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A novel experimental approach to investigate the effect of different agitation methods using sodium hypochlorite as an irrigant on the rate of bacterial biofilm removal from the wall of a simulated root canal model

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ABSTRACT

Objective. Root canal irrigation is an important adjunct to control microbial infection. This study aimed primarily to develop a transparent root canal model to study *in situ Enterococcus faecalis* biofilm removal rate and remaining attached biofilm using passive or active irrigation solution for 90 s. The change in available chlorine and pH of the outflow irrigant were assessed.

Methods. A total of forty root canal models ($n = 10$ per group) were manufactured using 3D printing. Each model consisted of two longitudinal halves of an 18 mm length simulated root canal with size 30 and taper 0.06. *E. faecalis* biofilms were grown on the apical 3 mm of the models for 10 days in Brain Heart Infusion broth. Biofilms were stained using crystal violet for visualization. The model halves were reassembled, attached to an apparatus and observed under a fluorescence microscope. Following 60 s of 9 mL of 2.5% NaOCl irrigation using syringe and needle, the irrigant was either left stagnant in the canal or activated using gutta-percha, sonic and ultrasonic methods for 30 s. Images were then captured every second using an external camera. The residual biofilm percentages were measured using image analysis software. The data were analyzed using Kruskal–Wallis test and generalized linear mixed model.

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Results. The highest level of biofilm removal was with ultrasonic agitation (90.13%) followed by sonic (88.72%), gutta-percha (80.59%), and passive irrigation group (control) (43.67%) respectively. All agitation groups reduced the available chlorine and pH of NaOCl more than that in the passive irrigation group.

Significance. The 3D printing method provided a novel model to create a root canal simulation for studying and understanding a real-time biofilm removal under microscopy. Ultrasonic agitation of NaOCl left the least amount of residual biofilm in comparison to sonic and gutta-percha agitation methods.

1. Introduction

Root canal treatment describes the dental procedure used to either prevent apical periodontitis by the treatment of diseased or infected soft tissue contained in the root canal system, or the procedure used to resolve established apical periodontitis [1], which is caused mainly by bacteria [2]. Bacteria adhere to the root canal surfaces and rapidly form biofilms [3]. A biofilm is defined as a community of microorganisms of one or more species embedded in an extracellular polysaccharide matrix that is attached to a solid substrate [4]. Thus, the essential aim of the root canal treatment involves the microbial control of the root canal system through instrumentation and irrigation. Instrumentation aims to give the canal system a shape that permits the delivery of locally used medications (*e.g.*, irrigant), as well as a root canal filling, which helps to entrap the remaining microbiota [5]. Irrigation also aims to lubricate the instruments, and, remove pathogenic microorganisms (microbiota) in the root canal system through the flushing action [6]. However, as the lubricated instrument is rotated along its long axis to sculpt the inner canal surface which it engages with, the most apical part of the canal remains untouched [7]. Thus, the use of a final irrigation regimen, after the completion of a chemo-mechanical canal preparation, with high volumes of various chemically active solutions may contribute to removing residual biofilm in the non-instrumented part of the root canal system [8].

The debridement action of an irrigant within the root canal system may remain elusive when using a needle and syringe alone [9]. Two phenomena are inherent to irrigant penetration and debridement action in the confined space of a closed root canal system. First, the stagnation of the irrigant flow beyond the irrigation needle tip [10]. Second, the gas bubbles or vapor locks effect ahead of the advancing front of the irrigant [11]. These phenomena may limit the delivery of irrigant to the canal terminus [12]. For the above mentioned reasons, attempts to improve the efficacy of irrigant penetration and irrigant mixing within the root canal system are therefore important [13] since they may improve the removal of residual biofilms. Irrigant agitation may be applied to aid the dispersal of the irrigant into the root canal system, especially into the periapical terminus of the canal [14]. Agitation techniques for root canal irrigants include either manual agitation [13,15–18] or automated agitation [17,18].

Manual agitation of the irrigant could be achieved by using a file [19] or a tapered gutta-percha cone [16], which is achieved by moving the master file or gutta-percha cone up and down in short strokes within an instrumented canal [20]. Automated devices for agitation of the irrigant in the root canal system include ultrasonic and sonic devices [17].

During ultrasonic agitation, a file oscillates at frequencies of 25–30 kHz in a pattern of motion consisting of nodes and anti-nodes along its length [21]. During sonic agitation of the irrigant, the file oscillates at frequencies of 1–6 kHz [22], and it produces lower shear stresses compared to ultrasonic agitation [23]. The EndoActivator system is a sonic device with polymer tips with a cordless electrically driven hand-piece [24].

The issue of the efficacy of irrigation protocol to remove bacterial biofilm has received considerable critical attention. It has been investigated by the immersing of samples in a static irrigant that neglect irrigant flow within the confinement of a root canal system [25–27]. Other studies used Computational Fluid Dynamics models to measure the physical parameters associated with irrigant flow within the root canal system, that lack the ability to estimate the chemical action of irrigant as they provide a virtual view of the root canal irrigation [28,29].

Although the use of extracted teeth might be clinically relevant, it may not be the optimum method as the root canal components (dentin, cementum) are concealed body compartments [30], making them unavailable for direct visualization. In addition, the use of extracted teeth of a different size introduces many variables to the studies [31].

Attempts to mimic the root canal anatomy using gypsum converted to hydroxyapatite [32,33] have shown promising anatomical features, but such opaque materials did not allow direct visualization. The use of 3D printing models to study root canal disinfection has been explored in a preliminary study [34], but the tested steriolithography material, Visijet® EX200 Plastic did not allow bacterial colonization and was not transparent. It seems justifiable to develop an *in vitro* model that provides transparency and generation of multiple samples with the same anatomical features to investigate the real-time interaction between the activated irrigant and biofilm removal during the irrigation process.

This study aimed primarily to develop and utilize transparent test models to facilitate an investigation into the influence of NaOCl agitation on the removal rate of *Enterococcus faecalis* biofilm subjected to sodium hypochlorite irrigation. A further aim was to compare the residual biofilm and removal rate of biofilm when subjected to passive (stagnant) and active irrigation (2.5% NaOCl). Finally, the outcomes of chemical