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Influence of model system parameters on the sodium hypochlorite susceptibility of endodontic biofilms

Aim To evaluate the influence of different biofilm model system parameters on the sodium hypochlorite (NaOCl) susceptibility of endodontic biofilms.

Methodology Biofilms were formed in 96-well microtitre plates. First, monospecies *Enterococcus faecalis* biofilms were incubated for 24 h aerobically or anaerobically. In a second experiment, monospecies *E. faecalis* biofilms were anaerobically cultured for 1 or 11 days. Finally, a monospecies *E. faecalis* biofilm and a

multispecies biofilm including *E. faecalis*, *Fusobacterium nucleatum*, *Prevotella intermedia* and *Porphyromonas gingivalis* were grown anaerobically for 11 days. Biofilms were subjected to NaOCl treatment (0.025%, 0.1%, 0.5%, 2.5%, contact time 1 min), and control groups included treatment with purified water. After treatment, biofilms were harvested and the number of CFU was quantified by plate counting using general (monospecies biofilms) or selective media (multispecies biofilms). A one-way ANCOVA was conducted to explore the effect of the model parameters on biofilm eradication.

Results Anaerobically grown *E. faecalis* biofilms were eradicated to a greater extent than aerobically grown *E. faecalis* biofilms, demonstrating a significant effect of oxygen presence during

incubation on biofilm CFU count ($P < 0.05$). For all NaOCl concentrations (except 0.025%), one-day-old biofilms were more susceptible than 11-day-old biofilms, showing a significant effect of biofilm age on biofilm survival after NaOCl treatment ($P < 0.05$). Finally, *E. faecalis* was significantly more tolerant to NaOCl treatment when grown in a multispecies biofilm ($P < 0.05$), suggesting the presence of other microorganisms can affect the outcome of treatment of endodontic biofilms with NaOCl.

Conclusions The parameters incubation atmosphere, biofilm age and biofilm composition had a significant influence on the NaOCl susceptibility of the biofilm. These findings emphasize the importance of selecting relevant parameters when designing a biofilm model system.