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Radiographic Outcome of Regenerative Endodontic Treatment Using a TCP Scaffold

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ABSTRACT

The purpose of this study is to assess the radiographic outcome of the novel TCP scaffold OSTEON III Collagen in regenerative endodontic procedures. Apical periodontitis was induced in the immature dogs' teeth, followed by a revascularisation procedure. The disinfection protocol used 1.5% sodium hypochlorite irrigant, and a double antibiotic paste was applied for 15 days. In the second visit, the scaffold under investigation was placed, and a coronal seal of mineral trioxide aggregate and glass ionomer restoration was placed. Follow-up radiographs and immediate postoperative radiographs were compared. Results showed that regenerative endodontic procedure is an effective treatment option for immature teeth with apical periodontitis. Based on this study, the following recommendations are made to help with the regenerative endodontic technique: (1) Longer follow-up studies are needed in the field of pulp regeneration, (2) an Optimum disinfection protocol is a key factor for the success of the regenerative endodontic process. (3) Preserving the coronal seal throughout interappointment time and after completion of treatment.

Keywords: *Immature, scaffold, hydroxyapatite, mineral trioxide aggregate, regeneration*

1. INTRODUCTION

Over the years, endodontic treatment of immature necrotic teeth has presented a significant challenge due to the thinness of dentin and the fragility of root walls, which can impact long-term tooth survival.[1] Regenerative endodontic procedures (REPS) offer innovative, biologically based clinical treatments to revitalize necrotic teeth, stimulating continuous root development and preserving their structural integrity. Revascularization of the necrotic dental pulp is the most applied treatment form of regenerative endodontic procedures (REPS). Advances in tissue engineering research focused upon thre. [2]

Scaffolds can be identified as biocompatible structures that support cell growth and provide a suitable environment for tissue formation. Good scaffolds should allow cell attachment, proliferation, migration, differentiation, and provide mechanical support for the extracellular matrix generation[3]. Ideally, scaffolds must be biodegradable as a native tissue and should degrade in a controlled manner, which is consistent with the formation of the new tissue. Among the other properties of scaffolds are conductivity, suitable porosity, sterilizability, and economic cost.

Blood clot is one of the natural scaffolds that revealed evidence of success for pulp tissue revascularisation in immature teeth, as it yields autologous growth factors, in addition to host compatibility, cost effectiveness, and clinical simplicity. However, some shortcomings occurred, including instability, inconsistent outcomes, bleeding challenges, and insufficient mechanical strength.[4] Osteon III collagen scaffold is one of the recently introduced scaffolds in the market. It is mainly a bone void filler composed of synthetic bone graft (OSTEON™ III) and natural Type I Collagen, which is moldable to various defect shapes after being wet. The manufacturer claims that highly interconnected macro and micro-pores allow new bone formation and collagen absorption within several weeks after application.[5] Therefore, this study aimed to investigate the outcome of regenerative procedures using commercially available Osteon III collagen scaffold radiographically. A null hypothesis of no significant difference in outcomes between the conventional blood clot scaffold and osteon III collagen scaffold was postulated in this study.

2. MATERIALS AND METHODS

Six immature dogs with a total of 48 teeth were chosen for regenerative treatment and met the inclusion criteria. The animals were of both sexes, and their weight and age ranged between 10 and 15 kg (mean 12.5 ± 0.5) and 6–9 months (mean 7.5 ± 0.5), respectively. The Research Ethics Committee, Faculty of Dentistry, Ain Shams University, reviewed these criteria and assigned approval FDASU-Rec/072005. The dogs were randomly allocated to one of the two groups: a control group and an experimental group (24 participants per group) following a simple randomization procedure.

The control group was subdivided into two subgroups: positive control, pulpectomy without any further intervention; and negative control**,** no intervention, normal pulp development. The Experimental group was subdivided according to the scaffold material applied: the Scaffold subgroup, in which root canals were filled with Osteon III Collagen scaffold after blood clot induction during revascularization, and the blood clot subgroup, in which root canals were filled with blood clot scaffold only after the conventional revascularization procedure.

The dogs were premedicated by using 0.05 mg/kg of atropine sulphate.[1] injected subcutaneously and 1mg/kg by weight Xylazine HCl[2] injected intramuscularly. The anaesthesia was induced by using Ketamine HCl[3] injected intravenously after using an intravenous cannula in the cephalic vein at a dose of 5 mg/kg by weight. The anaesthesia was maintained by using Thiopental sodium[4] , at a dose of 25 mg/kg by weight, 2-5% injected intravenously (dose to effect)[6].

In the first visit, before intervention, a preoperative digital x-ray was obtained using custom bite registrations and radiograph paralleling technique to confirm the presence of an immature apex and the lack of preexisting pathosis[6]. A full pulpectomy procedure was performed on all subgroups, except the negative control[7], as follows: Endodontic access was achieved by removing 2–3 mm from the incisal edge of each experimental tooth to expose the pulp with a sterile #4 fissure bur in a high-speed handpiece; the orifice was then enlarged with a tapered diamond bur. Pulp extirpation was done using broaches (size 40). The teeth were left exposed for 15 days to develop apical periodontitis[6,8]. The animals were given analgesics postoperatively and were monitored in the postoperative period.

In the second visit, after administration of 2% lidocaine without epinephrine[5] and teeth isolation, working length was determined using the Root ZX-II apex locator [6]. Gentle irrigation with 20 ml 1.5% Sodium Hypochlorite using a side-vented needle was done. The needle was placed 3mm shorter than the working length. The canals were dried, and a double antibiotic paste was packed using a plugger. Finally, dry cotton was placed, and the access cavity was sealed with a temporary restoration. In the positive control group, teeth underwent pulpectomy without any other intervention, and the access cavities were sealed using white WellRoot MTA, followed by composite resin restoration.

During the third visit (15 days after the initial visit), irrigation with 17% EDTA was applied[9]. Blood was induced inside the root canals by over-instrumentation using a K file size 30. Furthermore, 0.025 g of Osteon III collagen moldable scaffold was packed inside the root canals of the scaffold subgroup. A layer of 3-4 mm of white MTA was placed 3 mm below the CEJ in all subgroups, except the negative control group, and then covered with composite as a final restoration.

Regarding follow-up, animals were sacrificed with an intravenous overdose of sodium pentobarbital [10]at six different time intervals: 1 month, 2 months, and 3 months. All animals were euthanized and perfused with 10% buffered formalin. After

[1] Atropine Sulphate: ADWIA Co., Egypt
[2] Xylaject: ADWIA Co., Egypt
[3] Ketamine HCl EIMC pharmaceuticals Co., Egypt.
[4] Thiopental sodium: EIPICO, Egypt
[5] Dentsply International, York, PA
[6] J. Morita MFG corporation, Fushimi-ku, Kyoto, Japan.

perfusion, block sections of the maxillary and mandibular jaws were removed and placed into 10% buffered formalin for 2 weeks. The block sections were then decalcified in 10% EDTA. Radiographic outcome was assessed using successive digital radiographs taken with EZ sensor Vatech. [7]. Immediate postoperative radiographs were compared with follow-up radiographs taken according to each group, at 1 month, 2 months and 3 months to monitor root development at different time intervals[11,12]. Digital image files were converted to 32-bit TIFF files using Image-J analysis software[8]. TurboReg plug-in [9] was used to transform immediate postoperative and follow-up radiographs into standardized images.[11,13]

The following criteria were assessed:

A. **Percentage increase in length of root:** Root lengths were measured as a straight line from the cement–enamel junction to the radiographic apex of the tooth in millimetres[14]. Percentage increase in root length was calculated according to Nagy et al[15] as follows:

([follow-up root length – immediate postoperative root length]/ immediate postoperative root length) x100

B. **Percentage increase in root thickness:** The level of the apical third was determined and fixed relative to the cement–enamel junction. Dentine thickness was measured at three levels in the apical one-third, and the mean was calculated. The percentage increase in root thickness was calculated as follows: [15] ([follow-up dentin thickness - immediate postoperative dentin thickness]/ immediate postoperative dentin thickness) x 100

C. **Percentage decrease in the apical diameter:** The diameter of the apical foramen was measured in millimeters[14]. The percentage decrease in apical diameter was calculated as follows: [15]

([follow-up apical diameter – immediate postoperative apical diameter]/ preoperative apical diameter) x 100

Statistical analysis for comparison between groups was performed by applying the Kruskal-Wallis test, followed by the Mann-Whitney test for multiple comparisons between different groups and time intervals. A P-value ≤ 0.05 was considered statistically significant (95% significance level), and a P-value ≤ 0.001 was considered highly statistically significant (99% significance level). The Shapiro-Wilk test was used to test the normality of the data. Data was analyzed using the statistical software SPSS (version 25, IBM Co., USA).

3. Results

All experimental groups demonstrated a progressive increase in root length over the observation period (Table 1). Statistical analysis revealed no significant differences between 1- and 2-month measurements in any group (P>0.05), nor between 2 and 3 months in the Negative Control, Positive Control, and Collagen Scaffold groups. However, significant differences were observed between 1- and 3-month measurements in all groups, as well as between 2 and 3 months in the Blood Clot group specifically. While in comparative analysis between groups, the highest percentage was recorded in the negative Control group across all time intervals. In contrast, the Collagen Scaffold group showed the lowest values after 1 month, while the Blood Clot group exhibited the smallest increases after both 2 and 3 months.

Table 1: Mean ± SD, intra- and inter-group comparison of percentage root length increase (%) for the four studied groups at the three time intervals.

	1 Month	2 Months	3 Months	P-value*
Negative Control	15.87±7.62^{Ab}	22.36±6.55^{Aab}	31.92±6.61^{Aa}	**0.003^{NS}**
Positive Control	11.67±3.66^{Ab}	18.92±11.66^{ABab}	25.21±12.26^{ABa}	**0.026^{NS}**
Collagen Scaffold	8.18±5.87^{Bb}	11.1±5.7^{Cb}	18.02±9.4^{Ba}	**0.002^{NS}**
Blood Clot	7.94±4.56^{Bb}	14.59±9.8^{BCab}	20.35±10.06^{Ba}	**0.016^{S}**
P-value**	**0.009^{S}**	**0.043^{S}**	**0.018^{S}**	

-* *Overall P-value for Intra-group comparison between the three time intervals (Kruskal-Wallis test).*

-** *Overall P-value for Inter-group comparison between the four groups studied (Kruskal-Wallis test).*

- *Small letters for pairwise comparison between different time intervals, while the capital letters are used for pairwise comparison between the different groups (Mann-Whitney test), and there is no significant difference between means that*

[7] Vatech Europe, Praha, Czechia.
[8] Image-J v1.44, US National Institutes of Health, Bethesda, MD, USA.
[9] Biomedical Imaging Group, Swiss Federal Institute of Technology, Lausanne, Switzerland,

shared at least one letter at a significant level of 0.05.

- S= Statistically significant at P ≤ 0.05 *- NS= Non-significant P > 0.05.*

- HS Highly significant at P ≤ 0.001

Regarding the percentage increase in root thickness among different time intervals, the four groups showed the lowest percentage after 1 month and the highest mean after 3 months of treatment. However, there was no significant difference between 1 and 2 months in all groups (P-value > 0.05). A substantial difference was observed between 3 months and the other time intervals. While in the Intergroup comparison of percentage of root thickness increase, the Negative Control group achieved the highest growth in root canal thickness at the three time intervals ; however, the Blood Clot group achieved the lowest one (Table 2).

Table 2: Mean ±SD, Intra, and Inter group comparison of percentage of root thickness increase (%) for the four studied groups at the three time intervals.

	1 Month	2 Months	3 Months	P-value*
Negative Control	13.98±7.48^{Ab}	16.33±8.73^{Ab}	27.37±14.96^{Aa}	**0.013^{S}**
Positive Control	10.71±4^{ABb}	12.57±4.28^{ABb}	24.77±22.47^{Aa}	**0.003^{S}**
Collagen Scaffold	7.85±2.34^{Bb}	7.01±4.84^{Bb}	12.82±3.28^{Ba}	**0.001^{S}**
Blood Clot	8.44±1.97^{Bb}	10.15±3.77^{Bb}	18.53±6.74^{ABa}	**< 0.001^{HS}**
P-value**	**0.024^{S}**	**< 0.001^{HS}**	**0.039^{S}**	

-* *Overall P-value for Intra-group comparison between the three time intervals (Kruskal-Wallis test).*

-** *Overall P-value for Inter-group comparison between the four groups studied (Kruskal-Wallis test).*

- Small letters for pairwise comparison between different time intervals, while the capital letters are used for pairwise comparison between the different groups (Mann-Whitney test), and there is no significant difference between means that shared at least one letter at a significant level of 0.05.

- S= Statistically significant at P ≤ 0.05 *- NS= Non-significant P > 0.05.*

- HS Highly significant at P ≤ 0.001

Regarding the percentage change in apical foramen diameter, the lowest percentage was achieved after 1 month and the highest mean was achieved after 3 months of treatment for all groups. Also, according to the Tukey Mann-Whitney test for pairwise comparison between the different time intervals, there was no significant difference between 1 and 2 months in all groups (P-value > 0.05). However, there was a significant difference between 3 months and the other time intervals. While Inter-group comparison revealed that the Negative Control group achieved the highest decrease in apical diameter at the three time intervals, the Blood Clot group achieved the lowest one.

Table (3): Mean ±SD, Intra, and Inter group comparison of percentage of decrease in apical foramen diameter (%) for the four studied groups at the three time intervals.

	1 Month	2 Months	3 Months	P-value*
Negative Control	16.55±6.17^{Ab}	20.08±5.43^{Ab}	35.33±7.61^{Aa}	**0.013^{S}**
Positive Control	12.42±10.79^{Ab}	16.17±11.28^{Ab}	25.92±7.27^{Ba}	**0.003^{S}**
Collagen Scaffold	7.67±3.82^{Bb}	9.04±4.79^{Bb}	15.57±3.37^{Ca}	**0.001^{S}**
Blood Clot	9.24±4.84^{ABb}	11.89±4.33^{ABb}	19.52±11.89^{BCa}	**< 0.001^{HS}**
P-value**	**0.024^{S}**	**< 0.001^{HS}**	**0.039^{S}**	

-* *Overall P-value for Intra-group comparison between the three time intervals (Kruskal-Wallis test).*

-** *Overall P-value for Inter-group comparison between the four groups studied (Kruskal-Wallis test).*

- *Small letters for pairwise comparison between different time intervals, while the capital letters are used for pairwise comparison between the different groups (Mann-Whitney test), and there is no significant difference between means that shared at least one letter at a significant level of 0.05.*

- *S= Statistically significant at P ≤ 0.05* - *NS= Non-significant P > 0.05.*

- *HS Highly significant at P ≤ 0.001*

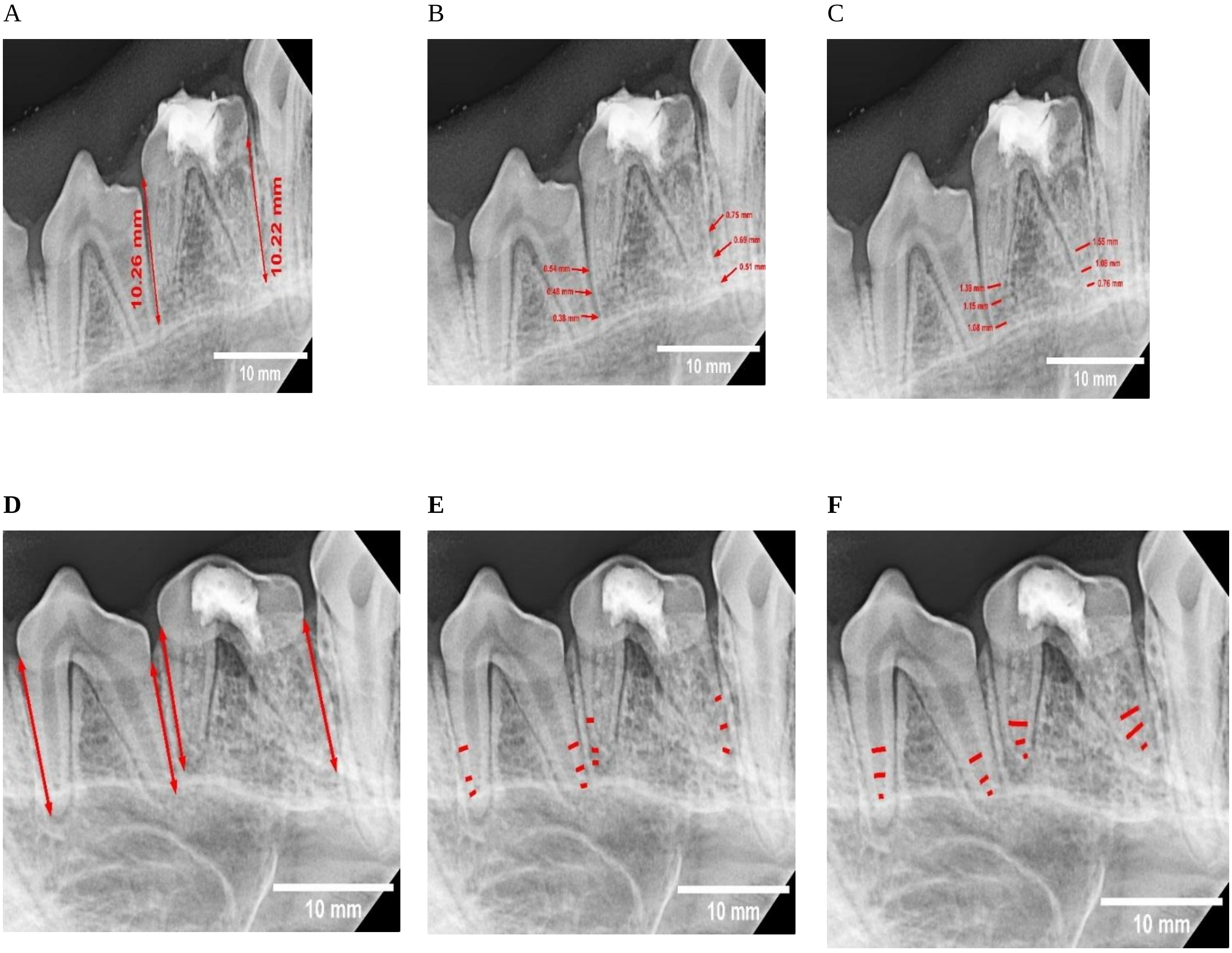

Figure 1. (A-C) Immediate postoperative radiographs for dog 1 showing blood clot and negative control subgroups measurements; A) Root length, B) Dentine thickness, C) Apical diameter (D-F) 1-month follow-up radiographs for dog 1 showing blood clot and negative control subgroups measurements; D) Root length, E) Dentine thickness, F) Apical diameter

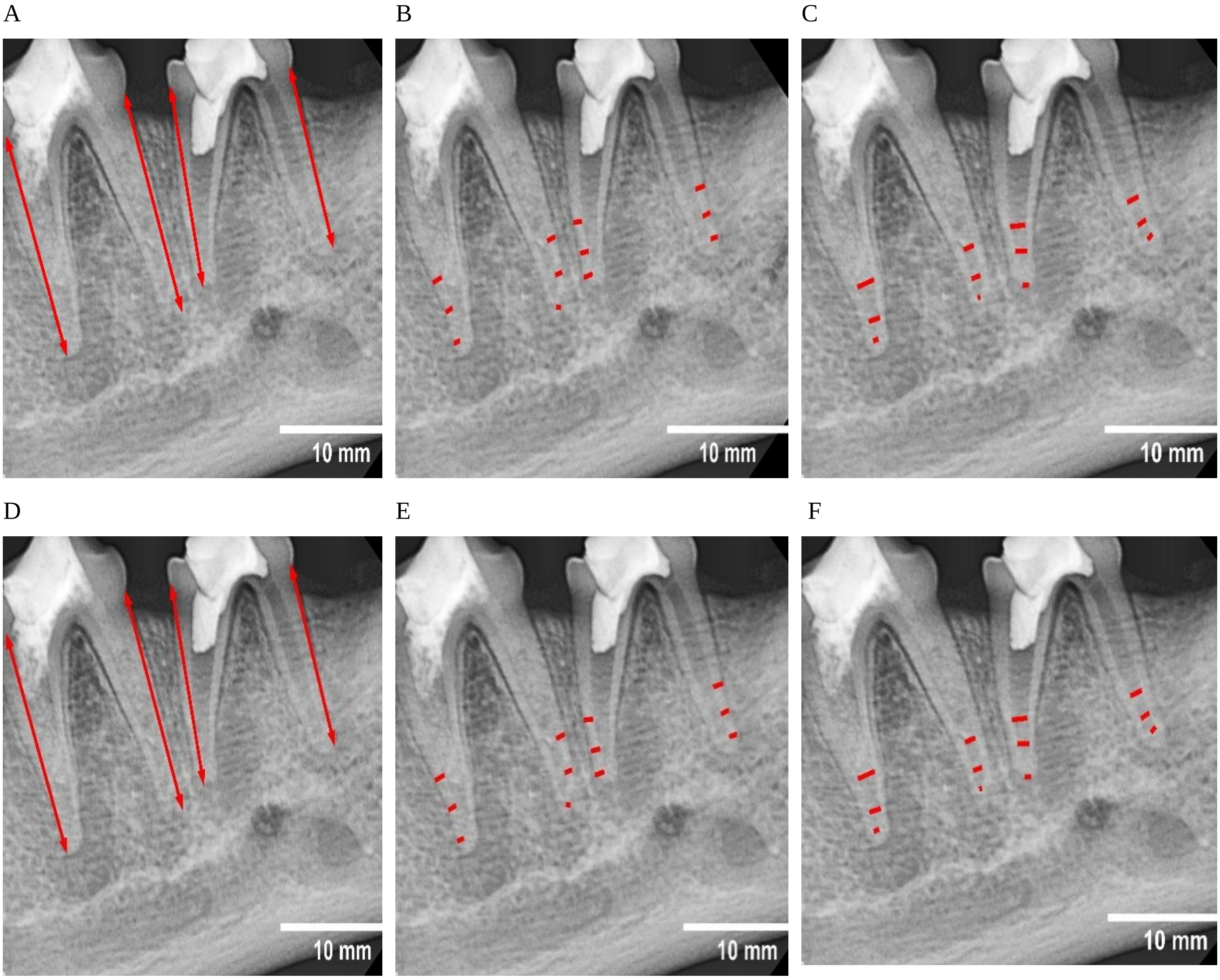

Figure 2. (A-C) Immediate postoperative radiographs for dog 2 showing measurements for blood clot and scaffold subgroups; A) Root length, B) Dentine thickness, C) Apical diameter (D-F) 2-month follow-up radiographs for dog 1 showing measurements for blood clot and scaffold subgroups; D) Root length, E) Dentine thickness, F) Apical diameter

3. DISCUSSIONS

Tissue engineering is a promising treatment modality that allows biological substitutes to replace or regenerate human cells and tissue and restore their normal function[16]. There are three main factors for tissue engineering: stem cells, scaffolds and growth factors. The use of bioactive inorganic scaffolds has been reported in many previous studies with clinically satisfactory results. In a study reported by **Sonoyama et al**, [17] a root/periodontal complex could be generated by transplanting human SCAP and PDLSCS to an HA/TCP scaffold, resulting in normal tooth function. In another study, bone and dentin-like mineralized tissues were generated when DPSCS were mixed with HA or HA/TCP and transplanted subcutaneously in nude mice [21]. OSTEON™II (HA: β-TCP 30:70), OSTEON™III (HA: β-TCP 20:80), OSTEON™II Collagen, and OSTEON™III Collagen are examples of HA/β-TCP scaffolds.[18] The collagenated synthetic bone graft Osteon III collagen showed its potential for guided bone regeneration in many previous studies.[18–20] The study aimed to evaluate the radiographic outcomes of immature teeth with necrotic pulps using revascularisation protocols that were either simple or enhanced with an external scaffold. The choice of dogs as an animal model for biological experiments in endodontics is based on the rapid apical repair compared with humans over short durations due to the high growth rate[21]. Two rooted premolars were selected, increasing the total number of samples for a reliable statistical analysis. Premolars are considered accessible for endodontic procedures and have average-sized canals for endodontic manipulation. The age ranged between 6 and 9 months, which was suitable for the study of immature teeth, as premolar teeth are immature at this age range and the animal can withstand general anaesthesia. In the present study, the revascularisation procedure was done following American Association of Endodontics guidelines[22], and the double antibiotic paste has been demonstrated to eradicate bacteria from infected root canals within 2 weeks. [23] Although longer placement of DAP can increase the amount of DAP adsorbed to dentin and improve the residual antibacterial effect of dentin after its removal, another study has shown that longer exposure times result in significantly greater demineralization of dentin, which causes negative effects on mechanical properties. [24] Wellroot MTA was the material of choice as a coronal plug due to its bioactivity. Bioactivity includes ion release, alkalization of the surroundings, and apatite formation that furnishes a favourable media for the mineralizing cells to form new dentin and bone. Mineralized tissue deposition depends on ph and Ca ion release. [25–27] Moreover, MTA upregulates osteo/odontogenic markers such as Osteocalcin (*OCN*), Alkaline phosphatase (*ALP*), Bone sialoprotein (*BSP*), Osteopontin (*OPN*), Collagen I (*COL*1), Runt-related transcription factor 2 (*RUNX2)*, Osterix (*OSX)*, and *DSPP*. [28] In the current study, the highest percentage increase in root length and thickness, along with the highest percentage decrease in apical diameter, was recorded in the negative Control group across all time intervals. In contrast, the Blood Clot group exhibited the smallest increases after both 2 and 3 months and the lowest decrease in apical diameter. Most of the studies showed that Regenerative Endodontic Treatment (RET) had the potential to promote thickening of the canal walls and/or continued root development of immature permanent teeth with necrotic pulp. However, these observations are not always predictable.[29],[30] The apparent increase in the dentin thickness and root length in our results was in agreement with many authors who reported an increase in length and thickness in the blood clot group in comparison to the scaffold groups [31,32]. There was no significant difference between the blood clot and scaffold group, which is comparable to results published by Thibodeau et al.[33] who reported the importance of the scaffold in tissue growth and hard tissue deposition. Our results disagree with **those of Matsui et al.**[34] and **Zhu et al.**[35], who reported that the low resorption rate of HA/TCP adversely affected the quality of the regenerated tissues [36]. However, a study reported by **Nosrat et al**, [80] using the same scaffold material, correlated failure of RET to scaffold placement without being intermingled with blood, highlighting the fact that blood is crucial in RET as it delivers stem cells, immune cells, growth factors, and signalling molecules inside the root canal. Regarding the effect of the length of the follow-up period on results, all groups showed an increase in root length and thickness and a decrease in apical diameter in a 3-month interval compared to 1- and 2-month intervals, with a statistically significant difference. Our results disagree with Nagy et al[15], who mentioned that only a significant increase in root length occurred at the 18-month follow-up. However, long-term follow-up studies in the literature that are concerned with reporting the outcome of pulp regeneration are scarce. Most of the reported results are based on short-term observations[37,38]. A long-term assessment is needed.

4. CONCLUSIONS

Under the circumstances of this study, it can be concluded that Regenerative endodontic procedures are an effective treatment modality for necrotic immature permanent teeth. Designing and fabricating external scaffolds may aid in the success of regenerative treatment, but long-term follow-up studies are needed to confirm the true added value of such scaffolds.

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