHNA) in vulnerable groups from a population perspective were included. Papers identified as relevant were reviewed to identify data of relevance to the (OHNA) process.

Results Of 1426 unique articles identified, 59 were deemed relevant for full review. The heterogeneity of the studies precluded numeric summary, so a narrative report was deemed most appropriate. Key findings were as follows. No publications described an Oral Health Needs Assessment which was taken forward via a strategy, implemented and evaluated. The majority of oral health needs assessments are conducted largely in the form of simple cross-sectional epidemiological surveys of dental caries prevalence. While socio-dental indicators have been extensively described, this has largely been in one-off studies and not as part of an on-going evaluated OHNA process. Access to dental care using simplistic measures of direct-line access were reported, as were more sophisticated forms of geographic information systems. Most interesting was a health equity audit approach, one of the few mechanisms suggested to address oral health inequalities within the OHNA process.

Conclusions Evidence on a systematic approach to OHNA was lacking. This work was used to inform the development of National Public Health Guidance for Local Authorities on how to assess local needs as part of the oral health improvement agenda.

Notes
The investigation of the effect of non-syndromic cleft lip and palate SNPs on lip morphological traits in an epidemiological study

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Objectives Non-syndromic cleft lip / palate (NSCLP) is thought to have a complex multifactorial aetiology, encompassing polygenic as well as environmental factors, such as smoking, alcohol and certain medications or nutrient deficiencies. Several genetic variants have been implicated in the aetiology of NSCLP, including common variants identified through genome wide association studies. However, little is known as to how these common variants affect normal morphology of the lip.

The aim of this study is to investigate normal lip morphological traits and their association with seventeen previously identified single nucleotide polymorphs (SNPs) implicated in NSCLP.

Methods High resolution 3D surface facial scans were taken of 4,474 15 year-old children in the Avon Longitudinal Study of Parents and Children (ALSPAC). A robust, comprehensive scale for identifying lip and peri-oral traits has been developed. The morphological traits of the 4,747 ALSPAC subjects have been classified according to this scale. Seventeen genetic variants implicated in NSCLP were selected and tested for their association with the twenty-five lip and peri-oral morphological traits using genome-wide association (GWAS).

Results Genetic data was available for 3,181 individuals out of the 4,747 15 years olds. Fifteen genetic variants achieved statistical significance with nineteen lip traits. Skeletal pattern was significantly associated with SNP rs227731 at 17q22 (p = 1.58 x 10⁻⁶). The allele C of the SNP rs227731 represents the risk allele for NSCLP, achieving a GWAS significance of 1.78 x 10⁻⁸, suggesting that a skeletal II pattern (relative retrusion of the lower jaw) may be associated with NSCLP common variants. Cupid’s bow shape and lower lip shape were also significantly associated with SNP rs987525 at 8q24 (p = 2.48 x 10⁻³) and (p = 2.11 x 10⁻²) respectively. SNP rs987525 has previously achieved robust GWAS significance (5.12 x 10⁻³⁵) and the allele A is the risk allele; suggesting an exaggerated V-shaped Cupid’s bow and thin lower lip may be associated with NSCLP common variants. Logistical regression analysis of the combined risk alleles showed an association with four lip features.

Conclusions Non-syndromic cleft lip and palate common variants influence normal lip shape and facial skeletal pattern in the general population

Notes
The feasibility of alcohol misuse screening and treatment in a primary care general dental practice setting.

Roked, Z. Y., Moore, S., Shepherd, J.
Cardiff School of Dentistry

Objectives The aim of this study was to determine the feasibility of screening for alcohol misuse and providing brief intervention in a primary dental care setting.

Methods In this randomised controlled trial, patients aged 18–65 years were recruited from a local general dental practice. Patients were stratified according to appointment (with a dentist or hygienist). Reception staff administered envelope packs containing screening materials (the Modified Single Alcohol Screening Question [M-SASQ]), consent forms, and a short survey collecting contact details to patients who agreed to take part in the study. Packs were randomly pre-allocated to control and intervention groups by strata using block randomisation before the start of the study. Consenting patients scoring positively on the M-SASQ for drinking hazardously and allocated to the intervention group received a motivational intervention to reduce alcohol intake from either the hygienist or dentist. Patients in the control group received usual care. The outcome assessor and patients were masked to allocations. The outcome measure at 3 months was M-SASQ score. This trial is registered with the ISRCTN registry, number ISRCTN18745862.

Results One hygiene patient and 106 dental patients were recruited. The hygiene patient did not score positively on the M-SASQ for alcohol misuse. Of the 106 dental patients, 46 (43%) scored positively, with 26 allocated to the intervention group and 20 to the control group. Follow-up data were available for 22 (48%) of the 46 patients (12 intervention, 10 control). M-SASQ scores changed from positive to negative for two patients in the intervention and five in the control group.

Conclusions Alcohol misuse screening and treatment was feasible in a primary dental care setting; this suggests a new approach involving the general dental team, which could potentially reduce burdens on specialist dental services. Overall, in this practice, the dentist was best placed to deliver the intervention rather than the hygienist since these healthcare professionals saw most of the patients recruited into the trial. Contamination might have been a problem because more patients in the control group changed M-SASQ score. Building on these findings, a multicentre, cluster randomised controlled trial is planned.

Notes
Do outcomes in orthodontic research of cleft lip and palate patients reflect patient values?
Tsichlaki, A., Johal, A., Fleming, P.
Queen Mary University of London

Objectives The aim of this systematic review was to identify existing outcomes used in studies of orthodontic treatment in children with cleft lip and palate. The objectives were to categorize research outcomes into outcome domains and to explore whether any relevant outcome domains were underrepresented.

Methods The following electronic databases and grey literature were searched until March 2015 to identify all studies of orthodontic treatment interventions in children with cleft lip and palate: MEDLINE, EMBASE, CENTRAL, CDSR, PsycINFO, LILACS, BBO, ClinicalTrials.gov. Abstracts and subsequently eligible full-text articles were screened independently and in duplicate by two reviewers using systematic review methodology. Outcome measures were identified and categorised into six pre-determined outcome domains: morphological changes of treatment; adverse effects of treatment; quality of life; health resource utilisation; physical consequence of malocclusion; and functional status.

Results The search identified 665 abstracts, of which 53 eligible articles were retrieved in full. Forty two studies met the inclusion criteria and were included in the review. Morphological features of treatment were measured in the majority of studies, whereas the remaining five outcome domains were infrequently evaluated. A diverse range was outcomes was used with little consistency in the outcomes selected amongst studies of similar context to measure these domains.

Conclusions Most of the outcomes used in orthodontic research involving young people with cleft lip and palate are concerned with measuring morphological changes of treatment and do not reflect patient perspectives. Outcomes between studies were varied and heterogenous. Developing a core standardised set of outcomes reflecting the perspectives of all stakeholders will help to inform patients, parents, clinicians and commissioners about the effects of orthodontic treatment on young people with cleft lip and palate and would help overcome these issues.

Notes
The relationship between adolescent’s participation in community groups and dental caries in a deprived area in Brazil.
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1Federal University of Amazonas, 2School of Clinical Dentistry, University of Sheffield, 3Municipal Health Secretariat of Manaus (SEMSA)

Objectives This study assessed the association between adolescents’ participation in community groups and dental caries.

Methods A cross-sectional household-based study was carried out involving 200 subjects aged 15 to 19 years living in a deprived area in the state of Amazon, Brazil. Dental caries was assessed through dental examinations using the DMFT index conducted by a single examiner previously calibrated. Four dental caries outcomes were investigated including caries experience (DMFT score), current caries (number of current decayed teeth), missing teeth due to caries and the Care Index (ratio between number of filled teeth and DMFT score). Adolescent’s participation in community groups, demographic and socioeconomic data, and dental visiting were obtained through individual interviews.

Results All caries measures were significantly higher in adolescents who did not participate in community groups compared to their counterparts. Multivariate Poisson regression showed that adolescent’s participation in community groups was independently associated with all dental caries outcomes. After adjusting for confounders, participation in community groups was statistically associated with lower DMFT score (Ratios means (RM): 0.33, 95% CI: 0.24-0.46), less decayed teeth (RM: 0.23, 95% CI: 0.11-0.47), less missing teeth (RM: 0.28, 95% CI: 0.17-0.47) and higher Care Index (RM: 1.69, 95% CI: 1.24-2.29) than those who did not participate in community groups.

Conclusions Adolescents’ participation in community activities was related to lower levels of dental caries.

Notes
Surface Roughness Measurement of Carious Dentine Using Non-contact Optical Profilometry

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**Objectives**
Dentine surfaces undergo changes during a carious episode. Previous studies show that non-restorative management methods can arrest dentinal lesions. Non-contact optical profilometer (NCOP) has been used as a proxy to assess mineral loss of enamel following de and re-mineralisation. However, there have been no corresponding studies using NCOP for investigating carious dentine. The aim was to evaluate NCOP for measurement of the surface roughness of carious dentine.

**Methods**
90 extracted teeth with root caries were selected. Specimens containing only the lesion site were prepared by removing the coronal and apical third. Each specimen was embedded onto a customized tray to standardize the scanning process. A 1.5mm line (1500 points, 1.0 microns apart) on each lesion was selected and NCOP scanned. The mean value of the surface roughness parameter (termed Ra value) with its corresponding standard deviation was calculated from the NCOP scans on each of the 90 samples. A frequency distribution of Ra was also calculated in order to categorize the roughness values into ranges of values.

**Results**
The average Ra value was 10.9 µm with a standard deviation of 5.7 µm, and between 3.0 µm and 33.0 µm. However, the distribution of values is positively skewed.

**Conclusions**
NCOP measurements can be used to provide surface roughness values for carious dentinal lesion studies.

**Notes**
Interim results investigating frequency of dietary acid intake and erosion.
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Objectives To investigate the frequency of dietary acid intake in those with severe erosive tooth wear using an interim analysis of a questionnaire-based study.

Methods Patients attending King’s College London Dental Institute were recruited, from specialist clinics for tooth wear and general clinics, following informed consent (REC Ref 14/EM/1171). A single, trained interviewer and examiner questioned participants on their frequency and timing of dietary acid intake in addition to habits associated with dietary acid consumption. Erosive tooth wear was assessed using Basic Erosive Wear Examination (BEWE). Participants with an accumulative score >12 and a score of 3 in any one sextant were allocated into the severe wear category. Preliminary data were analysed using multivariable binary logistic regression models in SPSS vers 22.

Results Data from 216 participants consisting of 94 with erosive tooth wear and 127 controls were analysed. The mean age of the total sample population was 41yrs (sd=14). A statistically greater number of males had more severe wear compared to females (24% and 18.6% respectively; p=0.012). Wear patients consumed fruit an average of 2.07 (sd=1.55) times/day compared to 1.74 (sd=1.25) times/day for controls but this difference was not statistically significant (p=0.08). Wear patients consumed acidic drinks an average of 2.54 (sd=2.42) times/day and controls 0.98 (sd=1.09) and this difference was statistically significant (p<0.0001). Wear patients were also more likely to sip, swish or hold drinks in the mouth than controls (p=0.027).

Conclusions Frequency of dietary drink intake was significantly associated with erosive tooth wear. Participants with erosive tooth wear were significantly more likely to have a habit associated with their acidic beverage intake. An increase in sample size will allow further analysis of associated risk factors.

Notes
Antimicrobial Hydrogels For The Control Of Pulpal Disease

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1Cardiff University, 2Cardiff University

Objectives Persistence of polymicrobial infection within the dental pulp is a common reason for the failure of dental restorations. Systemic antibiotics are ineffective against pulpal infections and therefore there is a need to develop new treatments. Hydrogels have potential as a restoration material due to their tuneable physical properties and the ability to carry and deliver therapeutic agents. This study investigates the rheological properties of a polymer hydrogel and considers its suitability for use in dental restorations.

Methods Methylcellulose solutions were prepared at concentrations 1-10 % (w/v) and in different buffer solutions, including NaCl and glycerophosphate. Methylcellulose blends were also produced with other polymers, including carboxymethylcellulose and chitosan in order to tune the gelling behaviour. Solubility and gelation were studied as a function of temperature and sample composition. Rheological analyses were performed: viscometry and oscillatory shear measurements were used to assess the viscosity, gelation temperature and time-to-gel of the hydrogel formulations.

Results Methylcellulose could be prepared under mild conditions, without the need for non-aqueous solvents, high temperature or harsh pH conditions. Solutions were found to be viscous liquids at ambient temperature, and formed a gel at higher temperatures. Viscoelastic characteristics indicated that the gelation temperature was influenced by polymer concentration (increasing the concentration lowered gelation temperature) and ionic strength. Based on these data, synergistic effects between methylcellulose and additional polymers are being investigated.

Conclusions A potential hydrogel has been identified that can be prepared under mild conditions and has tuneable gelation properties, according to rheological analyses. Further work aims to assess the drug release properties of these gels and how the rheology relates to drug release rates.

Notes
Porphyromonas gingivalis preferentially invades oral keratinocytes in S phase of the cell cycle.

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Objectives Invasion of eukaryotic cells by Porphyromonas gingivalis is thought to be an important virulence trait. Previous work has shown that P. gingivalis does not invade all cells in a population equally but the reason for this selectivity is not fully understood. We hypothesise that at any one time within a culture cells are at different stages of the cell cycle and that this may explain this phenomenon. The aims of this study were to investigate whether P. gingivalis adhesion and invasion varies according to which phase of the eukaryotic cell cycle the host cells are in.

Methods H357 oral keratinocytes were serum starved for 24 hours and then brought into synchronous growth by re-introducing serum for differing times before exposure to P. gingivalis NCTC 11834 (MOI 100, 90 minutes). The percentage of cells in the cell cycle phases at each time point was determined by flow cytometry with propidium iodide and expression of integrin α5 (ITGA5) assessed using qPCR. Invasion of the synchronised cultures by P. gingivalis was measured using a metronidazole antibiotic protection assay and FITC labelled P. gingivalis was used to assess whether bacteria adhesion/invasion was dependent on the stage of the cell cycle.

Results The proportions of cells in S phase differed according to the length of time after serum was re-introduced; 2 hours (1.1%), 9 hours (6.7%), and 16 hours (33.2%). P. gingivalis invasion was higher in cells synchronised for 16 hours (5.5%) compared to those for 9 hours (2.5%), and 2 hours (1.1%) relative to unsynchronised controls. ITGA5 expression was increased two fold in synchronised cells at 16 hours compared to unsynchronised controls. When the level of FITC fluorescence (a measure of P. gingivalis adhesion/invasion) was expressed in relation to the proportion of cells in each phase of the cell cycle, the highest values were for cells in S phase (109.8 AU +/- 28.6) followed by G2 (67.6 +/- 32.7) and G1 (16.7 +/-32.7).

Conclusions P. gingivalis invasion positively correlates with the proportion of cells in S-phase and the highest level of adherence/invasion of FITC labelled P. gingivalis was seen in S phase cells. This suggests that P. gingivalis may preferentially target cells in S phase which may be explained by increased levels of integrin α5 which is known to be a receptor for P. gingivalis.

Notes
*Porphyromonas gingivalis* Lipopolysaccharide Isoforms Initiate Cytokine Tolerisation Responses in M1 and M2 Macrophages.

Strachan, A.1, 2, Jackson, S. K.2, Harrington, Z.1, 2, McIlwaine, C.1, 2, Foey, A.2, Zaric, S.1, 2

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**Objectives** Periodontitis is a chronic inflammatory disease characterised by excessive innate immune response to the oral biofilm bacteria. The pathogenesis of the disease manifests as periods of rapid progression and conversely, periods of remission. The “keystone” periopathogen *Porphyromonas gingivalis* has the ability to synthesise LPS isoforms, which differ in levels of endotoxin activity and in turn affect host immune response. Both pro-inflammatory M1 and regulatory M2 macrophages (MΦ’s) are hypothesised to be associated with the differential host response to this persistant pathogen. The objectives of this study are two-fold; i) to determine the sensitivity of M1 and M2 MΦ’s to endotoxin tolerance induced by LPS isoforms, and ii) to compare cytokine profiles to those produced by MΦ’s challenged with LPS extracted from subgingival plaque samples obtained from both healthy patients and those with chronic periodontitis.

**Methods** The pro-monocyte THP-1 cell line was differentiated using PMA and Vitamin D3 into M1- and M2-like MΦ’s (respectively). These were pre-treated with *Porphyromonas gingivalis* LPS isoforms (m/z 1690 and 1435) and *Escherichia coli* K12 LPS for a period of 24 hours. Cells were then washed and stimulated for a further 18 hours. Macrophages were also treated with phenol-extracted LPS derived from the subgingival plaque samples. Supernatants were collected and levels of TNFα, IL-8, IL-10 and IL-18 secretion determined by sandwich ELISA.

**Results** Our results indicate a differential LPS isoform-dependent cytokine profile between M1 and M2 MΦ’s in both the pre-stimulation and stimulation stages. Cytokine production suggests a link between LPS isoform-specific endotoxin tolerance and the response seen in M1 and M2 MΦ’s exposed to LPS extracted from patients.

**Conclusions** The divergent cytokine production observed with tolerised M1 and M2 MΦ’s, identifies a mechanism whereby the pathogen is able to subvert and influence the immune response to its benefit by manipulating its LPS structure, potentially driving the progression of the disease.

**Notes**
Novel Therapies Against *Candida albicans* Invasion And Biofilm Formation

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¹Cardiff University , ²AlgiPharma AS

**Objectives** Oral infections caused by *Candida albicans* are of growing concern due to the rise in anti-fungal resistance, limited availability and toxicity of anti-fungal drugs. The oligosaccharide OligoG CF-5/20 has been shown to modify the formation of fungal biofilms through inhibition of hyphal formation and potentiation of anti-fungal therapies (Tøndervik *et al.*, (2014) PLoS ONE 9:e112518). This study investigated the anti-fungal properties of OligoG in models of hyphal invasion including an *in vitro* reconstituted human oral epithelial (RHE) tissue model (SkinEthic®, France).

**Methods** Growth curves (24 h) of *C. albicans* (ATCC 90028) were carried out +/- OligoG (0.2-6 %). Hyphal invasion of *C. albicans* was then monitored using (a) a Yeast Peptone Dextrose agar assay +/- OligoG and (b) the RHE model +/- OligoG (0.2 %, 12 h). Tissue samples were stained with Periodic acid–Schiff or LIVE/DEAD® stains and immunohistochemistry performed on fixed samples (concanavalin A–Alexa 594, pan-cytokeratin antibody–Alexa 488 and Hoechst nucleic acid dye) for microscopic analysis. Phospholipase activity was determined using an egg-yolk agar assay +/- OligoG (0.2-6 %).

**Results** Growth curves were reduced in a dose-dependent manner with OligoG. OligoG treatment induced marked reductions in hyphal invasion, in both the agar-based and RHE models (P<0.001). Confocal microscopy showed the modified intra-epithelial invasion of *C. albicans* and increased hyphal death with increasing OligoG concentration. No change in lactate dehydrogenase activity was observed after OligoG treatment. However, a significant reduction of phospholipase activity was seen at 6% OligoG (P<0.05).

**Conclusions** These *in vitro* studies demonstrate the utility of this model to study the ability of therapies to modulate the attachment, growth and invasion of pathogens to the skin. Biofilm modification and inhibition of hyphal formation are important mechanisms wherein OligoG may affect the previously observed *in vitro* potentiation of anti-fungal agents.

**Notes**
Unconventional protein secretion in innate immunity: The role of P2X7R in transglutaminase 2 export and activation
Griffiths, R. M., Adamczyk, M., Jones, A. T., Aeschlimann, D.
1Cardiff University, 2Cardiff University, 3Cardiff University

Objectives Transglutaminase 2 (TG2)-mediated stabilization of extracellular protein assemblies has a pivotal function in tissue repair. However, aberrant TG2 activity has been linked to fibrosis and autoimmunity. TG2 is secreted via an unconventional and enigmatic mechanism. Our group has shown that TG2 export is linked to purinergic signalling, and implicated P2X7 receptor activation. Investigating this process will unravel a novel secretory pathway potentially used by select proteins, including potent signals regulating inflammation. We aim to elucidate mechanistically the process by which cells export TG2 and control its activation.

Methods P2X7R variants were stably expressed in HEK293 cells. P2X7R pore formation was assessed using YO-PRO1 uptake by cells. TG2 and thioredoxin externalization was assessed by Western blotting of conditioned medium. TG2 enzymatic activity was measured using an isopeptidase assay.

Results Pharmacological agents and site directed mutagenesis were used to investigate which activity of P2X7R was required for TG2 export. Pannexin-1 inhibition demonstrated that pannexin hemichannels are unlikely to be the pore-forming component coupled to P2X7R in our cell model and are not involved in TG2 secretion. In contrast, a gain-of-function mutation in P2X7R that caused enhanced TG2 externalization from cells, correlated with increased pore activity thereby implicating P2X7R itself. Using various TG2 mutants we showed that GTP binding is essential for its externalization, however, transamidation activity is not required. Thioredoxin, a TG2 activator, is co-secreted.

Conclusions We show that P2X7R induced membrane pore activity directly correlates with TG2 export. Hence, P2X7R polymorphisms affecting membrane pore formation may also affect extracellular levels of proteins secreted via this pathway. Thioredoxin is co-secreted with TG2 and may therefore act as a molecular chaperone in TG2 export enabling adoption of the active enzyme conformation. This begins to identify components of a mechanism for unconventional protein secretion crucial to innate immunity.

Notes
IL-34 suppresses *Candida albicans*-induced TNF-α production in M1 macrophages by down-regulating expression of Dectin-1 and TLR2

Xu, R., Wei, X., Song, B., Williams, D. W.
Cardiff University

**Objectives** The aim of this study was to investigate the role of IL-34 in regulating macrophage response following *C. albicans* challenge.

**Methods** Mouse bone marrow macrophages (BMM) and a mouse macrophage cell line (RAW264.7) were cultured with GM-CSF to drive M1 macrophage development and treated with increasing doses of IL-34 before stimulation with heat killed Candida (HKC). TNF-α production was evaluated by ELISA. The mRNA and cell surface protein expression of TLR2 and Dectin-1, which are key pattern recognition receptors (PPRs) for β-glucan in the yeast wall, were also determined using a combination of qPCR and FACS.

**Results** M1 macrophages produced TNFα after HKC stimulation. IL-34 was found to suppress TNFα production in a dose dependent manor. In addition, IL-34 suppressed both TLR2 and Dectin-1 mRNA expression as detected by RT-qPCR. The suppression of TLR2 and Dectin-1 protein expression was confirmed by FACS in BMM. IL-34 was also found to suppress mRNA and protein expression of TLR2 and Dectin-1 which was confirmed in RAW264.7 cells.

**Conclusions** IL-34 suppressed HKC induced TNFα production in inflammatory M1 macrophages by down regulation of Dectin-1 and TLR2 expression. The constitutive expression of IL-34 by keratinocytes may maintain the tolerance of resident macrophages in skin. Transient blocking IL-34 may enhance immune responses of Langerhans cells which may be of benefit during vaccination.

**Notes**
An *in vitro* model for evaluating denture cleaning toothpaste regimes

Marsh, L. L.¹, Williams, D. W.¹, Milward, P.¹, Wilson, M.¹, Lewis, M.¹, Rowe, W.¹, Bamford, S.¹, Bradshaw, D.², Roy, P.²

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**Objectives** Persistence of microorganisms in the form of biofilms on the non-shedding surfaces of acrylic dentures occurs in cases of poor denture-cleansing. The biofilms may then be responsible for oral infection such as *Candida*-associated denture stomatitis (DS). As a result, both the development and evaluation of denture cleaning products is important. The objective of this study was to identify appropriate methodology for the *in vitro* evaluation of denture-cleansing using physical brushing and an experimental denture cleansing paste.

**Methods** A 200-µl volume of human saliva, artificially contaminated with *Candida albicans* was overlaid on the surface of acrylic squares which had previously been immersed in an artificial saliva for 24 h at 37°C. The acrylic was incubated at 37°C for 72 h, with addition of artificial saliva at 24 h intervals. The acrylic was immersed in sterile water for 5 min and selected samples were also air dried at 37°C for a further 72 h. Brushing with cleansing paste or water for 2 min, used a powered toothbrush positioned so the bristles just made contact with the acrylic surface. Cleansing agent was removed by immersion and gentle agitation of the acrylic in sterile water for 1 min. Microorganisms on acrylic were fixed in 4% (v/v) formalin for 24 h, stained with propidium iodide and imaged using confocal laser scanning microscopy or scanning electron microscopy (SEM). CLSM images were converted to binary black and white to determine the percentage area colonised using ImageJ software.

**Results** An extensive biofilm (mean percentage area colonised=3.9%) was generated on non-brushed control acrylic. Compared with unbrushed controls, significantly reduced colonisation (mean percentage area colonised=0.6%) occurred following brushing with cleansing paste (P<0.001). Brushing with water (mean percentage area colonised=3.9%) did not reduce colonisation compared with unbrushed controls.

**Conclusions** An *in vitro* method of evaluating the efficacy of denture cleaning approaches has been developed. Using this model system, it was evident that when coupled with toothbrushing, use of a cleansing paste provided added value to removal of microbial biofilm on acrylic surfaces.

*The study was funded by GlaxoSmithKline Consumer Healthcare, Weybridge.*

**Notes**
Lethal Photosensitisation of Prevotellaceae Under Anaerobic Conditions
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1University of Liverpool, 2University of Liverpool, 3University of Liverpool

Objectives Oxygen is considered to be essential for lethal photosensitisation by photodynamic processes. The black-pigmented oral anaerobes Prevotella intermedia and Prevotella nigrescens are known to be photosensitive, but are also extremely sensitive to the cytotoxic effects of oxygen. It was considered prudent to study their photosensitivity under strict anaerobic conditions to determine the feasibility of this process within the anoxic confines of a periodontal pocket.

Methods A series of experiments were undertaken to induce lethal photosensitisation in P. intermedia (ATCC 25611) and P. nigrescens (ATCC 25261) using 405 nm light sources under anaerobiosis. Samples of these bacteria were grown on a blood–containing, solid growth medium before being suspended in saline and then exposed to 405 nm light delivered by either a hand-held light-emitting diode source (Toothcare™) (19.1 mW/cm²; 15 mW) or a laser pointer (328.5 mW/cm²; 45 mW). Viable counts of the light exposed samples were compared to light-free controls to determine the proportion of bacteria killed. All of the experimental procedures were carried out within a validated anaerobic chamber.

Results Lethal photosensitivity was demonstrated against P. intermedia and P. nigrescens. 60 seconds exposure to the LED source elicited a 0.73 log₁₀ reduction in viable counts of P. nigrescens and a 1.70 log₁₀ reduction in P. intermedia. The 405 nm laser pointer resulted in a 3.03 log₁₀ reduction in P. intermedia. The proportions of bacteria killed by either the LED or laser pointer were similar at any given energy density (J/cm²).

Conclusions Lethal photosensitivity was demonstrated in two species of Prevotella under anaerobic conditions. Further research is required to improve the efficacy of the photodynamic process, elucidate the photochemical mechanisms at play and determine the potential clinical significance of a periodontal treatment based upon the intrinsic photosensitivity of putative periodontal pathogens.

Notes
Investigation Into The Rate of Bacterial Biofilm Removal by NaOCl Irrigant
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Objectives Irrigation is an important part of successful root canal treatment. It has several important functions, among them to disrupt biofilms. The present work aimed primarily to assess the removal rate of Enterococcus faecalis biofilm and organic films (hydrogel, collagen) during irrigation using 2.5% NaOCl using artificial canals. Secondly, to evaluate the mimic behaviour of organic films to biofilm.

Methods Thirty-six Endo-Vu blocks were prepared to size 30, 0.06 taper with open and close ends. Each model was sectioned into two sagittal halves and sterilized. The 3.5mm apical of one half either biofilms were grown for 7 days or received organic films. The models were reassembled and attached to a glass slide. Irrigation (9mL NaOCl or water for 60 seconds) was performed and under fluorescence microscopy and images were captured every second using a camera. The residual film or biofilm percentage, and the number of bubbles were measured using image analysis software. The outflow irrigant was collected to evaluate available chlorine and pH.

Results Biofilm removal rate was significantly less (P<0.001) than of collagen. Hydrogel removal was significantly more (P<0.001) than of collagen. A difference at the 5% significance level was identified in the removal rate of the film or biofilm between the models (closed, open). Open canals containing hydrogel were associated with the highest number of bubbles, while those containing biofilm were the lowest. Percentages of the available chlorine (1.5-1.9) and pH values (10.3-12.8) of NaOCl from closed canals were significantly more (P=0.001) than that from open canals. The removal rate by NaOCl was more effective that water.

Conclusions The proposed biofilm model provided a viable mean to investigate biofilm or film removal under microscopy. Biofilm proved was more resistant than organic films. Closed canals adversely influenced removal efficacy of irrigant, and NaOCl was more effective than water.

Notes
Characterisation of a Temperate Phage Residing in the Genome of the Anaerobic Bacteria *Fusobacterium nucleatum polymorphum* ATCC 10953


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**Objectives** Characterisation of a prophage residing in the genome of *Fusobacterium nucleatum polymorphum* ATCC 10953 and an assessment of its presence in periodontal patient plaque DNA. In addition, we are investigating three potential lysin proteins via recombinant production to assess potential antimicrobial activity as therapeutics.

**Methods** Mitomycin was used to induce the 10953 prophage with visualisation by TEM. Three prophage genes with potential lysis activity were identified using the PHAge Search Tool (PHAST) server: FNP_1707 (amiC), FNP_1699 and FNP_1700. These genes were commercially synthesised for expression in *E. coli*. Cloning of the genes and overexpression of its protein were attempted in BL21 and C41. Furthermore, plaque samples from chronic periodontitis patients in Sheffield were screened for *F. polymorphum* and the occurrence of its prophage using PCR primers.

**Results** Despite good expression of amiC-pGEX (FNP_1707 protein), it was found to be mainly insoluble. Cloning and overexpression of FNP_1699 and FNP_1700 are ongoing alongside attempts to further improve production AmiC. 23/45 patients tested screened positively for the presence of *Fusobacterium nucleatum polymorphum* ATCC10953 strain and its prophage, which represent 405 plaque samples, tested. In addition induction of the prophage has proved inconsistent with visualisation of a tailed phage in some samples.

**Conclusions** We have identified that the prophage of FNP10953 is present in a large number of patients, indicating that it is present not only in a lab strain but also in the general population. Attempts to isolate this prophage and its potential lysins are ongoing but present potential novel antimicrobials that would target *Fusobacterium nucleatum spp*, and might aid in the treatment of gum disease.

**Notes**
The Localisation Of Fluorescently Labelled OligoG In A Pseudomonal Biofilm

Powell, L. C., Pritchard, M. F., Ferguson, E., Onsøyen, E., Rye, P. D., Hill, K. E., and Thomas, D. W.

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Objectives Biofilm-associated infections are an important cause of morbidity amongst patients, as biofilms are innately resistant to antibiotics and host immune defences. Recently, a novel antimicrobial agent, OligoG CF-5/20, based on alginate oligosaccharide, has been shown to potentiate the effect of antibiotics against multi-drug resistant bacteria, whilst also disrupting the formation of pseudomonal biofilms. To evaluate the anti-biofilm properties of OligoG, we sought in this study, to characterise and quantify the ability of fluorescently-labelled OligoG to both prevent biofilm formation and disrupt established biofilms.

Methods The ability of OligoG to penetrate and disrupt mucoid Pseudomonas aeruginosa (NH53788A) biofilms was assessed in vitro. OligoG was conjugated to the fluorophore Texas Red (TxRd) cadaverine and used to study biofilm formation and disruption of established biofilms when treated with 0.5-6% OligoG (24 h). Biofilms were then stained with SYTO 9® and visualised using confocal laser scanning microscopy. Controls with TxRd only were performed to test for possible dissociation of the conjugate and any inherent anti-biofilm properties of the TxRd itself. COMSTAT image analysis software was used to quantify the physical properties of the biofilms.

Results TxRd-labelled OligoG demonstrated a dose-dependent effect on NH53788A biofilm formation through inhibition of growth, as well as disruption of 24 h established biofilms. COMSTAT analysis revealed a significant decrease in biofilm volume and height with increasing OligoG dose (0.5-6%; p<0.05). The TxRd-labelled OligoG was able to diffuse through the entire biofilm depth without dissociation of TxRd from OligoG, whilst TxRd alone showed no biofilm disruption.

Conclusions This study supports the previously reported in vitro inhibition and disruption properties of OligoG, and for the first time, quantifies the extent of this disruption. The fluorescently-labelled conjugate provided an insight into the interaction of OligoG with the structure of the mucoid biofilm, visualising the widespread diffusion of OligoG through the biofilm.

Notes
Trends in the antimicrobial susceptibility of anaerobic isolates from dentoalveolar abscesses

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Objectives Dentoalveolar abscesses (DAAs) are polymicrobial in nature, with a predominance of strictly anaerobic bacterial species often of the *Prevotella*, *Fusobacterium* and *Porphyromonas* genera. The use of antimicrobials as part of DAA management should only be considered in immunocompromised patients or if there are signs of spreading systemic infection. However, there is evidence that antibiotics are often used inappropriately in dentistry and this will contribute to the global burden of antimicrobial resistance. The WHO recommends that susceptibility surveillance takes place in all avenues of healthcare, including dentistry. The objective of this study was to compare antibiotic susceptibility of bacteria from DAAs obtained from patients attending the University Dental Hospital in Cardiff in 2007 and 2012.

Methods Species identification and susceptibility of microbial isolates (n=275) from DAAs in 2007 (n=177) and 2012 (n=98) were retrospectively collated. Isolates were identified to species level by phenotypic methods, 16S rRNA gene sequencing and MALDI-TOF-mass spectrometry. Susceptibility to clindamycin, erythromycin, penicillin and tetracycline was recorded.

Results The percentage of resistant isolates to all antimicrobials showed a statistically significant change between the years studied. Increased resistance was recorded for erythromycin (29.7% to 44%), clindamycin (2.1% to 7.1%) and tetracycline (10.6% to 29.8%). However, a decrease in penicillin resistance (53.2% to 27.4%) was documented.

Conclusions For the majority of antibiotics studied, an increase in the percentage of resistant isolates from DAAs was noted. Surveillance of antimicrobial resistance amongst disease causing bacteria in the oral cavity is essential to inform empirical prescribing and to monitor impact of any changes to prescribing behavior.

Notes
The Candida albicans protein Ece1p promotes activation of human microvascular endothelial cells in vitro and drives systemic candidiasis in a zebrafish model.

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Objectives Objective: In susceptible individuals the usually commensal fungal organism C. albicans is capable of becoming pathogenic causing a range of systemic infections with high mortality. However, little is known about the mechanisms involved in interaction of C. albicans with the endothelial cells that line blood vessels during systemic infection. This study aimed to examine the role of the C. albicans protein, Ece1p, in driving the initial immune response in microvascular endothelial cells in vitro and virulence in vivo.

Methods Methods: Primary human microvascular endothelial cells (HuDMEC) and the human microvascular endothelial cell line HMEC-1 were stimulated with either wild-type C. albicans strain BWP17, an ECE1 deleted mutant or ECE1 reintegrated mutant strains at increasing MOI for up to 24 hours post-infection. Activation of several pro-inflammatory transcription factors by endothelial cells upon stimulation with these stains of C. albicans was examined by Western blot. Expression of CXCL8 was measured by qPCR and ELISA and cell damage analysed by lactate dehydrogenase (LDH) release. A zebrafish model of systemic candidiasis was used to measure virulence of wild-type and ECE1 mutants.

Results Results: Incubation of HuDMEC and HMEC-1 cells with the ece1 null mutant resulted in significantly reduced gene and protein expression of CXCL8, and LDH release compared to BWP17 and ECE1 reintegrant strains. In addition, activation of HMEC-1 by the ece1 null mutant showed markedly less activation of c-Fos, phospho-c-Jun, phospho-MAPK compared to wild-type and ECE1 reintegrant strains. In vivo, ECE1-deficient C. albicans was significantly less virulent then both wild-type BWP17 and ECE1 reintegrant strains in a zebrafish systemic candidiasis model.

Conclusions Conclusion: The C. albicans cell wall protein Ece1p is a key molecule in driving endothelial cell activation and virulence during systemic candidiasis.

Notes
Fungal bacterial interactions: from opportunistic pathogen to mutualistic biofilm communities

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**Objectives** Fungi and bacteria co-colonise in variety of niches within the human body, including the oropharynx and airways, biomaterials and wounds, and there is evidence to suggest these affect clinical outcomes, through synergized pathogenicity and enhanced antimicrobial resistance. We hypothesize that bacterial and fungi interact physically and chemically, supporting one another pathogenicity and protecting one another from environmental stressors. The aim of this study was to investigate the clinical implications of cross kingdom interactions and investigate this experimentally *in vitro*.

**Methods** We evaluated the association of fungi in a chronic disease, i.e. denture induced stomatitis (DIS), using prospective and retrospective approaches, respectively, and applied next generation sequencing and culture based methodologies. Biofilm biomass was assessed by crystal violet assay. We then investigated the association of *Candida albicans* (Ca) with *Staphylococcus aureus* (Sa), using a combination of phenotypic, biochemical and molecular based methodologies. Furthermore, we used untargeted hydrophilic interaction liquid chromatography and high resolution mass spectrometry metabolomics approach to detect thousands of chemical features in mono-species and multispecies biofilms.

**Results** *Candida* species were shown to associate with defined bacterial genera, and clinical outcomes were negatively associated in DIS. The interaction between Ca and Sa showed that Sa required Ca for enhanced biofilm formation, adhering to its hyphae. Moreover, transcriptional analysis of Ca in co-culture showed a significant up-regulation of the cell wall protein *HWP1* and adhesin *ALS3*, suggesting a specific interaction. Analysis of metabolites suggested a significant modulation in the activation of different metabolic pathways in co-culture model compared to mono-species biofilms.

**Conclusions** Polymicrobial infections are common in oral cavity and interaction between the microbes modulates myriad of virulence factors, which in turn influence the biofilm formation. Understanding the interactions between bacterial fungal species may result in new therapeutic strategies and effective management of DIS.

**Notes**
Development of a sampling matrix and topic guide for qualitative in-depth interviews exploring the landscape of child protection in dentistry

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Objectives To devise an appropriate sampling matrix and topic guide for exploratory in-depth interviews with members of the dental team in Scotland, in order to map the landscape of child protection in dentistry.

Methods A purposive sampling matrix was selected to give a heterogeneous/broad cross section of members of the dental team to allow comparative understanding. The target population included dental practitioners, dental nurses and dental hygienists/therapists working in dental primary care in Scotland.

The topic guide for in-depth exploratory interviews was developed using a “bottom up” approach. All topics of interest were listed then sorted under main and sub topic headings.

Results Fifteen initial purposive selection criteria were identified and refined to 12 primary and 3 secondary criteria. Primary criteria were entered into a sampling matrix table to give a sample size of 18 to 50 participants which is in keeping with average participant numbers in qualitative research.

Topic guide development resulted in 8 main headings to explore the research question of “What is involved in the decision by a member of the dental team to refer a paediatric patient or not, and what influences the decision?” Each main heading was followed by between 2 to 14 sub topic headings. The topic guide will now be piloted before being learned by the main researcher for use in the final study.

Conclusions Creating a sampling matrix for this qualitative research project has allowed the consideration of various selection criteria to ensure adequate mapping of the views of dental team members regarding child protection. The construction of a topic guide provides a checklist of essential topics that must be covered during in depth interviews. It provides the ground a researcher wishes to cover but does not dictate the questions to be asked, therefore giving flexibility in the question order and open question wording.

Notes
Can Dental Service Utilization Decrease The Occurrence Of Dental Pain?
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Objectives This study tested whether the pattern of dental attendance mediates the association between educational attainment and dental pain.

Methods This is a cross-sectional analysis conducted with 1,099 adults from a prospective cohort study, in Southern Brazil. Educational attainment was the exposure of interest. The mediators were the pattern of dental services utilization and the reason for the last dental visit. Dental pain in the last six months was the outcome, while sex and age were covariates. The mediating effects were assessed by including interaction terms in logistic regression equations, as well as by the use of the KHB method, which estimated the direct, mediated and total effects of educational attainment on dental pain.

Results The prevalence of dental pain was 17.5%. Education was negatively related to dental pain, although this association was not statistically significant. Both mediators – pattern of dental services utilization and reasons for the last dental visit – were associated with the dental pain, and educational attainment was associated with both mediators. Inconsistent mediation was detected. Interaction terms showed that individuals with less than 12 years of study who seek dental services occasionally, and who visited the dentist to solve dental problems had 20% higher odds of reporting dental pain than those with 12 or more years of study, who regularly seek the dentist for preventative reasons.

Conclusions A regular pattern of dental services utilization and the use of services for preventative purposes may decrease the frequency of dental pain in less educated adults.

Notes
A New Fluoride Containing Bioactive Glass Additive For Toothpastes

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1Barts & The London, 2Queen Mary University of London, 3BioMin Technologies, 4QMUL, 5QMUL

Objectives To characterize a new fluoride containing bioglass intended for use as a remineralising toothpaste.

Methods The bioglass was synthesized by a high temperature melt quench route. The glass was ground to a <38 micron powder. The glass composition is given in Hill R and O’Donnell M “Multicomponent Glasses for Use in Personal Care Products” WO 2011/000866A2. The composition contains fluoride and a high phosphate content.

The glass (75mg) was immersed in 50ml of Tris buffer at pH 7.3 and in artificial saliva (AS) at pH 6.5. The solutions were filtered after the designated time period. The ion release into solution from the glass in Tris buffer over 24 hours was followed by ICP-OES for Si, Ca, Na, P and fluoride was measured using an ion selective electrode.

The precipitate from the Tris buffer and AS was collected, dried and characterized by XRD, FTIR, 31P and 19F MAS-NMR.

The ability of the bioglass when formulated as a toothpaste to occlude dentinal tubules was assessed using SEM and hydraulic conductance using a modified Pashley Cell, with mid coronal dentine discs cut from human molars. The hydraulic conductance was measured before and after application of the toothpaste.

Results The BioMinF glass dissolved rapidly over approximately 6 hours releasing Na, Ca, PO43- Si and F-. The glass formed apatite in under 6 hours in Tris buffer and under 30 mins in AS as evidenced by the XRD and FTIR results. The apatite formed exhibited a peak in the 19F MAS-NMR spectrum at -103ppm corresponding to fluorapatite. The SEM studies showed the dentinal tubules to be largely occluded and the apatite to form preferentially on the peritubular dentine. The percentage reduction in hydraulic conductance was >90%.

Conclusions BioMinF is a promising additive for remineralising and sensitivity toothpastes. The prolonged release of fluoride in addition to calcium and phosphate and the formation of fluorapatite, as opposed to the more soluble hydroxyapatite formed with existing bioactive glasses are attractive features.

Notes
Dentifrice Delivery into Dental Plaque Biofilms by High-velocity Microsprays


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Objectives Dental plaque biofilms can act as a barrier to the transport of dentifrices inside the biofilm, thus antimicrobial agents are limited in their effectiveness. We assessed the ability of high-velocity microsprays generated by the Philips Sonicare AirFloss to enhance the delivery of dentifrices into *Streptococcus mutans* biofilms, the primary etiologic agent of caries.

Methods Microscope slides colonized with 3-days old *S. mutans* biofilm grown in a 1% mucin-containing medium supplemented with 2% sucrose were exposed to microsprays delivered at a 90° or 30° impact angle using an AirFloss containing 1-µm carboxylate-modified polystyrene fluorescent tracer beads. In addition a 0.2% Chlorexidine (CHX) solution microspray was delivered at 90° impact. For comparison, static diffusive transport (30 sec) and a simulated shaking mouth-washing (30 sec at 200 rpm) were performed. Confocal microscopy was used to determine the number and relative bead penetration depth (PD) into the biofilm. For CHX, the killing depth was found from the resultant zone of killing by live (green)/ dead (red) viability staining.

Results The 30° impact delivered approximately 10 times more microbeads than the 90° impact (Fig. 1). However, both microsprays delivered significantly more beads deeper (P<0.05, n=2) into the biofilm than static or shaking conditions (Fig. 1). The 90° microspray impact resulted in significantly greater killing depth (28.3% of the total biofilm thickness) compared to shaking (1.2%) and static (3.4%) experiments (P<0.05, n=2).

Conclusions These data suggest that high-velocity water microsprays can be used as an effective mechanism to deliver microparticles and dentifrices inside *S. mutans* dental biofilms. Further, the impact angle has potential to be optimized both for biofilm removal and dentifrice delivery inside biofilm in those protected areas where some biofilm might remain.

Notes
Stain Control Efficacy and Tolerability of an Oral Hygiene Regimen

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1GlaxoSmithKline Consumer Healthcare, 2Salus Research

Objectives To determine whether an Experimental toothpaste, toothbrush and post-brushing mouthrinse (TTM) regimen prevented tooth stain accumulation and promoted stain removal versus a conventional marketed toothpaste and toothbrush (TT) regimen, and to study the Experimental regimen’s oral tolerability.

Methods This program involved a stain control study (1) and an oral soft tissue (OST) tolerability study (2): both were single-centre, examiner-blind, healthy-volunteer designs. Study 1 had a 4-week stain-induction phase, followed by a 6-week stain-reduction phase (3 cells, N=423). After dental prophylaxis, subjects were randomised to an Experimental TTM regimen (1 cell) or a Reference TT regimen (2 cells). The Experimental toothpaste and mouthrinse contained the stain-control agent, sodium tripolyphosphate, at 5% and 1.2% respectively. Subjects used their allocated treatment 2x/day for 4 weeks, rinsing with fresh tea 3x/day to encourage stain development. After 4 weeks, subjects’ stain levels were assessed using the modified Lobene stain index (intensity), and tea-rinsing stopped. Subjects using the Reference regimen were then divided: one cell continued as before, the other switched to the Experimental regimen. Stain was re-assessed after 6 weeks. In study 2, the Experimental regimen or a marketed whitening TTM regimen (2 cells, N=208) were used 2x/day for 28 days. An OST examination was completed at baseline, 7 and 28 days.

Results In study 1, stain accumulation in the Experimental TTM group was 8.3% lower (p=0.0364) and stain reduction was 28.7% greater than the Reference TT group (p<0.0001). There were 11 treatment-related adverse events (TRAEs). In study 2, there were 4 TRAEs in the Experimental TTM group, and 20 in the Reference TTM group. In both studies, mild desquamation and ulceration were the most frequent TRAEs.

Conclusions The Experimental toothbrushing-mouthrinising regimen prevented stain accumulation and promoted stain removal, compared to conventional toothbrushing with toothpaste alone. The Experimental regimen was well-tolerated. Studies funded by GSK Consumer Healthcare.

Notes
A novel intervention for oral malodour reduction: A randomized clinical trial on the effect of Philips Sonicare TongueCare+ and BreathRx

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Objectives Oral malodour is a widespread problem in adults. Approximately 80% of the cases are of intra-oral origin caused by bacteria inhabiting the tongue. The objective of this investigation was to test the effectiveness on oral malodour of the new Philips Sonicare TongueCare+ technology (TC+). TC+ is a powered sonic motion tongue brush with soft silicone bristles, designed to optimally clean between the tongue papillae and working synergistically with the antibacterial spray BreathRx (BRx, 0.09% cetylpyridinium chloride, 0.7% zinc gluconate).

Methods 20 participants with detectable oral malodour took part in this randomized cross-over clinical trial, comprising four treatment arms: TC+ with BRx, TC+ with water, BRx, and water with one week washout period. Malodour levels were monitored immediately before tongue brushing and at 1 hr, 3 hrs and 6 hrs after a single treatment by organoleptic score and bacterial density.

Results TC+ with BRx showed a significantly (p-value< 0.01) higher reduction in organoleptic score and bacterial density at 6 hrs than all alternative treatments. TC+ with BRx showed better performance at 6 hrs than BRx at 1hr. Both measurements were significantly lower at all-time points after treatment: they reduced from levels characteristic of high oral malodour to barely noticeable. Organoleptic score was reduced from 3.6 ± 0.4 before treatment to 2.3 ± 0.6 at 6 hrs. Bacterial density significantly decreased from 8.9 ± 0.5 Log_{10} CFU cm^{-2} before treatment to 7.7 ± 0.4 Log_{10} CFU cm^{-2} at 6 hrs. We identified a significant positive correlation between bacterial density and organoleptic score, confirming that tongue biofilm is the dominant cause of oral malodour.

Conclusions The results of this investigation show that the Philips Sonicare TongueCare+ tongue brush in combination with the antibacterial spray BreathRx delivers more than 6 hrs of fresh breath following a single use.

Notes
The effect of toothbrush abrasion force on dentine hypersensitivity in-vitro
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Objectives This study investigated the effect of tooth brushing force on changes in dentine tubule patency in an erosion-toothbrush abrasion model.

Methods 60 dentine samples were prepared and polished with an artificial smear layer and divided randomly into control (no tooth brushing), 100g, 200g or 400g brushing force groups. Tandem Scanning Microscopy (TSM) (x40) imaged the centre of each sample. Samples were immersed in 3:1 artificial saliva/NaF 1450ppm and either brushed, using a standard protocol (p35 soft tooth brush; 120 strokes) at the specified force, or not brushed. Then samples were subjected to an agitated acid challenge (0.3% citric acid pH2.6 for 2 minutes). Finally, samples were re-brushed using the same protocol. TSM images (x40) were retaken after each stage. Previously validated software calculated the numbers of patent dentine tubules. Profilometry was used to determine tooth wear.

Results At baseline, mean patent tubules in all samples were 188 per image (SD 54) with no significant inter-group differences. Following the first brushing cycle, mean patent tubules from baseline decreased using 100g to 150 (SD 32) (p<0.01) and increased using 400g to 215 (SD 45) (p=0.02). Differences in patent tubules between 100g and 400g groups were significant after first brushing (p<0.001). Following acid challenge, mean patent tubules increased to 218 (SD 40) in all samples (p<0.01) with no significant inter-group differences. Following further brushing, mean patent tubules decreased using 100g to 175 (SD 72) (p<0.01), but increased with 400g to 232 (SD 52). Differences in patent tubules between 100g and 400g groups were significant after second brushing (p<0.001). Tooth wear on all samples was a mean 2µm (SD 2µm).

Conclusions Tooth wear was minimal (2µm+2µm) and likely to be within the smear layer (10µm+5µm). At higher brushing forces (400g), more tubules were exposed whereas at lower brushing forces (100g), tubule patency decreases even post-acidic challenge.

Notes
Polymer Therapeutics for Sustained, Controllable Release of Growth Factors to Promote Cranial Nerve Repair and Regeneration by Stem Cells.

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Objectives Nerve injuries in the craniofacial region are common, difficult to treat and often result in unpredictable sub-optimal functional outcomes. Attempts to induce healing using stem cell implantation have shown some success. To facilitate stem cell proliferation and differentiation, growth factor (GF) supplementation is essential. However, rapid clearance of exogenous GFs from the target site and premature inactivation by proteolytic enzymes and reactive oxygen species have limited clinical success. This study investigated the ability of two model dextrin-GF conjugates (dextrin-EGF and dextrin-bFGF) to support in vitro stem cell proliferation and differentiation by sustained, controllable GF release and demonstrate their potential as a supplement for stem cell therapy.

Methods Dextrin-EGF and -bFGF conjugates were synthesized (using 51,000 g/mol dextrin with ~30 mol% succinoylation) having a protein content (BCA assay) of 3.9 and 6.7% w/w and a molecular weight (by GPC) of 190,000 and 180,000 g/mol, respectively. Proliferation and apoptosis of mouse neural stem cell (mNSC) was assessed over 7 days by MTT and TUNEL assays, respectively. Multipotency and differentiation of mNSCs was assessed immunocytochemically and quantified by fluorescent confocal microscopy.

Results In vitro supplementation of mNSC monolayer and 3-dimensional spheres with dextrin-GF conjugates led to greater and prolonged proliferation compared to unbound GF controls. Cells grown with a combination of dextrin-EGF/dextrin-bFGF conjugates showed the greatest and most prolonged proliferation profile, with no detectable apoptosis after 7 days of treatment, demonstrating their ability to controllably release GFs over time. Immunocytochemical detection of multipotency (nestin) and differentiation (olig2, MAP2, GFAP) markers of dextrin-GF-treated mNSCs verified that controlled release of GFs preserves the multipotency of mNSCs and enhances their ability to differentiate into nerve cells, compared to ‘free GF’-treated cells.

Conclusions These results show the improved stem cell survival and differentiation in the presence of dextrin-GFs, and demonstrate the potential that dextrin-GFs may offer for localised delivery of therapeutic agents for nervous tissue repair, including cranial nerve repair and regeneration.

Notes
Influence Of Nanopatterning On Oral Bacteria Adhesion onto Surfaces
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Objectives Adhesion of oral bacteria to artificial surfaces such as dental implants and restorations is the initial step in the process of biofilm formation and infection. Surface topography has been shown to play an important role in cellular attachment; however, the effect of nanoscale topography on oral bacteria adhesion and early biofilm formation has yet to be fully understood. Therefore, the aim of this research is to evaluate the effect of surface nanopatterning on the adhesion of Streptococcus sanguinis and Staphylococcus aureus at the nanoscale.

Methods Engineered nanostructured surfaces consisting of 120nm pits with 300nm centre-centre separation were employed, either in square arrangement (SQ) or with random offsets of up to 50nm in x and y (NSQ). Cultures of S. sanguinis and S. aureus in growth media were inoculated onto the surfaces and allowed to grow for 48hr (37ºC - 5% CO₂). After incubation, bacterial attachment to surfaces was assessed by focused ion beam (SEM-FIB) milling and atomic force microscopy (AFM) force spectroscopy.

Results SEM-FIB exploration of the bacteria-substrate interface showed intimate relationship between bacteria and surface nanopits for both studied groups. Further exploration with AFM force-spectroscopy demonstrated important differences in nanoadhesive behaviour for both S. sanguinis and S. aureus, which can help explain their clinical relevance in surface colonisation.

Conclusions Surface nanotopography plays an important role in the adhesion and colonisation of S. sanguinis and S. aureus to substrates at the nanoscale, as the presence of nanopatterning impacts bacterial nanoadhesion and colonisation. Therefore, design of intraoral artificial materials should take into account potential infection complications due to bacterial colonisation.

Notes
**Dentures: a pathogenic reservoir for respiratory pathogens**


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**Objectives** Pneumonia is the leading cause of death attributable to infection in patients aged 65 years and older, costing the NHS in excess of £440 million annually. Denture wearers may be at increased risk of aspirating opportunistic pathogens from the denture into their lungs. Dentures provide an ideal environment for biofilm formation, and microorganisms from this biofilm may disperse and be inhaled into the respiratory tract, potentially causing pneumonia in susceptible individuals. Few studies have inspected the denture surface for the presence of respiratory pathogens. Therefore, the aim of this study was to develop a specific and sensitive quantitative PCR assay to analyse denture plaque for the presence of known respiratory pathogens.

**Methods** 130 patients attending a prosthodontic clinic at Glasgow Dental Hospital were examined for the presence of denture stomatitis and the severity recorded using Newton’s Classification. The removable dentures of these patients were sonicated in phosphate buffered saline to remove denture plaque biofilm. The total DNA was extracted from the sonicate and specific primer pairs representing 9 known respiratory pathogens were used to perform qPCR.

**Results** 64.6% of dentures were found to carry respiratory pathogens. Six species were identified: *S. aureus, S. pneumoniae, P. aeruginosa, H. influenza B, S. pyogenes* and *M. catarrhalis*. 20% of dentures were colonised by two or more pathogens. *S. aureus* was identified from 67 dentures, which when re-tested using primers specific for the *mecA* gene demonstrated that 3% were MRSA. *P. aeruginosa* was the most abundant species with a mean count of $4.3 \times 10^6$ CFU’s. None of the samples were shown to be positive for *L. pneumophila, C. pneumoniae* or *K. pneumoniae*.

**Conclusions** Dentures are a reservoir for respiratory pathogens. These pathogens have the potential to be aspirated into the respiratory tract and cause infection.

**Notes**
Development of an In-Vitro Tissue Model for Biofilm Infection

Morse, D. J., Williams, D., Wei, X., Wilson, M., Lewis, M., Bradshaw, D.

1Cardiff University, 2Cardiff University, 3Cardiff University, 4Cardiff University, 5GlaxoSmithKline, 6Cardiff University

Objectives Denture-associated stomatitis presents as areas of inflammation on the palatal mucosa as a result of a biofilm on the denture-fitting surface. Our previous research has shown that bacteria in Candida biofilms influence Candida virulence and pathogenicity, but associated effects on host cells and responses are poorly understood. To facilitate research in this area, this study aimed to develop cost-effective 3D tissue models representative of oral palatal mucosa. The ultimate goal was to incorporate a basal layer of fibroblasts and monocytes in collagen, with overlying keratinocytes. Such a model would allow cytokine profiling and monocyte migration to be measured in response to biofilms.

Methods Fibroblasts were mixed with type 1 collagen at varying ratios, and added to cell culture inserts and incubated for 48-72 h. Medium was then aspirated from the surface of the membrane and keratinocytes added. After 24 h, the inserts were raised ensuring that the keratinocytes were at the liquid:air interface. Tissues were then cultured with medium changed every 2-3 days, prior to histological analysis.

Results Single culture fibroblast tissues were obtained by seeding at 1x10^6 cells with 10% collagen and incubation for 48 h. A well-established basal layer was observed under these conditions, which was less developed when lower numbers (≤5x10^5 cells) of cells were used. Single culture of keratinocytes (1x10^6 cells) over a five-day period was optimal for promoting tissue thickness, cell morphology and structural integrity. Co-culture was achieved with establishment of fibroblasts in collagen followed by addition of keratinocytes and lifting to the liquid:air interface for maturation.

Conclusions Single culture of keratinocytes generated a 3D tissue, which was similar in appearance to that of commercially available constructs. The model was cost effective and could be used as an alternative in tissue infection experiments. Single culture fibroblast and co-culture fibroblast/keratinocyte tissues were also established allowing more complex interaction analysis.

Notes
Interleukin-24 Is Expressed In Gingival Fibroblasts And Promotes Keratinocyte Interleukin-17

Williams, R. C.1, 2, Serrage, H.1, Rowan, A. D.2, Preshaw, P.1, 2, Taylor, J. J.1, 2
1Newcastle University, 2Newcastle University

Objectives The novel interleukin (IL)-10 family cytokine IL-24 is elevated in the skin and intestinal mucosa during psoriasis and inflammatory bowel disease, respectively, and is implicated in inflammatory processes. However, little is known about the cellular targets and functions of IL-24 in the oral cavity. We previously found IL-24 to be synergistically upregulated in human gingival fibroblasts (HGFs) by IL-1 and the adipokine leptin. Therefore, we investigated the signalling pathways responsible for IL-24 up-regulation in HGFs, and the effects of IL-24 on oral keratinocytes and HGFs.

Methods Primary HGFs isolated from healthy gingiva were pre-treated for 30 min with pharmacological inhibitors of signalling pathway intermediates or an anti-IL-24 antibody/isotype control before stimulation with combinations of the following recombinant proteins for 24 h: leptin (10 µg/ml), IL-1 (0.05 ng/ml), oncostatin M (OSM) (5 ng/ml) and IL-24 (10-100 ng/ml). Oral keratinocytes (OKF6 cell line) were stimulated with IL-24 (10-100 ng/ml) for 24 h. Gene expression was measured by RT-PCR, protein expression by Western blot and protein secretion by ELISA and Proteome Profiler Cytokine arrays (R&D Systems).

Results IL-1 and OSM synergistically increased IL-24 expression by HGFs. The JNK inhibitor SP600125, ERK inhibitor U0126, p38 inhibitor SB203580 and STAT3 inhibitor VI all significantly (p<0.001) reduced IL-24 gene expression in leptin+IL-1-stimulated HGFs, whilst Akt inhibitor VIII had no effect. Both HGFs and oral keratinocytes expressed the IL-24 receptor subunits IL-20R1, IL-20R2 and IL-22R1 at the mRNA level, and IL-22R1 at the protein level. IL-24-stimulated oral keratinocytes secreted increased IL-17A, cystatin C and extracellular matrix metalloproteinase inducer compared to unstimulated cells. In contrast, IL-24 did not affect basal or leptin±IL-1-stimulated IL-6 secretion by HGFs.

Conclusions IL-24 is produced by HGFs under pro-inflammatory conditions in a MAPK- and STAT3-dependent manner. It is possible that gingival fibroblast-derived IL-24 promotes pro-inflammatory responses in oral keratinocytes.

Notes
Cholinergic modulation of Candida albicans pathogenic potential
Rajendran, R.¹, O’Donnell, L. E.², Lappin, D. F.³, Ramage, G.⁴, Nile, C. J.¹
¹University of Glasgow, ²University of Glasgow, ³Dental School, ⁴Dental School

Objectives The opportunistic pathogen Candida albicans is the main aetiological agent of Denture Stomatitis (DS). The ability of C. albicans to switch between yeast and hyphal morphologies and form biofilms are major virulence factors. Evidence suggests that C. albicans can synthesise and respond to acetylcholine (ACh) signalling by as yet undefined mechanisms. The aims of this study therefore were to: (i) investigate associations between salivary levels of ACh and DS pathogenesis; (ii) investigate the role of ACh in dictating C. albicans yeast to hyphae transition and biofilm formation and (iii) investigate the receptor based mechanisms by which C. albicans can respond to ACh.

Methods Salivary levels of ACh were measured in a cohort of 68 denture wearers with and without DS using a choline/acetylcholine assay. The effect of ACh on C. albicans yeast to hyphae transition and biofilm formation were determined using crystal violet assays, phenotype microarray technology and scanning electron microscopy. The effect of a range of specific cholinergic receptor agonists and antagonists on C. albicans yeast to hyphae transition and biofilm formation were also investigated using the same methodologies.

Results ACh levels were found to be elevated in the saliva of DS patients and levels correlated significantly with levels of palatal inflammation, as determined by Newton’s score. Acetylcholine was also found to inhibit C. albicans yeast to hyphae transition and biofilm formation in vitro. Furthermore, preliminary pharmacological evidence suggests that C. albicans possesses specific cholinergic receptors which mediate ACh signalling.

Conclusions Elevations in salivary levels of ACh are associated with DS severity. C. albicans possesses functional receptors for ACh which can regulate the yeast to hyphae transition and biofilm formation. These data suggest a colonised host may upregulate ACh production in an attempt to inhibit the pathogenic potential of C. albicans.

Notes
Periodontal Pathogens and their Sialidases

1 The University of Sheffield, 2 University of Sheffield, 3 GlaxoSmithKline, 4 The University of Sheffield, 5 The University of Sheffield

Objectives The pathogens Porphyromonas gingivalis and Tannerella forsythia are associated with severe periodontitis and both possess sialidase enzymes (PGsia and NanH respectively) that contribute to their virulence. This work aims to investigate the molecular properties of these sialidases and their pharmaceutical inhibition in relation to host-pathogen interactions and biofilm formation.

Methods Sialidase activity of whole bacteria and purified sialidases was characterised using fluorescence based methylumbelliferyl or colourimetric thiobarbituric acid (TBA) assays. Sialic acid release from oral epithelial cells was investigated using fluorescence microscopy, and the sialidase inhibitor zanamivir was provided by GlaxoSmithKline, GSK, UK. In addition, the effect of zanamivir on host-pathogen interactions and biofilms was quantified using antibiotic protection assays and crystal violet staining and cell counting to quantify biofilm formation, respectively.

Results Pathogen sialidases were capable of acting on a variety of host substrates with varying efficacy, and enzymes were broadly active at a number of pH conditions with optimum at pH ~5.5. Furthermore, NanH and PGsia contain novel carbohydrate binding motifs that were characterised. While zanamivir was shown to inhibit sialidase activity of whole T. forsythia and P. gingivalis, and their purified sialidases, only P. gingivalis and PGsia were significantly inhibited (~70% and ~90% reduction in activity, respectively). Cleavage of cellular sialic acid by purified enzymes was reduced by the presence of zanamivir, and zanamivir was also shown to reduce host-bacteria association in P. gingivalis, T. forsythia, and sialidase negative Fusobacterium nucleatum.

Conclusions Novel periodontal pathogen sialidases release sialic acid from host sources, with variable efficacy of PGsia and NanH for different substrates perhaps indicating different roles for the two sialidases in the host. The effect of zanamivir on enzyme activity, biofilm formation, and host-bacteria association highlights both the importance of sialidases during disease, and the potential application of sialidase inhibitors during periodontitis therapy.

Notes
A Review of Approaches for Dental Practice Teams for Promoting Oral Health
1Plymouth University, 2Plymouth university, 3British Dental Association

Objectives In order to determine the circumstances in which oral health promotion (OHP) in General Dental Practice is at its most effective, a systematic review was conducted to identify, critically appraise, and synthesise the available evidence. The research question was: Is oral health promotion within dental practice effective and how can its effects be optimised?

Methods Systematic searches of 20 online resources (including Ovid Medine and Embase) were conducted. A call for evidence was also issued, and citation lists of other relevant systematic reviews were included. All studies published since 1994 that were set in the context of general dental practice and investigated promoting good oral health in adult or child patients were considered.

Results 44 studies reported in 52 papers were included in the review. The evidence was heterogeneous and the quality of reporting was variable. Results showed that oral health promotion based on behavioural and psychological models was effective for improving oral health. Verbal advice affected knowledge as well as reported behaviour, and written advice promoted oral health knowledge. There was moderate evidence that the attributes of the ‘sender’ of an oral health promotion message influenced its effectiveness. Many barriers and facilitators were shown to influence the effectiveness of OHP in dental practice.

Conclusions The results of this review suggest that the psychology of behaviour change is the key to oral health promotion and greater emphasis on teaching oral health professionals about health psychology would make oral health promotion in the dental surgery more effective.

Notes
Economic Evaluation of public health interventions to reduce caries in children at high risk

Kay, E. J.1, Owen, L.2, Sheppard, L.2, Taylor, M.3, Claxton, L.3

1Plymouth university, 2National Institute for Health and Care Excellence, 3York Health Economic Consortium

Objectives This study assessed the cost effectiveness of preventive oral health interventions to reduce caries in children. To date there are no published economic evaluations of the cost effectiveness of dental disease prevention.

Methods A decision analytic model was developed in which five key parameters could be varied. The model examined the costs and potential quality of life benefits associated with delivering fluoride varnish and toothbrushing schemes to 5 and 12 year old children in the UK. Reductions in caries risk were derived from published data. Baseline risk of caries for 5 and 12 year olds in the most deprived quintile (by Index of Multiple Deprivation) were obtained from national survey data. In order to address uncertainties around the key parameters, the model examined a range of alternative values for QALY loss associated with tooth decay/extraction and the costs of treatment.

Results For 5 year old children in the most deprived quintile, if all extractions are carried out under GA, the QALY loss is high (0.007) and the cost of treatment is £225, then spending up to £46 per child on toothbrushing or up to £62 per child on fluoride varnish can be considered cost effective at the NICE threshold of £20,000 per QALY. If QALY loss is low (0.002) and cost of treatment is £175, supervised brushing is still cost effective up to £27 per child and fluoride varnish up to £37 per child. For 12 year olds, for interventions that reduce caries risk by 11% and if GA extractions are common (80%) a spend of up to £23 per child is justifiable on the basis of £20,000 per QALY. If however a relative risk reduction of 39% can be achieved, spending up to £81 per child is justifiable. If 50% of extractions are carried out under GA, QALY loss is low (0.002) and treatment costs low (£175) spending more than £9 per child would not be considered cost effective.

Conclusions The analysis is limited by the need to make assumptions where data is absent. However, this model shows that for children at high risk of disease, supervised brushing and fluoride varnish schemes are cost effective public health interventions in terms of benefit to quality of life. The challenges of undertaking sound economic evaluation of oral health research will be demonstrated and discussed.

Notes
Feasibility of screening for diabetes in General Dental Practice
Bould, K. J., Dunne, S., Scott, S., Asimakopoulou, K.
King’s College London

Objectives The objective of this study was to determine the uptake of patients using the Finnish Diabetes Risk Score (FINDRISC) and HbA1c information as preliminary screening tools in general dental practice, in screening for possible diabetes, and determine the number of patients at risk of diabetes.

Methods Dental patients attending one of two dental surgeries for routine appointments who did not already have diabetes, who were aged 45 and over, and spoke fluent English, were offered the chance to be screened for diabetes using the self-report FINDRISC screening tool to assess risk of development of diabetes in the next 10 years. If a patient’s score showed them to be at risk, they were offered an instant HbA1c finger-prick test where they are given their result instantaneously. Patients found to be at risk on the FINDRISC, were referred to their GP for formal diagnostic testing.

Results N=1292 patients eligible for inclusion based on age, attended dental appointments over 118 days of recruitment. N=221 of these patients were then found to be ineligible due to not speaking fluent English and having a history of diabetes. N=515 patients refused to participate whilst N=520 patients consented for screening. N=259 patients were found to be ‘at risk’ of developing diabetes based on FINDRISC scores, and were referred to their GP for subsequent diagnostic testing.

Conclusions The study demonstrates a feasible method of diabetes screening that shows an acceptable rate of uptake by dental patients. It demonstrates a high number of patients ‘at risk’ of developing diabetes being referred to their GP.

Notes
Urinary fluoride excretion in children aged 4-5 years living at low and high altitude in Nepal
Sah, O. P.¹, Atkinson, G.², Maguire, A.³, Zohoori, V.⁴
¹Health and Social Care Institute, Teesside University, ²Health and Social Care Institute, Teesside University, ³Centre for Oral Health Research, School of Dental Sciences, Newcastle University, ⁴Health and Social Care Institute, Teesside University

Objectives A higher prevalence of dental fluorosis has been reported in high altitude communities vs low altitude communities. Therefore, specific changes in the body’s fluoride balance may occur in humans living at high altitude. To quantify the effects of altitude on urinary fluoride excretion (UFE) in children living at high and low altitude in Nepal.

Methods One-hundred healthy children, aged 4-5 years, were recruited from schools in a low altitude (100m) town, Rajbiraj, and a higher altitude (1500m) town, Banepa, in Nepal. Complete datasets were available for 43 low-altitude-living (LAL) children and 48 higher-altitude-living (HAL) children. Participants were visited at home to collect 24-hour urine, food and drink, and tooth-brushing expectorate samples. Fluoride concentrations of samples were measured by fluoride-ion-selective electrode using an acid-diffusion method for foods and toothbrushing expectorates and a direct method for urine and drinks. Nutritional status was estimated using WHO growth standards. A multivariable-adjusted general linear model was used to quantify mean HAL vs LAL difference in UFE, with covariates of nutritional status, sex and total daily fluoride intake (TDFI).

Results The mean (SD) TDFI for LAL children was 0.696 (0.361) mg/d vs 0.446 (0.223) mg/day for HAL children (95%CI: 0.131-0.369 mg/day, P<0.0005). In the multivariable adjusted model, mean (SD) UFE was 0.573 (0.321) mg/day for LAL children vs 0.313 (0.316) mg/day for HAL children (95%CI: 0.110-0.411 mg/day, P=0.001). When normalised to body mass, mean (SD) daily UFE was 0.038 (0.022) and 0.021 (0.022) mg/kg/day in LAL and HAL children, respectively (95%CI: 0.007-0.027 mg/kg/day, P=0.001).

Conclusions Urinary fluoride excretion of Nepalese children living at 1500m was substantially lower than children living at sea level, even after adjusting for differences in TDFI. Therefore, the risk of dental fluorosis for HAL children may be greater than for LAL children due to lower excretion and consequently higher body retention of fluoride. Supported by a grant from The Borrow Foundation.

Notes
Head Injuries in Early Childhood: Is There a Social Gradient?
Letelier, A.
University College London

Objectives Injuries are important causes for children's morbidity and mortality worldwide. Head injuries are the most prevalent type of injuries during early childhood, and are largely preventable. Evidence regarding the existence of a social gradient is inconclusive. This study aimed to examine if there is a social gradient in early childhood head injuries among UK children at ages three, five and seven.

Methods Data came from the UK Millennium Cohort Study, a UK prospective project that follows the lives of approximately 19,000 children. Logistic regression models were used to examine the association between head injuries and parental social class; household income, maternal education and area deprivation at ages 3, 5 and 7.

The sample size for the analysis for each sweep was: 15,382; 15,042 and 13,682 respectively.

Results No linear social gradients were observed for any marker of family socioeconomic position. At ages 3 and 5, head injuries were associated with area deprivation, low income and low social class. At age 7, only area deprivation was weakly related to head injuries. Head injuries were more prevalent among males at all ages. At age 3 and 5 the child's age was not significant, but at age 7 the association became significant. There was an association between head injuries and the mother's age at all sweeps.

Conclusions Prevalence of head injuries in early childhood was not socially graded. There appeared to be threshold effects in that children from the most disadvantaged backgrounds were most likely to experience head injuries, however these associations were weak. Potential limitations in relation to data quality will be discussed. These findings suggest that in the aetiology of head injuries, environmental factors such as area deprivation have an important effect in early-childhood head injuries prevalence; suggesting a threshold effect. Therefore socio-demographic determinants should be taken into account.

Notes
The impact of oral health risk indicator labels on prevention uptake

Sharma, S., Vernazza, J., Steele, J., and Finch, T.

1NEWCASTLE UNIVERSITY, 2Newcastle university, 3Oral Health Services Research, 4Institute of Health and Society

Objectives Informing patients about their disease risk may initiate behaviour change. Proposed changes in NHS dentistry (England) include the use of red, amber and green (RAG) oral health risk indicators for patients as part of a mandatory oral health assessment. These labels might have several different effects, including impacts on how patients engage with services as well as personal (home based) preventive interventions. This study aims to investigate the impact of oral health risk labelling (RAG) on patients’ understanding of risk and their motivation towards preventive behaviours.

Methods Semi-structured qualitative interviews were conducted with patients (11 Males & 9 Females, range: 22-83 yrs, avg: 40yrs) attending Newcastle Dental Hospital who had been given results of a RAG assessment. A focussed topic guide covered: Previous dental experiences, experience, emotional response and views on the presentation of RAG and intention to change behaviour. Interviews were recorded, transcribed and analysed using thematic analysis.

Results Findings suggest a range of different understandings of the risk assessment, attitudes towards risk reduction behaviours, and preferences for presentation of risk information. The colour system was seen as a series of “stages”. Patient responses towards their oral health behaviour on receiving risk information included statements of intent and feelings of empowerment. Aesthetics and a desire to change oral health emerged as their main motivating factors. The concept of shared responsibility emerged as an important aspect, with expectations of oral health care providers and of themselves differing between patients.

Conclusions The data provides an insight into patients’ explicit understanding of the RAG score assigned to their risk. The RAG system has the potential to elicit emotional responses which can create an intention to change behaviour, but with mixed views on responsibility. Nevertheless, the evidence suggests that improved understanding of risk can help improve patient engagement in preventive behaviour.

Notes
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PROSCAN 2100
SCANTRON IS THE WORLD LEADER IN NON-CONTACT PRECISION MEASUREMENT FOR DENTAL RESEARCH. LABORATORIES AND RESEARCH ORGANISATIONS AROUND THE WORLD CHOOSE PROSCAN 2100 FOR PILOT PROJECTS AND CLINICAL STUDIES.

Being ultimately practical, Proscan suits many applications and replaces expensive instruments capable of just one specific task. The measurements you need are achieved quickly and inexpensively, by equipment proven to last for many years with minimum maintenance and calibration.

EROSION

Proscan measures the direct effect of erosion on enamel and dentine by acid foods and drinks. Small specimens are mounted onto, or into, acrylic holders for in-vitro testing. The surface profiles are measured both before and after exposure to the erosive element.

Once scanned, a datum profile is produced for each sample showing sufficient detail to identify the enamel rod structure. The difference between the two scans is qualified either as height deviations or as a volume measurement.

ABRASION

Proscan is fast becoming the benchmark for enamel abrasion measurement. Favoured for its ease of use, the system easily captures the true surface profile of enamel both before and after abrasion testing.

Fast, accurate surface topography allows the user to qualify the exact amount of material abraded away, expressed either as height deviations or as a volume measurement.

ATTRITION

The entire topography of occlusal, incisal and proximal surfaces are captured through either in-situ modelling or in-vitro testing.

Accurate 3D measurement of the polished facets formed on the cusp tip or the flattening of the incisal edge allows qualification of wear levels, especially where analysis over time is concerned.

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