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SCANNING ELECTRON MICROSCOPIC EVALUATION OF ROOT SURFACE ALTERATION INDUCED BY DIFFERENT ROOT BIOMODIFICATION AGENTS: AN IN-VITRO COMPARATIVE STUDY

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INTRODUCTION

Successful periodontal regeneration requires the establishment of a biologically compatible root surface that facilitates the attachment, migration, and proliferation of periodontal ligament cells and gingival fibroblasts. Mechanical instrumentation by scaling and root planing (SRP), although considered the gold standard for periodontal debridement, produces a smear layer composed of organic and inorganic debris, microorganisms, endotoxins, and contaminated cementum.[1,2] This smear layer may interfere with cellular attachment and compromise periodontal wound healing.

The concept of root biomodification was introduced to enhance the biologic acceptability of periodontally diseased root surfaces. Root biomodification refers to the chemical alteration of the root surface after mechanical instrumentation with the objective of removing the smear layer, exposing collagen fibrils, opening dentinal tubules, and creating a biologically favorable surface for periodontal regeneration.[3,4]

The modern concept of root conditioning emerged from the pioneering work of Register and Burdick (1975)[5] who demonstrated that citric acid treatment could demineralize root surfaces and potentially enhance connective tissue attachment. Subsequently, numerous agents including tetracycline hydrochloride, ethylenediaminetetraacetic acid (EDTA), citric acid, and more recently biologically active materials such as injectable platelet-rich fibrin (i-PRF) and oxygen-releasing BlueM gel have been investigated.

Tetracycline hydrochloride has been extensively studied because of its antimicrobial properties, root demineralizing effect, and ability to expose collagen fibers.[6] EDTA, a neutral pH chelating agent, has gained popularity due to its ability to remove the smear layer without causing excessive root surface damage.[7,8] Citric acid has demonstrated effective root demineralization but may produce variable results because of its highly acidic nature.

Recently, novel agents such as i-PRF and BlueM gel have attracted interest in regenerative dentistry. i-PRF contains platelets, leukocytes, and growth factors that promote tissue healing and angiogenesis.[9,10] BlueM gel, an oxygen-rich formulation containing active oxygen-releasing compounds, has been reported to possess antimicrobial and wound-healing properties.[11] However, their efficacy as root biomodification agents remains inadequately investigated.

Therefore, the present study aimed to compare the effectiveness of tetracycline hydrochloride, EDTA, citric acid, BlueM gel, i-PRF, and saline in modifying root surface morphology and removing the smear layer using scanning electron microscopy (SEM).

MATERIALS AND METHODS

Study Design

An in vitro comparative SEM study was conducted using freshly extracted human teeth affected by periodontal disease.

Extracted teeth

Armamentarium

Sample Preparation
Extracted teeth were thoroughly cleaned to remove blood, soft tissue remnants, and visible debris. Scaling and root planing were performed using hand curettes and ultrasonic scalers.

Following instrumentation, the teeth were sectioned using a diamond disc under copious irrigation. The crown portion and apical one-third of the root were removed, retaining the middle third of the root for analysis.

The specimens were randomly allocated into six experimental groups:

- Group I – Tetracycline Hydrochloride
- Group II – EDTA
- Group III – Citric Acid
- Group IV – BlueM Gel
- Group V – Injectable Platelet-Rich Fibrin (i-PRF)
- Group VI – Saline (Control)

Root Biomodification Procedure
Tetracycline hydrochloride solution was prepared by adding a standard 1 ml of sterile water with the contents of one capsule. The solution was thoroughly mixed in a sterile dappen dish until producing viscous slurry simulating clinical technique.

The prepared root specimens were treated with the assigned root biomodification agent for 5 minutes. After 5 minutes, all specimens were rinsed with distilled water and prepared for SEM examination.

These treated samples were then sealed and sent to the laboratory for SCM analysis.

Scanning Electron Microscopic Analysis
The primary evaluation was performed at 20000 magnifications because these magnifications provide optimal visualization of dentinal tubule patency and smear layer removal.

The specimens were evaluated for:

- Presence or absence of smear layer
- Dentinal tubule exposure
- Surface roughness
- Surface deposits and debris
- Evidence of collagen exposure

RESULTS
SEM evaluation demonstrated varying degrees of root surface modification among the tested agents.

Tetracycline Group
Tetracycline-treated specimens exhibited the most pronounced root surface alterations. The smear layer was markedly reduced, and numerous dentinal tubule openings became visible. Surface demineralization and collagen exposure were evident. Among all groups, tetracycline demonstrated the greatest potential for smear layer removal.

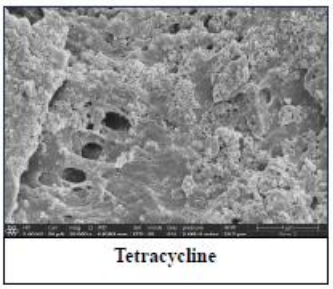
EDTA Group
EDTA-treated specimens showed substantial smear layer removal with a relatively uniform root surface. Dentinal tubule openings were observed in some areas. Although slightly less effective than tetracycline, EDTA produced favorable root conditioning while preserving surface integrity.

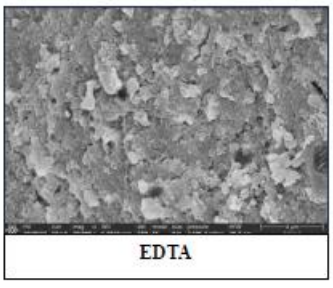
Citric Acid Group
Citric acid produced moderate root demineralization and partial smear layer removal. Surface roughness increased following treatment; however, dentinal tubule exposure was less pronounced compared with tetracycline and EDTA.

BlueM Gel Group
BlueM gel produced surface modification characterized by mild surface roughening and partial disruption of the smear layer. Although some cleaning effect was observed, complete smear layer removal and widespread dentinal tubule exposure were not consistently evident.

i-PRF Group
i-PRF-treated specimens demonstrated fibrin-like biological deposits on the root surface. While these deposits might be advantageous for cellular attachment and regenerative healing, they obscured direct visualization of dentinal tubules. Smear layer removal appeared limited compared with conventional chemical conditioners.

Saline Group
Saline-treated specimens exhibited persistent smear layer and surface debris with minimal dentinal tubule exposure. This group demonstrated the least root surface modification.


Tetracycline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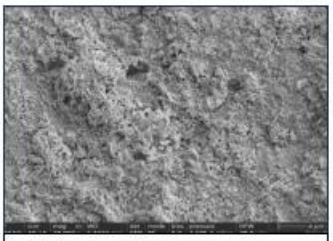
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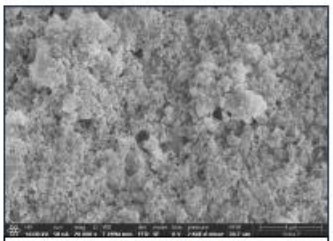
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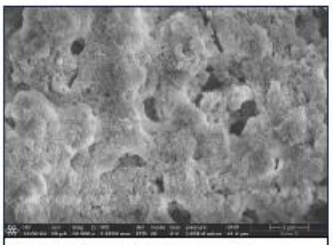
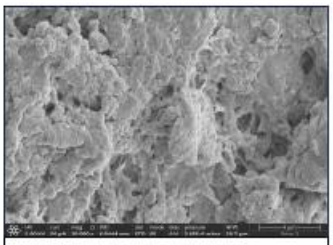

i-PRF


Saline

Overall Ranking
1. Tetracycline
2. EDTA
3. Citric Acid
4. BlueM Gel
5. Saline
6. i-PRF

DISCUSSION
The objective of root biomodification is to remove the smear layer generated during mechanical instrumentation and expose a biologically receptive root surface capable of supporting periodontal regeneration.

The present study demonstrated that tetracycline produced the greatest degree of smear layer removal and dentinal tubule exposure. These findings are consistent with those reported by Madison JG et al. (1997)[12] Terranova et al. (1986)[13] and Babay (2000)[14] who observed enhanced root demineralization and collagen exposure following tetracycline application.

EDTA demonstrated results comparable to tetracycline. Blomlöf et al. reported that EDTA effectively removed the smear layer while preserving collagen architecture because of its neutral pH.[7] Similar findings were observed in the present study, where EDTA-treated surfaces appeared cleaner and more uniformly conditioned.

Citric acid produced moderate smear layer removal. Register and Burdick first demonstrated the regenerative potential of citric acid-treated root surfaces. However, later investigations reported inconsistent outcomes because of variable demineralization and potential tissue irritation. The present findings support these observations.

BlueM Gel
BlueM gel represents a relatively novel oxygen-releasing biomaterial used in wound healing and implant dentistry. Active oxygen enhances local tissue metabolism and may reduce anaerobic bacterial load. However, limited literature exists regarding its role in root biomodification.

The present study demonstrated partial smear layer disruption but failed to show the degree of dentinal tubule exposure observed with tetracycline and EDTA. This suggests that BlueM gel may be more beneficial as a biologic adjunct rather than a primary root-conditioning

agent.

Future studies investigating different concentrations, application durations, and combinations with conventional conditioners may further clarify its role in periodontal regeneration.

Injectable Platelet-Rich Fibrin (i-PRF)

i- PRF is rich in platelets, leukocytes, fibrinogen, and growth factors including PDGF, VEGF, and TGF-β. Unlike conventional chemical conditioners, its primary objective is not demineralization but biologic enhancement of wound healing.

In the present study, fibrin deposits were observed on the root surface, indicating biologic coating rather than smear layer dissolution. Similar observations have been reported in regenerative studies evaluating platelet concentrates.

The findings suggest that i-PRF may serve as a biologically active regenerative adjunct rather than a direct smear-layer-removing agent.

Studies Supporting Similar Findings related to the root biomodification agents are,

- Register and Burdick (1975)
- Wikesjö et al. (1986)
- Terranova et al. (1986)
- Blomlöf et al. (1996)
- Babay (2000)
- Madison and Hokett (1997)

These studies generally reported:
Tetracycline > EDTA > Citric Acid > Control

Studies Reporting Contradictory Findings:
Several clinical studies have questioned the long-term benefits of root biomodification despite favorable SEM findings.

- Mariotti (2003)
- Polson and Proye
- Hanes and O'Brien

These authors suggested that although root conditioning may improve root surface morphology, its clinical superiority over conventional SRP alone remains uncertain.

Thus, while SEM studies consistently demonstrate improved root surface characteristics, translation into predictable clinical regeneration remains a subject of debate.

CONCLUSION

Within the limitations of this in vitro SEM study, tetracycline hydrochloride demonstrated the greatest effectiveness in smear layer removal and dentinal tubule exposure, followed closely by EDTA. Citric acid produced moderate root conditioning, whereas BlueM gel exhibited limited smear layer removal. I-PRF showed deposition of biological products instead of removing the smear layer. Saline-treated specimens showed persistent smear layer and minimal surface modification.

The findings suggest that conventional chemical root biomodification agents remain superior for smear layer removal. However, novel biologic agents such as BlueM gel and i-PRF may offer additional regenerative benefits through mechanisms other than direct root demineralization. Further studies incorporating cellular attachment assays and clinical outcomes are warranted to establish their role in periodontal regeneration.

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