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Cementum histomorphology after chemical and mechanical root surface treatments for residual periodontal ligament removal

Marzieh Sahebnasagh [1], Maryam Kourepaz [2], Fatemeh Mazhari [3], Zahra Sadeghi [4] *

Abstract

Objective: Effective removal of necrotic tissue while minimizing cementum damage is essential before replanting avulsed teeth. This study compared different root surface treatments in terms of their effectiveness for removing residual periodontal ligament (PDL) and their impact on histomorphometric properties of cementum.

Methods: In this in vitro study, 90 extracted premolars were randomly assigned to nine group based on the root surface treatment applied: Group 1: sterile gauze, Group 2: 5.25% sodium hypochlorite (NaOCl) for 5 minutes, Group 3: 1% NaOCl for 15 minutes, Group 4: 5.25% NaOCl with surfactant for 2 minutes, Group 5: 5.25% NaOCl with surfactant for 5 minutes, Group 6: 1% NaOCl with surfactant for 15 minutes, Group 7: 10% calcium hypochlorite ($Ca(OCl)_2$) for 35 minutes, Group 8: 10% $Ca(OCl)_2$ with surfactant for 35 minutes, and Group 9: electric brushing with pumice. Following treatment, the roots were evaluated histomorphometrically for residual PDL and cementum integrity.

Results: Significant differences were observed in residual PDL and cementum integrity among groups ($P<0.05$). The highest removal of PDL tissue was achieved with 5.25% NaOCl with surfactant applied for 2 minutes (Group 5) or 5 minutes (Group 4), as well as 10% $Ca(OCl)_2$ with surfactant applied for 35 minutes (Group 8) ($P<0.05$). Cementum integrity was best preserved in Groups 4 and 5.

Conclusions: The addition of surfactant to 5.25% NaOCl and 10% $Ca(OCl)_2$ improved the removal of residual PDL tissue. Among the tested protocols, 5.25% NaOCl with surfactant achieved the most effective PDL removal while maintaining optimal cementum integrity.

Keywords: Dental cementum, Periodontal ligament, Sodium hypochlorite, Surfactants, Tooth avulsion, Tooth injuries

Introduction

Traumatic dental injuries (TDIs) are common in children and adolescents, accounting for approximately 5% of all reported bodily injuries (1). Avulsion represents 0.5% to 3% of TDIs and is considered one of the most severe forms of dental trauma (2). The ideal treatment for an avulsed permanent tooth is immediate replantation, which maximizes the chance of preserving viable periodontal ligament (PDL) cells on the root surface (3).

Delayed replantation of avulsed teeth can cause PDL remnants on the root surface to undergo necrosis. The presence of these necrotic tissues can act as a potent inflammatory stimulus, triggering root resorption. These processes may lead to progressive destruction of the root, osseous replacement, and ankylosis of the root, compromising the long-term tooth survival (4, 5).

On the other hand, an intact cementum layer serves as a protective biological barrier. It shields the underlying dentin, supports potential periodontal healing, and prevents direct bone-to-root contact that can accelerate ankylosis (6). Therefore, thorough decontamination of the root surface, while preventing cementum damage, is essential (7).

Several mechanical and chemical methods have been proposed for removing PDL tissue from the root surface (5, 8-10). Mechanical approaches include the use of curettes, surgical blades, diamond burs, stone discs, sterile gauze, and toothbrush bristles with or without abrasive pastes such as pumice. However, these methods frequently cause varying degrees of cementum

[1] Department of Pediatric Dentistry, School of Dentistry, North Khorasan University of Medical Sciences, Bojnurd, Iran
[2] Private Dental Clinic, Mashhad, Iran
[3] Department of Pediatric Dentistry, School of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran
[4] Department of Pediatric Dentistry, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran

*Corresponding Author: Zahra Sadeghi
Email: sadeqizahra2@gmail.com

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abrasion or compromise its integrity, which adversely affects long-term periodontal healing.

Chemical methods use tissue-dissolving solutions to remove necrotic PDL remnants. Various agents have been investigated, including citric acid, phosphoric acid, fluoride, sodium hypochlorite (NaOCl), and calcium hypochlorite ($Ca(OCl)_2$). These chemical agents can be combined with surfactants to enhance their effectiveness in residual PDL removal.

Sodium hypochlorite (NaOCl) has been extensively studied at various concentrations and exposure times for its strong ability to dissolve PDL remnants while minimizing damage to root cementum. However, NaOCl is highly cytotoxic if extruded into periapical tissues, degrades the collagen matrix in mineralized dentin, and can adversely affect the mechanical properties and structural integrity of root dentin (11). Calcium hypochlorite ($Ca(OCl)_2$) has emerged as a potential alternative, offering comparable tissue-dissolving capacity with a potentially gentler impact on surrounding structures (6, 12-21).

Surfactants are amphiphilic compounds that reduce surface and interfacial tension, thereby improving the wettability, penetration, and spreading of aqueous solutions into organic tissues. Commonly used surfactants include cetrimide and polypropylene glycol. Several studies have shown that combining surfactants with hypochlorite solutions enhance their efficacy by reducing surface tension, and increasing both tissue dissolution and antimicrobial efficacy (12-16, 22, 23). Therefore, necrotic PDL removal can be obtained in shorter application times (12-16, 22, 24).

The literature contains various protocols for effective tissue removal without cementum damage in avulsed teeth. Given the variability of existing methodologies and the lack of a standardized protocol, the present study compared the effectiveness of electric toothbrush and several chemical protocols with or without using surfactant to identify an optimal protocol that maximizes PDL removal and preserves root surface integrity.

Materials and methods

Study design and ethical approval

This in vitro experimental study was conducted on sound premolar teeth extracted for orthodontic reasons. The study protocol was approved by the ethics committee of North Khorasan University of Medical Sciences (IR.NKUMS.REC.1398.056). The patients consented to the use of their extracted teeth for research purposes.

Sample size calculation

The sample size was determined using G*Power software (version 3.1.9.7; Heinrich Heine University Düsseldorf, Düsseldorf, Germany). Based on the findings of Bai et al. (3), and assuming a significance level (α) of 0.05 and a statistical power (1–β) of 80, a minimum of 8 samples per group was required (3). To increase the statistical power, 10 samples were included in each group, resulting in a total of 90 samples.

Eligibility criteria

The inclusion criteria comprised intact, single-rooted premolars extracted for orthodontic reasons within the previous month. Teeth exhibiting cracks, structural defects, grooves, or root surface caries were excluded. To establish a consistent and standardized baseline for all specimens, the teeth were disinfected by immersion in 0.2% thymol solution for 24 hours. This procedure ensured that subsequent measurements of residual periodontal ligament (PDL) reflected solely the efficiency of the cleaning protocols, as thymol inhibits microbial growth without altering the structural properties of dental tissues.

Specimen preparation

The teeth were mounted upside down in red dental wax. They were then allowed to dry at room temperature for 60 minutes. After immersion in saline for 10 minutes for rehydration, they were numbered and randomly assigned to 9 groups (n=10) using a random sequence table, based on the treatment applied for root surface dessication. Group 1 underwent minimal mechanical decontamination and served as the control group. Groups 2-8 were exposed to chemical cleaning methods, and group 9 was cleaned using a mechanical method. The study groups were as follows:

Group 1 (control): The PDL tissue attached to the root surface was removed using a wet gauze.

Group 2 (NaOCl 5.25%-5 min): The PDL tissue was removed with 5.25% NaOCl for 5 minutes (3). The NaOCl solution was obtained from a pure product (Tirak, Iran) without any additives.

Group 3 (NaOCl 1%-15 min): The PDL tissue was removed with 1% NaOCl for 15 minutes (3). The 1% NaOCl solution was prepared by diluting 19 mL of 5.25% NaOCl with 81 mL of distilled water.

Group 4 (Sur- NaOCl 5.25%-2 min): The PDL tissue was removed with 5.25% NaOCl solution supplemented with a surfactant mixture (3).

Group 5 (Sur-NaOCl 5.25%-5 min): The PDL tissue was removed using 5.25% NaOCl solution supplemented with a surfactant mixture for 5 minutes (3, 22).

Group 6 (Sur- NaOCl 1%-15 min): The PDL tissue was removed with 1% NaOCl solution supplemented with a surfactant mixture for 15 minutes (3, 22).

Group 7 ($Ca(OCl)_2$ 10%-35 min): The PDL tissue was removed with 10% calcium hypochlorite ($Ca(OCl)_2$) for 35 minutes (20, 21). The solution was prepared by dissolving 10 g of $Ca(OCl)_2$ powder in 90 mL of distilled water.

Group 8 (Sur-$Ca(OCl)_2$ 10%-35 min): The PDL tissue was removed using 10% $Ca(OCl)_2$ solution supplemented with a surfactant mixture for 35 minutes (20, 21, 24).

Group 9 (Electric toothbrush): The PDL tissue was removed using an electric toothbrush and pumice paste. Pumice powder (NikDarman Co., Tehran, Iran) was mixed with saline to form a paste. The paste was applied to the root surface, and the buccal, lingual, mesial, and distal surfaces were brushed from coronal to apical regions for 30 seconds. The roots were then thoroughly rinsed with saline (8).

In the surfactant-containing groups, a mixture of cetrimide (1%), polypropylene glycol (1%), and potassium sorbate (0.1%) was added to the NaOCl or $Ca(OCl)_2$ solutions.

For Groups 2–8, the teeth were placed in containers filled with 11 mL of the respective solutions. For these groups, a 5-minute cycle of continuous ultrasonic agitation was performed, followed by replacement of the solution as needed.

For groups requiring longer immersion periods (e.g., 15 or 35 minutes), this 5-minute cycle was repeated until the target treatment time was completed (25, 26). Groups 1 and 9 were not subjected to this process as they did not involve chemical immersion.

Finally, the root surfaces were wiped with a moist gauze. Each surface (buccal, lingual, mesial, and distal) was cleaned with four movements, once from coronal to apical and once from apical to coronal, followed by a final rinse with saline (3).

After root surface treatment, the teeth were decoronated at the cementoenamel junction (CEJ) using a high-speed rotary disc (Diatech Dental, Coltène-Whaledent, Altstätten, Switzerland).

All root surface treatments were performed by a single, trained operator to ensure consistency. For the electric toothbrush group, the operator applied light, controlled pressure in a standardized motion pattern.

Histomorphometric assessment

The roots were rinsed with distilled water and fixed in 10% neutral buffered formalin at 25 °C for 72 hours. Subsequently, they were decalcified in formic acid for four weeks (25, 26). Following complete decalcification, the specimens were dehydrated through ascending concentrations of ethanol, cleared in xylene, and embedded in paraffin. All reagents were obtained from Merck (Darmstadt, Germany). Using a microtome, consecutive 6-µm thick sections were prepared from the paraffin-embedded samples. Sections were cut horizontally, parallel to the long axis of each root. The sections were then stained with hematoxylin and eosin (Merck) and examined under a light microscope (BX51; Olympus, Tokyo, Japan).

The percentage of residual PDL was calculated in three sections per root, and the mean value was recorded. In each section, the total root periphery (µm) was measured using Analysis LS Starter software (Version 2.2; Olympus Soft Imaging Solutions GmbH, Münster, Germany), followed by measurement of the root periphery covered with PDL. The percentage of residual PDL was then calculated.

Cementum integrity was assessed qualitatively under a light microscope, with specimens categorized as either “intact” or “damaged”. The cementum was ranked as damaged in the presence of visible surface discontinuities. The observer was blinded to the group allocation.

Statistical analysis

The normality of the data was assessed using the Kolmogorov-Smirnov test. Residual PDL data were analyzed using one-way ANOVA followed by Tukey’s post hoc test. Differences in cementum integrity among groups were evaluated using the Chi-square test. All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA) at a significance level of $P < 0.05$.

Results

Residual PDL

Table 1 presents the descriptive statistics for the percentage of residual PDL tissue on the root surfaces in the experimental groups. ANOVA revealed a significant difference in the percentage of residual PDL among the study groups ($P < 0.001$). The highest residual PDL coverage was observed in the control group (15.76 ± 4.48%), which was significantly higher than all other groups ($P<0.05$). The surfactant-free NaOCl groups (groups 2 and 3) ranked second, with residual PDL values significantly higher than other groups ($P < 0.05$)

Table 1. Means and standard deviations (SD) of the percentage of residual PDL tissue on the root surface in the study groups

Group	Definition	Mean ± SD* (%)	Minimum (%)	Maximum (%)
Group 1	Control	15.76 ± 4.48[a]	9.89	24.43
Group 2	NaOCl 5.25%-5 min	8.99 ± 1.85[b]	5.45	11.49
Group 3	NaOCl 1%-15 min	10.26 ± 1.46[b]	7.74	12.02
Group 4	Sur-NaOCl 5.25%-2 min	1.98 ± 0.70[d]	1.05	3.03
Group 5	Sur-NaOCl 5.25%-5 min	1.26 ± 0.44[d]	0.67	2.05
Group 6	Sur-NaOCl 1%-15 min	6.54 ± 0.66[c]	5.33	7.38
Group 7	$Ca(OCl)_2$ 10%-35 min	3.92 ± 1.34[c,d]	2.09	6.32
Group 8	Sur-$Ca(OCl)_2$ 10%-35 min	2.06 ± 0.63[d]	1.04	3.03
Group 9	Electric toothbrush	6.79 ± 0.94[c]	5.28	8.18
P-value	<0.001			

*Different lowercase superscript letters indicate statistically significant differences between groups at P < 0.05.
NaOCl: Sodium hypochlorite; Ca $(OCl)_2$: Calcium hypochlorite; Sur: Surfactant; min: Minute

except the control. The lowest PDL tissue was found in the Sur-NaOCl 5.25%-5 min group (group 5: 1.26 ± 0.44%), Sur-NaOCl 5.25%-2 min (group 4: 1.98 ± 0.70%) and Sur-$Ca(OCl)_2$ 10%-35 min (group 8: 2.06 ± 0.63%) groups, which showed significantly lower PDL values than all other groups (P < 0.05) except $Ca(OCl)_2$ 10%-35 min (group 7: 3.92 ± 1.34) (P > 0.05; Table 1). The comparison of residual PDL tissue on root surfaces in the experimental groups is illustrated in Figure 1.

The surfactant-free NaOCl groups (NaOCl 5.25%-5 min and NaOCl 1%-15 min groups) exhibited comparable residual PDL values (P > 0.05), which were significantly higher than all surfactant-containing hypochlorite groups (P < 0.05). Residual PDL was comparable between the Sur-NaOCl 5.25%-2 min group and Sur-NaOCl 5.25%-5 min groups (P > 0.05). The Sur-NaOCl 1%-15 min group performed significantly better than its surfactant-free counterpart (group 3), but was less effective than the surfactant-containing 5.25% NaOCl groups (P < 0.05).

The Sur-$Ca(OCl)_2$ 10%-35 min group showed a lower percentage of residual PDL than the $Ca(OCl)_2$ 10%-35 min group, but the difference was not statistically significant (P > 0.05; Table 1).

The mechanica method (electric toothbrush) showed residual PDL that was significantly lower than the control and surfactant-free NaOCl groups (P<0.05).

Cementum integrity

Cementum integrity was preserved in all specimens in the Sur-NaOCl 5.25%-2 min (group 4), Sur-NaOCl 5.25%-5 min (group 5), Sur-NaOCl 1%-15 min (group 6), and control (group 1) groups, whereas damage was observed in other groups (Figure 1, Table 2).

Discussion

The primary goal of root surface treatment in delayed replantation of avulsed teeth is the near-complete removal of necrotic PDL remnants while preserving an intact cementum layer. This study compared the effects of various root surface treatments on the histomorphometric properties of cementum to identify methods that maximize PDL removal while minimizing

Table 2. Frequency (Number) and percentage (%) of cementum integrity of root surfaces in the study groups (n=10)

Groups		No N (%)	Yes N (%)	P-value*
1	Control	0 (0)	10 (100)	<0.001
2	NaOCl 5.25%-5 min	10 (100)	0 (0)	
3	NaOCl 1%-15 min	10 (100)	0 (0)	
4	Sur- NaOCl 5.25%-2 min	0 (0)	10 (100)	
5	Sur- NaOCl 5.25%-5 min	0 (0)	10 (100)	
6	Sur- NaOCl 1%-15 min	0 (0)	10 (0)	
7	$Ca(OCl)_2$ 10%-35 min	10 (100)	0 (0)	
8	Sur- $Ca(OCl)_2$ 10%-35 min	10 (0)	0 (0)	
9	Electric Toothbrush	10 (100)	0 (0)	
Total		50 (55.6)	40 (44.4)	

* Chi-square test
NaOCl: Sodium hypochlorite; $Ca(OCl)_2$: Calcium hypochlorite; Sur: Surfactant; min: Minute ()

cementum damage in delayed replantation of avulsed teeth.

In this study, sterile gauze was used in two contexts: as a control group and as part of the standardized root surface preparation protocol in the experimental groups. The sterile gauze group was selected to represent minimal mechanical intervention, in accordance with IADT guidelines, thereby providing a baseline level of physical debridement against which the efficacy of chemical and mechanical treatments could be compared. Additionally, the use of sterile gauze for standardized wiping of all specimens ensured uniform removal of loose debris and improved consistency across samples without causing significant alteration to the cementum.

The different treatment durations for NaOCl and $Ca(OCl)_2$ were selected based on previously established protocols reported in the literature (3, 20, 21), allowing comparison of the most effective published regimens rather than testing equal exposure times.

A surfactant composed of cetrimide and polypropylene glycol was used in combination with hypochlorite solutions in the present study. The findings indicated that the control, NaOCl 1%-15 min, and NaOCl 5.25%-5 min groups had the highest residual PDL. On the other hand, the Sur-NaOCl 5.25%-5 min, Sur-NaOCl 5.25%-2 min, and Sur-$Ca(OCl)_2$ 10%-35 min groups exhibited the lowest residual PDL.

Notably, surfactant-free NaOCl groups (NaOCl 5.25%-5 min and NaOCl 1%-15 min) demonstrated significantly greater residual PDL compared to surfactant-containing NaOCl groups (Sur-NaOCl 5.25%-5 min, Sur-NaOCl 5.25%-5 min, and Sur-NaOCl 1%-15 min). The Sur-NaOCl 5.25%-5 min and Sur-NaOCl 5.25%-2 min groups achieved the lowest residual PDL percentages among all NaOCl groups, but were comparable to both $Ca(OCl)_2$ groups. The $Ca(OCl)_2$ groups demonstrated effective PDL removal, performing better than the surfactant-free NaOCl groups, the electric toothbrush group, control, and Sur-NaOCl 1%-15 min group.

NaOCl has long been recommended for the removal of necrotic PDL tissue due to its strong tissue-dissolving properties; however, the optimal concentration and application time remain under debate (3, 6, 17-19, 27). Several studies have reported successful outcomes using NaOCl for root surface treatment of avulsed teeth and reported effective inflammation control and preservation of cementum integrity (3-6, 17, 19, 21, 28, 29). In the present study, 5.25% NaOCl applied for 5 minutes and 1% NaOCl applied for 15 minutes showed better performance than the control group. However, these groups retained significantly more residual PDL compared with surfactant-containing NaOCl groups. Similarly, several studies have shown that the addition of surfactants reduces NaOCl's surface tension, enhancing its tissue-dissolving capacity and antimicrobial activity (12-17, 19, 22, 23, 28, 29).

In the present study, the addition of surfactants to both 1% and 5.25% NaOCl enhanced their tissue-dissolving capacity compared to their surfactant-free counterparts, which is consistent with the findings of Almeida et al. (22), and Bolfoni et al. (15). No significant difference was observed in residual PDL between the 5.25% NaOCl plus surfactant groups applied for 2 and 5 minutes, but these groups performed significantly better than the 1% NaOCl plus surfactant applied for 15 minutes. Therefore, the concentration of NaOCl and the presence of a surfactant may play a more critical role in tooth decontamination than the duration of immersion in the solution.

Calcium hypochlorite ($Ca(OCl)_2$) can be used for the removal of necrotic tissue from root surfaces due to its antimicrobial and tissue-dissolving properties (20, 21). In the present study, both $Ca(OCl)_2$ groups (with or without surfactant addition) showed the least amount of residual PDL, performing better than surfactant-free NaOCl solutions at various concentrations and exposure times. In contrast to the present findings, Dutta and Saunders (21) and Taneja et al. (20) found no significant difference between $Ca(OCl)_2$ and NaOCl applied for 30 minutes in terms of antimicrobial activity and tissue dissolution. Iglesias et al. (24) demonstrated that adding a surfactant to $Ca(OCl)_2$ significantly enhanced both its tissue-dissolving capacity and antimicrobial efficacy.

Cementum integrity was compromised in all surfactant-free NaOCl groups, the $Ca(OCl)_2$ containing groups, and the Electric toothbrush group. The control group and all surfactant-containing NaOCl groups maintained intact cementum. Among these, 5.25% NaOCl with a surfactant, applied for 2 or 5 minutes, demonstrated the optimal combination of effective PDL removal and preservation of cementum integrity. Consistent with these results, Bai et al. (3) reported that cementum remained intact following exposure to NaOCl containing a surfactant.

Evidence indicates that most mechanical cleaning methods, such as the use of sterile gauze or a toothbrush with pumice paste, may cause damage to the cementum. However, in the present study, sterile gauze preserved cementum integrity, although it was less effective in removing necrotic periodontal ligament remnants. Other studies suggested that chemical

methods should be preferred for cleaning the root surfaces of avulsed teeth that have been out of the socket for more than one hour (3, 6, 32).

In the present study, sterile gauze was used as the control group in accordance with International Association of Dental Traumatology (IADT) guidelines (1, 2). Although sterile gauze preserved cementum integrity, large amounts of residual PDL tissue remained on the root surface, often detectable by the naked eye. Similarly, some studies reported that the sterile gauze group showed no significant difference compared with a no-cleaning control group (3, 30). However, Krug et al. (31) observed minimal ankylosis and replacement resorption in long-term follow-up after cleaning avulsed teeth with sterile gauze, which may reflect differences in study design.

In the present study, an electric toothbrush with pumice paste was evaluated as a mechanical method for root surface cleaning. While this technique removed a substantial portion of the necrotic PDL tissue, it was less effective than several chemical protocols in achieving near-complete elimination of residual PDL remnants. Furthermore, the cementum integrity was not preserved in teeth cleaned with an electric toothbrush. Previous studies by Kenny et al. (32) and Esper et al. (8) suggested that this method preserved cementum integrity, which contrasts with the present findings. This discrepancy may be attributable to differences in study design, brushing technique, or pressure applied.

The present results indicated that 10% $Ca(OCl)_2$ combined with surfactant for 35 minutes was highly effective in removing necrotic PDL tissue, showing no significant difference compared with 5.25% NaOCl plus surfactant. The 10% $Ca(OCl)_2$ without surfactant also showed effective results in removing PDL tissue, which was lower but statistically comparable to that of all surfactant-containing groups. However, despite its acceptable tissue removal capacity, 10% $Ca(OCl)_2$ caused visible damage to the cementum and, therefore, it cannot be recommended as a suitable alternative to NaOCl with surfactant. Accordingly, 5.25% NaOCl combined with surfactant remains the preferred protocol, providing both superior PDL removal and better preservation of cementum integrity.

This study has several limitations. Information regarding the patients from whom the teeth were extracted was unavailable, which may influence the results, as systemic conditions can affect the quality of cementum and PDL. Additionally, assessing PDL cellular viability and differentiation potential after root surface treatments was not performed in this study. Future studies with larger sample sizes are needed to evaluate the cleaning efficacy of 10% $Ca(OCl)_2$ combined with surfactant when applied for less than 35 minutes, in order to determine the shortest application time that achieves optimal PDL removal without compromising cementum integrity.

Conclusions

Within the limitations of this study, the following conclusions can be drawn:

1- The highest residual PDL was observed in the control, NaOCl 1%-15 min, and NaOCl 5.25%-5 min groups. On the other hand, the Sur-NaOCl 5.25%-5 min, Sur-NaOCl 5.25%-2 min, and Sur-$Ca(OCl)_2$ 10%-35 min groups exhibited the lowest residual PDL.

2- Cementum integrity was preserved in the control group and all surfactant-containing NaOCl groups.

3- The use of 5.25% NaOCl with surfactant for 5 minutes provided optimal PDL removal while preserving the integrity of the root cementum.

Author contributions

M.S. contributed to study design, supervision, data analysis, and critical revision of the manuscript. M.K. contributed to data acquisition and analysis. Z.S. drafted the initial manuscript and conceptualized the study. F.M. contributed to study administration, supervision, and data interpretation.

Conflict of interest

The authors declare that they have no competing interests.

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Ethical Considerations

The study protocol was approved by the ethics committee of North Khorasan University of Medical Sciences (IR.NKUMS.REC.1398.056). The patients consented to the use of their extracted teeth for research purposes.

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