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The effect of root surface conditioning on smear layer removal in periodontal regeneration (a scanning electron microscopic study)

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Abstract. The role of root surface conditioning treatment on smear layer removal of human teeth is affected by periodontitis in periodontal regeneration. The objective of this study is to analyze the smear layer on root surface conditioned with 2.1% minocycline HCl ointment (Perioline), and 24% EDTA gel (Prefgel). A total of 10 human teeth indicated for extraction due to chronic periodontitis were collected and root planed. The teeth were sectioned in thirds of the cervical area, providing 30 samples that were divided into three groups – minocycline ointment treatment, 24% EDTA gel treatment, and saline as a control. The samples were examined by scanning electron microscope. No significant differences in levels of smear layer were observed between the minocycline group and the EDTA group ($p=0.759$). However, there were significant differences in the level of smear layer after root surface treatment in the minocycline and EDTA groups, compared with the control group ($p=0.00$). There was a relationship between root surface conditioning treatment and smear layer levels following root planing.

1. Introduction

Periodontitis is one of the most common tooth supporting tissue inflammation diseases [1]. Calculus and tooth plaque are removed by scaling and root planing (SPA), which plays a major role in periodontal treatment [2]. According to Blomlöf and Lindskog, as well as Baker, the cementum almost always remains contaminated with bacteria in the SPA procedure and creates a smear layer. The smear layer acts as a physical barrier between periodontal tissue and the root surface, and will inhibit the formation of new attachments [3]. Mariotti asserted that several procedures can be used to increase periodontal regeneration, one of which is the use of chemicals for bio modification of root surface conditioning [4]. The objective of this procedure is the detoxification, decontamination, and demineralization of the root surface [4,5]. Minocycline HCl is an active derivative of the broad-spectrum tetracycline antibiotic against Gram-negative anaerobic bacteria, and is one of the most potent antibiotics in the elimination of periodontal pathogens. Zhang stated that 2.1% minocycline HCl (Perioline) can be used as a root surface conditioning agent to remove endotoxin bacteria and the smear layer on the root surface in periodontitis, stimulate the attachment of fibroblast cells, protect periodontal healing, and increase the regeneration of periodontal tissue [6]. Using method immersion (passive) in 10mg/ml, 50 mg/ml, and 5 mg/ml minocycline solution for 10 minutes, previous study showed that 10mg/ml minocycline is effective in eliminating endotoxin, but no more so than the active method (rubbed/swabbed) [7]. EDTA with a neutral pH produces a teeth root surface that is more bio



compatible than EDTA of a lower pH, making the tissue process attachment easier [5]. In vitro research using a scanning electron microscope (SEM) showed that the use of EDTA gel at neutral pH as a root surface conditioner is superior in smear layer removal, soluting the mineralized surface root, and stimulating the production of collagen fibers, so it eases migration and fibroblast attachment [8,9]. The ultimate objective treatment is periodontal treatment by repairing the long junctional epithelial attachment and regenerating (new attachment).

2. Materials and Methods

A total of 10 single and multiple root human teeth extracted due to periodontal disease were used. The extractions were performed in the Teaching Dental Hospital Faculty of Dentistry Universitas, Indonesia, and the samples were taken from non-smoking individuals. Refinement of the surface of the root teeth was carried out using a Gracey curette, in order to remove the contaminants and create a smear layer. The teeth were cut, creating 30 samples - 10 samples per treatment. The teeth samples had no caries or restoration below the cemento-enamel junction, normal roots, and no cervical abrasions. Sample preparation was carried out in the National Nuclear Energy Agency of Indonesia laboratory, Serpong, Indonesia. For each sample, a Gracey curette 5/6 (Osung) was used to perform 50 apices-coronal pulls in one direction to remove contaminant and create a smear layer. The 30 samples were then separated into three groups, each consisting of 10 samples. The first was the minocycline group, in which the root surfaces were blocked and scrubbed/brushed with light pressure using a micro brush for 1 minute; this process was then repeated twice (3 minutes in total), with irrigation using 10ml phosphate buffered saline (PBS) between each repetition. In the second group, EDTA was dropped onto the samples until it covered the entire surface, and then left for 2 minutes, before irrigation with 10 ml PBS. The third group was a control group and was irrigated with 10 ml saline solution only. After rinsing, fixation was carried out to stop the chemical reaction, prevent autolysis, and increase the stability and durability of the tissue samples. Dehydration, drying, and coating, using a conductive material, was carried out such that the samples could be evaluated using an SEM. In the SEM analysis, each sample was placed on a specimen plate and placed inside a Zeiss EVO MA10 SEM unit. The images were digitally saved and given score by two experienced observers in a double-blind manner. The criteria for the level of presence of a smear layer using SEM were based on a previous study that assessed the level of existence of a smear layer.

3. Results and Discussion

3.1 Results

Chi-square test; score 1: root surface without smear layer, opened dentinal tubules; score 2: surface root without smear layer, opened dentinal tubules, followed by smear layer on dentinal tubule entrance; score 3: root surface without smear layer, partially opened dentinal tubules; score 4: root surface uniformly covered with smear layer, opened dentinal tubules; score 5: root surface uniformly covered with smear layer, without opened dentinal tubules; score 6: root surface irregularly covered with smear layer, followed by debris. As shown in Table 1, the use of EDTA material gel as a root surface conditioner provided a better result, producing five samples with score 1 (16.7%), than 2.1% minocycline, which produced two samples with score 1 (6.7%).

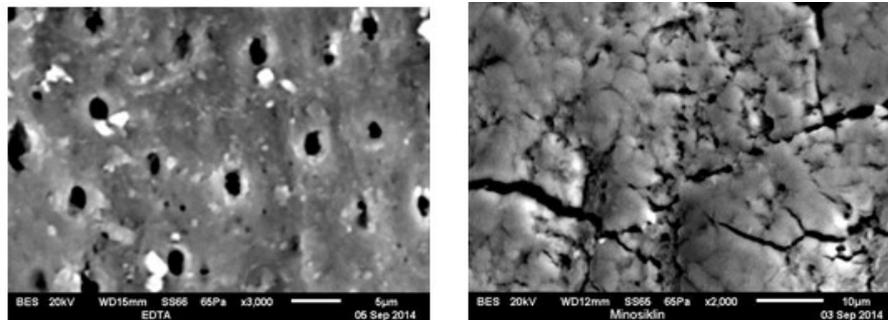
As shown in Table 2, there were no statistically significant differences between the use of 24% EDTA chemical gel and 2.1% minocycline ointment as root surface conditioning materials ($p=0.759$), although Table 1 shows that the EDTA gel gave a superior result. SEM was used to obtain morphologic photos that captured a 2000x–3500x range of magnification for every sample.

Table 1. The distribution of a smear layer after root surface conditioning treatment using 24% EDTA buffer (Prefgel), and 2.1% minocycline (Periocline)

Score	Presence score of smear layer on root surface						Total N (%)
	1	2	3	4	5	6	
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	
24% EDTA (Prefgel)	5 (50)	1 (10)	3 (30)	1 (10)	0 (0)	0 (0)	10 (33.33)
2.1% minocycline (Periocline)	2 (20)	2 (20)	5 (50)	1 (10)	0 (0)	0 (0)	10 (33.33)
Saline (control)	0 (0)	0 (0)	0 (0)	0 (0)	5 (50)	5 (50)	10 (33.33)
Total	7 (23.33)	3 (10)	8 (26.67)	2 (6.67)	5 (16.67)	5 (16.67)	30 (100)

Table 2. Analysis of the material used in root surface conditioning according to presence level of smear layer

	2.1% Minocycline	24% EDTA	Saline
Minocycline	-	0.759	0.00
EDTA	0.759	-	0.00
Saline	0.00	0.00	-

**Figure 1.** Scanning electron microscope images of root surface without smear layer, opened dentinal tubules (score 2, EDTA)

3.2 Discussion

In vitro research using SEM has shown that EDTA gel at neutral pH is able to solute a mineralized root surface, stimulate the opening of dentinal tubules, and stimulate the production of collagen fibers, thereby easing the migration and attachment of fibroblasts [10]. Although minocycline is a semisynthetic antimicrobial tetracycline derivative, it can react as a calcium chelator, and application to the root surface can remove endotoxin bacteria and result in a demineralization process in the root surface that has undergone periodontics [7]. An application of 1–2 minutes was used, as the root surface conditioning material will inhibit the healing process of periodontal tissue after 3 minutes [11]. Application technique used also affects the result; a previous study applied EDTA using the rubbing technique, via a cotton pallet, which results in a mechanical reaction, as well as a chemical reaction that will clean the smear layer from the root surface, stimulate the opening of dentinal tubules, and simplify the demineralization process. EDTA is considered superior to minocycline and other tetracycline derivatives in removing the smear layer from the root surface. Although minocycline has a lower pH than EDTA, a better result is obtained using 24% EDTA at normal pH.

4. Conclusion

The presence level of a smear layer is not significantly different, irrespective of whether 2.1% minocycline gel (periocline) or buffered 24% EDTA gel (Prefgel) is used.

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