

# Histological evaluation of local application of collagen I and /or vascular endothelial growth factor (An experimental study in rats)

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## ABSTRACT

**Background:** The study was designed to evaluate the effect of local application of exogenous VEGF/collagen I separately and as a combination in socket healing. Sixty male Albino Wistar rats were subjected for a surgical tooth extraction of upper 1<sup>st</sup> molar of both sides (right side was considered as experimental site, while left be the control one, treated with 1 $\mu$ L of normal saline). The rats were scarified at 3, 7, 14, 28 days post extraction. Socket healing was histologically examined with immunohistochemistochemical localization of ALP&FGF2.

**Materials and Method:** Sixty male Albino Wistar rats were subjected for a surgical tooth extraction of upper 1<sup>st</sup> molar of both sides (right side was considered as experimental site, while left be the control one). The animals were divided into following groups according to the applicable of biomaterials.

A. Control group the tooth socket treated with 1 $\mu$ L of normal saline

B. Experimental group includes

- Group I contains (20) rats, the tooth socket treated with 1 $\mu$ L of VEGF
- Group II contains (20) rats the tooth socket treated with 1 $\mu$ L of collagen type I
- Group III contains (20) rats, the tooth socket treated with 1 $\mu$ L of a combination of VEGF and collagen I.

**Results:** At 28 days all groups show re-epithelization but in different thickness, and with newly bone apposition and with different maturity. For positive cells expressed ALP, VEGF group records a high mean values at 3, 14, 28 days periods and with high differences in comparison to other groups while control group reports a high mean value at 7 days. For positive cells expressed FGF2. Control group illustrates a high record for the mean of positive cells expressed FGF2 at 3,7days periods and with high differences in comparison to other groups, while combination group reports a high mean value at 14 days .

**Conclusion:** Results, high lighted on the effect of local application of VEGF in extracted tooth socket that facilitate epithelization, while combination of (collagen and VEGF) shows a high mineralization zone.

**Keywords:** Exogenous VEGF/collagen I, healing, extraction. (J Bagh Coll Dentistry 2015; 27(3):79-84).

## INTRODUCTION

Tooth in the maxillary or mandibular alveolar process is surrounded and anchored by tissues that make up the periodontium. The periodontium includes gingiva, connective tissue, cementum, periodontal ligament, and alveolar bone. The alveolar bone consists of cortical bone, cancellous trabeculae, and the alveolar bone proper, which is compact bone that composes the alveolus (tooth socket).

The normal healing response to the tooth extraction procedure results in a significant loss of bone and collapse of the surrounding gingival tissue, followed by regeneration of epithelial and connective tissues<sup>(1)</sup>.

Healing process at extraction sites, including bone resorption and remodeling, which are fundamental events in socket healing. Changes occur at molecular, cellular, and tissue levels. Extraction of a tooth commences a cascade of inflammatory reactions<sup>(2)</sup>.

Blood from severed vessels fill the socket creating a mixture of proteins and damaged cells. Blood platelets initiate a series of events that will ultimately lead to the formation of a fibrin clot,

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filling the entire socket, within the first 24 hours<sup>(3)</sup>. The coagulum, facilitated by growth factors, acts as a physical matrix and directs the movement of the inflammatory cells, neutrophils and macrophages enter the socket to phagocytize bacteria and tissue debris released growth factors and cytokines induce and amplify the migration of mesenchymal cells and their synthesis within the coagulum<sup>(4)</sup>.

Several growth factors are expressed in distinct temporal and spatial patterns during repair. Of these, vascular endothelial growth factor, VEGF, is of particular interest because of its ability to induce neovascularization (angiogenesis) and its role to promote healing of bone defects<sup>(5)</sup>.

Collagen 1 is the most abundant collagen of the human body. It is present in scar tissue, the end product when tissue heals by repair. It is found in tendons, skin, artery walls, cornea, the endomysium surrounding muscle fibers, fibrocartilage, and the organic part of bones and teeth.

Collagen has the correct properties for tissue regeneration such as pore structure, permeability, hydrophilicity and it is stable in vivo. Collagen scaffolds are also ideal for the deposition of cells, such as osteoblasts and fibroblasts and once inserted,

growth is able to continue as normal in the tissue<sup>(6)</sup>. Therefore; the present research was designed for application of VEGF / collagen 1 in rat tooth socket and determine their roles in healing process.

## MATERIALS AND METHODS

All experimental procedures were carried out in accordance with the ethical principles of animal experimentation.

### Study Design

Sixty male Albino Wistar rats weighting (250-300) gm, aged 5-6 months were used and maintained under control conditions of temperature, drinking and food consumption. The animals were subjected for a surgical tooth extraction of rat upper 1st molar of both sides (right side was considered as experimental site, while left be the control one).

The animals were divided into following groups according to the applicable of biomaterials.

**A. Control group** the tooth socket treated with 1 $\mu$ m of normal saline and its number represented the all number of the following experimental groups as the left side of each animal considered to be the control.

### B. Experimental group includes

**-Group I contains (20) rats**, the tooth socket treated with 1 $\mu$ m of VEGF

**-Group II contains (20) rats** the tooth socket treated with 1 $\mu$ m of Collagen type 1

**-Group III contains (20) rats**, the tooth socket treated with 1 $\mu$ m of a combination of VEGF and collagen type-1.

Each group is composed of 20 rats that will be studied in four periods 3, 7, 14, 28 days (5 rats for each period).

### Materials

- VEGF 165A protein (Rat) (ab51967), lyophilized form from Abcam company
- Collagen type-1 protein. Abcam company (ab7533).
- \*Alkaline phosphatase Antibody (ALP) from Abcam Company UK (ab56023).
- Fibroblast growth factor antibody (anti – FGF2), from Abcam Company UK (ab106245).

## Methods

It was performed under a well sterilized condition and gentle surgical technique. Each animal was weighted to calculate the dose of general anesthesia and antibiotic, the general anesthesia was induced by Intra muscular injection of xylazine 2% (0.4 mg/kg B.W.), plus ketamine HCL 50mg (40 mg/kg- B.W.). Then the animal was placed on the surgical table and the gingivae of upper posterior 1<sup>st</sup> molar tooth is separated with dental probe. An enamel hatchet was then positioned between the first and second molars allowing avulsion of the dentalreminiscent.

Tooth socket cleaned with saline irrigation, then dryness with air. Application of 1 $\mu$ L of VEGF and/or collagen type-1 was applied by micropipette for experimental sockets while 1 $\mu$ L of normal saline applied to control one. The socket was closed with gentle pressure by fingers.

### Assessment of Immunohistochemistry results

Positive reading was indicated when the cells display a brown cytoplasmic stain, while negative reading was indicated for absence of immune-reactions depends on positive and negative control.

### Immunohistochemical scoring of FGF2, ALP:

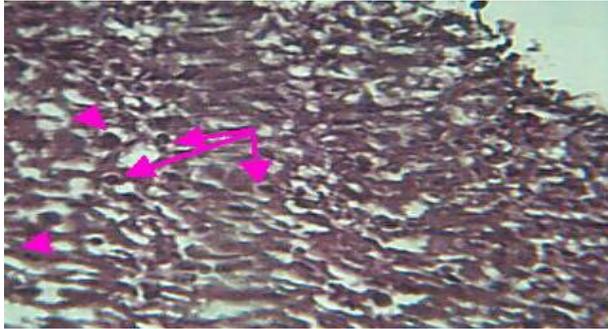
Quantification method of Immunoreactivity was semi-quantitatively estimated the immunostaining score that was calculated as the sum of a proportion score and an intensity score. The proportion score reflects the estimated fraction of positively stained infiltrating cells. For FGF, ALP, it was assessed by identifying and scoring 100 cells in five fields (X40) along bone defect area of different sections (Score 0, none; score 1, <10%; score 2, 10-50%; score 3, 51-80%; score 4, >80%)<sup>(7)</sup>.

## RESULTS

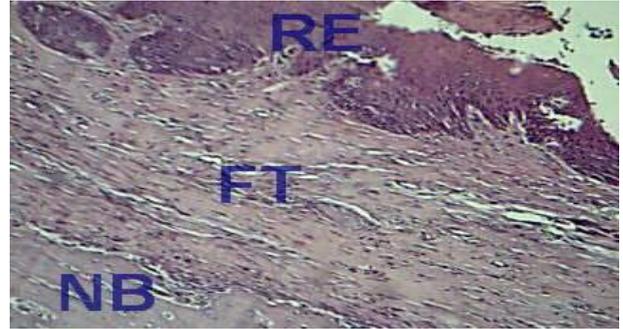
### Histological findings

Control group at 3 days duration shows blood clot illustrates bypresence of inflammatory cells include polymorphic nuclear cell, monocyte cell, plasma cell (Figure 1). Socket healing site for7days duration shows filled with fibrin clot, illustrates fibroblast and lymphocytes (Figure 2).

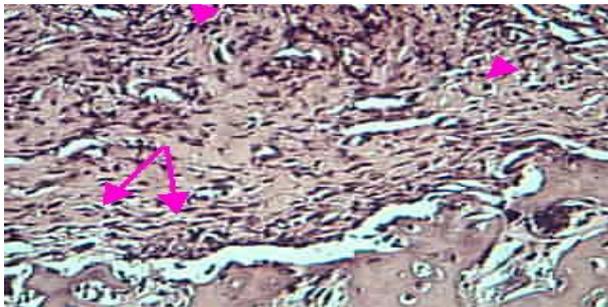
Re-epithelization, bone trabeculae filled the base of the socket with fibrous tissue are detected at 14 days post extracted period (Figures 3 and 4). At 28 days duration shows re-epithelization cover a fibrous tissue, then new bone (Figure 5).



**Figure 1:** Microphotograph view for control group shows blood clot illustrates plasma cell (arrow heads), lymphocyte (arrows). H&E X20.



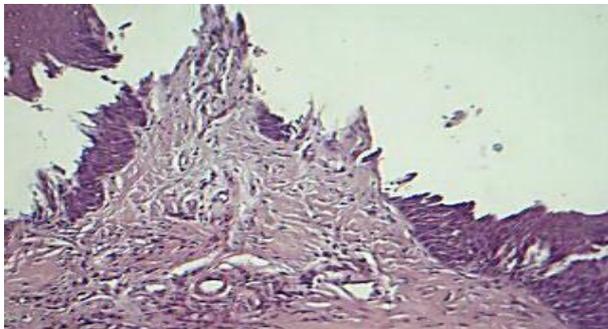
**Figure 5:** View for control (28 day) shows re-epithelization (RE) cover the healing socket fibrous tissue (FT), then new bone (NB) H&EX10.



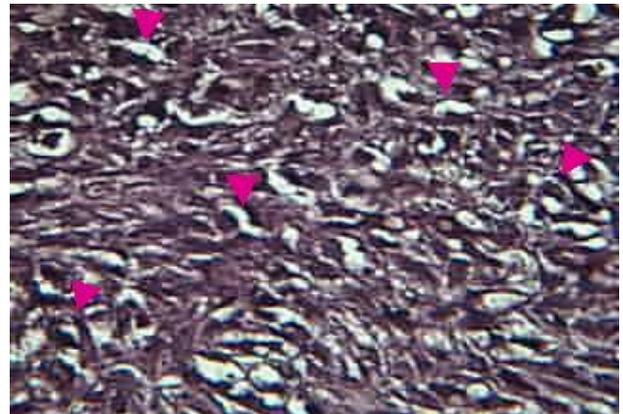
**Figure 2:** View for socket healing shows fibrin clot, illustrates fibroblast (arrows), lymphocyte (arrow heads) control group (7days) .H&ExX20

**Experimental group:** Alveolar socket treated with VEGF illustrated fibrin clot, new blood vessels, and osteoclast in bone socket is detected at 3 days (Figures 6 and 7). At 7 days duration shows osteocyte occupies resorptive Howship's lacunae, osteoblast and new bone apposition (Figure 8). Bone trabeculae filled the base of the socket coalesce with bonesocket re-epithelization of the socket surface .Proliferating osteoblasts, pre osteocyte and osteocytes are detected in histological sections of 14 days duration (Figures 9 and 10).

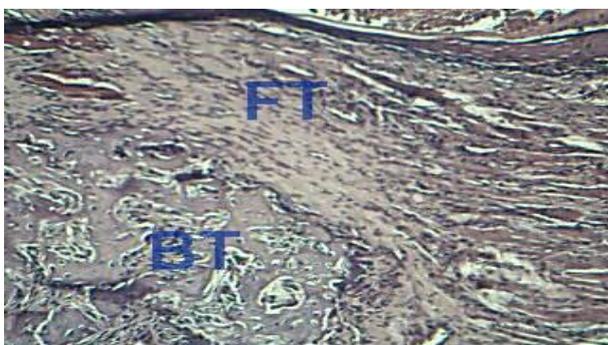
The period 28 days duration illustrated new bone with multiple Harvasian canals filled all socket covered by new epithelium, proliferating basal cell of epithelium tissue (Figures 11 and 12).



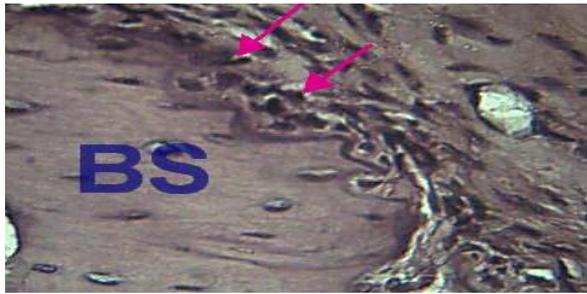
**Figure 3:** Re-epithelization is detected in control group (14 days). H&EX 10.



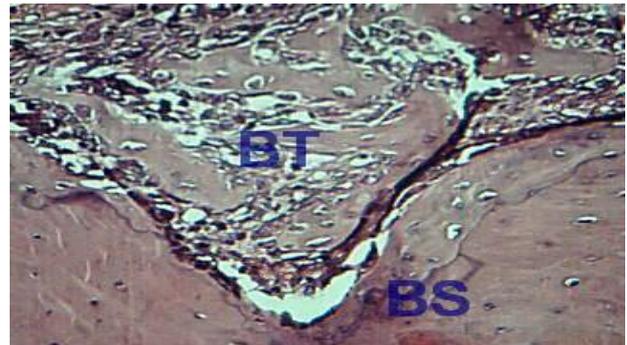
**Figure 6:** Angiogenesis illustrates clot (3 day) for VEGF group, blood vessels (arrow heads) .H&EX20



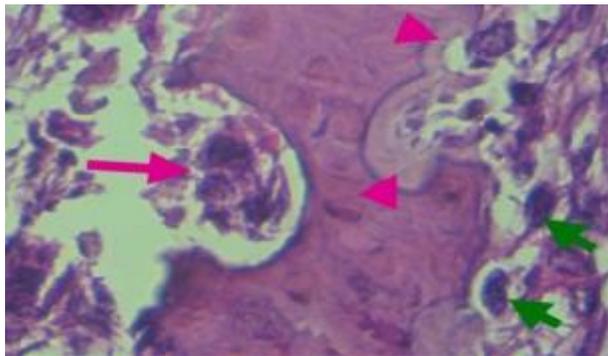
**Figure 4:** Bone trabeculae (BT) filled the base of the socket with fibrous tissue (FT), control group (14 days). H&EX20



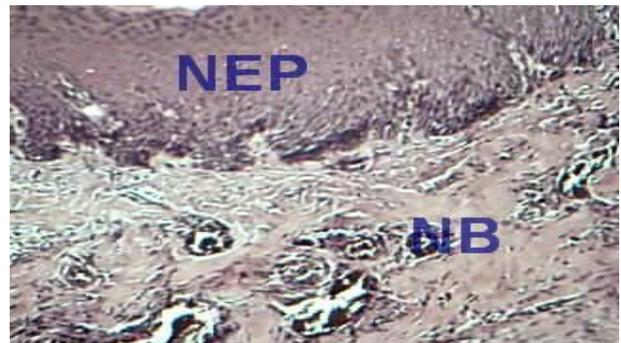
**Figure 7: Osteoclasts (arrows) illustrate in VEGF (3 days) in bone socket (BS). H&EX40**



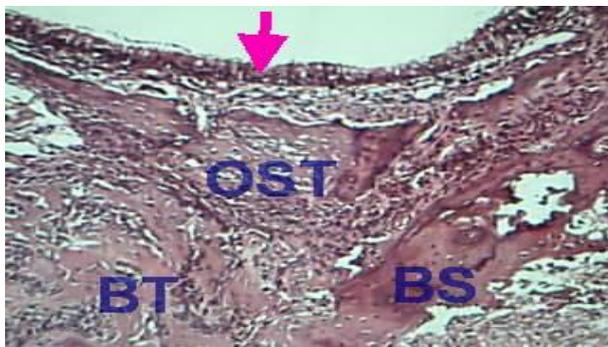
**Figure 10: Base of the socket illustrates bone trabeculae (BT) that surrounded by bone socket (BS) at 14 days. H&E X20**



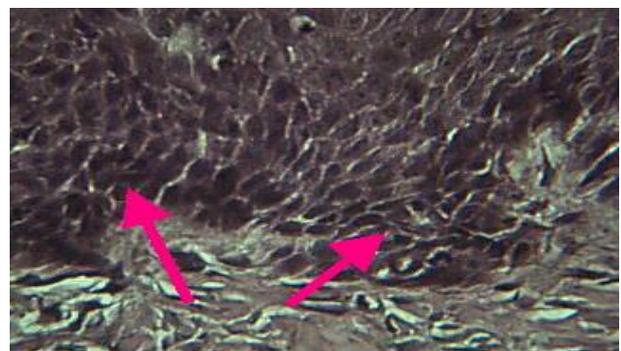
**Figure 8: View for VEGF group (7 day) shows osteoclast (pink arrow), osteoblast (green arrows), new bone apposition occupies Howship's lacunae (pink arrow heads) H&EX100.**



**Figure 11: View for socket of VEGF group (28 day) shows new bone (NB) filled all socket covered by new epithelium (NEP) H&E X20**

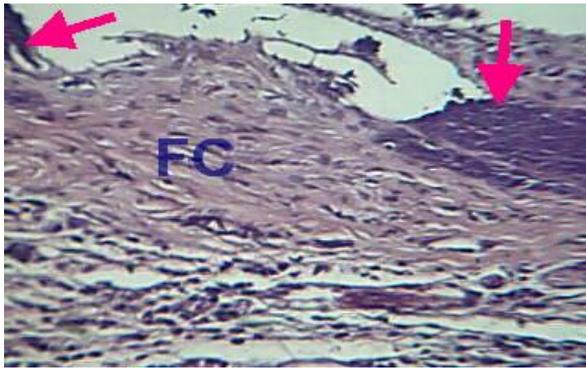


**Figure 9: View for extracted socket of rat in VEGF group (14 days) shows bone trabeculae (BT) filled the base of the socket, bone socket (BS) and osteoid tissue (OST), re-epithelization of the socket surface (arrow). H&EX10.**

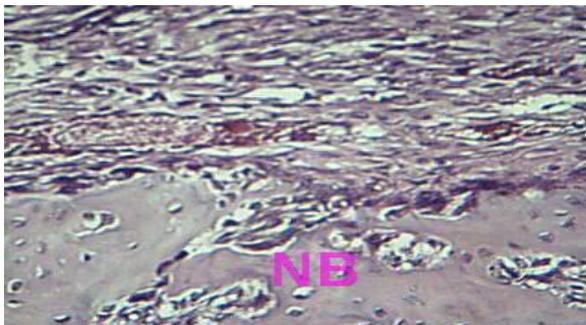


**Figure 12: Proliferating basal cell of epithelium tissue (arrows). H&EX40.**

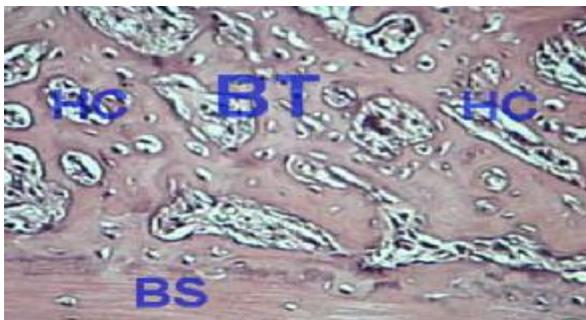
**Experimental group:** Alveolar socket treated with Collagen I shows fibrin clot, with cutting epithelial edges, osteoid tissue apposition, and identification of osteoclast, at 3 days (Figure 13). New bone apposed in base of the socket in collagen I group at 7 days (Figure 14). Alveolar socket for collagen group at 14 days duration shows bone trabeculae coalesce with bone socket (Figure 15). At 28 days, new epithelization (NEP), and fibrous tissue recognized (Figure 16).



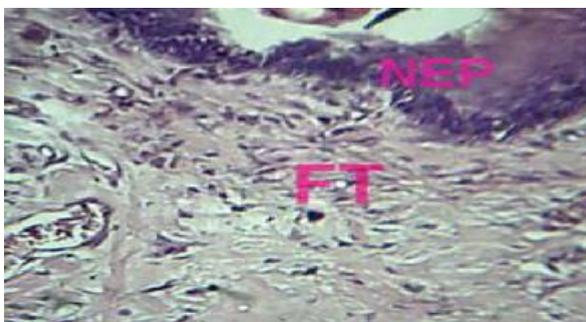
**Figure 13:** View for fibrin clot, with cutting epithelial edges, osteoid tissue apposition, identification of osteoclast at (3days).



**Figure 14:** New bone apposed in base of the socket in collagen I group at 7 days



**Figure 15:** Alveolar socket for collagen group at 14 days duration shows bone trabeculae coalesce with bone socket.



**Figure 16:** New epithelization (NEP), fibrous tissue recognized underneath.

## DISCUSSION

In the present study, the effect of application of VEGF in extracted rat socket was investigated histologically. The result illustrates a formation of blood clot at 3 days, although some samples show a fibrin clot too. Osteoclast cell with obvious angiogenesis is detected early in VEGF group, this may explained as **van Bruggen** <sup>(8)</sup> reported that VEGF is mitogenic, angiogenic, and a potent mediator of vascular permeability. VEGF causes extravasations of plasma protein and increases hydraulic conductivity in isolated perfuse micro-vessels, that help mono-nucleated cell to recruit and aggregate as osteoclast and begins to resorbed old bone and enhanced bone matrix apposition which appears early in VEGF group.

**Cornelini et al.** <sup>(9)</sup> demonstrated that angiogenesis is an important feature of inflammation and healing and its role in the development and progression or in the wound healing related to vascular endothelial growth factor (VEGF) as a potent inducer of endothelial cell proliferation. The present findings shows that VEGF group illustrates osteoclast with high mean value (at 3 days) regarding to other groups.

According to **Yang et al.**, <sup>(10)</sup> findings that show VEGF is directly targets osteoclasts, thereby playing a novel role in bone development and angiogenesis. Collagen is a natural product, therefore it is used as a natural wound dressing and has properties that artificial wound dressings do not have. It is resistant against bacteria, which is of vital importance in a wound dressing. It helps to keep the wound sterile, because of its natural ability to fight infection. When collagen is used as a burn dressing, healthy granulation tissue is able to form very quickly over the burn, helping it to heal rapidly **Singh et al.** <sup>(11)</sup>.

In present study application of exogenous collagen 1 demonstrated a fibrin clot with early detection of osteoid tissue at 3 days, this result could be explained that healing of the rat tooth extraction socket occurs rapidly, indicating a mechanism for cancellous bone formation occurring swiftly throughout the matrix. The residual periodontal ligament is evident at 2 days after extraction and its rich collagen type III fiber content, while collagen type I fibers were formed later, and were especially evident at 6 days after extraction, as normal healing events **Devlin** <sup>(12)</sup> therefore application of exogenous collagen I appears to be affected at 3 days instead of 6 days and may form an early template for future cancellous bone formation. After 7 days the pattern of distribution of both collagen type I and III fibers were similar as they passed from the

bone margin towards the centre of the socket - in the same direction as the forming bone trabeculae. Bone formation occurs by rapid movement of the osteoprogenitor cells along these collagen fibers to allow a rapid healing. Socket healing constitutes a complex and delicate physiological process. Local vascularity at the site of the extracted teeth has been identified as one of the most significant parameters influencing the healing procedure. VEGF is the most important component of the regeneration of the vascular system at the healing site.

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