Title: Protocol for collecting mature biofilms from the root canal system for in vitro study in dentistry

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Abstract

Microorganisms, when colonizing the root canal system (RCS) after pulp necrosis, can form biofilms attached to the dentin walls and also leave the pulp cavity, reaching other regions through planktonic cells that detach from the biofilms. In dentistry, one of the major problems in evaluating the amount of bacteria present in the RCS is the way in which these bacteria are collected for subsequent counting. Bacterial biofilms are usually collected from the root canals by employing sterilized absorbent paper cones to promote bacteria absorption. This *in vitro* procedure is carried out this way in order to reproduce the *in vivo* collection procedure. However, certain regions of the RCS happen to be hardly reached by the paper cone as well as by instrumentation and irrigating solutions. The biofilm itself, when in dense and mature form, given around 21 days with inoculum changes from 24 to 48 hours, is well adhered to the walls of the RCS and also inside the dentinal tubules, presenting some bacteria, such as *Enterococcus faecalis*, which has greater penetration into the interior of the dentinal tubules. For *in vitr*o experimental procedures, in addition to absorbent paper cone, biofilm collection is performed by using sonication or hedstroem files. However, this mechanical approach can lyse many bacterial cells inside the root canal, leading a lower count of colony-forming units (CFU). In this sense, these procedures could bias the analytical results concerning the evaluation of the number of viable cells in bacterial biofilms. Therefore, this study aimed to evaluate the process of collecting mature bacterial biofilms formed in the SCR, using vortex agitation and ultrasonic vibration to detach the biofilm from the interior of the root canal, followed by centrifugation to collect the detached biofilms, avoiding lysis of bacterial cells. The collection with an absorbent paper cone provided approximately 7,590,000 CFUs, evidenced by scanning electron microscopy (SEM). The proposal experimental collecting obtained approximately 22,920,000 CFUs and showed by SEM few bacteria adhered to the entrance and interior of the dentinal tubules. The present study demonstrated that the proposal method is more efficient than the usual methods to collect and study viable biofilms in RCS.

Keywords: Dentistry; Endodontic; root canal; *Enterococcus faecalis*; biofilm.