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## RESEARCH ARTICLE

### A NOVEL ROOT BIOMODIFIER CONTAINING CHLORHXIDINE AND EDTA—AN ESEM ANALYSIS

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#### ABSTRACT

**Objective:** EDTA (Ethylenediaminetetracetic acid) and Chlorhexidine are potent chelating and bactericidal in nature. QMIX<sup>®</sup> (Dentsply Tulsa Dental, USA) has been developed as a two in one single irrigation solution containing EDTA and Chlorhexidine and to be used after a final rinse post bleach. Due to the properties of its composition, QMIX has the potential to modify as well as disinfect the intracanal root surface. Thus the aim of this in vitro study was to evaluate the efficacy of QMIX<sup>®</sup> on the removal of smear layer from periodontally affected root surfaces.

**Materials and Methods:** 30 longitudinally sectioned specimens from 15 freshly extracted teeth diagnosed with advanced periodontal disease were divided into two groups. In group A and B, the root surfaces were scaled and irrigated using normal saline and with QMIX<sup>®</sup> respectively. All specimens were prepared for ESEM (Environmental scanning electron microscope) and scored according to the presence of smear layer.

**Results:** QMIX<sup>®</sup> significantly increased smear layer removal as compared to the associated control group, in which only saline was used

**Conclusion:** QMIX<sup>®</sup> increased the removal of the smear layer from periodontally affected root surfaces. Thus use of QMIX can be of therapeutic value and may be suggested as a root conditioner on diseased root surfaces.

**Clinical Relevance:** A novel Agent with known chelating and bactericidal properties to be used for root biomodification on diseased root surfaces in order to facilitate attachment of periodontal fibers and aid in periodontal regeneration.

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## INTRODUCTION

Periodontitis is a chronic inflammatory disease induced by microbial biofilm. This inflammation causes destruction of Periodontal fibers allowing down growth of junctional and pocket epithelium. Thus, the root surface becomes exposed to the periodontal pocket. This exposed root surface undergoes substantial alterations such as degeneration of Collagenous remnants of Sharpey's fibres, creation of an environment favourable to penetration of bacteria leading to fragmentation and breakdown of cemental surface and areas of necrotic cementum, separated from the tooth by masses of bacteria (Adriaens *et al.*, 1988). Bacterial products, such as endotoxins penetrate the cemental wall of the periodontal pockets. (Fine, 1978), there is an increase in the mineral content of the exposed cementum including that of Ca, Mg, P and F (Selvig, 1977) also there are evidences showing Surface coating of inflammatory origin seen as calcified state (Eide, 1983).

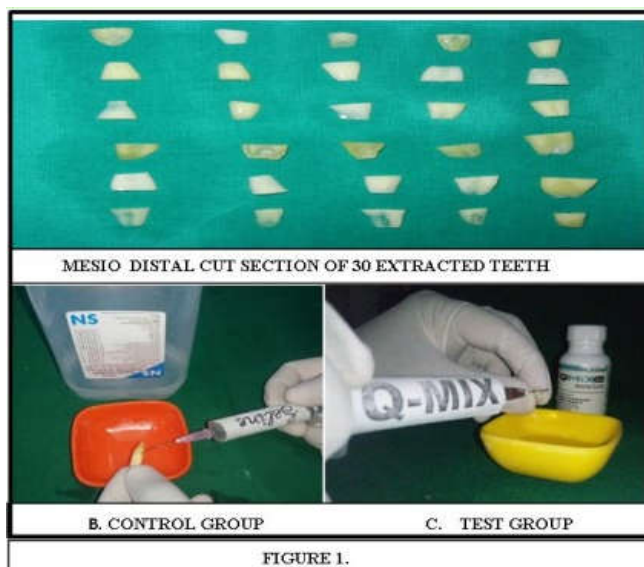
Thus the ultimate goal of periodontal regeneration is to alter the periodontitis affected root surface and make it a hospitable substrate to support and encourage migration, attachment, proliferation and proper phenotypic expression of periodontal connective tissue progenitor cells. The routine scaling and root planing is considered as a method to decontaminate this root surface however it was shown in studies by Jones *et al.* (1978) that it is not possible to decontaminate a periodontitis affected root surface completely by mechanical means. Polson *et al.* (1984) suggested that instrumented surface will inevitably be covered by a smear layer following root planing. Thus the concept of acid demineralization in periodontal therapy was first introduced in the 1800s as a substitute for scaling and calculus removal following the case presented by Marshall (1833) of pocket eradication with "presumable clinical reattachment" after the use of aromatic sulfuric acid. Later on with his Series of experiments, Urist (1965, 1973) suggested that acid demineralization causes inorganic component of dentin to obscure potential inductive proteins associated with the organic component which can aid in reattachment.

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Following this numerous agents have been tried to achieve root biomodification like Citric acid (Register, 1973), Tetracycline (Terranova, 1986), Fibronectin (caffessey, 1985) and EDTA (Ethylene diamene tetraacetic acid). Other mediums like Lasers have also been tried to conditioned the root surfaces and promote reattachment (Hess and myers, 1990). Adding to this the present In vitro Study aims to evaluate the smear layer removal efficacy of a novel irrigating agent – Qmix, containing EDTA and Chlorhexidine which has chelating and bactericidal properties respectively on periodontally affected root surfaces

## MATERIALS AND METHODS

15 human single rooted teeth diagnosed with severe chronic generalized periodontitis judged as hopeless prognosis and planned for extraction by clinicians with no association to this *in vitro* study, were used. The included teeth fulfilled the following criteria; attachment loss of more than 5 mm on all surfaces, bone loss of more than 50%, visible calculus on all root surfaces from the CEJ and Grade III mobility (Miller's mobility index) or, presence of no root caries or restorations and absence of any root defect. Following extraction, the teeth were placed in a normal saline solution. Each root was sectioned longitudinally in the mesiodistal direction using a diamond disc to form two halves (Figure 1A).



The specimens were divided into two groups with organized pairing of the related specimens. A horizontal shallow groove, 5 mm beneath the CEJ, was placed on each specimen to allow identification of the working area, which extended from the CEJ to this depth. In accordance with the selection criteria, the tooth surfaces had visible calculus and diseased cementum in this area. The efficacy of QMIX in its removal of smear layer were investigated under Environmental Scanning electron microscope after complete removal of calculus from the root surface.

### Group A (Figure 1B)

The root surfaces of the 15 samples were scaled using Gracey curette (1/2 Hu- Friedy, Chicago) to remove attached debris or calculus and the working area was then flooded with 1ml normal saline solution. The specimens were then kept in a

solution of 2.5% glutaraldehyde for 24 hours. Followed by this the specimens were kept in ethanol solution with graded concentration beginning with 30 % to 100 % for 10 minutes each. The specimens are then air dried. The dried specimen were then analysed under Environmental Scanning Electron Microscope (QUANTO 200). The micrograph images from the SEM analysis were then examined concerning the presence of a smear layer. The SEM images of the working root surfaces were observed at 1500 X, 3000 X and 3200 X. under the following setting Working Distance (WD) – 10 mm, High voltage (HV) – 20.00 Kv, magnification detected using Large field detector, Pressure of 65 Pa and horizontal field width (HFD) of 199  $\mu$ M to 93.3  $\mu$ M as per desired magnification required. The qualitative nature of the surface and degree of smear layer removal was evaluated and assessed qualitatively using a 1 to 6 Scoring system (Sampaio *et al.*, 2005) mentioned below.

**Score 1** Root surface without smear layer with the dentinal tubules completely open without evidence of smear layer in the dentinal tubules.

**Score 2** Root surface without smear layer with the dentinal tubules completely open, but with some evidence of smear layer in the dentinal tubules

**Score 3** Root surface without smear layer with the dentinal tubules partially open

**Score 4** Root surface covered by a uniform smear layer, with evidence of dentinal tubules opening

**Score 5** Root surface covered by a uniform smear layer without evidence of dentinal tubules opening

**Score 6** Root surface covered by an irregular smear layer, with the presence of grooves and / or scattered debris

### Group B (Figure 1C)

The 15 samples were scaled as described for group A. The working areas of the specimens were then exposed to 1 ml of QMIX as per the manufacturers instruction. The specimens were then prepared for SEM as done in group A. Finally, the samples were examined using the above template system.

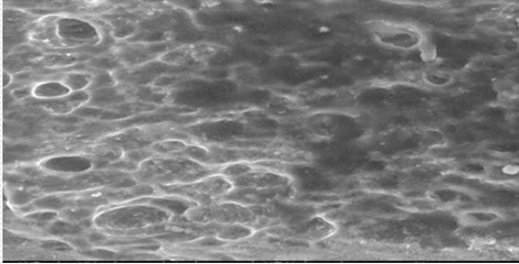
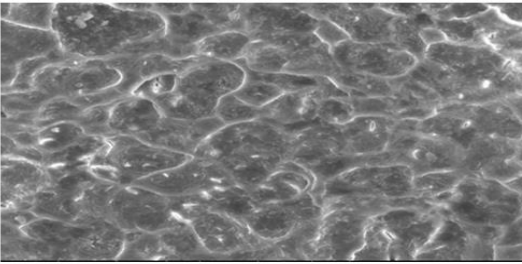
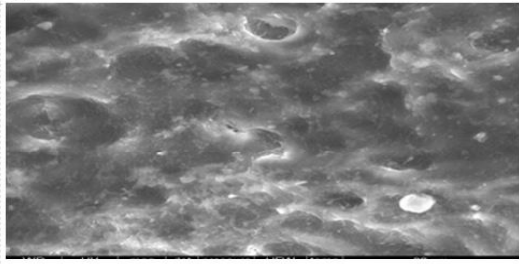
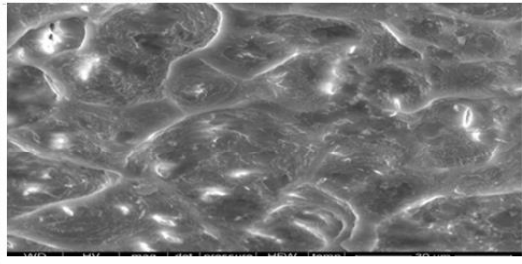
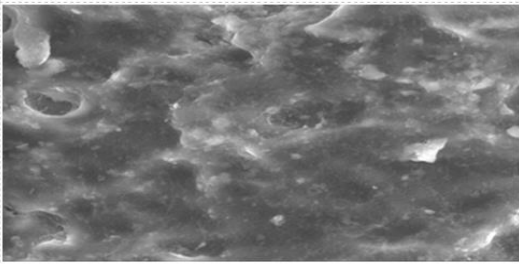
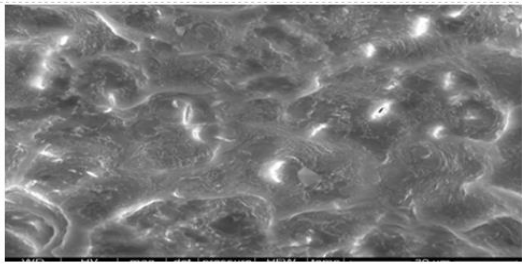
## RESULTS

The results were calculated on the basis of scores obtained following evaluation of ESE Mexamination Table 1 and categorized according to the scoring system. The scores of the groups are mentioned in Table 2. As from the results it can be evident that Group B has shown promising results than Group A in terms of smear layer removal whereby most of its score lies between 1-3 of the above mentioned criteria.

### Statistical Analysis

The statistical analysis was carried out using Graphpad prism 6.0 software. The two-tailed unpaired test is used to analysed the difference between the two groups. P value obtained from comparing the total score of two groups is < 0.001. Thus the data obtained suggested a statistically significant difference between the result of Group A and Group B.

**Table 1**

<b>FIGURE A (CONTROL)</b>	<b>FIGURE B. (CASE )</b>
	
<b>(1500 X)</b> Root surface covered by an irregular smear layer , with the presence of grooves and / or scattered debris	<b>(1500 X )</b> dentinal tubules completely open without evidence of smear layer in the dentinal tubules
	
<b>(3000 X)</b> Root surface covered by an irregular smear layer , with the presence of grooves and / or scattered debris	<b>(3000 X )</b> Root surface without smear layer with the dentinal tubules completely open, but with some evidence of smear layer
	
<b>( 3200 X )</b> Root surface covered by a uniform smear layer without evidence of dentinal tubules opening	<b>( 3200 X )</b> Root surface without smear layer with the dentinal tubules partially open
<b>SEM photographic images taken at respective magnification</b>	

**Table 2.**

Grading	Group 1 (No. of samples )	Group 2 (No. of samples )
Score 1	0	5
Score 2	0	8
Score 3	0	2
Score 4	0	0
Score 5	7	0
Score 6	8	0
TOTAL	83	27
SCORE		

## DISCUSSION

Root planning and removal of the smear layer may lead to exposure of collagen fibres which may increase the migration and attachment of periodontal ligament cells to the root surface improving periodontal healing (Ishi *et al.*, 2008). A number of chemotherapeutic agents have been shown to be successful in removal of the smear layer including the use of lasers or chemicals such as citric acid, EDTA and tetracycline.

QMIX (mixture of EDTA and Chlorhexidine) was developed by Dentsply Tulsa Dental, USA. Which has got its FDA approval for use in the year 2005. The use of QMIX has, so far, been recommended for endodontics, although its properties may also be useful for periodontal conditioning. The use of intracanal irrigant on periodontally affected root surface was first suggested by Houshmand *et al.*, 2011 using MTAD (root canal irrigant) as a root conditioner. He suggested that a statistically significant ( $p=0.001$ ) difference were seen in smear

layer removal from periodontally affected root surface when compared with saline. Tandon *et al.*, 2014 concluded that MTAD as a root biomodifier have a significant role in periodontal wound healing and future new attachment both in vitro and in vivo. The smear layer efficacy of EDTA from root surfaces were very well documented by studies carried out by Pitaru *et al.* (1983,1987) and Samapao *et al.* (2005) whereas Bogle *et al.* (1974) in an animal study observed that significantly more bone had regenerated in the bifurcation defects following use of chlorhexidine. So the present study was carried out with the same intention of using an intracanal irrigant as a root biomodifier but using a different agent having both the agents and perhaps the first study as far as the author's knowledge using Q mix for the same purpose. The results showed significant effect of Q-Mix on removing smear layer than the control group.

## Conclusion

In the above in vitro study it can be concluded as Q mix can be used as a potential root biomodifying agent to remove the smear layer. However further microbiologic studies are required to provide its chemotherapeutic action on periodontal pathogens owing to its composition. Also Further In vivo studies are necessary to provide its role in periodontal regeneration.

## Conflict of interest

The authors declare that they have no conflict of interest.

## Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors.

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