International Journal of Health Sciences and Research
Vol.10; Issue: 1; January 2020
Website: www.ijhsr.org
Original Research Article *ISSN: 2249-9571*

The Effect of Intermittent Negative Pressure on the Amputation Stump of Adult Mice: A Pilot Study

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ABSTRACT

Overview: Limb amputation could occur accidently or due to an inevitable medical indication such as cancer, vascular damage, or traumatic destruction. Whatever the cause, a lost limb does not regenerate in mammals and most animals; however, studies showed that young mice have an ability to restore a distally amputated finger tip. There are evidences the in literature supporting that applying intermittent negative pressure to tissues could trigger the release of growth factors; which are important for tissue regeneration.
Objectives: To document the effects of the intermittent negative pressure-INP in an amputation stump of albino mice, particularly if there are evidence of cellular proliferation or tissue remodeling.
Methods: Two albino mice, three weeks of age, were first cut at the wrist level on their right arms. One mouse was subjected to manual INP by a fifty milliliter pump syringe, ten minutes daily for three weeks, the other mouse left to heal spontaneously, as a control.
Results: There was an excessive fibrous tissue formation within the stump, seen by the naked eye. Microscopic examination showed thinning of the epithelial lining, areas of fluid and inflammatory cells collection, and excessive fibrous tissue formation.
Conclusion: Negative pressure does not enhance the growth or regeneration of amputated limbs in adult mice; instead, it caused an inflammatory-like response and fibrous tissue formation.

Keywords: Amputation, Negative pressure, Albino mice.

INTRODUCTION

Amputation is the act of removal- especially by cutting- of part of the body. The term is used mainly to the limbs, but it could include other body parts such as the nose, ears, tongue, penis, and sometimes for the breasts. Among all species, amphibians have a distinctive ability to reform lost parts of their body, [1,2] and this might be due to the late onset of maturation of limb buds, which make their development to be independent from other embryonic roots. [1] Other amniotes cannot regenerate a limb, but mice embryos shown to restore only the tip of the digit. [3,4] The real difference between the amphibians and other organisms that they retain their abilities to produce Blastema cells; cells that can grow and differentiate to different cell lineages. [4] Other animals usually loose theses cell clusters after birth. [3,4] The limb regenerative capacity of mice is very limited, as for all mammals, [4] and the ability of the fingertip of mice to regenerate is explained by its pattern of ossification: fingertips ossify at the very distal tip of bone surface, while the other proximal bony tissues ossifies and gain length through the embryonic end plates. [5]

The use of negative pressure had become a cornerstone in the treatment of chronic wounds. The negative pressure wound therapy (NPWT) is a mechanical system uses the application of negative

pressure to heal and drain a preformed ulcer. It is used in the treatment of diabetic foot wound and any chronic ulcerated wound of the foot. [6] The conventional NPWT system is composed of a suction device to give the negative pressure potential, a sucking tube connected between the device and the wound surface, and at the wound surface there is foam gauze shaped to fit the ulcer area, and a plaster cover to ensure the sealing and isolation of the negative pressure area. [7] It is hypothesized that NPWT increases local expression of anti-inflammatory factors such as IL-8 and IL-10 and growth factors such as PDGF, VEGF, TGF-β, and PDGF. [8] The molecular responses to NPWT are attributed to the Mechano-transduction and chemical transduction of the cells. [8, 9]

We were aiming in this thesis to study the changes that could possibly affect the amputation stump in the mice model by administration of intermittent negative pressure; would it accelerate the healing, remodel the stump site for better placement of the prosthesis, or induce any form of tissue growth.

MATERIALS AND METHODS

This was a case-control experimental study, performed in the laboratories of the veterinary research centre, Khartoum city, Sudan. The study duration was three weeks. Two mice, of the genus Mus *musculus*, were chosen; one mouse was tested, and the other was a control. Mice were anaesthetized by Di-ethyl Ether inhalation, cut with a scalpel above the wrist, and then given three days for wound healing and recovery. Each mouse was cut at the right anterior limb. Intermittent negative pressure was delivered daily to the test mouse for ten minutes. Negative pressure was performed by a small suction pump that fit perfectly into the stump. The suction was performed in intervals as follows: four seconds negative suction, four seconds to allow the syringe handle to recoil, and four seconds rest. Intervals are important to avoid the development of edema and pain. Three weeks later, results were documented by naked eye examination and photography. The skin in the negative pressure area was excised for further histological examination. (10×15) millimeters of skin and muscle tissues, from the stump, were taken from each mouse. The two mice were sent back to the veterinary research center. Histological staining that had been performed was:

1. Hematoxylin and Eosin (H&E) stain: It is a general stain that visualizes the cytoplasm of the cell as a light pink colored fluid, and the nucleus as a purple or a blue dot.
2. Mayer's Hematoxylin: A method derived from the above H&E stain. The nucleus here appears better defined, and could be blue or black.

All practical procedures on mice were performed following the regulations of the Institutional Animal Care and Use Committees (IACUCs). [10]

RESULTS

- **Macroscopic examination:**

In all the four mice, no visible growth was appreciated, neither in the bones or the soft tissues. However the two test mice formed what appeared as a fibrous plug in the amputation stump, see figures (1). Erythema was noticed after the application of negative pressure, and disappeared after the session in an average of five minutes.

- **Microscopy:**

In H&E stain, epithelial lining looked more irregular and decreased in thickness; collagen looked denser in some areas, with evidences of fibrosis. There were signs of inflammations- fluid accumulation and presence of inflammatory leukocytes, around the fat cells; revise figures (2) and (3). Mayer's Hematoxylin also showed loss of epithelial lining, and decreased girth, also there was increased fibrous tissue formation, but no evident inflammatory signs. There was loss of continuity in the sub-dermal matrix. See figures (4) and (5)

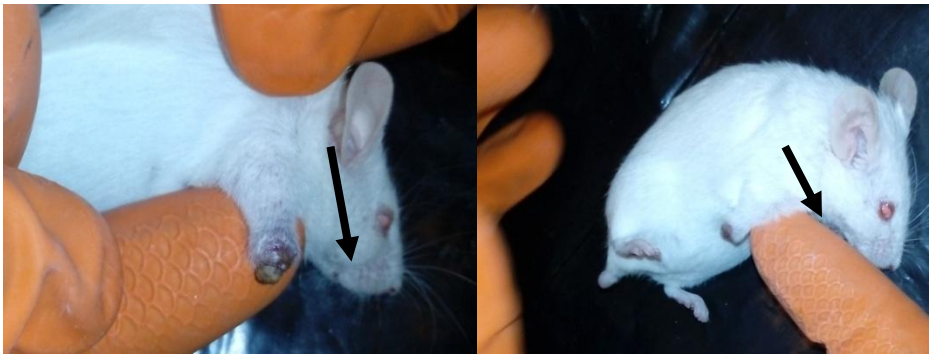

Figure (1): The test mice (Left): The stump became elevated and hypertrophied. Fibrous tissue was formed excessively (Shown by the black arrow).The control mice (right): The stump is blunt as seen, and no excessive fibrous tissue was formed (Shown by the black arrow).

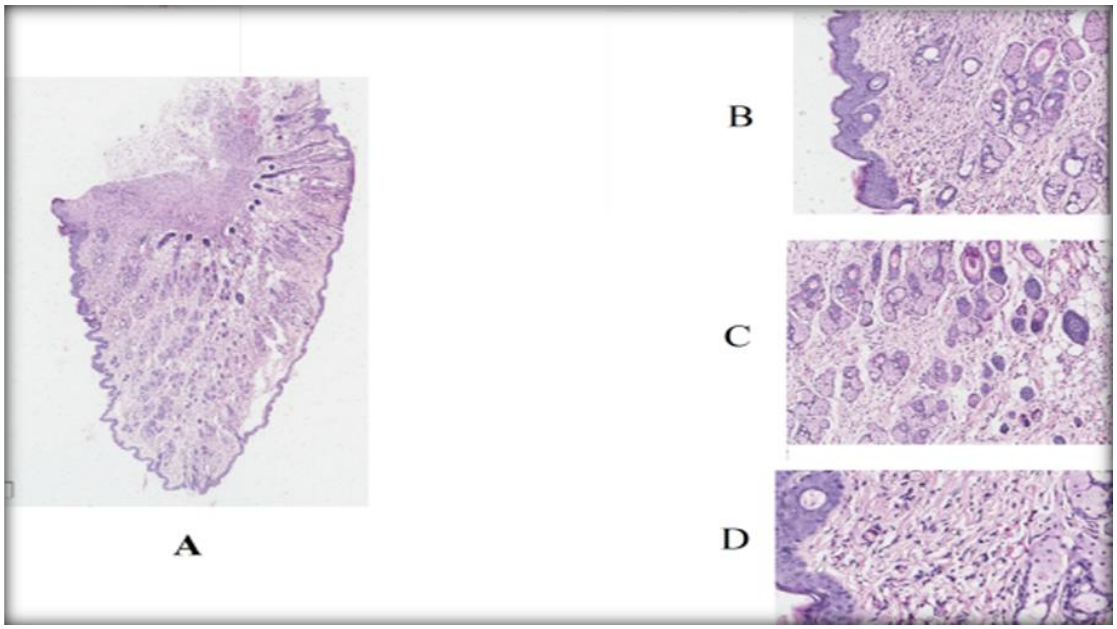

Figure (2): Control H&E, showing the original slide A. B shows normal epithelium thickness (4×). C shows the regular distribution of fibrous tissue and sebaceous glands (4×). D demonstrates the normal connective tissue (10×).

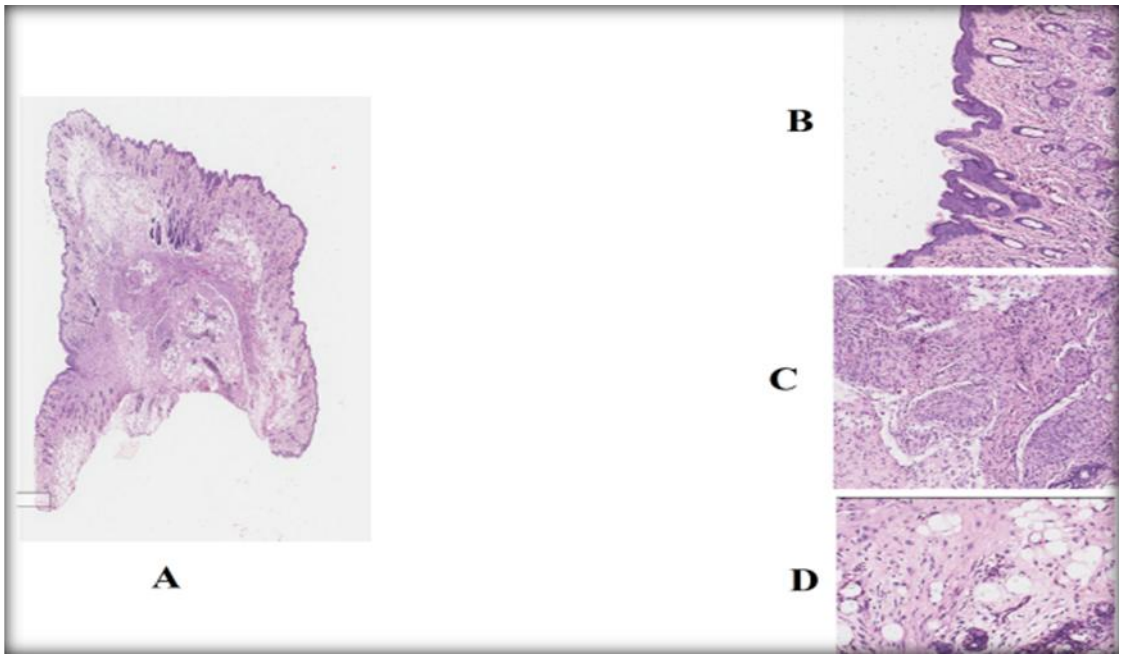

Figure (3): Test H&E, where A resembles the original slide. B shows thinned epithelial lining (4×). C shows the extensive fibrous tissue formation and hyperdiensity of collagen (4×). D shows the accumulation of fluid and inflammatory cells surround the adipocytes (10×).

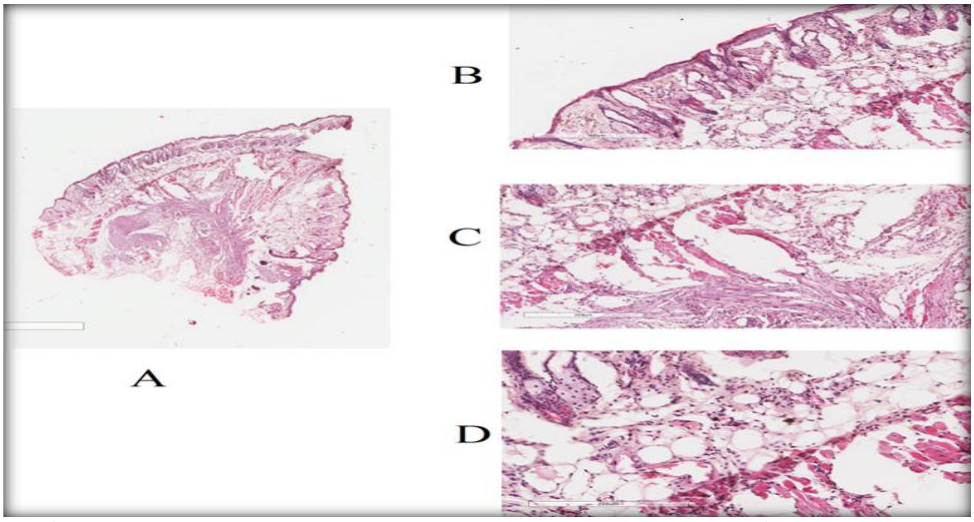

Figure (4): Control Mayer's Hematoxylin. A shows the original slide. B shows the regular lining of the epithelium. C shows the dermal layer. D shows the peri-adipocyte area; no signs of inflammation could be detected (10×).

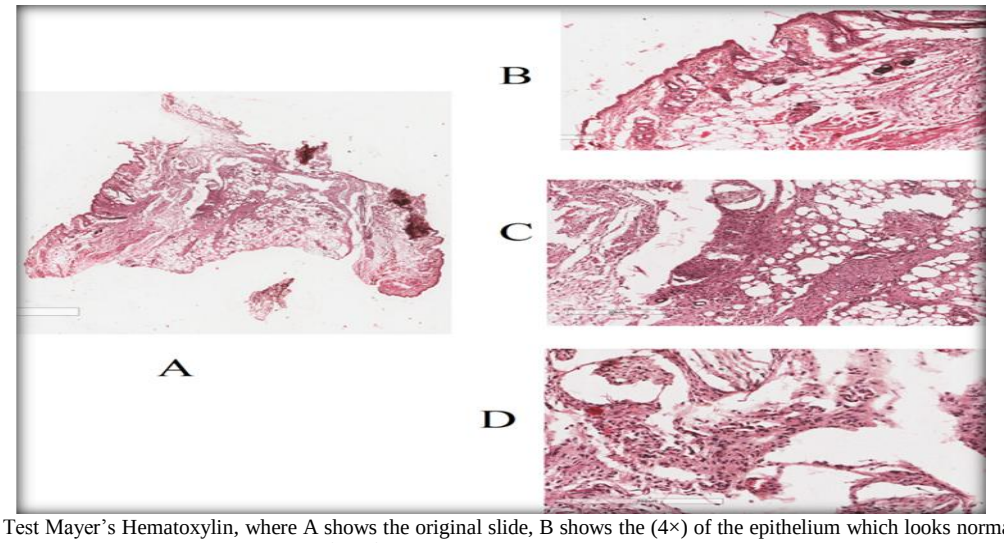

Figure (5): Test Mayer's Hematoxylin, where A shows the original slide, B shows the (4×) of the epithelium which looks normal. C shows fibrotic areas in the dermis. D shows (10×) view of the fibrous thickening in the dermis.

DISCUSSION

To summarize the results, it is convenient to say that the tested mice formed a fibrous plug within the stump area while the control mice did not. In the histology slides, there were mild clues of edema. These findings reflect the traumatic nature of the negative pressure, as seen in many researches, particularly those in the field of space medicine, in relation to decompression sickness. [11, 12] The inflammatory signs could also be attributed to the initial amputation process that might not healed completely, or due to minor trauma occurring inside the experiment cage, however these two possibilities cannot explain the reduction in hair follicles and sebaceous glands. One possible explanation is the interference of the repetitive INP sessions with the normal blood circulation, which had lead to the atrophy of the glands and follicles. When compared with the results of the Accessory limb model (ALM) researches, [4, 13, 14] it is obvious that the INP is disappointing, but may get benefit from combination of the ALM methodology, such as local BMP injection, [3, 4] or MMP administration prior to the INP. [15] However, it is expected to have negative results as mice are mammals and known to have a poor regenerative abilities. [4] Things to be taken against this study include the lack of the sufficient sample size, the short duration of the experiment and experiment preparation, the absence of accurate measuring means of negative pressure, and

the dependence only on macroscopic remarks and histology. It is suggested to carry out the same research on amphibians that are characterized by their high regeneration capacity, where more promising results are expected. Synthetic growth factors should be added for better outcomes. The experiment should also contain important diagnostic tools such as radio-imaging, Immunohistochemistry to detect inflammatory responses, and Flowcytometry for the detection of non viable cells.

CONCLUSION

This was an experimental study to document the effects of intermittent negative pressure on the amputation of an adult mouse. Amputated stump was subjected to INP sessions ten minute daily for three weeks. INP did not induce any form of growth; instead, it resulted in the formation of a massive fibrotic mass, and an inflammatory-like response in the histological examination.

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How to cite this article: Mohammedahmed Abbadi OS, Nafie KM. The effect of intermittent negative pressure on the amputation stump of adult mice: a pilot study. Int J Health Sci Res. 2020; 10(1):201-205.

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