ORIGINAL RESEARCH

A Scanning Electron Microscope Evaluation of the Adhesion of Fibrin Clot to the Periodontally Compromised Teeth after Exposed to Different Root-conditioning Agents: An *In Vitro* Study

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Abstract

Aim: The aim of this research was to assess the binding of fibrin clot to the teeth affected by periodontal disease following exposure to different root conditioning agents.

Materials and methods: A total of 60 human teeth with a solitary root that were subjected to extraction following severe periodontal disease were used as study samples in this research. Two analogous grooves were prepared on the proximal radicular surface of every sample employing a diamond-tapered fissure bur using an aerator handpiece beneath abundant irrigation. Every sample was assigned to one of the following groups:

- Group I: Tetracycline hydrochloride solution
- Group II: Ethylenediaminetetraacetic acid (EDTA) gel
- Group III: Biopure MTAD™

Subsequent to conditioning, the samples were rinsed for 3 minutes with phosphate-buffered saline (PBS) and permitted to air-dry for 20 minutes. A drop of fresh human whole blood procured from a hale and hearty volunteer was coated onto the dentin blocks in all three groups. A scanning electron microscope under 5000× magnification at 15 kV was used to examine the samples. Kruskal–Wallis test and Mann–Whitney *U* test were performed to procure the inter- and intragroup assessments.

Results: The greatest fibrin clot union was noted in the EDTA gel group at 2.86 ± 0.14 in pursuit by Biopure MTAD™ group at 2.39 ± 0.08 as well as tetracycline hydrochloride solution group at 1.82 ± 0.10. A statistically significant difference was noted between the investigational groups ($p < 0.001$).

Conclusion: This research arrived at a conclusion that the dentinal surfaces subjected to conditioning with EDTA gel group as well as coated with human whole blood resulted in appreciably superior fibrin clot bonding to dentin vs Biopure MTAD™ as well as the tetracycline hydrochloride solution group.

Clinical significance: Connective tissue attachment subsequent to surgical procedures causing the adhesion of a fibrin clot to the radicular surface as a result of initial wound healing processes is directly related to periodontal regeneration. It depends on biocompatibility for the fibrin clot and the periodontal pathosis-affected radicular surface to stick together, which can be procured with the aid of a variety of root conditioning measures in course of periodontal treatment.

Keywords: Fibrin clot, Periodontal regeneration, Root conditioning agents, Scanning electron microscopy.

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Introduction

Pathological exposure of the root surface occurs due to periodontitis which is distinguished by an inflammatory process of the numerous parts of the periodontium resulting in considerable alterations in the tooth and its radicular surface. Pathological exposure of the root surface occurs due to the destruction of the external and internal periodontal fibers caused by an inflammatory process that is stimulated by plaque, permitting a downward growth of the junctional epithelium. Plaque, calculus, and cytotoxic materials infiltrate the pathologically exposed root surface that operates as a physical obstacle, restraining a fresh attachment and offers a medium to aid in microbial growth.[1]

Procurement of a spotless, even, and disinfected radicular surface in periodontal surgery is very important for aiding in novel connective tissue reattachment as well as augmentation of the tissue healing procedure.[2] Although, ultrasonic/manual scaling, as well as root planing are relatively popular treatment procedures in a clinical scenario for physical cleansing of the radicular surface during periodontal treatment, these techniques do not possess the capability to completely cause decontamination of the dental hard

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tissues. Furthermore, owing to physical debridement, a condensed smear coating is produced in close contact with the surface subjected to treatment, comprised of a combination of organic and inorganic substances, bacterial toxins, and debris. Histologically, the existence of a smear layer interjected amid the root surface as well as the neighboring connective tissue may perhaps refrain from the connective tissue re-attachment procedure.[3]

The radicular surfaces of periodontally affected teeth are greatly infected with bacteria as well as their endotoxins, and this contagion may hamper the consequences of periodontal regenerative techniques by deferring a novel connective tissue attachment. As a result, to augment periodontal regeneration, root conditioning is the technique to re-establish the biological acceptability of the radicular surface to encourage the movement, attachment, proliferation, and production of connective tissue macromolecules by connective tissue cells. Root conditioners are frequently utilized to eliminate the smear layer and expose collagen fibers rendering the radicular areas biocompatible which is consequently essential for the accomplishment of regenerative actions. A substance called Biopure mixture of doxycycline, citric acid and a detergent (MTAD)™ was initially created to clean the root canal walls of the smear layer. Many of MTAD's special characteristics aid in the conditioning process during periodontal therapy.[4]

The demineralizing effect of EDTA is achieved by chelating divalent cations at neutral pH, which is why it is favored for application. The matrix proteins' ability to adhere to the dentin is improved by tetracycline hydrochloride, and fibroblast attachment and growth are stimulated. The development of connective tissue connection subsequent to regenerative treatment is straightforwardly linked to the binding of the fibrin clot to the radicular surface at the time of initial wound healing proceedings. Fibrin clot catalyzes the fibrin matrix and the gingival tissues' initial attachment to the radicular surface and as a scaffold for collagen formation, cell migration, and attachment. The biological receptive capacity of the radicular surface as well as the tensile strength of the healing wound are connected to the bond of the fibrin clot to the periodontitis-affected radicular surface. Biological alteration of the root using root conditioning substances eliminates the smear coating, thereby exposing the intra- and peri-tubular dentinal collagenous matrix along with the dentinal tubules.[5] Therefore, the aim of this research was to assess the binding of the fibrin clot to the teeth affected by periodontal disease following exposure to tetracycline hydrochloride solution, EDTA gel, and Biopure MTAD™ root conditioning agents.

Materials and Methods

The present *in vitro* study was conducted in the Department of Periodontology and Implantology, Buddha Institute of Dental Sciences and Hospital, Patna, India.

Estimation of Sample Size

The sample size was calculated using the following formula:

$$n = \frac{z^2_{1-\alpha/2}}{d^2}$$

where n is the required sample size, $z_{1-\alpha/2}$ is a constant, two-sided test, that is, 1.96 for 95%, and d is the absolute precision 20% = 0.2.

A total of 60 human teeth with a solitary root that were subjected to extraction following severe periodontal disease were used as study samples in this research.

Grade III mobile teeth or those with a discouraging prognosis, bleeding on tender probing, as well as radiographic evidence of proximal bone loss, constituted the inclusion criteria.

Individuals with a history of systemic pathologies, oral prophylaxis in the preceding 6 months, dental caries with periapical infection, plus a history of sharp pain, as well as swelling requiring tooth extraction, were excluded from this research. After extraction, the teeth were subjected to washing with distilled water and storage in 0.9% normal saline at room temperature until future investigation.

Preparation of Specimen

Two analogous grooves were prepared on the proximal radicular surface of every sample employing an aerator handpiece with a diamond-tapered fissure bur was used underneath copious irrigation. The second groove was created around 3 mm apical to the first, which was constructed at the cementoenamel junction (CEJ). The region amid both grooves was subjected to debridement and planed employing 50 apico-cervical strokes, with a pointed Gracey curettes No. 5–6. Subsequent to root planing, the region amid both grooves was sectioned to attain dentinal blocks of ample size. The blocks were then subjected to storage in normal saline till further utilization.

Application of Root-conditioning Agents

All 60 samples were allocated into one of the three investigational groups using the random number method:

Group I: Tetracycline Hydrochloride Solution

Commercially obtainable tetracycline hydrochloride 4,000 mg capsule (Hostacycline®, Aventis Pasteur) was dissolved in 80 mL of distilled water with continuous stirring at 37°C for 10 minutes to render a 50 mg/mL solution of tetracycline hydrochloride. The solution was blended using a magnetic agitator. A pH meter was used to measure the solution's pH, which was 1.11. The samples were conditioned with tetracycline hydrochloride solution by introducing cotton balls inundated with it and replaced every 20 seconds for 3 minutes.

Group II: EDTA *Acid Gel*

Commercially available 24% EDTA gel at pH 7.3 was used (PrefGel, Biora, Malmo, Sweden). The samples were subjected to conditioning with 24% EDTA gel by placing cotton balls inundated with it and replaced every 20 seconds for 3 minutes.

Group III: Biopure MTAD™

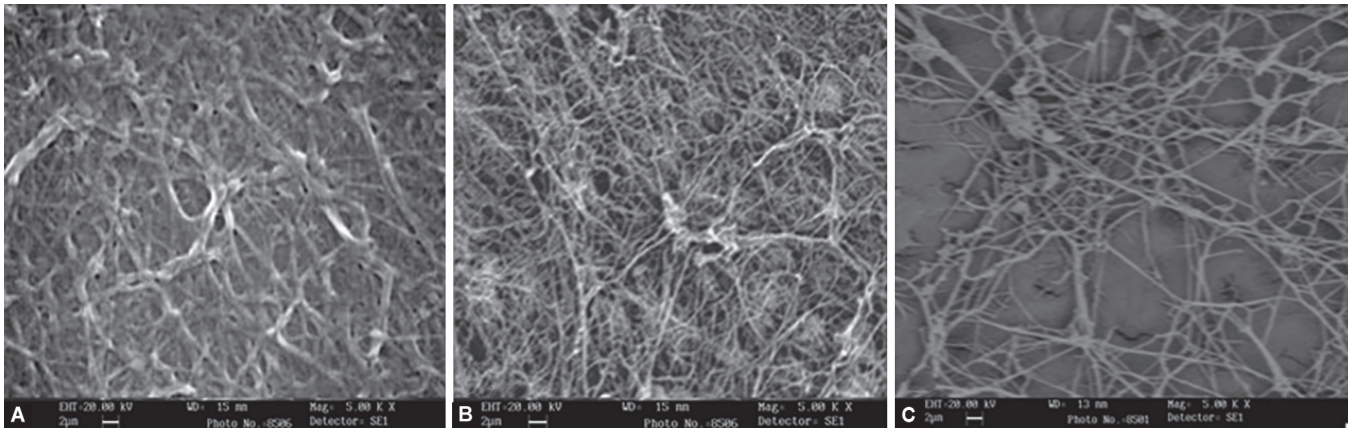
Samples were subjected to conditioning with Biopure MTAD™ (Dentsply Tulsa Dental, Tulsa, OK, USA) by placing cotton balls inundated with it and replaced every 20 seconds for 3 minutes.

Formation of Fibrin Clot Adhesion to the Specimens

Subsequent to conditioning, the samples were rinsed for 3 minutes with PBS and permitted to air dry for twenty minutes. A drop of fresh human whole blood procured from a hale and hearty volunteer was coated onto the dentin blocks in all three groups. The blood was allowed to clot in a humidified chamber for approximately twenty minutes. The samples were then rinsed for 5 minutes in PBS thrice.

Evaluation of SEM Analysis

The dentinal blocks were subjected to fixing in 3% glutaraldehyde for twelve hours at 4°C. Subsequent to fixation, the blocks were rinsed with PBS. The samples were subjected to dehydration through



Figs 1A to C: The SEM images of (A) Tetracycline hydrochloride solution; (B) EDTA gel; (C) Biopure MTAD™

a graded series of ethanol at 30, 50, 70, 90, and 100% concentrations. The samples were then dried using liquid carbon dioxide in a critical point dryer. The dehydrated samples were mounted on metallic stubs and subjected to the gold coating as well as desiccation at room temperature. Scanning photomicrographs were acquired at a magnification of 5000× at 15 kV employing a scanning electron microscope; the SEM images shown are tetracycline hydrochloride solution (Fig. 1A), EDTA gel (Fig. 1B), and (Fig. 1C) Biopure MTAD™. To establish the quantity of fibrin clot union to the radicular surface, the subsequent scores were employed.[6]

- Score 0: Lack of fibrin network as well as blood cells.
- Score 1: Scant fibrin network or blood cells.
- Score 2: Moderate fibrin network as well as a moderate amount of blood cells.
- Score 3: Dense fibrin network as well as entrapped blood cells.

Statistical Analysis

A descriptive statistical analysis was performed in this research. The results on constant measurements are depicted as mean ± standard deviation as well as results on categorical dimensions are delineated in number (%). A 5% level of significance was used to assess the significance. Kruskal–Wallis test and Mann–Whitney *U* test was performed to procure the inter- and intragroup assessments.

Results

Table 1 and Figure 2 show the mean fibrin clot adhesion amid the investigational groups. The highest fibrin clot bond was noted in the EDTA gel group at 2.86 ± 0.14 in pursuit by Biopure MTAD™ group at 2.39 ± 0.08 as well as the tetracycline hydrochloride solution group at 1.82 ± 0.10.

Table 2 delineates the contrast assessment of mean fibrin clot bond amid the investigational groups. The lowest fibrin clot union was noted in the tetracycline hydrochloride solution group at 1.82 ± 0.10 and a superior fibrin clot bond was noted with the EDTA gel group at 2.86 ± 0.14 in pursuit by Biopure MTAD™ group at 2.39 ± 0.08 with a mean rank of 22.68 as well as 19.87, in that order. A statistically noteworthy disparity was noted amid the investigational groups (p <0.001).

Table 1: Mean fibrin clot adhesion between the experimental groups

		95% Confidence interval (CI) for mean	
Experimental groups	*Mean ± SD*	*Lower*	*Upper*
Group I: Tetracycline hydrochloride solution	1.82 ± 0.10	1.50	2.24
Group II: EDTA gel	2.86 ± 0.14	2.48	3.10
Group III: Biopure MTAD™	2.39 ± 0.08	2.04	2.74

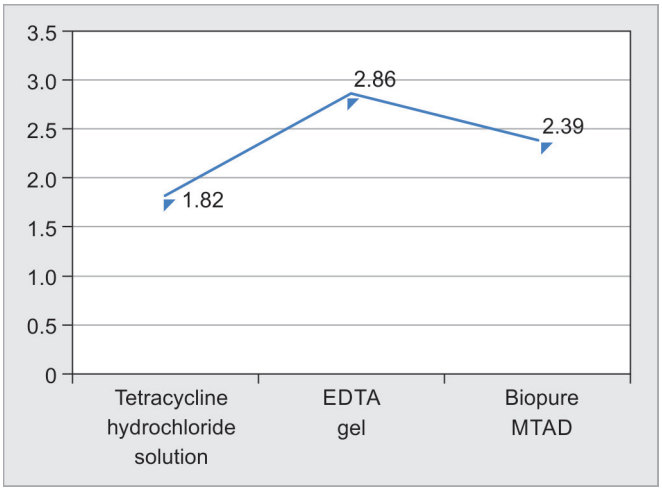


Fig. 2: Mean fibrin clot adhesion of different study groups

Table 2: Comparison of mean fibrin clot adhesion between the experimental groups

		95% CI for Mean			
Experimental groups	*Mean ± SD*	*Lower*	*Upper*	*Mean rank*	*p-value*
Group I: Tetracycline hydrochloride solution	1.82 ± 0.10	1.50	2.24	13.42	
Group II: EDTA gel	2.86 ± 0.14	2.48	3.10	22.68	0.001
Group III: Biopure MTAD™	2.39 ± 0.08	2.04	2.74	19.87	

Table 3: Pairwise comparison between the experimental groups

Groups	*Mean difference*	*Significance*
Tetracycline hydrochloride solution vs EDTA gel	1.04	0.001
Tetracycline hydrochloride solution vs Biopure MTAD™	0.57	0.502
EDTA gel vs Biopure MTAD™	0.47	0.682

Pairwise contrast among the investigational groups is depicted in Table 3. The mean difference between tetracycline hydrochloride solution as well as EDTA gel was 1.04, and it exhibited an exceedingly significant disparity between the groups. The mean difference among tetracycline hydrochloride solution as compared to Biopure MTAD™ (p >0.502) and EDTA gel vs Biopure MTAD™ (p >0.682) was 0.57 and 0.47, in that order. There were no significant dissimilarities between these groups (p >0.05).

Discussion

The principle aim of every periodontal management is the conversion of a periodontally afflicted radicular surface to a substrate that is biocompatible for receipt of epithelial cells thereby permitting connective tissue adhesion. Nevertheless, the radicular surfaces afflicted with periodontitis are hypermineralized as well as infected with cytotoxic plus various bioactive agents that render them non-biocompatible with the neighboring periodontal cells that play a significant part in periodontal wound healing. It is not feasible to disinfect the periodontally afflicted radicular surface by mechanical means only.[7]

Scaling and root planing methods leave behind a 2.15 µm broad smear covering of microcrystalline debris that is closely related to the radicular surface and is essentially only removed by demineralizing agents. The fibrin from the blood clot causes a preliminary adhesion to the radicular surface during periodontal healing, establishing a scaffold for the development of a cell plus collagen fiber attachment as well as avoiding a downward growth of the junctional epithelium. Radicular surface demineralization employing a variety of chemical substances has been hypothesized to eradicate cytotoxic substances from the afflicted radicular surface, decontaminate exposed dentinal surface as well as decalcify the planed radicular surface with exposure of the dentine/cementum matrix collagen, thereby promoting attachment amid the radicular surface as well as the connective tissues of the healing flap. Therefore, radicular conditioning may balance the shortcomings of mechanical radicular debridement.[8]

In an effort to imitate the circumstances found *in vivo*, scaling and root planing preceded chemical treatment of the radicular surfaces. Nevertheless, a broad smear deposit of microcrystalline debris closely linked with the radicular surface arises from the mechanical treatment. Thus, acidic conditioners have been employed to disinfect and cause demineralization of the radicular surface, eliminate the smear layer, expose certain constituents of the extracellular dentinal/cementum matrix, like type I collagen, and assist adherence amid the radicular surface and healing connective tissues.[9] This is in harmony with earlier research by Isik et al.[10] Cavassim et al.,[11] and Amaral et al.[12] The technique of applying the root conditioner employed in this study caused root surface demineralization, smear layer elimination, and exposure of dentinal tubules as well as collagen of intra- and peritubular dentinal matrix, that aids in fibrin clot union.

In this research, superior fibrin clot bond was noted in the EDTA gel group, in pursuit by Biopure MTAD™ group and use of tetracycline hydrochloride solution. The EDTA has the ability to get rid of the smear coating on the radicular surface, expose the dentinal tubules plus intertubular collagenous matrix, and augment the wettability of the radicular surface causing superior adherence and adsorption of the blood components to the radix. The exposure of dentinal/cemental collagenous fibers at the radicular surface augments movement, proliferation, union, and matrix production of the cells engaged in the process of healing of the periodontium. However, the collagen fibers improve the stabilization of the fibrin network of blood which is very important for periodontal tissue reparative and regenerative processes.[13]

Our findings are also in concordance with the research by Blomlof and Lindskog,[14] who noted that etching with EDTA appears to enhance cell and tissue colonization near the beginning, by rendering a more biologically acceptable surface for cellular/tissue adhesion. Additional research by Gamal [15] exhibited that EDTA gel root conditioning augments β-TCP combined clot adherence to periodontally affected radicular surfaces. In disparity, Leite et al.[16] discussed that there can be unfinished elimination of the gel off the radicular surface, and as EDTA causes calcium chelation; its remains may inhibit/delay the clotting process.

In this research, fibrin clot union was enhanced with the use of Biopure MTAD™ vs tetracycline hydrochloride solution. This is akin to research performed by Zia et al.[17] and Tandon et al.[4] The improved results for MTAD can be ascribed to its reduced pH (1.28) and the existence of a detergent (TWEEN® 80), promoting infiltration and hence improved elimination of the smear coating with the superior connection of the fibrin clot in due course.

In the present study, the highest fibrin clot bond was noted in the EDTA gel group followed by Biopure MTAD™ group and tetracycline hydrochloride solution, respectively. The EDTA has demonstrated that the root surface's wettability is increased by removing the smear layer, which also exposes the dentinal tubules and intertubular collagenous matrix. This increases the blood components' adherence and adsorption to the root surface.

Lesser sample size may be a limitation for this research. From the clinical point of view, radicular biomodification may be utilized as in addition to conservative periodontal treatment as they amend periodontal wound healing positively. Yet, additional research with bigger sample sizes is necessary to verify the results of root conditioners on periodontal wound steadiness and curing.

Conclusion

Amid the confines of the inherent limitations in this research, the investigators arrived at a conclusion that the dentinal surfaces subjected to conditioning with EDTA gel group as well as coated with human whole blood resulted in appreciably superior fibrin clot bonding to dentin vs Biopure MTAD™ as well as the tetracycline hydrochloride solution group.

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