ABSTRACTS
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Co-aggregation studies on bacteria from infected root canals

Aim Co-aggregation phenomena may play a crucial role in root canal colonization and subsequent infection, and may be an important aspect of the selection process taking place. The aims were to determine intracanal and intercanal co-aggregation partners in bacterial strains isolated from infected root canal systems.

Methodology Bacterial strains isolated from the root canals of four intact teeth, and one strain of *Fusobacterium nucleatum* from a separate tooth were selected for the study. The isolates were grown in static culture at 37 °C in brain heart infusion broth. The cells were harvested by centrifugation and prepared for the co-aggregation study in an appropriate buffer. Aliquots (100 μL) of each pair of isolates (all combinations) were placed in a durham tube, mixed and left to settle. A visual co-aggregation assay was performed after 1–2 h after pairing and again 6–7 h later. Co-aggregation was judged by a predetermined 5-point visual assessment scale, which showed good reproducibility.

Results Co-aggregation results were identical at the different time points. Physical interaction between bacterial cells within a genus and between different genera was surprisingly infrequent; where it manifested in auto-aggregation (within strain) or co-aggregation, it was found to be species- and strain-specific. *Actinomyces viscosus, Rothia dentocariosa, Streptomyces scabei, Neiserria mucosa* and two unidentified strains displayed auto-aggregation. Intrageneric and intergeneric co-aggregation was observed both between strains from the same tooth and those from different teeth. Sometimes, strains that showed no intracanal co-aggregation were identified as co-aggregation partners with strains from different teeth. Intrageneric co-aggregation occurred between *Streptococcus* species, and intergeneric co-aggregation involved *Abiotrophia adiacens, A. naeslundii, Pediococcus urinaeequii, R. dentocariosa, S. scabei, Streptococcus gordonii* and *S. mitis*. The *F. nucleatum* strain tested for co-aggregation did not auto-aggregate or co-aggregate with any of the isolates it was paired with.

Conclusions This study showed that co-aggregation is species- and strain-dependent, and was surprisingly infrequent among the various pairings. The renowned associations between root canal isolates may be dominated by interactions other than physical, such as nutritional and physiological dependence.

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Utilization of relevant nutritional resources by root canal isolates

Aim To identify nutritional sources potentially available within the root canal systems that may allow the growth of bacteria.

Methodology All the cultivable bacterial species (*Aerococcus viridans, Actinomyces viscosus, Rothia dentocariosa, Streptococcus mitis, Corynebacterium durum, Pediococcus urinaeequi, Campylobacter gracilis, Fusobacterium nucleatum* and *Prevotella corporis*) from a single tooth infection were used. The nutrient sources tested included those potentially available from teeth (enamel, dentine, cementum, pulp tissue, saliva, serum and blood), artificial media (reduced transport fluid (RTF), brain heart infusion (BHI) and nutrient broth) and a control (phosphate-buffered saline (PBS)). Each medium was inoculated with each of the bacterial isolates in microtitre plates in doubling dilution series. All experiments were carried out in triplicate. The plates were incubated either aerobically or anaerobically, as appropriate. Bacterial growth was assessed as a function of relative optical densities (ODs) at different nutrient dilutions measured by spectrophotometry at 24 and 48 h. Mean values were calculated ($n = 3$) and the resultant data were analysed to determine the presence or absence of growth.

Results The effectiveness of artificial media in supporting the growth of the isolates was confirmed; BHI supported the growth of all isolates, but nutrient broth supported the growth of only six strains. As expected, PBS and RTF did not support bacterial growth. Of the dental tissues, enamel and dentine did not support bacterial growth, but cementum supported the growth of *C. durum and C. gracilis.* Pulp tissue only supported the growth of *A. viscosus and C. gracilis.* The body fluids

supported bacterial growth well. Blood supported growth of all bacterial isolates except *C. gracilis.* Serum supported the growth of all isolates except *P. urinaeequi and C. gracilis.* Saliva supported the growth of *A. viridans, A. viscosus, R. dentocariosa, C. durum* and *P. urinaeequi.*

Conclusions The differential support of growth by the nutrient sources revealed in the present study support the conclusion that during root canal infection, the causative bacteria may derive nutrition from a variety of locally abundant key tissues, but the principal components of hard and soft tissue present within teeth support bacterial growth only to a limited extent or does not support at all. The principal requirements are body fluids.

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Effect of customization of master gutta-percha cone on apical control of obturation using different techniques: an *in vitro* study

Aims (i) To compare the prevalence of root filling extrusion placed using three different obturation techniques, each with or without customization of the master gutta-percha cone; and (ii) to investigate the effects of various factors on the prevalence of root filling extrusion.

Methodology A total of 180 roots were selected and randomly allocated into three groups. Five general dental practitioners were recruited; each obturated one group of the roots using three techniques, namely cold lateral condensation ($n = 20$), Schilder's warm vertical condensation ($n = 20$) or continuous wave condensation ($n = 20$). Each technique was completed with ($n = 10$) or without ($n = 10$) customization of the master gutta-percha cone using chloroform. Two groups of the roots were recycled to allow all five operators to use them. Two observers examined the preinstrumentation, working length, master apical file and postobturation radiographs, and determined the presence of root filling extrusion and voids independently; they were blinded regarding the obturation technique used. The presence of root filling extrusion was also assessed by inspecting the root apex after obturation. The data were analysed using logistic regression models.

Results A total of 300 root fillings were performed and 291 were included for analysis. Most of the root fillings were placed within 0.5 mm of the working length (80%, $n = 233$); only 20% ($n = 58$) were placed >0.5 mm beyond the working length. The odds of prevalence of extrusion >0.5 mm were significantly reduced by approximately 50% when cold lateral condensation (OR = 0.50; 95% CI = 0.26, 0.99; $P = 0.04$) or customization of master gutta-percha cone (OR = 0.55; 95% CI = 0.30, 0.99; $P = 0.04$) was used. One operator produced 2.5 times more extruded root fillings than the other operators (OR = 2.50; 95% CI = 1.31, 4.78; $P = 0.006$). Other factors, such as root canal curvature and length, apical size of the prepared canal, as well as the operator's preferred obturation technique, were shown to have no significant influence on the prevalence of extrusion.

Conclusions The prevalence of extrusion was significantly lower when cold lateral condensation and customization of the master cone were used. The 'operator' emerged as a significant factor affecting the prevalence of root filling extrusion.

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Identification of *Enterococcci* isolated from canals of root filled teeth with periapical lesions and their antimicrobial susceptibility to different antibiotics

Aim The objective of the present study was to investigate the occurrence of *Enterococcus* spp. in root filled teeth with periapical lesions and the *in vitro* antimicrobial susceptibility of the isolates.

Methodology Sixty teeth with failed root canal treatment were included in the study. During nonsurgical endodontic retreatment, the root filling material was removed and the canals were sampled and microbiologically examined. *Enterococcus* ssp. isolates were tested for their antibiotic susceptibilities using the *E*-test system (AB BIODISK, Solna, Sweden). The following antibiotics were used: benzylpenicillin, amoxicillin, amoxicillin-clavulanic acid, erythromycin, azithromycin, vancomycin, chloramphenicol, tetracycline, doxycycline, ciprofloxacin and moxifloxacin. The strains were also tested for β-lactamase production with nitrocefin (Oxoid, Hampshire, England).

Results Microorganisms were recovered from 51 of 60 teeth. *Enterococcus faecalis* was recovered from 27 of the 51 canals with bacteria, 18 times in pure culture. All strains were susceptible to penicillins; however, the MICs of amoxicillin and amoxicillin-clavulanic acid ($MIC_{90} = 0.75\ \mu g\ mL^{-1}$) were lower than that for