Histomorphological Evaluation of Teeth After Three Months Follow up of Pulp-Cell Homing Procedure in Teeth with Incomplete Apex Formation in Dogs

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ABSTRACT

Aims: To evaluate the tissues formed by the pulp stem cell homing technique through the histopathological sections, after a three months follow-up period. Materials and Methods: The study was performed on twelve right second premolars of twelve local breed female dogs, that were randomly selected form different areas of Mosul City. The study was divided into four stages, stage I (Induce infection), stage II (Disinfection), stage III (Treatment), and stage IV (3 months follow-up). In the stage IV the tooth and the surrounding bone segment was sectioned and routinely processed to produce H&E stained tissue sections that were evaluated under the light microscope. The histological examiners were evaluated presence or absence of 1st normal pulp-like tissue, 2nd newly hard tissue (bone,cementum, dentin), 3rd empty/necrosis, 4th intra-canal inflammation, 5th periapical inflammation, 6th apical narrowing and wall thickening. Results: The results have shown that 85.71% of specimens had normal pulp-like tissue, 100% had newly hard tissue structure, 14.28% had an empty canal, 57.14% had intra-canal inflammation, 71.42% had periapical inflammation, and 85.71% had apical narrowing. Conclusion: The pulp stem cell homing procedure showed an effective way to treat an open apex tooth and this procedure was increased the root length and thickness and also filled the pulp space with pulp-like tissue. The procedure did not regenerate the tooth structure, but formed a tissue like dental structure (dentin, bone and cementum) and these tissues did not for regeneration, but for tissue repair.

Key words: Stem Cell Homing, Immature Apex Tooth, Open Apex Tooth, Dogs

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INTRODUCTION

One of the most critical cases that will make endodontic and pediatric dentists confused about the best treatment plan are those cases with periapical periodontitis and an open apex tooth.\(^1(1,2,3)\) Pulp necrosis can be managed by root canal treatment in teeth with a mature apex; however, if the formation of root is not completed, a combination of dentin thin walls and an open apex, make the accomplishment of root canal treatment challenging.\(^4\) Pulpless teeth with immature apexes are also more susceptible to injury, losing the capability to sense environmental alteration (loss of sensation) and are more disposed to root fracture.\(^5\)

There are different treatment strategies to treat apical periodontitis with immature apexes by apexification, which requires to induce a calcified barrier in an open apex root.\(^6\) As a conventional treatment modality either by the use of calcium hydroxide paste, or mineral trioxide aggregate (MTA) also can be used as an apical barrier to achieve the apical closure.\(^7\) Although the high success rate of apexification which has been reported to be between 74% and 100%,\(^7\) it may result in abnormal morphology of the immature formed root (calcified tissue formation inside the root canal). Also calcium hydroxide placing for a long-term may weaken the dentin and may induce root fracture.\(^7\) The second biological strategy, is to replace the space of root canal with vital tissue, this has recently attracted great attention under the term of “Regenerative Endodontics”\(^2\)

Two strategies can be used for dental pulp regeneration: either cell transplantation or cell homing. The cell transplantation approach is cell-based, which means collecting of exogenous stem cells from the same host which are called (autologous) or from other individuals called (allogenic) and they are may, either by separating them from tissue or by growing them on a culture to increase the number of these stem cells, then transplant to the root canal system, being carried onto a suitable scaffold and incorporated with suitable signaling molecules.\(^3\) However, cell-based therapy faces many obstacles in clinical translation because the intricate procedures need to be followed, such as extraction of tooth (frequently third molar), pulp extirpation, in-vitro cell culture, stem/progenitor cell population selection, cell expansion in ex-vivo, high-cost related with storage (cell cryopreservation), and shipping.\(^8\) Also, difficulties in obtaining regulatory approval (WHO approval), there are other concerns including potential contamination, development of tumorigenesis during ex-vivo cell manipulation, and risk of immune-rejection.\(^8\)

The other strategy for dental pulp regeneration (termed cell homing or revitalization technique) is defined as attraction and recruitment of endogenous
cells, can include stem/progenitor cells, into a region to be regarded or into an anatomic compartment.\(^9\)

The connotation of cell homing is to obtain tissue repair/regeneration employing chemotaxis of endogenous host cells to injured tissue by biological signaling molecules. The cell homing strategies might be easier to perform clinically, as compared with stem cell transplantation, this ease way is because of no need to isolate, manipulate and transplant the stem cells in vitro and then in vivo.\(^{10}\)

Techniques for revitalization in endodontics do not depend on stem cell population expansion that will transplant inside the root canal, but actually on the use of recruitment factors or “mobilization” factors, which include chemotactic agents, growth factors, and other signaling factors. These factors use to “home” the cells from the periapical area or “apical vasculature” to the site of injury, inside the root canal system. Stem cell (SC) homing is defined as the recruitment of endogenous SCs from bone marrow and other niches by signaling “mobilization” factors to the site of injury to induce repair\(^{11}\).

The aim of this study is to evaluate the tissues formed by SC homing technique through the histopathological sections after completion of the procedure.

**MATERIALS AND METHODS**

The study was approved by Research Ethics Committee board (University of Mosul, College of Dentistry, REC reference No. D.B.S./4/2432019-3)

Approximately 5 months aged local breed female dogs (n=12) were used in this experiment after vaccination (BIOCANDHPPI + L, LYOPHILISATE, England). The animals had been cared, and kept in separate standardized cages with the good entrance of sunlight and good air ventilation and have received soft food (well cooked meat). The lower right permanent second premolar was appointed in this study, a radiograph was taken to ensure the state of the apex (incomplete apex formation). According to Kao et al. (2000) and Chieruzzi et al.(2016), the anesthesia protocol used, atropine (0.04mg/kg) intramuscularly (IM), then a mixture of xylazine (1mg/kg) with ketamine (0.1mg/kg), with the same syringe, was performed intramuscularly (IM). A maintenance (with the same mixture) was performed every 30 minutes\(^{12,13}\).

This experiment was divided into four steps, step I (Induce infection), step II (Disinfection), step III(Treatment), and step IV (three-months follow up) . After 3 month follow up the teeth with their surrounding tissue were removed as biopsies and prepared into histopathological slides to be evaluated under a light microscope (Olympus, Japan).

This study started on 19 August 2019, and completed on 9 June 2020 in the animal house of the College of Dentistry in Mosul University.
The step I (induced infection) began by covering the dog with surgical towels allowing the mouth to be unwrapped. Utilizing a 1/4 round bur with a high-speed hand-piece, an access cavity was performed, a cotton pellet soaked with a supra-gingival plaque was inserted into the pulp chamber, the access cavity was closed with a glass ionomer filling material as a temporary filling. The teeth were maintained for nearly (2-4) weeks to ensure existence of periapical radiolucency by means of a periapical radiograph performed every 1week. Figure (1,A)

Step II (disinfection) after 2-4 weeks from induce infection. The target tooth was isolated with a rubber dam, also the tooth surface was disinfectant with chlorhexidine 2 %, and then with Povidone-iodine 3 % until drying. The temporary restoration was removed by round bur and cotton pallet was removed by the dental probe.

Before the disinfection procedure was established, the working length of both mesial and distal canals of the tooth were determined by using a size of 20-file with radiograph. as in Figure (1,B)

According to the American Association of Endodontist (AAE) and European Society of Endodontology(ESE), the root canal system was disinfected by 20ml of sodium hypochlorite(NaOCl) 1.5% using 20ml disposable syringe and 27 gauge double sided vent needle in order to prevent or reduce NaOCl extrusion to the periapical area, and to minimize the cytotoxic effect on cells at periapical area (periapical papillae stem cells), the needle was inserted 1 mm shorter than the working length. A 20ml of 1.5% sodium hypochlorite used to irrigation for about 10 minutes, followed by normal saline and another 20 ml Ethylenediaminetetraacetic acid (EDTA) 17%, then for about 5 minutes with 5ml of normal saline. The canals were then dried with a size 80 paper-points, the canals were filled with calcium hydroxide paste (calasept ® plus, Sweden) in order to complete disinfection of the canals, and covered by a cotton pellet. Glass ionomer filling material was used to close the access cavity. Figure (1C).

Figure (1): Radiograph of Stage II (Disinfection)
After about 4-5 weeks from the disinfection step, the step III (treatment) was started. After proper anesthesia, also, rubber dam isolation and disinfection as in previous steps, the temporary filling material was removed by the aid of a round bur to expose and remove the cotton pallet, 20ml of 17% EDTA solution was used to irrigate (agitate) and remove the calcium hydroxide which was presented inside the root canals and also to open the dentinal tubules which contain signaling factors that will stimulate the cell migration. The irrigation procedure lasted for about 10 minutes. In order to reduce the toxicity of EDTA 5ml of 0.9% normal saline was used to irrigated the canals. The canals were dried with a size 80 paper points.

Then the induction of bleeding and allowing blood to flow inside the canals. This step was performed by entering a head-storm file (size 25), 2mm beyond the working length. This step was caused by injuring the periapical region and allowing the blood to flow inside the canals.

The level of blood was not allowed to exceed the cementoenamal junction (CEJ) level by aid of paper point, the blood was let to clot for 15 minutes. Figure (2). White mineral trioxide aggregate (MTA) (MTA ANGLUS, Brazil) powder with a sterile distill water has mixed in a ratio of 1:1 on a sterile glass slab, and with sterile MTA applicator, the mixed (MTA) was applied over the blood clot, with wet head of size 80 paper point, the mixed MTA was spread and condensed over the blood clot. The adequate wet cotton pellet was placed over the MTA thus allowing it to complete the setting reaction. Glass ionomer filling material was used to close the access cavity.

After 12 weeks (3 months) of treatment step, the follow up step began. In this step, the tooth and its surrounding structures (periodontal ligament and alveolar bone) were removed surgically for histopathological analysis without scarifying of the animal.
The surgical procedure was started by two-sided, full thickness flap extending from the third premolar to the first premolar, was performed by the No. 15 surgical blade. With a slow speed hand piece and a small surgical fissure bur with a copious amount of normal saline for irrigation, the alveolar bone around the tooth was cut vertically on the mesial side of the tooth until reaching 3 mm below the apex of the tooth, and the distal side and finally cut the bone horizontally below the apex of the tooth. Then with the aid of a chisel elevator the entire segment was mobilized, completely removed, and fixed in 10% formaldehyde. The wound side was well irrigated with normal saline and the edges of the flap were approximated and sutured by the gauge (0) chrome cat-gut suture. One week after surgery, these animals were released after ensuring they were healthy, assisted by a consultant Veterinarian. The Figure (3) shows the surgical procedure of biopsy taken.
The samples were decalcified and routinely proceeded to prepare Hematoxylin-Eosin stained slides for histomorphological evaluation, according to Suvarna, et al. (2018). The slides were evaluated blindly and subjectively by two examiners considering presence or absence of normal pulp like tissue, newly formed hard tissue (bone, dentin, cementum), empty/necrosis, intra-canal inflammation, periapical inflammation, and apical narrowing and root wall thickening under the light microscope (Olympus, Japan).

**RESULTS**

The final samples size diminished to seven since two experimental tooth were missed during the second stage due to severe mobility related to primary endodontic lesion with secondary periodontal lesions. Also three samples were distraction after histopathological preparation.

The results after evaluating the parameters in experimental teeth in comparison to control Figure (4a and b) are shown in Table (1).

**Table (1): Contains of Root Canal System After Three Month Follow Up of Cell -Homing Procedure**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Normal tissue</th>
<th>Hard tissue</th>
<th>Empty/necrosis</th>
<th>Intra-canal inflammation</th>
<th>Periapical inflammation</th>
<th>Apical narrowing and wall thickening</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
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<tr>
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<td>0</td>
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<td>1</td>
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</tr>
<tr>
<td>7</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Percentage</th>
<th>85.71%</th>
<th>100%</th>
<th>14.28%</th>
<th>57.14%</th>
<th>71.42%</th>
<th>85.71%</th>
</tr>
</thead>
</table>

0 = Absence
1 = Presence
* 1= Dog

The histopathological teeth sections showed the presence normal pulp-like tissue structures in six out of seven sample (85.71%) specimens Table (1), with Chi-Square statistical test ($X^2$) show no significant difference at 5% between these specimens Table (2), Figure (6f) this meant that the treatment procedures have been executed equally to the all animals. All the specimens (100%) showed hard tissue structures deposition Table (1). It was mostly bone–like tissue structure that was seen in the apical area of the root canals, also there was newly formed cementum-like tissue that seen was attached to the internal dentine of the canal in the pulp space as in Figure (5c). However, some slides clearly showed dentine-like structure has formed over the original root dentine Figure(5c). There were no evidence for the
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presence of debris inside the canals in all teeth sample except one (14.28%), and there is no significant difference between these samples that also analyzed by Chi-Square statistical test ($X^2$). Table (2) Figure (5c).

The presence of intra-canal inflammation (inflammatory cells infiltration) predominated lymphocyte which was the most frequently cell found). There is no significant difference has been seen between the samples in presence of intra-canal inflammation which also analyzed by Chi-Square statistical test ($X^2$). Table(2), Four of seven teeth samples (57.14%) has shown persistence of inflammation inside the root canals, near the apical third of the root. Table (1), Figure (5c,d) and Figure (6e).

The periapical inflammation was seen in five of seven teeth samples (71.42%) Table (1), Figure (6f). Samples have been analyzed by Chi-Square statistical test ($X^2$), shown no significant difference between the sample in presence of periapical inflammation Table (2).

Six of the seven teeth samples (85.71%) apical narrowing and little increase in wall thickness Table(1). Figure (5c,d) and Figure (6e) Samples have been analyzed by Chi-Square statistical test ($X^2$), show no significant difference between the samples in the presence of apical narrowing and wall thickness Table (2).

<table>
<thead>
<tr>
<th></th>
<th>Normal tissue</th>
<th>Empty debris</th>
<th>Intra-canal inflammation</th>
<th>Periapical inflammation</th>
<th>Apical narrowing and wall thickening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chi-Square</td>
<td>3.571*</td>
<td>3.571*</td>
<td>.143*</td>
<td>1.286*</td>
<td>3.571*</td>
</tr>
<tr>
<td>df***</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Asymp. Sig., **</td>
<td>.059</td>
<td>.059</td>
<td>.705</td>
<td>.257</td>
<td>.059</td>
</tr>
</tbody>
</table>

* 2 cells (100.0%) have expected frequencies less than 5. The minimum expected cell frequency is 3.5.
** The level of significance $\alpha=0.05$, $p=0.05$
*** Degree of freedom

DISCUSSION

According to this study, the histopathological evaluation was performed depending on six parameters

I. Presence of normal pulp tissue: In this study the histopathological examination showed that 85.71% of slides have normal pulp-like tissue with the surrounding of fibrous connective tissue, multiple fibroblast cell, and newly formed blood vessels as in Figure (5). This study is in agreement with different studies in that the presence of normal pulp tissue or normal pulp- like tissue during pulp revascularization$^{[17,18,19]}$, and this may result from disruption of the pulp tissue during the induction of infection (stage I) as in the study of Torabinejad et al. who disrupted part of pulp tissue to evaluate the success of revascularization when vital tissue structure (vital pulp) present, and the study
concluded that the presence of vital pulp tissue structure may increase the possibility of successful revascularization.\(^{(19)}\)

II. Presence of hard tissue structure: In this study, different types of hard tissue were observed in the histological sections in all specimens (100%) are noticeably occupying different newly formed hard tissues as bone-like structure which occupy the canals space as in Figure(5a,c) and Figure (6a,b) as in study Torabinejad et al. \(^{(20)}\), new-formed cementum-like tissue or internal cementum occur clearly near the apical foramen Figure (5c) as in the study Wang et al. \(^{(17)}\), also, newly-formed dentine-like structure also near the apical foramen Figure (5,c) this study is in agreement with different studies that evaluated the histological changes after pulp revascularization as in \(^{(18,21,22,23)}\).

III. Presence of empty/debris: The canal debris or empty (there are no type of tissues inside the canal) canals was detected in 14.28% of histopathological slide samples. In this slide also there is no normal tissue-like structure, this result is in agreement with the study of Torabinejad et al.(2015) (in of the presence of debris) and also absence of normal tissue in the same sample\(^{(24)}\).

IV. Presence of intra-canal inflammation: The inflammation is one step toward tissue healing, four processes toward tissue healing, first, hemostasis, after the induction of the bleeding inside the root canals and then allowing the blood to clot inside the canals, this process stimulated the second step which is inflammation, the body will infiltrate the acute inflammatory cells (neutrophil and tissue macrophage) at the beginning then the chronic inflammatory cells (the most frequently occur is lymphocyte cells), third, there is the proliferation of tissue cell and fourth, healing of the tissue or may healing to tissue-like structure.\(^{(25)}\)

In this study about 57.14% of samples have shown intra-canal inflammation and 42.85% did not show intra-canal inflammation Table (1), Figure (5c,d), and Figure (6a). The result of this study is in agreement with study of Torabinejad et al.(2015) which represent the inflammatory cell infiltration after three months of pulp revascularization,\(^{(24)}\) and this result means that the time for complete repair was not enough and needed more time for follow up. But most studies that took the dogs as a model of experiment lasted in 3 months,\(^{(18,21,22)}\) Also, there are different studies that increase the period of the follow up to six to seven month,\(^{(23,26)}\), in those two studies the follow up for about 6-7 months and represented 20% of cases after 6 months follow up showed inflammatory cell infiltration\(^{(23)}\). And about 50% of cases showed inflammatory cell infiltration\(^{(26)}\). These results suggest that the present of inflammation means more tissue will repair as mentioned above.

V. Presence of periapical inflammation: The periapical inflammation is present in the samples. about 71.42%of samples which seen to have the granulation tissue
formation Figure (5c), Figure (6a) and 28.75% of samples have no granulation tissue and the state of the tissues prone to complete healing of the area Figure (5d), Figure (6f), as mentioned above the inflammation is part of the tissue healing process, and formation of the granulation tissue is the preparatory step toward the complete healing process. The formation of granulation tissue was described in different studies, and most of the studies that histologically evaluate the pulp revascularized tissues when described the soft tissue present in the root canals, were mentioned the presence of the granulation tissue formation as a part of the tissue healing process different studies agree with this study as they observed the granulation tissue formation after different revascularization protocols.\(^{(21,24,27)}\)

VI. Presence of apical narrowing and wall thickening: The increase in apical narrowing and wall thickening are the most important parameters in evaluating the success of pulp revascularization process (pulp stem cell-homing). The increase of the apical narrowing, wall thickness and root length these will increase the prognosis of tooth survival by decrease the susceptibility of root fracture in comparison with apexification.\(^{(7,28,29)}\)

In this study about 85.71% of samples showed to have apical narrowing and increase of root thickness, and these outcomes are in agreement with different studies \(^{(27,30)}\) that used different protocols for pulp revascularization, the outcome of Zhang et al. who mentioned that about 64.71% of pulp revascularization with blood clot was observed with apical closure, and about 76.47% observed with wall thickening.\(^{(30)}\) Another study by Khademi et al. observed that about 60% of cases occur to have apical closure and 40% of cases had wall thickening.\(^{(27)}\) This study is in agreement with these studies which found that the pulp stem cell-homing may increase the wall thickness and also promote the apical closure of open apex with periapical periodontitis.

(a,b) represent how normal tooth look like after completion of root development stained with Hematoxylin-eosin (H&E) under magnification 2X\(^{(17,18)}\). Dentine demarcated with (D), (NFC) Newly formed Cementum, (AB) Alveolar bone, (PDL) Periodental Ligament, The black arrows represent the apical foramen closure.

**Figure (4):** Normal Tooth Histological Images After Complete Root Development

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Histological analysis after pulp cell homing of incomplete apex formation in dogs

(a,b) Represent the apical area of the root. Present of plug structure of bone-like ingrowth (B) as healing in the area of root apex (↔) with granulation tissue (GT) representing by proliferation of fibrous connective tissue (F), newly blood vessels (BV), infiltration of inflammatory cells (i) and also pulp-like tissue (PLT). newly formed dentine (ND), newly formed cementum (NCE), and empty or debris area (ED). 100X, H&E stain

(c) present of bone-like tissue (B) ingrowth that narrowing the apical foramen, and also newly cementum tissue (NCE) present, the black arrows represent the true margins of tooth .40X H&E stain

(d) Proliferation of fibrous connective tissue (F) with newly blood vessels (BV) in the area of root of tooth, and represent pulp-like tissue (PLT). 100X, H&E stain

Figure (5): Histological Images After Three Month Follow Up of The Procedure

(a) Present of granulation tissue (GT) as healing in the area of puncture of apex of root of tooth (↔) representing by proliferation of fibrous connective tissue (F), newly blood vessels (BV) and infiltration of inflammatory cells (i) with structure of bone-like ingrowth (B). 40X, H&E stain

(b) Present of granulation tissue (GT) as healing in the area of puncture of apex of root of tooth (↔) representing by proliferation of fibrous connective tissue (F), newly blood vessels (BV) and infiltration of inflammatory cells (i) with structure of bone-like ingrowth (B). 100X, H&E stain

Figure (6): Histological Images After Three Month Follow Up of The Procedure
CONCLUSION

The pulp stem cell homing procedure is an effective way to treat the open apex teeth and this procedure will increase the root length and thickness and also fill the pulp space with pulp-like tissue. The procedure does not regenerate the tooth structure but forms dental tissue-like structure (dentin, bone and cementum) and these tissues are not for regeneration but, tissue repair.

REFERENCES
23. Altaii, M., Cathro, P., Broberg, M., Richards, L. Endodontic regeneration


