

## Effects of Rat's Licking Behavior on Cutaneous Wound Healing

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### Abstract

#### Objective(s)

Wound licking has been shown to advance wound healing among humans and many other animals. The present study evaluates the licking effects on healing of skin wound in rats.

#### Materials and Methods

Twenty four rats were assigned to 4 different groups randomly and two 3 cm longitudinal full thickness incisions were made on each dorsal and ventral side of rats. The ventral incisions were considered as treated wounds because of contact to saliva as rats lick them easily and dorsal incisions as control wounds. Clinical changes and histopathological effects of rat saliva on wound healing were evaluated every day and on 3, 7, 14 and 21 days post-operation respectively.

#### Results

Histologic and clinical evaluation of treated wounds showed better healing than control wounds.

#### Conclusion

This study showed that licking behavior can promote wound healing. Thus salivary compounds could be isolated, be mass produced and may have potential to become as common as antibiotic cream.

**Keywords:** Licking behavior, Rat, Saliva, Wound healing

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### Introduction

Wound healing is a dynamic, interactive process involving soluble mediators, blood cells, extracellular matrix and parenchymal cells. Wound healing has three phases: i) an inflammatory phase ii) a proliferative phase and iii) tissue remodeling phase (1). In inflammatory phase, blood clot causes hemostasis; some mediators release and recruit neutrophils, which are rapidly replaced by mononuclear cells to the site of injury. Infiltrating neutrophils and macrophages clean the wounded areas of foreign particles, bacteria, and damaged tissue. The proliferative phase is characterized by the proliferation of fibroblasts, extracellular matrix deposition and re-epithelialization. Remodeling and organization of collagen occurs during the transition from granulation tissue to scar formation. Excess collagen in the wound site is degraded by several proteolytic enzymes and leads to the completion of tissue repair (1). It is well known that biological substances such as cytokines, chemokines and growth factors are closely involved in every phase of wound healing process (2).

The salivary gland is considered to be a reservoir of many growth factors in rodents. Saliva has some components including epidermal growth factor (EGF), nerve growth factor (NGF) and secretory leukocyte protein inhibitor (SLPI) that seems to play an important role in the wound healing of skin. This is based on the behavioral observations (increased self and communal licking) among animals following wounding (3). These substances stimulate infiltration of inflammatory cells into the wound space; induce proliferation of keratinocytes and fibroblasts, lead to angiogenesis and granulation tissue formation. Saliva also contains other biologically active substances such as kallikrein (4, 5), amylase (6), lysozyme (7), immunoglobulins (8) and renin (9). Lysozyme is believed to enhance healing by suppressing infection (10). In fact topical application of some growth factors is successful to accelerate healing of full thickness in rat. Therefore, probably wound healing of skin enhances by licking in animals such as rat because of growth factors deposit onto the wound area (11). This study was aimed

to investigate the effect of rat salivary glands on the healing process of skin wounds which rat could lick them naturally.

### Materials and Methods

All experiments were performed on 24 almost one-year-old Wistar rats, weighing 200-250 g, in accordance with the Animal Ethics Committee for the Isfahan Medical University. All groups of rats were allowed to equilibrate in standard conditioned animal houses at the Isfahan Medical University for one week before use. Animals were randomly assigned to four treatment groups (each consisting of 6 rats) before wound induction procedure was initiated. All animals were housed one per cage.

Rat skin was shaved and sterilized using 70% ethanol in dorsal and ventral regions of each animal. The animals were anesthetized by intraperitoneal injection of ketamine (50 mg/kg) and xylazine HCl (5 mg/kg). Using scalpel blade, two 3 cm longitudinal full thickness incisions with the same depth and diameter were made on each dorsal and ventral side of rats. At the end of the procedure, wound was closed in double layer in a continuous method (skin with 3-0 silk suture). The ventral incisions were considered as treated wounds because of contact to saliva as rat lick them easily and dorsal incisions (control wounds) didn't have any contact with saliva.

Clinical changes in the appearance of the wounds were evaluated each post-operative day in order to document edema, hyperemia, reepithelialization, debris, exudation and quality of the healed wound. Six animals in each group were sacrificed by ether inhalation. Skin samples were removed from dorsal and ventral wounds of animals on 3, 7, 14 and 21 days post-operation and fixed in 10% neutral buffered formalin, dehydrated with graded ethanol and embedded in paraffin. Tissue sections of 5  $\mu$ m in thickness were stained with hematoxyline-eosin and studied with an ordinary light microscope.

### Results

#### *Macroscopic findings*

Clinical evaluation of treated wounds on the 3rd day of post-operation (PO) showed more hyperemic areas and edema but less crust and

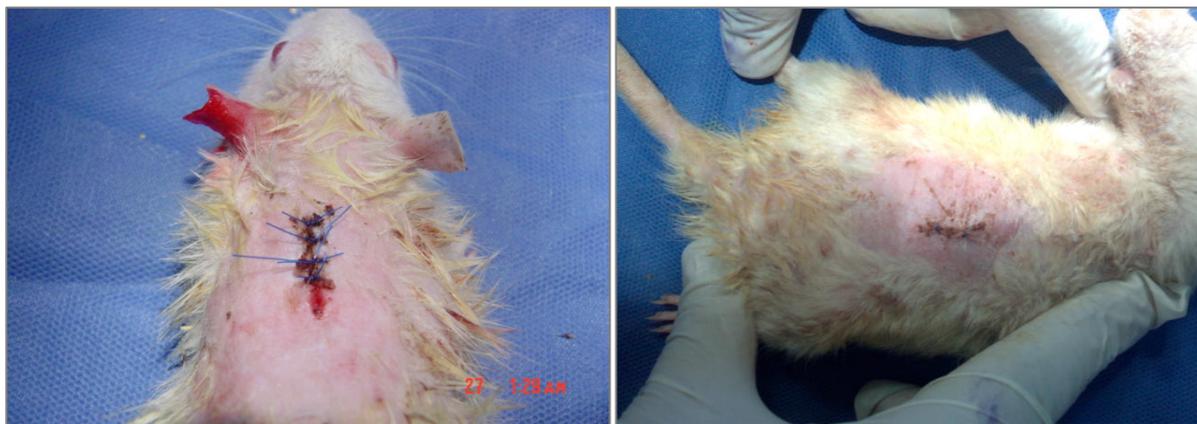


Figure 1. Macroscopic evaluation of control and treated wounds at 7 days after incision. Control wound shows more hyperemia, edema and crust on wound surface in comparison with treated wound which shows a better re-epithelialization



Figure 2. Macroscopic evaluation of control and treated wounds at 21 days after incision. Complete new epithelium formation with hairs that line wound surface.

hyperemic areas and edema but less crust and debris on the wound borders in comparison with control wounds.

On the 7<sup>th</sup> day PO, a quantity of crusts and debris was detected surrounding the control wound's margins. These wounds were more hyperemic and edematous. We observed faster regeneration of the new epithelium and better debriding effect in ventral wounds (Figure 1). No secretion, edema or hyperemia was observed on the 14<sup>th</sup> day wounds, although the treated wounds presented better clinical evolution than control wounds. On the 21<sup>th</sup> day PO, ventral wounds showed complete reepithelialization with hair follicles lining the surface of wounds (Figure 2).

#### **Microscopic findings**

On the 3<sup>rd</sup> day (PO), a few neutrophils and more infiltration of macrophages and

lymphocytes were observed in wound areas of dorsal and ventral incisions. There were blood clot and debris on the wound surface. Reepithelialization was started from wound edges. No significant differences in population of inflammatory cells and reepithelialization were observed in two incisions but vascular dilation and dermal edema were prominent features of ventral incisions.

On the 7<sup>th</sup> day (PO), ventral incisions showed more advanced re-epithelialization and layering with continuous basement membrane in addition to a better angiogenesis, proliferation of fibroblasts, deposition of extracellular matrix and a decrease of wound macrophages. No crust was observed on wound area. Control wounds had a presence of crust, and infiltrated granulation tissue with active fibroblasts. Fourteen days after wound induction, both incisions showed deposition of

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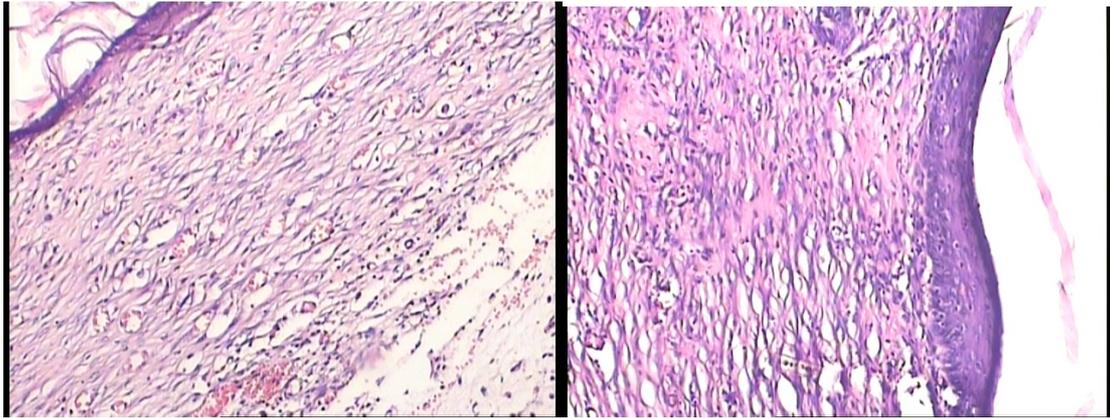


Figure 3. Histopathologic evaluation of control and treated wounds at 14 days after incision. Treated (ventral) wound (left) shows denser collagen fibers deposition, decreased angiogenesis and hyperemia as compared to control (dorsal) wound with dilated new capillaries, more hyperemia and edema in dermis.

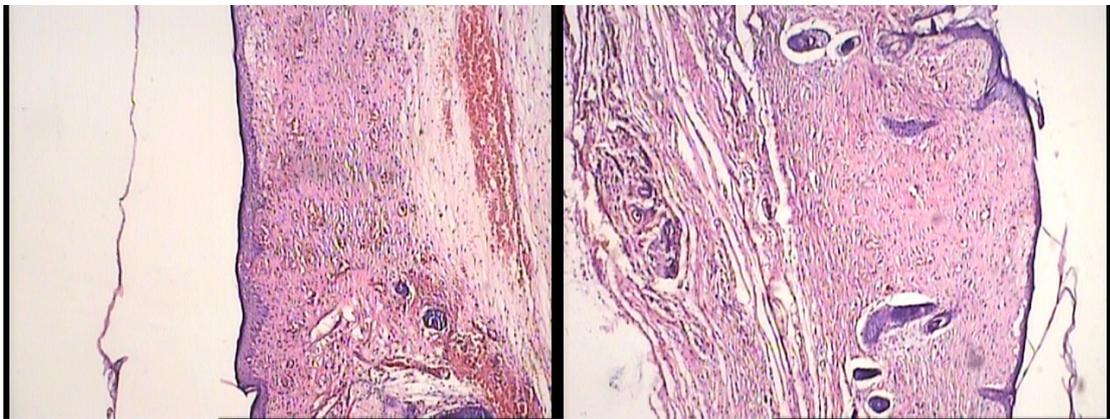


Figure 4. Histopathologic evaluation of control and treated wound at 21 days after incision. Treated (ventral) wound (left) shows thin well-formed epidermis, hair follicle formation in the dermis, decreased hyperemia and new capillaries compared to control (dorsal) wound with no hair follicle formation, hyperemic granulation tissue and thicker epidermis.

collagen but the granulation tissue was more dissolved than control wounds (Figure 3).

Twenty first days after operation, ventral incisions were filled by organized connective tissue rich in fibrocytes and fewer fibroblasts and lesser neoformed capillaries than dorsal incisions (Figure 4). The progressive increase was observed in the thickness of collagen fibers and hair follicles and a greater decrease in the number of fibroblasts and blood vessels in the ventral wounds.

### Discussion

The wound healing process can be divided into epithelialization, contraction, collagen synthesis and scar remodeling. Tissue repair process occurs in an ordered sequence of events, beginning with an inflammatory response of the wounded area (that lasts about 3 days), followed by the formation of

granulation tissue (3-7 days after lesion); then the accumulation of new connective tissue (7-14 days after lesion) and finally the remodeling phase (which may take some months to be completed) (12). A variety of biologic substances can accelerate wound repair. Saliva has been shown to play an important role in promotion of healing of oral or cutaneous wounds. Hotson *et al* (1979), studied the effect of salivary glands on wound contraction in mice and suggests that contraction is affected by the salivary glands (3). Grossman *et al* (2004), investigated the effect of crude extracts of rat salivary glands extracts on proliferation of cultured skin cells as a wound healing model (13). They suggested that each salivary gland had a specific effect on wound healing and the combination of the three extracts had an additive effect.

Saliva contains numerous bioactive molecules which were classified according to their various functions: lubrication, antimicrobial activity, antioxidant activity, inflammatory and immune responses and wound healing (14). The exact mechanism through which the salivary gland extracts might affect cell proliferation and wound healing is not yet known. The salivary gland growth factors include epidermal growth factor (EGF), nerve growth factor (NGF), transforming growth factor- $\alpha$  (TGF- $\alpha$ ), insulin, insulin-like growth factors I and II (IGF-I, IGF-II), transforming factor- $\beta$  (TGF- $\beta$ ), and basic fibroblast growth factor (bFGF) which help in reconstitution and healing of injured area (15). Epidermal growth factor (EGF) has been first identified in secretion of mouse submaxillary salivary glands known to promote the reepithelialization stage in wound healing (16). Noguchi *et al* (1991), studied the effect of salivary epidermal growth factor (EGF) on wound healing of tongue in mice (17). They removed submandibular glands (which are the major source of salivary EGF) 2 weeks before infliction of wound on the tongue and showed a significant delay in sialoadenectomized mice wound healing compared with control group. They suggested that salivary EGF was involved in the promotion of wound healing of the tongue in both male and female mice. Sialoadenectomy and selective desalivation of experimental animals has showed that the function of submandibular and sublingual glands are the most active in promoting cutaneous and oral wound healing (13). It is suggested that the stimulatory effect of the submandibular and sublingual extracts on wound healing were stronger than parotid extract in desalivated rats (18, 19).

Present study investigated the effects of rats' licking behavior on their wounds. Two longitudinal full thickness skin incisions were made on each dorsal and ventral side of rats. The rats could lick ventral incisions (treated wounds) but dorsal incisions (control wounds) were not available for animals for licking. After 3 days PO, the treated wounds showed

more hyperemia and edema than control wounds. By light microscopy, edema was assessed through the presence of spaces between granulation tissue. Also, more dilated capillaries and congestion were observed in treated wounds.

On the 7th PO, reepithelialization, angiogenesis and number of fibroblasts were more commonly observed in the treated wounds than control ones. On the 14th PO, histopathologic findings such as increase in the degree of re-epithelialization, thickness of the granulation tissue, and the density of extracellular matrix in treated wounds as compare to the controls showed a better healing. Twenty first days PO, treated wounds showed better organization and maturation of collagen fibers and hair follicles formation. Our findings provided evidence that saliva had a biological ability to improve the degree of the parameters of wound healing in treated wound. Histopathologic parameters such as the increase in the degree of reepithelialization, the thickness of the granulation tissue and the density of extracellular matrix are evaluated for improving of wound healing in this study.

## Conclusion

Our findings answer the biological question of why animals lick their wounds. In fact, wound licking is successful to accelerate healing of full thickness wounds. We suggest that the salivary compounds can be isolated and be mass produced. They have the potential to become as common as antibiotic creams and rubbing alcohol. It is needed to do further studies to evaluate the effect of topical application of salivary-derived biological factors such NGF, EGF, FGF on wound healing.

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