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Original Article

Comparative evaluation of antimicrobial substantivity of different concentrations of chlorhexidine as a root canal irrigant: An in vitro study

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ABSTRACT

Introduction: The aim of this in vitro study was to evaluate and compare the antimicrobial substantivity of different concentrations of chlorohexidine as root canal irrigant by microbiological assay using *mitis salivarius bacitracin streptomycin*, MSBS agar plate.

Methods: Extracted single rooted permanent human teeth were selected as samples and randomly divided into four groups (three experimental, one control). The samples of the three experimental groups used different concentration of chlorhexidine, CHX (0.1%, 1% and 2%), deionized water used as an irrigant served as control. In each group the apices of teeth were sealed with composite and mounted on plaster blocks. Root canals were prepared using step back technique and enlarged upto no 80. With each change in the file size the corresponding irrigant was used and final irrigation was done with deionized water. Samples were taken with paper points at 12 h, 1 day, 2 days and 3 days respectively and stored in sterile phials which were then arranged on MSBS agar plates for microbiological assay.

Results and conclusion: Results were analysed by ANOVA and Tukey's HSD test showed that antibacterial substantivity of 2% CHX was best followed by 1% CHX and 0.1% CHX in decreasing order respectively.

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1. Introduction

The aim of endodontic treatment is to preserve the tooth in the dental arch in healthy/return the tooth to health, therefore allowing it to be functional. Endodontic treatment can be divided into three main phase: biomechanical preparation of the root canal (cleaning and shaping), disinfection and obturation. The initial step for cleaning and shaping the root canal is proper access to the pulp chamber that leads to straight-line penetration of the root canal orifices. The next step is exploration of root canal, extirpation of the remaining pulp tissue or gross debridement of necrotic pulp tissue, and verification of the instrument depth. This step is followed by proper instrumentation, irrigation, debridment and disinfection (sanitation) of root canal. Obturation usually completes the procedure, if any of above described step is faulty, root canal treatment may fail. During biochemical preparation, the use of an irrigant is mandatory to wipe out the remnants of pulp tissue and resultant organic debris.[1]

An ideal irrigant should be biocompatible, antimicrobial, be able to lubricate the canal walls, prevent smear layer formation and have substantivity.[2] The most widely used irrigating solutions are physiological saline solution, 30% urea, sodium hypochlorite (NaOCl), urea-peroxide, 15% EDTA, Endo PTC combined with Dakin's solution, a mix of sodium hypochlorite (NaOCl) and hydrogen peroxide, 2% chlorhexidine and many more but no single irrigant meets all these requirements.[1,3]

Chlorhexidine gluconate is an effective oral antimicrobial agent. It has been used in periodontal therapy in caries prevention and as a therapeutic agent for oral infections in general. Chlorhexidine possesses many properties i.e. a broad-spectrum, substantivity (extended residual activity) and a relative absence of toxicity, that suggest it may be useful as an endodontic irrigant.[4]

2. Methods and aims

The aim of the study was to evaluate and compare the antimicrobial substantivity of different concentrations of chlorohexidine (CHX) as root canal irrigant by microbiological assay using *mitis salivarius bacitracin streptomycin* (MSBS) agar plate on extracted human permanent teeth.

This study used forty single rooted human permanent teeth with close apices were randomly divided into four different groups viz. three experimental (0.1%, 1%, 2% CHX) and one control (sterile deionized water) having ten samples each as shown in Table 1. In each tooth of Group 1, before biomechanical preparation the apex was sealed with composite material (Filtek, 3M ESPE St Paul, U.S.A) using light cure as per manufacturer's instructions and mounted on plaster blocks. The access opening was done using a high speed hand piece and root canal instrumented using a step-back technique with K-file (Denstply India pvt. Ltd) to a size of no. 80 and the canal was irrigated with the 1 ml of corresponding irrigant with each change in file size. Final irrigation was done with sterile deionized water.

Canal was dried with absorbent paper points and filled with sterile deionized water and teeth placed in the humidifier (NuAire, Plymouth, Minnesota (MN)) for 6 h. After 6 h, paper point kept in the canals for 2 min, removed and stored in sterile phials. Same procedure was repeated at 12 h, 1 day, 2 days and 3 days respectively and samples stored in phials. Similarly the procedure was done for the other groups (2, 3 and 4).

Table 1 – Distribution of samples and groups.

Group	Irrigants	Number of samples
1 [Experimental]	0.1% Chlorhexidine	10
2 [Experimental]	1% Chlorhexidine	10
3 [Experimental]	2% Chlorhexidine	10
4 [Control]	Sterile deionized water	10

Within 24 h of the last sample taken from each tooth, paper points were tested for antimicrobial activity. Freshly prepared Todd Hewitt broth culture of *Streptococcus mutans* (*S. mutans*) strain ATCC 25175 (American Type Culture Collection) was used for cultivation. Bacitracin (200 units/ml, HiMedia) and streptomycin (200 mg/ml, HiMedia) were added in Mitis Salivarius agar base to form MSBS agar plate before cultivation. *S. mutans* was spread over MSBS agar plate with sterile swab and allowed to dry for 30 min at room temperature. Then paper points were removed from the phials and placed on the MSBS agar plate in clock-face pattern, which were then incubated in an anaerobic jar (McIntosh Jar) at 37 °C for 48 h. The metallic scale calibrated in millimeters and centimeters was kept at 90° to the absorbent paper point and inhibitory zones were measured in millimeters, with the help of magnifying lens. The data collected was tabulated and subjected to statistical analysis using Analysis of Variance [ANOVA] and Tukey's HSD multiple comparison test (Figs. 1–4).

3. Results

The effect of three groups (CHX concentrations) on zone of inhibition (mm) were observed over the periods (0–6 h, 6–12 h, 12–24 h, 24–48 h and 48–72 h). A parallel control (Group 4) i.e. of deionized water was done which showed 0.00 mm zone of inhibition at all periods therefore was not included in the analysis of variance (ANOVA).

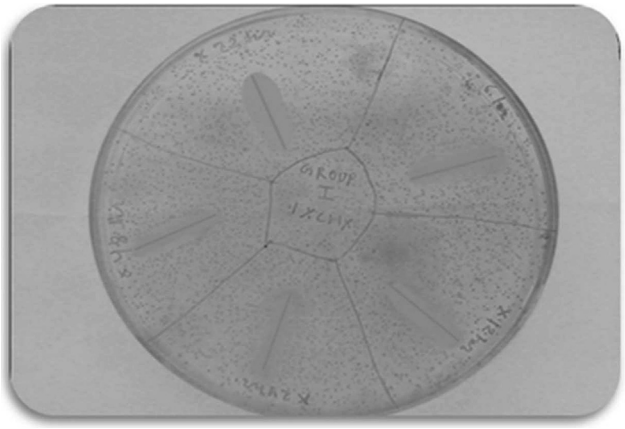

Fig. 1 – Group 1 (0.1% CHX).