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ORIGINAL ARTICLE

The buffy coat platelet-rich fibrin concentrate on chelated root dentin. An in-vitro model in regenerative endodontics

ABSTRACT

Aim *To characterize in scanning electron microscopy (SEM), the morphology and structural conformation of the buffy coat – platelet-rich fibrin concentrate on root dentin conditioned with different chelation schemes.*

Materials and methods *An in vitro study was conducted on 35 human hemi-sected roots, randomly assigned to 7 groups. The samples were immersed in different solutions as follows: G1 distilled water (DW); G2 17% EDTA+DW; G3 17% EDTA; G4 chitosan (CS)+DW; G5 Cs; G6 nanoparticulate chitosan (CSnp)+DW; G7 CSnp. A sample of human blood was collected by venipuncture, then centrifuged to obtain a PRF matrix from which the buffy coat was extracted and placed on dentin surfaces for SEM analysis. The Blood Element Adhesion Index (BEAI) categorised each sample according to the arrangement and number of essential scaffolding elements. The Kruskal-Wallis nonparametric and Dunn's multiple-comparisons tests established statistical significance.*

Results *Significant differences were observed between groups (p = 0.011). Group G1, showed a complete absence of fibrin-matrix formation, with cellular dispersion on the surface. In groups G2-G3 (EDTA), a weak interconnection between fibrin, cells, and dentin was observed. Groups G4-G5 (CS) exhibited the greatest cellular adhesion to a consolidated fibrin-matrix. Finally, the variability for G6 and G7 (CSnp) resulted in an adequate fibrin network and cellular dispersion. The final rinse decreased matrix consolidation in CS-CSnp.*

Conclusions *The chelating agent and final rinse protocol significantly influence the initial stability of the buffy coat platelet-rich fibrin. Chitosan enhanced the stability and interaction of the essential elements of the scaffold compared to EDTA.*

Clinical Relevance *An approach to regenerative treatments is to examine the role of chelation in dentin, which supports the stability of a solid matrix, such as the buffy coat, which contains bioactive factors and cellular elements essential for regeneration.*

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Introduction

Regenerative endodontic procedures (REPs) have been developed to integrate biological mechanisms that enable the physiological replacement of damaged dental tissues and the restoration of pulp–dentin complex function (1). In vital teeth, an initial inflammatory response activates cells and signalling molecules that promote angiogenesis and the formation of reparative dentin in the presence of a bioactive material (2–4). In contrast, regeneration in non-vital teeth relies on intracanal conditioning through disinfection and chelation, followed by the incorporation of a scaffold that supports cell adhesion, proliferation, nutrition, and signalling (5).

The use of low-concentration sodium hypochlorite (1%) in combination with intracanal medication has been established as a disinfection strategy (6). Chelation, as an adjunct, presents significant biological challenges in contemporary endodontics. The release of signalling molecules conserved in the dentin matrix, such as fossilized morphogens, following the use of chelating agents, activates pro-regenerative interaction mechanisms in immature teeth or, more recently, pro-reparative mechanisms in necrotic teeth with periapical pathologies and complete root formation (7). Regarding the use of 17% ethylenediaminetetraacetic acid (EDTA), considered the standard chelating agent in REPs, it acts by coordinating with Ca^{2+} ions in root dentin (8). However, its high affinity for dentin leads to residual chelating agent within the intracanal space (9), which may reduce collagen matrix exposure and the availability of calcium ions, thereby interfering with the adhesion of coagulation factors and platelets present in the scaffold (10,11).

Natural cationic polymers, such as chitosan (CS), either in solution or in nanoparticulate form (CSnp), constitute chemical structures containing amino and hydroxyl groups capable of forming covalent bonds with dentinal collagen through the establishment of mineral bridges with Ca^{2+} ions and phosphate groups present in dentin. These interactions allow chitosan to act as a bioactive, antimicrobial, and chelating agent in root dentin (9,12). Nevertheless, although dentin chelation is considered a key process in configuring the intracanal microenvironment prior to regeneration, the interaction between conditioned dentin and biological scaffolds in REPs may depend on the chelating agent's mechanism of action and the final irrigation protocol. These aspects remain unclear in the available evidence (13) and are not yet standardized in clinical practice (13,14).

The development of autologous scaffolds has progressed significantly. Although the induction of a blood clot through the apical foramen may provide a biological scaffold containing stem cells, fibrin, platelets, and coagulation factors (15), its clinical acquisition is unpredictable, leading to non-standardized variations in specific conditions such as bleeding volume and the morphology of the cellular and protein components that constitute the fibrin network (16). In this context, platelet concentrates, including platelet-rich fibrin (PRF), may represent an autologous leukocyte-based biomaterial that provides a stable fibrin network and a slow, sustained release of growth factors over 7 to 28 days (17–19). Choukroun et al. (2001) identified, within PRF, an intermediate layer that concentrates the highest number of platelets and leukocytes, termed the buffy coat. This layer, with a thickness of up to 2 mm, is considered the primary site of growth factor release, thereby enhancing its regenerative scaffold effectiveness (17, 20).

Given this background, understanding how different dentinal chelation protocols influence the morphology and structural organization of the buffy coat–PRF concentrate is a clinical need. Therefore, the aim of the present study was to characterize, by scanning elec-

tron microscopy, the morphology and structural organization of the buffy coat–platelet-rich fibrin concentrate on root dentin conditioned using different chelation protocols.
Ho: Dentinal chelation protocols used in regenerative endodontics do not produce significant changes in the morphology and structural organization of the buffy coat–platelet-rich fibrin concentrate.

Materials and methods

An in vitro, quantitative experimental study approved by the Ethics and Research Committee of the Faculty of Dentistry at the Universidad Nacional de Colombia (FOUN), (ACT No. 03-25). The study design complied with the ethical standards established in the Declaration of Helsinki and with the technical recommendations of ISO 15189:2022 regarding the handling of body fluid samples for laboratory testing.

Study unit and sample selection

A total of 18 human permanent single-rooted teeth were included. All specimens were healthy, with complete root formation and without developmental defects or fracture lines and were extracted for orthodontic reasons. The sample was obtained from donors aged 18 to 24 years who attended the oral surgery clinics of the FOUN. Dental integrity was confirmed by stereomicroscopic examination (SMZ1000, Nikon Corp., Tokyo, Japan). The teeth were individually stored in Eppendorf tubes containing 0.9% sodium chloride (NaCl) with 0.02% sodium azide (NaN_3) at 4 °C for a period not exceeding three months (9). Teeth that exhibited coronal or apical fractures during extraction or laboratory handling were excluded.

Processing of the study unit

The hemi-sected root was defined as the unit of analysis. Sectioning was performed using an IsoMet cutting system (Buehler Ltd., Lake Bluff, IL, USA), operated at low speed under continuous irrigation. Each tooth was decoronated at the cemento-enamel junction, and each root was standardized to a length of 12 mm. Subsequently, the roots were hemisected by a transverse cut perpendicular to the long

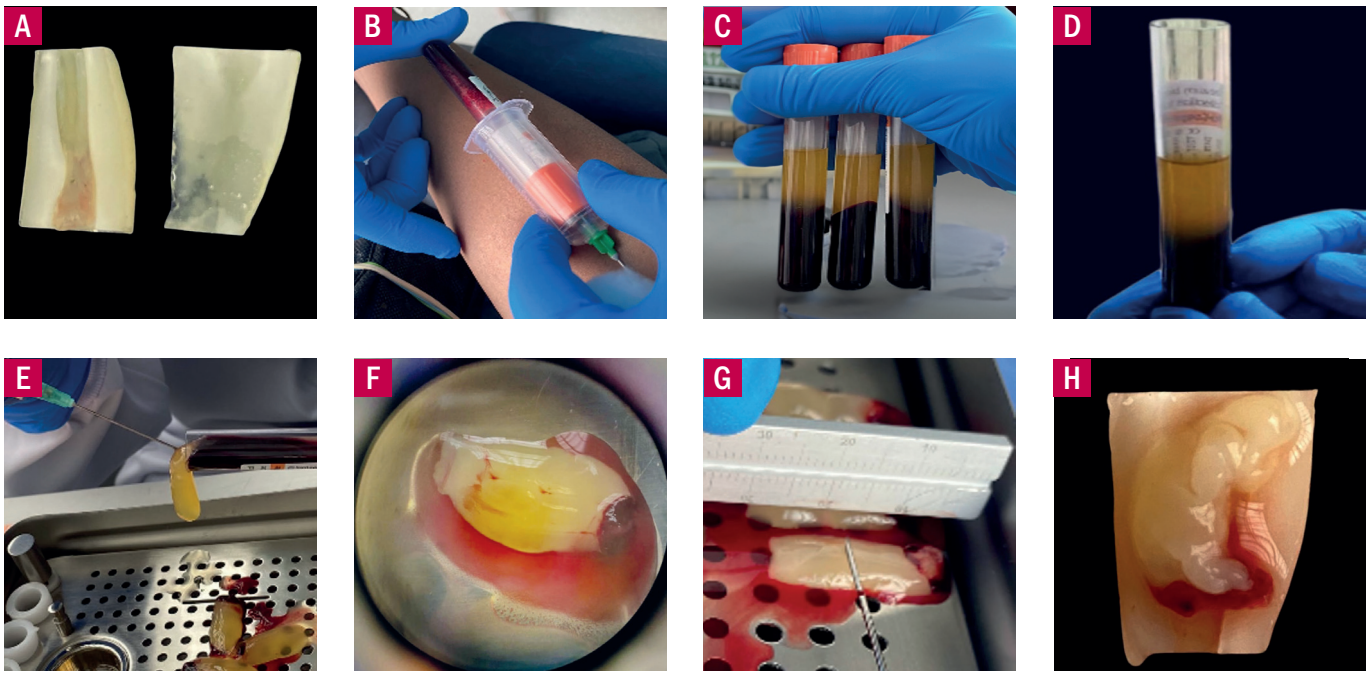


Figure 1
Sample preparation and buffy coat acquisition: **A** Decoronation and root hemisection; **B** Blood sample collection; **C** PRF obtained after centrifugation; **D** PRF maturation at body temperature; **E** Separation of PRF from the sediment; **F** Observation of the PRF clot formed by the fibrin yellow portion, constituting the main body, and a red portion located at the end of the clot. Between these two areas, a whitish layer called the ''buffy coat'' **G** Extraction of the buffy coat; **H** Application of the buffy coat onto conditioned root dentin.

axis of the root, in a buccal–palatal/lingual direction, until a total of 35 observational units (n = 35) (Figure 1A). Organic tissue remnants within the root canals were removed using a #25 K-flexofile (Dentsply Sirona, Maillefer, USA). The integrity of the dentinal surfaces was reconfirmed under stereomicroscopic examination. To eliminate residual smear layer, ultrasonic irrigation was performed for 8 minutes with distilled water using a Vita Sonic II for In-Ceram unit (Vita Zahnfabrik H. Rauter GmbH & Co. KG). The samples were subsequently stored under the same conditions previously described.

Chelating solutions

The chelating agents included 17% EDTA solution (Eufar®, Bogotá, Colombia) with a pH of 7.5 and a molecular weight of 292.24 g/mol; chitosan in solution (CS) with a pH of 3.2 and a molecular weight ranging from 50 to 190 kDa; and nanoparticulated chitosan (CSnp) with a pH of 4.58 and a nanoparticle size of 248.1 ± 4.07 nm. Chitosan-based solutions were prepared in the Department of Pharmacy at the Faculty of Sciences, Universidad Nacional de Colombia. Specifically, the CS was prepared by mixing 2 g of chitosan (Sigma-Aldrich, St. Louis, MO, USA) with 100.0 mL of 1% v/v acetic acid under magnetic stirring for 2 hours until a homogeneous, transparent solution was obtained. To obtain the nanoparticles, sodium tripolyphosphate (Sigma-Aldrich, St. Louis, MO, USA) was added to the CS at a concentration of 1 mg/1 mL using the ionic gelation dropwise method, followed by homogenization with an Ultra-Turrax® homogenizer (IKA, Staufen, Germany) at 5000 rpm for 15 minutes. Once prepared, the solutions were stored at 4 °C.

Seven experimental groups were established, each containing the study units (n = 5), and were assigned using Excel's randomization function (version 2412; Microsoft, Washington, USA). The groups were organized as follows: Group 1 (G1): distilled water (DW); Group 2 (G2): 17% EDTA with final irrigation using DW; Group 3 (G3): 17% EDTA without final irrigation; Group 4 (G4): CS with final irrigation using DW; Group 5 (G5): CS without final irrigation; Group 6 (G6): CSnp with final irrigation using DW; and Group 7 (G7): CSnp without final irrigation. Each specimen was placed in an Eppendorf tube to ensure complete immersion in 2 mL of each solution for 5 minutes at each step (14). Following the chelation protocol, the specimens were kept dry and immediately prepared for scaffold application.

PRF preparation

At the LIMACIB laboratory of the FOUN, a healthy adult volunteer under 30 years of age was recruited with informed consent. The volunteer had no history of comorbidities, no medication, and did not exhibit addictive behaviors, two weeks prior to sample collection. A total of 5 mL of blood was obtained by venipuncture of the median cubital and cephalic veins in the right and left forearms (Figure 1B). The sample was collected in orange-cap glass tubes, internally coated with silica as a procoagulant to reduce coagulation time during centrifugation (8 minutes at 1300 rpm and 223 relative centrifugal force (g) using an LC-04R centrifuge (Scientific, Zenith Lab, Jiangsu, China) (21) (Figure 1C). After centrifugation, the sample in the tube was exposed to ambient air for 10 minutes at 37 °C to allow stabilization (Figure 1D). Once stabilized, the PRF was extracted, and the scaffold components were identified by visual inspection. The basal portion containing red blood cells, identifiable by its color, was separated and discarded (Figure 1E). Subsequently, the buffy coat was identified as a slightly paler zone adjacent to the upper fibrin-rich portion (Figure 1F). A 5 mm-long section was then obtained (Figure 1G), which was divided into equal parts and immediately placed onto each previously chelated hemi-sected root (Figure.

1H). Each specimen was placed in a dry Eppendorf tube and stabilized in a Hygrobath (Whip Mix, Louisville, USA) for 15 minutes at 37 °C. The samples were then immediately prepared for SEM.

Preparation for SEM analysis

At room temperature, each specimen was fixed by immersion in 2.5% glutaraldehyde for 1 hour and subsequently rinsed with three changes of distilled water for 10 minutes each. Dehydration was performed through a graded ethanol series (70%, 75%, 80%, 85%, 90%, 95%, and 100%), with each step lasting 2 minutes. The process continued with gold coating of the specimens using the sputtering technique, applying a current of 8 mA for 150 seconds in a vacuum metallization unit (SPT-20, COXEM, South Korea; target: Au). All specimens were examined under SEM (EM-30AX PLUS, COXEM, South Korea), and micrographs were obtained at 2500× and 5000× magnifications at the Department of Physics, Faculty of Sciences, ?????.

Observational topographic analysis

To standardize the observations, the area at the interface between the buffy coat and dentin was selected. Topographically, four essential elements were identified: platelet conglomerates; the presence and morphology of leukocytes and erythrocytes; and the presence and density of the fibrin network (19,20).

Quantitative analysis

Two evaluators selected two representative images per group, ensuring one image for each magnification (2500× and 5000×). The observers recognize the four categories of the Blood Elements Adhesion Index (BEAI) proposed by Theodoro et al. (2006) (22) and classified the images according to the arrangement and number of essential elements observed, as follows: Category 0, absence of fibrin network and blood cells; Category 1, scarsely distributed fibrin network and/or blood cells; Category 2, a moderate number of blood cells and a thin fibrin network with poor interlacing; and Category 3, a dense fibrin network with rich interlacing and the presence of blood cells.

To standardize visualization conditions, an observation window of 1,280 µm × 960 µm was used in ImageJ version 1.52a (Wayne Rasband, National Institutes of Health, USA; Java 1.8.0_112 (64-bit)), under similar lighting and

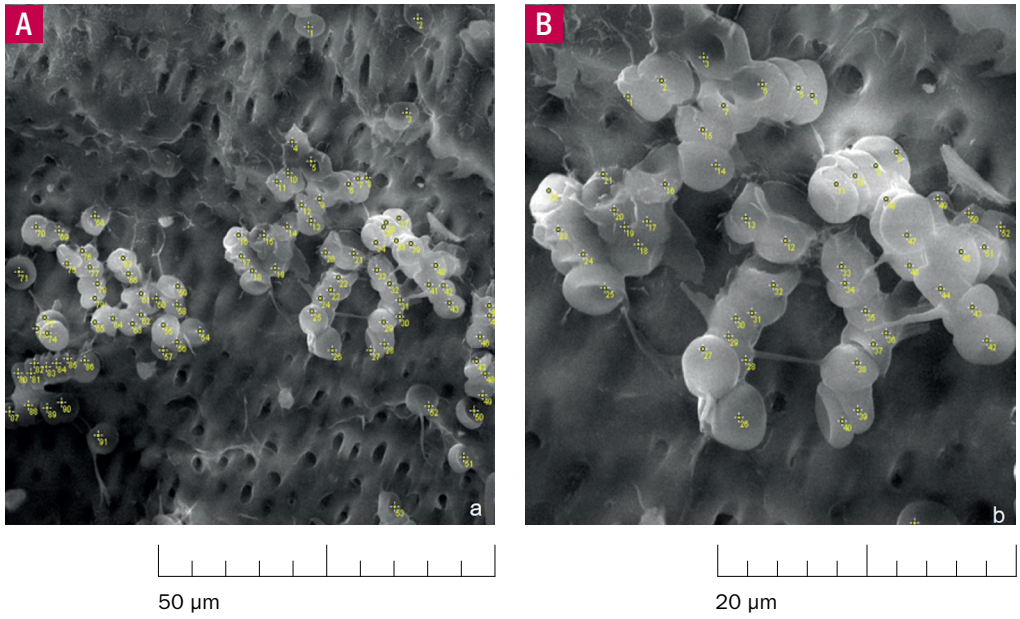


Figure 2
Observation windows for cell counting and fibrin network identification. **A** Field of view of 50 µm at 2500× magnification. **B** Field of view of 20 µm at 5000× magnification. Yellow dots indicate cell counts performed in ImageJ.

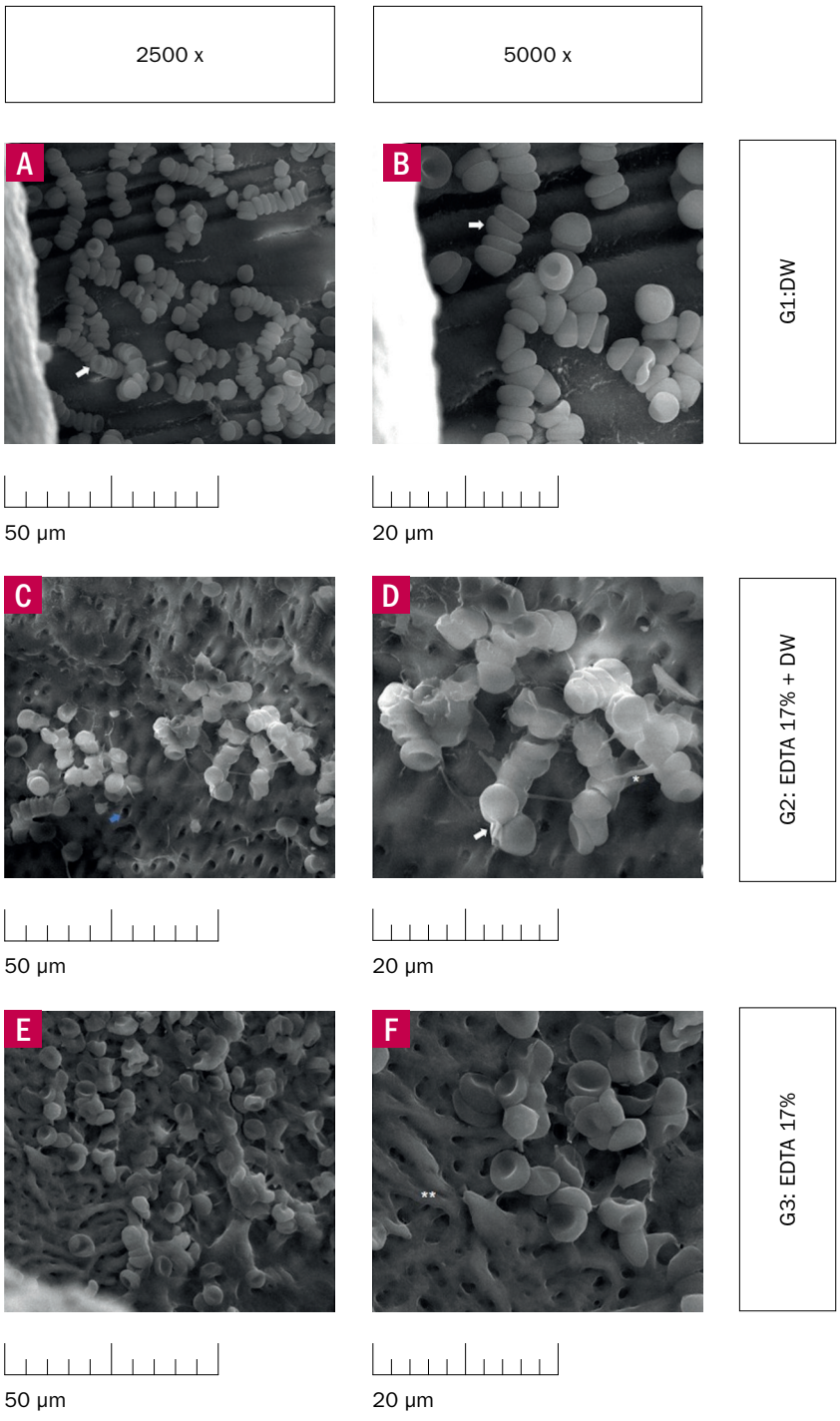


Figure 3 G1 – G3 fibrin network and cell layout. **A**. DW at 2500x, the white arrow indicates the conglomerate erythrocytes. **B**. DW at 5000x shows erythrocytes arranged on the root dentin without conditioning. **C**. EDTA 17% + final rinse at 2500x, the blue arrow denotes the exposed dentinal tubule. **D**. EDTA + final rinse at 5000x, the white arrow indicates conglomerate red blood cells, * Thin fibrin network interconnected with cells. **E**. EDTA 17% at 2500x cellular clusters superimposed on the fibrin network. **F**. EDTA 17% at 5000x,** thicker fibrin network.

timing conditions. For each field of view, a 50 µm grid was applied to micrographs acquired at 2500× magnification, and a 20 µm grid was applied to micrographs acquired at 5000× magnification (Figure 2A, 2B). According to the index, the objective was to categorize the fibrin mesh arrangement and the expected number of blood cells per field.

Statistical analysis

A descriptive analysis of the blood element adhesion index (BEAI) was performed by estimating position measures for each treatment or experimental group. The data were verified for normality using the Shapiro-Wilk statistic. Subsequently, a comparison of the BEAI between groups was performed with the nonparametric Kruskal-Wallis test. As a complement, a multiple-comparison analysis was performed using the Dunn test (with Benjamini-Hochberg correction). All tests were performed using IBM SPSS Statistics, version 27.0 (© 2020 IBM Corp., United States), with a significance level of $p < 0.05$.

Results

Morphological findings of three-dimensional architecture – SEM

Overall, the topographic description identified the presence of the four essential elements: cross-linked fibrin network scaffold, erythrocytes, platelets and leukocytes, representing the "buffy coat", an intermediate section between the fibrin network of the PRF and the red portion. Now standing, the SEM images revealed variability in the chelation patterns on the dentin surface as follows: In the control group with DW (G1), the absence of a chelating agent resulted in a smooth dentin surface without dentinal tubules and no evidence of fibrin deposition. Only biconcave erythrocytes in association with platelets were identified in clusters (Figure 3A, 3B). In the 17% EDTA (G2 and G3), dentinal tubules opened due to chelation. In G2, with a final rinse, a

scaffold characterised by a weak fibrin matrix and a cellular disposition similar to G1 was observed (Figures 3C and 3D). The G3 group, without a final rinse with DW, formed a denser fibrin network, showing some interaction with the cellular elements; however, the scaffold did not completely cover the chelated dentin surface (Figures 3E and 3F). In groups (G4 and G5), CS with and without a DW final rinse, a consolidated fibrin matrix was observed in close contact and significant interaction with the entire surface of the chelated dentin. A scaffold formed by fibrin filaments was observed, supporting the concentration of erythrocytes, platelets, and leukocytes with normal morphology (Figure 4A, B). In group G5 without a final rinse, the CS solution on the dentin surface promoted the formation of an extensive fibrin meshwork, enabling adequate accommodation of blood cells within the network and providing greater stability to the scaffold at all levels of the chelated dentin surface (Figure 4C, D). For CSnp (G6 and G7), variability in the results was observed. In general, high-density fibrin networks were identified, hindering the identification of the embedded cellular elements (Figures 5A and 5B). In the case of G7, without final washing, a denser scaffold was observed (Figures 5C and 5D). The comparison of group ranks showed significant differences between Groups 5 and 1 and 2 ($p = 0.011$), indicating variation in the distribution of BEAI values by treatment type (Figure 6). In group G1 (DW), fibrin network formation was not promoted, and some cellular elements were confirmed. In the four remaining groups, variable fibrin network formation was observed, confirming that dentin chelation is important for scaffold placement on the surface; however, scaffold and cell cluster stability is mediated by the chelating solution type and the final rinsing protocol. In this regard, the G4 and G5 (CS with and without final rinse) showed a blood component adhesion score of 3 (Figures 4A-D and 6). The presence of a dense fibrin network and trapped blood cells covering the dentin surface supports this result.

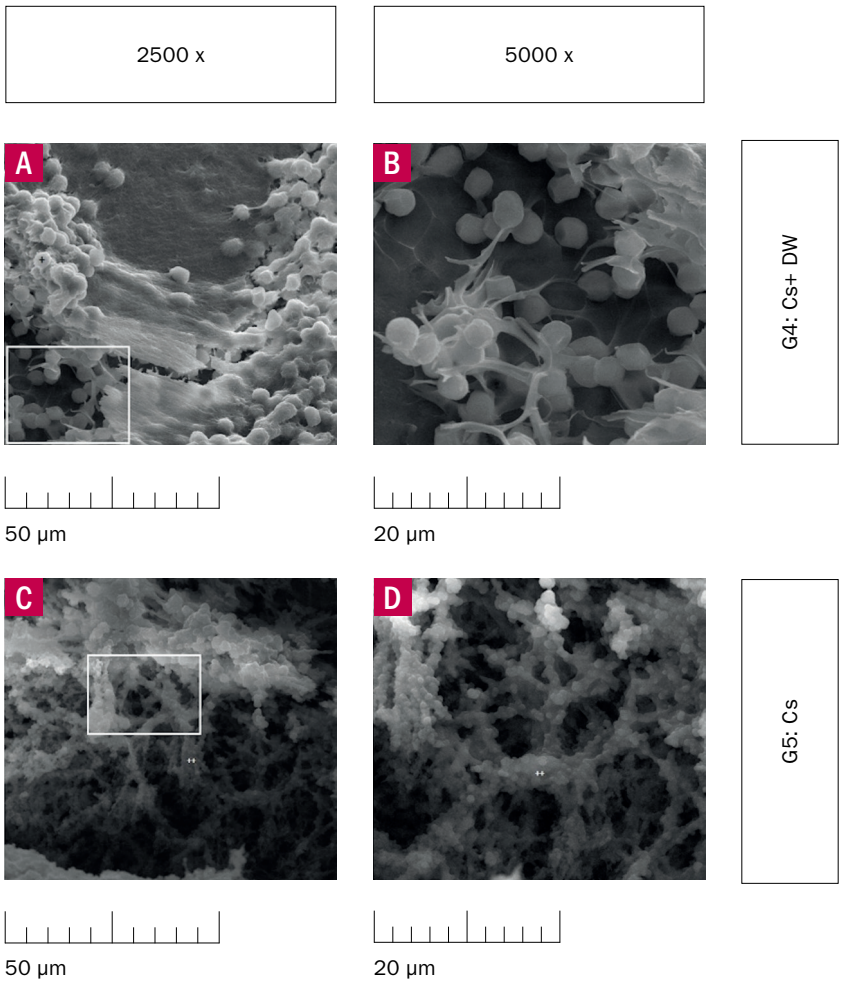


Figure 4
G4, G5 fibrin network and cell layout. **A**. Cs + final rinse at 2500x. + Presence of a leukocyte immersed in the fibrin network. **B**. Magnification at 5000x of the area indicated in image A. The marked interconnection of fibrin and blood cells is shown. **C**. Observation of Cs at 2500x, ++ Blood cells introduced into the fibrin network. **D**. Magnification to 5000x of the area indicated in the image C.

Figure 6 shows that the effect of 17% EDTA on buffy coat stability results in an insufficient fibrin network and/or blood cells, demonstrating great variability in findings within the same group (Score 1-2) (Figure 3A-F). Finally, for CSnp G6 and G7, the final wash made a difference, identifying moderate to high stability of the elements and the fibrin network for G7 CSnp without a final wash (Figure 5A-D), (Figure 6). Finally, for CSnp G6 and G7, the final wash made a difference, identifying moderate to high stability of the elements and the fibrin network for G7 CSnp without a final wash (Figure 5A-D), (Figure 6).

Discussion

In regenerative endodontics, the use of

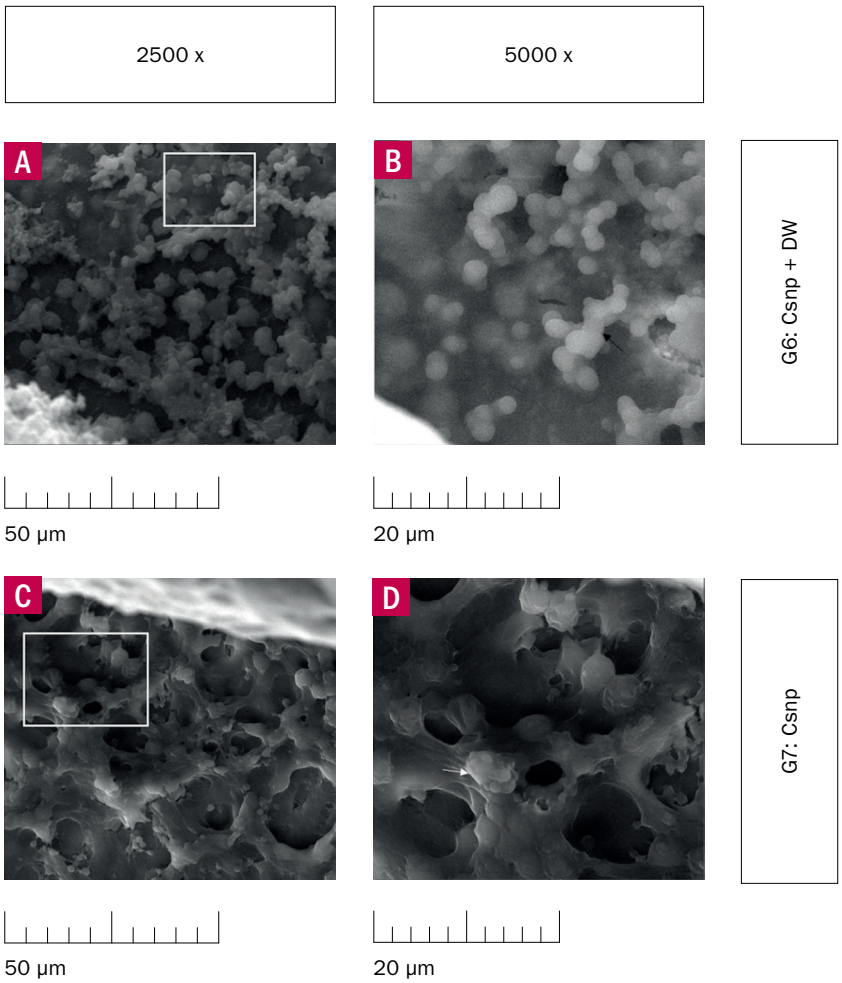
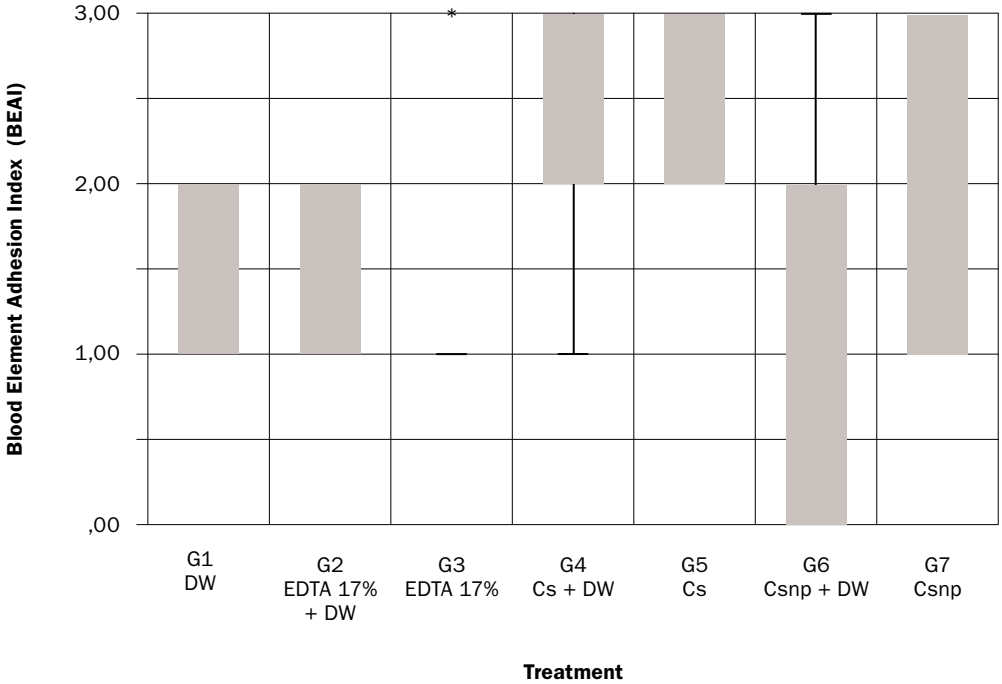


Figure 5
G6 and G7 fibrin network and cell layout. **A**. Csnp + final rinse (2500x), the black arrow indicates a large number of cells trapped within the fibrin network. **B**. Magnification of the area indicated in image a (5000x) reveals cells retained in clusters within the fibrin mesh. **C**. Csnp at 2500x, a thicker fibrin network is observed. **D**. Magnification of the area indicated in image C (5000x) shows that the thick fibrin network covers cells. The white arrow indicates the appearance of cell entrapment under the net.

autologous scaffolds is justified to facilitate interactions among essential elements and a chemically conditioned dentinal surface during disinfection and chelation (23 - 25). In the present work, the comparative analysis between the groups showed significant differences in the incorporation and distribution of platelets, leukocytes and erythrocytes within the fibrin meshwork architecture, with distributions and appearances that could be modified by the chelation protocol. In this regard, CS without a final rinse significantly promoted the adhesion of a dense fibrin network that integrates essential cellular elements to the dentinal surface, thereby allowing rejection of the null hypothesis.
The principle of intracanal chelation involves the formation of bridges between free electrons provided by a chelator and calcium-type metal ions of the dentinal inorganic substrate (26).

Figure 6
Blood element adhesion index (BEAI) for the different experimental groups.



The chelation process will expose the collagen fibers within the matrix, thereby modifying the surface energy of the dentin, promoting the initial stability of the scaffold (27). Three chelating solutions were analysed: 17% EDTA, CS and CSnp, which induced opening of the dentin tubules on the surface and scaffold attachment, with some variation depending on the chelating agent or final rinse (28). Dentinal conditioning increases the "biological acceptance" of the scaffold (29), in this regard, the negative control group (DW) showed a smooth dentinal surface, without tubular openings or structural modifications, and a limited or almost absent fibrin network, with the presence of small, morphologically conserved cell conglomerates, but without apparent adherence to the surface or a defined organizational pattern. This observation, consistent with the literature (29, 30), recognizes a determining role of the chelating agent in the initial scaffold stability as an early healing event.

The differences observed at the microscopic level between the analysed groups revealed how the nature of the chelating agent and the final rinse protocol affect the results. Clinical standards in endodontics recommend using EDTA as an adjunct to endodontic irrigation. However, the experimental results identified a weak fibrin conformation on the dentin, with low adhesion of blood elements, suggesting limited interaction between the buffy coat and the dentinal substrate; EDTA in the intracanal space increases the surface energy of the dentin substrate (12); however, its performance with respect to scaffold stability is limited (29, 31). Taweewattanapaisan et al. (2018) reported that the presence of residual EDTA increases the likelihood of trapping more Ca^{2+} ions, which are necessary for fibrin network stability and proper cell integration (32). The affinity of platelet aggregates for dentin collagen can be affected by poor scaffold adhesion, triggering premature platelet fragmentation (33). This aligns with the observation in G3 and supports the recommendation to perform a final rinse with a sterile solution after EDTA use in clinical practice (32).

In contrast, the results showed that the performance of chitosan in solution, without a final rinse, was significantly superior for forming a consistent fibrin network firmly adhered to the dentinal surface, capable of integrating blood elements and preserving their morphology and organization in clusters. From this perspective, the cationic nature of the CS polymer supports its chelating action on the anionic components of the mineral phase of dentin (9), exposing the dentin collagen after chelation without altering its integrity (34). Although evidence suggests that higher surface energy on a substrate may favor initial contact with biological fluids (14), the adhesion and stability of a less liquid matrix, such as buffy coat, appear to depend on the integrity of an organic matrix on the dentinal surface (27). In this regard, CS, as a dentinal conditioner, maintains the collagen structure and remains on the surface, protecting sites of collagen binding to fibrin in the buffy coat (35). Therefore, based on its cationic character, it is possible to suggest that the presence of CS on the dentinal surface could form electrostatic bonds with negatively charged components of the buffy coat, whose amphoteric behavior allows them to establish other surface-binding mechanisms to achieve the initial stability observed in this experimental group (36). This analysis, as a product of in vitro observation, raises hypotheses that must be confirmed.

In contrast, dentin chelation with CSnp resulted in weak adhesion of essential elements, which is explained by the arrangement of the nanostructured components. These components accumulate on exposed dentin collagen, interfering with the scaffold's interaction with the N-terminal domain of the dentin collagen triple helix (35).

The structural and rheological charac-

teristics of the consolidated fibrin networks and integrated cellular elements are directly influenced by the type of chelating agent and its specific effect on dentin (37).
In vivo studies have demonstrated that PRF production is a simple, rapid, and cost-effective procedure that does not require the addition of anticoagulants or exogenous activators that could interfere with healing and tissue regeneration. Physically, the three-dimensional PRF matrix exhibits mechanical properties that enable the retention of cellular elements and the progressive release of platelet-derived growth factors, which promote cell chemotaxis and angiogenesis (38). These biological events, in a clinical setting, determine dentin formation and increase root length by up to 50% (39), compared to blood clot (40). However, evidence supports promoting controlled microbleeding in the apical third to complement the process (41), so the chelating agent must prepare the intracanal space to receive scaffolds of varying consistencies. Finally, the role of leukocytes, identified in this study and integrated into the buffy coat, could be related to the regulation of the healing process, from promoting an initial inflammatory response to the stages of proliferation and tissue remodeling. Therefore, they are considered a key biological component that transcends the contributions of platelets and growth factors. Leukocytes could exert the antimicrobial and immunomodulatory effect needed to facilitate an orderly transition to more efficient repair phases (42).
In conclusion, the chelation, as the final complement to chemical conditioning in regenerative endodontics, must transcend the concept of "smear layer removal" and focus on chemically modifying the dentin substrate. The in vitro results obtained demonstrated that the initial adhesion of the scaffold depends on the protocol and the nature of the chelating agent used. Regarding, the preservation of the dentinal organic fraction promoted by chitosan, irrespective of the final rinse protocol, resulted in a more highly organized fibrin architecture and a greater abundance of cellular components compared to EDTA. Furthermore, the use of buffy coat as a biologically active fraction of PRF exhibited distinct structural advantages, supporting its efficacy as a robust scaffold.

Clinical relevance
This study is part of an ongoing research line focused on developing a safe microenvironment for regenerative endodontic procedures. Chelation is a critical step in endodontic treatment, and both previous evidence and our research experience indicate that chelating agents significantly influence the interaction between the dentin substrate and endogenous or exogenous components involved in regenerative processes.
In this context, the present manuscript investigates the interaction between autologous scaffolds and radicular dentin under the influence of different chelating agents. The study was conducted under strict methodological rigor, and the images presented accurately reflect the analyzed outcomes, which were interpreted in accordance with current scientific evidence.
The reported findings provide novel insights with potential clinical applicability, supporting the translational perspective emphasized by Giornale Italiano di Endodonzia and contributing evidence to advance knowledge to improve oral health.

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Data declaration
We certify that this work did not use any additional data, whether published or from other available sources. The

data used for the statistical analysis are shown in Figure 6. If sharing the data collection grids is required, we, as authors, are willing to provide them.

Author contribution declaration
The authors declare that they have participated sufficiently and voluntarily in the preparation of the work; therefore, we take public responsibility for the document's overall content. Authorship criteria are supported by contributions (C.G; L.F; S.Q) to the conception, design, analysis, critical revision of the intellectual content, (J.D; L.F; S.Q) verification of laboratory good practices, image evaluation, interpretation of the data, (C.G) and drafting and revising the entire document.
All authors declare no conflicts of interest, and the approval of the ethics committee of the Faculty of Dentistry guaranteed the development of this research under all the ethical and scientific integrity recommendations indicated by international and national regulations.

References

1. *Xie Z, Shen Z, Zhan P, et al. Functional dental pulp regeneration: basic research and clinical translation. Int J Mol Sci. 2021;22:89-91.*
2. *About I. Dentin regeneration in vitro: the pivotal role of supportive cells. Adv Dent Res. 2011;23:320–324.*
3. *Schmalz G, Widbiller M, Galler KM. Clinical perspectives of pulp regeneration. J Endod. 2020;46:161–174.*
4. *Cao Y, Song M, Kim E, et al. Pulp-dentin regeneration: current state and future prospects. J Dent Res. 2015;94:1544–1551.*
5. *Pérez VB, Inostroza CS, Saint Jean N, Brizuela C. Spatial root canal distribution of blood clot and membrane obtained from platelet-rich fibrin. Canal Abierto. 2019;40:4–9.*
6. *García C, Mora T, Quijano S, et al. Clinical, histological, and molecular perspective on regenerating nonvital immature teeth. Contemp Clin Dent. 2023;14:220–226.*
7. *Virdee SS, Bashir NZ, Grant MM, Cooper PR, Tomson PL. Therapeutic Irrigant Procedures for Treating Apical Periodontitis (TIPTAP): A triple-blinded parallel-group randomized controlled phase I/II trial. Int Endod J. 2025;58:965-990.*
8. *Baron M, Morales V, Fuentes MV, et al. The influence of irrigation solutions in the inorganic and organic radicular dentine composition. Aust Endod J. 2020;46:217–225.*
9. *Quijano-Guauque S, Bernal-Cepeda LJ, Delgado FG, et al. Effect of chitosan irrigant solutions on the release of bioactive proteins from root dentin. Clin Oral Investig. 2023;27:691–703.*
10. *Nakashima M, Reddi AH. The application of bone morphogenetic proteins to dental tissue engineering. Nat Biotechnol. 2003;21:1025–1032.*
11. *Aksel H, Albanyan H, Bosaid F, Azim AA. Dentin conditioning protocol for regenerative endodontic procedures. J Endod. 2020;46:1099–1104.*
12. *Arias Alvarado F, Iriarte MR, Jordan-Marino F, et al. Experimental Solution of Chitosan and Nanochitosan on Wettability in Root Dentine: In Vitro Model Prior Regenerative Endodontics. Int J Biomater. 2021;2021:1–10.*
13. *Galler KM, Krastl G, Simon S, et al. European Society of Endodontology position statement: revitalization procedures. Int Endod J. 2016;49:717–723.*
14. *Murray P. Review of guidance for the selection of regenerative endodontics, apexogenesis, apexification, pulpotomy, and other endodontic treatments for immature permanent teeth. Int Endod J. 2022;55:188–199.*
15. *Dohan Ehrenfest DM, de Peppo GM, Doglioli P, Sammartino G. Slow release of growth factors and thrombospondin-1 in Choukroun's platelet-rich fibrin (PRF): A gold standard to achieve for all surgical platelet concentrates technologies. Growth Factors. 2009;27:63–69.*
16. *Diogenes A, Ruparel NB, Shiloah Y, Teixeira FB. Regenerative endodontics: a way forward. J Am Dent Assoc. 2016;147:372–380.*
17. *He X, Chen WX, Ban G, et al. Development of dental pulp cells and PRF complex. J Endod. 2016;42:1634–1639.*
18. *Ghanaati S, Booms P, Orlowska A, et al. Advanced Platelet-Rich Fibrin: A New Concept for Cell-Based Tissue Engineering by Means of Inflammatory Cells. J Oral Implantol. 2014;40:679–689.*
19. *He L, Lin Y, Hu X, et al. A comparative study of platelet-rich fibrin (PRF) and platelet-rich plasma (PRP) on the effect of proliferation and differentiation of rat osteoblasts in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009;108:707–713.*
20. *Choukroun J, Diss A, Simonpieri A, et al. Platelet-rich fibrin: a second-generation platelet concentrate. Part IV: Clinical effects on tissue healing. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;101:56–60.*
21. *Yamaguchi S, Akashi M, Kondo H, et al. Concentrated growth factor matrices prepared using silica-coated plastic tubes are distinguishable from those prepared using glass tubes in platelet distribution: application of a novel near-infrared imaging-based, quantitative technique. Front Bioeng Biotechnol. 2020;8:1–11.*
22. *Theodoro LH, Sampaio JE, Haypek P, et al. Effect of Er:YAG and Diode lasers on the adhesion of blood components and on the morphology of irradiated root surfaces. J Periodontal Res. 2006;41:381-90.*
23. *Liu H, Lu J, Jiang Q, et al. Biomaterial scaffolds for clinical procedures in endodontic regenera-*

tion. Bioact Mater. 2022;14:24–47.
24. Pavlovic V, Ciric M, Jovanovic V, et al. Platelet-rich fibrin: basics of biological actions and protocol modifications. Open Med (Wars). 2021;16:1086–1096.
25. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). Biotechnol. 2009; 27:158–167.
26. Hülsmann M, Heckendorff M, Lennon A. Chelating agents in root canal treatment: mode of action and indications for their use. Int Endod J. 2003;36:810–830.
27. Baker DL, Stanley SP. Fibrin clot adhesion to dentin conditioned with protein constructs: an in vitro proof-of-principle study. J Clin Periodontol. 2005;32:561–566.
28. Abdelkafy H. Efficacy of using chitosan and chitosan nanoparticles as final irrigating solutions on smear layer removal and mineral content of intraradicular dentin. J Indian Soc Pedod Prev Dent. 2023;41:170–177.
29. Preeja C, Janam P, Nayar BR. Fibrin clot adhesion to root surface treated with tetracycline hydrochloride and ethylenediaminetetraacetic acid: A scanning electron microscopic study. J Periodontol. 2008;79:1880–1886.
30. Subramanian S, Appukuttan D, Tadepalli A, et al. Root conditioning with citric acid and ethylenediaminetetraacetic acid and their effect on fibrin clot adhesion to dentin: a scanning electron microscopic study. J Clin Diagn Res. 2017;11:82–85.
31. Chakravarthy Y, Chellamuthu M, Senthamilselvan A, et al. Comparative Evaluation of fibrin density with Chitosan, Papain and 17% EDTA-Normal saline combination as irrigants in teeth with open apices: Anex vivoSEM study. J Conserv Dent. 2022;25(2):140-144.
32. Taweewattanapaisan P, Jantarat J, Ounjai P, Janebodin K; Effects of EDTA on blood clot in regenerative endodontic procedures. J Endod. 2019;45:281–286.
33. Dantas AAR, Fontanari LA, Ishi EP, et al. Blood cells attachment after root conditioning and PRP application: an in vitro study J Contemp Dent Pract. 2012;13:163–169.
34. Stefano C, Gianguzza A, Piazzese D, Sammartano S. Speciation of chitosan–phosphate and chitosan–nucleotide systemsin NaCl aqueous solution. Chem Speciat Bioavailab. 2010;22:99–107.
35. Reyhani V, Seddigh P, Guss B, et al. Fibrin binds to collagen and provides a bridge for αVβ3 integrin-dependent contraction of collagen gels. Biochem J. 2014;462:113–123.
36. Minami S, Östblom M, Johansson J, et al. Blood protein adsorption onto chitosan. Biomaterials. 2002;23:2561–2568.
37. de Araújo L, Goulart TS, Gil ACK, et al. Do alternative scaffolds used in regenerative endodontics promote better root development than that achieved with blood clots?. Braz Dent J. 2022;33:22–32
38. Guerreiro SR, Marto CM, Paula A, et al. Platelet-Rich Plasma and Platelet-Rich Fibrin in Endodontics: A Scoping Review. Int J Mol Sci. 2025;26:54–79.
39. Torabinejad M, Milan M, Shabahang S, et al. Histologic examination of teeth with necrotic pulps and periapical lesions treated with 2 scaffolds: An animal investigation. J Endod. 2015;41:846–852.
40. El Halaby HM, Abu-Seida AM, Fawzy MI, et al. Evaluation of the regenerative potential of dentin conditioning and naturally derived scaffold for necrotic immature permanent teeth in a dog model. Int J Exp Pathol. 2020;101:264–276.
41. Brizuela C, Meza G, Urrejola D, Quezada MA, Concha G, Ramírez V, Angelopoulos I, et al. Cell-Based Regenerative Endodontics for Treatment of Periapical Lesions: A Randomized, Controlled Phase I/II Clinical Trial. J Dent Res. 2020;99:523-529.
42. Blanco J, García A, Hermida-Nogueira L, Castro AB. How to explain the beneficial effects of leukocyte- and platelet-rich fibrin. Periodontol 2000. 2025;97:74–94.