

# The effect of chitosan-based gel on second degree burn wounds

**Objective:** Chitosan has been widely used in wound healing, including burn wounds. ChitoHeal gel, a novel chitosan-based gel, is studied to assess the effectiveness and rate of wound healing in second degree burns compared with traditional wound dressings.

**Method:** New Zealand rabbits were divided into two groups, control and treatment. Second degree burn wounds were created using a hot round aluminium stamp. It was heated up to 80°C and applied 14 seconds without additional pressure. Morphological, histological, and molecular parameters were used to evaluate the effects of the chitosan-based gel on burn healing.

**Results:** The study exhibited that in the control group with an increase

in the number of keratinocytes due to the papillary growth of the epidermis, melanocyte pigmentation increased, and some melanin was observed in the dermis. In the treatment group, however, there was no hyper pigmentation nor overgrowth of the epidermis. Transmission electron microscopy micrographs of healed epidermis in treatment group showed melanocyte with normal morphology.

**Conclusion:** The result of the present study is likely to be important for the development of novel strategies for wound healing using chitosan gel.

**Declaration of interest:** S.S. Kordestani is the Managing Director of ChitoTech and ChitoHeal wound dressing used in this project is one of the products of this company.

burn • melanocyte • hyper pigmentation • structure change • ChitoHeal gel

**P**olysaccharides and its derivatives may actively participate in the process of wound healing. Hyaluronic acid and chitosan can be used for this purpose.<sup>1</sup> Recent progress in wound management is mainly in providing physiological support to aid healing. Chitosan has been employed in many areas in wound care, such as artificial skin, wound dressings, and contact lens. [AQ3 provide reference].<sup>2</sup> The main biochemical activities of the chitosan-based materials are: polymorphonuclear cell activation, fibroblast activation, cytokine production, giant cell migration and stimulation of type IV collagen synthesis.<sup>2,3</sup>

Normally, dressings are used for the stabilisation of the wound site and may have therapeutic effects. As technology advances, the role of wound dressings for burns is no longer simply protection against infection, but also effective treatment of burn wounds and reduction of patients' suffering.<sup>1</sup> Materials used in dressings vary from cotton gauze to biological materials such as chitosan, which has been used for dermal burn wound healing.<sup>4-7</sup>

In this study, the effect of a chitosan-based topical gel, called ChitoHeal gel, was investigated on healing and scar formation of superficial burns. The aim was to study the effectiveness and the rate of wound healing in second degree burns compared with traditional wound dressings.

## Materials and methods

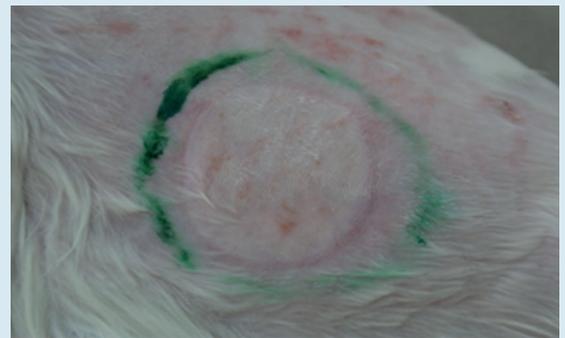
### Animals

Following the guidelines and recommendations of the Animal Research Board of the American University of Beirut,<sup>8</sup> New Zealand rabbits with a weight range of 1.8 to 2.0 kg were used in this study, designed to continue for 30 days after the experimental burns were inflicted. The animals' backs were shaved with a standard electric shaver three days before the experimental burn. Just before inflicting the burn, the area was depilated with a commercial depilatory cream to obtain smooth and hairless skin. The animals were kept under standard laboratory conditions with veterinary supervision and no restrictions on water and food.

### Anaesthesia

The animals were anaesthetised by intramuscular injection of 3mg/kg ketamine and 5mg/kg xylazine 2% (Chanazine). Booster injections of up to half of the

**Fig 1.** Burn wound immediately after infliction

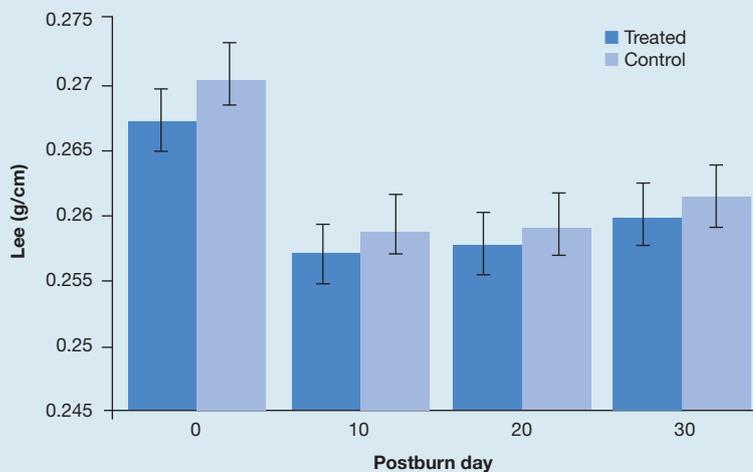


S. Honardar,<sup>1</sup> S.S. Kordestani,<sup>1,2</sup> M. Daliri,<sup>3</sup> F. NayebHabib,<sup>1</sup>

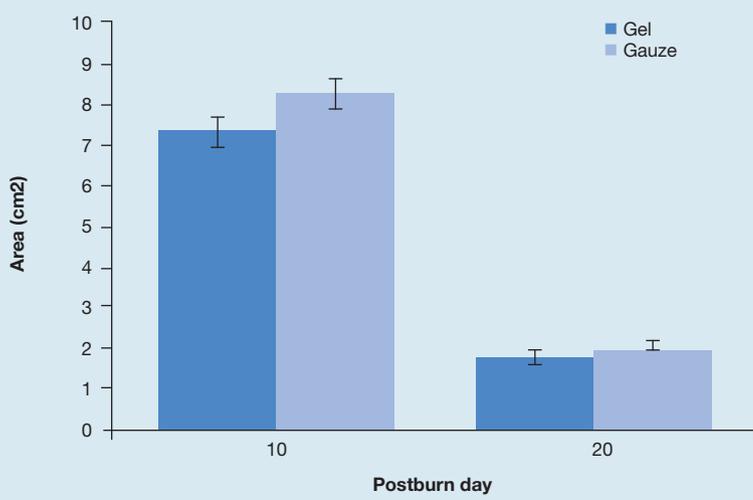
E-mail: ss.kordestani@chitotech.com

**1** Biomaterial Group, Medical Engineering Department, Amirkabir University of Technology, Tehran, Iran. **2** ChitoTech Company, Khaghani Building, Somayrh Avenue, Tehran, Iran. **3** National Institute of Genetic Engineering and Biotechnology, Tehran, Iran.

**Fig 2.** Lee index for control group



**Fig 3.** Wound area-post burn day



initial dose were administered as needed, in order to ensure that the rabbits were pain-free during the procedure and subsequent application of the dressing.<sup>10</sup>

**Burn injury**

A round aluminium stamp, described by Knabl et al.,<sup>9</sup> measuring 4 cm in diameter and 81 g in weight was heated to 80°C. It was then applied for 14 seconds with no extra pressure on the skin to produce a superficial, partial-thickness burn (SPT). In each animal, one SPT burn was produced on the vertebral and paravertebral area 10 cm from the tail and 14 cm from the last cervical vertebrae (Fig 1).

**Treatment**

The animals were divided into two groups. Group one was the control group, in which the burn wounds were covered by sterile gauze dressing. Group two received a daily application of a chitosan-based gel covered with sterile gauze dressing. The chitosan-based gel was

applied with a thickness of about 1 mm. Both treatment and control groups wounds were then covered by a wrap-around bandage.

**Change of dressing**

The dressings were changed daily, after sedating the rabbits with an intramuscular injection of 0.4 ml/kg of 2% xylazine, for the 30-day treatment period. Before the application of a new dressing, the wounds were cleansed with saline solution for both groups.

**Burn tissue assessment**

Punch biopsies were taken from the centre of the burn areas along with small bits of normal skin from around the wounds on days 10, 20, and 30 from both groups. The biopsied tissues were cut into two halves; one was fixed in 10% formalin for routine microscopy staining and the other half was fixed in 2.5% glutaraldehyde for analysis by scanning electron microscope (SEM) and transmission electron microscopy (TEM), respectively. The specimens for light microscopy were processed according to standard methods and stained with Hematoxylin and Eosin. The harvested specimens were fixed in 10% formalin for 24 hours. After conventional ethanol gradient dehydration, the tissues were embedded in paraffin and sectioned at 5 mm for Hematoxylin and eosin staining.<sup>10</sup>

**Other assessments**

Morphological, histological, and molecular parameters were used to evaluate the efficacy of chitosan-based gel on SPT burns in the treatment group, compared with a gauze dressing used in the control group.

Immediately after the burn was inflicted on the rabbits, the burned tissue was biopsied. The biopsied tissue was fixed in 10% formalin. After light microscopy assessment, the pathologist confirmed an SPT burn.<sup>11</sup>

Based on the TEM protocol, the cell nucleus was stained with uranyl acetate, and the cell cytoplasm was stained with lead citrate.

Body mass index (BMI) was calculated using the Lee formula:<sup>12</sup>

$$BMI = BW1/3(g)/\text{nasoanal length (cm)}$$

**Results**

**Macroscopic assessments**

On day 10, the Lee index (BMI) noticeably declined in both groups. The decline, resulting from trauma-based weight loss, was expected. In time, the Lee index improved in all groups, due to the progression of wound healing and reduction of stress (Fig 2).

**Wound edge migration rate assessment**

The area and periphery of the wounds were calculated using digital photographs and the AUTO CAD programme. Wound edge migration rate was calculated using Gilman's modified equation:<sup>12</sup>

$$WEMR (\text{mm/day}) = \{ (Ab - Aa) / ([Pa + Pb] / 2) \} / (b - a)$$

In this equation, A represents area, P represents

periphery, a and b represent the start and end days of observations. The area and periphery of wounds treated with chitosan-based gel were smaller than the wound area and periphery in the control group (Figs 3 and 4).

Wound edge migration in the treatment group was noticeably higher than that in the control group (Fig 5).

### Histopathology

In the light microscopy assessment of normal skin, intact epidermis and dermis were distinguished. Melanocytes were irregularly present among keratinocytes. Since rabbit skin is more flexible than human skin,<sup>9</sup> a large number of elastin fibres were observed.

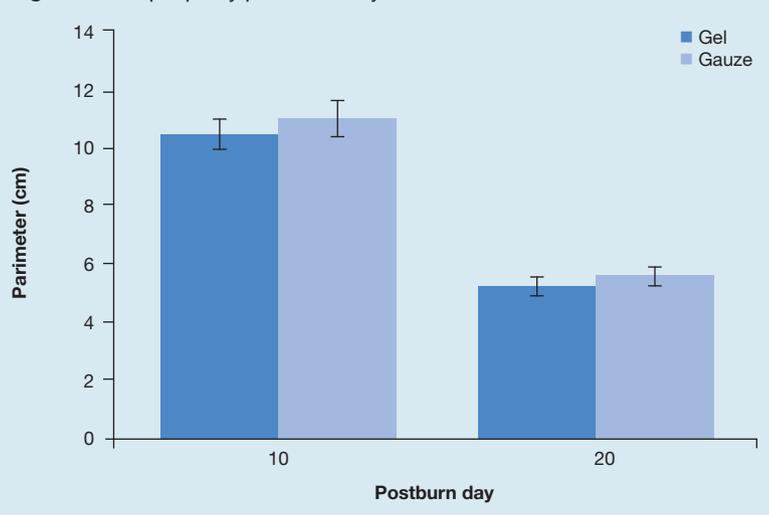
On day 10, no re-epithelialisation was observed. Epidermis was present only in necrotic and ulcer forms. In the dermis, both fibrous and granular tissue were observed, which is a sign of the progression and matrix deposition processes. Since haemostasis and inflammation precede epidermal cell proliferation, the presence of inflammatory cells and coagulation phase residue was considered as normal (Fig 6).

There was less inflammation (33%) in the treated group on day 10 compared with the control group, suggesting that the chitosan-based gel decreased the inflammatory reaction. Less inflammation results in less collagen synthesis and deposition, and therefore, less scar formation (Fig 6b). In the control group on day 10, granulation was observed. The epidermis was not reconstructed, ulceration was noticeable, and inflammation continued.

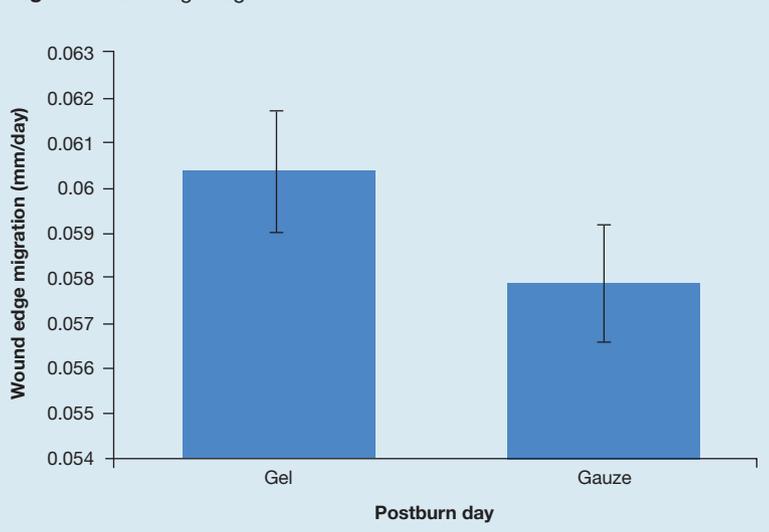
On day 20, in the treated group, proliferation of epidermal cells was observed. Re-epithelialisation was completed, and the epidermis was healed and had a near normal appearance. Furthermore, all wounds showed almost complete epidermis reconstruction, while 50% of the control group specimens showed ulcers and poor or partial reconstruction of the epidermis. The acceleration of healing in the treatment group was confirmed microscopically. In the group, 67% of wounds healed; however, there was no trace of hair follicles. In the control group, 50% of wounds healed (Fig 7). The epidermis in the control group appeared to be partially reconstructed; however, ulceration was observed. The epidermis thickness seemed to be thin and inflammatory cells were present in the dermis. The formation of wound bed, before epidermis formation in the expected healing direction, from depth to surface, was observed (Fig 7).

In the treated group, by day 30, the epidermis was thoroughly reconstructed and was a normal thickness. There was no melanocytes overgrowth, indicating absence of hyper-pigmentation and the restoration of normal colour of the skin. In some cases, swelling in keratinocytes was observed; that was due to their moisture absorption as a result of the moist wound-healing environment provided by chitosan-based gel. Inflammation had mostly disappeared. Epidermis

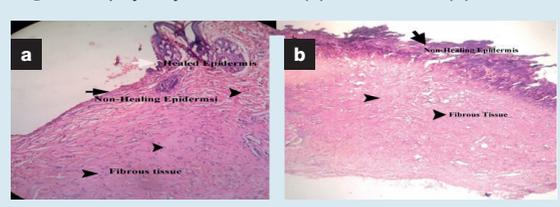
**Fig 4.** Wound periphery post-burn day



**Fig 5.** Wound edge migration rate

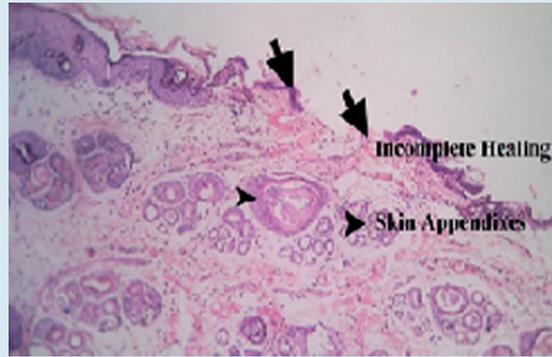


**Fig 6.** Biopsy day 10. Treated (a) and control (b)

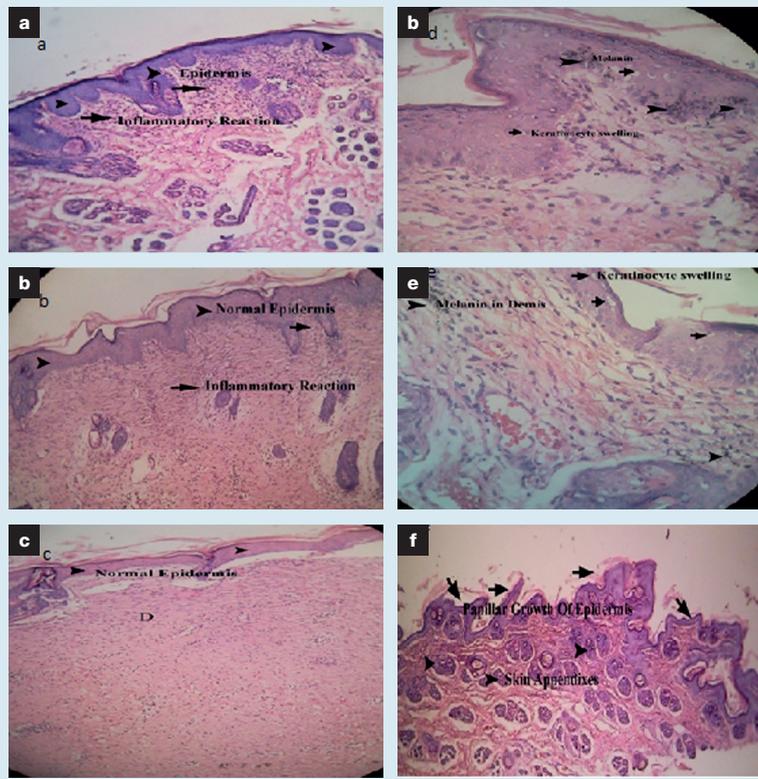


growth and the number of keratinocytes appeared to be normal (Figs 8a–c). In the control group, by day 30, the epidermis was reconstructed. There was papillary growth in the wound bed, which could be associated with hyper-keratinocytes expression.<sup>13</sup> There were numerous active melanocytes, and melanin was dispersed in the stratum spinosum of the epidermis and dermis.<sup>13</sup> A number of keratinocytes appeared swollen. The epidermis displayed a higher than normal thickness in the control group, comparing the light microscopy

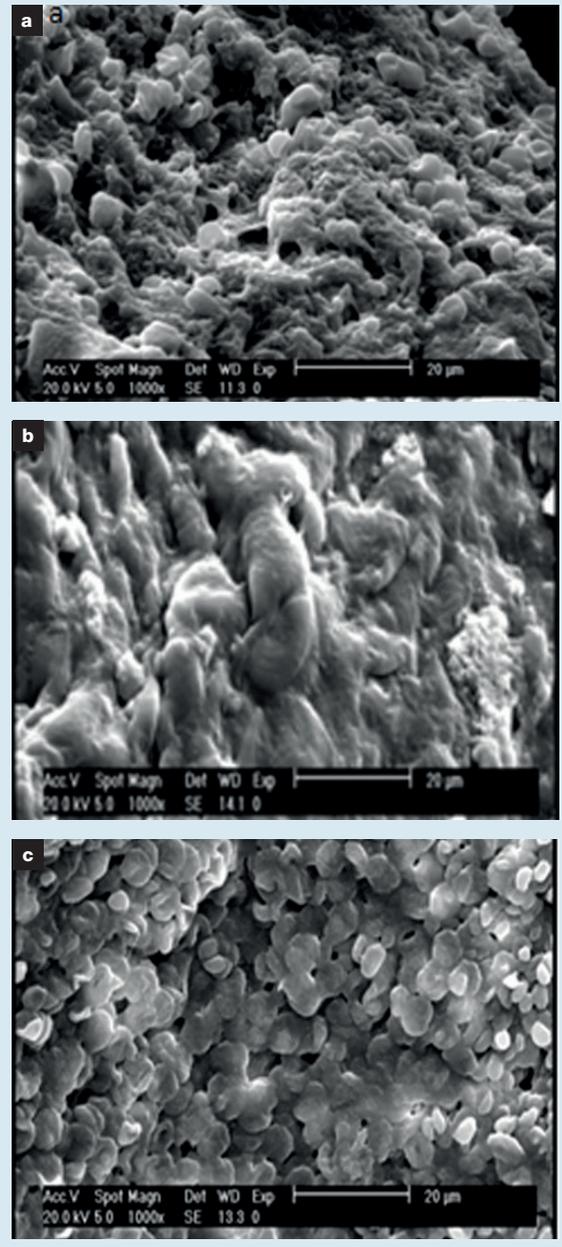
**Fig 7.** Treated specimen, day 20



**Fig 8.** Treated specimen, day 30



**Fig 9.** SEM images of control group days 10 (a), 20 (b) and 30 (c)



images to that of the treatment group. This indicated an abnormal healing in the control group (Fig 8d-f).

**Clinical assessment**

On days 2 and 5, the scabs on the wounds in the treatment group were thicker than those in the control group. The necrotic tissue, however, was completely adherent to the wound and its underside was soft and flexible. This indicated the rapid healing process, since the faster the damaged tissue degrades, the more quickly the wound bed clears and healing enters the next phase. The softness of the scab in the treatment group in comparison with that of the control group may well be associated with the application of the chitosan-based gel dressing.

**Microscopic assessment**

On day 10 using SEM, it was noted that fibrous tissue was present in both treatment and control groups. Fibroblasts were the main cells present, and the inflammatory phase was almost over. In the images for both groups on day 10, small immature cells together with tissue granules were noticeable, indicating the start of the cell proliferation and tissue granulation phases. In the control group, the presence of blood and inflammatory cells was more noticeable, indicating a longer inflammatory phase in the control group (Fig 9a).

On day 20, the wounds were mainly healed in the treatment group. As the epidermis was reconstructed, keratin fibres were detectable. In the dermis of the treatment group, some immature granules were present, showing the accelerated re-epithelialisation and formation of epidermis. The epidermis in the treatment group was more intensified before the dermis was completely mature. In the control group, less keratin was observed (Figs 9b).

On day 30, keratin, representing reconstructed epidermis, and contracted mature granular tissue, representing reconstructed dermis, were noticeable in the treatment group. Red blood cells indicated effective angiogenesis. In the control group, tissue granules were still visible. There were little keratins as compared with the treatment group, indicating poor epidermis reconstruction (Fig 9c).

Using TEM, we observed melanocytes in the treatment group (Fig 10). As a result of re-epithelialisation, melanocytes were present among the keratinocytes. The morphology of the melanocytes was normal, implying that, in appropriate wound healing, epidermal melanocytes are reconstructed.

As illustrated in Fig 10b, some melanin was observed inside the melanocytes. This pigment, in addition to melanocytes being present in the reconstructed epidermis, might indicate stimulated melanin production, since growth factors and cytokines released during the wound healing process include melanogenic components such as  $\alpha$ -melanocyte-stimulating hormone and endothelin-I as well as prostaglandins.<sup>14</sup>

## Discussion

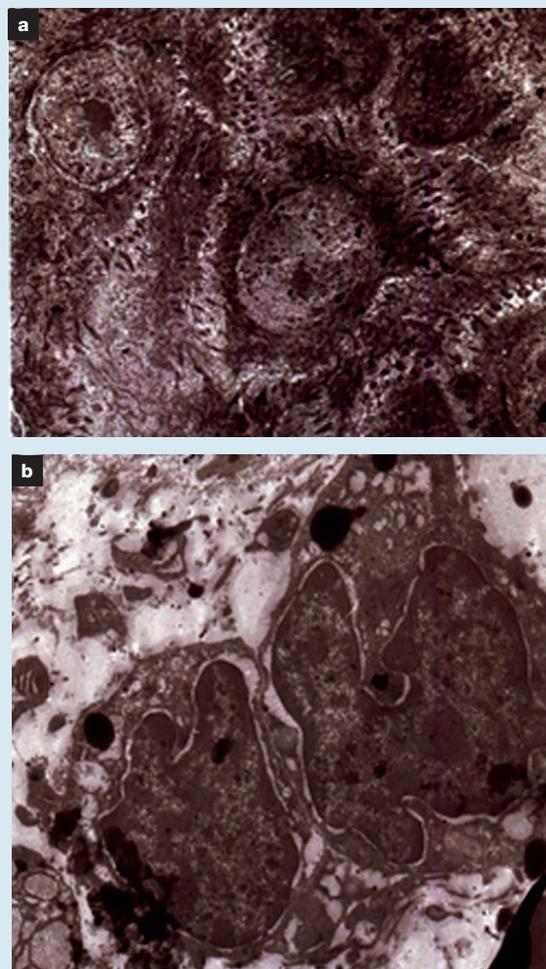
The use of bioactive dressings consisting of different biomaterial, natural or synthetic, is becoming more widespread in modern wound care treatment.<sup>15,16</sup> As has been demonstrated by Archana et al., application of chitosan and chitosan blend dressing is effective in accelerating burn wound healing.<sup>17</sup>

Results in all animals in the treatment group compared with the control group indicated that chitosan-based gel had accelerated the rate of wound healing, the formation and deposition of fibrous matrix as well as the proliferation and migration of epithelial cells towards the centre of the wound. Since epithelial cells proliferate from the wound's edges toward its centre, as the wound heals, its dimensions decrease. Thus, a faster wound closure, with recovering epithelial, shows a satisfactory rapid healing.

In the proliferation phase, which occurs after synthesis and deposition of intracellular matrix, the division, differentiation, and migration of epithelial cells begins. As chitosan-based gel accelerated wound closure, it seems it induced all stages of the proliferation phase, especially cell migration, possibly because of the moist environment which it provides, allowing the cells to move more easily and farther.

The application of the chitosan-based wound dressing in the treatment group led to the appearance

**Fig 10.** Transmission electron microscopy images of treatment group on day 30 (a); images of melanocyte cytoplasm in treatment group day 30 (b)



of normal morphological and structured melanocytes in the finely reconstructed epidermis, which contrasted with the papillary, rough reconstructed epidermis in the control group.

## Limitations

A limitation of this study is that the animals had to be eliminated shortly after the biopsy and therefore the final remodelling of the dermis and epidermis could not be studied. Furthermore, as this is an animal model work is required to determine if the same response is seen in a clinical situation.

## Conclusion

The outcomes of the present study are likely to be important for the development of novel strategies for wound healing, since it sheds light on the potential of the therapeutic regimens. Faster wound healing and re-epithelialisation of burn wounds could result in the reduction of collagen deposition, hence preventing severe scar formation.<sup>18-27</sup> JWC

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