

Evaluation of the Success Rate of Revascularization Technique Using Leukocyte-Platelet-Rich Fibrin (L-PRF) Concentrate Compared to Blood Clot as a Scaffold

Ahmed Kamel Abd El Nasser^{1*}, Nagwa Mohamed Ali Khattab² and Ehab Saeed Abd Elhamid³

¹Assistant Lecture in Pediatric and Community Dentistry Department, Faculty of Dentistry, Assuit University

²Professor and Head of Pediatric Dentistry and Community Dentistry, Faculty of Dentistry, Minia University

³Professor of Oral Pathology, Faculty of Dentistry, Ain Shams university

***Corresponding Author:** Ahmed Kamel Abd El Nasser, Assistant Lecture in Pediatric and Community Dentistry Department, Faculty of Dentistry, Assuit University.

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Abstract

Introduction: Regenerative endodontic treatment on immature teeth with apical periodontitis is rapidly becoming an accepted treatment alternative to conventional apexification.

Blood Clot (BC) and platelet concentrates [1] have been used as scaffold in regenerative endodontic treatment (RET).

Aim: This study was designed as an experimental study to determine histological findings of the regenerative procedure in immature dogs teeth.

Methods: An Experimental study of A total of sixty incompletely formed roots were divided into III experimental groups study and control groups (negative and positive) according to the treatment protocol in addition to specimen for normal pulp of the dog's teeth.

Results: There was an increase in the mean area fraction of blood vessels and in the mean number of odontoblasts in the study group compared to the other two groups with a statistical significant difference (P value < 0.001), (P = 0.003). Concerning the number of inflammatory cells, there was an increase in the number of inflammatory cells in the negative control group compared to the study and control group.

Conclusions: Blood clot and PRP show comparative results in periapical healing in terms of vascularity and number of odontoblast.

Keywords: Revascularization; Blood Clot; Immature Teeth; Platelet-Rich Fibrin; Scaffolds

Introduction

Pulp necrosis of immature teeth is a major concern because it can stop root development and result in weak, vulnerable teeth [1].

The necrosis may be the result of caries or traumatic injury of the teeth. Apexification using calcium hydroxide and the placement of an artificial barrier are the two most commonly used procedures to treat this problem [2,3].

Treatment of immature permanent teeth with pulp necrosis and apical pathosis constitutes a challenge for endodontists. Teeth with a necrotic pulp are commonly encountered in cases of trauma to the anterior teeth or untreated carious lesions. Such conditions are challenging, not only in root canal debridement and filling, but also for the thin dentinal walls increasing the risk of subsequent fracture [4].

Management of such cases was achieved historically by apexification procedures using calcium hydroxide. Such treatment requires long-term placement of calcium hydroxide inside the root canal to induce formation of an apical hard tissue barrier [5,6].

Regenerative endodontic procedures are biologically based procedures which deals with the regeneration of pulp like tissue, more idealistically the pulp-dentin complex, damaged coronal dentin such as that following a carious exposure or trauma; and regenerate re-sorbed root, cervical or apical dentin [7].

The mechanics behind the revitalization endodontic procedure is that, despite the tooth being necrotic, some pulp tissue can survive apically which under favorable conditions proliferate to aid in the process of regeneration [8,9].

There are three factors that govern the regenerative endodontic treatment regimens stem cells, signaling molecules, and a 3-dimensional physical scaffold without which an empty canal space would not support ingrowth of new tissues from the periapical area [10].

The conventional method of revitalization procedure was done by inducing bleeding into the pulp canal by mechanically irritating the periapical tissues [11].

The increase understanding of the physiological roles of platelets in wound healing led to the idea of using platelets as therapeutic tools. Platelet-Rich Plasma (PRP) consists of a limited volume of plasma enriched with platelets, which is obtained from the patient. The use of PRP as a potentially ideal scaffold for regenerative endodontic therapy has been documented in the literature [12].

PRF was developed in France by Choukroun., *et al.* in 2001. This technique is very simple and inexpensive. PRF contains platelets, growth factors, and cytokines that might enhance the healing potential of both soft and hard tissues [13].

Literature survey reveals that there is a few documentation regarding the application of PRF in the field of regenerative endodontics.

This study was done to Evaluate the success rate of revascularization technique using leukocyte-platelet-rich fibrin (L-PRF) concentrates compared to blood clot as a scaffold clinically and radiographically *in vivo* and Histological *in vitro*.

Subjects and Methods

Animal model

Three purpose-bred mixed breed canine model males dogs aged approximately 6 months were obtained from Department of Veterinary Surgery, Faculty of Veterinary Medicine, Assuit University and was quarantined in separate cages.

A total of sixty incompletely formed roots were divided into III experimental groups study and control groups (negative and positive) according to the treatment protocol in addition to specimen for normal pulp of the dog's teeth.

Grouping

- Group I: (Positive control): Blood Clot (18 roots).
- Group II: (Study group): Leukocyte-platelet-rich fibrin (L PRF) concentrate (18 roots).
- Group III: Negative control (18 roots).

And normal pulp (6 roots) for comparison.

Each experimental group was further subdivided into 3 subgroups (subgroups A, B and C) according to the post treatment evaluation period (6 roots each).

Procedures

Induction of periapical pathosis

Under general anesthesia, supplemented with local anesthesia, endodontic access cavity was done in all experimental and control teeth. The pulp was accessed by a sterile file used to disrupt the pulp tissue in the canals. Supra gingival plaque from dog teeth was mixed with sterile saline then a sterile sponge soaked in the plaque suspension and then inserted into the pulp chamber. Dogs were monitored radiographically under general anesthesia after three weeks to confirm the evidence of development of periapical pathosis.

Dogs were kept under soft diet and analgesics (Cetal syrup) to relief pain during this period.

Disinfection

After the infection period, the previously infected experimental teeth were re-entered after 10 days from the evidence of development of periapical pathosis. under completely aseptic conditions irrigation of 17% EDTA (17% EDTA Prevest Denpro Limited, India) was done then by 1.5% sodium hypochloride then a prepared antibiotic paste were inserted into the canals and access cavity was double sealed with glass ionomer restoration (Cream II PSP Dental.Co.Ltd).

Regeneration

The teeth were re-entered, after one week the antibiotic paste was removed by a sterile K file and canals irrigated with 10 mL of sterile saline per tooth. Then canals were dried with paper point. Nine teeth (18 roots) per group I and II were randomly assigned and treated according to different treatment modalities as mentioned in the sample classification.

Blood clot group: (Positive control)

A sterile hand file (suitable size) was used to induce bleeding into the root canal by passing the file beyond the apex. After blood clot formation the canal was sealed by White MTA which was applied gently over the blood clot then the cavity was sealed by glass ionomer restoration (Cream II PSP Dental.Co.Ltd).

L- PRF group: (Study group)

Sample of the whole blood was drawn intravenously and the centrifuged to obtain the L- PRF in jelly like consistency.

L-PRF was cut into a small pieces by a sterile scalp then grasping it with a forceps and placed in the pulp. The PRF was condensed into the canal using a finger plugger (Dentsply Maillefer Ballaigues) till the level the cemento-enamel junction. MTA (ProRoot MTA, Dentsply, Tulsa, OK, USA) was used following application of L- PRF and the cavity was sealed by glass ionomer restoration.

Negative control group

The 18 roots assigned to group 3 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 glass ionomer restoration.

All of the teeth were monitored after the treatment on a monthly basis for 3 months before the animals were sacrificed at intervals, (3 weeks, 10 weeks and 15 weeks) and tissues were harvested for histologic examination. All animal procedures followed a protocol approved by Department of Veterinary Surgery, Faculty of Veterinary Medicine, Assuit University.

After animal scarification the specimen undergo Histological Processing and Image Analysis and stained with Hematoxylin and Eosin [14] then Sections were cleared in xylol then were placed on glass slides and then viewed and photographed in a digital light microscope

Photomicrographs were captured at a magnification (x200) by a digital camera mounted on research microscope.

Image analysis software was used for analysis. The software was downloaded from (<http://imagej.nih.gov/ij/download.html>) with Java package.

Images were corrected automatically for color and brightness and then transferred into 8-bit monochrome image. Color code threshold was adjusted to calculate the parameter of interest.

Parameters were as follows:

- Surface area, area fraction and volume of blood vessels.
- Inflammatory cell count.
- Number of odontoblasts at the apical one third of the root.

Before calculation, the microscopic field was adjusted for the default width and length of the digital image (W = 366.22 μm and L = 652.22 μm) and the surface area for the microscopic field (238856 μm^2) was unified for each image prior to calculation.

Data was tabulated in Excel Sheets (Microsoft Office14®) and mean values were used for statistical analysis

Results

Table 1 and figure 1 show that there was an increase in the mean area fraction of blood vessels in the study group compared to the other two groups with a statistical significant difference (P value < 0.001).

Parameter	Control group	Negative Control group	Study group	P value*
	Mean \pm SD	Mean \pm SD	Mean \pm SD	
Area fraction	9.39 \pm 5.5	7.81 \pm 1.8	20.4 \pm 1.37	0.001
Odontoblasts	121.9 \pm 54.0	53.88 \pm 7.4	226.2 \pm 133.3	0.003
Inflammatory cells				
Lymphocytes	73.69 \pm 34.5	77.65 \pm 76.5	38.25 \pm 12.1	0.043
Plasma cells	26.54 \pm 14.3	34.8 \pm 32.4	11.75 \pm 3.8	0.004
Histiocytes	11.92 \pm 6.6	15.3 \pm 11.8	5.90 \pm 2.6	0.003
Total	112.15 \pm 53.4	127.7 \pm 119.2	53.90 \pm 17.4	0.018

Table 1: Difference in histologic parameters in the 3 study samples.

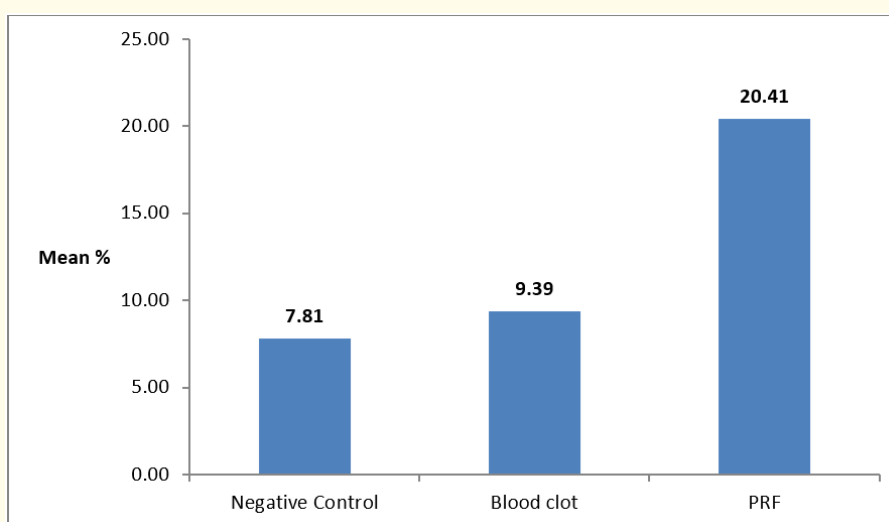


Figure 1: Area fraction of blood vessels percentage in the three study groups.

As for the number of odontoblasts, it was also found that there was an increase in the mean number of odontoblasts in the study group compared with the other 2 groups (Table 1 and Figure 2) with a statistical significant difference ($P = 0.003$).

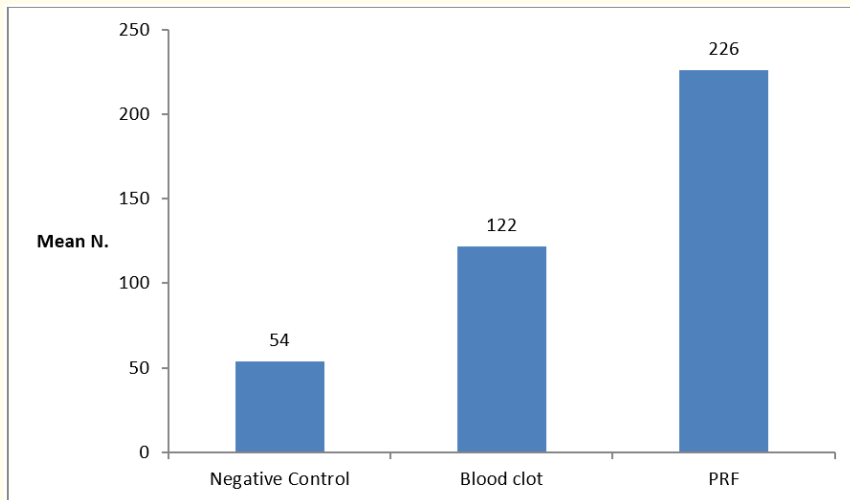


Figure 2: Mean number in odontoblasts in the three study groups.

Concerning the number of inflammatory cells, there was an increase in the number of inflammatory cells in the study group and control group compared to the negative control group. However, the number of inflammatory cells in the study group was less than the number in the control group (Figure 3).

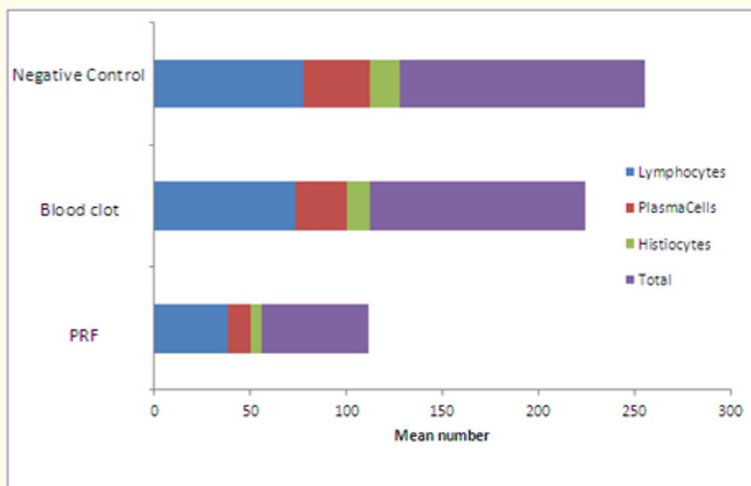


Figure 3: Mean number of inflammatory cells in the three study groups.

All the results showed statistical significant difference in the mean number of the lymphocytes, plasma cells and histiocytes among the three study groups (Table 1).

Discussion

Due to the lack of specimens of human teeth showing revascularization this study is concerned with the underlying histological basis for revascularization.

Dogs samples were divided into many groups based on the treatment protocol. Each group was divided into subgroups based on the evaluation periods; one month, two months and three months. The one month period is equivalent to six months in the human analogue. The two-month and three-month periods were equivalent to twelve and eighteen months respectively in relation to their human analogue.

Histological evaluation included assessment of vascularity and number of odontoblasts inside the pulp space it also included inflammatory tissue reaction.

In our study there was an increase in the mean area fraction in the PRF group compared to the other two groups (negative and BC) with a statistical significant difference (P value < 0.001).

The tissue in the canal space was cell rich and well vascularized. This tissue is similar to cell-rich, well-vascularized connective tissue in the canal space reported by Claus, *et al.* 2004 in autotransplanted immature teeth after removal of the original pulp tissue in beagle dogs at 4 weeks of histologic observation and described by Skoglund and Tronstad, 1981 and also similar to cell-rich, well-vascularized connective tissue [15,16].

Regarding the presence of vascularity inside the pulp after one month evaluation period in both PRF group and BC group, the mean area fraction in the PRF group compared to the other two groups (negative and BC) with a statistical significant difference (P value < 0.001). This could be attributed to that PRF provides ideal scaffold and prolonged source of growth factors and better cell organization and diffusion than blood clot [13].

This was supported by another study which stated that PRF is considered as a fibrin biomaterial. Its molecular structure with low thrombin concentration is an optimal matrix for migration of endothelial cells and fibroblasts. Moreover, it permits a rapid angiogenesis and an easier remodeling of fibrin in a more resistant connective tissue.

As for the amount of odontoblasts, it was also found that there was an increase in the mean number of odontoblasts in the study group compared with the other 2 groups with a statistical significant difference (P = 0.003).

One month after the/regeneration procedure, loose connective tissue filled the canal space up to the coronal MTA plug in both study and control group. A layer of flattened cells similar to root odontoblasts were polarized along the pre-dentin in the canal in both groups. It is not known if these odontoblast-like cells were newly differentiated odontoblasts from the apical papilla or preexisting primary odontoblasts after regeneration procedure.

However histological study done by Lei, *et al.* 2015, on regarding the nature of newly formed tissues after tooth revascularization Ten months after regeneration; the tooth was extracted for orthodontic reasons and processed for histological observation. In the canal space, neurons and nerve fibres were observed histologically and were confirmed by immunohistochemical examination [17].

However PRF group showed significant increase in number of odontoblast-like cells than that of blood clot which may attributed to PRF has a very significant sustained yet slow release of various growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor (TGF) extending up to 28 days [18].

Regarding the results of inflammatory scores, after one month evaluation period, all the experimental groups showed a statistically significant difference compared to negative control. These findings could be attributed to the inflammatory reaction of the peri-radicular

tissues to all of the performed treatment protocols superimposed by the immunological reaction against the previously induced infection [19].

All the results showed statistical significant difference in the mean number of the lymphocytes, plasma cells and histiocytes among the three study groups.

The persistent presence of lymphocyte infiltration indicates the existence of unresolved irritating materials including microbes or foreign bodies. However, adjacent to the heavy infiltrates, new odontoblast-like tissue was able to be laid down by the odontoblast-like cells, suggesting that the presence of those inflammatory cells does not interfere or may even serve as a stimulus of the involved cells to make odontoblast-like tissue.

Wang, *et al.* 2010 observed inflammatory cells which may provide factors to guide the differentiation of stem/progenitor cells in the healing soft tissue into cementoblasts [20].

At two and three months, study and control specimens had mild to moderate inflammation. The inflammatory cell count was significantly higher in negative control group than the other groups. This might be attributed to the empty canal space in which bacterial invasion via anachoresis increased with time and may be also due to continued bacterial irritation recorded the same finding [21].

Since the mean inflammatory cell count was higher in BC group than the PRF group. This might be attributed to the traumatic insult of periapical tissues via over instrumentation to induce bleeding [22].

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