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THE POTENTIAL ROLE OF PRP IN STEM CELLS FROM THE APICAL PAPILLA (SCAP)

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ABSTRACT

Aim of the Study: The aim of this study was to evaluate the potential role of Platelet Rich Plasma (PRP) in the stem cells from the apical papilla through the histopathological changes after using PRP to aid regeneration of non-vital immature teeth. **Materials and Methods**: Twenty local breed 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 was introduced in treating teeth. Histopathological analyses carried out after 2, 4, 8 and 12 weeks. **Results:** At different time intervals, all teeth of Group (1) (antibiotic group) failed to demonstrate any increase thickening of canal walls, continue root development, apical closure or pulp like tissue in the root canal, meanwhile, there were differences in the images at different time intervals among treated teeth of Group (2). Appropriate outcomes of this treatment determined by the histopathological evaluations of this study which revealed that immature teeth with necrotic pulp and apical periodontitis after revascularization procedure of using PRP could increased thickening of canal walls, continued root development, apical closure with pulp like tissue in the root canal space in teeth of Group (2). **Conclusions:** Apical maturation of treating teeth continued in much the same fashion as in the untreated control teeth. PRP has increased concentrations of growth factors that can attract stem cells present in the apical tissues, aid in revascularization and stimulation of undifferentiated mesenchymal cells leading to the deposition of a calcific material at the apex as well as on the lateral dentinal walls of the canal.

INTRODUCTION

It is well known that dental papilla is derived from the ectomesenchyme induced by the overlaying dental lamina during tooth development.[1,2] This developing organ evolves into dental pulp after being encased by the dentin tissue produced by odontoblasts that come from this organ. Most information regarding tooth development comes from studies using animal models. Recently, we described the physical and histologic characteristics of the dental papilla located at the apex of developing human permanent teeth and termed this tissue apical papilla.[3] Apical papilla is apical to the epithelial diaphragm, and there is an apical cell-rich zone lying between the apical papilla and the pulp. Importantly, there are stem/progenitor cells located in both dental pulp and the apical papilla, but they have somewhat different characteristics.[3,4]

Platelet Rich Plasma (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.[5]

Because of the apical location of the apical papilla, this tissue may be benefited by its collateral circulation, which enables it to survive during the process of pulp necrosis. The aim of this study was to evaluate the potential role of PRP in regeneration of pulp – dentin complex of immature non – vital teeth.

MATERIALS AND METHODS

The experimental procedures in this study were confirmed by the ethics committee. Twenty local breed 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 in 2013.

Sixty immature upper incisor teeth from twenty dogs were included in this study. The dogs were randomly divided into four groups. Upper right first and second 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.[6]

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).[7]

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.* [8]. 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. 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 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. Upper anterior part of jaws with the incisor teeth were resected and placed in 10% buffered formaldehyde. The upper teeth separated using 0.15 mm diamond disc mounted on a slow speed hand-piece and cutting carried out under distilled water cooling were placed separately in transparent screw-top polyethylene tubes (30ml) contained 10% buffered formaldehyde. Each specimen kept inside plastic histology cassettes and gave it specific number, then decalcified with buffered 10% dilute hydrochloric acid[9] The specimen was then removed from the storage cassette and embedded in paraffin and was sectioned on a microtome. Sections were made longitudinally every 5µm through the apical foramen of the roots. Tissues were stained with Hematoxylin and Eosin and evaluated by three specialists under light microscopy at up to X100 and X400 magnifications for the presence or absence of healthy, vital tissue and revascularization pattern.

RESULTS

Apical root histopathological evaluations of all groups' teeth demonstrated in Figures (1) to (3).

Figure (1) showed the apical root area of Group (1) (antibiotic group). There were loss of the entire pulp, apical papilla tissues and the Hertwig's epithelial root sheath. The teeth with large lumen and immature root apex, thin root dentin walls and dentin stop growing with evidence of resorption of apical alveolar bone without appearance of any inflammatory cells or reaction. No odontoblastic cell layer and pulp-like tissue were detected and nearly the same histopathological appearance those not showed differences after 2, 4, 8 and 12 weeks.

Figure (2) revealed Group (2) (PRP group) teeth. After 2 weeks, deposition of materials used was seen the lumen of root canal but not pass to the periapical area. There was no any change within the teeth of this group when compared with Group (1). After 4 weeks, there was a vital tissue extend from periapical area and filled the canal space up to the coronal MTA plug**.** This vital tissue considered as pulp-like tissue of a loose connective tissue with few collagen fibers devoid of inflammatory cells and the majority of cells in the canal space and in the periapical area were spindle-shaped fibroblasts or mesenchymal cells and few blood vessels. No mature nerve-like tissue was seen in the canal space. No hard tissue was seen in the canal space. Layers of epithelial-like cells, similar to Hertwig's epithelial root sheath, surrounded the root apex. After 8 weeks, lesser amount the vital tissue found than in 4 weeks revealed the presence of a mildly cellular fibrous connective tissue, fibroblasts, and some blood vessels. However, there were presence of odontoblast-like cells adjacent to the predentin and gain of thickness of the root canal dentin walls and continued root development was attributed to growth of root canal dentine walls. After 12 weeks, there was an evidence of increase in the thickness of canal dentin walls and apical closure that revealed apical maturation with narrowing pulp space. There was a layer of odontoblast-like cells adjacent to predentin. The canal space contained fibrous connective tissue densely infiltrated with fibroblasts and well vascularized.

Figure (3) revealed Group (3) (negative control group) teeth were allowed to develop normally and the roots reached their maturity. After 2 weeks, the tooth with immature open apex and still thin dentin walls with large root canal lumen. Pulp tissue in the root canal composed of loose connective tissue fibrous connective tissue densely infiltrated with fibroblasts, undifferentiated mesenchymal cells as well as few lymphocytes, vascular, lymphatic and nervous. After 4 weeks, the tooth with immature open apex. The dentin walls appeared thicker and there was narrowing of root canal lumen. The pulp tissue consisting of numerous spindle shaped fibroblasts and undifferentiated mesenchymal cells, many blood vessels, dense collagen fibers, and nerve. The canal walls became quite bulky near the apex and curved toward each other**.** Layers of epithelial-like cells, similar to Hertwig's epithelial root sheath, surrounded the root apex. After 8 weeks, pulp tissue revealed the presence of a mildly cellular fibrous connective tissue, fibroblasts, and some blood vessels. However, there was a gain of thickness of the root canal dentin walls. There were more blood vessels and cellular components in the canal than that at the apical area. There was a layer of flattened odontoblast-like cells polarized along the predentin in the apical canal. However, there was an increase in the thickness of the root canal dentin walls and the apically the root canal walls curved toward each other. After 12 weeks, the root dentin acquired its functional thickness, which resulted in narrowing of the pulp space with apical closure that revealed apical maturation. The canal space contained fibrous connective tissue densely infiltrated with fibroblasts and undifferentiated cells, well vascularized and nervous.

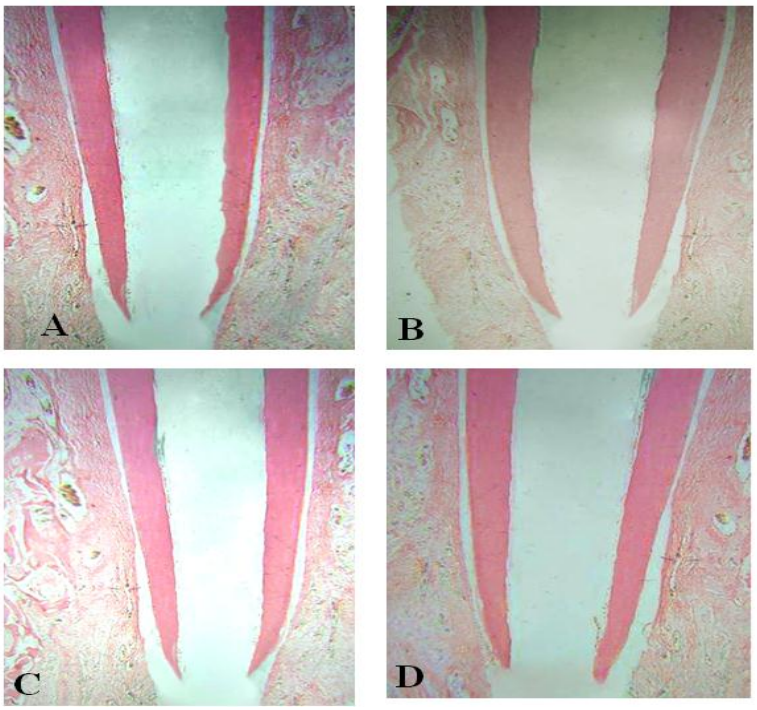

Figure (1): Histopathological assessment of Group (1) antibiotic group at different time intervals (X100) - hematoxylin-eosin staining. (A) after 2 weeks. (B) after 4 weeks. (C) after 8 weeks. (D) after 12 weeks.

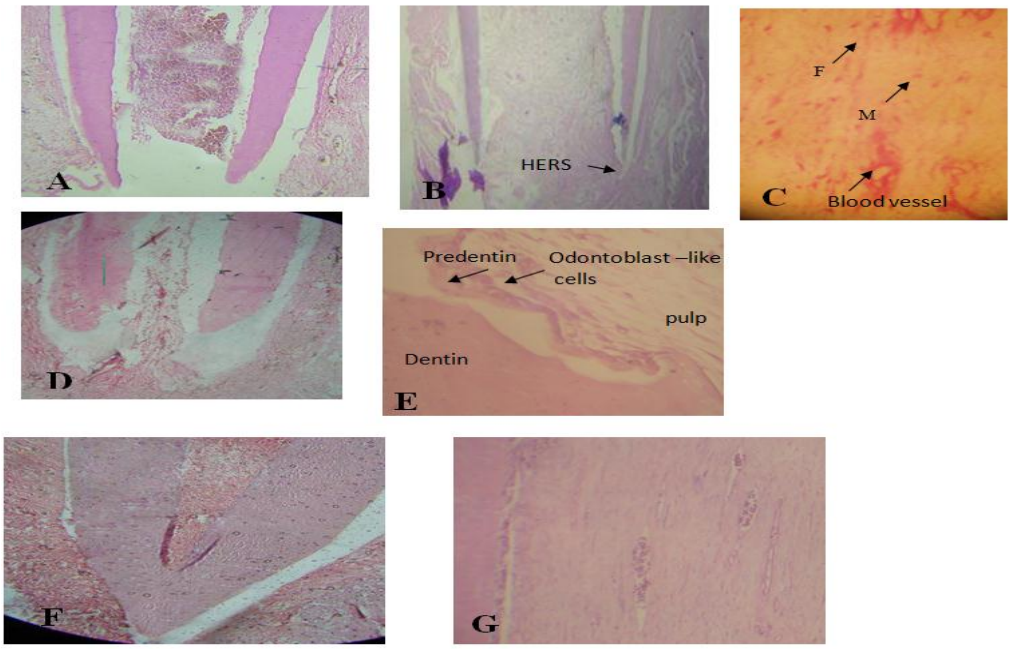

Figure (2): Histopathological assessment of regeneration procedure of immature non-vital teeth of Group (2) (PRP group) at different time intervals - hematoxylin-eosin staining. (A) after 2 weeks, (X100). (B) after 4 weeks, (HERS) Hertwig's epithelial root sheath (X100). (C) after 4 weeks,(X400) (D) after 8 weeks, (X100). (E) after 8 weeks, (X400). (F) after 12 weeks, (G) after 12 weeks, (X400).

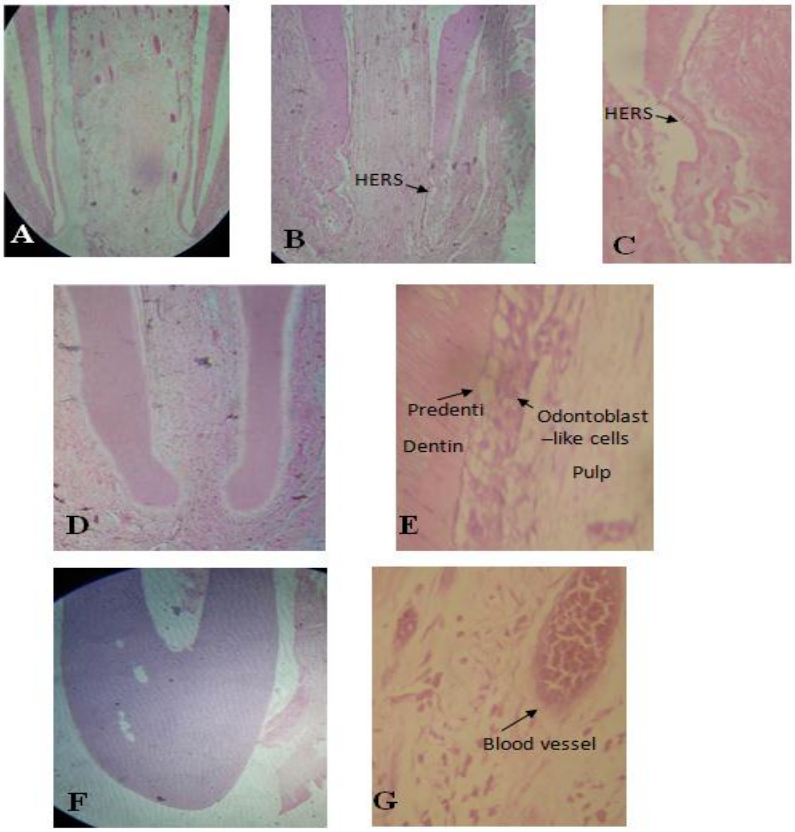

Figure (3): Histopathological assessment of regeneration procedure of immature non-vital teeth of Group (3) (negative control group) at different time intervals - hematoxylin-eosin staining. (A) after 2 weeks (X100) (B) after 4 weeks (HERS) Hertwig's epithelial root sheath (X100). (C) after 4 weeks, (HERS) Hertwig's epithelial root sheath.(X400). (D) after 8 weeks, (X100). (E) after 8 weeks, (X400). (F) after 12 weeks, (X100). (G) after 12 weeks, (X400).

DISCUSSION

Group (1) that's failed to show any vital pulp tissue in the root canal without evidence of continued root development or radicular wall thickening at all time interval. Concerning Group (2), after 2 weeks, deposition of material used were observed in the root canal. After 4 weeks, demonstrated vital tissue extend from periapical area and extend into the canal. This vital tissue considered as pulp- like tissue of a loose connective tissue with few collagen fibers and the majority of cells in the canal space and in the periapical area were spindle-shaped fibroblasts or mesenchymal cells and few blood vessels. No hard tissue was seen in the canal space. Layers of epithelial-like cells, similar to Hertwig's epithelial root sheath, surrounded the root apex. After 8 weeks, the presence of odontoblast-like cells was found to be the most important factor influencing the thickness of the root canal dentin walls and continued root development was attributed to growth of root canal dentine walls. After 12 weeks, there was evidence of increased in the thickness of canal dentin walls with narrowing pulp space. The canal space contained fibrous connective tissue densely infiltrated with fibroblasts and well vascularized. Apical closure was observed in Group (2) and negative control group (Group 3) showed apical closure after 12 weeks.

Potential effects of PRP with the absence of inflammatory cells infiltrates suggested the biocompatibility of the applied material or their number was very minimal. The newly formed blood vessels were observed in dental pulp, indicating that revascularization and remodellation of pulp tissue occurred. Inserting scaffold systems with growth factors in the root canal on immature non-vital teeth may create a system that promotes angiogenesis for the formation of a vascular network that's agreed with Hargreaves *et al*.[10] and Ding *et al*.[11] who mentioned that PRP has been suggested as a scaffold for regenerative endodontic procedures. Tissue has been produced in the canal is a result of the presence of PRP. It has increased concentrations of growth factors that can attract stem cells present in the apical tissues (vital pulp cells, periodontal ligament, apical dental papilla, bone marrow) and even from periapical lesions.

In this study, there were continued growth of the root and canal narrowing, thickening of radicular walls and apical closure may related to stimulation of mesenchymal cells or undifferentiated cells in the pulp – like tissue to differentiate into odontoblast – like cells that's responsible in deposited reparative dentin-like structure on the dentin and forming additional new dentin on existing dentin. These findings disagreed with other animal studies which mentioned that the hard tissue formed in the canal spaces was described as bone-like or cementum-like tissues, and continued root development was attributed to apical cementum deposition after revascularization procedures of immature necrotic teeth with apical periodontitis.[12,13] These findings are consistent with the observations of other investigator.[14] They concluded that these types of tissues are not of pulpal origin and the whole revitalization process is not tissue regeneration but tissue repair.

The cellular source of regenerated tissue and precise mechanisms behind biologic root development still needs to be identified in the future. Whether the regenerated tissue is periodontal ligament tissue which grows into the canal from the apical foramen and lays down cementum onto the inner walls of the root canal or is pulp tissue which has grown from surviving pulp stem cells in the lateral canals is still not clear. According to many researchers, PRP has increased concentrations of growth factors that can attract stem cells present in the apical tissues (ie, vital pulp cells, periodontal ligament, apical dental papilla, and bone marrow)[15] and even from periapical lesions.[16] As has been shown by Lovelace *et al*.[15] the periapical tissue contains a higher concentration of stem cells compared with the blood from the systemic circulation. It is likely that PRP facilitated the migration of the stem cells from the periapical tissues.

Theoretically, the development of normal, sterile pulp-like tissue within the root canal is thought to aid in revascularization and stimulation of undifferentiated mesenchymal cells at the periapex, leading to the deposition of a calcific material at the apex as well as on the lateral dentinal walls of the canal.

CONCLUSIONS

Understanding the biology of dental stem cells and the principle of tissue engineering/ regeneration provides us with a better knowledge base on which the clinical treatment plans can be established. Although further research is needed to verify the role of SCAP in the continued root formation after treatment, the clinical observations of this great healing potential of immature teeth favors the possibility that SCAP in the apical papilla and in some cases perhaps along with DPSCs in the survived dental pulp are important in this healing process. With regard to the regeneration/engineering of pulp/dentin or bioroot engineering using SCAP, the issue of cell source needs to be resolved simultaneously. Banking teeth as an autologous cell source and the potential use of allogenic stem cells both require further research to determine the ultimate benefits to our patients.

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