ORIGINAL RESEARCH

Impact of Different Chelating Agents on Fibrin Clot Adhesion to the Exposed Root Surface: A Comparative Study

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Abstract

Aim: The aim of the current study was to assess the influence of three different chelating agents on fibrin clot union to the exposed radicular surface.

Materials and methods: A total of 40 recently extracted human teeth with a solitary root afflicted with periodontal disease were chosen for this research. Postroot planing, every sample was divided to procure two dentinal blocks more or less 3 × 3 × 1 mm in dimensions. Consequently, 80 radicular specimens were attained that were subjected to individual storage in saline solution. A total of 20 specimens were assigned to one of the following groups: group I—control group, group II—radicular surface subjected to treatment with doxycycline (10%), group III—radicular surface subjected to treatment with 24% ethylenediaminetetraacetic acid (EDTA) gel, and group IV—radicular surface subjected to treatment with saturated citric acid. Following the above, a single drop of fresh human whole blood was included in every dentin block while permitting clot formation for roughly 20 minutes. The blocks were then subjected to rinsing thrice for 5 minutes in 10 mL of phosphate-buffered saline (PBS). Ultimately the specimens were evaluated beneath a scanning electron microscope (SEM) unit.

Results: The highest mean fibrin network value was noted with the use of saturated citric acid at 2.88 ± 0.06, in pursuit by the use of 24% EDTA gel at 2.62 ± 0.12, doxycycline (10%) at 2.18 ± 0.18 while 1.28 ± 0.02 for the control group. The difference between the groups was statistically significant, with a $p < 0.001$.

Conclusion: It may be inferred that the highest fibrin network was found with the use of saturated citric acid group compared to 24% EDTA gel, doxycycline (10%), as well as control group.

Clinical significance: Applying chelating agents on periodontally-afflicted radicular surfaces subject to instrumentation augments the union of the fibrin clot while proving to be vital in the organization of a novel connective tissue attachment in periodontal rejuvenation techniques.

Keywords: Chelating agents, Fibrin clot, Root conditioning, Smear layer.

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Introduction

Procuring a spotless, even radicular surface that is free from contamination at the time of periodontal surgical procedures is crucial for the formation of a novel connective tissue reattachment plus augmentation of the curative tissue procedure. Although ultrasonic/manual scaling along with root planing is frequently employed procedures in clinical practice for the mechanical cleansing of the radicular surface all through periodontal treatment, they cannot fully decontaminate the dental hard tissue.[1] Furthermore, owing to mechanical debridement, a solid smear coat is created in close contact with the surface subjected to treatment, comprising a blend of organic plus inorganic agents, and microbial toxins, in addition to debris. The connective tissue reattachment procedure is limited from a histological perspective by the existence of the smear coat amid the radicular surface, as well as adjoining connective tissues.[2]

Periodontal treatment refers to the expected restoration of the periodontal tissues in regions formerly afflicted with periodontal disease. Radicular surfaces are probably vulnerable to hyper-mineralization and infectivity by dissimilar types of bacterial microorganisms as well as their endotoxins. Radicular surface contagion/infection is capable of altering the outcomes of rejuvenating periodontal treatment strategies and so to attain the most favorable and apt results, the amendment plus disinfection of the infected radicular surfaces is mandatory.[3]

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To compensate for such inherent drawbacks of mechanical radicular surface treatment, radicular conditioning by chemical means was established. Such radicular conditioning brought about by chemical means supports clot stability in the infant phases of periodontal healing by augmenting the bond of blood corpuscles plus fibrin to the radicular surface. Radicular conditioning is anticipated to enable detoxification of the radicular surface by getting rid of the smear coat while causing its demineralization, thereby causing exposure of the collagen matrix that upholds movement as well as the proliferation of cells engaged in the process of healing of periodontal tissues. A lot of chelating agents, such as citric acid, phosphoric acid, EDTA, tetracycline, fibronectin, antiformin, as well as sodium deoxycholate, have to be utilized. Citric acid, as well as tetracycline hydrochloride, have been expansively subjected to research and employed in clinical practice owing to their capacity to exhibit greater tissue tolerance in addition to ease of storage.[4]

These enormous labors were put in place to enhance the clot linkage power, as it is documented that the connection between clot-fibrin and radicular surface collagen can avoid epithelial down-growth. This can thereby lead to development of a scaffold that promotes cell growth and enhanced mature collagen fiber adhesion.[5] Therefore, the current study was performed to evaluate the influence of three different [doxycycline (10%), 24% EDTA gel, and saturated citric acid] chelating agents on fibrin clot union to the exposed radicular surface.

Materials and Methods

The current *in vitro* investigation was performed in the Department of Periodontics, Kalinga Institute of Dental Sciences, Kalinga Institute of Industrial Technology (KIIT) (Deemed to be University), Bhubaneswar, Odisha, India. A total of 40 recently extracted human teeth with a solitary root afflicted with periodontal disease were chosen for this research. Postremoval, the sample teeth were scrupulously subjected to washing as well as root planing with Gracey curettes. The samples underwent decoronation with the aid of carborundum disks. They were subjected to longitudinal division with a water-cooled high-speed fissure bur. The primary groove was placed at the cementoenamel junction, while the second was roughly about 3 mms apical to the first. Postroot planing, every sample was divided to procure two dentinal blocks more or less 3 × 3 × 1 mm in dimensions.[6] Consequently, 80 radicular specimens were attained that were subjected to individual storage in saline solution.

Chelating agent's application on exposed root surface:

- A total of 20 specimens were assigned to one of the following groups,

Group I—control group:

- A 3 minutes saline treatment of the radicular surface was performed in the control group.

Group II—root surface treated with doxycycline (10%):

- Doxycycline (10%) was applied with the aid of cotton balls that were replaced after 30 seconds each to ensure a uniform spread. Following the coating of the radicular surfaces, they were subjected to washing in distilled water to end every reaction.

Group III—root surface treated with 24% EDTA gel:

- The EDTA employed was commercially obtainable as 24% EDTA gel of pH 7.3 (PrefGel, Biora, Malmo, Sweden). This substance was coated on the sample for approximately 3 minutes using a cotton ball that was replaced after 30 seconds each.

Group IV—root surface treated with saturated citric acid:

- Saturated citric acid solution (Sigma-Aldrich, Bengaluru, India) was employed to treat the radicular surface with the aid of cotton balls drenched with it and replaced every 30 seconds for 3 minutes.

Addition of Human Blood for Fibrin Clot Formation

Following the above, a drop of a healthy male volunteer's venous blood was applied to each surface of the chemically treated roots, and these dentin blocks were allowed to clot for roughly 20 minutes. The blocks were then subjected to rinsing thrice for 5 minutes in 10 mL of PBS. These steps were performed at 36°C (that is the normal body temperature) and rinsing was done in petite Petri dishes with a tender swirling movement.

Assessment of Scanning Electron Microscopic Analysis

The dentinal blocks were subjected to fixation for 20 minutes in 2.5% glutaraldehyde. Afterward, the blocks were exposed to PBS 3 times and then for 5 minutes in graded ethanol (70, 90, and 95%) to cause dehydration. Two ultimate dehydration cycles, each with duration of 5 minutes were done using hexamethyldisilazane. The specimens were subjected to overnight drying within a dehydration jar positioned on metallic stubs by means of adhesive tape, along with gold sputter coating. Ultimately the specimens were evaluated beneath an SEM (JEOL, Tokyo, Japan). The radicular surfaces were subjected to scanning after which indicative photomicrographs were procured at 2000× magnification. The below scores were employed in determining the extent of fibrin network connection to the conditioned radicular surface:[7]

- Score 0—no fibrin network connection to dentinal surface
- Score 1—scant fibrin network connection to dentinal surface
- Score 2—modest fibrin network connection to dentinal surface
- Score 3—thick fibrin network connection to dentinal surface

The photomicrographs obtained by means of SEM were assessed followed by scoring by one examiner.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS)—SPSS PC version 20.0 was employed in performing the statistical assessments. The normality test Kolmogorov–Smirnov as well as the Shapiro–Wilks test, was employed in evaluating the normal distribution. Kruskal–Wallis test was used to contrast the score values amid the study cohorts. Pair-wise comparative assessment amid the study cohorts was performed with the Mann–Whitney test in the company of Bonferroni correction. $p < 0.05$ was set as the level of significance in this research.

Results

Table 1 and Figure 1 delineate the effectiveness of chelating agents on the fibrin network values amid the investigational groups. The control group exhibited a mean fibrin network score of 1.28 ± 0.02, while it was 2.18 ± 0.18 for doxycycline (10%), 2.62 ± 0.12 for 24% EDTA gel, and 2.88 ± 0.06 for saturated citric acid.

Table 2 depicts the relative effectiveness of chelating agents on fibrin network values amid the investigational groups. The highest mean fibrin network value was noted with use of saturated citric acid at 2.88 ± 0.06, in pursuit by use of 24% EDTA gel at 2.62 ± 0.12, doxycycline (10%) at 2.18 ± 0.18 while 1.28 ± 0.02 for the control group. The difference between the groups was statistically significant, with a $p < 0.001$.

On the whole, contrast evaluation of the effectiveness of chelating agents on fibrin network values amid the investigational groups was performed. The difference amid the groups depicted statistically significant differences with $p < 0.001$, with the exception amid 24% EDTA gel, as well as saturated citric acid with $p > 0.001$ (Table 3).

The inference of the current study indicates that the maximum fibrin network was found with the use of saturated citric acid group followed by 24% EDTA gel, doxycycline (10%), and control group.

Discussion

The pathologically affected radicular surfaces do not favor novel attachment. Conventional management of the disease-modified radicular surfaces depends only on scaling and root planing.[8] Multiple research has documented that development of a smear coat following radicular surface debridement avoids movement and union of blood constituents from the periodontal wound area. The sticking together as well as steadiness of the blood clot amid the gingival flap as well as the radicular surface is critical for the healing course, particularly in regenerative as well as mucogingival processes for the creation of novel connective tissue attachment. Therefore, chelating agents have been established to eliminate the smear coat while augmenting the exposure of dentinal collagen fibers, which symbolize the substrate for the association with the fibrin clot.[9]

Baker et al.,[10] utilized an *in vitro* model to depict imitation of the most primitive healing processes in regenerating periodontium on a dentine-like surface after radicular instrumentation, dentinal conditioning to eliminate smear coat, creation of a fibrin clot as well as exposure of the fibrin clot to gentle unsettling forces. It is plausible that these circumstances that may encourage or unfavorably influence fibrin clot union in the model scheme may as well generate comparable consequences *in vivo*.

In the current research, the highest mean fibrin network values were noted with use of saturated citric acid in pursuit by 24% EDTA gel, doxycycline (10%), and the control group. Similarly, Blomlöf et al.,[11] elucidates that radicular conditioning with saturated citric acid results in fractional demineralization that seemingly augments mesenchymal cell union, perhaps by biochemical means.

The findings of the current research implicate that the allocation of fibrin network to the dentinal surface was appreciably higher in the investigational groups, that is, citric acid and 24% EDTA gel groups. Denser fibrin connection was noted over dentine that was conditioned with citric acid in addition to coating with whole human blood. Subramanian et al.,[12] depicted through their *in vitro* research that citric acid conditioning leads to fractional dentinal-demineralization that apparently augments mesenchymal cell union, perhaps by biochemical means. EDTA is not as efficacious as citric acid since it portrays reasonable fibrin network association when enclosed with whole human blood. EDTA exercises demineralizing actions in the course of chelating divalent cations at neutral pH. Nevertheless, no noteworthy disparities were noted in the allocation of fibrin network amid the citric acid as well as EDTA group. Manzolli Leite et al.,[13] in their research, depicted that citric acid conditioning augments clot development, which bolsters the results of this research.

Approximately 24% EDTA gel was employed to condition dentin blocks since, as per Blomlöf et al.,[14] the concentration of EDTA must be between 15 and 24% so as to procure a suitable smear-eliminating as well as collagen-exposing action amid a clinically adequate time duration. Also, Babay[15] has documented that supersaturated EDTA at 24% promotes the union of gingival fibroblasts to the radicular surface. Moreover, in the research by de Vasconcellos et al.,[16] 24% EDTA gel did not intervene with periodontal reparative procedures when employed along with standard periodontal therapy.

Doxycycline behaved as a radicular conditioning agent while enhancing elimination of the smear coat post-application. Likewise, Chahal et al.,[17] documented that elimination of smear coat with tetracycline as well as citric acid was superior versus doxycycline.

Table 1: Assessment of the efficacy of chelating agents on fibrin network scores between the study groups

Study groups	Mean ± standard deviation	95% confidence interval for mean	
		Lower	Upper
Group I: control group	1.28 ± 0.02	1.22	2.36
Group II: doxycycline (10%)	2.18 ± 0.18	2.14	3.04
Group III: 24% EDTA gel	2.62 ± 0.12	2.54	2.88
Group IV: saturated citric acid	2.88 ± 0.06	2.78	2.96

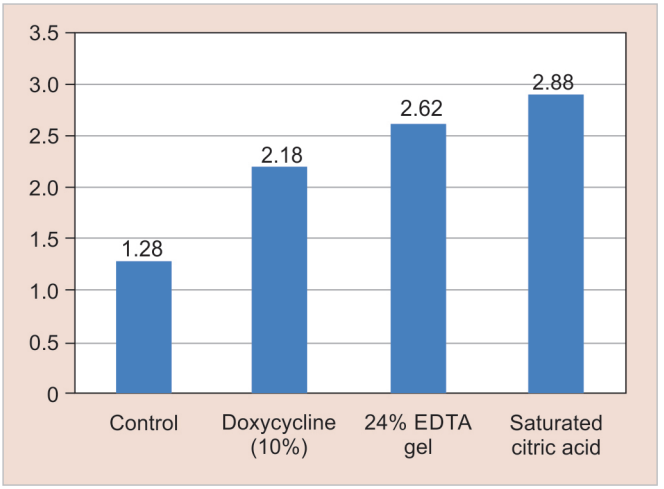

Fig. 1: Mean efficacy of chelating agents on fibrin network scores between the study groups

Table 2: Comparison of the efficacy of chelating agents on fibrin network scores between the experimental groups

Study groups	Mean ± standard deviation	95% confidence interval for mean		Mean rank	p-value
		Lower	Upper		
Group I: control group	1.28 ± 0.02	1.22	2.36	12.84	**0.001**
Group II: doxycycline (10%)	2.18 ± 0.18	2.14	3.04	20.08	
Group III: 24% EDTA gel	2.62 ± 0.12	2.54	2.88	21.16	
Group IV: saturated citric acid	2.88 ± 0.06	2.78	2.96	22.34	

Bold value denotes statistical significance.

Table 3: Overall comparison of the efficacy of chelating agents on fibrin network scores between the study groups

Study groups	*Compared with*	*Mean difference*	*Significance*
Control	Doxycycline (10%)	−0.90	0.001
	24% EDTA gel	−1.34	0.001
	Saturated citric acid	−1.6	0.001
Doxycycline (10%)	Control	0.90	0.001
	24% EDTA gel	−0.44	0.04
	Saturated citric acid	−0.70	0.001
24% EDTA gel	Control	1.34	0.001
	Doxycycline (10%)	0.44	0.04
	Saturated citric acid	−0.26	0.754
Saturated citric acid	Control	1.6	0.001
	Doxycycline (10%)	0.70	0.001
	24% EDTA gel	0.26	0.754

Also, the number of open tubules as well as their diameter was lesser with use of doxycycline.

Fibrin clot behaves as a crucial factor in catalyzing the premature stages of wound healing. Blood elements that develop on the radicular surface at the time of surgery and during wound approximation ascertain the attachment, which tolerates normal physiologic and other possibly tearing forces that act on the tooth-gingival flap border. This attachment should stay steady at the time of initial wound healing course so that the edge gets ample tensile potency on maturation to counterbalance the brunt faced by the disturbing forces. The acting together of parameters like the radicular surface, clot union, and connective tissue is essential for novel connective tissue development as it conflicts with a lengthy junctional epithelium.[6]

Petite sample size may be regarded as a limitation of this current research. Besides, citric acid should be cautiously utilized as it is likely to generate an acidic atmosphere in the adjacent tissues. Randomized clinical trials with adequate statistical power bolstered by quantitative histological assessment are recommended to verify and authenticate these *in vitro* interpretations.

Conclusion

Within the limitations of this research, it may be inferred that the highest fibrin network was found with the use of saturated citric acid group compared to 24% EDTA gel, doxycycline (10%), as well as control group.

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