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**Histological and Immunohistochemical
Evaluation of the Effect of Local Application
VEGF /TGF β_1 on Periodontium, Using VEGF
Collagen I and ALP Markers
(An Experimental Study In Rats)**

A thesis Submitted to the Council College of Dentistry
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**In Partial fulfillment of Requirements for the Degree
Doctor of Philosophy in Oral Histology and Biology**

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Abstract

Back ground: Growth factors are classified as biological mediators that have multiple effects on wound repair in almost all tissues, including the periodontium.

After periodontium injury occurs, healing proceeds in a succession of cell-cell and cell-extracellular matrix interactions. In the process of normal wound healing, the growth factors act in conjunction to form a complex arrangement of molecules that regulate cellular activity and bordering the wound.

TGF β 1 exhibits both pro-inflammatory and anti-inflammatory properties besides its ability to stimulate synthesis of extracellular matrix (ECM) molecules and to inhibit the breakdown of ECM, it has been intensively evaluated in relation to all types of gingival overgrowth.

Regarding TGF β 1 expression in chronic periodontitis, it is noticed a positive reaction in some keratinocytes from the gingival basal epithelial layer and pro-inflammatory cells infiltrating lamina propria .

VEGF is a key regulator of physiological and pathological angiogenesis, because it induces endothelial cell proliferation, stimulates angiogenesis and increases vascular permeability. VEGF is related to both maintenance of periodontal health and periodontal tissue destruction

Aims of the study: (1)To identify the biological events, illustrates as histological feature by using growth factors VEGF,TGF β 1 and a combination of the two factors ,in periodontial healing. (2) Immunohistochemical evaluation for the expression of VEGF, collagen 1 and ALP, in regenerative periodontal tissue.

Materials and Methods: Fifty four male Albino rats, weighting (300-400)gram, aged(6-8)months were used. The animals were subjected for non surgical operation of distal sides of both lower anterior teeth(right side was considered as experimental site ,while left be the control one).

The animals were divided into control and experimental group, the experimental group was subdivided into following groups according to the applicated growth factors.

A. Contol group the periodontium defect treated with $1\mu\text{m}$ 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 1contains (18) rats**, the periodontium defect treated with $1\mu\text{m}$ of VEGF
- **Group 2contains (18) rats** ,the periodontium defect treated with $1\mu\text{m}$ of TGF
- **Group 3contains (18) rats**, the periodontium defect treated with $1\mu\text{m}$ combination of (VEGF&TGF).

Every single group will composed of 18 rats that will be studied in three periods 3,7,14days(6 rats for each period).

All the samples studied histological, histometry and immunohistochemistry for identification of VEGF, collagen 1 and tissue non specific alkaline phosphatase.

Results

1. Concerning to the result of H&E ,the present study concludes the followings

- a. All study groups (control, VEGF treated group, TGF β 1 treated group and combination (VEGF&TGF) group), show organized blood clot and formation of granulation tissue at 3 day period.
- b. Edematous gingiva identified in control and VEGF groups, While TGF and combination groups illustrate gingiva with mitotic basal cells .
- c. Osteoclast cell identify early in VEGF only, while osteoid tissue recognized early in combination group.
- d. Mature bone detected in combination group only, at 14 day period
- b. Secondary junctional epithelia recognized in control group only, at 14 day period

Statistic analysis records the followings.

1. The mean value of amount of new bone and the mean of the length of junctional epithelia is showed to be higher in VEGF group, while the mean of periodontal ligament width and mean of rate of bone maturation are reported to be higher in combination group .High mean value for the width of cellular cementum is recorded in TGF group.

2. At day 7 ,osteoblast mean count is recorded to be higher in VEGF group, While osteocyte mean number showed to be higher in TGF- β 1 group, and osteoclast is found to be higher in combination group.

At 14 day ,osteoblast and osteocyte are recorded to be higher in mean number in VEGF group, while osteoclast showed to be higher in mean number in control group

3. At 7 day period density of fibroblast-like cell is showed to be higher in TGF- β 1 group. Mesenchymal cell and blood vessel densities show a high reading in VEGF group.

At 14 day period densities of fibroblast-like cell, Mesenchymal cell ,blood vessel show to be high in combination, TGF- β 1,VEGF groups ,respectively.

2. immunohistochemical evaluation for VEGF,Collagen1 and ALP

For expression of VEGF the results conclude the followings

- a. Higher records of positive Keratinocyte cell and stromal cell of gingiva for VEGF in VEGF group
- b. Higher records of positive cell of PDL and bone cell for VEGF in combination group.

For expression of collagen 1 the results conclude the following

- a. Higher records of positive stromal cell ,PDL cell and bone cell for collagen 1 in VEGF group in comparison to others

For expression of ALP The results conclude the following

- a. Higher records of positive keratinocyte cell, stromal cell ,PDL cell and bone cell for ALP in VEGF group in comparison to others.

Conclusion .The present study suggested that single use of VEGF in injured periodontium is a powerful tool in regenerative of periodontium tissue ,rather than TGF β 1 or their combination.