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ASSESSMENT OF THE EFFECTIVENESS OF REVASCULARIZATION METHODS UTILIZING PLATELET-RICH FIBRIN (PRF) CONCENTRATE VERSUS CLOT FORMATION AS A SUPPORTIVE MATRIX

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ABSTRACT

Introduction: Revascularization therapy for young permanent teeth with apical pathology has gained widespread acceptance as an alternative to traditional apexification procedures. In the context of regenerative endodontic treatment (RET), both PRF and clot formation [1] have been used as scaffolds.

Objective: This study aimed to evaluate the microscopic tissue-level outcomes of the revascularization process in young permanent premolar dog teeth in vitro. This experimental study aimed to evaluate histological outcomes of the process in immature dog teeth.

Methods: Sixty roots that were not fully developed were divided into three experimental groups (control and study) and each group divided again into subgroups (positive and negative) based on the treatment protocol. Moreover, samples from the normal pulp of dog teeth were incorporated.

Results: The study group showed a statistically significant increase the percentage of blood vessels and odontoblasts numbers in comparison to the other two groups (P value < 0.001, P=0.003). In terms of inflammatory count, the negative control group showed a higher count in comparison the other two groups

Conclusions: Clot formation and Platelet-Rich Plasma (PRP) demonstrated comparable outcomes in periapical healing, specifically regarding mean area fraction of blood vessels and odontoblasts numbers.

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

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INTRODUCTION

Necrotic pulp in young permanent teeth poses a considerable challenge, potentially hindering the development of the root and resulting in teeth that are vulnerable and weak. [1]. which can be a consequence of caries or traumatic injuries to the teeth. Historically, the predominant methods employed to tackle this issue involve traditional apexification using calcium hydroxide and the placement of an artificial barrier [2, 3].

Managing pulp necrosis and apical pathosis in immature permanent teeth poses a complex task for endodontists, particularly when dealing with trauma or untreated carious lesions in anterior teeth. The thin dentinal walls further compound the challenge by increasing the risk of subsequent fractures [4].

Traditionally, handling such cases entailed the use of apexification procedures with calcium hydroxide, requiring an extended stay within the canal cavity to prompt the formation of an apical hard tissue barrier[5,6]. On the contrary, revascularization procedures emphasize biologically-driven approaches, aiming to regenerate pulp-like tissue, specifically addressing damage to the pulp-dentin complex caused by carious exposure or trauma. Furthermore, these procedures aspire to facilitate the regeneration of root with more cervical and apical dentin [7].

The fundamental concept behind revascularization procedures is that, even in the presence of pulp necrosis, pulp tissue can survive apically can undergo proliferation under favorable conditions, thereby contributing to the regeneration process [8, 9]

The success of regenerative endodontic treatment hinges on three crucial elements: stem cells, growth factors, and a three-dimensional scaffold. Without these components, the growth of cells from the apical area into the root canal would be impeded [10]. Conventional revascularization procedures involve inducing bleeding into the pulp canal through mechanical irritation of periapical tissues [11].

The evolution of our understanding regarding the physiological functions of platelets in the healing process has prompted an exploration of platelets as therapeutic agents. Platelet-Rich Plasma (PRP), which involves enriching plasma with platelets sourced from the patient, is being considered as an ideal scaffold for regenerative endodontic therapy [12]. Platelet-Rich Fibrin (PRF), introduced in 2001 by Choukroun et al. in France, offers a simple and cost-effective technique. PRF contains platelets, growth factors, and cytokines, potentially enhancing the healing capabilities of both soft and hard tissues [13]. Despite its potential, there is limited documentation on the application of PRF in regenerative endodontics.

This study seeks to assess the clinical and radiographic success of revascularization techniques employing leukocyte-platelet-rich fibrin (L-PRF) concentrates compared to blood clots as scaffolds, both histologically in vivo and in vitro

SUBJECT AND METHODS

Three males' purpose-bred dogs, around 6 months old, were sourced from the Anatomy Department of Faculty of Veterinary Medicine. They were individually placed in quarantine cages. Sixty roots, which were incompletely formed, were allocated across three experimental groups (study and control) and a normal pulp group for comparison.

Grouping:

1. Positive Control (Group I): Clot formation (18 roots).
2. Study (Group II): Platelet Concentrate (18 roots).
3. Negative Control (Group III): Untreated (18 roots).

Furthermore, six normal pulp specimens were incorporated into the study.

The division of each group into three subgroups (A, B, and C) was determined by the post-treatment evaluation period, with six roots allocated to each subgroup

Procedures:

Induction of Periapical Pathosis:

- Endodontic access cavities were created under general anesthesia.
- Pulp tissue disruption and induction of periapical pathosis were achieved using a plaque suspension.
- Radiographic monitoring confirmed periapical pathosis development after three weeks.

Disinfection:

- After the infection period, teeth were re-entered under aseptic conditions.
- Irrigation with 17% EDTA and 1.5% sodium hypochlorite was performed.
- Antibiotic paste was inserted, and access cavities were sealed.

Regeneration:

- Antibiotic paste removal and saline irrigation were conducted after one week.
- Different treatment modalities were applied based on group assignments.

a. Blood Clot Group (Positive Control):

- Bleeding induced into the canal, followed by White MTA application.
- Glass ionomer restoration sealed the cavity.

b. L-PRF Group (Study Group):

- Leukocyte-Platelet-Rich Fibrin obtained and placed in the canal.
- MTA application followed by glass ionomer restoration.

c. Negative Control Group:

- Pulp tissue disrupted, supragingival plaque placed in the pulp chamber.
- Temporarily sealed with glass ionomer restoration.

Monitoring and Sacrifice:

- Monthly monitoring for 3 months before sacrificing animals at intervals (3 weeks, 10 weeks, and 15 weeks).
- Tissues were harvested for histologic examination.

Histological Processing and Image Analysis:

- Specimens underwent histological processing, staining with Hematoxylin and Eosin.
- Image analysis software (http://imagej.nih.gov/ij/download.html) was utilized.
- Parameters analyzed included surface area, area fraction, volume of blood vessels, inflammatory cell count, and number of odontoblasts.

Statistical Analysis:

- Data tabulated in Excel Sheets (Microsoft Office 14®), mean values used for statistical analysis.

RESULTS

Statistical Analysis and Comparative Results:

Vascularity (Table 1, Figure 1): The collective data from Table 1 and Figure 1 indicate a marked rise in vascularity percentage within the study group when contrasted with the other two groups, revealing a statistically significant distinction (P value < 0.001).

Odontoblasts Count (Table 1, Figure 2): Analysis of Table 1 and Figure 2 reveals a statistically significant increase in the odontoblasts numbers in the study group compared to the control groups (P = 0.003).

These findings elucidate the dynamic changes over time and underscore the statistical significance in vascularity, odontoblast count, and inflammatory cell assessment across the study, control, and negative control groups.

TABLE (1) Histologic parameters in the study samples

Criterion	Group 1 Control	Group 2 Negative Control	Group 3 Study	P value*
	Mean ± SD	Mean ± SD	Mean ± SD	
Vascularity	9.40 ± 5.5	7.7 ± 1.9	21.5 ± 1.37	0.000
Number of Odontoblasts	120.8± 54.0	55.2 ± 7.3	224.2±133.3	0.003

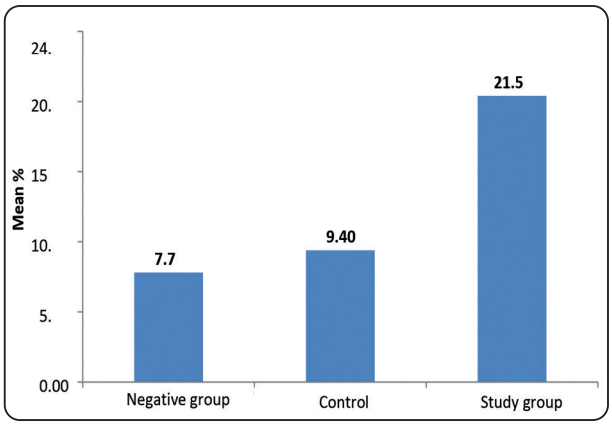

Fig. (1): Vascularity percentage in the study groups.

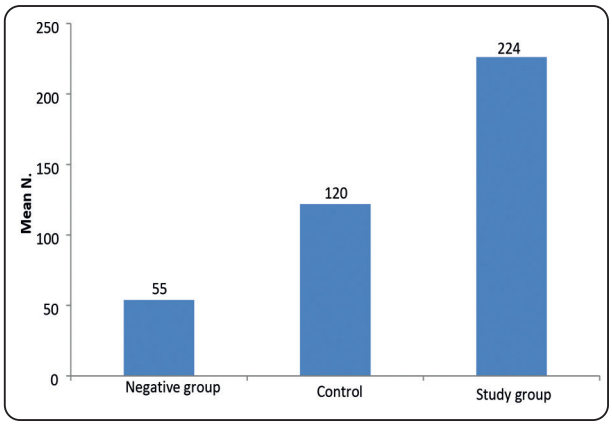

Fig. (2): odontoblasts percentage in the study groups

DISCUSSION

Study Focus and Specimen Selection:

The primary focus of this study is the histological basis for revascularization, given the scarcity of human teeth specimens displaying this phenomenon. Utilizing a canine model, the samples were meticulously categorized based on the treatment protocol, followed by further subdivision into subgroups aligned with distinct evaluation periods.

Equivalency with Human Analogues:

To establish relevance, the evaluation periods in canines were judiciously equated to human analogues: one month in canines corresponded to six months in humans, while two and three months equated to twelve and eighteen months, respectively.

Vascularity and Histological Assessment:

A noteworthy finding was the increased mean area fraction in the LeukocytePlatelet-Rich Fibrin (PRF) group compared to the negative and Blood Clot (BC) groups, indicating a well-vascularized and cell-rich canal space. This aligns with previous studies in autotransplanted immature teeth [15, 16].

PRF as an Optimal Scaffold:

The enhanced vascularity within the PRF group can be attributed to several factors. Firstly, PRF serves as an optimal scaffold, promoting cell adhesion, migration, and proliferation. Secondly, it acts as a sustained reservoir of growth factors, providing a prolonged release that stimulates angiogenesis. Additionally, PRF facilitates superior cell organization, leading to improved diffusion and nutrient transport. These favorable conditions collectively contribute to the observed increase in vascularization within the PRF group. This aligns with existing literature, which recognizes PRF as a fibrin-based biomaterial possessing an optimal molecular architecture for supporting cell migration, angiogenesis, and the remodeling of connective tissues [13].

Odontoblasts and Tissue Regeneration:

The greater odontoblast count in the study group, along with the presence of flattened cells resembling root odontoblasts, poses intriguing questions regarding the source of these cells following the regeneration procedure. A study conducted by Lei et al. in 2015 offers insights into the nature of newly formed tissues after tooth revascularization, notably the observation of neurons and nerve fibers[17].

PRF's Sustained Release of Growth Factors:

The PRF group demonstrated a significant increase in odontoblast-like cells compared to the Blood Clot group. This can be attributed to PRF's sustained yet slow release of various growth factors, including platelet-derived growth factor (PDGF) and transforming growth factor (TGF), which can extend up to 28 days [18].

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