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Research Article

Effect of Root Instrumentation on Fibroblast Viability: Hyaluronic Acid, Ethylenediaminetetraacetic Acid (EDTA), and Enamel Matrix Efficacy

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ABSTRACT

Background: Scaling and root planing (SRP) is an established and efficient procedure for treating periodontitis-associated compromised root surfaces. SRP raises challenges, such as forming a smear layer, increasing surface roughness, and potentially destroying the cementum layer, which can profoundly impact the behavior and attachment of fibroblast cells. In conjunction with SRP, various chemical agents have been employed as adjuncts to achieve an optimized surface structure conducive to fibroblast cell attachment. This study aimed to investigate the effects of SRP on healthy root surfaces with and without the addition of different adjunct root-conditioning materials, and to evaluate the varying impact on fibroblast adhesion.

Methods: A total of 60 single-root teeth were collected from individuals who exhibited no signs of periodontitis. The preparation process of these teeth yielded 120 root samples in total. Soft tissue samples were procured from patients undergoing a crown-lengthening procedure. These samples served as the source for fibroblast extraction. The root samples were systematically divided into two primary groups, SRP and non-SRP, and were further categorized based on the duration of chemical root surface applications. The chemical applications included hyaluronic acid (HA), ethylenediaminetetraacetic acid (EDTA), enamel matrix derivatives (EMD), and a combination of EDTA and EMD. Following the division and treatment, the root samples and extracted fibroblasts were cultured for a period of 72 hours. The adhesion efficacy of the fibroblast cells was evaluated through a cell viability assessment.

Results: The comparative analysis demonstrated an increase in cell viability in the group without SRP compared to the group with SRP. This increase was significant across all groups that underwent different chemical material applications, for both short and long durations. However, when data from short and long chemical surface treatments were compared, no statistically significant differences were observed among the treatment groups. This was the case regardless of whether the comparison was made within the non-SRP or SRP groups independently.

Conclusions: SRP significantly influenced the effectiveness of surface conditioning agents. The combination of SRP with root-conditioning materials resulted in a significant reduction in fibroblast attachment compared to using the root-conditioning materials alone.

INTRODUCTION

Scaling and root planing (SRP) are non-surgical periodontal therapies widely used to treat periodontitis (Duran-Pinedo *et al*., 2023; Guru & Aghanashini, 2022). This procedure involves the meticulous removal of dental plaque and calculus attached to the root surfaces, smoothing the root surface, reducing inflammation, and improving periodontal health (Fridus *et al*., 2019). However, during this process, there is a risk of inadvertent removal of the cemental layer and the outer covering of the root surface, which exposes the underlying dentin (Stähli *et al*., 2021). Removal of the cemental layer or a portion thereof adversely affects fibroblast attachment, consequently reducing the total number of fibroblasts on the root surface (Gürsoy *et al*., 2020). Fibroblasts play fundamental roles in periodontal healing and tissue regeneration. Their attachment and colonisation on the root surface are essential for re-establishing the functional periodontal ligament (PDL) and forming new connective tissue(Chang *et al*., 2023; Ko *et al*., 2023; Zarrough *et al*., 2023). Previous studies have investigated the attachment of fibroblasts to various modified root surfaces, including superficially curetted, demineralised, and root-planned surfaces. Notably, fibroblast cells exhibited distinct

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behaviours and morphologies on these surfaces, indicating the influence of root surface microstructure on fibroblast behaviour (Nishimura *et al*., 1989).

Several clinical studies have suggested a novel treatment approach for gingival recession aimed at augmenting exposed root surfaces through soft tissue graft periodontal surgery. In this method, the exposed root surface is cleaned meticulously using manual instruments, followed by surface treatment with ethylenediaminetetraacetic acid (EDTA) and enamel matrix derivative (EMD) materials (Dias et al., 2022; França et al., 2018; Mercado et al., 2020a, 2020b). The combined use of EDTA and EMD materials in this treatment approach aims to optimise the periodontal surgical procedure by improving the long-term stability of the soft tissue grafts. Furthermore, by creating an ideal surface for healing and promoting a strong bond between the graft and root surface, this technique holds promise for achieving satisfactory root coverage outcomes in individuals with gingival recession.

A recent study has assessed the effects of various root surface modifications on fibroblast attachment. (Babgi et al., 2021). The experimental groups included root surface alteration achieved by SRP alone and SRP combined with root-conditioning materials. Interestingly, the results revealed that the root surfaces modified with SRP alone exhibited significantly higher fibroblast attachment than the other experimental groups. However, it is essential to note that the study did not specifically investigate fibroblast attachment on surfaces modified solely by root-conditioning materials. These findings highlight the potential benefits of SRP in enhancing fibroblast attachment to the root surface and highlight its significance in periodontal therapy. Further investigations are needed to explore the specific effects of the root-conditioning material alone on fibroblast attachment, thereby providing a more comprehensive understanding of the effects of different root surface modifications on root coverage surgical therapy.

This study investigated the effects of various root surface modifications on fibroblast viability, including chemicals alone and SRP combined with root-conditioning materials. The objective was to compare fibroblast viability on modified root surfaces and determine any significant differences among the experimental groups.

MATERIALS AND METHODS

Sample selection

Teeth were obtained from healthy individuals who were non-smokers and did not exhibit signs of periodontitis. These individuals were referred for orthodontic tooth extraction, and the extracted teeth were carefully examined for root damage, caries, cracks, or calculus deposition before inclusion in the study. Following the extraction procedure, residual tissues were gently removed from the root surface using sterile wet gauze, and the teeth were stored in containers filled with saline solution.

Fibroblast cell retrieval

Soft-tissue samples were obtained from patients scheduled for crown-lengthening surgery under the supervision of a periodontist. Before surgery, patients received a 6-week plaque control regimen. Soft tissue samples were collected only from patients with firm and resilient tissues devoid of inflammation or bleeding upon probing. Written informed consent was obtained from all participating patients. During the surgical procedure, the keratinised gingival soft tissue was collected, preserved in saline solution, and promptly transported to the laboratory for subsequent fibroblast extraction.

Gingival tissues were thoroughly rinsed with phosphate-buffered saline (PBS) and incubated in dispase (1 mg/mL; Sigma, USA) at 4°C for 12 h. Following incubation, the epithelial layer was carefully removed, and the connective tissue was sliced into small fragments. These tissue fragments were then cultured in a 25 mL flask containing a suitable cell growth medium. The flask was placed in a controlled environment at a temperature of 37°C in a humidified atmosphere supplemented with 5% CO_2. The complete growth medium utilised for cell culture consisted of Dulbecco's Modified Eagle Medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA), supplemented with 10% fetal bovine serum (FBS; HyClone Thermo Fisher Scientific, Logan UT, USA), as well as 100 U/mL penicillin, 100 mg/mL streptomycin and 2.5 mg/mL amphotericin B (Thermo Fisher Scientific, Waltham, MA, USA).

Experimental groups

The specimens were categorized into two main groups: SRP and non-SRPs. Each group was further divided into subgroups based on the chemical root surface application duration: short (1 or 2 min) and long exposure (2 or 4 min). Within each subgroup, the root samples were treated with a specific root-conditioning material, including hyaluronic Acid (HA), ethylenediaminetetraacetic acid (EDTA), enamel matrix derivatives (EMD), and a combination of EDTA and EMD (EDTA/EMD) (Figure 1).

Root surface preparation

The study design consisted of two control groups, the first of which included root samples without mechanical or chemical modification of the root surface. The second control group underwent SRP mechanical alteration of the root surface without the application of root-conditioning agents. In addition, eight test groups were established: four test groups had chemical root-conditioning material on the root surface, and the other four had mechanical and chemical root surface modifications. Each test group received a specific root-conditioning agent to modify the root surface. The first test group was treated with HA Gel (Regedent AG, Zurich, Switzerland), the second test group was subjected to 24% EDTA gel (Biodinâmica, Lisbon, Portugal), the third test group received EMD (Straumann, Basel, Sweden), and the fourth test group

went through a combined treatment of EDTA 24% and EMD (Figure 1).

Cell *in vitro* cultivation

Gingival fibroblasts were seeded onto each root sample at a density of 2 × 10^4 cells per well in 500 µL of complete growth medium. After a 72-hour incubation period, the root samples with attached fibroblasts were transferred to a 48-well plate for the cell viability assay.

Fibroblast cells viability assessment

A 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) assay was performed. Fibroblasts that adhered to the root samples were incubated in a 48-well plate containing 500 mL of DMEM supplemented with 0.5 mg/mL of MTT. This incubation took place at a temperature of 37°C for 3 h. Following incubation, the samples were carefully separated from the medium, and a solubilisation solution consisting of a 1:1 mixture of dimethyl sulfoxide (DMSO) and isopropanol was added to dissolve the formazan crystals. The solubilised solution was transferred to a 96-well plate at 100 mL/well. Each well's optical density (OD) was measured at a wavelength of 570 nm using a spectrophotometer, which allowed the quantitative evaluation of the cellular response.

Statistical analysis

SigmaPlot (version 14.0; Systat Software Inc., San Jose, CA, USA) was used for the data analysis. Depending on the normality test, either independent-sample t-tests or Mann–Whitney rank sum tests were used to compare teeth treated with conditioning agents together with SRP and those without SRP during long and short exposures. The same tests were used to detect statistical differences between long and short exposures to conditioning agents treated with and without SRP. The level of significance was set at P<0.05.

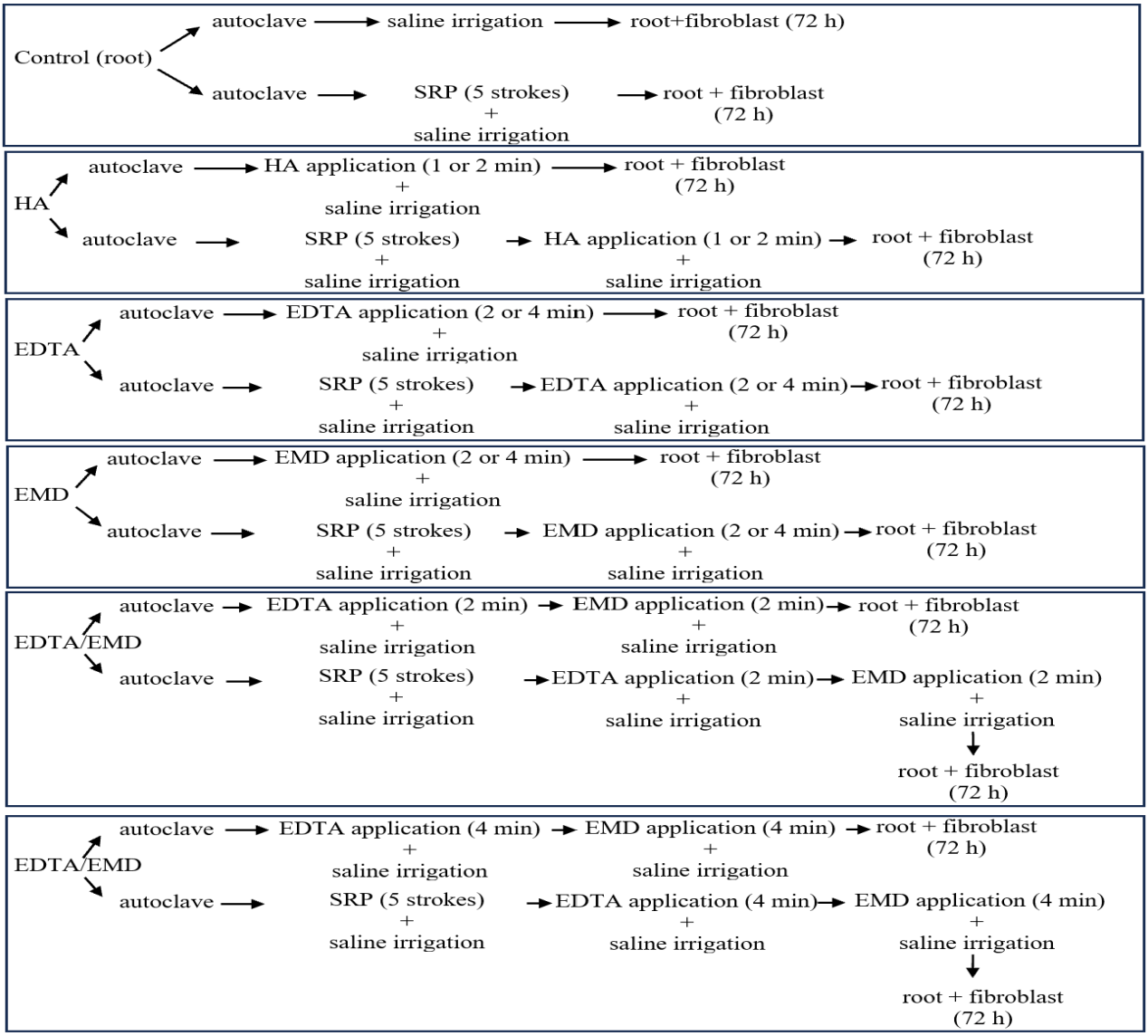

Figure 1. *Root Surface Preparation Protocol (SRP=Scaling and Root Planing; HA=hyaluronic Acid; EDTA= ethylenediaminetetraacetic acid; EMD= Enamel Matrix Derivatives.*

Table 1: *Mean (SD) values of the cell viability assay, demonstrating fibroblast viability following treatment with conditioning agents in the presence or absence of scaling and root planing (SRP) at varying exposure durations.*

	Without Mechanical instrumentation (SRP)				
	Control group (n=12)	HA (n=12)	EDTA (n=12)	EMD (n=12)	EDTA/EMD (n=12)
Duration (Short)	(n=6)	1 min (n=6)	2 min (n=6)	2 min (n=6)	2 min/2 min (n=6)
	1.063 (0.471)	1.065 (0.085)	0.903 (0.164)	1.058 (0.133)	0.740 (0.460)
Duration (Long)	(n=6)	2 min (n=6)	4 min (n=6)	4 min (n=6)	4min/4min (n=6)
	1.063 (0.471)	0.666 (0.286)	1.316 (0.322)	1.121 (0.311)	1.601 (0.213)
	With Mechanical instrumentation (SRP)				
	Control group (n=12)	HA (n=12)	EDTA (n=12)	EMD (n=12)	EDTA/EMD (n=12)
Duration (Short)	(n=6)	1 min (n=6)	2 min (n=6)	2 min (n=6)	2 min/2 min (n=6)
	0.045 (0.002)	0.052 (0.016)	0.049 (0.014)	0.069 (0.001)	0.042 (0.007)
Duration (Long)	(n=6)	2 min (n=6)	4 min (n=6)	4 min (n=6)	4min/4min (n=6)
	0.045 (0.002)	0.038 (0.011)	0.061 (0.018)	0.090 (0.013)	0.038 (0.004)

RESULTS

The mean, standard deviation (SD) of each conditioning agent used to treat teeth with or without SRP during short and long exposures is presented in Table 1. When comparing the conditioning agents with SRP to conditioning agents without SRP in the short-exposure analysis, a statistically significant difference ($P = 0.008$) in the median values was observed, where teeth treated with conditioning agents without SRP showed higher cell viability of fibroblasts than teeth treated with the same conditioning agents but with SRP. The median and interquartile range (IQR) values were 1.058 (0.243) and 0.049 (0.017), respectively. Furthermore, in the extended exposure analysis, a statistically significant difference ($P = 0.001$) in the mean values was found when comparing conditioning agents with and without SRP. Teeth treated with conditioning agents without SRP also showed higher fibroblast cell viability than teeth treated with the same conditioning agents but with SRP alone. The mean and SD values were 1.153 (0.344) and 0.054 (0.022).

No significant differences were found between short and long exposures in the cell viability assay of fibroblasts when comparing teeth treated with conditioning agents with or without SRP ($P > 0.05$).

DISCUSSION

The results obtained from this experimental investigation provide evidence of a significant decline in the adhesion capacity of fibroblasts to root samples following the implementation of root-planing techniques, regardless of the concurrent application of chemical modifications. These findings highlight the vital role of root planing in modulating cellular interactions at the root surface and reducing fibroblast adhesion. The observed decrease in fibroblast attachment emphasises the potential impact of root planing procedures on the biological response of periodontal cells.

Clinical evidence strongly supports the positive clinical outcomes achieved through SRP, as it effectively eliminates calculus and toxic substances from the root surface, resulting in reduced gingival inflammation and improved clinical attachment (Heitz-Mayfield *et al*., 2002). However, it is essential to acknowledge the inherent limitations associated with root planing, including the formation of a smear layer (Rocha et al., 2015), loss of the root cementum structure (Karacaoglu & Orhan, 2022), and increased surface roughness (Yıldız *et al*., 2023)The presence of a debris-laden smear layer and an altered root structure can hinder the re-establishment of periodontal tissues and impede optimal periodontal healing. Additionally, the removal of cementum during root planing can lead to an irreversible loss of root structure. Furthermore, the mechanical instrumentation involved in root planing can introduce surface irregularities, further exacerbating the root coverage challenges. Therefore, although SRP remains an effective treatment approach for periodontal diseases, it is crucial to address its potential limitations.

Several studies have explored the effects of HA, EDTA, and EMD on root surface modification and their potential to enhance fibroblast cell attachment. These studies have consistently demonstrated that root-conditioning materials can induce beneficial alterations on the root surface, leading to improved fibroblast cell attachment. Comprehensive research has illuminated the remarkable ability of HA to enhance fibroblast attachment by creating a highly favourable microenvironment at the root surface. Due to its unique properties, HA has demonstrated its ability to modulate surface topography, making it optimal for fibroblast adhesion. Moreover, HA's superior biocompatibility ensures the survival and vitality of the adhered cells. HA's multifaceted mechanisms of action promote cellular migration and proliferation, thereby enhancing the overall adhesion of fibroblasts to the root surface (Mueller *et al*., 2017). EDTA has the potential to modify root surfaces and enhance fibroblast attachment (Zhan *et al*., 2021). EDTA, a chelating agent, effectively removed the smear layer and exposed the underlying root structures, thereby improving cellular adhesion. The removal of the smear layer through EDTA application promoted a more biocompatible root surface, creating a conducive environment for fibroblast attachment and subsequent periodontal healing. EMD consists of bioactive proteins that mimic the natural components of enamel

and promote periodontal regeneration. These proteins interact with the root surface and stimulate fibroblast attachment, proliferation, and metabolism (Lyngstadaas *et al*., 2001), thus supporting periodontal tissue regeneration.

Scientific studies have consistently demonstrated the efficacy of HA, EDTA, and EMD in modifying the root surface and enhancing fibroblast attachment. These studies underscore the significant impact of HA, EDTA, and EMD in creating a favourable microenvironment that promotes robust fibroblast adhesion. However, in contrast to previous findings, the present study revealed intriguing results. The application of HA, EDTA, and EMD in this study did not yield a statistically significant improvement in fibroblast attachment compared to the untreated control group. These unexpected findings warrant further investigation to elucidate the underlying factors that may have influenced the outcomes. Factors such as variations in concentration, treatment duration, and specific characteristics of the study population may have contributed to the disparity in results. Additionally, it is essential to consider the complexity of cellular interactions and the potential interplay between the various molecular mechanisms involved in fibroblast attachment.

Despite the study's limitations, such as the lack of alternative methods to confirm fibroblast adhesion to the root surface, this experiment provides valuable insights into the effect of root-conditioning substances on fibroblast surface adhesion. Despite the use of various root-conditioning agents such as HA, EDTA, EMD, and EDTA/EMD, no significant improvements were observed in fibroblast adhesion compared to the groups that underwent mechanical surface alteration alone or in conjunction with chemical agents. This suggests that the root-conditioning materials used in this study did not effectively enhance fibroblast attachment to the root surface.

CONCLUSION

In conclusion, the most notable difference in fibroblast adhesion was observed between the groups that underwent root planing and the group that did not, irrespective of the duration of material application (short or long exposure). This finding demonstrates the significant impact of root planing on fibroblast behaviour and adhesion. As a mechanical treatment approach, root planing appeared to have a more pronounced effect on fibroblast adhesion than the specific root-conditioning agents used in this study.

DECLARATIONS

Ethical Approval

The local biological and medical ethics committee approved this study (UDZT270521). The consent form was obtained for the study purpose.

Participants Consent

Written informed consent was obtained from all participating patients.

Source of Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest

Author have declared that no financial support was received from any organization for the submitted work. Author have declared that no other relationships or activities could appear to have influenced the submitted work.

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