Mechanical removal of necrotic periodontal ligament by either Robinson bristle brush with pumice or scalpel blade. Histomorphometric analysis and scanning electron microscopy

Esper HR, Panzarini SR, Poi WR, Sonoda CK, Casatti CA. Mechanical removal of necrotic periodontal ligament by either Robinson bristle brush with pumice or scalpel blade. Histomorphometric analysis and scanning electron microscopy.

Abstract – One of the important factors accounting for successful delayed replantation of avulsed teeth is seemingly the type of root surface treatment. Removal of necrotic cemental periodontal ligament remnants may prevent the occurrence of external root resorption, which is the major cause of loss of teeth replanted in such conditions. The purpose of this study was to compare the efficacy of two mechanical techniques for removal of root-adhered periodontal ligament. Preservation or removal of the cementum layer concomitantly with these procedures was also assessed. Forty-five roots of healthy premolars extracted for orthodontic purposes were selected. After extraction, the teeth were kept dry at room temperature for 1 h and then immersed in saline for rehydration for an additional 10 min. Thereafter, the roots were assigned to three groups, as follows: group 1 (control) - the cemental periodontal ligament was preserved; group 2 - removal of the periodontal ligament by scraping root surface with a scalpel blade (SBS); group 3 - periodontal ligament remnants were removed using a Robinson bristle brush at low-speed with pumice/water slurry (RBP). The specimens were analysed histomorphometrically and examined by scanning electron microscopy. The quantitative and qualitative analyses of the results showed that the RBP technique was significantly more effective than the SBS technique for removal of the periodontal ligament remnants adhered to root surface. Both techniques preserved the cementum layer.

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Studies on delayed tooth replantation have sought to elucidate the several yet unanswered questions regarding the treatment management of accidentally avulsed teeth (1–4). Even when the circumstances are far less than ideal, replantation of an avulsed tooth should be attempted because it yields, at least temporarily, the reestablishment of function and esthetics (5), and therefore has an important psychological impact on patient's recovery.

The prognosis of a replanted tooth relies upon a wide array of factors, including the type of resorption occurring after replantation, degeneration of periodontal ligament (PDL) remnants (which is directly related to the trauma sustained and/or extra-alveolar handling of the root), desiccation of root surface and the storage medium in which the tooth is maintained until its replantation (6–9).

When an avulsed tooth is replanted with devitalized cemental periodontal ligament adhered to root surface, the granulation tissue may either be replaced by osseous tissue (10, 11) or trigger a resorption process (7, 12–14). In view of this, several techniques have been proposed for removal of necrotic PDL remnants prior to delayed replantation (9, 10, 15–17) including mechanical removal by scraping of the root surface with a scalpel blade (16), enzymatic techniques (18) or chemical removal by immersion of the avulsed tooth in a sodium hypochlorite solution (15, 19–22). It has been advocated (13, 15, 20, 23) that whereas the use of sodium hypochlorite does not seem to affect the cementum layer, scalpel blade scraping may result in removal of cementum together with the PDL remnants. The implications of such removal rely on the fact that the cementum layer seems to be more resistant to inflammatory resorption than dentin.

Nevertheless, it has been reported that the roots of teeth treated with sodium hypochlorite before replantation appeared surrounded by a fibrous connective tissue with fibres organized parallel to the root surface without insertion areas (21). This fibrous capsule-like structure has been shown to compromise the stability and maintenance of the replanted tooth into the socket. The findings of a previous study investigating delayed rat teeth replantation after treatment of root surfaces with sodium hypochlorite at high concentrations (5% and 10%) showed premature exfoliation of the replanted teeth with no sign of root resorption (24).

On account of these issues, the purpose of this study was to investigate, by means of histomorphometric analysis and scanning electron microscopy (SEM), the efficacy of two mechanical techniques for removal of root-adhered necrotic cemental periodontal ligament: Robinson bristle brush with pumice (RBP) or scalpel blade scraping (SBS). Preservation or removal of the cementum layer concomitantly with these procedures was also assessed.

Material and methods

Forty-five roots of noncarious, unrestored maxillary and mandibular premolars extracted for orthodontic reasons were utilized in this study. Immediately after extraction, the teeth were fixed by their crowns with wax on a flat base (Polidental Ind. e Com. Ltda, São Paulo, SP, Brazil) and left undisturbed at room temperature for 1 h. Thereafter, the teeth were individually immersed in disposable plastic recipients containing 50 ml of saline (Ariston Inds. Químs e Farms. Ltda, São Paulo, SP, Brazil), allowed for hydration for 10 min and assigned to three groups ($n = 15$), as follows.

Group 1 (Control): the periodontal ligament was preserved. Group 2 (SBS): the periodontal ligament was removed gently by scraping root surfaces with a #15 scalpel blade (Medico International Trading Co. LTD., China) mounted on a #3 scalpel handle (J.O.N. Comércio de Produtos Odontológicos Ltda, São Paulo, SP, Brazil). Each root surface was scraped only once with the blade positioned perpendicularly to the long axis of the root and moved in a crown-apex direction. Group 3 (RBP): the periodontal ligament was removed using Robinson bristle brushes (Labor Dental Ltda, São Paulo, SP, Brazil) at low-speed and a polishing paste prepared with fine-grain pumice (Dental AG Ltda, São Paulo, SP, Brazil) and filtered water. Each root surface was polished in a crown-apex direction for 30 s and the roots were thoroughly rinsed with air-water spray to remove pumice residues completely.

In all groups, two roots were processed for SEM examination. The other 13 roots were fixed in 10% neutral formalin for 24 h and then decalcified in EDTA solution (distilled water - 250 ml; EDTA - 50 g; sodium hydroxide - 6.1 g) for 60 days. After decalcification, 10 roots were sectioned longitudinally and three roots were sectioned transversally at the middle third, and the fragments were included in paraffin. Semi-serial 6-μm-thick sections were obtained and stained with hematoxylin and eosin (H&E). All sections were examined histologically but only the longitudinal cuts were used for histomorphometric analysis.

Histomorphometric analysis

Ten longitudinal sections from different glass slides, each representative of one of the 10 teeth selected for histomorphometric analysis in each group, were chosen. To evaluate the entire root surface, the sections were further divided in smaller fragments and examined by an optical microscope with ×5 magnification (Axiolab, Zeiss, Jena, Germany). The images were captured by a video camera (JVC TK-1270, Tokyo, Japan) and analysed using ImageLab 98 software. In each fragment, the cementum and periodontal ligament perimeters were measured (in pixels) and the values were analysed statistically using one-way ANOVA and the Tukey's test.

Scanning electron microscopy

The teeth selected for SEM analysis were fixed in 2.5% glutaraldehyde solution (ESM Electron Microscopy Sciences, Hatfield, PA, USA) and 4% paraformaldehyde (Sigma Chemical CO., St. Louis, MO, USA) in a 0.1 M sodium phosphate buffer at

pH 7.4, for 12 h at 4°C. The specimens were washed in sodium phosphate buffer for 20 min and immersed in this solution for additional 12 h. Thereafter, the specimens were postfixed with 1% osmium tetroxide (ESM Electron Microscopy Sciences, Hatfield, PA, USA) for 2 h and received four 20-minute washes with phosphate buffer solution. They were then dehydrated in a graded ethanol series (50, 70, 80, 90, 95 and 100%; 20 min each) and the critical point was obtained with liquid carbon dioxide (ESM Electron Microscopy Sciences, Hatfield, PA, USA). The specimens were mounted on metallic stubs with double-faced adhesive tape, sputter-coated with gold (Desk II, Denton Vacuum, Moorestown; NJ, USA) and kept in a vacuum chamber until examination with a scanning electron microscope (JSM 5410, JEOL, Tokyo, Japan).

Results

Histomorphometric analysis

The results were obtained after qualitative analysis of dentin, cementum and periodontal ligament.

Group 1 (Control): in all specimens, cemental periodontal ligament was observed throughout the root surface. The cementum layer was intact and the insertion of PDL fibres could be clearly identified (Figs. 1 and 2).

Group 2 (SBS): in both transversal (Fig. 3) and longitudinal sections, small areas with periodontal ligament adhered to the cemental surface were observed. The cementum layer appeared preserved in all specimens (Fig. 4).

Group 3 (RBP): both transversal (Fig. 5) and longitudinal (Fig. 6) sections exhibited small areas of periodontal ligament remnants adhered to the root surface. In this group, however, lesser amount of PDL fibres was noted. The cementum layer appeared preserved in all specimens (Fig. 6).

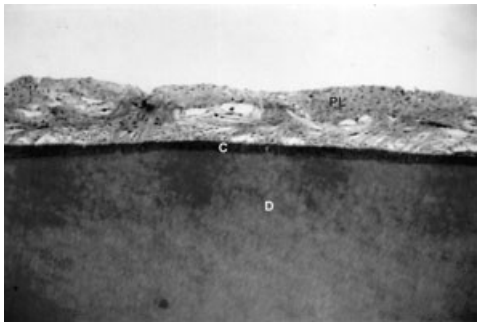

Fig. 1. Group 1 (Control) - Intact periodontal ligament (PL) and cementum (C). D = Dentin. Longitudinal section. H&E (Original magnification ×63).

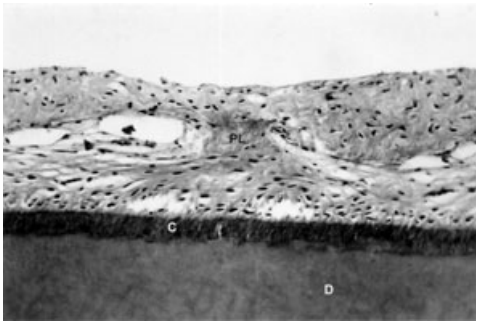

Fig. 2. Group 1 (Control) - Intact periodontal ligament (PL) and cementum (C). D = Dentin. Longitudinal section. H&E (Original magnification ×160).

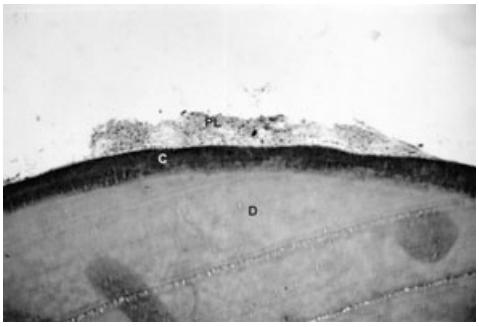

Fig. 3. Group 2 (Scalpel blade scraping) - Small areas of periodontal ligament (PL) adhered to cementum (C). D = Dentin. Transversal section. H&E (Original magnification ×160).

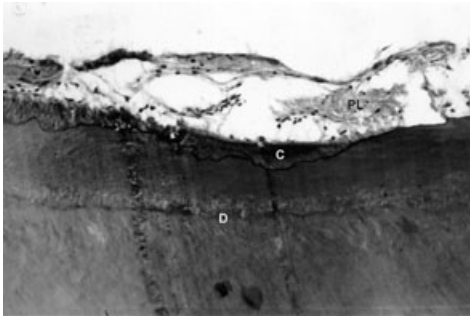

Fig. 4. Group 2 (Scalpel blade scraping) - Small areas of periodontal ligament (PL) adhered to cementum (C). D = Dentin. Transversal section. H&E (Original magnification ×160).

Scanning electron microscopy

The middle third area of each specimen was observed at ×1000 magnification and the same structures, i.e., dentin, cementum and periodontal ligament, were examined in all groups (Fig. 7). The SEM micrographs were consistent with the histomorphologic findings. In the control group, an extensive area of periodontal ligament fibres adhered to root surfaces was observed (Fig. 8). In

Fig. 5. Group 3 (Robinson bristle brush with pumice) - Small areas of periodontal ligament (PL) adhered to cemental surface (C). D = Dentin. Transversal section. H&E (Original magnification ×63).

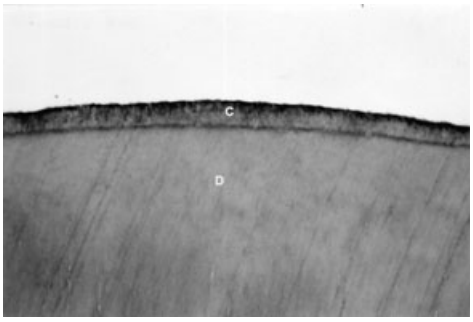

Fig. 6. Group 3 (Robinson bristle brush with pumice) - Absence of periodontal ligament fibres and intact cementum (C). Transversal section. D = Dentin. H&E (Original magnification ×160).

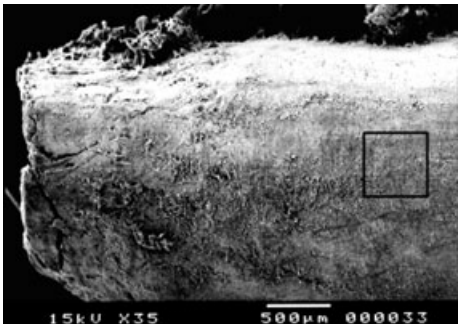

Fig. 7. Scanning electron micrograph of root surface. The demarcated area corresponds to the middle third.

the SBS group, although the scraped root surface exhibited grooves caused by the scalpel blade, still a large amount of adhered periodontal ligament remnants were observed. In the areas with absent periodontal ligament, an intact cementum lining was clearly identified without dentin exposure (Fig. 9). The RBP group showed considerably lesser

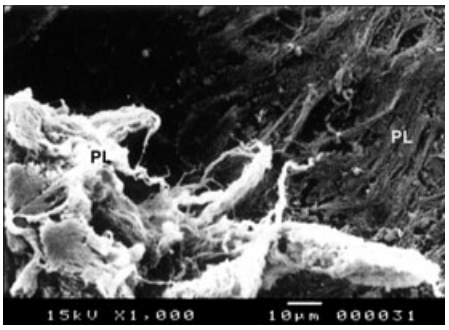

Fig. 8. Group 1 (control) - Extensive root area covered by periodontal ligament (PL) fibres.

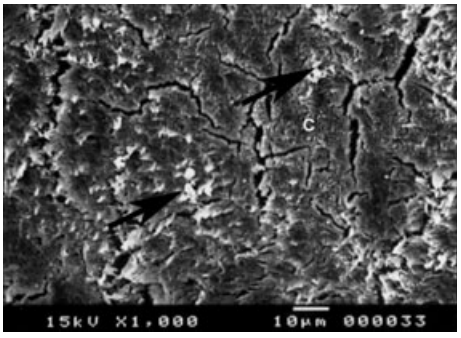

Fig. 9. Group 2 (Scalpel blade scraping) - Great amount of periodontal ligament remnants (arrows). Preserved cementum layer (C).

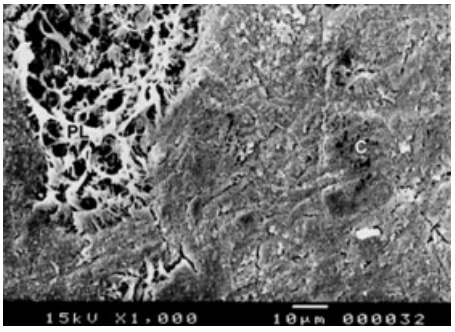

Fig. 10. Group 3 (Robinson bristle brush with pumice) - Smaller amount of periodontal ligament (PL) remnants. Preserved cementum layer (C). No exposed dentinal tubules can be identified.

amount of PDL remnants adhered to cementum surface and no exposure of dentinal tubules (Fig. 10).

Statistical analysis

One-way ANOVA and the Tukey's test showed highly significant difference between the surface treatments ($P < 0.0001$), the RBP technique presenting better results.

Discussion

The treatment guidelines for avulsed teeth recommend that first assistance be provided at the accident site immediately after sustaining the traumatic injury. The findings of a prospective study (9) evaluating the periodontal ligament healing in 400 avulsed and replanted permanent teeth confirmed that immediate replantation provides the best prognosis for avulsed teeth and clearly has an overwhelming influence on the outcome of replantation. Nevertheless, delayed replantation is so far a clinical reality because trauma patients are not usually seen by a dentist readily enough to allow for an optimal treatment to be rendered. Clinical practise has shown that most avulsed teeth are replanted after long extra-alveolar times. Furthermore, in the majority of the cases, the first procedures are not correctly performed due to lack of knowledge of dental trauma management and replantation protocol (4, 25–27) and unawareness of the best media for transportation of the avulsed tooth to the dental office. It is well documented that the survival rate of avulsed permanent teeth following replantation is affected primarily by the duration of the extra-buccal period and the nature of the storage conditions.

Studies have shown that even after a short extra-alveolar dry storage period (up to 30 min), only a small percentage of cells remain viable, because of the rapid decrease in the proliferating capacity of periodontal ligament cells (7, 28, 29). There is a consensus in the literature that the devitalized periodontal ligament should be removed when tooth replantation is delayed because the necrotic remnants seem to stimulate the occurrence of external root resorption (9, 10, 15, 16, 30, 31). According to the treatment guidelines for avulsed teeth replanted after an extended extra-alveolar period (31), removal of cemental PDL fibres should be the first step in root surface treatment prior to replantation.

Several techniques have been described for the removal of necrotic periodontal ligament, including scaling of root surfaces with scalpel blades (16) and periodontal curettes (32–34). However, there are no reported studies attesting the efficacy of such methods with respect to not only the removal of PDL remnants but also the preservation of the cementum layer, which is an important barrier against external root resorption (15, 23).

In addition to mechanical procedures, chemical agents, such as sodium hypochlorite at different concentrations, have been indicated for removal of necrotic periodontal ligament (19, 21, 22, 24). Even though it is an excellent solvent of organic material that efficiently removes PDL remnants (22) preserving the cementum layer, sodium hypochlorite is considered to be toxic for the periodontal tissues (35), possibly because of its high pH. Experimental studies in dog and rat teeth using sodium hypochlorite for root surface treatment prior to replantation, revealed the formation of a connective tissue similar to a fibrous capsule that interfered with the maintenance of the replanted tooth into the alveolus (21, 24).

This study was designed to investigate whether two mechanical techniques were able to remove root-adhered periodontal ligament remnants without causing any alterations to root surface pH, and preserve the cementum layer.

Kenny et al. (36), in a study on the effect of Emdogain in delayed replantation, evaluated the removal of root-adhered cemental periodontal ligament using conventional rubber prophylaxis cups loaded with wet pumice. They advocated that this method produces minimal root surface damage and does not leave any organic residues. The methodology of the present study was designed based on these outcomes, however we used Robinson bristle brushes instead of rubber cups because the morphology of the brushes would yield a more effective removal of PDL remnants.

Pumice is a siliceous material of volcanic origin that may be satisfactorily used both as an abrasive material and as a polishing agent, depending on the particle size (37). For this study, fine-grain pumice slurry was applied at low-speed for 30 s on each face of the roots to avoid unnecessary cementum abrasion, as removal of root-adhered PDL remnants could be observed macroscopically. The methodology adopted attempted to reproduce the clinical conditions as close as possible, i.e., the teeth were kept dry for 1 h and then hydrated by immersion in saline to facilitate the removal of necrotic cemental PDL. Pumice and Robinson bristle brushes mounted in a low-speed handpiece are routinely found in dental offices.

In this study, scalpel blade scraping was chosen amongst other mechanical techniques because it is a feasible procedure to be done at hospitals and emergency rooms, where first-aid care is usually provided in trauma cases, and was based on the methodology of a previous *in vivo* investigation (16). We evaluated the efficacy of a #15 blade to remove root-adhered PDL fibres (preserving the cementum layer), and compared its performance to that of a presumably less aggressive technique using Robinson bristle brushes and pumice. Control group served as a reference of the amount of periodontal ligament present and the aspect of the cementum layer without any root surface treatment.

The longitudinal root sections selected for histomorphometric analysis allowed for thorough

visualization of root extension, from the amelocemental junction to tooth apex. SEM examination of the treated root surfaces at different magnifications (×35, ×100, ×500 and ×1000) revealed the presence of both cementum and periodontal ligament fibre remnants.

The findings of this study showed that, unlike previously assumed, the cementum layer was preserved when the periodontal ligament fibres were removed by scraping with a scalpel blade. Although the SEM micrographs revealed the presence of grooves produced by the blade in few areas, a continuous cementum lining was clearly identified in all specimens. The technique using Robinson brush plus pumice, in addition to preserve the cementum layer, was more effective in removing the periodontal ligament than the scalpel blade. Lesser amount of root-adhered PDL remnants was observed in the specimens of the RBP group. A noteworthy observation, however, was the limitation of both methods to detach periodontal ligament fibres from root surface close to enamel.

The outcome of this study indicates that for delayed tooth replantation requiring removal of necrotic periodontal ligament, both scalpel blade scraping and Robinson bristle brush with pumice preserved the cementum layer while removing PDL remnants. However, brush/pumice technique yielded better efficacy in ligament removal.

Conclusions

The findings of the histomorphometric analysis and scanning electron microscopy examination revealed that:

1 The cementum layer remained intact in the control and experimental groups alike.

2 The use of Robinson bristle brush with pumice was more effective than scalpel blade for removal of root-adhered periodontal ligament remnants.

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