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Autologous Transplantation of Dental Pulp Tissue - a Radiographic Evaluation

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Abstract

The study used decellularized autologous pulp tissue as a scaffold for regeneration of root canals of immature infected teeth. Four dogs' premolar teeth were used. 96 roots of premolars were divided into four groups according to the treatment protocol. Group I: Dental pulp tissue transplantation and blood clot. Group II: Dental pulp tissue transplantation. Group III: Conventional regeneration. Group IV: Negative control group. Each subgroup was radiographically evaluated. Results revealed that after one as well as three months, there was no statistically significant difference between percentage increase in root lengths in the four groups, while in all groups, the percentage increase in root length after three months showed statistically significantly higher value than one month (P-value <0.001). Regarding dentin thickness, after one as well as three months, there was no statistically significant difference between percentage increase in dentin thickness in the four groups, while in all groups, the percentage increase in dentin thickness after three months showed statistically significantly higher value than one month (P-value <0.001). After one as well as three months, there was a statistically significant difference between percentages of apical closure in the four groups. Pair-wise comparisons between groups revealed that Group IV showed the statistically significantly highest percentage of apical closure. In all groups, the percentage of apical closure after three months showed statistically significantly higher value than one month (P-value <0.001). Decellularized pulp tissue can be successfully used as a scaffold in promoting pulp tissue regeneration.

Keywords: Dental Pulp, Decellularization, Regeneration

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1. Introduction

In the recent years, there has been a significant interest in dental pulp regeneration for both immature and mature permanent teeth among dental practitioners [1]. Since damage to the dental pulp at an early age result in the loss of biological functions, this prompted researchers to develop tissue engineering strategies to restore these functions and achieve reliable clinical outcomes [2]. The primary goal of regenerative endodontics is to restore normal pulp function in cases of reversible pulpitis and to regenerate irreversibly inflamed or necrotic pulps [3]. Currently, pulp revascularization is the only known regenerative endodontic procedure widely practiced, as clinicians continue to seek alternatives to devitalizing traditional root canal treatments [4]. Advancements in understanding and diagnostic methods have advanced, yet current approaches to dental pulp regeneration do not fully replicate natural development of dentin-pulp complex [5]. Regenerating neural tissue is notably more complex than mature connective tissue and neo-vascularization, with inflammatory response being a critical factor [6]. Modulating inflammation to promote an anti-inflammatory environment, notably through induction of M2 macrophages, is essential for successful regeneration [7]. Thus, employing tissue engineering processes worked well in field of root canal regeneration and vascularization [8]. One of triads of regeneration scaffold-guided cell arrangement, is a critical factor in regeneration and tissue maturation [9].

Several attempts made towards scaffold fabrication using either natural or synthetic materials. Natural scaffolds had the drawback of low mechanical strength and disorderly degradation of natural materials [10]. Yet synthetic polymers provided more improved mechanical properties and more predictable outcomes but with possibility of provoking unwanted inflammation and undesirable tissue formation

[11]. Decellularized tissue embodies current state-of-the-art in scaffold production with natural histoarchitecture and mechanical properties, similar to the native tissue [12]. The use of extracellular matrix scaffolds by tissue decellularization provided more promising outcomes in regeneration [13]. These types of matrices proved to be biologically superior in terms of preferred cellular activities, architectural composition, mechanical stability, preserved natural microenvironment, as well as minimal immune response. These scaffolds help in vascular tissue formation and the enhancement of inflammation modulation [14]. The idea of tissue transfer has been long tested, with ancient beliefs in organ replacement. Although the process used to be difficult and was faced with challenges, current advances led to remarkable advancement today. The aim of this study was to evaluate the efficacy of auto transplanted decellularized dental pulp tissue in regeneration of immature infected teeth.

2. Materials and Methods

2.1 Animal Study

The study carried out in the Faculty of Veterinary Medicine, Cairo University. A total of four healthy male mongrel dogs aged approximately four to six months old with permanent dentition were selected for this study. While dogs were quarantined, they were examined both clinically for diseases or injury, and radio graphically to observe the root condition (Figure 1). Diseased animals were excluded from the study. The study was conducted according to the ethical committee protocol of the Faculty of Dentistry, Ain-Shams University, Egypt. (FDASU – RecID 041909). The five freedoms of animals under human care respected: freedom from hunger and thirst, freedom from discomfort, freedom to express normal behavior, and freedom from fear and distress [15]. The 3Rs guiding use of animals in research also respected: Replacement, Reduction, and Refinement [16].

2.2 Sample Classification

Sample size was calculated using G*Power version 3.1.9.2. Premolar teeth in both jaws were used in the study. The total number of roots used was 96 roots of 56 teeth. Roots were divided equally into four experimental groups as follows: Group 1: Pulp transplantation + Blood Clot (n=24), Group 2: Pulp transplantation (n=24), Group 3: Conventional regenerative endodontic procedure (Positive control) (n=24), Group 4: Negative Control (n=24). (Figure 2).

2.3 Sample Preparation

General anesthesia was administrated after fasting the dogs for 12 hours, but water was allowed and libitum [17]. Dogs were pre medicated using 0.05 mg/kg weight subcutaneous atropine sulphate and 1mg/kg weight intramuscular Xylazine-HClbk. Anesthesia was induced using intravenous 5 mg/kg by weight Ketamine HClcl. Anesthesia was maintained using 25 mg/kg by weight 2-5 % intravenous Thiopental sodium [18] (Figure 3). The work environment was kept sterile, and teeth were disinfected by 0.5% povidone iodine solution.

2.4 Induction of Periapical Infection

Access cavities were prepared in all experimental teeth using sterile #6 round carbide burs and low speed hand piece. Pulp tissues were extirpated and were transferred to the laboratory within one hour in phosphate buffered saline solution (PBS). Each pulp tissue was placed in a micro tube with PBS corresponding to the experimental tooth and was transferred in an insulated bag with cold ice packs. Access cavities left exposed for three weeks. During this period, dogs given a soft diet and Xylaject analgesic to relief pain [17].

2.5 Root Canal disinfection

Dogs were re-anesthetized, and experimental teeth were treated under completely aseptic conditions under rubber dam application. Minimal instrumentation was done with file #35 to disrupt the present biofilm and remove bacteria on the root dentin surface [19]. Canals were irrigated using 20 ml of 1.5 % NaOCl for 5 minutes and then irrigated with 20 ml of 0.9% saline for 5 minutes, with irrigating needle positioned about 1mm away from the root, followed by drying with paper points. Calcium hydroxide was placed inside the canals and the access cavity sealed using a cotton palette topped with a 3-4 mm glass ionomer restoration [20].

2.6 Treatment Protocol

Three weeks later, calcium hydroxide paste was removed from the root canals and radiographs were taken to assure periapical pathosis were healed. Decellularized dental pulp tissues were brought back from laboratory and were allowed to thaw in a refrigerator at the workspace. Treatment initiated according to the proposed protocol as follows:

Group I: A #35 k manual k hand file was inserted 2 mm beyond the root apex. The decellularized pulp tissue was placed inside the root canal space 2mm away from the working length by using sterile hand pluggers and tissue forceps. Time was given for blood clot to form, and an MTA coronal plug was placed. A composite restoration was used for coronal seal.

Group II: Using sterile hand pluggers and tissue forceps, the pulp tissue was carefully pushed 2mm short of the apical terminus. MTA was placed as a coronal plug, and a composite restoration placed on top for coronal seal.

Group III: Conventional Revascularization was done according the *AAE Guidelines*. Over instrumentation was done using a #35 k endodontic manual hand Once the blood reached the level of the cemento-enamel junction, 15 minutes were /given for the blood clot to form and then MTA was placed coronally as a capping material. MTA powder and liquid were mixed in a ratio of 3:1 according to the manufacturer's instructions. A cotton pallet was placed on top of the MTA to absorb any excess moisture. A periapical radiograph was taken to assure the location of the MTA coronal plug. A Composite restoration was then placed on top of the MTA after complete setting.

Group IV: Teeth in this group were left untouched as they served to compare and assess the normal physiological development of teeth.

2.7 Pulp Tissue Decellularization Protocol

The decellularization process followed a published protocol in which the pulp tissue was rinsed with PBS to remove any debris. A decellularization solution was prepared by combining PBS with detergents including Triton X-100 (1%) and Sodium dodecyl sulfate (SDS) (1%) [21]. Pulp tissues were fully immersed in the solution and the container was placed in a shaker and incubated overnight at room temperature (20-25 °C). Afterwards, the decellularization solution was removed from container, and the pulp tissue was

rinsed with PBS to remove residual detergents. A solution of DNase (100U/mL) prepared, and tissues were immersed in it. The container was placed back on shaker and incubated at room temperature (20-25 °C) for four more hours. Pulp tissue removed and rinsed several times with PBS to remove any residual DNase. Pulp tissues then added to fresh sterile containers with PBS and protease inhibitors for a final rinse to inhibit any remaining protease activity. Decellularized pulp tissue scaffolds finally suspended in 1 ml. of PBS and stored in a sterile container at -80 °C until further use.

2.8 Methods of Evaluation

Radiographic images were taken after treatment according to the study groups to follow up in one- and three-months duration periods. Turbo Reg plug-in was used to transform non-standardized pre-operative and post-operative radiographs into standardized images. Three fixed points identified on all pre-operative and post-operative radiographic images. These points were cusp tips, cemento-enamel junction, and the mesial marginal bone. Samples evaluated for statistical analysis for following parameters [22] by 2 examiners blinded to experimental groups:

1. Increase in root length

$$\text{Percentage of increase in length} = \frac{\text{Postoperative length - Preoperative length}}{\text{Preoperative Length}} \times 100$$

2. Increase in dentin thickness

$$\text{Dentin thickness} = \text{root thickness - pulp width}$$

$$\text{Percentage of increase in thickness} = \frac{\text{Postoperative thickness - Preoperative thickness}}{\text{Preoperative thickness}} \times 100$$

3. Decrease in apical diameter

$$\text{Percentage of apical closure} = \frac{\text{Postoperative apical diameter - Preoperative apical diameter}}{\text{Preoperative apical diameter}} \times 100$$

2.9 Statistical Analysis

Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). All data showed non-normal (non-parametric) distribution. Data were presented as median, range, mean and standard deviation (SD) values. Kruskal-Wallis test used to compare between the groups. Dunn's test was used for pair-wise comparisons when Kruskal-Wallis test is significant. Wilcoxon signed-rank test was used for comparisons within each group. Qualitative data were presented as frequencies and percentages. Chi-square test and Fisher's Exact were used for comparisons related to qualitative data. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

3. Results and discussions

Data were calculated, tabulated, and statistically analyzed.

3.1 Percentage Increase in root length

Comparison between groups revealed that after one as well as three months, there was no statistically significant difference between percentage increase in root lengths in the four groups (P-value = 0.642, Effect size = 0.005) and (P-value = 0.497, Effect size = 0.027), respectively. While comparisons within each group revealed that in all groups, the percentage increase in root length after three months showed statistically significantly higher value than one month (P-value <0.001, Effect size = 3.334), (P-value <0.001, Effect size = 3.616), (P-value <0.001, Effect size = 3.078) and (P-value <0.001, Effect size = 3.081), respectively (Figure 4).

3.2 Percentage increase in root dentin thickness

Comparison between groups showed that after one as well as three months, there was no statistically significant difference between percentage increase in dentin thickness in the four groups (P-value = 0.431, Effect size = 0.012) and (P-value = 0.416, Effect size = 0.027), respectively. Comparisons within each group showed that in all groups, the percentage increase in dentin thickness after three months showed statistically significantly higher value than one month (P-value <0.001, Effect size = 3.325), (P-value <0.001, Effect size = 2.193), (P-value <0.001, Effect size = 3.078) and (P-value <0.001, Effect size = 3.002), respectively (Figure 5).

3.3. Percentage of apical closure

Comparison between groups revealed that after one as well as three months, there was a statistically significant difference between percentage of apical closure in the four groups (P-value = 0.001, Effect size = 0.178) and (P-value = 0.015, Effect size = 0.120), respectively. Pair-wise comparisons between groups revealed that Group IV (43.3 ± 11.7) showed the statistically significantly highest percentage of apical closure. There was no statistically significant difference between Groups I and II, both showed statistically significantly lower percentages of apical closure. Group III showed the statistically significantly lowest percentage of apical closure. Comparisons within each group revealed that in all groups, the percentage of apical closure after three months showed statistically significantly higher value than one month (P-value <0.001, Effect size = 2.863), (P-value <0.001, Effect size = 3.337), (P-value <0.001, Effect size = 3.328) and (P-value <0.001, Effect size = 3.331), respectively (Figure 6). The concept of dental pulp regeneration in immature permanent teeth has raised a massive interest among dental practitioners over last years. Researchers are on a continuous quest to come up with new tissue engineering strategies to restore dental pulp's biological function and come up with more predictable outcomes [23].

Pulp tissue as a natural scaffold principle was initiated by using autologous deciduous tooth pulp transplant, which is a vital 3D scaffold, as well as a rich source of stem cells and growth factors in the root canals of necrotic young permanent teeth. The use of decellularized scaffolds in regenerative medicine proved to promote vascularization and offer a productive environment for stem cell differentiation. The introduction of decellularized extracellular matrix (dECM) scaffolds provided a successful alternative to the long-standing use of synthetic scaffolds, as they help imitate the optimal non-immune environment with 3D structures and different bioactive components [24]. Decellularization is the process of eliminating cells and their components such as DNA and RNA from the extracellular matrix (ECM) to provide a natural matrix while preserving its mechanical structure [25]. Dental pulp tissue extracellular matrix is a loose connective tissue matrix made up of collagen fibers and adhesive proteins. The extracellular matrix is believed to have dual benefits.

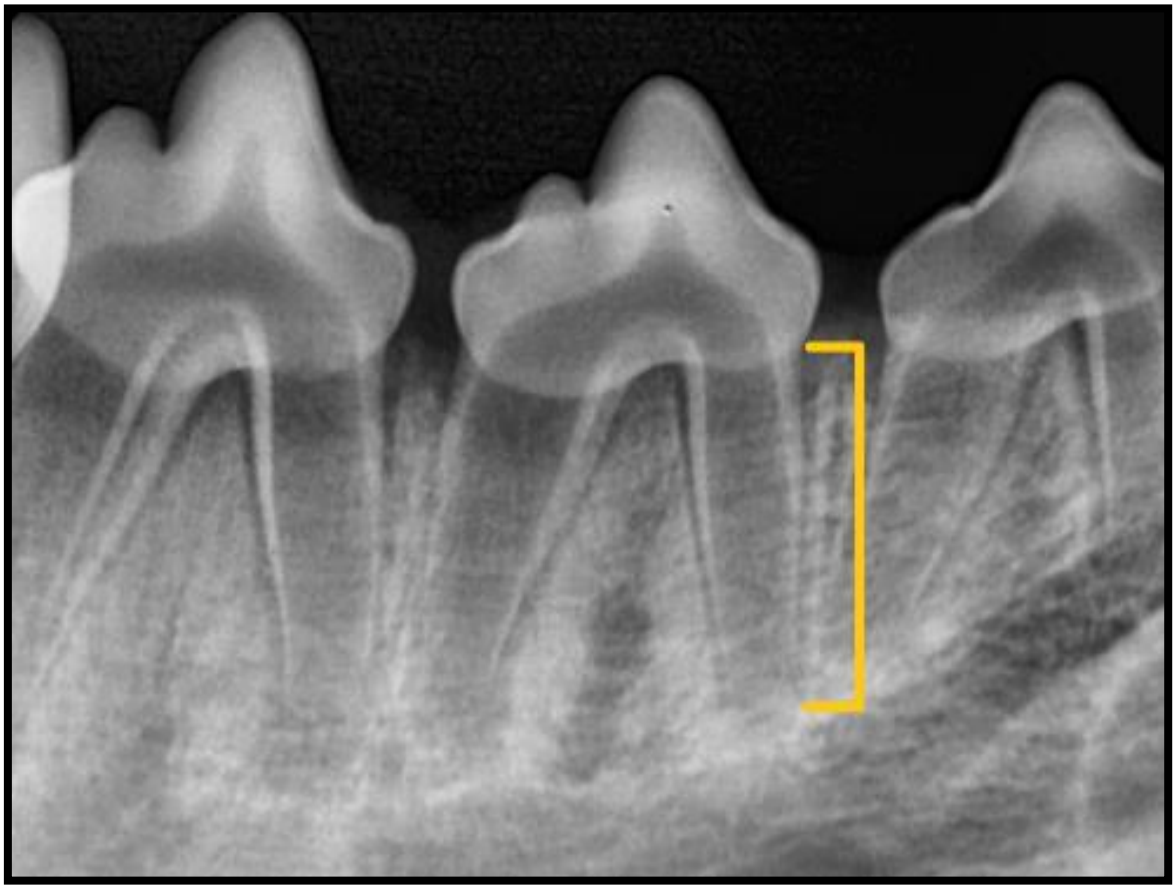

Figure 1: Radiographic image showing the preoperative root length estimation of dogs' teeth

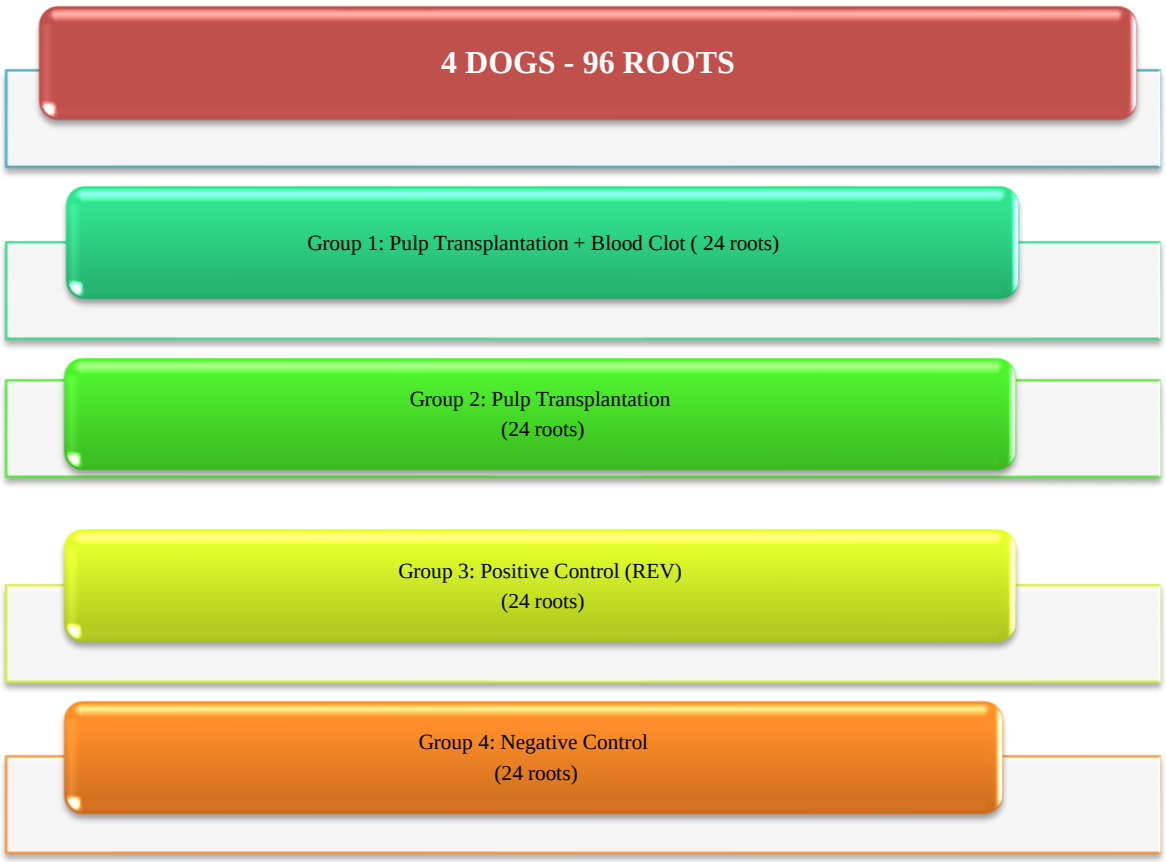

Figure 2: Schematic Diagram for Sample Classification

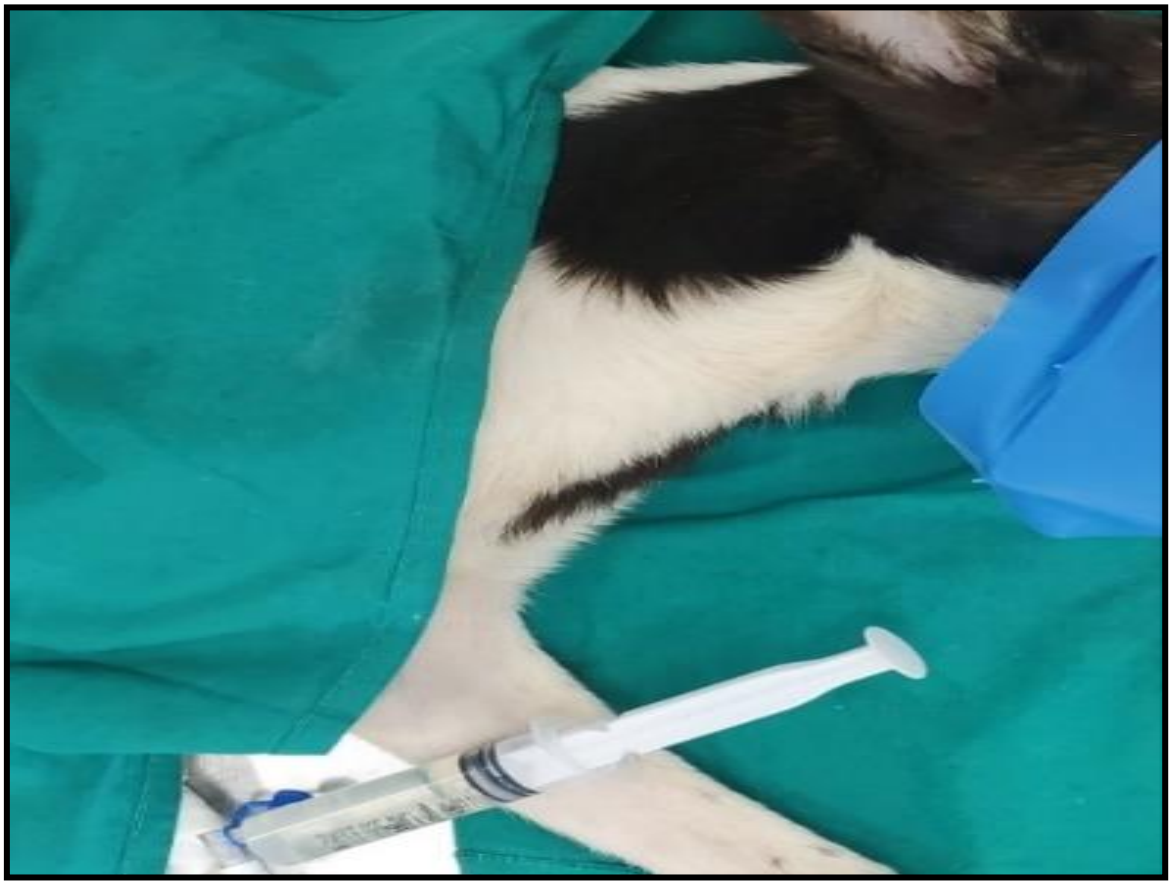

Figure 3: Photograph showing administration of General Anesthesia

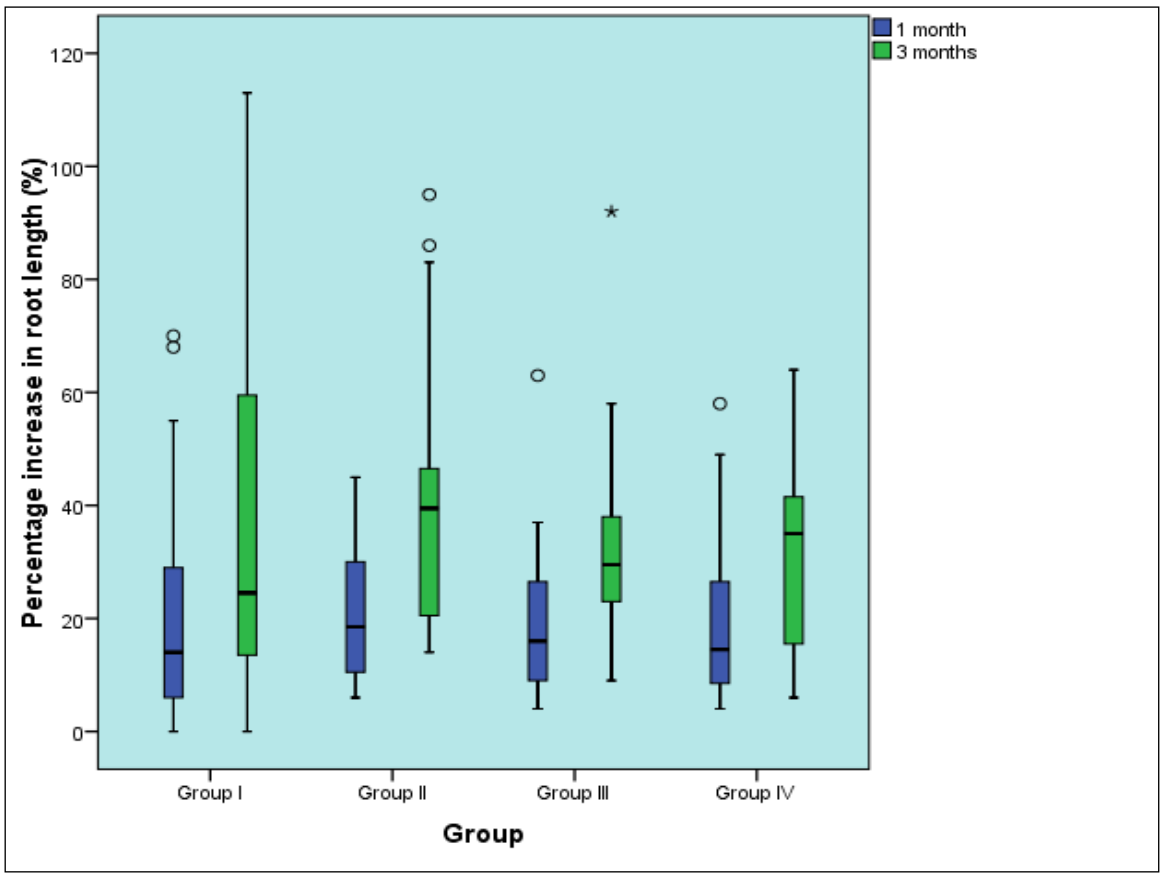

Figure 4: Box plot representing median and range values for percentage increase in root length in the four groups (Circles and star represent outliers)

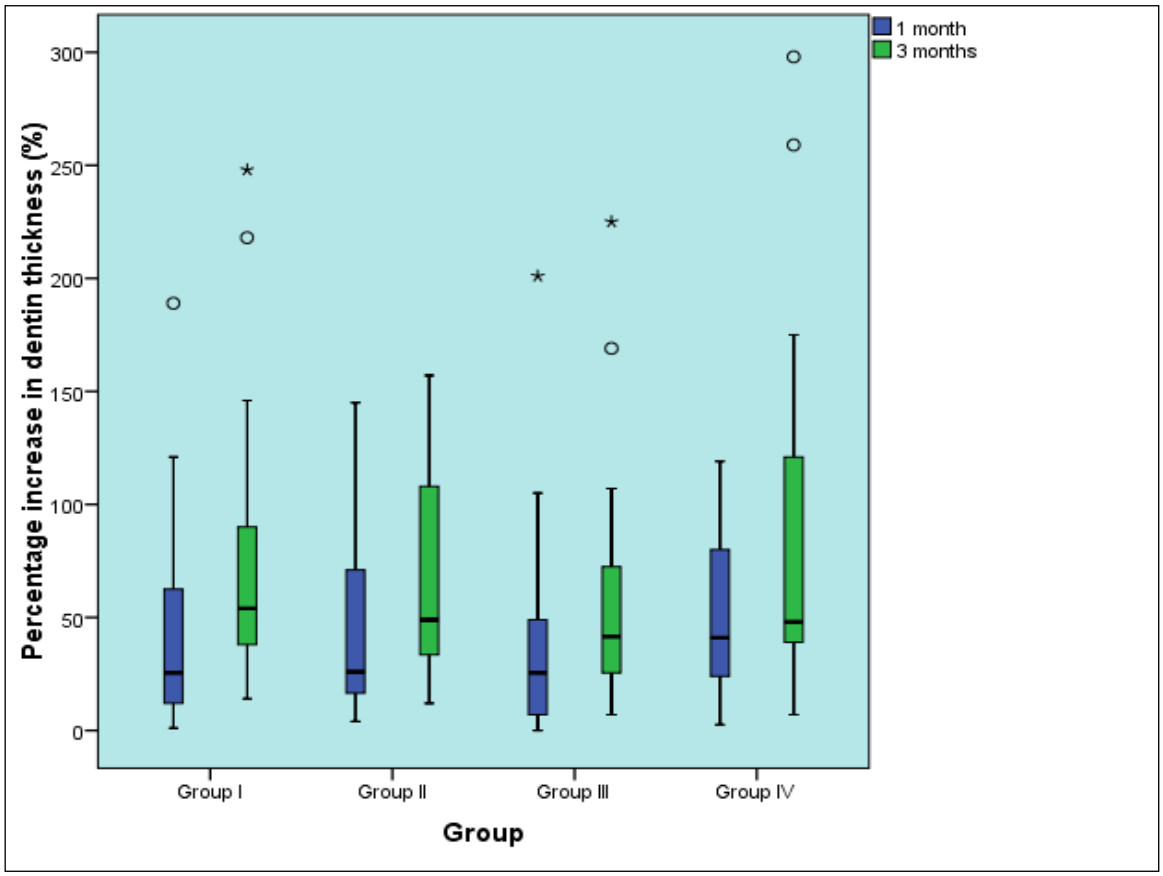

Figure 5: Box plot representing median and range values for percentage increase in dentin thickness in the four groups (Circles and stars represent outliers)

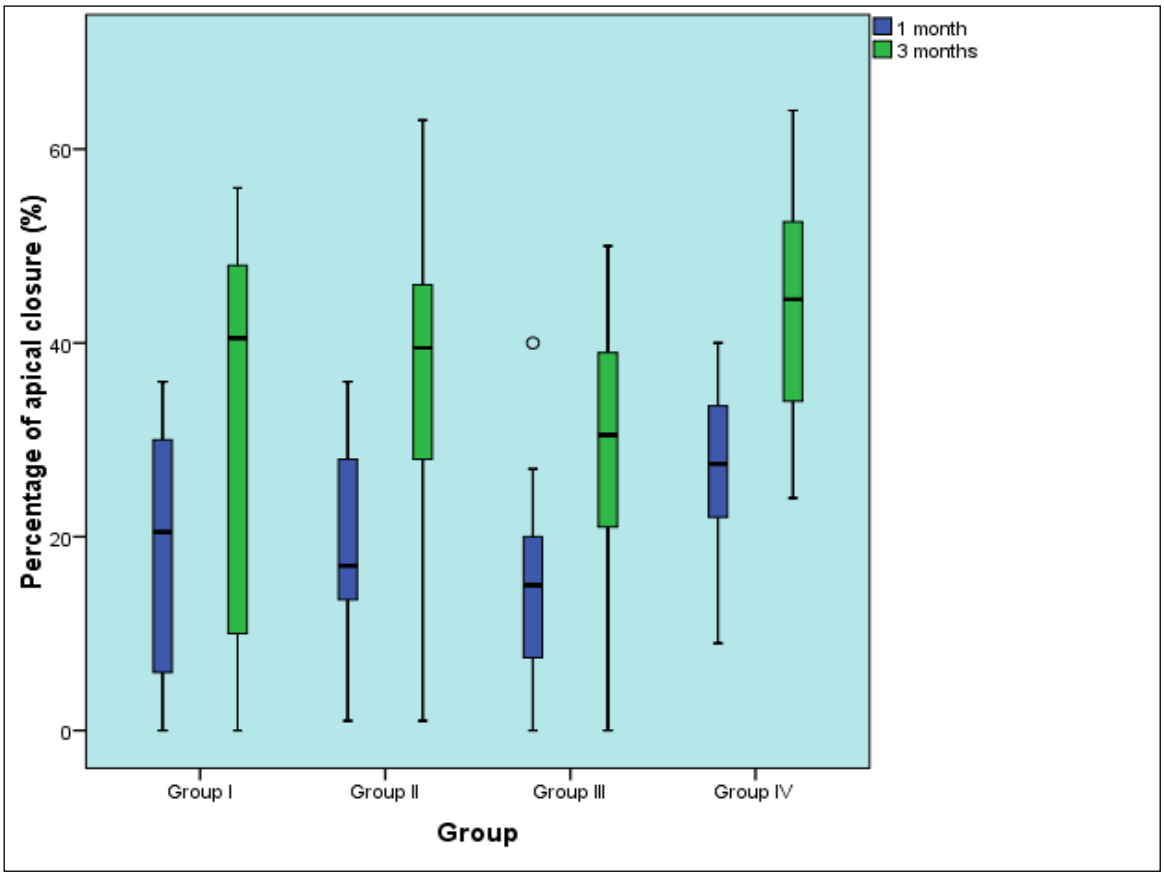

Figure 6: Box plot representing median and range values for percentage of apical closure in the four groups (Circle represents outlier)

First, it would trigger periapical progenitor/stem cell trafficking; and second, modulate inflammation through triggering an anti-inflammatory host response that supports pulp regeneration [26]. In the current study, four male dogs were chosen for experimentation since dogs considered more physiologically like humans in their pattern of growth and comparable apical repair to humans but with a faster growth rate [27]. The selected age ranged from 4-6 months, which is the suitable age for studying permanent with incompletely formed mature teeth roots. This age as well is appropriate so that the dogs can withstand GA procedure at different visits and different interventions [22]. Double rooted premolar teeth were selected as they are easily accessible for endodontic procedures, wider occlusal table for lower chance of teeth fracture during follow up periods, as well as average sized canals for endodontic manipulation and pulp tissue extirpation. A high number of samples were employed in the study to achieve reliable statistical analysis [28]. The root canal disinfection phase is a major requirement for the success or failure of REP [29]. Several studies demonstrated that irrigating solutions and intra-canal medicaments are not fully capable of eliminating bacteria in biofilms in infected root canals during root canal treatment. Hence, minimal mechanical debridement was done in this study as a part of disinfection to disrupt the biofilms on infected canal walls.

Disinfection carried out in accordance with guidelines which recommend use of 1.5% NaOCl followed by 17% EDTA solution [30]; these concentrations have proved to be less cytotoxic on stem cells from apical papilla (SCAP), which is crucial for regenerative process [31]. Final irrigation with 20 mL normal sterile saline after EDTA promoted SCAP proliferation without proposing any change in stem cell differentiation [32]. Calcium hydroxide was used as an intra-canal medicament according to the *AAE protocol for regeneration* [33]. Exposure of SCAP to calcium hydroxide is thought to help induce their osteogenic differentiation, proliferation, increases cell survival, and acts against all endodontic pathogens. Alkaline pH of calcium hydroxide facilitates dissolution of organic tissues and bacterial products such as endotoxins [34]. MTA Angelus used in this study as it has excellent sealing capacity, prevents bacterial leakage, biocompatible, and exceptional marginal adaptation, and overcame drawbacks of regular MTA regarding high setting time due to high calcium sulfate concentration and difficult manipulation. MTA angelus has low setting time and easy handling properties [35]. In this study, the decellularization protocol adapted was done by Hsieh et al. [36], which proved to be effective in complete decellularization of the dental pulp tissue.

In our study, the most common detergents for decellularization were used, which are the nonionic detergent Triton X-100 and the ionic detergent SDS. Triton X-100 helps remove the tissue cellular component by disrupting lipid-lipid and protein-lipid interactions without affecting protein-protein interactions. While SDS disrupts protein-protein interactions and solubilizes cell membranes, SDS is proven to effectively remove nuclear materials faster than other different chemical treatments and can effectively remove 90% of tissue cellular content [37]. Radiographic images standardized using Image J (Fiji) software and Turbo Reg plug-in. This method helped in standardization of data for accurate quantitative evaluation for treatment outcomes [38]; root length and root thickness and reduction in apical diameter. All tested teeth revealed changes in radiographic appearance regarding length, thickness and apical closure. Regarding radiographic increase in root length and thickness, there was no statistically significant difference between four groups, yet in each group, there was a significant difference between root lengths in both one as well as three months evaluation periods. This discrepancy may be due to short evaluation period, the difference in preoperative lengths of roots in different dogs' teeth due to their different sizes and ages, and lack of preoperative length standardization.

This could be also due to the difference between operator interpretations of data using the computer software to measure root lengths. Results were in agreement with Panda et al. [39] who stated that the lack of significant difference may be due to the intentional induction of bleeding from the periapical region and blood clot formation, which supports angiogenesis, providing a pathway for stem cells migration from periapical area, inducing pulp regeneration and root maturation. Remnants of pulp tissue and Epithelial Root Sheath of Hertwig may render in infected teeth with open apices, and after canal disinfection, inflammation stops and tissues can thrive. The diameter of apex is another important factor, as open apices help in mesenchymal stem cell migration which allow tissue formation in root canal space [40]. Meanwhile, results of apical closure showed statistically significant difference between the four groups. Several studies revealed that regenerative endodontic treatment of immature permanent teeth with necrotic pulps can help in root maturation [41-44], yet these studies have also demonstrated that root maturation is not predictable [45-46]. Difference in reporting can be owed to different factors, according to Almutairi et al. [47] and Lee et al. [48], who stated that no standardized technique was followed; maybe due to inefficient disinfection protocols, lack of healing process knowledge, and finally the type of scaffold used.

4. Conclusion

In the highlight of the current study, it can be concluded that regeneration using decellularized autologous dental pulp tissue scaffold was a successful treatment option alternative to blood clot. Decellularized dental pulp tissue scaffold can be also used successfully with blood clot. The regeneration protocol used was successful in promoting root dentin thickness, increasing root length, and apical closure. Natural scaffolds can successfully replace artificial scaffolds in regenerative endodontic procedures.

Conflict of interest: The Authors declare that there is no conflict of interest.

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