Research Paper

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# Experimental Evaluation of Healing Process of Burn-wound Treated by Lyophilized *Aloe vera* Dressing

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In this research work, 30 albino rats were tested to evaluate the effectiveness of lyophilized *Aloe vera* dressing on wound healing process. The treatments were applied on the deep partial thickness burn wounds that were induced by modified electric solder. Three rats from each group were euthanatized on day 5, 10, 15, and 20 for tensile strength investigation. Another three rats were randomly selected from each group to be euthanatized on 24 hours (day 0) post-burn to determine the level of damage by histological study. Generally, the tensile strength increased on day 5 and 10 because of fibroblast activity and decreased on day 15 and 20 due to the rearrangement of the collagen. However, there is no significant difference ($P<0.05$) in the tensile strengths of both treated and control groups. The treated groups healed faster as compared to the control. The lyophilized *Aloe vera* dressing showed scar formation after 7 to 10 days of post-burn, and control showed scarring after 8-12 days of post-burn. However, all the wounds did not show any sign of infection, and no pustule was observed during the experimental period. Epithelialisation was observed in lyophilized *Aloe vera* dressing on day 5 and almost completed on day 10, whereas the control group showed signs of epithelialization only on day 15 and completed on day 20.

**Key words:** Burn-wounds, lyophilized, *Aloe vera*

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## Introduction

Several times, burn wounds are caused by accidents, suicidal attempts etc. Treatment of burn wounds with reduced risk of inflammation and pain has been a matter of concern (Kaufman *et al*., 1990). Therefore, this investigation is undertaken to evaluate the healing process of burn wounds. *Aloe-vera* is a perennial succulent native plant in East and South Africa, but also cultivated in the West Indies and other tropical countries. The strong, fibrous root produces a rosette of fleshy basal leaves. The tissue in the center of the leaf contains a mucilaginous gel that yields aloe gel or *Aloe vera* gel. *Aloe vera* or *Aloe barbadensis* has been recognized as having the most effective healing power in wounds. It contains 96% water, enzymes, trace of sugars, protein containing, 18 amino acids, vitamins and minerals i.e., sulfur (S), silicon (Si), iron (Fe), calcium (Ca), copper (Cu), sodium Na), potassium (K), manganese (Mn) etc. (Heinerman, 1998).

Now-a-days, doctors have used *A. vera* successfully for X-ray burns, sunburn, chemical and first degree burn, traumatized tissue, decubitus ulcers, primary candidal dermatitis, stomach ulcers, herpes simplex, periodontal surgery, insect bites and stings, irritating plant stings and other minor dermatological manifestations. It is believed that *Aloe vera* can relieve pain immediately, reduce swelling, stimulate cell growth and circulation, balance nutrition, reduce inflammation, kill bacteria, fungus and viruses and, in essence, it can normalize anything that is abnormal without causing toxic effect.

In this investigation the *Aloe vera* is used as a dressing material to test its efficacy in healing process of burn-wound in the rats.

## Materials and Methods

**Experimental animals:** Thirty adult laboratory rats of both sexes, weighing between 250 to 350gm were used. The rats were housed according to the treatment, one rat in one cage. The rats were given regular standard feed and drinking water.

**Lyophilized *Aloe vera* dressing:** Fresh *A. vera* were picked and cleaned with saline solution and soaked in 1% sodium hypochloric solution (NaOCl) for 10 minutes (the methods were modified from, Kaufman *et al*., 1990). Outer layer of the skins of *A. vera* was removed to expose the flesh, the flesh was cut into pieces of 5-6cm length, sliced into 0.5cm thick and layered with gauze. The flesh was refrigerated for 2 hours and then it was dried in 'freeze dryer' for 10 to 12 hours until the moisture content dropped to < 10 %. Lyophilized *Aloe vera* was triple packed and gamma (γ) irradiated (25kGy) for sterilization at the Malaysian Institute for Nuclear Technology Research.

**Wound inducing technique:** The rats were anaesthetized with intramuscular injection of xylazine (3-5mg $kg^{-1}$) / ketamine (40-90mg $kg^{-1}$). The hairs on the back and flanks on both the left and right sides were shaved. This area was disinfected with 70% ethanol ($C_2H_5OH$) before inducing the wound. A modified commercial soldering iron (200V/240V, 60W) with a round bronze plate (2cm diameter) on the top was used to induce burns. A constant (240V) electric current was supplied to the soldering iron for 10 minutes as to maintain suitable initial temperature to induce burns. After each injury (burn) the electric solder was elapsed for 5 minutes before reused, to ensure maintenance of the desired temperature of the bronze plate. The anaesthetized rats were restrained and kept stretched on a paraffin board. The heated electric solder was applied to the skin between the twelfth rib and the horizontal upper limits of the sacroiliac joints as described by Kaufman *et al*. (1990) for a period of 2 seconds. This location has loose skin and can prevent over-stretching that could affect healing process.

**Experimental design:** Thirty rats were used in this experiment, they were divided into 2 groups with 15 animals in each group.

1. Control group (untreated) : The burn wounds were not given any treatment. However, bandage and plaster were applied on the wound.
2. Lyophilized *A. vera* dressing (treated): The burn wounds were covered by lyophilized *A. vera*. The dressing was changed whenever the wound appeared moist and dressing continued until the rats were euthanatized.

**Determination of tensile strength:** The tensile strength determination was done on day 5, 10, 15 and 20 post-burn. The skin was cut into dump-bell shape (wounded part in the center) by a special cutter. Instron tensiometer was used to measure the tensile strength of the skin with 15.00 mm $min^{-1}$ of crosshead speed.

**Histological study:** The skin of 1cm wide and 1cm length (containing both normal and healing sides) were collected on day 0, 5, 10, 15 and 20 for histological studies. Paraffin sections of 5$\mu$m thickness were stained by hematoxylin and eosin (H and E) and examined under light microscopy.

## Results

**Tensile strength:** The treated group (Table 1) always showed higher tensile strength (mean ± S. D.) compared to the control during the experimental period. On day 5 post-injury, tensile strength in the treated group was 6.707 ± 2.914 while in the control it was 4.746 ± 1.893. On day 10 post-injury, tensile strength in the treated group slightly increased to 7.412 ± 0.687 but in the control group it decreased to 4.170± 1.332. On day 15 post-injury the tensile strength in the control and the treated groups were 1.664 ± 0.648 and 2.321 ± 0.33 MPa, respectively. Tensile strength of both the control and the treatment groups on day 20 post-injury were 1.345 ± 0.85 and 2.249 ± 1.175, respectively.

**Histological studies**

**Control**: These are typified by the illustration in Fig. 1. Immediately after wound induction (day 0, post-burn), entire epidermis was damaged, sebaceous gland and hair follicles were abnormal. No sign of epithelialisation and blood vessel was observed. Moderate numbers of polymorpho-nuclear leucocytes accumulated in the region of the deeper part of the dermis and a small collection of normal fibroblasts was found in the dermis (Fig. 1a). By day 5 post-burn, there was no sign of epithelialisation and hair follicle regeneration. Only mild numbers of polymorpho-nuclear leucocytes and fibroblasts dispersed in dermis (Fig. 1b). Beside that, normal sebaceous gland and blood vessel were not clearly observed. Inflammation process was still progressing during day 10 post-burn. Several polymorpho-nuclear leucocytes and moderate numbers of fibroblasts were accumulated in dermis. However, epithelialisation, regeneration of hair follicle, normal sebaceous gland etc., were not observed (Fig. 1c). By day 15 post-burn, newly formed epithelium that appeared to have migrated from hair follicle, was observed (Fig. 1d). Polymorpho-nuclear leucocytes, and sebaceous gland were not clearly observed. However, epithelialisation and mitosis of hair follicle were continually observed during day 20 post-burn. Mild collection of

fibroblast was found in dermis, but sebaceous gland, blood vessel and stratified epithelium were still not well developed (Fig. 1e).

**Lyophilized *Aloe vera* dressing:** The histological investigation in the treated group are typified by the photomicrographs shown in Fig. 2. On day 0 post-injury, moderate numbers of polymorpho-nuclear leucocytes were accumulated in the upper dermis region (Fig. 2a). Arrector pili muscle and sebaceous glands showed signs of damage (Fig. 2b). However, there was no sign of regeneration in hair bulb papilla region and epidermis. Only a few numbers of normal fibroblasts were found in the dermis and blood vessel was not observed. By the fifth day of post-burn, hair follicles were found in active mitotic form (compact collection of cells indicated active cell proliferation) and newly formed cells surrounded the bulb of hair follicle (Fig. 2c). Moderate numbers of polymorpho-nuclear leucocytes were found accumulated in granulation tissue and the upper region of dermis. But only mild epidermal repair with abundance of fibroblasts was found on day 5. The effect of the lyophilized *A. vera* dressing by day 10 post-burn was obvious, several basal cells originate from bulb of hair follicles migrated superiorly to form cellular layers to replace damaged epidermis. New regenerated epidermis consisted of stratified squamous epithelium and the cells become more flat superficially (Fig. 2d). Besides that, inflammatory cells were not clearly observed and moderate numbers of fibroblasts were distributed in dermis region. By day 15 after injury, regeneration of epidermis was almost completed and the experimental area showed relatively less inflammation as indicated by the absence of polymorpho-nuclear leucocytes (Fig. 2e). The structure of hair follicle and sebaceous gland became normal (Fig. 2f) with moderate numbers of fibroblasts and blood vessels were distributed in dermis. The healing process had completed within day 20 post-burn. No inflammatory cell was observed, while the hair follicles, sebaceous glands and epidermis were normal and well defined (Fig. 2g).

## Discussion

A day after the burn-injury, the wounds seemed to be clean and healthy in both control and experimental groups. The wounds treated with lyophilized *A. vera* dressing healed faster compared to the controls. In lyophilized *A. vera* dressing treatment, scars were formed 7-10 days after burn injury while control animals showed scaring after 8-12 days. Furthermore, there was no sign of infection and no pustule in all the wounds (both in the control and experimental animals) throughout this work..

**Tensile strength:** Table 1 depicts that the tensile strengths in treatment and control animals, lyophilized *A. vera* treated wounds continues to increase between day 5 and 10 compared to control which decreased in tensile strength during this period. Kenyon and Michaels (1983) have defined a fibroblastic phase (between day 3 and 10 post-injuries) in which fibroblasts entered the vicinity of the wounds so as to deposit tropocollagen that interacts with reticular fibers that caused to increase this tensile strength. However, the tensile strengths of both groups started to decrease on day 15 and 20 during differentiation phase. This is in accordance with the observation of Lee *et al.* (1986) where in the differentiation phase, re-arrangement and re-absorption of unaligned fiber processes reduce the tensile strength.

There is no significant difference ($P<0.05$) of tensile strengths in control and experimental groups during various intervals.

Table 1: Tensile strength of the skin in the control and treated groups

| Time post-injury | Tensile strength | |
|---|---|---|
| | Control (Mpa) (Mpa) | Lyophilized *A. vera* Dressing |
| Day 5 | 5.121 | 6.469 |
| | 2.694 | 9.733 |
| | 6.424 | 3.920 |
| | 4.746 ± 1.893 | 6.707 ± 2.914 |
| Day 10 | 5.292 | 7.584 |
| | 2.697 | 6.655 |
| | 4.522 | 7.997 |
| | 4.170 ± 1.332 | 7.412 ± 0.687 |
| Day 15 | 1.916 | 2.321 |
| | 2.149 | 1.991 |
| | 0.929 | 2.650 |
| | 1.1664 ± 0.648 | 2.321 ± 0.33 |
| Day 20 | 1.160 | 1.482 |
| | 2.273 | 3.601 |
| | 0.603 | 1.663 |
| | 1.345 ± 0.850 | 2.249 ± 1.175 |

This result is found to be different from the incision wounds investigation (Al-Sadi and Grourley, 1977; Kenyon and Michaels, 1983; Lee *et al.*, 1986) in which the entire dermis and epidermis are involved.

**Histology**

**Dermis:** On day 0, in the treated group moderate numbers of polymorpho-nuclear leucocytes accumulated in the upper dermis compared to less numbers of polymorpho nuclear leucocytes distributed in lower dermis region in control group indicated that healing process in this control group was slower compared to the treated group. The reduction in the numbers of polymorpho-nuclear leucocytes caused a corresponding decrease in the numbers of eosinophils, basophils and lymphocytes. Eosinophils and basophils are believed to be important in autacoid (a critical aspect of early response to injury concerning increased blood flow to the injured site to provide enhanced delivery of blood-borne solutes and cells important to the repair process) to stimulate cell growth and DNA synthesis during healing process (William, 1981). Polymorpho-nuclear leucocytes observed in control group increased greatly in number only on day 10, compared to the treated group in which this condition was observed earlier on day 5. However, during later stages (on day 10, 15 and 20) when the repair process became actively functional, the polymorphonuclear leucocytes tend to disappear, possibly because the initial protective role had completed. This is similar to the observation described by William (1981).

On day 0, abundant numbers of inflammatory cells were found around glossy membrane of the root sheath of the hair follicles, and it was difficult to differentiate them from the accumulation of inflammatory cells in lower dermis of control group. There was no sign of regeneration until day 15 in the control group compared to the treated group in which regeneration started on day 5 and almost complete by day 15. Less collection of cells were observed around hair follicle during day 0, 5 and 10 in the control group indicating that the tissue was inactive and did not showed any proliferation. In lyophilized *A. vera* treated wounds a compact collection of cells was observed in the dermis even on day 5 and continued on day 10 which indicated that cells of hair bulbs were in active proliferation. However, in the control group mitosis was observed in the papilla of the hair follicle only on day 15 and

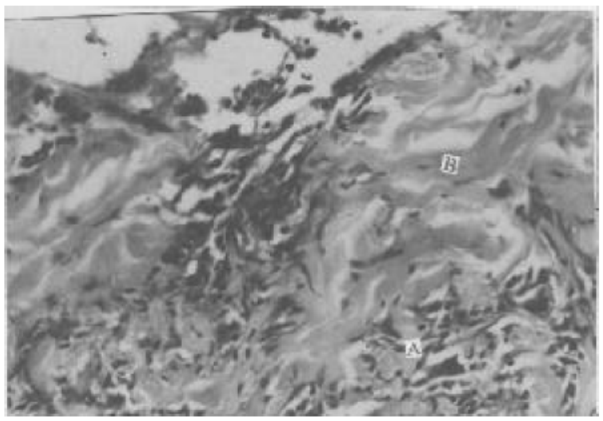


Fig. 1a: Control on day 0. Entire epidermis was damaged. No sign of epithelialisation and blood vessel was observed. Moderate number of polymorpho nuclear leucocytes (A) accumulated in the region of the deeper part of the dermis and a small collection of normal fibroblasts (B) was found in the dermis. H & E stain, X400.

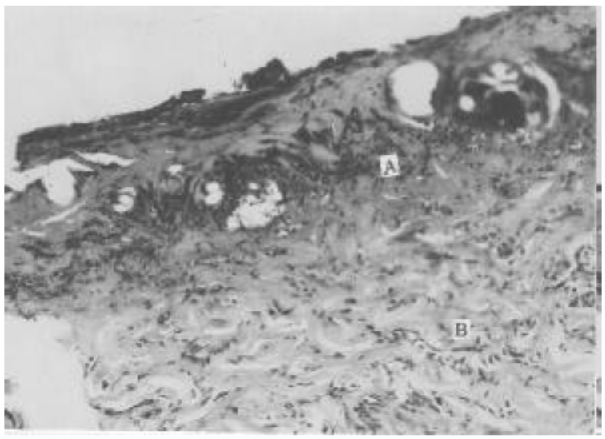


Fig. 1b: Control on day 5. There was no sign of epithelialisation and hair follicle regeneration. Only mild numbers of polymorpho-nuclear leucocytes (A) and fibroblasts (B) dispersed in dermis, H & E stain, X200.

this process continued even on day 20. According to Marks (1981), hair follicle was one of the sources of the migrating epithelial cells. If the hair follicle can recover faster after post burn, epithelialisation can occur earlier. In control group epithelialisation was observed only on day 15 due to the delay in the onset of regeneration process of hair follicle.

In both control and treated groups, arrector pili muscles appeared abnormal on day 0. However, the structure of arrector pili muscles became normal on day 15 in treatment group. Delay of epithelialisation in the control group possibly had caused a delay in the regeneration of arrector pili muscle so that this muscle was not normal during the experimental period.

The sweat glands was not clearly observed during the short experimental period in both the control and treated groups. Possibly the regeneration of sweat gland is the last step in the repair process.

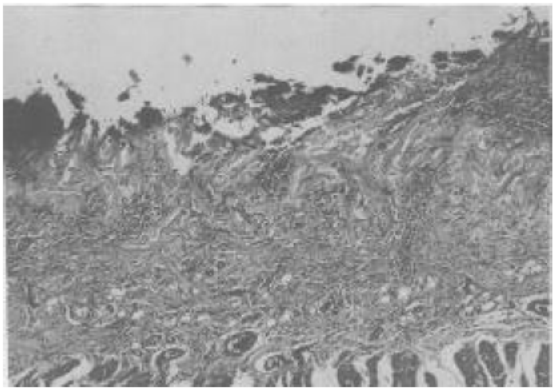

Fig 1c: Control on day 10. Inflammation process still progressing, epitheliasation, hair follicle regeneration and normal sebaceous gland were not observed, H & E stain, X 100.

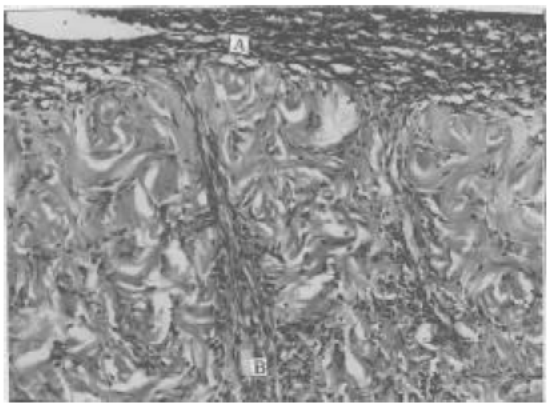


Fig. 1d: Control on day 15. Newly formed epithelium (A) which appears to have migrated from hair follicle (B), H & E stain, X 200.

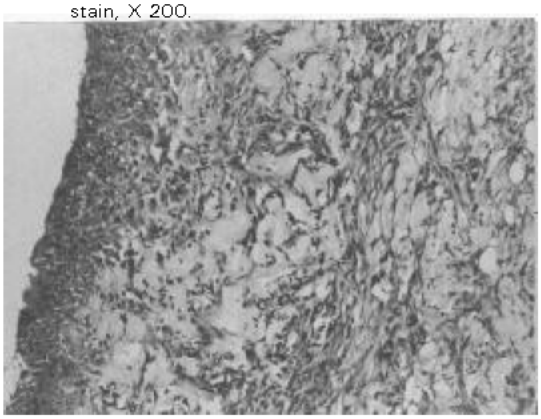

Fig. 1e: Control on day 20. Mild collection of fibroblast was found in dermis, but sebaceous gland and stratified epithelium (epidermis) have still not well developed, H & E stain, X 200.

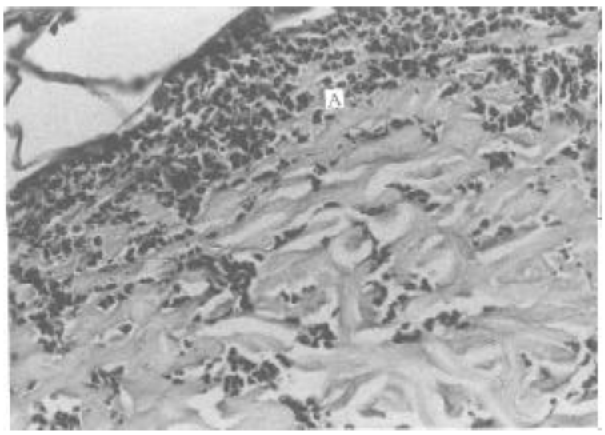


Fig. 2a: Treated group on day 0. Moderate numbers of polymorpho-nuclear leucocytes (A) accumulated in the upper dermis region, H & E stain, X 200.

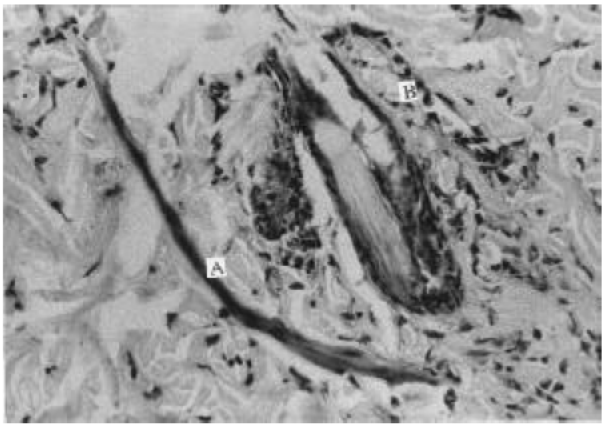


Fig. 2b: Treated group on day 0. Arrector pili muscle (A) and sebaceous glands (B) showed signs of damage, H & E stain, X 400.

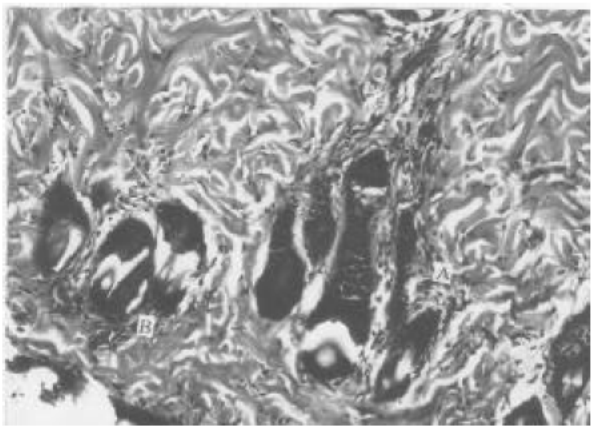


Fig. 2c: Treated group on day 5. Hair follicles (A) were found in active mitotic form (compact collection of cells indicated active cell proliferation) and newly formed cells surrounded the bulb of hair follicle (B) although the bordes between the cells of the sebaceous gland were not clear, H & E stain, X 200.

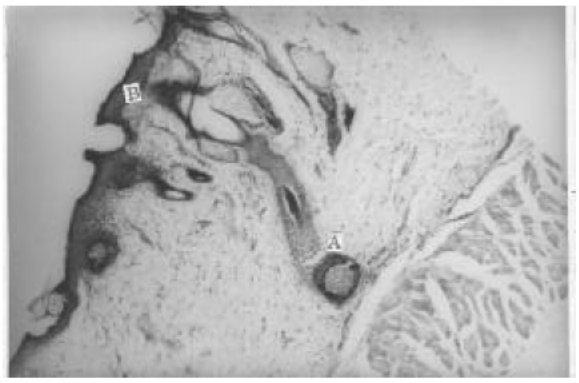


Fig. 2d: Treated group on day 10. Several basal cells originate from bulb of hair follicles (A) migrated superiorly to form cellular layers to replace damaged epidermis (B). New regenerated epidermis was consisting of stratified squamous epithelium and the cells gradually become more flat superficially, H & E stain, X 100.

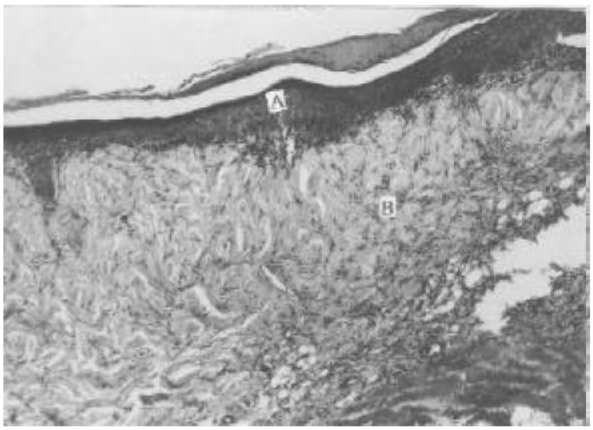


Fig. 2e: Treated group on day 15. Regeneration of epidermis (A) was almost complete and the experimental area showed relatively less inflammation (B) as indicated by the absence of polymorpho-nuclear leucocytes, H & E stain, x 100.

In both the control and treated group, a few numbers of fibroblasts were observed in the dermis on day 0. In the treated group the fibroblasts increased in number on day 5 and slightly reduced to moderate numbers on day 10 and 15. Because of the reduction in the fibroblast numbers on day 15 in the treated group, the tensile strength was also reduced from 7.412 MPa ± 0.687 on day 10 to 2.321 MPa ± 0.330 on day 15 in the treated group. However, in the treated group mild collection of fibroblast was observed on day 20 during which the tensile strength was 2.249 MPa ± 1.175, and almost equal to day 15. In the control group the fibroblasts in the dermis increased on day 5 (score 1) to moderate (score 2) on day 10 and 15. However, the fibroblasts decreased in the density on day 20. Since the fibroblasts did not increase in numbers in the control group, the tensile strength that might depend on the packing of the fibroblasts (Mark, 1981) remained low during experimental period.

In the treated group few blood vessels were observed on day 10 and they increased in numbers on day 15 and 20. These newly proliferated blood vessels supplied necessary

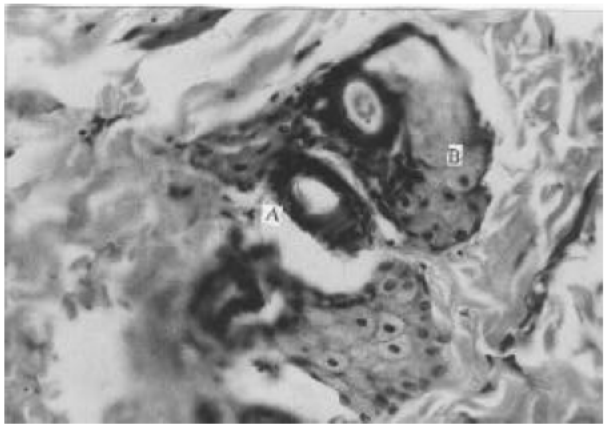


Fig. 2f: Treated group of day 15. The structure of hair follicle (A) and sebaceous gland (B) became normal, H & E stain, X 400.

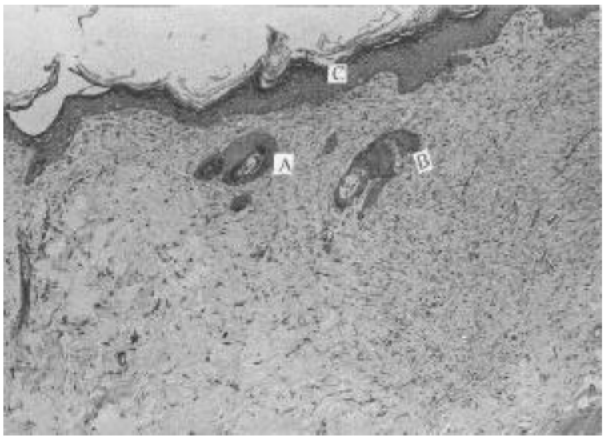


Fig. 2g: Treated group on day 20. No inflammatory cell was observed and hair follicles (A) and sebaceous glands (B) looked normal. Epidermis C) consists of a well defined stratified squamous epithelium, H & E stain, X 100.

nutrient and elements to promote healing process. Blood vessel proliferation was believed to be stimulated by polymorpho-nuclear leucocytes (Fromer and Klintworth, 1976) and fibroblasts (Polverini *et al.*, 1977). This indicated that the control group which showed less numbers of polymorpho-nuclear leucocytes and fibroblasts during the experimental period, resulted in few blood vessels unlike the treated group and therefore, the healing process was slower.

**Epidermis**: There was no sign of epithelialisation in the control group until day 15 and 20 compared to the treated group, where the regeneration of epidermis was clearly observed even on day 5. In the treated group on day 5, mild epithelialisation was observed in certain locations. Single layer of cells covered some of the exposed outer layer of skin. On day 10, clear and well-identified epithelium consisting of different types of cells in several layers were observed. The structure of the epidermis became normal on days 15 and 20 by which time epithelialisation process was almost complete in the treated group.

The results of histological studies on day 0 depicted that 80% of the rats were successful in creating deep partial thickness burn and the remaining (20%) had either superficial partial thickness or full thickness burn. So it may be concluded that burns inducing method, i.e., modified electric solder employed in this experiment is suitable and acceptable. Epithelialisation was observed earlier in the lyophilized *A. vera* treated group. Moreover, the control group showed signs of onset of regeneration of epidermis only on day 15 and 20, while during this period the epithelialisation process was almost complete in the treated group. The *A. vera* treated wounds also showed earlier regeneration of hair follicle, earlier normalization of arrector pili muscles and sebaceous gland, and faster proliferation of blood vessels compared to the control group. The occurrence of polymorphonuclear leucocytes and blood vessels induce fibroblasts regeneration that help faster recovery of burn wounds in the treated group.

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