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(RESEARCH ARTICLE)

ROOT SURFACE BIO MODIFICATION USING NATURAL FRUIT JUICE EXTRACTS: AN IN VITRO SCANNING ELECTRON MICROSCOPIC STUDY

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ABSTRACT

Background: Scaling and root planning is cornerstone of periodontal therapy; however, it produces smear layer that may limit biological interaction between periodontal tissue and treated root surface. Root surface biomodification has been advocated to remove smear layer, expose collagen matrix and create a favorable surface for periodontal healing. Natural fruit extract rich in organic acid have recently gained attention as potential alternative to conventional chemical root conditioning agents.

Objective: To explore and assess the efficacy of freshly prepared citrus fruit extracts as a root surface conditioning agent over the conventional agents.

Methods: Single rooted teeth indicated for extraction due to periodontal diseases, were sectioned, instrumented with curettes and treated with test and control agents. Treated samples were observed and studied under scanning electron microscope.

Results: SEM evaluation demonstrated that all three root conditioning agent effectively modified instrumented root surface. The 10% citric acid group exhibited smear layer removal with complete dentinal exposure. Both fresh lemon and fresh carambola fruit juice extract immediately removed smear layer. Comparatively greater dentinal tubule exposure was observed in carambola group than in the lemon group, although complete tubule exposure was only achieved with citric acid.

Conclusion: Within the limitation of this exploratory in vitro study,10% Citric acid demonstrated most effective root surface bio modification. Fresh carambola fruit juice showed root conditioning potential with greater dentinal tubule exposure than fresh lemon juice, suggesting its application as a natural root conditioning agent. However, further quantitative laboratory investigations and clinical studies are required to validate these preliminary finding.

Keywords: Roots surface biomodification, Root conditioning; Scanning electron microscopy; Averroha carambola, citrus lemon, Smear layer, Dentinal tubule.

1 INTRODUCTION

Periodontitis is a chronic inflammatory disease initiated by complex interaction between dental plaque microorganism and host immune response, resulting in gradual breakdown of the tooth-supporting structures, including gingival tissue, periodontal ligament, cementum, and alveolar bone.[1,2] The primary goal of treatment of periodontitis is to halt the disease process through removal of bacterial deposits from the tooth structure and creation of a biological surface for periodontal regeneration.[3,4]

Scaling and Root Planing (SRP) remains gold standard non-surgical periodontal therapy for the debridement of plaque, calculus, bacterial endotoxins, and diseased cementum.[5–7] Despite its clinical effectiveness, instrumentation with hand or ultrasonic scalers inevitably produces smear layer that composed of organic and inorganic material, microbes, and degenerated collagen. The presence of this smear layer may occlude the dentin tubules, mask underlying collagen matrix and interfere the attachment, migration and proliferation of periodontal cells thereby compromising periodontal wound healing and regeneration.[8–10]

Root surface biomodification, also referred to as root conditioning, has been introduced as an adjunctive procedure to enhance the biological characteristics of instrumented root surfaces.[11–13] The application of chemical conditioning agents removes the smear layer, partially demineralizes the superficial root surface, exposes the collagen fibrillary matrix, and improves the surface characteristics required for fibroblast migration, adhesion, proliferation, and connective tissue attachment. [14,15] Consequently, root biomodification has become an important step in regenerative and mucogingival periodontal procedures.[16]

Chemical like citric acid, ethylenediaminetetraacetic acid (EDTA), tetracycline hydrochloride, phosphoric acid, and doxycycline are used to condition the root surfaces since ages.[17,18] While all the mentioned chemicals proved to be an effective removal of smear layer and revealing the dentinal tubules, issues regarding their availability, cost, ease of use and tissue irritation made people look out for alternative from nature. [19,20]

Natural products have increasingly been explored in periodontal research owing to their biocompatibility, cost-effectiveness, easy availability, and the presence of several bioactive compounds with antimicrobial, antioxidant, and wound-healing properties. [21,22] Fruit-based extracts are especially high in the number of naturally occurring acids, vitamins, flavonoids, and polyphenols which can help in root surface demineralization with good biological compatibility. [23–25] The fresh juice of lemon (Citrus limon) has low pH and citric acid and therefore is a good natural root conditioner.[26] On the other hand, fresh juice of carambola (Averrhoa carambola) fruit has various organic acids like oxalic and citric acid as well as antioxidant phytochemicals which can help in smear layer removal and root surface conditioning.[27–30] Although natural fruit extract possess diverse bioactive constituent, include organic acid and antioxidant compounds, their application s root surface biomodification agent has not been sufficiently investigated. [31]

Although plant derived biomaterial has received increasing attention in periodontal research, the application of natural fruit juice extracts as root conditioning agents remains inadequately explored. [32,33]Scanning electron microscopy is an effective technique in visualizing the root surface topography and also in assessing the removal of the smear layer and exposure of dentinal tubules after root conditioning.[34]

Therefore, the present exploratory in vitro study was undertaken to evaluate root surface biomodification potential of natural fruit juice extract using scanning electron microscopy. The root surface treated with fresh lemon and fresh carambola fruit juice extracts was qualitatively evaluated and compared based on the smear layer removal and dentinal tubule exposure.

2 MATERIAL AND METHODS

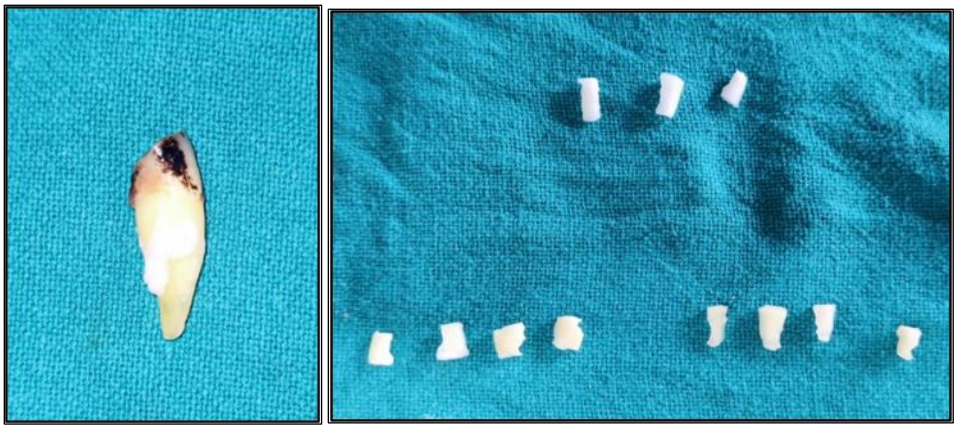
2.1 Study Design

The present study was designed as an exploratory in vitro scanning electron microscopic (SEM) study to evaluate root surface biomodification potential of natural fruit juice extract in comparison with citric acid.

2.2 Sample preparation

Periodontal compromised teeth indicated for extraction as a part of routine dental treatment were collected after extraction. The teeth were thoroughly cleaned with distilled water to remove blood, adhered soft tissue remnant and surface debris. Root surface were mechanically instrumented using periodontal curette to stimulate clinical scaling and root planning, thereby producing standard smear layer.

Subsequently, the root was sectioned into standardized specimen for root surface biomodification and SEM evaluation. (Figure.1a, b)



(a) (b)

Figure.1: a) Periodontal compromised extracted teeth b) Mechanical instrumentation and sectioning.

2.3 Study groups

- Group I (Control Group)- Citric acid solution
- Group II (Test 1)- Fresh lemon Juice(T1)
- Group III (Test 2)- Fresh Carambola (Averroha carambola) fruit juice T2 shown in Fig 2.

Figure. 2: (a)Control, (b)test 1 and (c)test 2 groups

2.4 Root surface conditioning procedure

Fresh lemon and carambola fruits were thoroughly washed with distilled water for juice extraction. The fruit were sectioned aseptically, and the juice was extracted immediately before application to ensure freshness. The designated conditioning agent was applied directly onto the instrumented root surface using a sterile cotton pellet. The 10% Citric acid solution (was prepared by 10g citric acid crystal in 100g of distilled water) applied for 3 min, whereas fresh lemon and carambola juice is applied for 7 min as a single continuous application. Following root surface biomodification, all specimens were gently rinsed with distilled water to remove any residual conditioning agent and allowed to air dry before SEM analysis.

2.5 Scanning electron microscopic evaluation

The conditioned root specimens were prepared for scanning electron microscopic evaluation according to standard laboratory procedure. The specimens were examined using JSM-5600 LV scanning electron microscope (JOEL, TOKYO, JAPAN). Digital photomicrographs were obtained at 1000x magnification with the specimen maintained at zero tilt angle. Root surface morphology qualitatively evaluated for presence or absence of smear layer and degree of dentinal tubule exposure.

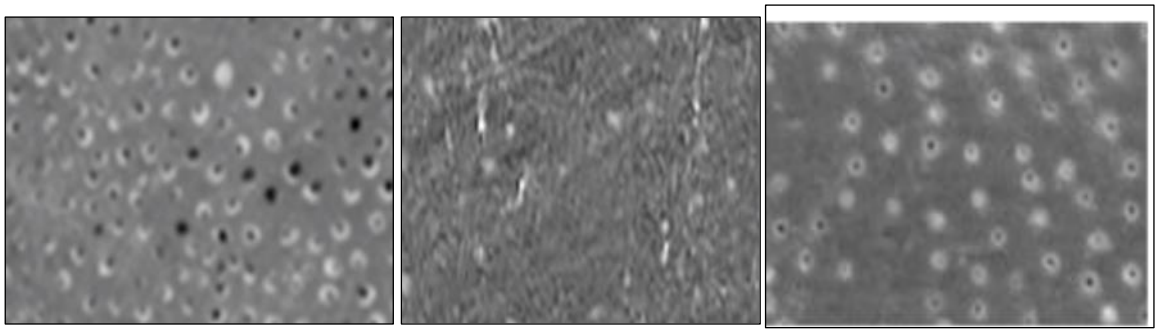
3 RESULTS

SEM examination revealed noticeable differences in root surface morphology among the three study group following root surface morphology among the three study group following root surface biomodification.

The control group (10% citric acid) demonstrated complete removal of smear layer with most dentinal tubules appearing completely exposed. The conditioned root surface appeared clean and well defined, indicating root surface conditioning (Figure.3 A)

In group II (fresh lemon juice), the smear layer was effectively removed from root surface. Most dentinal tubules appear partially exposed, while overall root surface morphology suggested satisfactory root conditioning. (Figure.3 B)

In group III (Fresh carambola fruit juice) exhibited effective smear layer following 7 minutes of application. Most dentinal tubules were partially exposed and conditioned root surface demonstrated morphological characteristics comparable to those observed in lemon juice group. (Figure.3 C)



(A) (B) (C)

Figure.3: Representative SEM micrograph of root surfaces following root surface biomodification A) control group (10% citric acid) B) lemon juice group and C) carambola fruit juice group

Overall, qualitative SEM observation demonstrated that both fresh lemon and carambola fruit juice extracts were effective in removing smear layer from instrumented root surfaces. Complete dentinal tubule exposure was observed in control group treated with 10% citric acid. Among two natural fruit juice extracts, the carambola group exhibited greater dentinal tubule exposure than lemon group, indicating comparatively better root surface biomodification following 7-minute application period.

4 DISCUSSION

Root surface biomodification has been advocated as an adjunctive procedure to improve biological compatibility of instrumented root surfaces by facilitating smear layer removal and exposing the underlying collagen matrix and dentinal tubules. Such surface modification are considered favorable for fibroblast attachment, migration and subsequent periodontal wound healing,[35]

In present exploratory in vitro study, qualitatively SEM observation demonstrated that all three conditioning agent effectively remove the smear layer from instrumented root surfaces. The control group treated with 10% citric acid exhibited complete smear layer removal with complete dentinal tubule exposure. Fresh lemon and fresh carambola fruit juice extract also produced satisfactory root surface biomodification although dentinal tubule remain partially exposed. Among the natural two fruit juice extracts, the carambola group qualitatively demonstrated greater dentinal tubule exposure than lemon group.

Citric acid has long been regarded as one of the most extensively investigated root conditioning agent because of its ability to demineralize the superficial root surface and expose collagen matrix. Previous studies[36–38] have reported effective smear layer removal and dentinal exposure following citric acid application, finding that are consistent observations of present study.

The favourable finding observed with fresh lemon juice may be attributed to its naturally acidic pH and citric acid content, which facilitate superficial demineralization of root surface and contribute to smear layer removal. In addition, lemon contains naturally occurring antioxidants and bioactive phytochemicals that may support a biologically compatible root surface.[39]

Fresh carambola (Averroha carambola) fruit juice also demonstrated effective smear layer removal and comparatively greater dentinal tubule exposure than fresh lemon juice. The favourable root surface changes observed with fresh carambola fruit juice may be attributed to its naturally occurring organic acids, including oxalic and citric acid, as well as rich content of flavonoids, polyphenols and antioxidant phytochemicals.[40] These bioactive constituents have been reported to exhibit antimicrobial, anti-oxidant and anti-inflammatory activities which may contribute biologically favourable root surface for periodontal healing. To best of our knowledge, this is first exploratory in-vitro to investigate the potential of fresh carambola fruit juice as a natural root conditioning agent root surface biomodification and may serve as a foundation for future research.

Although citric acid remained the most effective root conditioning agent in the present study, both natural fruit juice extract demonstrated encouraging root surface changes following application. These findings may suggest that naturally derived fruit juice extract may represent economical, readily available and biocompatible alternatives for root surface biomodification. However, their clinical effectiveness should be validated through additional laboratory and clinical investigations.

4.1 Limitation

The present study has certain limitations. The observations were qualitative in nature and based solely on scanning electron microscopic evaluation without quantitative image analysis or statistical comparison. In addition, the sample size was limited and biological effect of conditioning agent on periodontal cell reattachment and regeneration were not investigated. Further in vitro studies incorporating quantitative SEM analysis, cell culture experiments and well-designed clinical trials are required to validate the finding of present study.

5 CONCLUSION

Within the limitation of present exploratory in vitro study, all the evaluated root conditioning agent demonstrated ability to remove smear layer and modify the instrumented root surface. Among them,10% citric acid produced the most pronounced root surface biomodification, exhibiting complete smear layer removal with complete exposure of dentinal tubules on qualitative SEM evaluation. Fresh lemon (citrus lemon) and fresh carambola (Averroha carambola) fruit juice extracts also demonstrated favourable root surface changes following application. Qualitative observation suggested greater dentinal tubule exposure in the carambola group than lemon group.

The finding of present study suggest that fresh carambola fruit juice may represent a natural root conditioning agent for root surface biomodification. However, further in vitro and clinical studies with quantitative evaluation are required to confirm these preliminary observations and establish its potential clinical application in periodontal therapy.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

Statement of ethical approval

Ethical approval was obtained from the Institutional Ethics Committee of Siksha ‘O’ Anusandhan (SOA) University, Bhubaneswar, prior to the commencement of the study.

Statement of informed consent

Verbal informed consent was obtained from the patients for the use of their extracted teeth for research purposes.

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