Scanning electron microscopy evaluation of chitosan and carboxymethyl chitosan as retrograde smear layer removing agents

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Abstract

Background: The smear layer acts as a physical barrier against penetration of root canal medicaments and sealers, thus compromising the seal leading to microleakage.

Objectives: This study was conducted to evaluate the efficacy of 17% ethylenediaminetetraacetic acid (EDTA), 0.2% chitosan solution, and 0.2% carboxymethyl chitosan (CMC) used as smear layer removing agents in retrograde root canal preparation using scanning electron microscopy (SEM).

Materials and Methodology: Eighty single-rooted teeth extracted for periodontal reasons were collected for the study. Root canals were prepared and obturated with gutta-percha coated with AH plus resin sealer. Apical 3 mm of each root resected and Class I retrograde preparation carried out using ultrasonic handpiece and ultrasonic retro tips to a depth of 3 mm along the root long axis. In Group 1 (control), normal saline solution alone was used for smear layer removal. In Group II, 17% EDTA, Group III and IV were treated with 5 ml of 0.2% chitosan and 0.2% carboxyl methyl chitosan, respectively, for 3 min. Blinded evaluation of specimens using SEM was performed independently by two operators who registered the amount of the smear layer present on the surface of the canal walls based on the score described by Hülsmann et al.

Results: Group I (saline) was least efficient in the removal of the smear layer. Group II (17% EDTA), Group III (0.2% Chitosan), and Group IV (0.2% CMC) efficiently removed the smear layer from the retrograde cavity with mean scores 1.35, 1.60, and 1.35, respectively. Statistically, no significant difference found in Group II (17% EDTA), Group III (0.2% Chitosan), and Group IV (0.2% CMC).

Conclusions: About 0.2% CMC and 0.2% chitosan can be better alternatives to 17% EDTA for smear layer removal due to their biological advantages.

Keywords: Carboxymethyl chitosan; chitosan; ethylenediaminetetraacetic acid; smear layer

INTRODUCTION

When the root canals are instrumented during endodontic therapy, a layer of material composed of dentine, remnants of pulp tissue and odontoblastic processes, and sometimes bacteria, is formed on the canal walls. This layer has been called the smear layer. It has an amorphous, irregular, and granular appearance under the scanning electron microscope.

Surgical endodontic treatment should be considered when nonsurgical root canal treatment fails to treat...
periapical infections of endodontic origin. Apicoectomy and retrograde cavity preparation always associated with smear layer formation on dentinal surfaces. The smear layer consists of the necrotic pulp tissue, inorganic debris, microorganisms, and their by-products. The presence of such smear layer acts as a physical barrier against penetration of root canal medicaments and sealers, thus compromising the seal leading to microleakage. The smear layer also serves as a reservoir for bacterial proliferation.

Sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA), the mixture of tetracycline acid detergent, organic acids (citric acid), have been advocated for the removal of smear layer for both orthograde and retrograde root preparation. The alternating use of EDTA and NaOCl has been recommended for the efficient removal of the smear layer. However, this combined irrigation regimen causes inadvertent erosion of the intraradicular dentin. Silva et al. proposed that the use of 15% EDTA, 0.2% chitosan, and 10% citric acid effectively removed the smear layer from apical thirds of the root canal. The use of EDTA has been shown in vitro to inhibit the substrate-adherence capacity of macrophages when extruded into the periapical tissues, thus reducing both periapical inflammatory reactions and periapical healing. Hence aiming at minimizing its harmful effect on periapical tissues, the search for more biocompatible solutions than EDTA continues.

Chitosan is a natural copolymer of glucosamine and N-acetylg glucosamine produced by the alkaline and the partial deacetylation of chitin from shrimps and crustaceans shells. Chitosan has a high chelating capacity for various metal ions. It has properties of biocompatibility, bioadhesion, biodegradability, and antimicrobial activity. Carboxymethyl chitosan (CMC) was introduced to overcome the lower solubility of chitosan. Hence, this study was evaluated smear layer removal capacity of chitosan and CMC solutions from retrograde cavity preparation.

MATERIALS AND METHODOLOGY

The sample size calculated by keeping the power of the study at 80%, alpha error 5%, effect size 0.92, and mean standard difference (d) is 0.54. The formula used was:

\[ n = \frac{2(S^2)}{d^2} \left( Z_{0.02} + Z_{0.01} \right)^2 \]

Where, \( Z_{0.02} = Z\text{-} value \) for \( \alpha \) level = 1.96 and \( Z_{0.01} = Z\text{-} value \) for \( \beta \) level = 1.96

Eighty single-rooted teeth, extracted for various orthodontic and periodontal reasons have been chosen for the study. Teeth were stored in physiologic saline until the sample was completed. Teeth were decoronated at the level of the cementoenamel junction using a diamond saw. Root canal preparation was completed in a crown-down manner with ProTaper rotary Ni-Ti files (Dentsply Maillefer, Switzerland) up to F3 size. After each instrumentation, 3 ml of 3% NaOCl (Prime Dental Products Pvt., Ltd., Thane, Maharashtra, India. Lot No. 111130-01) was used for irrigating root canals.

Canals were obturated with 6% gutta-percha coated with AH plus resin sealer. Apical 3 mm of the root was resected using a carbide bur (Zekrya; Maillefer Dentsply, Baillagues, Switzerland) at a plane perpendicular to the long axis of the root. Following resection of the root end, a class I retrograde preparation carried out using ultrasonic handpiece (Mini Piezo; EMS, Nyon, Switzerland) and ProUltra (Maillefer Dentsply) ultrasonic retrotips to a depth of about 3 mm along the root long axis. Debris was initially washed off using a normal saline solution.

Based on the solution being used to treat the retro smear layer, the specimens were categorized into four groups of twenty teeth each. In Group 1 (control), normal saline solution alone was used for smear layer removal. In Group II, 17% EDTA was used for about 30 s. Experimental Groups III and IV were treated with 5 ml of 0.2% chitosan and 0.2% carboxyl methyl chitosan for 3 min. After treating with these agents, the specimens were sectioned longitudinally with the help of hard tissue microtome (Leica SP 1600, Leica Biosystem, Germany) for the scanning electron microscopy (SEM) (Carl Zeiss, Japan: Neon 40) evaluation.

The blind evaluation was performed independently by two operators who registered the presence of the smear layer on the surface of the canal walls based on the following score described by Hülsmann et al.,[10] score 1: Dentinal tubules completely open, score 2: More than 50% of dentinal tubules open, score 3: <50% of dentinal tubules open, and score 4: Almost all dentinal tubules are covered with the smear layer. When the scores attributed by the observers were not coincident, the worst score was chosen.

The recorded data were transferred into IBM SPSS software version 20.0 (IBM, Armonk, NY, USA) for the statistical analysis. The data were then analyzed statistically using Kruskal–Wallis ANOVA and pairwise comparison was performed using Mann–Whitney U-test.

RESULTS

The present in vitro study evaluated the smear layer removal efficacy of 0.2% chitosan, 0.2% CMC with 17% EDTA from retrograde root canal preparation using scanning electron microscope. Scores were noted from photomicrographs of SEM images, and results were tabulated [Figure 1].

Group I (saline) was least efficient in the removal of the smear layer and most of the specimens were covered with...
of smear layer [Table 1]. Group II (17% EDTA), Group III (0.2% Chitosan), and Group IV (0.2% CMC), resulted in the efficient removal of the smear layer from the retrograde cavity with mean scores 1.35, 1.60, and 1.35, respectively [Table 1]. Smear layer removal efficacy between groups was compared using Mann–Whitney U–test [Table 2]. Statistically, a significant difference was found in Group II (17% EDTA) and Group IV (0.2% CMC) when compared to Group I (saline) [Table 1]. Group II (17% EDTA) and Group IV (0.2% CMC) equally efficient in the removal of the smear layer followed by Group III (0.2% Chitosan). Statistically, a significant difference was not found between Group II (17% EDTA), Group III (0.2% Chitosan), and Group IV (0.2% CMC) [Table 2].

**DISCUSSION**

During apicoectomy and retrograde cavity preparation, a smear layer formed on the prepared root dentinal surfaces. This smear layer contains microorganisms and necrotic pulpal tissues; bacteria can survive and reproduce inside or below the smear layer.

The presence of the smear layer can also inhibit the penetration of intracanal irritants and medications into the dentinal tubules. Smear layer may also act as a physical barrier between obturating material and the canal wall that results in poor seal leading to microleakage.[3] Thus, removal of the smear layer from retrograde cavities enhances the adaptation of root-end filling material and potentially eliminates or minimizes microleakage.[11] EDTA combined with NaOCl is the most commonly used for effective removal of the smear layer from root canals.[12] EDTA reacts with calcium ions of smear and forms soluble calcium chelate, thereby removes the inorganic component of the smear layer. Crumpton et al. reported that the smear layer was removed most effectively by with 1 mL of 17% EDTA for one min.[13]

However, irrigation with a higher concentration of EDTA for prolonged time results in erosion of dentin and damages periapical tissue when extruded beyond the apex.[14,15] Drawbacks associated with EDTA led to the search for more biocompatible solutions.

Chitosan is a natural polysaccharide, which has attracted attention in dental research because of its biocompatibility, bioadhesion, biodegradability, and the lack of toxicity.[16] Chitosan is obtained by acetylation of chitin from crustacean shells.[17] It has a high chelating ability for various metal ions in acidic conditions and has been applied widely for the removal or recovery of metal ions in different industrial areas.[17]

Chitosan forms complexes with metal ions through adsorption, ionic exchange, or chelation. The type of reaction depends on metal ions involved and chemical nature and pH of chitosan.[18,19] Kamble et al. reported that 0.2% chitosan removed the smear layer with to greater extent than 17% EDTA.[20] Silva et al. reported that that 15% EDTA, 0.2% chitosan, and 10% citric acid removed the smear layer effectively from apical thirds of the root canal.[21]
Compared with chitosan, the solubility of CMC in aqueous solution was improved remarkably because of the introduction of the carboxymethyl group. CMC can dissolve in acidic, neutral, or basic aqueous solution when the degree of substitution of carboxymethylation for chitosan is > 60\%.\[21\]

In the current study, the efficacy of chitosan and CMC were investigated using a normal saline solution as negative control and 17\%EDTA as positive control. Statistically, no significant difference was found between 0.2\% chitosan and 0.2\% carboxyl methyl chitosan and EDTA. Low Hussmann scores were obtained for carboxyl methyl chitosan (mean score 1.35) compared to that of chitosan (mean score 1.6), indicating the improved ability of carboxyl methyl chitosan over chitosan. Improved efficiency of CMC over chitosan could be attributed to the increased solubility of carboxymethylation of the chitosan molecules.

Similar to the EDTA molecule, the chitin dimer shows two nitrogen atoms with pairs of free electrons responsible for the ionic interaction between the metal and the chelating agent. In an acid medium, the amino groups present in the biopolymer are protonated, resulting in an overall positive charge (-NH\(^3+\)). This form is responsible for the attraction of other molecules for adsorption to occur. The formation of complexes between chitosan and metal ions most probably is due to the mechanisms of adsorption, ion exchange, and chelation.\[22\]

Mathew et al. reported from quantitative analysis of root canal surface treated with EDTA and chitosan using atomic force microscopic that EDTA-treated teeth was associated with significantly higher surface alteration than chitosan.\[23\]

Mittal et al. reported that 0.2\% chitosan was very effective in removing smear and smear plug with less peritubular dentin erosion compared to apple cider vinegar and 15\% EDTA.\[24\]

It is known that the efficiency of a chelating agent depends on several factors such as application time, pH, concentration of the solution, and amount of solution. Thus, in the present study, the volume of chitosan (pH 3.2) used as the final irrigant was standardized at 5 ml for 3 min. Chitosan exhibits many advantages over EDTA, such as antibacterial property, healing of wounds, bioadhesive nature, chelating action, and can be used as an irrigating solution.\[25\] From the results of this study, 0.2\% chitosan and 0.2\% CMC at pH 3.2 can be a better alternative to EDTA as a smear layer removing solution because of more biological advantages of chitosan and CMC over EDTA.

CONCLUSIONS

Within the limitations of this in vitro study, 0.2\% chitosan, 0.2\% CMC, and 17\% EDTA effectively removed the smear layer from the retrograde root canal preparation. Considering adverse effects associated with 17\% EDTA as an irritant, it was concluded that 0.2\% chitosan and 0.2\% CMC are deemed to be a better alternative for the removal of the smear layer.

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Conflicts of interest
There are no conflicts of interest.

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