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Effect of VEGF on the Success of Dental Tissue Regeneration in Delayed Replantation of Avulsed Teeth

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ABSTRACT

Background: Avulsion of tooth constitutes an emergency case in dental management. It accounts 1%–16% of all traumatic tooth injuries. Avulsed tooth required immediate treatment and all efforts must be oriented to restore periodontal attachment and blood supplement to surrounding structures. Vascular endothelial growth factor (VEGF) is a potent angiogenic and vasculogenic factor that affects dental pulp and periodontal repair. The study was designed to investigate the effect of exogenous application of VEGF on regeneration of dental tissue in delayed replantation of avulsed tooth in rats.

Methods: Twenty-four adult male Wistar rats were used in this study, 12 rats with replanted of avulsed incisors without treatment, others replanted in their treated sockets with 0.5 µl VEGF. Histological and histomorphometric analysis were done at periods (21 and 45 days) postoperatively to estimate repaired cementum, periodontal ligament attachment and percentage of resorbed area.

Results: Histomorphometric analysis reported that replanted avulsed tooth with VEGF application showed lower areas of root resorption and ankylosis in comparison to control group with a significant difference value ($p > 0.05$). Histological assessment showed reattachment of periodontal ligament with obvious angiogenesis in experimental group.

Conclusion: The risk of root resorption, ankylosis and subsequent tooth loss is minimized by using VEGF in delayed replantation of avulsed tooth.

Keywords: VEGF, Tooth Replantation, Ankylosis, Root resorption, Periodontal ligament, Angiogenesis

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INTRODUCTION

Tooth avulsion is a complete displacement of a tooth from its socket and replantation is the treatment of choice to recover the optimal function and esthetics[1,2]. The prognosis is associated with the duration between the time of tooth displacement and when it is replanted. The success of replantation depends on the subject's general health, the maturity of the root, the period of extra-oral time, and the storage medium[3,4]. The achieved goal in treating an avulsed tooth is to preserve periodontal attachment and blood supplement to surrounding structures and restore the tooth attachment with alveolar bone, gingival tissue with formation of new cementum and synthesis of sharpy's fibers to reestablishing of new attachment[5,6]. The success of dental tissue restoration depends on avoiding of many complications including inflammation , root resorption that may resulted in loss of the tooth[7,8].

Growth factors are able to regulate many cellular events in dental tissue regeneration and repair[9]. Vascular endothelial growth factor (VEGF) is a powerful key that regulate the physiological and pathological angiogenesis, as it induces proliferation of endothelial cell and stimulates angiogenesis[10,11]. Angiogenesis involves in repair and healing processes; without adequate blood supply, tissue regeneration will impaired[12]. All those factors seem to be necessary and have important roles in replantation of avulsed tooth, and because there are few studies concerning application of growth factors in dental field, therefore, current study was conducted an experimental design for delayed replantation model in rats and studied the effect of local application of VEGF on the success of restore and regeneration of dental tissue and minimized tooth loss.

MATERIALS AND METHODS

Animal Models

Twenty four adult male Wistar rats, weighted 0.30 -0.35 kg, aged 4-5 months were used and kept in the Animal Department of National Center of Drug Control and Research, Iraq at a constant humidity and temperature of 23°C. Animal care was furnished in accordance with the National Council's guide. After 2 weeks of acclimatization, rats were randomly allocated into 2 groups:

Group I (control n=12) rats with replanted of avulsed incisors without treatment

Group II (experimental n=12) rats with replanted avulsed incisors with application of VEGF

Extraction and Replantation

Wistar rats were given anesthesia using ketamine combined with xylazine intraperitoneally (0.1 mL/10 g body weight).After the anesthesia stage was reached, the incisors and surrounding areas were cleaned with iodine, and then extraction was performed by using scalpel blade #15 and appropriately sized elevator placed between the central using gentle force to luxate the left central incisor without trauma. The dental pulp of the extracted teeth was removed using a pre-curved size 10, 15 and 20 Hedstrom file, followed by saline irrigation, then drying using paper points. The canal was then filled with calcium hydroxide paste[13]. The extracted incisors were placed on a piece of dry gauze for 45 minutes before replantation and then replanted. Twelve of animals were replanted their teeth into their respective sockets with no treatment as a control group. For the experimental group the socket hole was treated with 0.5 µl VEGF (Rat VEGF 165A protein (ab51967) by using micro-pipette and was left for 30 seconds, then the teeth replanted in their treated sockets. The replanted teeth were splinted using etching by phosphoric acid for 1 min, washing with distilled water and drying. Then bonding and application

of flowable composite resin. Intramuscular injection of antibiotic ceftriaxone (20,000 IU) was used at the day of the procedure. Animals of each group were subdivided into 2 subgroups (6 rats each) and according to time of scarification (21 and 45 days) from the replantation procedure. The rats were euthanized by an overdose of diethyl ether and the anterior portion of the maxilla dissected out.

Sample preparation and histomorphometric analysis

The specimens were decalcified, washed and then dehydrated in the ascending graded ethanol solution. Specimens were embedded in wax block and trimmed, the first undefined slice was removed from the saw blade and the selected 5 serial sections were obtained involved the middle third and apical third of the root. Mounting and staining of slides by hematoxylin and Eosin (H&E). From each section an image was taken and imported into a computer program software image lab 2.3 for histomorphometric analysis to estimate cementum, periodontal ligament attachment and percentage of resorbed area that was organized and analyzed based on the following formula: total area of tooth resorption times to 100 and divided by the total tooth area [14] .The data was tabulated and analyzed statistically with fissure test and student's t-test .

RESULTS

Histological findings for control group revealed resorbed cementum and dentin with presence of inflammatory cells at 21 days of replantation of avulsed tooth. At 45 days postoperative period the results show excessive dentin resorption with presence of Odontoclast cell occupies the pay like resorbed area. External root resorption was recorded in most study specimens of control group at 45 days period with evidence of necrotizing periodontal ligament and resorbed bone, figure (1). Microscopical study for the specimens related to experimental (VEGF) group at 21 days postoperatively showed resorption in cementum extends shortly to dentin. Periodontal ligament showed a hyalinization at 45 days of replantation period with new cementum deposition filled the resorbed area with presence of active proliferative cementoblast cells. Most microscopic slides of experimental group record an active new blood vessels formation with well reestablished periodontal ligament, figure (2). The observed frequency of histological parameters include root resorption ,PDL hyalinization, ankylosis, angiogenesis, inflammation and Success PDL attachment were estimated at 45 days period and recorded a significant difference value for experimental in comparison to control, except PDL hylinization showed a non-significant difference, table(1). Statistical analysis for the percentage of root resorption of study groups at 21 days and 45 days periods revealed a significant difference ($p<0.01$), table (2)

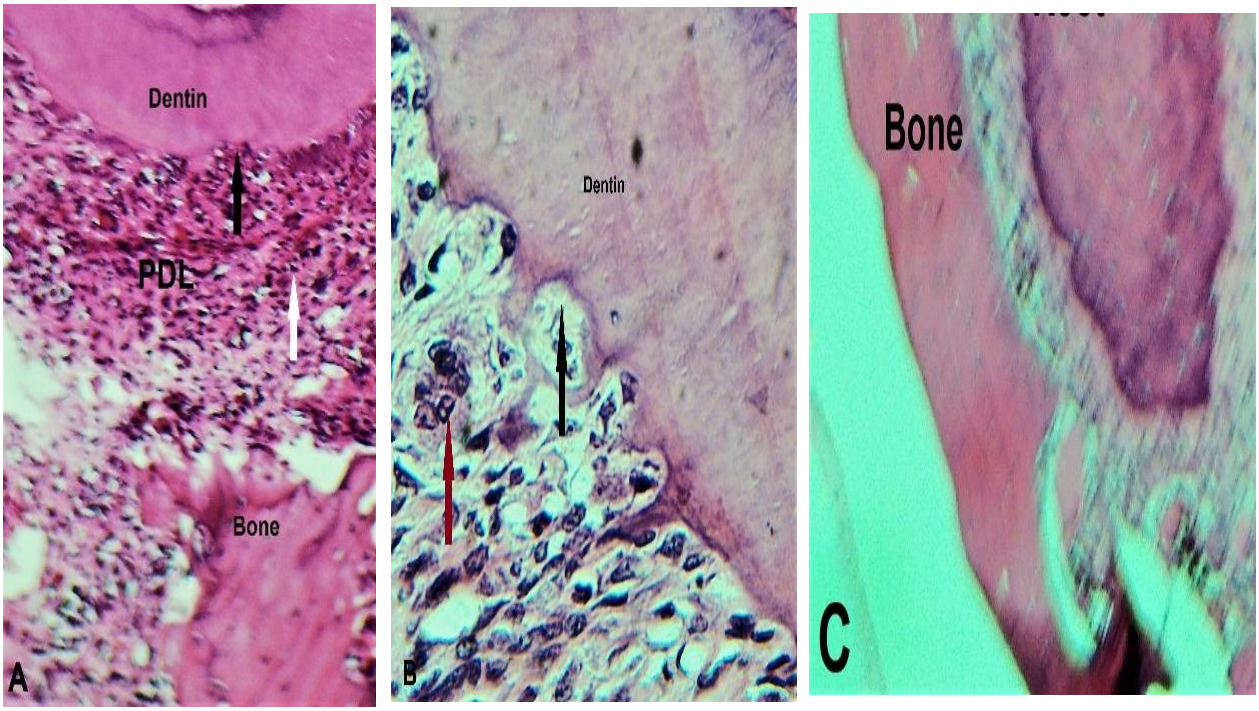

Figure 1. Microscopic views for control group: A; Resorbed cementum and dentin (black arrow), with inflammatory cells (white arrow) at 21 days of replantation of avulsed tooth. H&E x 10.B; Excessive dentin resorption (black arrow) with presence of Odontoclast cell (red arrow).H&E x40. C; Resorbed root, necrotic periodontal ligament (loss the natural architecture) and resorbed bone at 45 days of replantation. H&E x20.

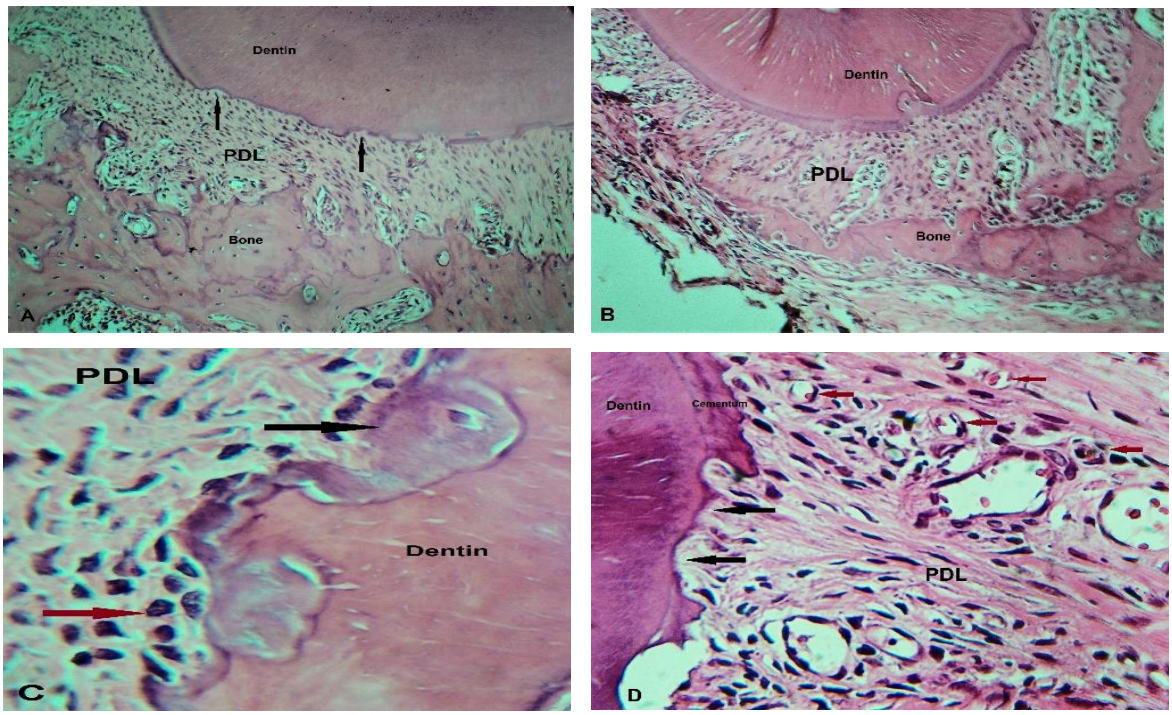

Figure 2. Microscopic views for experimental group: A; Resorbed dentin (arrows) at 21 days of replantation of avulsed tooth. H&E x20. B; Hyalinized periodontal ligament (PDL) at 45 days of replantation .H&E x10. C: New cementum filled the resorbed area (black arrow) with active cementoblast (red arrow). H&E x40. D; View for other specimen shows active new blood vessels formation (red arrows) with well reestablished periodontal ligament (PDL)(black arrows) H&E x20

Table 1. Observed frequency of histological parameters for the study groups at 45 days

Parameters	No.of specimen/group	Control	Experimental	F test	Sig.
Root resorption	12	8	2	6.521	0.001*
PDL Hyalinization	12	2	2	0.575	0.125
Ankylosis	12	3	0	5.330	0.001*
Angiogenesis	12	2	10	7.555	0.001*
inflammation	12	10	1	6.344	0.001*
Success PDL attachment	12	1	10	8.233	0.001*

* Statistically significant at p ≤ 0.05

Table (2): Statistic analysis for the Percentage of root resorption of study groups at 21 days and 45 days.

Periods	Control	Experimental	T test	P value
21 days	7.81± 0.65	4.58±0.60	6.770	<0.01*
45 days	17.56±1.21	5.22±1.11	8.532	<0.01*

* Statistically significant at p ≤ 0.05

DISCUSSION

The present study used incisor tooth of rat as an experimental model because it has a wide apical foramen that mimic the open apex of an immature tooth of human maxillary central incisors of children that are the main target to avulsion after trauma[15]. In this study Calcium hydroxide was used as an intra canal dressing, because it has well antimicrobial property and it is widely use in tooth replantation[16]. Several lines of evidence have revealed that topical application of recombinant cytokines can induce regeneration of periodontal tissues[17,18].

To recover damaged periodontal ligament, angiogenesis is an important key in healing process[19]. Periodontal ligament is enriched vascularized tissue and gained its supplement from the apical vessels, gingival vessels and intra-alveolar bone vessels that protects against frequent inflammatory insults[11] .Many studies reported that inflammation can result in a reduction of the number of blood vessels and VEGF expression levels[20]. In present study using of exogenous VEGF application enhanced new blood vessels formation resulted a reduction in an inflammation reaction in experimental group in comparison to control which showed high frequency of inflammation stroked replanted tooth. Moreover, histologically, results record an increment in the blood supply in the VEGF group with obvious evident of angiogenesis action filled the injured periodontal area.

Although injured periodontal cells secrete angiogenic growth factors to stimulate angiogenesis[21], exogenous VEGF seemed to add other trigger to cells and the sequences were continued until the healing process was completed.

Angiogenesis is important for successful tissue regeneration including repair and healing of dental tissue; without adequate blood supply, tissue regeneration will impaired and necrotic tissues are subsequently formed[22,23] as the present results for control group report excessive root resorption with necrotic periodontal tissue. The results revealed that group VEGF showed significantly lower (percentage and frequency) of root resorption and lower frequency for observed detected ankylosis. These findings suggest that exogenous VEGF may be a useful growth factor in the repair of damaged PDL and resorbed cementum as the histological views show deposition of new cemetenum filled the resorbed area with new reattachment of PDL. This study demonstrates that exogenous VEGF has positive influences on proliferation, differentiation as the examined slides recorded active proliferation of cementoblast .In addition, that VEGF stimulate angiogenesis, it modulate the external root resorption by formation of reparative dentin and cementum matrix formation [24].

Detection of PDL hyalinization in 4 cases for control and experimental groups may related to ischemic injury and cell death within the periodontal tissue accompanied with root resorption[25,26]. The present study demonstrated that application of exogenous VEGF in replantation of avulsed tooth stimulated angiogenesis and induced repaired dentin and cementum with reestablishment of periodontal attachment with better results in comparison to control.

CONCLUSION

The study concluded that exogenous VEGF enhance angiogenesis therefore, it reduces external root resorption, ankylosis and enhance reattachment of tooth to surrounding structures and increase the chance of replanted tooth to survive.

ETHICAL CLEARANCE

All work of this study had done according to the National Council's guide for the care of laboratory animals.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

FINDING

Self- funding

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