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Radiographical evaluation of 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 find the radiographical outcomes of using Platelet Rich Plasma (PRP) with or without hyaluronic acid and/or Osteon^{TM}II (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 Osteon^{TM}II were introduced in treating teeth. Radiographical examination carried out after 2, 4, 8 and 12 weeks.

Results: After 8 and 12 weeks of treatment, all the teeth groups (1-5) showed disappearing of periapical radiolucency that present before. The earliest and best results gain in Group (5) those contain a mixture of all materials through showing evidence of continued growth of the root and canal narrowing, thickening of radicular walls and apical closure before other treating groups.

Conclusions: Regeneration of immature non-vital teeth with apical periodontitis is a clinical possibility through using of a mixture of PRP with or without hyaluronic acid and/or Osteon^{TM}II. . A single matrix may not be an ideal scaffold material. Combination of two or more materials may be the best suited matrix material.

Key words: Radiographical, PRP, revascularization, 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 apexes 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 find the radiographical outcomes of using Platelet Rich Plasma (PRP) with or without hyaluronic acid and/or Osteon^{TM}II (30% hydroxyapatite and 70% beta-tricalcium phosphate) to aid revascularization and continued root development of infected immature dog root canals with apical periodontitis.

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 upper 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 Plus^{TM} Inj., LG Life Sciences, Korea) of (0.1ml) and (0.004)g of Osteon^{TM} II (GENOSS, Korea) were used. The teeth treated as following

- Group 1 (upper right third incisor): Infected - disinfected
- Group 2 (upper right second incisor): Infected - disinfected - PRP
- Group 3 (upper right first incisor): Infected- disinfected - PRP + Hyleronic acid
- Group 4 (upper left first incisor): Infected - disinfected - PRP + Osteon^{TM}II
- Group 5 (upper left second incisor): Infected - disinfected - PRP + Hyleronic acid + Osteon^{TM}II
- Group 6 (upper 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 inomer cement. Radiographical evaluation carried out after 2, 4, 8 and 12 weeks.

RESULTS

Figure (1) demonstrated presence or absence of different radiographical criteria among all upper incisor teeth of all groups involved in this study at different time intervals.

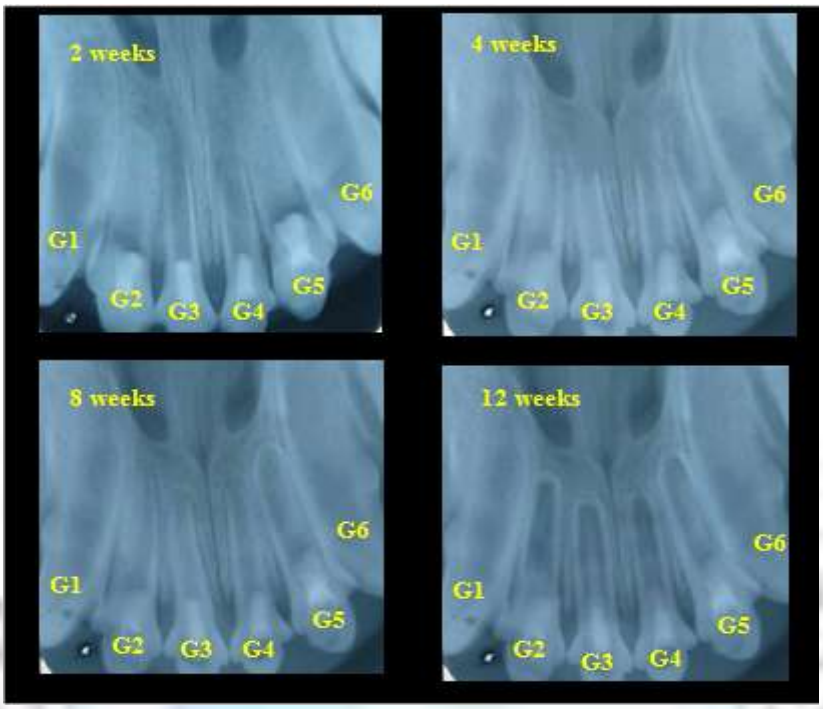

Figure (1): Radiographical views demonstrated the presence or absence of continued growth of the root, canal narrowing and thickening of radicular wall of different teeth groups after 2 weeks, 4 weeks, 8 weeks and 12 weeks time intervals. G1: group 1; G2: Group 2; G3: Group 3; G4: Group 4; G5: Group 5; G6: Group 6.

Table (1) Number of teeth in different groups with and without presence of a periapical radiolucency.

	Number of teeth							
Time interval	**2 weeks (Total no.120)**		**4 weeks (Total no.90)**		**8 weeks (Total no.60)**		**12 weeks (Total no.30)**	
Periapical radiolucency	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**
Group (1)	20	0	12	3	9	1	4	1
Group (2)	17	3	10	5	0	10	0	5
Group (3)	18	2	8	7	0	10	0	5
Group (4)	9	11	1	14	0	10	0	5
Group (5)	9	11	1	14	0	10	0	5
Group (6)	0	20	0	15	0	10	0	5
Chi-Square	81.945		37.683		44.116		31.099	
P - value	0.000*		0.000*		0.000*		0.000*	

df= 5. * Significant difference at P≤ 0.05

Table (1) demonstrated the number of upper teeth in different groups with and without periapical radiolucency. Chi-squared evaluation of all six groups with respect to presence or absence of aperiapical radiolucency showed a significant difference between the groups at different time interval. At 2 and 4 weeks, the majority of teeth of group 1, 2 and 3 showed presence of periapical radiolucency, meanwhile the majority of teeth of group 4 and 5 showed absence of it. All teeth of group 6 showed absence of periapical radiolucency. At 8 and 12 weeks, all the teeth of Groups (2-5) showed absence of periapical radiolucency except Group (1).

Table (2): Number of teeth in different groups with and without presence of continued growth of the root and canal.

	Number of teeth							
Time interval	**2 weeks (Total no.120)**		**4 weeks (Total no.90)**		**8 weeks (Total no.60)**		**12 weeks (Total no.30)**	
Continued root growth	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**
Group (1)	0	20	0	15	0	10	0	5
Group (2)	0	20	10	5	10	0	5	0
Group (3)	0	20	11	4	10	0	5	0
Group (4)	0	20	13	2	10	0	5	0
Group (5)	0	20	15	0	10	0	0	5
Group (6)	20	0	15	0	10	0	5	0
Chi-Square	119.000		50.490		59.000		29.000	
P - value	0.000*		0.000*		0.000*		0.000*	

df= 5. * Significant difference at P≤ 0.05

Table (2) demonstrated the number of teeth in different groups with and without presence of continued growth of the root and canal narrowing. Chi-squared evaluation of all six groups with respect to apical closure showed a significant difference between the groups ($p < 0.05$) at different time intervals. At different time intervals, all teeth of Group (1) showed no evidence of any continued growth of the root and canal narrowing; meanwhile all teeth of Group (6) showed evidence of them. At 2 weeks, all teeth of Group (2), (3), and (4) showed no evidence of continued growth of the root and canal narrowing. At 4 weeks, the majority of teeth of Group (2), (3) and (4) showed evidence of continued growth of the root and canal narrowing, meanwhile all the teeth of Group (5) showed evidence of them. At 8 and 12 weeks all teeth of Group (2), (3) and (4) showed evidence of continued growth of the root and canal narrowing, but all teeth of Group (5) showed these evidence at 8 weeks only those stopped to show them at 12 weeks

Table (3): Number of teeth in different groups with and without presence of thickening of radicular walls of the root .

	Number of teeth							
Time interval	**2 weeks (Total no.120)**		**4 weeks (Total no.90)**		**8 weeks (Total no.60)**		**12 weeks (Total no.30)**	
Thickening radicular walls	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**
Group (1)	0	20	0	15	0	10	0	5
Group (2)	0	20	7	8	9	1	3	2
Group (3)	0	20	5	10	6	4	3	2
Group (4)	0	20	5	10	6	4	4	1
Group (5)	0	20	15	0	10	0	5	0
Group (6)	20	0	15	0	10	0	5	0
Chi-Square	119.000		47.781		33.098		15.080	
P - value	0.000*		0.000*		0.000*		0.010*	

df= 5. * Significant difference at P≤ 0.05

Table (3) demonstrated the number of teeth in different groups with and without presence of thickening of radicular walls of the root. Chi-squared evaluation of all six groups with respect to thickening of radicular walls showed a significant difference between the groups ($p \leq 0.05$) at different time intervals. At different time intervals, all teeth of Group (1) showed no evidence of any thickening of radicular walls, meanwhile all teeth of Group (6) showed evidence of it. At 2 weeks, all teeth of Group (2), (3), and (4) showed no evidence of thickening of radicular walls. At 4 weeks, the majority of teeth of Group (2), (3) and (4) showed no evidence of thickening of radicular walls, meanwhile all of them showed the evidence at 8 weeks and 12 weeks. All teeth of Group (5), showed evidence of thickening of radicular walls at 4, 8 and 12 weeks

Table (4) Number of teeth in different groups with and without presence of apical closure.

	Number of teeth							
Time interval	**2 weeks (Total no.120)**		**4 weeks (Total no.90)**		**8 weeks (Total no.60)**		**12 weeks (Total no.30)**	
Apical closure	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**
Group (1)	0	20	0	15	0	10	0	5

Group (2)	0	20	0	15	7	3	5	0
Group (3)	0	20	0	15	7	3	5	0
Group (4)	0	20	0	15	8	2	5	0
Group (5)	0	20	0	15	10	0	5	0
Group (6)	0	20	0	15	10	0	5	0
Chi-Square	0.000		0.000		31.841		29.000	
P - value	1.000		1.000		0.000*		0.000*	

df= 5. * Significant difference at P≤ 0.05

Table (4) demonstrated the number of teeth in different groups with and without presence of apical closure. Chi-squared evaluation of all six groups with respect to apical closure showed no significant difference between the groups ($p > 0.05$) at 2 and 4 weeks, but there were significant difference at 8 and 12 weeks. At different time intervals, all teeth of Group (1) showed no evidence of any apical closure, meanwhile all teeth of other groups failed to reach apical closure after 2 and 4 weeks. At 8 weeks, the majority of teeth of Group (2), (3), and (4) showed evidence of apical closure and all teeth of these groups showed apical closure at 12 weeks. All teeth of Group (5), showed evidence of apical closure after 8 and 12 weeks.

DISCUSSION

Appropriate outcomes of this treatment determined by the radiographical evaluations of this study revealed that immature teeth with necrotic pulp and apical periodontitis after regeneration procedure of using PRP with or without hyleronic acid and Osteon^{TM}II could induce healing of periapical radiolucency, increased thickening of canal walls and continued root development in teeth of Groups (2-5) but with different degrees at different time intervals. Platelet-rich plasma (PRP) revealed results as a potentially ideal scaffold for regenerative endodontic treatment regimens.

At different time intervals, all teeth of Group (1) (antibiotic group) showed some healing of periapical radiolucency but fail to demonstrate any increase thickening of canal walls, continue root development and apical closure that's disagreed with Iwaya et al. [(14)]. They disinfected a necrotic immature mandibular second premolar with periapical involvement and sinus tract using triple antibiotic paste. Radiographic examination showed the start of apical closure five months after the completion of the antimicrobial protocol. Thickening of the canal wall and complete apical closure was confirmed 30 months after the treatment, indicating the revascularization potential of a young permanent tooth pulp into a bacteria-free root canal space.

Torabinejad and Turman[(15)] published a case report of using PRP in a 11-year-old boy whose maxillary second premolar tooth had been accidently extracted and immediately replanted developed pulpal necrosis and symptomatic apical periodontitis. After removal of the triple antibiotic paste, the PRP was injected into the canal space. Three millimeters of grey mineral trioxide aggregate was placed directly over the PRP clot and the tooth was double-sealed with permanent filling materials. Radiographic examination of this tooth showed resolution of the periapical lesion, further root development, and continued apical closure.

Jadhav et al.[(16)] evaluated and compared maturogenesis induced by revascularization with and without PRP. They concluded that revascularization is a conservative and an effective method for inducing maturogenesis in nonvital, immature teeth. Supplementations with PRP can potentially improve the desired biological outcome of this regenerative technique. Radiographically, after 8 and 12 weeks of treatment, all the teeth groups (1-5) showed disappearing of periapical radiolucency that present before. The earliest and best results gain in Group (5) those contain a mixture of all materials through showing evidence of continued growth of the root and canal narrowing, thickening of radicular walls and apical closure before other treating groups. The apical closure in Group (5) observed even before negative control group that's related to the anatomical differences. Group (5) included in the left third incisor, meanwhile the negative control group included in the left second incisor that's anatomically and radiographically appear longer requiring more time to complete root development.

This is the first study utilized Hyleronic acid and Osteon^{TM}II in combination with PRP and for regeneration and revascularization of immature non vital dogs' teeth. The radiographical benefits gained from groups contain Hyleronic acid in combination with PRP or contain Osteon^{TM}II in combination with PRP not differ from Group (2) that's contain PRP alone and the outcomes may attributed to PRP. But in Group (5) that's contain the three materials together demonstrated radiographical regeneration superior than others. PRP containing different growth factors such as platelet derived growth factor, transforming growth factors β, insulin like growth factor, vascular endothelial growth factor, epidermal growth factor, epithelial cell growth factor. These growth factors are released when platelets are de-granulated, which can be carried out by various methods; addition of thrombin, calcium containing products (e.g., — calcium chlorite, calcium sulfate etc.,) or even shaking the platelets[(16)].

As hyaluronic acid is a naturally occurring chemical already present in skin, there is little chance of an allergic reaction or the body rejecting the injection. Unlike artificial substances, hyaluronic acid is not only natural but known for its health-promoting qualities[17]. In this study Osteon^{TM}II was used that consisted of 30% hydroxyapatite and 70% beta-tricalcium phosphate. According to manufacturer's information (GENOSS, Korea) it's highly resorbable due to higher beta-tricalcium phosphate content, easy manipulation, excellent wettability, osteoconductive synthetic bonegraft, pore size is 200~300μm and porosity is 70%. Its applications include: ridge augmentation, extraction site and osteotomy, cystic cavities, sinus lift and periodontal defect. Koenigs[18] used tricalcium phosphate to induce apical closure in monkeys. He successfully found a mineralized tissue closing the open apices of monkeys' teeth. A small canal containing blood vessels and connective tissue was observed within the mineralized tissue. Roberts and Brilliant[19] carried out a study to evaluate clinically the effect of tricalcium phosphate and calcium hydroxide used as a canal dressing in human pulpless teeth with open apices. They concluded that apical closure occurred clinically when pulpless teeth were treated with tricalcium phosphate and when they were treated with calcium hydroxide.

CONCLUSIONS

Regeneration of immature non-vital teeth with apical periodontitis is a clinical possibility through using of a mixture of PRP with or without hyaluronic acid and/or Osteon^{TM}II. . A single matrix may not be an ideal scaffold material. Combination of two or more materials may be the best suited matrix material.

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