

## SEM Analysis of MTAD Efficacy for Smear Layer Removal from Periodontally Affected Root Surfaces

B. Houshmand<sup>1</sup>, M. Ghandi<sup>2</sup>, MH. Nekoofar<sup>3,4,5✉</sup>, Gh. A.Gholamii<sup>1</sup>, R. K. Tabor<sup>6</sup>, P. M. H. Dummer<sup>7</sup>

<sup>1</sup>Associate Professor, Department of Periodontics, Shahid Beheshti University of Medical Science, Tehran Iran

<sup>2</sup>Assistant Professor, Department of Periodontics, Shahid Beheshti University of Medical Science, Tehran Iran

<sup>3</sup>Assistant Professor, Dental Research Center, Tehran University of Medical Sciences, Tehran, Iran

<sup>4</sup>Clinical lecturer, School of Dentistry, Cardiff University, Cardiff, UK

<sup>5</sup>Assistant Professor, Department of Endodontics, Tehran University of Medical Sciences, Tehran, Iran

<sup>6</sup>Researcher, Geneva Dental Hospital, University of Geneva, Geneva, Switzerland

<sup>7</sup>Professor, School of Dentistry, Cardiff University, UK

### Abstract:

**Objective:** Biopure® MTAD (Dentsply Tulsa Dental, USA) has been developed as a final irrigant following root canal shaping to remove intracanal smear layer. Many of the unique properties of MTAD potentially transfer to the conditioning process of tooth roots during periodontal therapy. The aim of this ex vivo study was to evaluate the effect of MTAD on the removal of smear layer from root surfaces.

**Materials and Methods:** Thirty two longitudinally sectioned specimens from 16 freshly extracted teeth diagnosed with advanced periodontal disease were divided into four groups. In group 1 and 2, the root surfaces were scaled using Gracey curettes. In group 3 and 4, 0.5 mm of the root surface was removed using a fissure bur. The specimens in group 1 and 3 were then irrigated by normal saline. The specimens in groups 2 and 4 were irrigated with Biopure MTAD.

All specimens were prepared for SEM and scored according to the presence of smear layer.

**Results:** MTAD significantly increased ( $P=0.001$ ) the smear layer removal in both groups 2 and 4 compared to the associated control groups, in which only saline was used.

**Conclusion:** MTAD increased the removal of the smear layer from periodontally affected root surfaces. Use of MTAD as a periodontal conditioner may be suggested.

**Key Words:** MTAD (Intracanal Irrigant); Root Conditioning; Root Surface Debridement

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✉ Corresponding author:  
MH. Nekoofar, Department of Endodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran.  
nekoofar@yahoo.com

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### INTRODUCTION

Root surface debridement (RSD) is carried out with the aim of facilitating reattachment of connective and gingival tissue. RSD is recognized as an important contribution to the resolution of periodontal disease [1]. Instrumentation of the root surface has been shown to lead

to the formation of a smear layer of both organic and inorganic material [2]. This layer is believed to provide a physical barrier to periodontal reattachment and its removal may be considered beneficial [3]. Conditioning agents, such as citric acid, EDTA and tetracycline, have been used to remove the smear layer, re-

duce bacterial presence and alter the surface of roots of periodontally affected teeth [2-6]. Therefore, a more biologically compatible root surface for reattachment of the periodontium may be created [7-9]. In an in vitro study, Isik *et al* compared the efficacy of different application techniques of tetracycline HCL on root surfaces and examined the resultant surfaces under SEM [10].

The burnishing technique, involving vigorous rubbing of a saturated cotton pellet, was found to increase the dentinal tubule size most significantly and expose the largest amount of collagen fibrils.

Several studies using citric acid and tetracycline as root conditioners have shown increased periodontal healing [7, 11-13].

However, controlled clinical trials investigating the conditioning of periodontally involved root surfaces with citric acid found no significant improvement in healing compared to control groups [14, 15].

Biopure MTAD (Dentsply Tulsa Dental, USA) is a material originally developed as a final irrigant for endodontics and has been shown to be capable of the removal of the intracanal smear layer created during endodontic preparation [16, 17].

MTAD is a mixture of doxycycline (a tetracycline isomer), citric acid and polysorbate 80 (a detergent) [16]. This recently developed material is used during endodontic treatment as a final irrigant prior to obturation [18]. MTAD has been reported effective in killing *Enterococcus faecalis* [19].

Many of the unique properties of MTAD which contribute to its status as an endodontic irrigant seem likely to be transferable to the conditioning process in periodontal therapy. The individual components of citric acid and tetracycline have already been used in this role [3, 7, 20].

The efficacy of MTAD in the smear layer removal from periodontally affected root surfaces has not been investigated.

This *ex-vivo* study aims to evaluate the effect of MTAD on the removal of the smear layer from periodontally affected root surfaces.

## MATERIALS AND METHODS

Sixteen human single root teeth with advanced periodontal disease, already planned for extraction by clinicians with no association to this *ex-vivo* 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 to a depth of at least 5 mm and mobility of at least Grade III (Miller's mobility index). Following extraction, the teeth were placed in a normal saline solution. Each root was sectioned longitudinally in the buccolingual direction using a diamond disc to form two halves. The two related specimens were paired.

The specimens were divided into four 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.

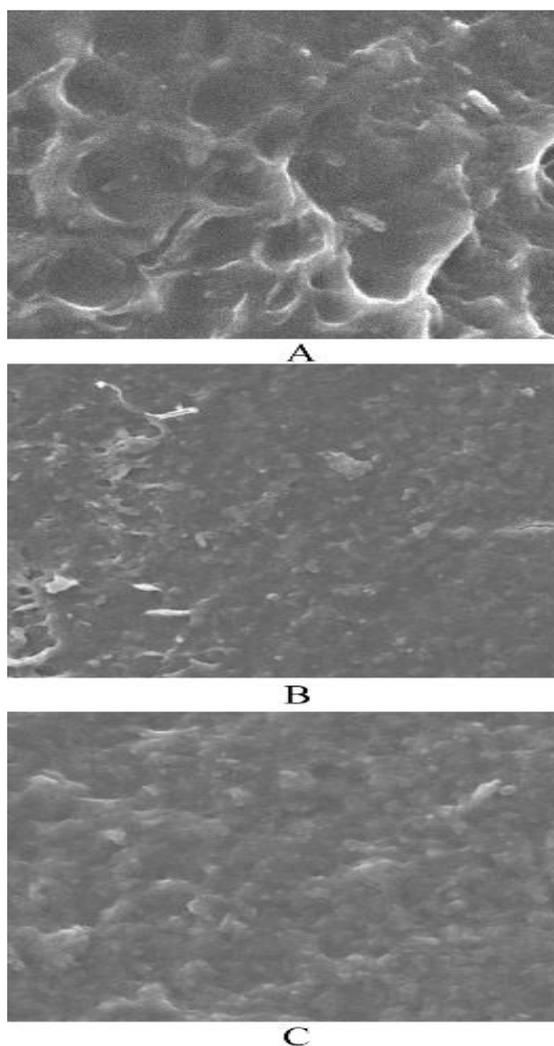
The production of the smear layer by instrumentation and the efficacy of MTAD in its removal were investigated by separation of the paired specimens into four groups.

### Group 1

The root surfaces of the eight samples were scaled using number 11-12 Gracey curettes (Nova Dental Instruments, Dentafix, UK) until a smooth surface was obtained.

This was completed by one operator to reduce variability. The specimens were exposed to 1 ml of 0.9% saline for 4 minutes and then irrigated with 4 ml of saline for 1 minute.

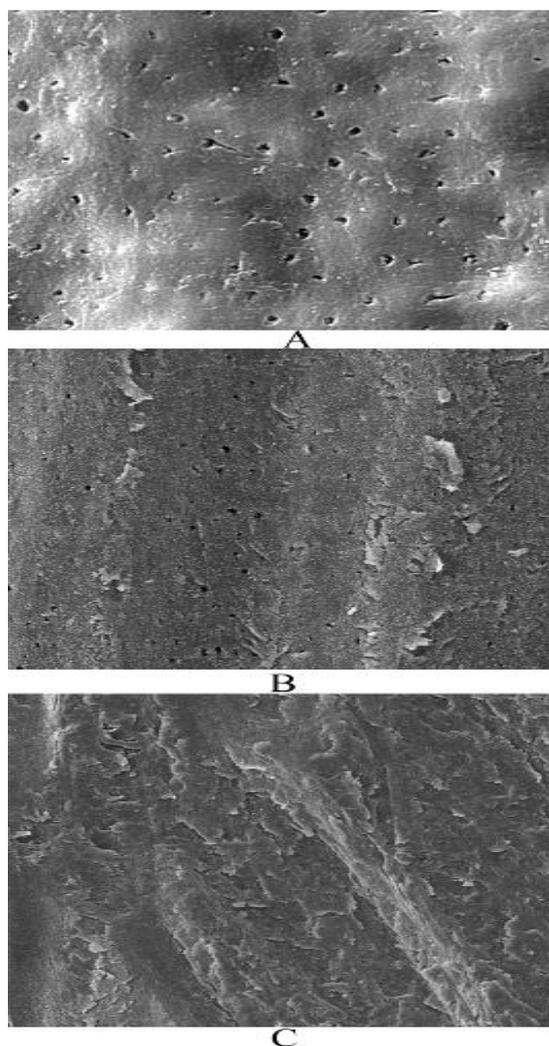
Preparation of specimens for Scanning Electron Microscope (SEM) included exposure to



**Fig 1.** Ranking system for removal of smear layer from cementum. Grade 3: Collagen structure could be seen and no debris present (A). Grade 2: Some collagen structure could be seen, although some debris is present (B). Grade 1: Significant debris may be seen and no collagen structure (C).

2.5% glutaraldehyde for a period of 24 hours and then freeze-drying for 12 hours using a Modulyo freeze-dryer (Edwards, Crawley, UK).

The surfaces were then sputter-coated with gold using a Polaron Sputter Coater (Quorum Technologies, Newhaven, UK) and analysed with an EBT1 (Electron Beam Technology) Scanning Electron Microscope (S.E.M. Tech Ltd, Woodbridge, UK).



**Fig 2.** Ranking system for removal of smear layer from dentine. Grade 3: No debris present, the dentinal tubules are easily visualised (A). Grade 2: The debris covers some of the surface, although some dentinal tubules may be seen (B). Grade 1: The smear layer entirely covers the surface and no dentinal tubules may be seen (C).

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 at a low magnification (X4) were overlaid with a template with five specific marked points to prevent operator bias (magnification,  $\times 350$ ). The qualitative nature of the surface and degree of smear layer removal was evaluated and assessed qualitatively using a 1 to 3 grading system.

- 1- Heavy smear layer. Significant debris was seen. It was not possible to see any collagen structure (Fig 1C).
- 2- Moderate smear layer. The mineralized cementum was seen and also some debris present. Some collagen structure could be seen (Fig 1B).
- 3- No smear layer. No debris was present and collagen structure could be seen (Fig 1A).

### Group 2

The eight dental samples were scaled as described for group 1.

The specimens were then exposed to 1 ml of Biopure MTAD for four minutes and then irrigated with 4 ml of Biopure MTAD for 1 minute, according to the regimen recommended by the manufacturer for intra-canal irrigation.

Brief irrigation with saline was carried out to remove the MTAD solution.

The specimens were then prepared for SEM as for group one.

Finally, the samples were examined using the same template system, SEM and classification.

### Group 3

The eight dental specimens were subjected to the removal of the surface layer in the working area to a depth of 0.5 mm using a straight fissure bur no 105 (Densply/Midwest, Des Plaines, IL, USA). The samples were then exposed and irrigated with saline as described for group 1, then treated and examined using the SEM in the procedure used for groups 1 and 2. The grading system used was slightly different to that used for cementum as removal of the surface layer using the bur had entirely exposed the dentine.

The qualitative ranking of the dentine surfaces was categorized as groups 1 to 3.

- 1- Heavy smear layer. The smear layer entirely covered the surface and no dentinal tubules could be seen (Fig 2C).
- 2- Moderate smear layer. The debris of the smear layer covered some of the surface, although some dentinal tubules could be seen (Fig 2B).
- 3- No smear layer. There was no debris present, the dentinal tubules were easily visualized (Fig 2A).

**Table 1.** Ranking of Smear Layer Removal from Root Surfaces. There was a total number of 40 sites on root surfaces examined for each group.

	Ranking 1 Heavy smear layer		Ranking 2 Moderate smear layer		Ranking 3 No smear layer	
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage
<b>RSD* + Saline (cementum surface) Group 1</b>	27	67.5%	13	32.5%	0	0%
<b>RSD* + MTAD (cementum surface) Group 2</b>	13	32.5%	24	60%	3	7.5%
<b>BA** + Saline (dentine surface) Group 3</b>	21	52.5%	19	47.5%	0	0%
<b>BA** + MTAD (dentine surface) Group 4</b>	11	27.5%	17	42.5%	12	30%

\*RSD = root surface debridement

\*\*BA = bur application

#### Group 4

The eight dental specimens had the surface layer removed with a fissure bur, as described for group 3. They were then exposed and irrigated with Biopure MTAD followed by a short irrigation with saline, as described for group 2. The samples were then treated with gluteraldehyde, dehydrated and examined using the SEM and grading system described for group 3.

The data were analyzed using the Wilcoxon statistical test.

#### RESULTS

The results of the rankings chosen based on qualitative analysis of the smear layer removal from all four groups are summarized in Table 1.

In both groups (2 and 4), in which MTAD was used, there was an increased smear layer removal compared to the associated control groups (1 and 3), in which only saline was used. This difference was statistically significant ( $P=0.001$ ).

#### 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 (21).

A number of techniques 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 [4, 22-25].

MTAD (mixture of tetracycline, acid and detergent) was developed by Torabinejad and co-workers as a final endodontic irrigant to disinfect the canal and remove the smear layer [16]. It is commercially available as BioPure™ MTAD (Dentsply Tulsa Dental, Tulsa, OK, USA). The use of MTAD has, so far, been recommended for endodontics [26], although

its properties may also be useful for periodontal conditioning.

Repeated episodes of root planning in clinical situations may lead to the exposure of dentine [27]. Previous studies examining the effects of periodontal conditioning have been carried out on cementum [21, 28] and/or dentine [3, 10]. The need to examine dentine surfaces was addressed within the method by removal of the cementum layer in groups 3 and 4 to create a dentine surface.

The effect of MTAD as a periodontal irrigant could then be evaluated by examining cementum and dentine surfaces. The method of root planning the cementum surfaces of the samples within groups 1 and 2 is comparable to clinical scenarios.

The use of a fissure bur in groups 3 and 4 to create a dentine surface did not attempt to simulate clinical conditions. However, the latter method allows the consistent removal of tissue to an exact depth and has previously been used by Wang *et al* (29) and Garrett *et al* (30). In contrast, some other studies such as the study conducted by Lasho, used root planning only to examine the effects of various agents on instrumented periodontally involved cementum [31].

However, it may be suggested that this method removes cementum to an inconsistent depth.

Therefore, in the present study, both preparation methods were used to allow clear examination of cementum and dentine surfaces.

A template with five points was used to facilitate random selection of areas for examination. This method has the advantage of eliminating operator bias. After their selection, the chosen regions were examined by one operator and ranked according to the system described in the methodology.

Different ranking systems were required to evaluate the cementum and dentine samples due to the different appearances of the two tissues (Figs 1-2).

The results showed that MTAD was more successful in the removal of the smear layer from both cementum and dentine than saline, with a greater proportion of ranking 3 (no smear layer) scores obtained, and fewer ranking 1 (heavy smear layer) scores (Table 1). The difference between the saline and MTAD specimens was significant ( $P=0.001$ ). The use of a ranking system to categorize the qualitative examination process was beneficial as it allowed some statistical analysis of the results, which was superior to simple descriptive analysis, as used in previous studies. However, the system of ranking has certain disadvantages, such as the difficulties experienced in ranking cementum, with its more amorphous surface appearance.

It is interesting that in both groups treated with MTAD (groups 2 and 4), the ranking score achieved by the specimens was predominately 2 (moderate smear layer), suggesting incomplete smear layer removal.

This is in accordance with Torabinejad *et al's* study, reporting that MTAD could not entirely remove the smear layer when it was used as the only canal irrigant, or as a final irrigant following distilled water [26]. The manufacturer recommendation, in the context of endodontics, is to use MTAD as a final irrigant after NaOCl irrigation [32]. The incomplete removal of the smear layer when MTAD was used prior to NaOCl is reported by Mancini *et al* [33]. However, MTAD is not recommended by the manufacturer or by previous investigators for intra-canal use prior to NaOCl, but as a final irrigant. In addition, Mancini *et al* did not follow manufacturer's recommendations such as the volume of MTAD to be used in irrigation [33]. Greater success in smear layer removal was noted by Torabinejad *et al* when MTAD was used as a final intra-canal irrigant following NaOCl irrigation [16, 26]. It may be suggested that topography and composition of root surfaces, such as those used in the present study, and intra-canal dentine as examined by

other investigators differ to the extent that direct comparison is not optimal. For example, external root surfaces have greater amounts of fluoride ions present due to the topical effect of saliva, which may possibly neutralize the effect of acids to some extent [3]. In addition, the use of MTAD as a periodontal conditioner has not been investigated previously, so there are no published studies for direct comparison. Moreover, the use of NaOCl was not considered for the current study as the caustic nature of this material was thought to be detrimental to periodontal reattachment. Additionally, MTAD used as a conditioner in groups 2 and 4 was rinsed with saline after use, since in clinical condition its presence might have negative effects on the periodontium [34].

## CONCLUSION

In both groups (2 and 4), in which MTAD was used, there was increased smear layer removal compared to the associated control groups (1 and 3), in which only saline was used. The difference was statistically significant ( $P=0.001$ ). Further research is required into the future potential use of MTAD as a periodontal conditioner.

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