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ABSTRACTS

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Enterococcus faecalis and its prevalence in the oral cavity

Aim To evaluate the prevalence of *Enterococcus faecalis* in the human oral cavity by undertaking an opportunistic survey of oral rinses submitted to a regional diagnostic oral microbiology laboratory over a 7-month period.
Methodology Oral rinse specimens were serially diluted and spiral plated onto a series of nonselective and selective agar for *S. aureus*, *C. albicans* and *E. faecalis* (Slanetz and Bartley agar) to allow enumeration of bacterial growth. Presumptive colonies were identified using Gram stain, catalase reaction and API 32 strep. Clinical data were collected from laboratory request forms which included demographics, referral source and clinical details.
Results *E. faecalis* was detected in 6/47 (13%) of samples. Counts ranged from 160–2.1×10^4 colony forming units per mL of oral rinse sample. The majority of specimens (39/47) were submitted by the oral medicine clinic with a provisional diagnosis of oral candidal infection. Both *E. faecalis* positive and negative rinses also co-cultured *C. albicans*. Although there was a noticeable difference in the mean age of the *E. faecalis* positive (mean = 63.3) and negative (mean = 54.1) groups, using a two sample *t*-test, this was not statistically significant ($P = 0.074$).
Conclusion This study confirms previous findings of a low prevalence of *E. faecalis* in oral specimens. The study cohort is biased by the referral specimens sent to the diagnostic laboratory; in this case, the majority of patients were attending oral medicine outpatient clinics for oral mucosal disease. The small number of positive isolates precluded any meaningful statistical analysis of clinical data between *E. faecalis* positive and negative groups. However, the findings from this study would support a hypothesis that acquisition of enterococci in a high proportion of failed root-treatment cases is more likely from an exogenous rather than an endogenous oral flora source.

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Susceptibility of a mature multispecies endodontic biofilm to different chemo-physical treatments

Aim To compare the effectiveness of commonly used disinfection techniques on a multispecies mature biofilm grown in the root-canal space and in artificial lateral canals created in the middle and apical third of the root canal.
Methodology Seventy-two single-rooted teeth were instrumented with ProTaper (size F3), sectioned into two halves and re-assembled in a silicone putty matrix. Lateral canals were artificially created in the middle and apical third of the roots using a Twisted Files TF (size 25, 0.08). Multispecies biofilm consisting of *E. faecalis*, *A. radicidentis*, *P. acnes*, *S. Epidermidis* and *P. micros* were grown for 2 weeks on one half of each sample. Following reapproximation, the roots were divided into six groups of 12 teeth each, which are as follows: (i) control, no treatment; (ii) irrigation with 10 mL of saline; (iii) O_3 treatment; (iv) irrigation with 10 mL of 1% NaOCl; (v)irrigation with 10 mL of 1% NaOCl + O_3 treatment; (vi) 10 mL NaOCl with passive ultrasonic activation. Five SEM images per specimen (×700) (coronal, middle and apical thirds; middle and apical lateral canals) were taken using a standardized protocol. The images were randomized, and biofilm presence was assessed independently by three calibrated examiners. Differences between tooth levels and between treatments at each level were analysed using Kruskal–Wallis analysis of variance (ANOVA) with multiple post-ANOVA contrasts performed using Mann–Whitney *U*-tests. Intracanal and lateral canal paper point microbial sampling, performed by serial dilution and replating, were also undertaken.
Results Within each group, the imaging of the root canals showed no difference between coronal, middle, apical thirds and lateral canals. Comparison between the different techniques revealed significant differences. Saline was as effective as NaOCl in removing the biofilm at all levels except in the apical third. O_3 alone was not effective in removing the biofilm from any area of the canal space. NaOCl activated by passive ultrasonic did remove more biofilm in the middle lateral canal compared with any other treatment. Intracanal bacterial CFUs were reduced by 96.8% (87.9% lateral canals) in Group 4 and 97.8% (96.8% lateral canals) in Group 6; the other treatment modalities showed significantly less bacterial reduction ($P < 0.05$).
Conclusions Irrigation with NaOCl and with passive ultrasonic activation was found to be the most effective techniques for the removal of the multispecies biofilm from the apical part of the root canal and from the artificial lateral canals. *In vitro* development of a mature multispecies biofilm within the root canal space associated with the creation of artificial lateral canals may represent a suitable model to study the effectiveness of root canal disinfection.

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Survival of advanced periodontally involved teeth following root canal treatment and periodontal treatment including root amputation

Aim To assess the survival probability of advanced periodontally involved teeth undergoing both periodontal and root canal treatment, and to identify significant prognostic factors for tooth survival.
Methodology This prospective study involved the annual follow-up for up to 4 years of 132 teeth with advanced periodontal involvement (≥5 mm probing defects) and both periodontal and root canal treatment in an Eastman cohort of 87 patients (132 teeth). Pre-, intra- and postoperative data were collected on pre-designed proformae. Tooth survival probabilities by each prognostic factor were estimated. Prognostic factors were investigated using Cox proportional hazard regression models separately for teeth with ($n = 40$) or without ($n = 92$) subsequent root amputation.

Results Tooth survival for teeth that had undergone root canal treatment with furcation involvement and subsequent root amputation (92.5%, 37/40) was significantly higher (HR = 0.10; 95% CI: 0.03, 0.31) than teeth that did not undergo amputation (51%, 47/92). For those teeth that did not undergo root amputation, three significant prognostic factors were identified: smoking history (HR = 0.38; 95% CI: 0.12, 1.17); clinical attachment loss (HR = 1.12; 95% CI: 0.99, 1.27) and the presence of pus within the periodontal pocket (HR = 2.40; 95% CI: 1.36, 4.21). Although multivariable Cox regression analysis was not possible for those teeth that underwent root amputation, clinical attachment loss (HR = 0.57; 95% CI: 0.39, 0.82), pocket depth (HR = 0.55; 95% CI: 0.33, 0.92) and radiographic depth of the intra-bony defect (HR = 0.84; 95% CI: 0.73, 0.98) were found to have a significant effect on tooth survival using univariable regression models.
Conclusion The long-term prognosis of advanced periodontally involved teeth that underwent periodontal and root canal treatment without subsequent root amputation was poor (51% 4-year survival probability). Smoking history, clinical attachment loss and the presence of pus within the periodontal pocket were identified as significant prognostic factors.

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The effect of temperature on tooth surface strain during irrigation and loading

Aim To evaluate the effect of root canal irrigation with 5% sodium hypochlorite (NaOCl) at two temperatures (with saline as controls), on tooth surface strain (TSS) during irrigation and cyclic loading.
Methodology Thirty-six single-rooted premolar teeth with a single canal each had their crown and enamel reduced and the root canal prepared following a standardized protocol. Teeth were randomly allocated to six experimental irrigation groups: (A1) NaOCl at room temperature (RT) (21 °C); (A2) saline RT followed by NaOCl RT; (A3) saline 60 °C followed by NaOCl RT; (B1) NaOCl 60 °C; (B2) saline RT followed by NaOCl 60 °C and (B3) saline 60 °C followed by NaOCl 60 °C. TSS (μ) was measured using electrical strain gauges bonded to the cervico-proximal part of acrylic mounted teeth. The root canal of each tooth in groups A1 and B1 received nine consecutive 10-min irrigation periods (IP) with NaOCl (RT, 60 °C, respectively). The teeth in groups A2, A3, B2 and B3 received nine 10-min IP with saline, followed by nine periods with NaOCl (temperatures as mentioned earlier). TSS was recorded during nondestructive loading 2 min after each irrigation period. The pre-loading strain data (strain shift) (μ) were recorded at three time points; pre-irrigation (3 min from previous loading except for initial irrigation), post-irrigation (immediately following irrigation) and pre-load (2 min following completion of irrigation and immediately before loading) for each IP. The data were analysed using paired *t* tests and linear regression models.
Results There was a small but significant ($P = 0.04$) reduction in loading TSS (μ) over time when irrigating with saline RT for teeth in group B2 but not in group A2. Irrigation with saline at 60 °C, NaOCl at RT or 60 °C did not result in any significant change in loading TSS. The pre-load strain shift (μ) increased with loading TSS during NaOCl irrigation of teeth in groups A3, B2, and B3, but this finding was only significant for the first group.
Conclusions Root canal irrigation with NaOCl at 21 or 60 °C did not increase TSS. The recording of strain shift changes during the nonloading phase brought new insight into the immediate impact of irrigation with NaOCl.

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Ex vivo FTIR investigation of the effect of NaOCl irrigation on dentinal collagen changes influenced by root maturity, periodontal involvement and irrigation protocol

Aims *Phase I* – To evaluate the effect of EDTA surface treatment of specimens on the efficacy of FTIR detection of dentinal collagen changes caused by NaOCl irrigation in an *ex vivo* model; *Phase II* – To determine the effect of NaOCl irrigation on dentinal collagen changes as influenced by root maturity, periodontal involvement or irrigation protocol.
Methodology *Phase I* – Extracted human teeth with mature roots were irrigated in standard fashion. They were sectioned into discs with intact canal lumens and the coronal surfaces polished. FTIR analysis was carried out at two sites (inner = 0.5 mm from the canal lumen; and outer = 0.5 mm from the root surface) on this surface before and after treatment with 17% EDTA. Amide I/phosphate and amide II/phosphate absorbance ratios were calculated. *Phase II* – Teeth with mature roots and no periodontal involvement were irrigated with saline ($n = 7$), 5% NaOCl ($n = 7$) or 5% NaOCl + 17% EDTA ($n = 7$); whilst teeth with immature ($n = 7$) or mature ($n = 7$) roots and periodontal involvement were irrigated with 5% NaOCl. After irrigation, dentine discs were prepared, coronal surfaces polished and surface-treated with 17% EDTA and FTIR performed. The effect on dentinal collagen amongst mature teeth subjected to the test irrigation protocols and amongst mature or immature teeth irrigated with 5% NaOCl were compared.
Results *Phase I* – FTIR analysis of the saline or NaOCl irrigated samples before and after surface EDTA treatment only revealed significant ($P < 0.05$) reduction in collagen bands near the canal lumen after NaOCl irrigation with surface EDTA treatment of disc. *Phase II* – The dentinal collagen changes amongst the three test irrigation protocols in the mature roots were significantly ($P < 0.05$) different. The collagen changes in the immature roots were significantly ($P < 0.05$) greater compared with the mature roots with or without periodontal involvement; however, there was no significant difference between the latter two groups.
Conclusions EDTA surface treatment of polished coronal surfaces enhanced the ability of FTIR to detect collagen changes in dentine resulting from root canal irrigation; both root maturity and irrigation protocol influenced the ability of NaOCl to alter dentinal collagen near the canal lumen.

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Validation of a collagen film irrigation model (Huang *et al.* 2008) using a stained bacterial biofilm *ex vivo* model

Aim To validate the collagen film irrigation model findings (Huang *et al.* 2008) using an *ex vivo* bacterial biofilm model.
Methodology Forty-two human teeth with single straight root canals were randomly allocated to two groups for (i) static ($n = 21$) or (ii) dynamic irrigation ($n = 21$). The root canals were prepared to the same apical size and taper (40/0.08) and were split sagittally. The teeth were incubated with a human saliva inoculant within a constant depth film fermentor for 2 weeks. The internal root canal surfaces were stained with a dye (toluidine blue), photographed in a

standard manner and reassembled prior to sequential irrigation with 9, 18, 27 and 36 mL of 2.5% sodium hypochlorite, with either static or dynamic irrigation. The reduction in percentage of stained bacterial biofilm coverage was imaged and quantified using ImagePro® software. The data were analysed using a paired *t*-test and multilevel regression analysis.

Results The percentage of initial canal surface coverage with bacterial biofilm ranged from 26.3% to 98.5% which was not directly comparable with the controlled application of collagen in the Huang model but was comparable with the *in vivo* condition. There was a significant difference in the reduction in percentage of biofilm coverage between sequential stages of irrigation, regardless of whether it was static or dynamic ($P < 0.0001$); a finding consistent with the Huang model. There was no significant difference in reduction in biofilm coverage between static or dynamic irrigation or between different corono-apical levels within the tooth, these findings were not consistent with the Huang model but could be explained by the differences in behaviour between collagen and bacterial films. The reduction significantly ($P = 0.01$) increased with the amount of coverage at baseline, a factor which was controlled in the Huang model.

Conclusions The pattern of biofilm reduction validates the behaviour of the collagen simulant, but not entirely in its response to agitation. The large variation in pre-treatment biofilm coverage did not allow direct comparison with the Huang model.

Huang T-Y, Gulabivala K, Ng Y-L (2008) A bio-molecular film *ex vivo* model to evaluate the influence of canal dimensions and irrigation variables on the efficacy of irrigation. *International Endodontic Journal* **41**, 60–71.

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The use of graphics editing software to facilitate quantitative evaluation of digital images used within an endodontic irrigation research model

Aim To assess the viability of two image analysis techniques, when utilized to evaluate digital photographs that were taken as part of an endodontic irrigation research protocol.

Methodology Distinctive irrigation protocols were applied to root canals that had been split and painted with ink-stained collagen using a standardized protocol (Huang *et al.* 2005, Bryce *et al.* 2010). The root halves were photographed pre- and post-testing to allow visual assessment of the degree of removal of the stained collagen. The resultant images were paired, converted to grey-scale values with the ink-stained root canal cropped from the surrounding root anatomy using Adobe Photoshop CS2®. Two similar but distinct techniques were employed to quantitatively evaluate the grey-scaled images. Technique 1 examined the entire pixel range of the imaged canal by measuring the number of grey-scale pixels for each value set over a range of 0 (absolute black) to 255 (absolute white). An original grey-scale pixel range was calculated for the untreated tooth, with the cleaning effect of the irrigation protocol assessed by calculating the percentage of pixels remaining at each grey-scale value following subtraction of the pre-test figures. Technique 2 utilized a process where a specific grey-scale value (e.g. 0–30) was calibrated from a series of test images to determine the value at which the stained collagen membrane was completely removed from the root canal. The threshold value was applied to all photographs to allow the determination of percentage areas 'cleaned/ not-cleaned' by the irrigation protocol. The data sets for both techniques were analysed using UTHCSA Image Tool Version 3® and Microsoft Excel®.

Results Technique 1 allowed examination of all 'grey-scale' pixels along the value range. This allowed insight into degree of partial and complete removal of the stained collagen layer. Technique 2 found that an optimum threshold value of 50 gave the most accurate representation of 'cleaned' and 'uncleaned' areas within pre- and post-tested images. When a threshold value of 50 was applied, percentages of the canal areas that were classed as completely 'cleaned' were obtained. When this data was compared with the data sets obtained using Technique 1, a correlation coefficient of 0.98 was calculated.

Conclusions It was judged that Technique 2 gave a better summary of the areas completely 'cleaned' by the test protocol but, by applying both techniques, a better evaluation of the effectiveness of the root canal irrigation was obtained.