Evaluation of the Efficacy of Root Surface Biomodification Agents on Periodontal Regeneration: An SEM Study

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Abstract
Aim: The aim of the current research was to appraise the effectiveness of three radicular surface biomodification agents on the regeneration of periodontal tissues.

Materials and methods: A total of 60 human permanent mandibular premolars with a solitary root that was subjected to extraction owing to complex periodontal pathosis were chosen for the purpose of this research. Decoronation and longitudinal splitting of the sample teeth were performed to create sections that were shaped into a rectangle having estimated proportions of 4 × 2 × 1 mm employing slow-speed diamond bur beneath water irrigation. Next, the chosen teeth were allocated at random using a simple random technique into one of the following three groups—group I, application of Carisolv™ root biomodification agent; group II, application of ethylenediaminetetraacetic acid (EDTA) root biomodification agent; group III, application of a mixture of tetracycline isomer, acid, detergent (MTAD™) root biomodification agent. Each sample was evaluated and observed beneath the scanning electron microscope (SEM) at 1000× magnifying power to appraise alterations on the radicular surfaces.

Results: Carisolv™ radicular biomodifiers exhibited the greatest radicular surface biomodification at 2.92 ± 0.12, in pursuit by EDTA radicular biomodifiers at 3.38 ± 0.09, finally followed by MTAD™ radicular biomodifiers at 3.52 ± 0.14. A statistically significant disparity was noted among the groups.

Conclusion: The current research arrived at a conclusion that the Carisolv™ radicular biomodifiers exhibited the highest radicular surface modifying properties in comparison to EDTA and MTAD™ radicular biomodifiers.

Clinical significance: Alterations in radicular surfaces in the surroundings of periodontal disease and the existence of periodontal pockets hamper the periodontal tissue’s regenerative process. Periodontal management should thus produce a radicular surface well-suited for the cells that arbitrate repair as well as the periodontal regenerative process.

Keywords: Periodontal diseases, Periodontal regeneration, Root Planing, Root surface biomodification, Scaling.

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Introduction
Periodontal inflammation, also known as “periodontitis,” has a bacteria etiology chiefly affecting the periodontal tissues leading to lamina propria attachment loss, resorption of the alveolar bone, as well as the relocation of the junctional epithelium. The inflammatory process provoked by the dental biofilm causes alteration of the radicular surface leading to detachment of the collagen fiber inclusion, radicular surface infectivity with bacterial endotoxins, enhanced density of minerals, as well as decreased chemotactic urge to the cells of the periodontium that are accountable for regenerative processes. Thus, periodontal treatment does not merely aim to seize the pathologic process but further aims to also cause regeneration of the missing periodontal constitution, which comprises the periodontal ligament, cementum, and plus alveolar bone.¹

Scaling and root planing are efficient in getting rid of the buildup along with accrual caused by bacteria and endotoxins from the uncovered radicular surface. Nevertheless, it is not feasible to entirely disinfect the radicular surface that is afflicted with periodontal inflammation by employing unaided mechanical techniques. A surface subject to instrumentation unavoidably gets coated with a smear film which is formed by nearly all root management practices and might possibly influence fibroblast adjustment in periodontal wound curing.² This smear coat comprises dental calculus remains, impure radicular cementum, in addition to a subgingival plaque that behaves as a physical hurdle amid

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periodontium and the radicular surface, consequently restraining the development of a novel attachment. Besides, the smear coat resists saline cleansing. Therefore, radicular conditioning has been suggested as an appendage to mechanical radicular surface debridging to eliminate the smear coat as well as the root-related endotoxins while exposing the dentinal collagen fibers.3

Radicular surface bioalteration, as well as conditioning, encompasses numerous tactics derived from physical/chemical therapies to stimulate cell adhesion as well as blood clot creation, consequently making better the healing of the diseased periodontium. The significant action of these techniques is the elimination of the smear coat, which can impede cell relocation plus bonding, and may comprise the substrate for bacterial plaque development along with growth, therefore depressingly influencing the cure of damaged periodontal tissues. The conditioning of the radicular surface, therefore, leads to uncovering of the dentinal tubules and of the intratubular/peritubular dentinal collagen matrix.4

Numerous substances have been predicted for radicular bioalteration, like citric acid as well as phosphoric acid, tetracycline hydrochloride (HCl), EDTA, fibronectin, hydrogen peroxide, enamel matrix proteins, recombinant human growth factors, specifically platelet-derived growth factor in addition to dentin bonding matrix proteins, recombinant human growth factors, specifically platelet-derived growth factor in addition to dentin bonding.

As a result, radicular bioalteration is yet arguable, and its relevance in regenerative periodontal treatment is uncertain. Furthermore, it is unclear which of these substances is efficient to a higher degree for radicular bioalteration. Thus, this in vitro research was intended to appraise the effectiveness of three radicular surface biomodification agents on the regeneration of periodontal tissues.

**MATERIALS AND METHODS**

The current research was performed in the department of periodontics, Kalinga Institute of Dental Sciences, Bhubaneswar, Orissa, India. A total of 60 human permanent mandibular premolars with a solitary root that was subjected to extraction owing to complex periodontal pathosis were chosen for the purpose of this research. While premolars with dental caries/affected pulp from any other cause, cervical abrasion, erosion as well as cervical restorations were subjected to exclusion. Postextraction, the sample premolars were rinsed under saline to abolish the attached soft tissues. This was followed by manual instrumentation of the sample to eradicate any attached calculus. The samples were subjected to storage in 10% formalin. The surface of the cementum was scrupulously subjected to root planing and irrigation with distilled water to procure an even and spotless surface.

**Sample Preparation**

Decoronation and longitudinal splitting of the sample tooth were performed to create radicular halves. Every half was additionally sectioned to eliminate the coronal plus apical 3rd of the radix in order to conserve the middle 3rd region of the root. The sections were shaped into a rectangle having estimated proportions of 4 × 2 × 1 mm employing slow-speed diamond bur beneath water irrigation. The specimens were subjected to storage in disinfected capped tubes full of sterile saline solution at 4°C till further utilization.

Next, the chosen teeth (20 samples in each group) were allocated at random using a simple random technique into one of the following three groups:

**Group I: Application of Carisolv™ Root Biomodification Agent**

Carisolv™ (Medi Team, Dentalutveckling AB, Savedalen, Sweden) was coated on the radicular surface for 3 minutes. This was done to ease the elimination of calculus by the dissolution of calculus with a chemical means and cause decontamination of the radicular surface.

**Group II: Application of EDTA Root Biomodification Agent**

Treatment with 17% EDTA solution (Merck, Darmstadt, Germany)—root conditioner was coated on the radicular surface of the premolars using a soft brush for 3 minutes.

**Group III: Application of MTAD™ Root Biomodification Agent**

Specimens were conditioned with MTAD™ (Dentsply Tulsa Dental, Tulsa, Oklahoma, United States of America) employing a soft brush for 3 minutes.

**Preparation of Samples for Scanning Electron Microscopic Evaluation**

The samples were subjected to fixing in 2% glutaraldehyde for 15 minutes and then washed beneath phosphate buffer solution (PBS), followed by additional postfixing for 12 hours at 4°C–6°C with 1% (w/v) osmium tetroxide in 0.1M PBS buffer for 30 minutes at 37°C and once more 0.1M PBS was employed to finally cleanse for roughly ten minutes. The root sections were then subjected to dehydration in changeable ethyl alcohol concentrations (70, 80, 90, 95, and 100%) for 10 minutes. The samples were made dry by means of SAMDRI PVT-3 critical point dryer equipment, followed by gold coating with the aid of a sputter finishing the appliance. Sputtering leads to the inclusion of a conducting surface (gold) in the nonconducting sample for it to be identified by an SEM. Afterward, each sample was evaluated and observed beneath the SEM (Carl Zeiss, Evo 40) at 1000x magnifying power to appraise alterations on the radicular surfaces. Photomicrographs were procured to scrutinize the morphological transformations on the surface (Fig. 1). This study involved two investigators. One calibrated investigator was involved in the application of the root surface biomodification agents. Another investigator was involved in the scoring.

The images were then subjected to scoring as per root surface modification index,5 which is as under:

- Score 1: Radicular surface devoid of smear coating, with the dentinal tubules totally open; no proof of smear coating in the dentinal tubule breaches.
- Score 2: Radicular surface devoid of smear coating, with the dentinal tubules totally open; proof of smear coating in the dentinal tubule breaches.
- Score 3: Radicular surface devoid of smear coating, with the dentinal tubules incompletely open.
- Score 4: Radicular surface enclosed with smear coating, with consistent characteristics; proof of dentinal tubule breaches.
- Score 5: Radicular surface enclosed with smear coating, with consistent characteristics; no proof of dentinal tubule breaches.
- Score 6: Radicular surface enclosed with smear coating, with uneven characteristics as well as the existence of grooves and/or sprinkled debris.

**Statistical Analysis**

The data were statistically evaluated using the Statistical Package for the Social Sciences software version 20. Results were documented
as standard deviation as well as mean. Analysis of variance (ANOVA) and Tukey post hoc statistical tests was used to establish the statistically significant disparities among each group. Statistical significance was set at a p-value of <0.05.

RESULTS

The evaluation of the effectiveness of different radicular surface biomodifiers is delineated in Table 1. Carisolv™ radicular biomodifiers exhibited the greatest radicular surface biomodification at 2.92 ± 0.12, in pursuit by EDTA radicular biomodifiers at 3.38 ± 0.09, and finally followed by MTAD™ radicular biomodifiers at 3.52 ± 0.14.

The contrast assessment of different radicular surface modifiers is depicted in Table 2. The greatest radicular surface bioalteration was noted in Carisolv™ radicular biomodifiers at 2.92 ± 0.12 in pursuit by EDTA radicular biomodifiers at 3.38 ± 0.09 as well as MTAD™ radicular biomodifiers at 3.52 ± 0.14. A statistically significant disparity was noted among the groups.

On the whole, groups contrast assessment of the effectiveness of different radicular surface biomodifiers is described in Table 3. A noteworthy disparity was noted amid Carisolv as well as EDTA and Carisolv as well as MTAD. However, there were no noteworthy dissimilarities between EDTA and MTAD.

DISCUSSION

Altering the radicular surface that has been uncovered by periodontal pathosis into a further suitable atmosphere for periodontal rejuvenation is an imperative primary stride in inducing regeneration. Investigators have tried to eliminate toxic accretions from dental radicular surfaces in numerous manners. Scaling and root planing produce microcrystalline debris in a smear coating of 2.15 mm thickness, resolutely adhering to the radicular surface, which may only be gotten rid of with radicular surface demineralization substances. Therefore, demineralizing the radicular surface has been pioneered with a number of resources to eradicate toxic substances in addition to the smear coat from the radicular surface. Subsequently, the radicular surface conditioner is coated to unwrap the cementum–collagen matrix as well as ease the bonding amid the radicular surface and initially the blood

Table 1: Assessment of the efficacy of different root surface biomodification agents

<table>
<thead>
<tr>
<th>Root surface biomodification agents</th>
<th>Mean ± standard deviation (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: Carisolv™ root biomodification agent</td>
<td>2.92 ± 0.12</td>
</tr>
<tr>
<td>Group II: EDTA root biomodification agent</td>
<td>3.38 ± 0.09</td>
</tr>
<tr>
<td>Group III: MTAD™ root biomodification agent</td>
<td>3.52 ± 0.14</td>
</tr>
</tbody>
</table>

Table 2: Comparison of the efficacy of different root surface biomodification agents

<table>
<thead>
<tr>
<th>Root surface biomodification agents</th>
<th>Mean ± SD</th>
<th>F value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: Carisolv™ root biomodification agent</td>
<td>2.92 ± 0.12</td>
<td>26.146</td>
<td>0.001</td>
</tr>
<tr>
<td>Group II: EDTA root biomodification agent</td>
<td>3.38 ± 0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III: MTAD™ root biomodification agent</td>
<td>3.52 ± 0.14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Overall group comparison of the efficacy of different root surface biomodification agents

<table>
<thead>
<tr>
<th>Groups Compared with</th>
<th>Mean difference (I – J)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carisolv</td>
<td>EDTA</td>
<td>−0.46</td>
</tr>
<tr>
<td></td>
<td>MTAD</td>
<td>−0.60</td>
</tr>
<tr>
<td>EDTA</td>
<td>Carisolv</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>MTAD</td>
<td>−0.14</td>
</tr>
<tr>
<td>MTAD</td>
<td>Carisolv</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>EDTA</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Bold values are statistically significant
constituent at the instance of surgical intervention and, next, the connective tissue during the healing period. Teeth that were subject to extraction due to periodontal diseases were included in this research. Additionally, samples with solitary roots were considered to prevent prejudice on the prophecy of results amid teeth with solitary and those with multiple roots. Teeth with dental caries were excluded as they could unfavorably influence the radicular surface topography. Teeth with a recent history of scaling and root planing actions were not included as these techniques may change the radicular surface topography. Teeth with attrition, abrasion as well as erosion were also not subject to inclusion, as secondary alterations in the tooth configuration, like the mineral constitution and creation of sclerotic dentin, take place in such circumstances.

In the current research, the utmost radicular surface biomodification was exhibited by Carisolv™ radicular biomodifiers versus EDTA plus MTAD™ radicular biomodifiers. The utility of chemical substances in alliance with mechanical management exemplifies the likelihood of a smaller amount of trauma, forestalling the unnecessary loss of radicular areas. Carisolv™ is amongst those agents that have been launched recently. In the specialty of periodontology, the likelihood of chemically disbanding calculus and infected radicular cementum to enable their mechanical elimination is among the greatly hopeful implications of Carisolv™ gel. Likewise, Banerjee et al. elucidate that it can eradicate the smear coat in the course of mechanical elimination as it behaves as a lubricating gel. Carisolv™ gel was additionally capable of eradicating the impure cementum while uncovering the hale and hearty structures. In addition, Lima and Oliveira have stated that Carisolv™ has bactericidal effects in opposition to Streptococcus mutans and Lactobacillus species.

In this research, the radicular surface biomodification was somewhat higher in EDTA versus MTAD™ radicular biomodifiers. This agrees with the study by Blomlöf and Lindskog, who illustrated that the etching action exhibited by EDTA augments cell/tissue colonization near the commencement by providing a further biologically satisfactory surface for cellular/tissue bonding. A supplementary investigation by Gamal inferred that EDTA gel radicular conditioning enhances β-tricalcium phosphate pooled clot adhesion to periodontally afflicted root surfaces. In discrepancy, Leite et al. proposed that there can be incomplete abolition of the gel from the root surface, as EDTA leads to calcium chelation, and its remnants may restrain/setback the coagulation process.

In research by Calt and Serper, the correct efficiency of MTAD has been established following the application of the gel for four minutes subsequent to scaling and root planing. This method not only eliminated the radicular surface smear coat but also exhibited unsurpassed results in adhesion as well as the growth of the periodontal ligament cells. Apart from the contact tenure, it has been revealed that the healing actions of MTAD swiftly become noticeable so that it can get rid of the smear coat in fewer than 60 seconds. Scalza et al. and Zehnder et al. confirmed that MTAD could decrease dentinal micro-hardness by 17.33–29.48%, and this outcome is significantly better than other dynamic as well as control solutions. In reality, the natural pH, in addition to the ability to get rid of the radicular surface smear coat, makes the utility of MTAD extremely advantageous in clinical practice.

A smaller sample size is one of the limitations of this research. Also, investigations that have depicted encouraging results in vitro have intermittently been unsuccessful in showing comparable results in clinical trials. Therefore in vivo research must be performed to corroborate the same.

**Conclusion**

The current research arrived at a conclusion that the Carisolv™ radicular biomodifiers exhibited the highest radicular surface biomodifying properties in comparison to EDTA and MTAD™ radicular biomodifiers.

**References**


