consecutive histological sections were obtained from the mid portion of the puncture site to act as the tests. Bone generation was quantified and the ratio of cross-sectional area of bone marrow to circumference of bone (outcome ratio) was determined amongst different groups using a software package, Image-Pro<sup>®</sup>Plus. The data were analysed using Mann–Whitney tests and Wilcoxon signed rank tests.

**Results** The outcome ratio in the test group was significantly (P < 0.001) smaller than the inter-bone and intra-bone control groups. There was evidence of bone formation directly over the carriers charged with pamidronate.

**Conclusions** The successfully developed test model was able to establish that local deposition of pamidronate had a positive effect on bone generation within a period of 7 days, regardless of the carrier used.

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## An audit of the technical quality of primary orthograde root canal treatment performed by undergraduate dental students

Aim To investigate the technical quality of primary orthograde root canal treatment performed by undergraduate dental students. Methodology A retrospective radiographic analysis of the technical standard of root canal treatment performed over 1 year of the undergraduate course was undertaken. All primary root canal treatments performed by late 4th and final year students between 1 April 2006 and 31 March 2007 were evaluated. All students had completed an introductory laboratory course using stainless steel hand instruments and Gates Glidden drills and a subsequent course using Nickel titanium instruments. Additionally, some students completed a voluntary one-term special study module (Endodontics) for 12 sessions. All radiographs of completed root canal treatments were assessed as to the technical quality of the root filling. Two calibrated observers assessed the radiographs under standard conditions using a lightbox and SDI radiograph viewer. The root fillings in each canal were assessed by length, satisfactory being within 0-2 mm of the radiographic apex, quality of obturation (absence of voids) classified as satisfactory, and taper, with the radiographic appearance of a uniform taper classed as satisfactory. The type of clinic, either a general restorative or endodontic was also recorded.

**Results** A total of 266 root canals were evaluated in 149 teeth. The final radiograph was unsuitable in four teeth and one tooth was extracted shortly after canal preparation. The remaining sample size was 253 canals in 144 teeth (61 anterior, 37 premolar, 46 molar). No endodontic instruments were fractured during the study. Overall, 163 individual root canals (64.4%) and 87 teeth (60.4%) were classed as satisfactory. Fifty-five canals and 21 teeth (one lower anterior, five premolars, 15 molars) were treated on the endodontic clinic, 46 canals (83.6%) and 15 teeth (71.4%) of these were satisfactory. A total of 198 canals in 123 teeth (60 anterior, 32 premolar, 31 molar) were

treated on the restorative clinic with 117 canals (59.1.6%) and 72 teeth (58.5%) being satisfactory.

**Conclusions** Overall, 60.4% of teeth met the criteria of root filling within 2 mm of the radiographic apex, no voids and uniform taper. A higher percentage of compliance was noted on the endodontic special study module.

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## Ion release characteristics, precipitate formation and sealing ability of a potential new root canal filling material

**Aim** To test a new polycaprolactone (PCL)/bioactive glass (BAG) composite as a root filling material capable of producing a seal under moist conditions by precipitation.

Methodology Formulations of PCL/BAG composites were made using the solvent casting method and then modified into a form physically deliverable into root canals. Root canals of single-rooted extracted human teeth were modified by de-coronation and removal of the apical 2 mm of the root. Canal instrumentation was performed with gates glidden drills (1% NaOCl and 17% EDTA irrigation) to achieve a degree of standardization of shape. The canals were filled with the composites using warm vertical compaction (the composites are thermoplastic and behave like gutta-percha) but without root canal sealer. A group of obturated teeth (n = 6 per composite formulation) was monitored for ion release (Na<sup>+</sup>, Ca<sup>2+</sup>, PO<sub>4</sub><sup>3-</sup>,  $P_2O_7^{4-}$ ,  $P_3O_9^{3-}$  and  $P_5O_{10}^{5-}$ ) into solution from the apical portion using ion chromatography. Three composite formulations were used to fill 10 roots each and half (n = 5)exposed to saline, whilst others were sectioned immediately; both were examined by light and scanning electron microscopy to evaluate adaptation, precipitate and film formation. A further group of 30 roots was obturated to evaluate seal formation by dve penetration; a control group (n = 10) consisted of conventional gutta-percha and Roth's sealer delivered in like manner to the test material; two test groups (n = 10 each) were obturated with a composite formulation producing the optimal precipitate, the apices of teeth in one group were immersed in saline for 7 days and the other not.

**Results** All samples released various amounts of ions which followed inversely to the concentration of iron oxide within the bioactive glass. Adaptation of the experimental material was better than the gutta-percha control groups. The formation of precipitate was evident in some specimens; in others film formation and dentine tubule entry by the material was observed. The dye penetration test showed that composite root filling specimens immersed apically in saline for 7 days had significantly less leakage (almost none) than those not immersed in saline at all (P < 0.001) or the gutta-percha control (P < 0.001).

**Conclusions** Polycaprolactone–bioactive glass composites may have potential as 'sealer-free' root filling material because of their ion release, precipitate formation and short-term seal. A. Iacovidou, N. Mordan, J. A. P. Figueiredo & K. Gulabivala Unit of Endodontology, Eastman Dental Institute, University College London, London, UK.

## Utility of the FISH technique in conjunction with confocal microscopy to study the intraradicular microflora of teeth associated with apical periodontitis

**Aim** To develop protocols to investigate the reliability of fluorescence in situ hybridization (FISH) using 16SrRNA probes to examine the composition and distribution of the biofilm along the length of the root canal using confocal laser scanning microscopy (CLSM).

**Methodology** Resin-embedded mixed cultures of *Porphymonas* gingivalis and *Fusobacterium*. *nucleatum* (n = 108) and artificially infected dentine specimens (n = 6) were used to optimize the FISH protocol. The following hybridization variables were tested: hybridization temperature, incubation time, probe concentration, wash regime and buffer composition. Ten extracted human teeth (13 roots) associated with apical periodontitis were fixed, processed and embedded in resin for generating sections to be viewed under conventional light microscopy (LM) and CLSM (FISH). Roots were sectioned transversely into thirds and each third sectioned longitudinally to reveal the canal; semi-thin sections were used in an

optimized protocol for bacterial detection and viewing of association between microbial flora and canal structures. Representative sections of each root segment were examined with each microscopy technique. Observations were recorded systematically.

Results After many trials and errors, a protocol for FISH was successfully refined for predictable application using the EUB338 probe but the specific probe (POGI) failed to give predictable and specific binding. Bacteria were detected in all 10 teeth by both microscopy techniques. A biofilm structure comprising of rods, cocci and filaments was observed in each sample. Overall, more bacterial cells were evident coronally than apically and penetration of the dentinal tubules was evident solely in the coronal and middle thirds. PMNs were found in some of the apical and middle root segments. An 'amorphous substrate' was consistently detected in the canal lumen using the CSLM but not using LM; it was contiguous with the visible cells and microorganism structures, implying that it formed an interface with these visible structures, possibly giving shape to their boundaries. Apparently loose-lying bacteria may therefore be 'enclosed' within a 'structure' unseen by LM, and therefore may correctly be termed biofilm rather than planktonic.

**Conclusions** A protocol for FISH was successfully developed for a universal 16SrRNA probe; its application to teeth with apical periodontitis gave interesting insights about the juxtaposition of bacteria and canal contents.

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