

# The effect of MTAD, an endodontic irrigant, on fibroblast attachment to periodontally affected root surfaces: A SEM analysis

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## Abstract:

**Background:** Root surface debridement (RSD) is necessary to create an environment suitable for reattachment of the periodontium. Root surface conditioning may aid the formation of a biocompatible surface suitable for cell reattachment. BioPure™ MTAD (mixture of Doxycycline, citric acid and a detergent) is an endodontic irrigant with antibacterial properties and the ability to remove smear layer. It was hypothesized that MTAD may be useful for root surface conditioning. The efficacy of MTAD as a conditioner was measured by examining fibroblast attachment to root surfaces. **Materials and Methods:** Thirty-two specimens of human teeth with advanced periodontal disease were used. The surfaces were root planed until smooth. Half of the specimens were treated with 0.9% saline and the other samples with Biopure MTAD. As a negative control group, five further samples were left unscaled with surface calculus. Human gingival fibroblast cells HGF1-PI1 were cultured and poured over the tooth specimens and incubated. After fixation, the samples were sputter-coated with gold and examined with a SEM. The morphology and number of attached, fixed viable cells were examined. The data was analysed using the Mann-Whitney-U statistical test. **Results:** There was no significant difference between the numbers of attached cells in the experimental group treated with MTAD and the control group treated with saline. Little or no attached cells were seen in the negative control group. **Conclusion:** RSD created an environment suitable for cell growth and attachment in a laboratory setting. The use of MTAD did not promote the attachment and growth of cells on the surface of human roots following RSD.

## Key words:

Fibroblast attachment, mixture of Doxycycline, citric acid and a detergent, root surface conditioning, root planning, smear layer

## INTRODUCTION

Root surface debridement (RSD) is carried out with the aim of facilitating reattachment of connective and gingival tissue to the periodontally affected root surface. RSD aims to remove soft bacterial deposits, calculus and endotoxins within the diseased cementum<sup>[1]</sup> and is known to create a surface conducive to cell adherence and attachment.<sup>[2,3]</sup> However, repeated episodes of RSD may expose root dentin.<sup>[4]</sup>

The practice of root surface conditioning following RSD in periodontal therapy is of interest. A number of materials have been used in an attempt to create a root surface that is compatible with periodontal reattachment. The efficacy of conditioners such as EDTA, citric acid and tetracyclines has been investigated.<sup>[5-8]</sup> The periodontal conditioners are generally acidic in nature and remove the surface smear layer, demineralize the surface of the dentin and expose collagen fibers.<sup>[9,10]</sup> The availability of

dentinal collagen for binding is thought to aid the migration and attachment of periodontal ligament cells and their subsequent growth.<sup>[11,12]</sup>

To evaluate the effect of root surface conditioning, study of the growth and attachment of cultured periodontal ligament cells has been suggested.<sup>[13]</sup> This experimental model, involving the monitoring of cultured fibroblast attachment to root surfaces has been used extensively.<sup>[5,13-18]</sup>

Biopure MTAD (Dentsply Tulsa Dental, Tulsa, OK, USA) [Figure 1] is a material originally developed for use during endodontic treatment as a final irrigant to remove the smear layer from the root canal walls prior to root canal filling.<sup>[19]</sup> It is a mixture of Doxycycline (a tetracycline isomer), citric acid and Polysorbate 80 (a detergent).<sup>[20]</sup> The efficacy of MTAD as a conditioner of root surfaces in periodontal treatment has not been investigated before. This *ex vivo* study aims to evaluate the effect of MTAD after RSD on the adherence of cultured fibroblasts, compared with RSD with saline treatment.

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**Figure 1:** The addition of the liquid component of MTAD, including citric acid, to powder to form active MTAD

## MATERIALS AND METHODS

Ethical approval for the study was obtained from the Medical Ethics Committee of the Shahid Beheshti University of Medical Science, Tehran, Iran. Sixteen human single root teeth with advanced periodontal disease were used. The teeth were already planned for extraction by clinicians not associated with this study. Inclusion criteria consisted of 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 Grade III (Miller's mobility index). Following extraction, the teeth were placed in normal saline solution.

Each root was sectioned longitudinally in the buccolingual direction using a diamond disc to form two halves. A horizontal shallow groove, 5 mm beneath the CEJ, was placed to allow identification of the working area, which extended from the CEJ to this depth. In accordance with the inclusion criteria, the tooth surfaces had visible calculus and diseased cementum in this area. The specimens were divided into two experimental groups to investigate the propensity for cell adhesion and growth in relation to root surface conditioning. The root surfaces of all the samples were planed using number 11-12 Gracey curettes (Nova Dental Instruments, Dentafix, Hook, UK) until a smooth surface was obtained, this was completed by one operator to reduce variability. Half of the specimens were exposed to 1 ml of 0.9% saline for four minutes and irrigated with 4 ml of saline for one minute. The other sixteen samples were exposed to 1 ml of Biopure MTAD (Dentsply Tulsa Dental, Tulsa, OK, USA) for four minutes and then irrigated with 4 ml of Biopure MTAD (Dentsply, USA) for one minute according to the regimen recommended by the manufacturer for intra-canal irrigation. The samples were then irrigated briefly with saline to remove the MTAD solution. Five further samples were left unscaled with surface calculus in the working area and received no irrigation, to provide a negative control group. All samples were then exposed to UV radiation for 24 hrs to allow sterilisation prior to cell culture.

Human gingival fibroblast cells HGF<sub>1</sub>-PI<sub>1</sub> (NCBI code: C165) cells were obtained in a frozen state from a Cell Bank, (National

Cell Bank, Pasteur Institute, Tehran, Iran). These cells were thawed and cultured in flasks containing Dulbecco's Modified Eagle's Medium (DMEM) (Life Technologies Inc., Grand Island, NY, USA) and Fetal Bovine Serum 10% (Gibco, Grand Island, New York, USA) with Penicillin 100 IU/ml (Sigma-Aldrich Corp., St Louis, MO, USA), Streptomycin 100 µgr/ml (Sigma, USA) and Amphotericin B 250 µgr/ml (Sigma, USA). The thawed cells were re-cultured and taken from the fifth cycle. All tooth specimens were then placed within wells and the fibroblast culture medium poured over the samples. To check the viability of the cells, a coverslip added to the same medium. The samples were then incubated for 48 hrs at 37°C at 95% humidity and 5% CO<sub>2</sub>.

The tooth samples were then placed in glutaraldehyde for 24 hrs and immersed in distilled water for five minutes. The specimens in the experimental group were then freeze-dried for 12 hrs 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 specimens were examined with an EBT1 (Electron Beam Technology) Scanning Electron Microscope (S.E.M. Tech Ltd, Woodbridge, UK). The SEM images of the working root surfaces at a low magnification (×4) were overlaid with a template with five specific marked points to prevent operator bias. Each point was magnified to ×350. The morphology of cells present was examined by two independent fully calibrated examiners blind to the code given to the specimens. The only cells counted by the examiners were attached, flat cells with elongated processes [Figures 2 and 3]. The cells with this specific appearance were considered to be viable before fixation. The control coverslip was also examined following similar preparation using an EBT1 (Electron Beam Technology) Scanning Electron Microscope (S.E.M. Tech Ltd, Woodbridge, UK).

The data was analysed using the Mann-Whitney-U statistical test.

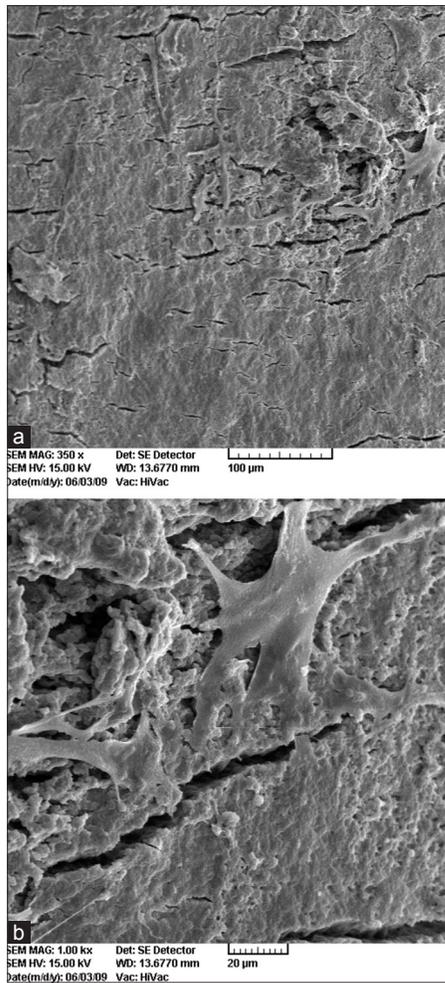
## RESULTS

The cells observed on the cover-slip positive control group were flat, well attached cells with elongated processes that were considered to be viable cells. The cells present on the specimens from the MTAD and saline conditioned samples were similar in shape, as were the viable cells identified in the positive control group. In contrast, little or no viable fibroblasts were attached to the samples in the negative control group in which calculus removal had not occurred.

The mean value of cell numbers found on the samples conditioned with MTAD was 14.69 ± 20.09. The mean value of cell numbers from the saline samples was 27.44 ± 36.34. The difference between the two groups was not significant ( $P > 0.05$ ). The median of the cell numbers found on the MTAD and saline samples was 6.5 and 13.0, respectively.

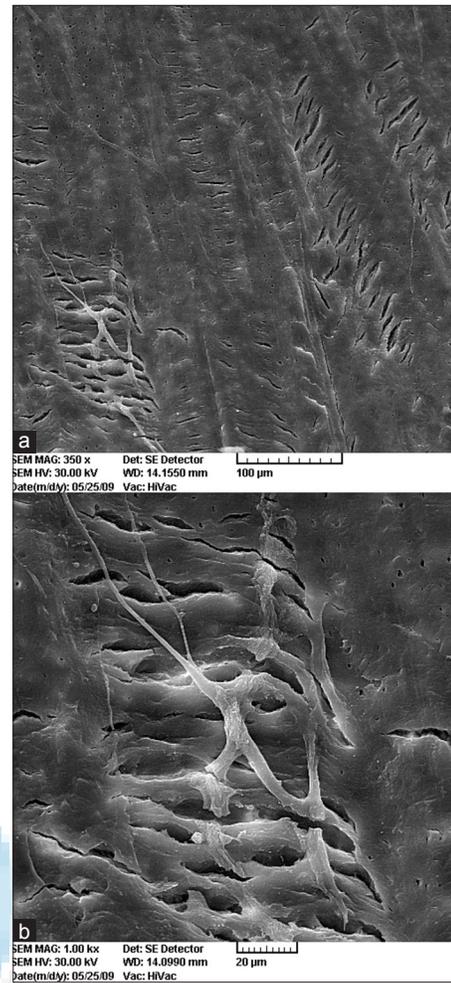
## DISCUSSION

The aims of root surface conditioning in periodontal therapy are to create a more biologically compatible surface for reattachment of the periodontium. Root surface conditioning removes the smear layer, exposing collagen fibers and should, ideally,



**Figure 2:** Root surface treated with saline after root surface debridement.

(a) (original magnification  $\times 350$ .) An example of one of the regions randomly selected by use of the template. (b) Selected region from (a) (original magnification  $\times 1000$ ). Viable, flat, well-attached cells with elongated processes attached to root surface covered by a heavy smear layer can be seen



**Figure 3:** Root surface treated with MTAD after root surface debridement.

(a) (Original magnification  $\times 350$ ) An example of one of the regions randomly selected by use of the template. (b) selected region from (a) (original magnification  $\times 1000$ ). Viable, flat, well-attached cells with elongated processes attached to root surface, following smear layer removal by MTAD, can be seen

reduce bacterial presence on the root surface.<sup>[12,21-24]</sup> A number of chemicals have been shown to be successful in the removal of the smear layer including EDTA, tetraacetic acid, citric acid, tannic acid, polyacrylic acid, bis-de-qualinium-acetate and the tetracyclines.<sup>[9,10,16,25]</sup>

MTAD (mixture of tetracycline, acid and detergent) was developed by Torabinejad and co-workers<sup>[19]</sup> as a final endodontic irrigant<sup>[26,27]</sup> to disinfect the canal<sup>[28-31]</sup> and remove smear layer.<sup>[20]</sup> The solution contains doxycycline, citric acid and Polysorbate and is commercially available as BioPure™ MTAD (Dentsply Tulsa Dental, Tulsa, OK, USA).<sup>[32]</sup> In addition to its ability to remove the smear layer in root canals<sup>[20]</sup> MTAD has other potentially beneficial properties. These include an antibacterial action<sup>[28]</sup> and the capability to solubilize pulp tissue,<sup>[33]</sup> particularly when used after sodium hypochlorite.<sup>[27]</sup> The use of MTAD has, so far, been confined to endodontics to aid bacterial removal from dentine tubules within the canal and the closer approximation of the sealant to the canal walls.<sup>[32]</sup>

In view of its many advantageous properties, this study investigated the possibility that MTAD could be useful as a root

surface conditioner during periodontal therapy. In particular, the ability of MTAD to remove successfully smear layer from the root surface of periodontally affected teeth and whether this produced an environment conducive to periodontal cell attachment and growth. The purpose of this study is to evaluate the ability of cultured fibroblasts to attach to periodontally diseased root surface conditioned with MTAD after root surface debridement. The results revealed that the use of MTAD following RSD created an environment suitable for cell attachment and did not lead to undesirable conditions for cell growth. However, MTAD did not promote cell attachment and growth. This is in accordance with previous work that demonstrated the relative non-cytotoxic nature of MTAD.<sup>[34]</sup>

Some of the individual components of MTAD have been used previously as periodontal conditioners. Chandra *et al.*, compared the effects of root surface conditioning with citric acid, EDTA and tetracycline hydrochloride.<sup>[5]</sup> Positive effects were seen with the use of citric acid and EDTA, although not with tetracycline hydrochloride. Babay *et al.*, also found increased attachment with conditioning of surfaces with tetracycline hydrochloride and EDTA.<sup>[8]</sup> These results are not

in accordance with the present study, in which no additional cell attachment was seen with MTAD surface conditioning.

Delazari *et al.*, carried out an SEM study investigating the formation of fibrin root surface networks *in vivo* as an indicator of the periodontal reattachment.<sup>[35]</sup> They compared qualitatively conditioned root surfaces after RSD with those on which only RSD had been carried out. No difference was seen between the groups, indicating RSD to be sufficient. In addition, Al-Nazhan *et al.*, in a SEM study, examined cultured fibroblast attachment to root surfaces in which no surface conditioning had been carried out and the smear layer was present.<sup>[16]</sup> These conditions were concluded to be desirable for cultured fibroblast attachment and are in alignment with the present study.

Biopure MTAD has been recommended for use in endodontic treatment as a final irrigant after the use of sodium hypochlorite.<sup>[27,36]</sup> In the present study, sodium hypochlorite was not considered for use in periodontal conditioning prior to MTAD because of its likely caustic effects. This is in alignment with the results of Ring *et al.*, who investigated the effect of various endodontic irrigants, including MTAD in combination with sodium hypochlorite, on cell growth and attachment to intra-canal dentin. The lowest average number of cells was found to be present on the samples irrigated with sodium hypochlorite and MTAD.<sup>[36]</sup> They also concluded that the presence or absence of the smear layer did not have a significant effect on cell attachment. The results of the present study are in accordance with their finding.

The method used in the present study of evaluating the effect of root surface treatment on the attachment of cultured fibroblasts to root specimens has been used previously<sup>[5,14,16,18,37]</sup> and is recognised as an appropriate methodology.<sup>[38,39]</sup> In an *ex vivo* study,<sup>[14]</sup> the effect of pre-conditioned root surfaces and application of fibroblast growth factor on attached fibroblast morphology and their proliferation was investigated. A qualitative index (developed by Jenkins *et al.*) was used to describe the attached, fixed viable cell populations.<sup>[40]</sup> Based on the attached cell density and morphology, the qualitative index graded the samples from 0-3. Other studies<sup>[5,18]</sup> used qualitative descriptive passages only to describe their findings. These descriptive methods could be considered to be less accurate than the quantitative system of counting attached fixed viable cells that was used in the present study. In addition, to reduce operator bias, a system of a 5-point template overlying the SEM image was used in the present study to provide random selection of points for magnification and cell counting. In contrast, the design of some previous studies<sup>[5,14,18]</sup> did not include a system to aid their selection of areas for magnification and examination. Silverio *et al.*, described the process of selection of examination area as 'random'. Therefore, the issue of operator bias seems not to have been addressed.<sup>[14]</sup>

The use of MTAD as a periodontal conditioner created a surface environment suitable for cell attachment and growth, as demonstrated by the presence of attached, fixed viable cells on the specimens treated with MTAD following RSD in this study. The study by Zhang *et al.*, found MTAD to be relatively non-cytotoxic, findings in alignment with the data from the current study.<sup>[34]</sup> The results of the present study revealed no significant difference in

cell numbers between MTAD and saline conditioning following RSD. This would suggest that the presence of the root surface smear layer formed by instrumentation causes no barrier to cell attachment. A similar conclusion was drawn on examination of cell attachment to intra-canal dentin.<sup>[36]</sup> Therefore, it may not be necessary to treat the root surface after RSD to gain satisfactory results in cell reattachment.

## CONCLUSION

Root surface debridement (RSD) created an environment suitable for cell growth and attachment. Although the use of MTAD following RSD did not lead to undesirable conditions, no significance difference in cell attachment was seen between MTAD conditioning of periodontally affected root surfaces following RSD, compared to saline conditioning. Overall, with the limitation of this laboratory study, use of MTAD did not promote cell attachment and growth.

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