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Effect of intracanal medicament gel materials separate and in combination in the elimination of *Enterococcus faecalis* biofilm

N.M. Moqbel [a,*], S.M. Ibrahim [a], G.E. Eid [a], H.K. Ibrahim [b]

[a] *Endodontics Department, Faculty of Oral and Dental Medicine, Cairo University, Egypt*
[b] *Faculty of Pharmacy, Cairo University, Egypt*

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Abstract

Objective: This study was to evaluate the antimicrobial efficacy of prepared medicament gels on induced *Enterococcus faecalis* biofilm using the fluorescent microscope.
Methods: The rheological determination was done for the prepared medicaments, namely: ECHXT (mixture of EDTA + CHX + Triss buffer), ENaOCl (2.5% NaOCl/18.6% EDTA gel) and ECHX (18.6% EDTA/17% CHX gel). Antimicrobial efficacy of each medicament gels was evaluated by bacterial viability method as examined using the fluorescent microscope. Fifty-five distal roots of extracted lower molars were contaminated with *E. faecalis* and incubated for 14 days at 37 °C. Scanning Electron Microscope was used to confirm the biofilm formation in five samples. After mechanical preparation, Forty five roots were divided into three equal groups according to the used medicament gel, and incubated for 1, 24 h and 1-week.
Results: In fluid samples, there was no statistically significant difference between the three medicaments after recommended time interval. While in dentin shavings samples; ENaOCl and ECHX groups showed the statistically significant lowest mean viability after 24 h. The statistically significant highest mean viability percentage was with the fluid samples when compared with the dentin shaving.
Conclusion: Variation in the physical nature of irrigant improved its ability to penetrate the dentinal tubules, while minimizing the time of application.

Keywords: Chlorohexidine; *Enterococcus faecalis*; Intracanal medicament; NaOCl

1. Introduction

Bacteria and their by-products play an essential role in the initiation and perpetuation of pulpal and periapical diseases [1]. *Enterococcus faecalis* occurs primarily in retreatment scenarios; it survives in root canals as a single organism without the support of other bacteria [2]. Various substances have been used during and immediately after root canal preparation to remove debris and necrotic pulp tissue and to help eliminate microorganisms that cannot be reached by mechanical instrumentation [3].

* Corresponding author. Box 71, El Malek El Salah, Cairo 11559, Egypt. Tel.: +20 1092880566.
E-mail address: nawal_mahfoud@hotmail.com (N.M. Moqbel).
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Sodium hypochlorite is to date, the most commonly used root canal irrigant. However, no general agreement exists regarding its optimal concentration, which ranged from 0.5% to 5.25%.The gel form overcomes the side effects of NaOCl solution when introduced beyond the tooth apex [4]. Chlorohexidine in a higher concentration has antimicrobial activity against a wide range of Gram positive and Gram-negative organism as well as yeast, facultative anaerobes and aerobes [5]. While in a lower concentration, it has a bacteriostatic effect [6]. Chlorohexidine when used as an intracanal medication in a gel form demonstrated a good performance [7]. EDTA as a chelating agent when used in 18.6% had a better cleaning effect [8].

The aim of the present study was to evaluate in-vitro the antibacterial effect of prepared intracanal medicament gel materials against induced *E. faecalis* biofilm through the presence of viable microorganisms using the fluorescent microscope.

2. Materials and methods

2.1. Part I: preparation of the medicament gel

Ethical committee, Faculty of Oral and Dental Medicine Cairo University, approved the present study. Intracanal medicament gel materials were prepared; namely ECHXT (0.01% EDTA[1] + 0.01% CHX[2] + 6% Triss buffer[3] + 12% HPMC 15 cps polymer[4]), ECHX (17% CHX with12% HPMC 15 cps polymer and 18.6% EDTA with 2% Sodium alginate polymer[5]), ENaOCl (2.5% NaOCl[6] with 15% Guar gum polymer[7] and 18.6% EDTA with 2% Sodium alginate polymer) and rheological determination was done. Using a cone and plate Brookfield viscometer for each of the freshly prepared medicament gel material and the stored for 2 h.

2.2. Part II: application of the medicament gel

Fifty-five distal roots of extracted lower molars were standardized to 12 ± 1 mm length and up to #20 K- file[8] apex's width. A cervical seat was created of 1 × 1 mm dimensions at the coronal third of the root. The root surfaces were coated with varnish and root apices were sealed with cyanoacrylate. Each root was placed separately inside Eppendorf tube and autoclaved for 15 min at 15 Psi and 121 °C. To confirm the procedure of sterilization; random sample ($N = 5$) from sterilized teeth were incubated in brain heart infusion broth for 48-h at 37 °C. All the next steps were performed under aseptic conditions inside class laminar flow cabinet with HEPA filter.[9]

2.2.1. Induced biofilm in initially prepared root canals

The canals were contaminated with 1 McFarland bacterial suspensions of *E. faecalis* (ATCC 29212) using sterile insulin syringe (gauge 27) and incubated for 14 days at 37 °C. Refreshing broth was added every 48 h throughout the incubation period. Randomly five roots were selected to confirm the formation of *E. faecalis* biofilm by SEM[10]. The roots were grooved vertically using a diamond disc without touching the canal. Then they were longitudinally splited into two halves.

Each half of root was immersed in 2.5% Glutraldehyde, pH 7.4, at 4 °C for 24 h for fixation, washed with Phosphate buffer solution (PBS) for 15 min, and post-fixation for 12 h at 4 °C to 6 °C in 1% (wt/vol). Osmium tetroxide. PBS was used as a final wash. Dehydration was performed with an ascending acetone series (30%, 60% and 100%) for 10 min each. Each sample was mounted and coated with a 200 Å layer of gold palladium. Canal observations were performed by using SEM. Representative samples were taken at 5000-X magnification for each third.

2.2.2. Mechanical preparation of the root canal

The root canals were prepared using K_3 Ni–Ti rotary system[11] using crown-down technique. The sequence used was as follows; #40, #35, #30, #25, #20 and #15. The K_3/0.6 taper sequence used in the apical third were from #25 up to #40. During instrumentation, the canals were irrigated with 2-ml of sterile distilled water.

[1] Ethylenediaminetetraacetic acid, Oxford, Mumbai.
[2] Chlorohexidine HCL, The Arabic drug company, Cairo A.R.E.
[3] Oxford, Mumbai.
[4] Hydroxyl propyl methyl cellulose polymer, Cairo Drug Company, Egypt.
[5] Oxford, Mumbai, India.
[6] Commercial Clorox, The Egyptian company for house cleanser, Cairo, Egypt.
[7] Loba Chemie, Colaba, Mumbai, India.
[8] Mani, Tochigi, Japan.
[9] Nuair, U.S.A.
[10] FEI, Holland.
[11] SybronEndo, Orange,CA.