ISSN 2349-7823

International Journal of Recent Research in Life Sciences (IJRRLS)
Vol. 6, Issue 4, pp: (27-30), Month: October - December 2019, Available at: www.paperpublications.org

The effect of intermittent negative pressure in mice wound healing; a pilot study

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Abstract: **Wound healing could be disfiguring, prolonged, and disappointing. The management of scar could be as simple as leave it alone, to surgical excision. The use of negative pressure in wound management proved to reduce the wound epidermal thickening for a transient period. This pilot study was performed to observe the difference in time and features of wound healing in albino mice with and without negative pressure, and also to perform histological analysis to the wound and report the changes, if found, at the cellular and extracellular level. Intermittent negative pressure (INP) application to the wound has been tried in this experiment to estimate its effects in mice skin with a recent wound. One albino mouse of the genus *Mus musculus* was purposefully wounded on abdominal wall. Two wounds were performed on the mouse; one as a test and the other was a control. The test wound was exposed to manually operated INP sessions ten minutes daily for two weeks. Wound healing was delayed by 48 hours in the test scar, as compared to control. The test histology showed signs of an inflammatory reaction and epithelial distortion. This study concluded that applying intermittent negative pressure in mice wounds delays the wounds healing and causes an inflammatory-like response.**

Keywords: **Negative pressure, wound, experimental mice.**

1. INTRODUCTION

Physical insults to the body cause injury. Injury causes wounds, and wounds heal by the formation of scar [1]. Mammalian fetuses, including humans, do not form scars during healing [2], [3], [4], [5]. Although the precise cause for this difference is unknown, the study of the healing mechanism between adults and fetal tissues showed characteristic differences in the proteins, growth factors, and cells that involved in tissue healing [2]. In general, we could say that wound healing in the vertebrates ranges from total restoring of tissue as in fetuses and amphibians to the formation of ugly discomforting hypertrophic and keloid scarring in humans [2], [6]. Scarred skin brings about figure acceptance issues, cosmetic issues, psychological and emotional disturbances, all of these affect the quality of life of the individual [7], [8], [9], [10]. Scars also could be painful, itchy, and disturb the movement. This thesis is a trial to observe the effects of intermittent negative pressure vacuum into the wound of adult mice and measure the outcome. Mouse was used to avoid the inconvenience of causing pain, discomfort, and any possible complications to human volunteers. Vacuum therapy, in the form of a massage, was introduced forty years ago to help treating burn scars [11], [12]. A French invention, Endermologie® system, was used for this purpose [11], [13]. It was supposed to modulate the scar appearance and make its surface smoother [13]. Another device, PRUS® vacuum device, was used for studying the efficacy of negative pressure in burn scar. Meirte et al [12], studied the PRUS device's effect. There was a significant reduction in the epidermal thickness and increase in the dermis layer girth. Unfortunately, the changes were transient, and diminished after about two hours; nevertheless, this 2016 study gave hope about the possibility of vacuum to enhance the extracellular matrix (ECM) remodeling and proliferation. The writers themselves attribute the increased dermal thickness to edema [12], which is probably due to the prolonged time interval used in this study. In this current study, we depended on manual vacuum device, and performed more frequent sessions with shorter vacuuming time, to avoid the development of edema. The model was an experimental mouse with two recently healed excision wounds.

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2. MATERIALS AND METHOD

An eight weeks old male *Mus musculus* was brought from the veterinary research center in Khartoum city. Two diamond-shape excisional wounds were performed under anesthesia in the lateral abdominal wall; see figure (1). Diethyl Ether was used as inhaled anesthetic. The wounds were (1.0 ×1.0) cm in size. One wound was used as a test, and the other as a control. After the primary closure of wound by contraction, a fifty ml syringe was used to apply the negative pressure at the site of scar. The syringe head was cut, so that the scar can be totally contained inside the syringe. Negative pressure was applied carefully by vacuuming the syringe in short intervals; four seconds vacuum, four seconds rest this was repeated ten minutes, daily for two weeks, after that, the test skin was excised for microscopy and histology. Types of histological stains used were:

1. Hematoxilin and Eosin (H&E) stain: This stain is prepared to demonstrate the cell nuclei that appears as blue foci, and cytoplasm that appears red or purple.
2. Mayer's Hematoxilin: This stain method demonstrates the Nucleus which appears clearly blue within the red- pink colored cytoplasm.

Ethically, all practical procedures were performed following the regulations of the 2002 Institutional Animal Care and Use Committees (IACUCs) [14].

3. RESULTS

1. Macroscopic notes:

The control scar healed earlier than the test scar by approximately 48 hours; see figure (2).

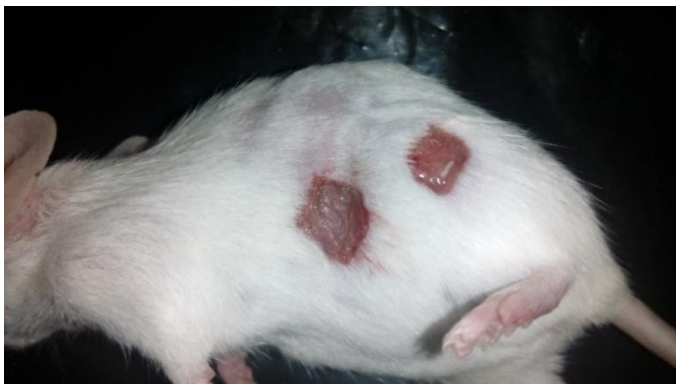

Figure 1: Shows the mouse with the diamond shaped scars after two days of skin excision. Within six days, the two wounds were almost fully contracted.

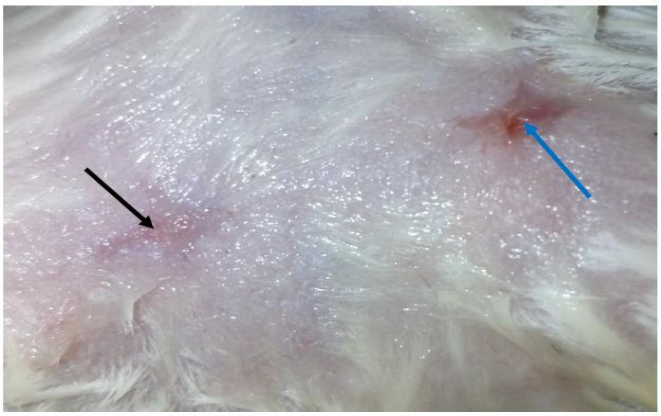

Figure 2: the control and the test scar at the end of the experiment; notice that the control scar (shown by black arrow) healed faster than the test scar (shown by blue arrow).

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2. Microscopy findings:

Hematoxilin and Eosin stain shoed increased infiltration of inflammatory cells with evidence of fluid accumulation, suggestive of edema and congestion. Epithelial cells look distorted and less organized at multiple areas of the test slides; revise figure (3). The fibrous tissue in test slides looked denser; however there were no detectable intracellular abnormalities. Mayer's Hematoxilin staining showed no significant difference between the test and control slides.

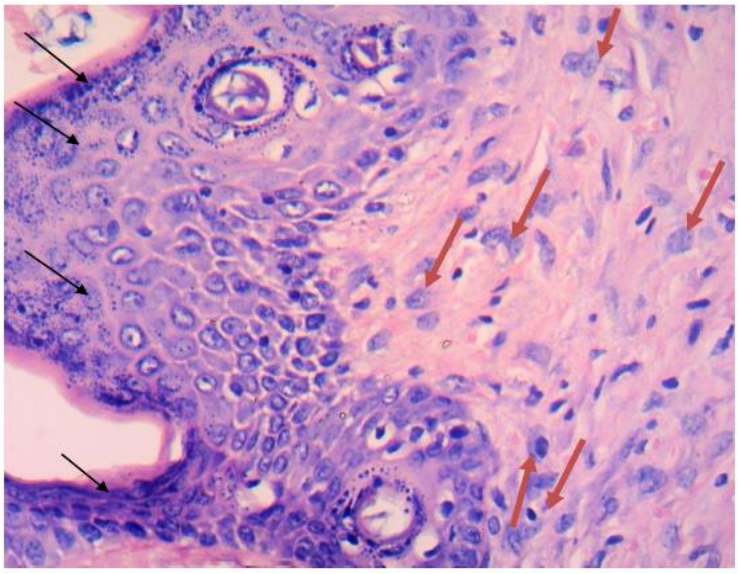

Figure 3: Test slide of scarred skin picked from the periphery where epithelium is not removed; notice the non organized epithelia (black arrows) and multiple inflammatory cells (brown arrows).

4. DISCUSSION

The results could be summarized as follows: applying intermittent negative pressure (INP), for two weeks into the excision wound of experimental mouse caused inflammatory-like changes, epithelial distortion, and delayed wound healing. The inflammatory-like response could be attributed to the traumatic effect of the negative pressure, likewise is the epithelial distortion. The delay of healing in the test scar may be due to the disturbed blood flow. The inflammatory results mimics the results obtained by the PRUS experiment; there was also an edematous response in the dermis[40]. However, the PRUS works in a different manner, where it applies a continuous vacuum for several minutes accompanied by massage movements. Mierte et al were seeking, through PRUS, for a permanent ECM remodeling response, but the response lasted for a maximum of two hours[40]. Major inconvenience in the study is that it was done on a small sample, short duration, and also the methods used were modest and lacking the negative pressure measurement facilities, and depended mainly on single calibration with a negative pressure gauge. It is suggested to perform more studies of negative pressure on animal models to be held on hypertrophic and keloid scars, taking in consideration the different levels and stages of the scar. Combined vacuum with topical drug management might be taken in consideration, such as steroids and INP, Cytotoxic drugs and PRUS, or physical compression combined with cryosurgery.

5. CONCLUSION

Intermittent negative pressure was applied to an inflicted wound in an experimental mouse. The wound healing was delayed for 48 hours, and histologic examination showed elements of inflammation.

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