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Potential systematic error in laboratory experiments on microbial leakage through filled root canals: an experimental study

Aim To assess the routes of bacterial leakage in a commonly used two-chamber model designed to evaluate root fillings.
Methodology Fifty-one intact human mandibular premolars with fully developed roots were used. They were left completely intact ($n = 23$), or were accessed, instrumented and either left open ($n = 5$) or root filled with gutta-percha and AH Plus ($n = 23$). All teeth were sealed between two chambers using sticky wax. The apical root aspects were left uncovered. The upper chamber was seeded with *Enterococcus faecalis*. An enterococci-selective broth was used in the lower chamber. Leakage was assessed for 120 days and compared using survival statistics ($\alpha < 0.05$). Subsequently, roots were trans-sected, stained using a 'live' DNA stain (Syto59) and inspected using confocal laser scanning microscopy. An *E. faecalis*-specific RNA probe was used for fluorescence *in situ* hybridisation (FISH).
Results Leakage started to occur from day 56, with further occurrence essentially identical between root filled teeth and intact counterparts ($P = 0.71$). All the trans-sections showed fluorescence related to Syto59 between the cementum layer and the sticky wax. Fluorescence was also observed between the root filling and the tubular dentine, whilst it was absent at the interface between root filling and sclerotic dentine. Secondary dentinal tubules, i.e. lateral branches connecting the main counterparts, contained fluorescent material. FISH revealed that Syto59 exclusively stained *E. faecalis*.
Conclusions The current experimental method proved to be unsuitable to compare root fillings. Histology revealed interesting observations regarding the relationship of dentine structure and bacterial leakage, which warrant further investigation.