Evaluation of the effect of local exogenous application of osteopontin on wound healing in rats

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ABSTRACT

Background: Wound healing is a complex dynamical interaction between various cell types, the extracellular matrix, cytokines, and growth factors. osteoponetin is a substance that acts as an anti-inflammatory.
Aims of study: The study was designed to identify the role of local exogenous applications of osteopontin on wound healing (in cheek skin).
Materials and methods: Thirty adult male albino rats weighting an average of (250-300gm) used in this study, incisional wounds were made in the skin of the cheek of rat and they were divided into the following groups:
A-Control group: 15 rats treated with 1µ l of normal saline
B-Experimental groups: 15 rats treated with topical application of 1µl osteopontin.
The scarification of animals were done for the healing intervals (1, 5 and 10 days) Histological analysis and assessment of number of inflammatory cell, Thickness and contraction of incision were performed for both experimental and control groups for all healing periods.
Results: Histological analysis revealed that osteopontin accelerate wound healing of cheek skin and there was highly significant difference among studied groups in periods 1,5,10 days. Regarding mean values of epithelial thickness, inflammatory cell count and contraction of wound area have reported a highest value different in 1, 5 and 10 days durations.
Conclusion: It can be concluded that application of osteopontin shown efficacy in the healing of skin wounds induced in rats.
Key words: osteopontin, Wound healing, inflammatory cell. **(J Bagh Coll Dentistry 2018; 30(2): 23-28)**

INTRODUCTION

Skin is the biggest external defense system (organ), it covers the outside of the body but has other functions beside the defense mechanism. It serves as a mechanical barrier between the inner part of the body and the external world (1). It consists of three layers, the outer layer is called epidermis, the middle layer is dermis and the inner most layer is hypodermis. A wound is defined as a break or damage in the skin, resulting from physical or thermal damage or as a result of the presence of an underlying medical or physical condition**,** Wound healing is a complex process consisting of four steps, hemostasis, inflammatory reaction, proliferation and remodeling, all of which are regulated by cytokines and growth factors released by cells in the wounded area. The phases are overlapping and linear for acute wounds, whereas the chronic wounds can be found at different stages of the healing process and do not heal in orderly manner, Osteopontin also known as secreted phosphoprotein 1 (SPP 1) (2). osteopontin is a negatively charged aspartic acid-rich, N-linked glycosylated phosphor- protein composed of 314 amino acid residues . The human gene for OPN has been localized on the long arm of chromosome 4q13 directly related to four similar genes encoding for bone sialoprotein (BSP), dentin matrix protein 1 (DMP1), dentin sialophosphoprotein (DSPP) and matrix extracellular phosphoglycoprotein (MEPE), A postulated role for OPN at wound sites is to function as a mediator of immune cell function and subsequent repair. (3).

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MATERIAL AND METHODS

Study Design:
Thirty adult male albino rats weighting an average of (250-300gm) were divided into following groups:
1- Group I (Control) contains (15) rats: the skin (incision in cheek) treated with 1µ l of normal saline.
2. Group II (osteopontin) contains (15) rats: the skin defect topical with 1µ l of osteopontin. Every group will will be studied in three periods 1,5,10 days (5 rats for each period).
Materials:
Osteopontin, normal saline, Alcohol.
Analysis of number of inflammatory cell, Thickness and contraction of incision. Counting of inflammatory cells, include neutrophils and thickness of epidermis by Ocular lens (5 histological sections), for each animal,and measurement of contraction of incision was done daily using the vernia.
Microscopic examination
Tissues were quickly excised and fixed in 10% formalin solution. The trimmed tissues were first washed with tap water followed by dehydration through a graded alcohol series and then passed

though xylol and paraffin series before being embedded in paraffin. The paraffin blocks were cut into 5-6 µm sections stained with Hematoxylin and Eosin and examined under a light microscope.

RESULTS

Histological findings (H&E stain) (Control groups)

At 1 day duration

After one day of skin incision, the histological view of wound site of control group shows the defect area, shown no epithelialization and migration of epithelial cells is formed yet in defect area, Microphotograph view of wound site at the dermis shows high infiltration by inflammatory cells (Figure 1, 2).

At 5 days duration

Histological findings at wound site of control group 5days duration shows complete epithelialization and there is decreased number of inflammatory cells together with formation of loose collagen fibers, The other view shows granulation tissue with irregular arrangement of collagen fibers and characteristic by proliferation of endothelial cell, new blood vessels.(Figure 3, 4).

At 10days duration

Histological section at wound site of 10days duration of control group shows re- epitheliazation with completely of the structure basal present, besides remodeling of collagen fibers can be detected, View shows formation new blood vessels and active fibrobalsts with remodeling of collagen fibers can be detected (Figure 5, 6).

Osteopontin group

At 1 days duration

Microphotograph view of skin of 1day duration at wound site of experimental group(Osteopontin) shown no epithelialzation and migration of epithelial cells is formed yet in defect area, Magnified view of skin section shows vacuolated (degeneration of stratum cell) of cell of epidermal layer(Figure 7, 8).

At 5 days duration

Histological findings of experimental group (osteopontin) 5 days duration showed reduction in inflammatory cells and replacement of granulation tissue by fiber with scattered fibroblasts and complete epithelization is seen too The other microphotograph shows condensed collagen fibers with signs of remodeling accompanied by active fibroblasts (Figure 9, 10).

At 10 Days duration

Histological section at wound site of (osteopontin) 10 days duration revealing complete epithelialzation, formation of new blood vessels, and thick keratinized layer with normal epidermal layer, Magnified of other view shows epithelial cell layers of the new epidermis and few fibroblasts are seen scattered throughout the fibrous of the underlying derim (Figure 11, 12).

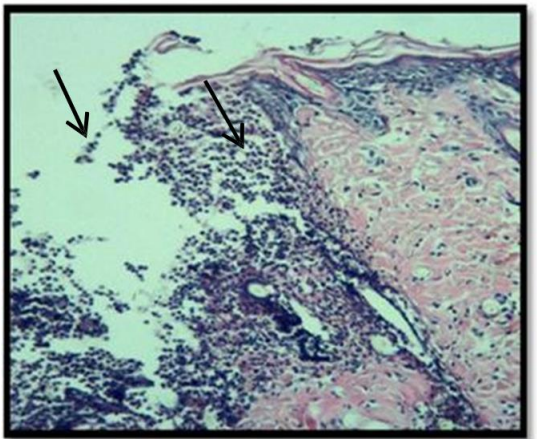

Figure 1: View at wound site of control group after 1day shows defect area with inflammatory cells (arrows). H&EX10.

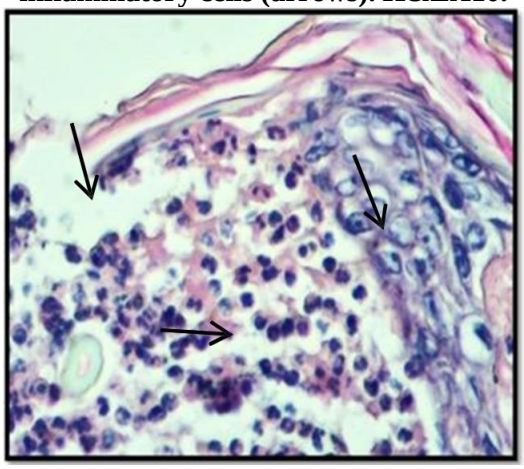

Figure 2: Magnification of previous Figure 1 shows, defect area with accumulation of inflammatory cells (arrows). H&EX40.

Assessment of number of inflammatory cell, thickness and contraction of incision.

Figure 13, shows descriptive statistics of inflammatory cell count/mm² of control and osteopontin groups at different healing periods (1, 5 and 10 days) at wound site.The mean values of cell count of control groups increased , and highest values were recorded with control group at one days. Regarding experimental groups (osteopontin) the mean values of cell count decreased values were noticed within ten days and in groups osteopontin compared with control group for all healing intervals. High significant difference was detected between all studied groups during each healing period. Fig-14, shows descriptive statistics of thickness of control and experimental groups (osteopontin) at different healing periods (1, 5 and 10 days) at wound site. The mean values

of thickness of control groups decreased values were recorded with control group at one days. Regarding experimental groups (osteopontin) the mean values of thickness increased for all healing intervals. High significant difference was detected between all studied groups during each healing period. Descriptive statistics of Contraction of control and experimental group (osteopontin) at different healing periods (1, 5 and 10 days) at wound site. It is noticed that the mean values of Contraction of control group increased and highest values were recorded with control group at one days. Regarding experimental groups (osteopontin) the mean values of Contraction decreased values were noticed in ten days. High significant difference was detected between all studied groups during each healing period.

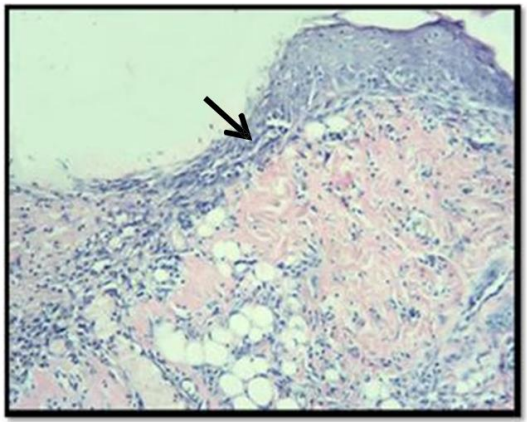

Figure 3: Microphotograph of wound site of 5 day duration in control group shows granulation tissue at defect area, migrating epithelial cells (arrow). H&EX10.

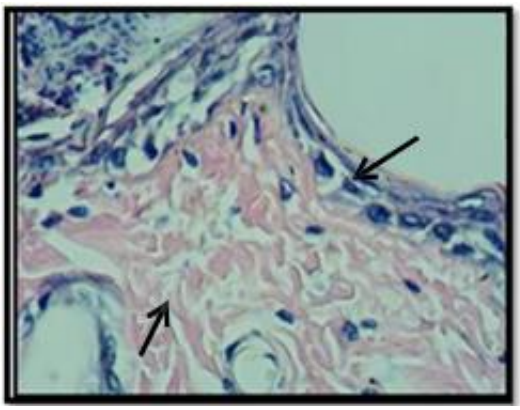

Figure 4: Microphotograph after 5day of control application shows. Migrating epithelial cells at wound area, collagen fiber. H&EX40.

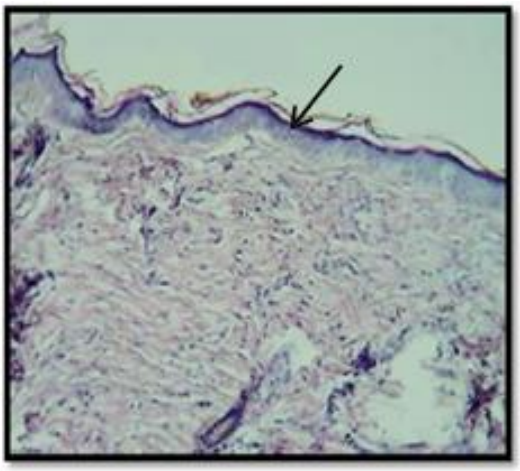

Figure 5: Microphotograph of wound site of Control group 10 day duration shows. Complete epitheliazation at defect site. H&EX10.

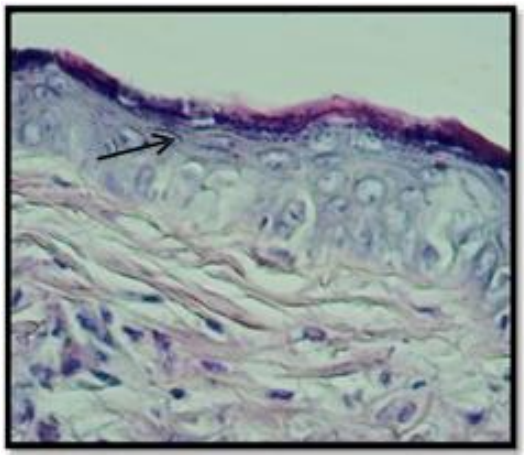

Figure 6: Microphotograph after 10day of control group shows Complete Epithelialazation at defect edge, H&EX40

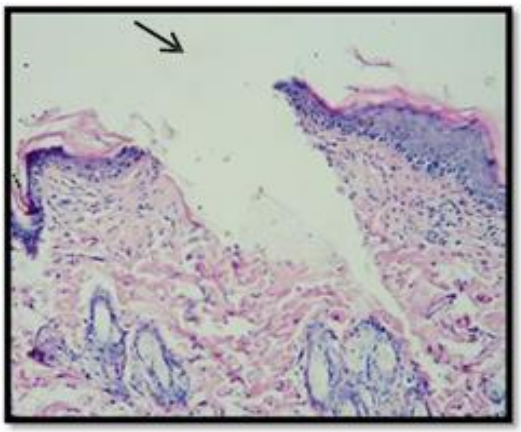

Figure 7: Microphotograph of wound site of 1 day duration osteopontin shows defect area, few inflammatory cells. H&EX10

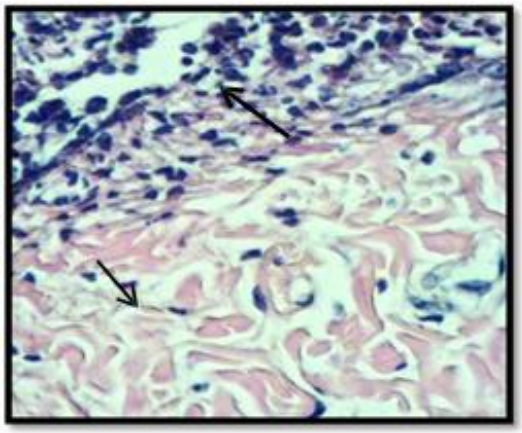

Figure 8: View of 1day duration in osteopontin group shows, few inflammatory cell, Fibroblast. H&EX40.

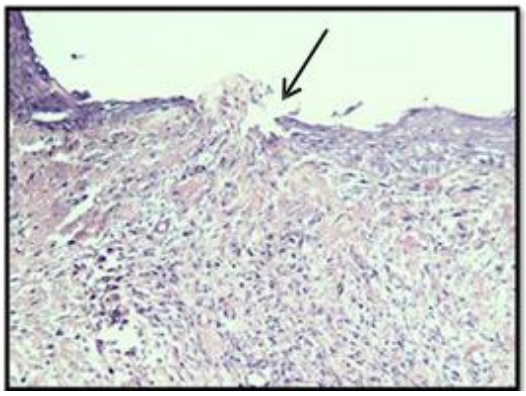

Figure 9: Microphotograph after 5day of osteopontin application shows. migrating epithelial cells at defect edge. H&EX10.

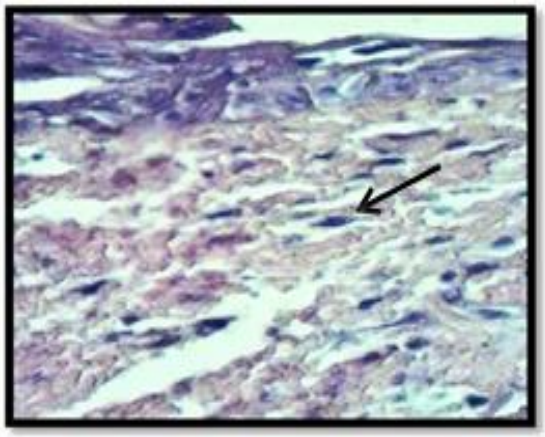

Figure 10: Microphotograph after 5 days of osteopontin application shows.Epithelial cells at defect edge, Fibroblast (arrow). H&EX40

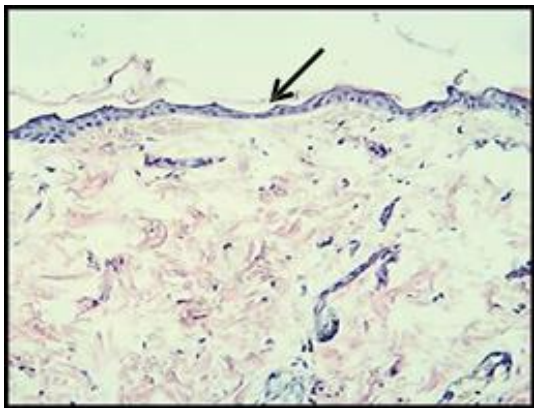

Figure -11: Microphotograph after 10 day of osteopontin application shows. Epithelial cells at wound edge, complete epithelialization. H&EX10

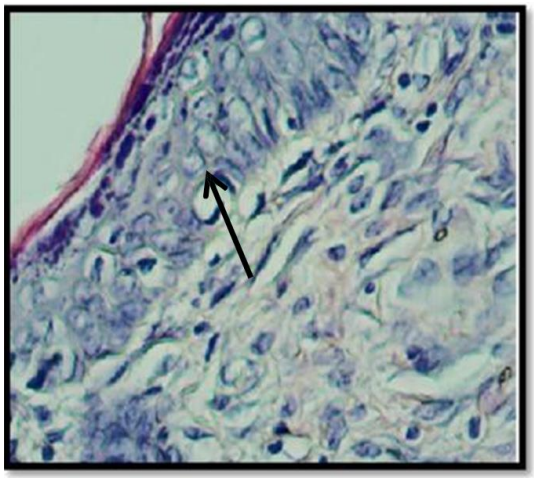

Figure 12: Microphotograph after 10day of osteopontin application shows. Epithelial cells at defect edge. H&EX40

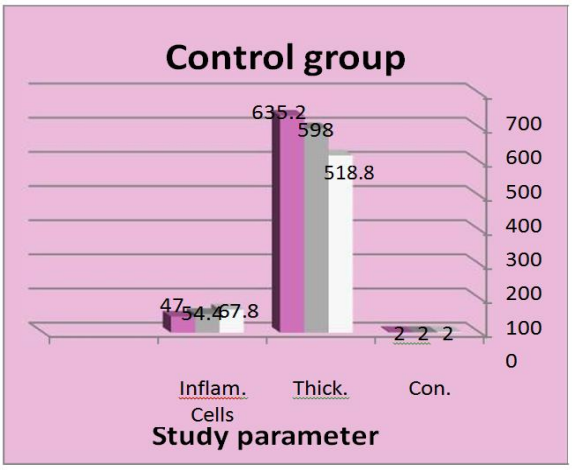

Figure 13: Control group of number of inflammatory cell, Thickness and contraction of incision

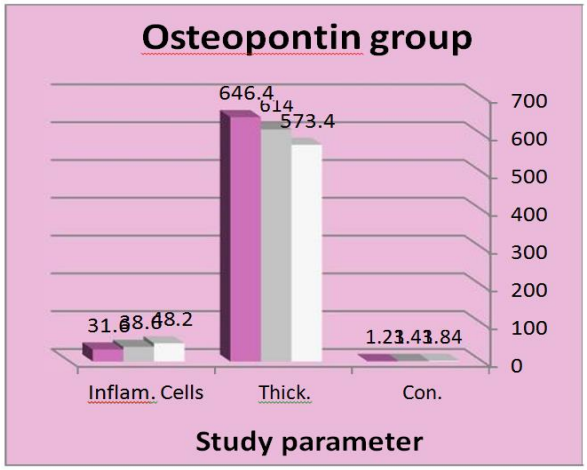

Figure 14: Osteopontin group of number of inflammatory cell, Thickness and contraction of incision.

DISCUSSION

Wound can be described as loss of tissue integrity which is a result of pathological change or physical trauma. Wound healing is a natural process to return the skin or mucosa back to it's normal state [(4)].

Epithelization is the process where epithelial cells (basal keratinocytes) arising from either the wound margins or residual dermal epithelial appendages within the wound bed with additional epithelial cells provided by the proliferation of immature keratinocytes in the basal layer begin to migrate over the underlying viable connective tissue [(5)].

The results of control group showed epidermis immediately adjacent to the wound edge begins thickening within 24 h after injury. Marginal basal cells at the edge of the wound lose their firm attachment to the underlying dermis, enlarge and begin to migrate across the surface.

The results of control group showed gradual decrease in epithelization. While for the experimental group the process of wound surface epithelization was enhanced and accelerated by topical (osteopontin) application [(1)]. Decrease in epithelization . While for the experimental group the process of wound surface epithelization was enhanced and accelerated by topical (osteopontin) application.

Also the results of epithelization for both control and experimental groups reach its peak at day 5 then declined 10 days and this result in agreement with previous study [(6)]. Who found epithelization reaches its peak at5days then decline may be due to remodeling process that associated with final stages of healing. Multiple studies have demonstrated that osteopontin is expressed by inflammatory cells such as macrophages and highly induced during inflammatory activation [(7)].

Osteopontin controls immune cell functions including monocyte adhesion, migration differentiation and phagocytosis, the induction of monocyte and macrophage chemotaxis and cellular motility as well as migration by OPN occurs via direct interaction with several different cell surface receptors [(8)]. This interaction is mostly mediated by two different binding domains.

Osteopontin further inhibits IL-10 expression by macrophages and thereby decreases anti-inflammatory signaling pathways**,** Collectively, these in vivo studies support altered innate immune responses in OPN mice, consistent with the well-described regulation of monocyte/ macrophage migration and invasion by OPN, and point to an underappreciated role of OPN as a potent mediator of cellular immunity [(7)].

As for as wound contraction in order to mimic the healing of human skin, some investigators have attempted to limit the effects of contraction during wound healing in rats, by creating large skin wounds and stretching the skin with adhesive bandages, ignoring the fact that contraction is an essential part of the healing process [(9)].

A Contraction of wound is the phenomenon in which the size of wound is reduced by the inward movement of wound margins, wound contraction begins 4 to 5 days after initial injury and actively continues for approximately 2 weeks. Wound contraction is characterized by a predominance of myofibroblasts at the wound periphery. However, the process of wound contraction is cell mediated and does not require collagen synthesis. In this study wound contraction was accelerated in experimental group and in 5 and 10 duration as a compared to the control group.

The result of present study shows that there was a marked increase in the number of inflammatory cells that infiltrated into the wound sites in the experimental group during period of 1 and 5 days as a compare with control group, while a higher count was detected at 10 days for control group than experimental group during histological scoring system, we found a higher rate of inflammatory cells in biopsy of control group while that used topical osteopontin application decrease than control group during early period of wound, then a lower rate of inflammatory cells for control group than experimantal group in late peroid of healing. The highest mean values of inflammatory cells were recorded at the 1 and 5 days for experimental and control groups and this result in agreement with previous study **[(10)].** Who improved that there was highly evidence of inflammatory cells in first week on wound done in rabbit skin and there is little evidence of inflammatory cells in 2 to 3 weeks.

The result of present study showed the defect area, no epithelium is formed and damage tissue at 1 days of skin incision in control group, this histological observation in agreement with previous study [11].

In experimental group at 1 days, histological observation showed area of granulation tissue formation, epithelial cells migration, new hair follicles were formed, numerous blood vessels and numerous inflammatory cells and this findings agree with previous study **[11]**. Who found after 4 days of wound healing there were migration of epithelial cells, the necrotic debris on wound surface was almost removed, the regeneration of hair follicles was also recorded also histological evaluation revealed the inflammatory phase during the first 5 and 10 days after surgery with blood cells.

CONCLUSION

It can be concluded that topical application of osteopontin showed efficacy in induced healing of wounds in rats.

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