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ABSTRACTS
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An investigation into the relationship between apical root Impedance and canal anatomy

Aim To investigate a possible relationship between apical root impedance and canal anatomy.
Methodology Twenty-three roots from human extracted teeth (mostly single rooted but also from molars) with different apical anatomy were selected. The apical anatomy was initially classified by staining the root tip to identify number of canal exits; after impedance measurements, the anatomy was confirmed by staining and clearing the dentine. The roots were divided into two groups; 12 had simple (S) anatomy (Vertucci type 1 with a single exit) and 11 had complex (C) anatomy (various Vertucci canal types with multiple exist).Impedance measurements were taken using a frequency response analyser at seven levels in the root (0.0, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mm short of the apical terminus) at 14 frequencies ranging from 1120 to 100 000 Hz. Care was taken to control the temperature and other variables that could confound measurement accuracy. The impedance characteristics of individual roots were compared with 37 equivalent circuits (based on a pool created from a previous study); the best fitting equivalent circuit was selected. The equivalent circuits were used as the single outcome measure describing the impedance characteristics and correlated with the canal anatomy (S/C). Generalized estimating equations were used to perform logistic regression to analyse the data.
Results Canal anatomy had a significant ($P = 0.046$) effect on the equivalent circuit model. One circuit (model 10) was found to be the commonest and occurred significantly more commonly in the simple canals. The odds of prevalence of circuit model 10 were 2.2 times (odds ratio 2.17, 95% confidence interval 1.01–4.63) higher in canals with simple anatomy compared with canals with complex anatomy.
Conclusions Canal anatomy had a significant effect on the equivalent circuit describing its impedance characteristics. It should be possible to use impedance spectroscopy to clinically predict and image apical canal complexities.

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The effect of purmorphamine on murine osteoclast activity

Aims The aim of the study was (i) to establish and characterize a model to evaluate the effect of purmorphamine on murine osteoclastic activity; (ii) to test the hypothesis that purmorphamine-stimulated osteoclasts would resorb test calcium phosphate surfaces more than controls. The alternative hypothesis was that purmorphamine-stimulated osteoclasts would be associated with significantly less resorption than baseline controls.
Methodology In the characterization phase, cultured osteoclasts were able to resorb a calcium phosphate coating (CaP) allowing a simple but effective model to assay their activity. Bone marrow from 50 neonatal mice provided the source of osteoclasts that were seeded onto 100 CaP-coated discs to evaluate the effect of purmorphamine on their activity. Culture medium was used as a baseline control and bisphosphonate as negative control.
Results The characterization phase of the study demonstrated that a suitable CaP coating could be reproducibly precipitated onto the discs and that resorption through the action of TRAP-positive, multi-nucleate cells was quantifiable. Bisphosphonate negative controls showed no resorption. From a starting sample of 100 CaP discs, attrition through development and infection problems left five experiment and control pairs, the analysis of which showed that purmorphamine-stimulated osteoclasts were associated with significantly ($P = 0.043$) less resorption than baseline controls, indicating that it probably had an inhibitory effect on osteoclast function.
Conclusions Purmorphamine is an important bone agonist that could possibly be combined with grafting materials and induce bone regeneration. Within the limitations of the study, it can be concluded that purmorphamine does not induce differentiation of precursors into osteoclasts, supporting the alternative hypothesis. The next step would be to evaluate the properties of purmorphamine in conjunction with grafting materials (e.g. hydroxylapatite) by using implantation and usage tests in animals. In this way, the effect of purmorphamine on bone regeneration can be assessed and conclusions about its usefulness can be drawn.

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Biofilm disruption by root canal irrigants and potential irrigants

Aim To investigate the disruption and bactericidal effect of root canal irrigants on single and dual-species biofilms.
Methodology Single-species (*Streptococcus sanguis, Enterococcus faecalis, Fusobacterium nucleatum, Porphymonas gingivalis)* and dual-species (*S. sanguis* and *F. nucleatum*) biofilms were grown on nitro-cellulose membranes and immersed in either a commonly used root canal irrigant; (NaOCl, EDTA, Corsodyl®, iodine) or potential root canal irrigant [sodium dodecyl sulphate (SDS), cetyl trimethyl ammonium bromide (CTAB) and Tween®80] for 1, 5 or 10 min. The number of viable and nonviable bacteria disrupted from the biofilm and those remaining attached to the biofilm were determined using a viability stain in conjunction with fluorescent microscopy. In addition, confocal laser scanning microscopy (CLSM) was used to allow a visual assessment of the disruptive effects of selected agents on the stained biofilms.
Results Gram-negative species were more susceptible to cell removal than their Gram-positive counterparts, *S. sanguis* being the least susceptible. The majority of the cell disruption occurred after the first minute of exposure as all of the agents exerted some effect on bacterial disruption and viability; however, the extent varied according to the agent. The most effective root canal irrigant for disrupting biofilms was NaOCl whilst in contrast iodine was generally effective at bacterial killing but not disruption. Of the

potential root canal irrigants, CTAB and SDS were both effective in disrupting the biofilm and at bacterial cell-killing.
Conclusions Biofilm disruption and cell viability were influenced by the species, their co-association in dual-species biofilms and the test agent. The effectiveness of NaOCl as an endodontic irrigant was reinforced.

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The effect of conditioning films on colonization of root canal dentine by endodontic bacterial isolates

Aim To determine if conditioning films have an effect on the initial attachment of primary colonisers to root canal pre-dentine.
Methodology Noncarious, unrestored, extracted human teeth with single root canals were prepared to expose the pre-dentine. Half of the samples (n = 50) were untreated (native), whilst the other half (n = 50) were treated with 2.5% NaOCl for 10 min and 17% EDTA for 2 min. The samples were then soaked in a designated conditioning solution; artificial saliva, human saliva, serum or blood for 90 min at 37 °C. The samples were then placed into a Constant Depth Film Fermentor and inoculated with either single [*Streptococcus oralis* (E01-07-008)] or dual species clinical isolates [*S. oralis* and *Actinomyces naeslundii* (E01-07-007)]. After 4 h, the samples were removed and prepared for SEM and viable counts to assess the bacterial attachment on their surfaces.
Results Bacteria were observed on all samples when incubated with *S. oralis*. NaOCl/EDTA-treated surfaces had less *S. oralis* colonization for all conditioning films. The number of bacteria ranged up to 4.4 × 104 CFU per sample; specimens not chemically treated but soaked in artificial saliva exhibited the most colonization. This was 17.4-fold higher than the serum/chemically treated group. Colonization of specimens was over 90% lower with dual species than with a single isolate. Blood or serum-treated surfaces had greater colonization (24-fold difference in CFUs) for dual isolates than those treated with artificial saliva. *Streptococcus oralis* and *A. naeslundii* were capable of invading dentinal tubules within 4 h. *Streptococcus oralis* colonization was more common than that by *A. naeslundii* at the selected time interval. There was a random distribution of bacteria over the entire pre-dentine surface but more bacterial attachment was observed in 'secluded areas' such as 'giant tubules', in voids in the conditioning film or around debris.
Conclusions Bacterial colonization of pre-dentine surfaces appeared to be influenced by species, surface pre-treatment and conditioning agent.

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Cutting ability of a KiS 2D tip with varying powers and loads

Aim To correlate the vibrations of a retro tip under a range of loads and generator powers with its cutting ability.
Methodology A scanning laser vibrometer was used to measure the maximum vibration displacement amplitude of a KiS 2D tip (Obtura Spartan, USA) under loads of 10–50 g (10 g increments). Loads were applied by contacting the tip against a polished root dentine surface (1200 grit) and were measured using a load cell (RDP Electronics, UK). A calibrated scale was placed over the power dial of the generator (Piezon Master 400; EMS, Switzerland) which enabled incremental power settings (1–5) to be selected between minimum and quarter power of the total generator output. Ten repeat scans were made for each load and generator power combination. The retro tip was then used to create cavities in polished dentine surfaces. The tip was operated using the same experimental conditions as described above (five power settings, 10–50 g loads). For each condition, the tip was contacted against surfaces for 5 s and used to make 10 repeat cavities. Cavities were scanned with a 3D laser profilometer (TaiCaan, UK), enabling the maximum width and length of the cavity to be measured. The same generator and handpiece were used throughout the experiment.
Results Load significantly reduced tip vibration displacement amplitude at 30–50 g ($P < 0.0001$) when compared with 10 and 20 g. Conversely, it was found that loads of 30–50 g led to either no significant difference ($P = 0.242$) or even significant increases ($P < 0.02$) in the length and width of the cavities produced when compared with those obtained at 10 and 20 g. Profile views of the cavities, obtained through laser profilometry, suggest that greater loads may cause chipping of the dentine near the surface.
Conclusions It is clinically recommended that low loads are exerted on tips to reduce the incidence of fracture. This recommendation is supported by this work which showed that, at loads greater than 20 g, tip vibration displacement amplitude was significantly reduced. Although the length and width of the cavities produced increased at greater loads, it is suggested that the instrument breaks up dentine distant to the periphery of the instrument with a chipping action, potentially causing the instrument to be less precise.

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Transcriptome analysis of odontoblasts in primary and secondary dentinogenesis.

Aim To determine whether odontoblasts demonstrate differential gene expression during primary and secondary dentinogenesis.
Methodology Coronal pulps were removed from immature developing and mature erupted bovine teeth extracted from 30-month-old animals. RNA from odontoblasts was selectively extracted [McLachlan *et al* (2003) *Archives of Oral Biology* **48**, 373–83]. Microarray analysis (Affymetrix) was used to compare the transcriptomes between the two odontoblast populations. One hundred nanograms of total RNA were initially double amplified to generate cDNA. Twenty-five micrograms of the cRNA generated by *in vitro* transcription reaction was fragmented using 5× Fragmentation buffer and RNase-free water at 94 °C for 35 min to generate 35–200 base fragments. Fifteen micrograms of fragmented cRNA was reconstitued in a hybridization cocktail, which was applied to each array and hybridized for 16 h at 45 °C. The two samples were analysed in duplicate using four GeneChip Bovine Genome arrays (900561). Bioinformatic analysis was performed using three software programmes (D-chip, Onto-Express and Pathway express). In parallel, the expression of 20 genes were compared between two populations of odontoblasts by RT-PCR [Early stage (ES) and late stage (LS)] (ADM, AMEL, BMP4, Clock Genes, CollagenIII, CollagenI, DMPI, DSPP, MEPE, Msx1, Msx2, OSTEOCALCIN, PGAP I, TGFβ1, TGFβ1R, NESTIN, Alkaline Phosphatase, SHH, NaNog, LPR15).
Results Of the 24 000 genes analysed by microarray, 1338 were differently expressed in both populations by at least twofold (Paired