

SEM Evaluation of Radicular Dentin after using Platelet Rich Plasma on Regeneration of non-Vital Immature Teeth

Running Title: Radiographical evaluation of regeneration of non-vital immature teeth

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ABSTRACT

Aims of the Study: The aim of this study was to evaluate the Scanning Electron Microscopy (SEM) analysis of radicular dentin after using Platelet Rich Plasma (PRP) with or without hyaluronic acid and/or OsteonTMII (30% hydroxyapatite and 70% beta-tricalcium phosphate) to aid regeneration of non-vital immature teeth.

Materials and Methods: Twenty local dogs of (4-6) months-old in good general health were used (approximately 6-9 kg body weight) in this study. The animals were anesthetized with a mixture of Xylazine 1mg/Kg body weight and Ketamine 5mg/kg. After infection and disinfection of teeth using triple antibiotic paste, PRP with or without hyaluronic acid and/or OsteonTMII were introduced in treating teeth. SEM analyses of radicular dentin carried out after 2, 4, 8 and 12 weeks.

Results: At different time intervals, Group (1) demonstrated the same morphological pattern. There were differences in the images at different time intervals among other entire groups.

Conclusions: The stimulation potential of PRP with Hyleronic acid and/or OsteonTMII for regeneration of non-vital immature to caused changes in the morphological pattern of radicular dentin.

Key words: Radiographical, PRP, regeneration, non-vital, immature.

INTRODUCTION

An open apex is usually found in a developing root of an immature tooth and, in the absence of pulp or periapical disease, is normal. In humans, apical closure takes place approximately three years after tooth eruption. When the pulp undergoes pathologic changes before root development is completed, dentin formation ceases, and root growth is arrested⁽¹⁾. Immature teeth that have open and often divergent apices are not suitable for complete cleaning and obturation with traditional techniques and materials. In addition, because of their thin dentinal walls, these teeth are susceptible to subsequent fracture after treatment. Teeth with necrotic pulps and immature apices present special challenges to clinicians during obturation⁽²⁾. The clinical decision as to whether to perform apexogenesis or apexification for immature teeth appears to be clear cut with the teeth deemed to contain vital pulp tissue being subject to apexogenesis and teeth deemed to have nonvital pulp tissue receiving apexification. However, certain clinical observations reported recently have broken this clear-cut guideline by showing that apexogenesis may occur in teeth which have nonvital pulps⁽³⁻⁵⁾. PRP enabled healing through the use of one's own natural growth factors. Studies suggest that platelets contain an abundance of growth factors and cytokines that can affect inflammation, postoperative blood loss, infection, osteogenesis, wound, muscle tear and soft tissue healing. Research now shows that platelets also release many bioactive proteins responsible for attracting macrophages, mesenchymal stem cells and osteoblasts that not only promote removal of degenerated and necrotic tissue, but also enhance tissue regeneration and healing⁽⁶⁾.

Hyaluronic acid can reduce nerve impulses and nerve sensitivity associated with pain. In experimental osteoarthritis, this glycosaminoglycan has protective effects on cartilage⁽⁷⁾. Synthetic hydroxyapatite (SHA) is a ceramic produced by

a sinterization process. The sintered sHA is osteoconductive, but it is relatively insoluble at neutral pH. Its slow rate of dissolution is considered by some surgeons to be a disadvantage in certain clinical applications. The porosity of the sHA must simulate or imitate the morphology of spongy bone⁽⁸⁾. Tricalcium phosphate is similar to sHA being a CaP with a different stoichiometric profile. Tricalcium phosphate has been formulated into pastes, particles or blocks, which have demonstrated an ability to be biocompatible and biodegradable^(9,10).

The aim of this study was to evaluate the Scanning Electron Microscopy (SEM) analysis of radicular dentin after using Platelet Rich Plasma (PRP) with or without hyaluronic acid and/or OsteonTMII (30% hydroxyapatite and 70% beta-tricalcium phosphate) to aid regeneration of non-vital immature teeth.

MATERIALS AND METHODS

Twenty local dogs of (4-6) months-old in good general health were used (approximately 6-9 kg body weight) in this study. The dental procedures carried out at Department of Surgery, College of Veterinary Medicine, University of Mosul/ Iraq. One hundred and twenty immature lower incisor teeth from twenty dogs were included in this study. The dogs were randomly divided into four groups. First and second incisors from each side and the upper right third incisors per dog were treated, while the upper left third incisors served as negative controls and were left to develop naturally for comparison. Every effort was made to minimize the discomfort of the animals involved in this project. The animals were anesthetized with a mixture of Xylazine (Xyla, interchemie, Holland) 1mg/Kg body weight and Ketamine (KEPRO, Holland) 5mg/kg⁽¹¹⁾. All experimental teeth were mechanically exposed and pulp tissue was disrupted by an endodontic file. Supragingival plaque scaled from the dogs' teeth was placed and sealed temporarily in the pulp chambers with light cured glass ionomer cement (Kavitan[®] LC, SpofaDental, Kerr, Holland). The animals were given dipyrone 500mg (AL-Shark, Syria) (100-200mg/kg twice a day for 3-4 days) post-operative procedures for analgesia. The incisor teeth of the dogs were monitored radiographically until such time as there was radiographic evidence of apical periodontitis (approximately 2 – 3 weeks)⁽¹²⁾.

All previously infected teeth were re-entered and disinfected. Each tooth underwent slow irrigation with 10 ml of 1 % NaOCl (sodium hypochlorite) (Bleach FAS, Babel Co., Baghdad, IRAQ), and was flushed with 10 ml of sterile saline (0.9% sodium chloride) (Hospira Inc., Lake Forest, Illinois) and dried with sterile paper points (Dentsply Maillefer, Tulsa, Oklahoma). This was followed by application of a triple antibiotic paste of metronidazole (Julphar, Gulf Pharmaceutical industries, U.A.E.), ciprofloxacin (Julphar, Gulf Pharmaceutical industries, U.A.E. Julphar, Gulf Pharmaceutical industries, U.A.E.) and minocycline (CIPLA LTD., India) in equal portions of each antibiotic mixed with sterile saline (0.9% sodium chloride) to a paste like consistency using a sterile K – file (Dentsply Maillefer, Johnson City, Tennessee). The triple antibiotic paste filled the root canal to the level of the canal orifice and completely removed from the access cavity. Then the access cavity adequately sealed with light cured glass ionomer cement.

After one month of disinfection procedure, the animals again anesthetized and PRP was prepared from the blood obtained from the experimental animals following the method developed by Weibrich et al.⁽¹³⁾. Peripheral Blood was obtained several minutes from jugular vein before starting treatment procedure. A total blood volume of 9ml was collected using a 10ml disposable syringe transferred to glass tube that contained 3.8% sodium citrate solution (Global, China) as an anticoagulant. The glass tube containing the blood was centrifuged at 1300 r.p.m. for 10 min, which resulted in the separation of three basic fractions. Platelet-poor plasma (PPP) was on top of the preparation, PRP in the middle, followed by the red blood cell (RBC) fraction at the bottom. Then the plasma should be separated. This plasma is then submitted to a second centrifugation of 2000 rpm for 8 minutes. Both centrifuging carried out at room temperature. The platelet poor plasma is separated and discharged leaving approximately 0.5 ml PRP. At the time of the application, one drop of PRP (0.05ml) and equal volume of a sterile saline solution containing 10% calcium chloride (Global, China) that were added. One drop of hyaluronic acid (Hyruan PlusTM Inj., LG Life Sciences, Korea) of (0.1ml) and (0.004)g of OsteonTMII (GENOSS, Korea) were used. The teeth treated as following

- Group 1 (lower right third incisor): Infected - disinfected
- Group 2 (lower right second incisor): Infected - disinfected - PRP
- Group 3 (lower right first incisor): Infected- disinfected - PRP + Hyleronic acid
- Group 4 (lower left first incisor): Infected - disinfected - PRP + OsteonTMII
- Group 5 (lower left second incisor): Infected - disinfected - PRP + Hyleronic acid + OsteonTMII
- Group 6 (lower left third incisor): negative control. Untouched teeth left to develop naturally for comparison.

The access openings were then closed with a coronal seal consisting of white MTA (PRO ROOT, USA) and light cured glass ionomer cement.

After 2, 4, 8 and 12 weeks, five dogs scarified per each time interval. Lower anterior part of jaws with the incisor teeth were resected and placed in 10% buffered formaldehyde. The lower teeth separated using 0.15 mm diamond disc mounted on a slow speed handpiece and cutting carried out under distilled water cooling were placed separately in transparent screw-top polyethylene tubes (30ml) contained distilled water. The crown of each isolated lower teeth was decapped at the level of cement – enamel junction, then stored in a distilled water. Then the roots were grooved longitudinally buccally and lingually without penetrating the canal. The roots were then split into two halves with the aid of a chisel and a surgical mallet for exposing the entire root canal lumen. The mesial half of each root was used for the study while the other half was discarded⁽¹¹⁾.

Each chosen half, dehydrated with ascending concentrations of ethyl alcohol (ethanol 50, 70, 95 and 100%) for 10 minutes each⁽¹²⁾ and placed in a desiccators for 24 hours⁽¹¹⁾. Each specimen was then mounted on an aluminum stub, then placed in a vacuum chamber and sputter – coated with 25 µm of gold palladium to increase the electrical conductivity and reflection of the samples through the use of sputter coater (Technics, Japan)⁽¹¹⁾ as a routine preparation for scanning electron microscopy, then examined at ×5000 with a scanning electron microscope (Hitachi S-4160, Japan) at Tehran University, Department of Electrical and Computer engineering operating at 20.0 kV. One photomicrograph was obtained from the center of the apical part of the intra-radicular surface.

RESULTS

Figure (1) showed the dentine surface of Group (1) teeth (antibiotic group). There were visible opened and numerous tubular orifices of different diameters and nearly the same tubular density those not showed differences after 2 weeks, 4 weeks, 8 weeks and 12 weeks. Also at different time intervals, there were the same appearances of peritubular dentin, inter tubular dentin and collagen fibrils network structure.

Figures (2) to (6) showed the dentine surfaces of all treated teeth of Groups (2) to (5). There were differences in the images at different time intervals among these entire groups. After 2 weeks, all teeth of Groups (2) to (6) demonstrated the dentine surface characteristics nearly resemble to that of Group (1).

Figure (2) showed the dentine surface of Group (2) teeth. After 4 and 8 weeks, there were some opened dentinal tubules and others partially occluded. The collagen fibrils became some fused together resulting in a reducing of interfibrillar space. After 12 weeks, some dentinal tubules were partially occluded meanwhile most of them completely occluded. The dentin surface appears heterogeneous or irregular but lesser degree of previous.

Figure (3) showed the dentine surface of Group (3) teeth. After 4 and 8 weeks, there were some opened dentinal tubules and most of them partially and completely occluded. The collagen fibrils became some fused together resulting in a reducing of interfibrillar space. The dentin surface appeared irregular. After 12 weeks, the dentinal tubules were not visible. The dentin surface appeared to be less irregular than previous.

Figure (4) showed the dentine surface of Group (4) teeth. After 4 and 8 weeks, there were some opened dentinal tubules and others partially or completely occluded. The collagen fibrils networks became unnoticed. The dentin surface appeared irregular and the dentin surface was covered by coherent deposits of material. After 12 weeks, the dentine surface changed to decrease in irregularity appearance than previous. Some dentinal tubules were visible.

Figure (5) showed the dentine surface of Group (5) teeth. After 4 weeks, the dentine surface characteristics began to differ from previous. There were some opened dentinal tubules and most of them partially and completely occluded. The collagen fibrils became fused together resulting in lack of interfibrillar space. The dentin surface appeared irregular and look like some material deposition was take place. After 8 weeks, most dentinal tubules were partially occluded and others completely occluded. The dentin surface appeared more irregular and was covered by coherent deposits of material. After 12 weeks, the dentine surface characteristics also differ from previous. Majority of dentinal tubules were completely occluded and some of them partially occluded. The dentin surface appeared to be less irregular than previous.

Figure (6) showed the dentine surface of Group (6) teeth (negative control). After 2 weeks, the dentine surface showed visible opened dentinal tubules of different diameters. Also, peritubular dentin, inter tubular dentin and collagen fibrils network structure were seen. After 4 weeks, the dentine surface characteristics began to differ from previous. The dentinal tubules showed decreasing in their diameters and most of them partially occluded and some of them completely occluded. The collagen fibrils became fused together resulting in decreasing of interfibrillar space. After 8 weeks, there was clear increasing in dentinal tubules density and decreasing in inter tubular spaces and dentinal tubules showed uniform pattern. After 12 weeks, the dentinal tubules were not visible. The dentin surface appeared to be without irregular appearance.

DISCUSSION

In this study, before introducing of materials in the root canal, the canals were flushed with 10 ml of 1 % NaOCl and dried with sterile paper points that's seen to cause planed root surfaces, eliminating the smear layer and debris, opening and widening the dentin tubules, that's seen in Groups (1 – 5) teeth that's to some degree agreed with Haapasalo and Orstavik⁽¹³⁾ and Perez et al.⁽¹⁴⁾, who mentioned that it is crucial to use NaOCl to remove the smear layer and increase the dentinal permeability through clearance of the canaliculi and any remaining organic tissue leaving a mesh of collagen on the prepared surface provided careful demineralization takes place and the surface remains wet facilitating incorporation of material applied with this mesh of collagen creating the micromechanical retention.

In general speak, the triple antibiotic paste failed to demonstrate any changes in the morphological pattern of dentin surface after different time intervals, there were visible opened and numerous tubular orifices of different diameters and nearly the same tubular density, the same appearances of peritubular dentin, inter tubular dentin and collagen fibrils network structure. Meanwhile with using PRP with Hyleronic acid and/or OsteonTMII in addition to Group (6) (negative control), there were differences in the images at different time intervals among these entire groups. After 4 weeks, there were some opened dentinal tubules but the dentinal tubules showed decreasing in their diameters and partially occluded. The collagen fibrils became some fused together resulting in a reducing of interfibrillar space and the latter more fused together resulting in a lack of interfibrillar space after 8 weeks. After 12 weeks, most of dentinal tubules completely occluded. The dentin surface appears heterogeneous or irregular but lesser degree of previous. Only Groups (3) and (5) after 12 weeks that were appeared to be the nearest to negative control group at that time interval.

These changes in the morphological pattern of dentin surface after different time intervals may related to the regeneration potential of materials used for enhancing regeneration and apical maturation of non- vital immature teeth resembling to acceptable degree to the negative control group. Regeneration procedure involved addition of new dentin deposited on existing dentin resulting in partially and completely occluding of dentinal tubules and irregular surfaces that become smoother with progression of time.

Lima et al.⁽¹⁵⁾ carried out a study and photomicrographs were taken of each examined surface and the diameter of the tubules was also measured on the pictures of Cebus paella monkey's canine teeth. The present study calculations, obtained from the measure of the dentinal tubules number, revealed that the average number of the dentinal tubules for each of the three locations was the following: apical root dentin, 74.800 tubules/mm²; mid-root dentin, 90.000 tubules/mm²; cervical root dentin, 91.600 tubules/mm². Concerning to the tubular diameter, the average number one each location was the following: apical root dentin, 4.30 µm; mid-root dentin, 4.37 µm; cervical root dentin, 5.23 µm. The dentinal tubules of the investigated species dentin do not differ much from the human dentin. These results suggest that the monkey teeth are a suitable substitute for human teeth in endodontics studies. Further studies are required to clarify this animal. Concerning dogs' teeth, dentin tubule numerical density information which was focused on the apical part of the tooth was scarce and among this study dentin tubule density was not numbering but just depend on general description. All experimental groups (2-5) failed to demonstrate changes in the dentinal tubules density after different time intervals, except Group (6) (negative control), after 8 weeks, there were clear increasing in dentinal tubules density and decreasing in inter tubular spaces.

CONCLUSIONS

No one of Group (1) teeth to Group (5) teeth exhibit exactly photomicrographs resemble to those of negative control group at different time interval, except Groups (3) and (5) after 12 weeks that were appeared to be the nearest to negative control group at that time interval. The stimulation potential of PRP with Hyleronic acid and/or OsteonTMII for regeneration of non-vital immature to caused changes in the morphological pattern of radicular dentin.

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FIGURES USED

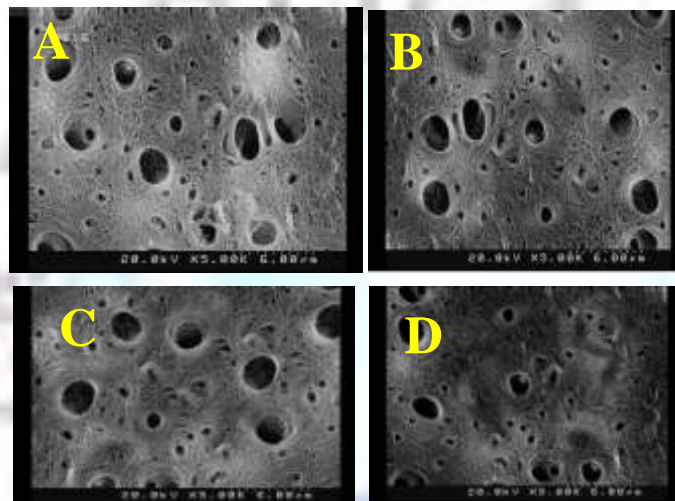


Figure (1) Scanning Electron Microscope Images of Radicular Dentine Surface of Group (1) teeth after different Time Intervals. A: after 2 weeks; B: after 4 weeks; C: after 8 weeks; D: after 12 weeks. Magnification (x5000) for 6.00 μm .

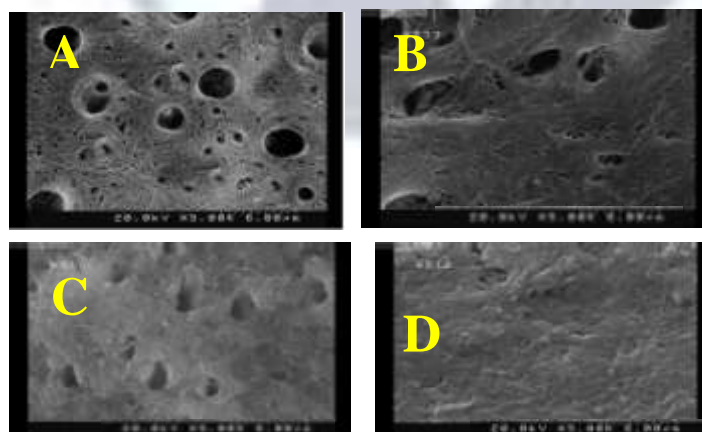


Figure (2) Scanning Electron Microscope Images of Radicular Dentine Surface of Group (2) teeth after different Time Intervals. A: after 2 weeks; B: after 4 weeks; C: after 8 weeks; D: after 12 weeks. Magnification (x5000) for 6.00 μm .

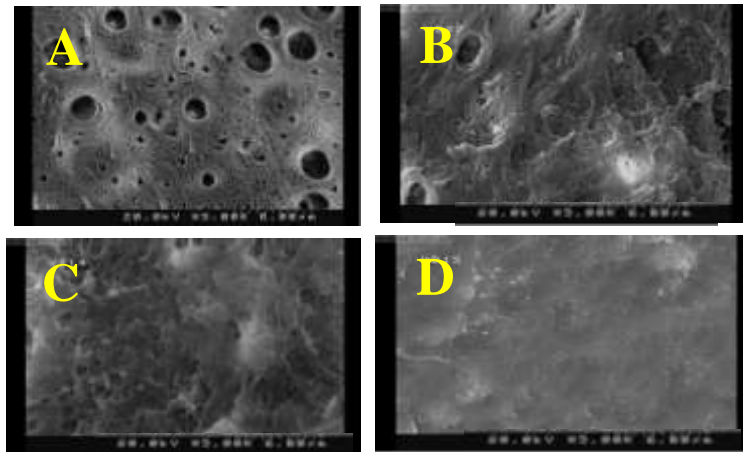


Figure (3) Scanning Electron Microscope Images of Radicular Dentine Surface of Group (3) teeth after different Time Intervals. A: after 2 weeks; B: after 4 weeks; C: after 8 weeks; D: after 12 weeks. Magnification (x5000) for 6.00 μm .

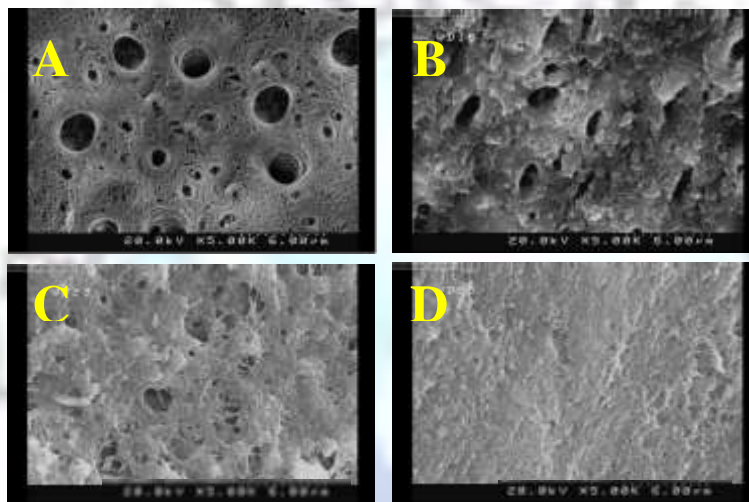


Figure (4) Scanning Electron Microscope Images of Radicular Surface of Group (4) teeth after different Time Intervals. A: after 2 weeks; B: after 4 weeks; C: after 8 weeks; D: after 12 weeks. Magnification (x5000) for 6.00 μm .

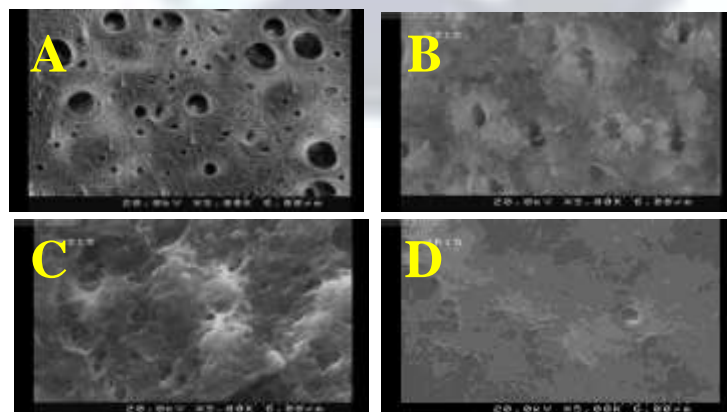


Figure (5) Scanning Electron Microscope Images of Radicular Dentine Surface of Group (5) teeth after different Time Intervals. A: after 2 weeks; B: after 4 weeks; C: after 8 weeks; D: after 12 weeks. Magnification (x5000) for 6.00 μm .

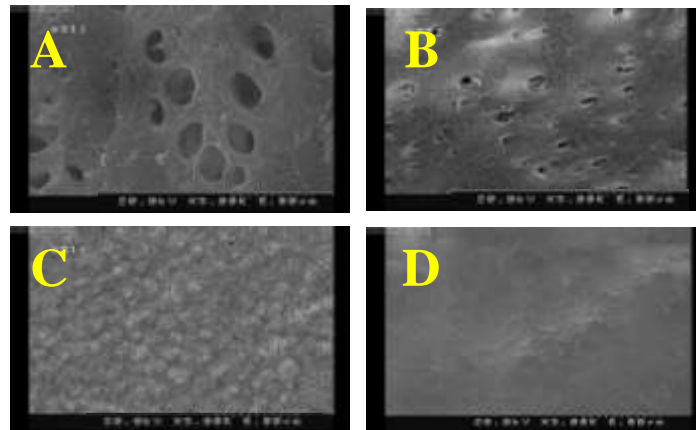


Figure (6) Scanning Electron Microscope Images of Radicular Dentine Surface of Group (6) teeth after different Time Intervals.
A: after 2 weeks; B: after 4 weeks; C: after 8 weeks; D: after 12 weeks. Magnification (x5000) for 6.00 μm .

