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PMA-QPCR to Quantify Viable Cells in Disinfectant-Treated Oral Biofilms

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Objectives Conventional qPCR amplifies DNA from viable and dead cells, which can lead to an overestimation of live bacteria. The addition of propidium monoazide (PMA) to samples prior to DNA extraction inhibits DNA amplification from membrane-compromised cells. In this study, we evaluated the PMA-qPCR method to distinguish between viable and dead cells in oral multispecies biofilms treated with chlorhexidine (CHX) and sodium hypochlorite (NaOCl). The cell counts obtained by PMA-qPCR were compared with those obtained by culture.
Methods Experiments were performed with biofilms consisting of *Actinomyces oris*, *Fusobacterium nucleatum*, *Streptococcus oralis*, *Streptococcus mutans* and *Veillonella dispar* grown under anaerobic conditions for 64 h. Two different disinfectant procedures were applied: biofilms were either immersed in 0.2% CHX or 3% NaOCl for 2 min before cell harvest at the 64-h time point, or they were treated six times with the disinfectants for 2 min during growth at specific time points (16.5 h, 20.5 h, 24.5 h, 40 h, 44 h and 48 h). Six biofilms were analyzed for each treatment and control group (0.9% NaCl) by culture (CFU) and qPCR from samples with or without 50 µM PMA.
Results A good correlation was observed between bacteria counts estimated from culture and PMA-qPCR in the control biofilms and mature biofilms treated once with 0.2% CHX for all species, except for *F. nucleatum*, where PMA-qPCR detected significantly more bacteria than culture. Single treatment of biofilms with 3% NaOCl and six-fold exposure of biofilms to disinfectants resulted in no viable cell detection by culture. However, PMA did not completely inhibit PCR amplification in most samples.
Conclusions PMA-qPCR suggested the presence of intact but not cultivable *F. nucleatum* cells in biofilms. In samples with disinfectant-killed bacteria, complete elimination of PCR signals using PMA remained a challenge. Limits of quantification and detection for PCR assays can help evaluate background PMA-qPCR signals.