

# Use of ovine acellular peritoneal matrix combined with honey and ovine fetal skin extract in the healing of full-thickness infected burn wounds in a rat model

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## Abstract

Treatment of infected burn wounds remains a challenge in burn units. Silver-sulfadiazine (SSD) is the most commonly used topical antimicrobial agent in managing these wounds. We aimed to accelerate the healing of burn wounds by combined application of ovine acellular peritoneal matrix (OAPM), honey (H), and ovine fetal skin extract (OFSE). Sixty-four standardized burn wounds were created on the dorsum of 16 rats and were subsequently inoculated with *Staphylococcus aureus* and *Pseudomonas aeruginosa*. After 48 hr, the wounds were surgically debrided and received either physiologic saline (control group) or SSD, OAPM+SSD, OAPM+H+SSD, and OAPM+H+OFSE+SSD. The healing wounds were evaluated for size, bacterial counts, histopathology, and biomechanical properties on days 3, 7, 14, 21, and 28 after surgery. All treatments had effectively reduced the level of *S. aureus* and *P. aeruginosa* on wounds compared to the control group by day 3 and 7. The wounds treated with combined application of OAPM+H+OFSE+SSD demonstrated considerable inflammation reduction, fibroplasia, complete re-epithelialization, and wound contraction together with significantly lesser scar tissue formation. Treatment with OAPM+H+OFSE+SSD showed superior biomechanical properties of the healing wounds. The findings suggested that the synergistic effect of dressing the wounds with OAPM, H, and OFSE was a very effective approach in accelerating the healing process of the experimentally induced infected full-thickness burn wounds in rats.

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## Introduction

Burns are partial- or full-thickness skin injuries caused by thermal sources.<sup>1-3</sup> Burn wounds are sterile immediately after creation, however, they will be colonized by superficial bacteria of normal skin flora within the first hours. Thus, the priority is focused on the prevention of septic complications by early removal of necrotic tissues.<sup>2-5</sup> *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the most frequently isolated pathogens from burn wounds. Infection with these pathogens can interfere with the healing process and delay wound repair.<sup>2-7</sup> Topical antibacterial agents such as silver sulfadiazine (SSD) are used routinely in the treatment of infected wounds, however, the application of SSD may retard healing or cause some side effects such as bacterial resistance and hepatic or renal toxicity.<sup>8,9</sup>

Wound healing is a dynamic biological process comprising interactions between cells, extracellular matrix (ECM), and growth factors that restore tissue integrity after injury.<sup>10-13</sup> Extracellular matrix, the major component of the dermal layer, plays an important role in the regeneration of tissues. This has led to the development of tissue-engineered products that are aimed to mimic the functional and structural criteria of native ECM.<sup>14-17</sup> When placed in the wound bed, the three-dimensional matrices accelerate the healing process by the provision of a temporary scaffold for cellular migration and proliferation in an organized manner.<sup>15,16,18</sup> Acellular matrices are animal- and human-derived tissues that are inactivated immunologically via decellularization while retaining the native collagen matrix. This process reduces inflammatory and immunogenic responses when implanted into recipient tissues.<sup>14,16,19-23</sup>

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Honey has been used for the treatment of wounds and burns injuries for centuries.<sup>24,25</sup> Using honey has shown promising results in accelerating the healing process by enhancing wound debridement, bacterial clearance, reducing inflammation, promoting granulation tissue formation, improving epithelialization, and minimizing scarring.<sup>24-26</sup> Also, honey has anti-microbial, immunomodulatory, and anti-oxidant effects.<sup>24,26,27</sup> The unique antibacterial effect of honey is related to its enzymatic production of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), high osmolarity, low pH, and high level of antioxidants.<sup>24-31</sup>

A fetus has been shown to regenerate the cutaneous wounds rapidly and without scar tissue formation.<sup>32,33</sup> Fetal wounds heal by regeneration of normal dermal architecture including restoration of dermal appendages and neurovasculature and by a relative absence of inflammation. Fetal cutaneous wound repair is characterized by a variety of growth factor profiles, reduced inflammatory reaction, particular ECM rich in type III collagen, attenuated biomechanical stress, and influence of multiple pluripotent stem cells.<sup>32-39</sup> From these observations, it has been proposed that the extract of fetal skin could be efficient in the treatment of burn wounds.

Although several studies have shown the effectiveness of acellular matrices, honey, and fetal skin cells, separately, in the healing of wounds, there is no single study to investigate the combined effect of these treatments on burn wound healing and in comparison with SSD. This study is in response to the need for a treatment approach that can optimize the healing of infected full-thickness burn wounds. We used ovine acellular peritoneal matrix (OAPM) as a dressing on burn wounds, honey (H) as a hydrogel to fill the wound defects, and ovine fetal skin extract (OFSE) as a healing enhancer. We hypothesized that this modality can promote efficient healing in infected burn wounds.

## Materials and Methods

### Preparation of ovine acellular peritoneal matrix.

The peritoneum was dissected out and removed from a healthy sheep from a local slaughterhouse. We used treatment with 1.00% sodium dodecyl sulfate (SDS; Sigma-Aldrich, St. Louis, USA) followed by repeatedly rinsing with deionized water. The decellularization process was completed by multiple freeze-thaw cycles. The peritoneum was cross-linked using 0.10 M polypropylene oxide (Haian Petrochemical Works, Jiangsu, China) and then was thoroughly rinsed with phosphate-buffered saline (PBS; Sigma-Aldrich). Then it was cut into 1.50 × 1.50 cm<sup>2</sup> rectangular pieces. The scaffolds were preserved in PBS and gentamycin (AdvaCare Pharma, Cheyenne, USA) was added to the solution at a dosage of 10.00 mg mL<sup>-1</sup> to prevent bacterial growth.<sup>16,19</sup>

**Honey.** Honey (Taksoo Co., Birjand, Iran) was used in this study. The selected honey was composed of 17.10% water, 42.90% fructose, 29.90% glucose, 7.20% maltose, 1.30% sucrose, 1.20% other higher carbohydrates, 0.40% other substances and had an energy density of 275 kcal 100 g<sup>-1</sup> (Foundation of Food Analysis Technology Center Sorentt, Mashhad, Iran). Silver-sulfadiazine powder was added, in the laboratory, to make 1.00% SSD in honey.

**Preparation of ovine fetal skin extract.** Fresh ovine uteri were collected from a local slaughterhouse. In the laboratory, the uteri were incised, and the fetuses were obtained. Approximately two-month-old fetuses were utilized in this study. Fetal skins were removed, collected in a container, and then cold extraction buffer was added. The tissues were homogenized and subsequently centrifuged to remove the cell debris. The supernatant was decanted carefully and the remaining sediments were re-centrifuged several times to improve the yield of extract. The extract was freeze-dried to obtain powder and then kept in a refrigerator at 4.00 °C until used.<sup>34,40</sup>

**Animals and experimental design.** Before performing the animal study, all the procedures were approved by the Institutional Animal Care and Use Committee of our laboratory. Animal care was accomplished for all rats by the Animal and Health Guidelines, published by the National Institutes of Health (NIH Publication No. 85-23). Sixteen male Sprague-Dawley rats weighing 325 ± 25.00 g were used in this investigation. The rats were caged individually and kept under the controlled condition at 24.00 ± 2.00 °C, the humidity of 50.00%, 12 hr light-dark cycle, and fed with a balanced diet (*ad libitum*).

**Infectious burn model.** Intramuscular 50.00 mg kg<sup>-1</sup> ketamine (Alfasan, Woerden, Netherlands) and 5.00 mg kg<sup>-1</sup> xylazine (Alfasan) were considered for induction and maintenance of anesthesia. The dorsum of each rat was shaved and disinfected with a 7.50% povidone-iodine scrub solution (Medline Industries Inc, Mundelein, USA) and 70.00% isopropyl alcohol (Techspray, Kennesaw, USA). A cylindrical metal rod (1.00 cm in diameter) was used for the creation of burn wounds. The rod was immersed in a flask of boiling water (100 °C) and then placed on the skin for 10 sec.<sup>41</sup> Four standardized full-thickness burns were created on dorsum of each rat.<sup>2,41,42</sup> After burn infliction, the wounds were inoculated with 1.00 × 10<sup>7</sup> colony-forming units (CFU) of each bacteria [namely *S. aureus* (ATCC 6538) and *P. aeruginosa* (ATCC 27853)] diluted in 100 µL saline. The bacterial suspension was injected intradermally in an oblique fashion.<sup>2</sup> The wounds were allowed to incubate for 48 hours and then were debrided using a standardized trephine like instrument (1.00 cm in diameter). Immediately after excisional debridement, each wound was swabbed for further bacterial counts. The wounds were irrigated, dried, and treated subsequently according to the experimental groups.<sup>2,41</sup>

**Experimental design.** In this study, two main experimental groups were considered: Treatment and control groups. In treatment group (n = 12 rats), the wounds (n = 48) were subdivided to four groups. In the control group (n = 4 rats), the wounds (n = 16) received only normal saline. In the treatment group, the treatments were randomly assigned to each of the four wounds as follows:

Group 1: SSD was applied to the wounds; group 2: OAPM was sutured to the surrounding skin with 3.0 Nylon suture (SMI, Vith, Belgium) and SSD was injected under the OAPM; group 3: The OAPM was applied and the mixture of H and SSD was then injected under it; group 4: The OAPM was sutured and the mixture of H, OFSE, and SSD was injected underneath. The topical agents (i.e. normal saline, SSD, H+SSD, and H+OFSE+SSD) were applied to the wound surface daily up to 10 days. The rats were euthanized with an overdose of anesthetic agents and intra-cardiac injection of potassium chloride (Hospira, Inc., Lake Forest, USA) on the 14<sup>th</sup> and 28<sup>th</sup> days after surgery and the specimens were retrieved. Each wound was incised longitudinally into two approximately equal parts. One strip of skin (1.00 cm × 5.00 cm) was excised for biomechanical tests and the other part was sampled with a small margin of healthy skin for histopathological examination.<sup>43,44</sup>

**Assessment of the wound size.** The wound size measurements were made on the 3<sup>rd</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>th</sup>, and 28<sup>th</sup> days after surgery using a digital caliper (Guanglu Electrical Co. Ltd., Wenling, China). All the data were recorded by a digital camera and the wound size was measured by image analysis software Digimizer (version 5.3; MedCalc Software bv, Ostend, Belgium).

**Quantitative bacterial cultures.** The wound area was sampled immediately after debridement, before subsequent treatment (day 0), and then on the 3<sup>rd</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup>, and 28<sup>th</sup> days after surgery, using sterile swabs. The swabs were placed in sterile cryogenic vials and bacterial counts were performed to evaluate infection levels of *S. aureus* and *P. aeruginosa* remaining on the wound bed. The samples were serially diluted in normal saline (1:10, 1:100, 1:1000, and 1:10000) and then plated on blood and nutrient agar plates.<sup>2</sup> The plates were then incubated overnight at 37.00 °C under a humidified atmosphere. All colony counts were expressed as log<sub>10</sub> CFU mL<sup>-1</sup>, while bacterial counts of > 1 × 10<sup>5</sup> CFU mL<sup>-1</sup> were considered to indicate a bacterial infection.

**Histopathological study.** The skin samples were immediately fixed in 10% neutral buffered formalin for 48 hours. Sections of 5.00 μm thickness were prepared from the paraffin-embedded tissues and stained with hematoxylin and eosin (H & E). The histological samples were evaluated using light microscopy (Olympus BX51; Olympus, Tokyo, Japan). Infiltration of inflammatory cells, epithelialization, fibroplasia, angiogenesis, and granulation tissue formation were assessed comparatively. Histologic

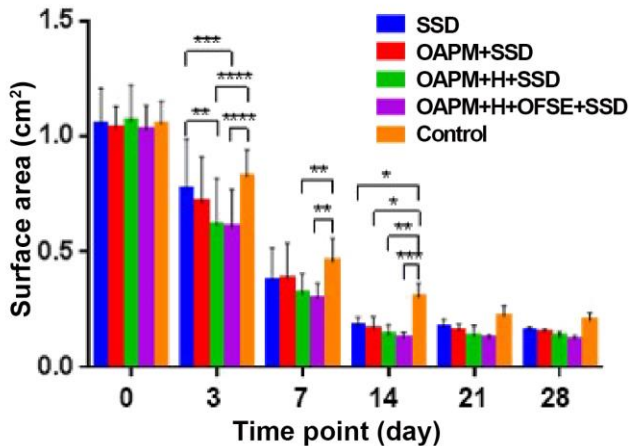
scores were made from 5 random fields per section for each specimen, at a magnification of 400×. Epithelialization was assessed semi-quantitatively on a 5-point scale, on days 14 and 28, and was assessed as 0 (without), 1 (25.00%), 2 (50.00%), 3 (75.00%), and 4 (100%) new epithelialization. Histomorphometric analysis was performed and the neovascularization was assessed, using computer software Image-Pro Plus® (version 6.0; Media Cybernetics Inc., Silver Spring, USA).

**Biomechanical analysis.** The frozen specimens were allowed to thaw at room temperature before the test. The width and thickness of all the skin samples were measured with a digital caliper before each test. The samples' extremities were grasped between special jaws of a universal testing machine (STM-20; Santam, Tehran, Iran) with a 20.00 kN load cell. Tensional loads were applied to each skin sample at a constant rate of 10.00 mm min<sup>-1</sup> until the failure occurred.<sup>43,44</sup> A force-extension graph was generated using software STM Controller (version 4.24, Santam). Then, the biomechanical parameters of healing wounds (i.e. ultimate load, stiffness, strain, modulus of elasticity, stress, and absorbed energy) were calculated and recorded.

**Statistical analysis.** All data were presented as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism (version 6.0; GraphPad Software Inc., San Diego, USA). Statistical comparisons were done using two-way ANOVA, multiple comparisons with a Dunnett posttest for wound size assessment, and microbial counts. For histopathological findings, the statistical analyses were accomplished using Kruskal Wallis for non-parametric and two-way ANOVA for parametric data. The biomechanical data were analyzed using a two-tailed *t*-test. The results with *p* < 0.05 were considered statistically significant.

## Results

**Macroscopic evaluation.** On the day of surgery, the wounds were created with an average size of 1.04 ± 0.12. Treatment with OAPM+H+OFSE+SSD and OAPM+H+SSD significantly decreased the wound size compared to the control (*p* < 0.0001, *p* < 0.0001, respectively) and the SSD treated (*p* < 0.001, *p* < 0.01 respectively) groups, on day 3 after surgery. In groups treated with OAPM+H+OFSE+SSD and OAPM+H+SSD the wound size was considerably reduced when compared to the control group on day 7 (*p* < 0.01). All the treatment regimens resulted in reduced wound size on day 14 (*p* < 0.05), however, the best results regarding the wound size reduction was observed in the OAPM+H+OFSE+SSD (*p* < 0.001) and OAPM+H+SSD (*p* < 0.01) treated groups. Furthermore, there were no statistically significant differences between the groups on days 21 and 28 (Fig. 1).



**Fig. 1.** Average wound size measurement in different groups on days 0, 3, 7, 14, 21, and 28 after treatment. On days 3, 7, and 14, the average wound size was generally reduced in all treatment groups relative to control group. However, the best results regarding to the wound size reduction were observed in the OAPM+H+OFSE+SSD and OAPM+H+SSD treated wounds (SSD: silver-sulfadiazine, OAPM: ovine acellular peritoneal matrix, H: honey, OFSE: ovine fetal skin extract, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ ). Error bars represent SD.

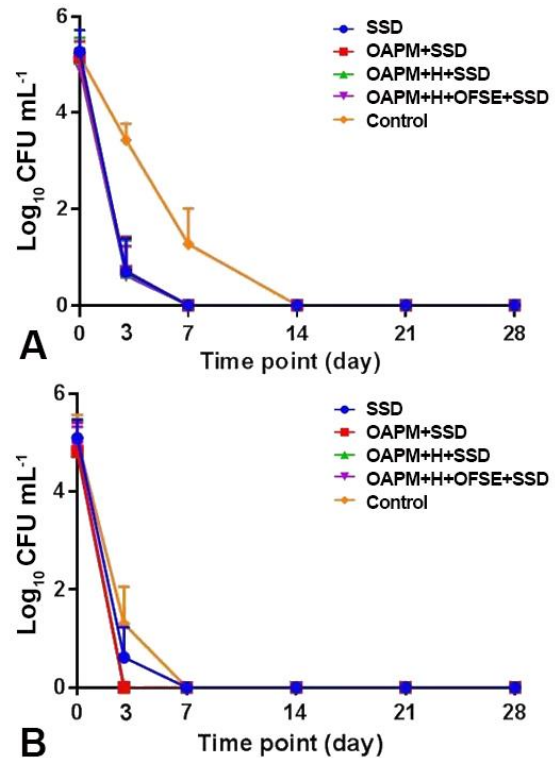
**Bacterial count.** An infection level of  $5.13 \pm 0.60$  logs CFU mL<sup>-1</sup> for *S. aureus* remained on the wound tissue after excisional debridement. In groups treated with OAPM+H+OFSE+SSD and OAPM+H+SSD, OAPM+SSD and SSD, the levels of *S. aureus* were decreased to  $0.61 \pm 1.20$ ,  $0.67 \pm 1.3$ ,  $0.71 \pm 1.4$ , and  $0.69 \pm 1.30$  CFU mL<sup>-1</sup> by day 3 of treatment, respectively. The *S. aureus* levels in all treated groups remained at the baseline of  $0.00 \pm 0.0$  CFU mL<sup>-1</sup> from day 7 to the terminal time point. In contrast, in the control group, the levels of *S. aureus* were reduced to  $3.43 \pm 0.60$ ,  $1.27 \pm 1.40$  and  $0.00 \pm 0.0$  CFU mL<sup>-1</sup> by day 3, 7 and 14, respectively (Fig. 2). The differences were statistically significant, on day 3 after surgery ( $p < 0.0001$ ).

Following the surgical debridement, an infection level of  $4.94 \pm 0.90$  CFU mL<sup>-1</sup> for *P. aeruginosa* remained on the wound bed. The *P. aeruginosa* levels in the wounds treated with OAPM+H+OFSE+SSD, OAPM+H+SSD and OAPM+SSD were reduced to the baseline level of  $0.00 \pm 0.0$  by day 3 and remained at this level to the terminal time point, day 28. Conversely, on day 3 after surgery, *P. aeruginosa* levels were reduced to  $0.61 \pm 1.20$  and  $1.30 \pm 1.50$  CFU mL<sup>-1</sup> in the SSD treated and control groups, respectively (Fig. 2). The differences observed between OAPM+H+OFSE+SSD, OAPM+H+SSD and OAPM+SSD treated groups and control group were statistically significant, on day 3 after surgery ( $p < 0.05$ ).

#### Histopathologic and histomorphometric findings.

Histopathological examinations of the control and SSD treated groups presented hemorrhage, hyperemia, and infiltration of mononuclear inflammatory cells on day 14 after surgery. The wound bed was generally filled with

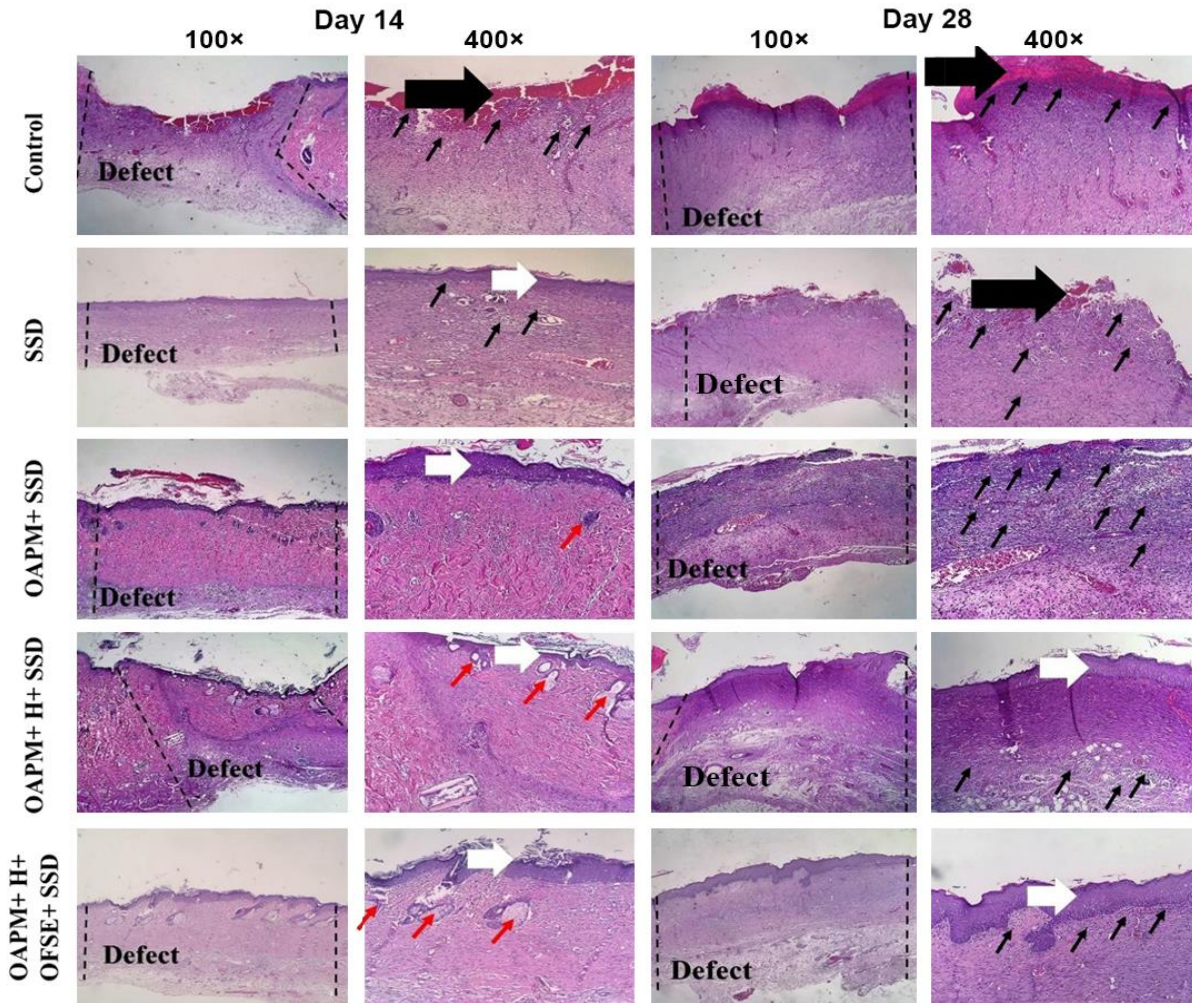
immature granulation tissue and covered with a crusty scab at 14 and 28 days after wounding. Re-epithelialization was minimal in these groups and the formation of the epidermal layer appeared to be incomplete by day 28. However, the inflammatory cells infiltration was significantly reduced in SSD treated group in comparison with the control group by days 14 and 28 ( $p < 0.05$ ). Angiogenesis was significantly higher in SSD treated group in comparison with the other groups ( $p < 0.01$ ), by day 14. Subsequently, on day 28, the number of blood vessels was considerably decreased in SSD treated group ( $p < 0.01$ ) that indicated the maturation of granulation tissue. Micrographs of the wounds treated with OAPM+SSD revealed a close similarity to the SSD treated wounds on day 14 of treatment, while moderate hyperemia and inflammation were still present. On day 28, the epidermal layer started to form, and epithelialization was significantly superior in this group in comparison with SSD treated and control groups, by days 14 and 28 ( $p < 0.05$ ,  $p < 0.01$ ).



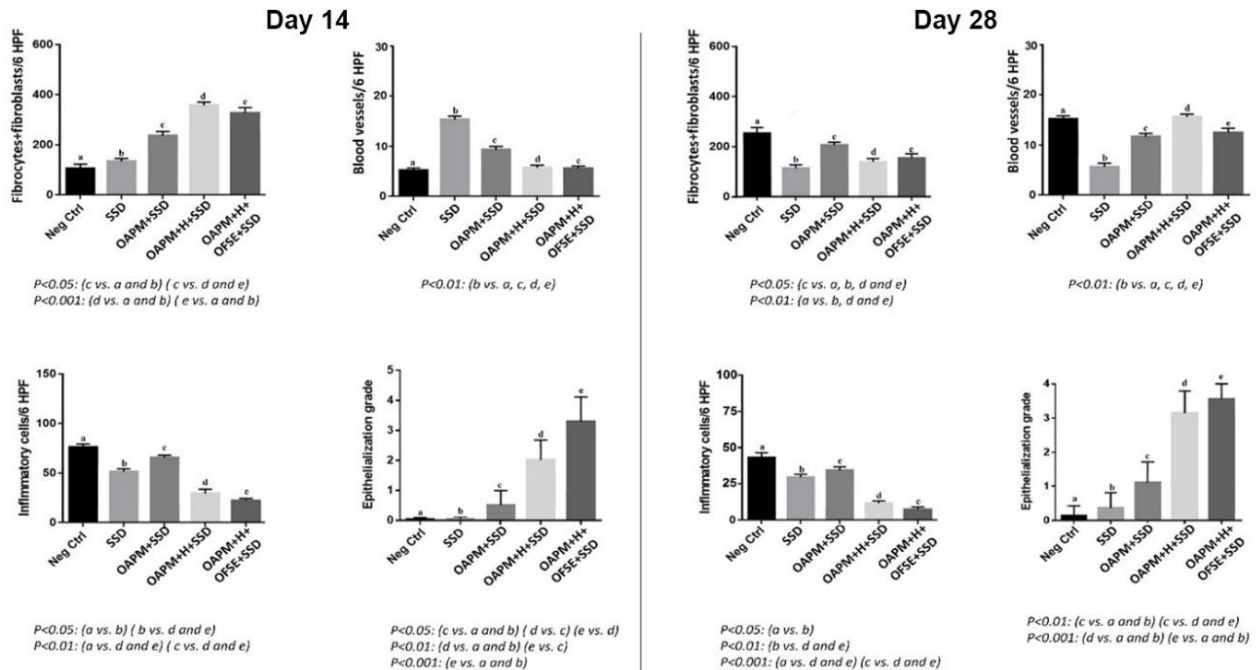
**Fig. 2.** Quantitative bacterial cultures on days 0, 3, 7, 14, 21, and 28 after wounding. The swabbed samples from the wounds infected with **A)** *S. aureus* and **B)** *P. aeruginosa* were analyzed for the amount of bacteria. All the treatment groups efficiently reduced *S. aureus* counts relative to control group, by day 3. Likewise, treatment with OAPM+H+OFSE+SSD, OAPM+H+SSD, and OAPM+SSD significantly reduced the *P. aeruginosa* levels in comparison with the control group, by day 3 (CFU: colony-forming unit, SSD: silver-sulfadiazine, OAPM: ovine acellular peritoneal matrix, H: honey, OFSE: ovine fetal skin extract). Error bars represent SEM.

The number of fibrocytes and fibroblasts (fibroplasia) was significantly increased compared to the SSD treated and control groups by day 14, although it was significantly reduced compared to control group on day 28 that showed wound maturation phase ( $p < 0.05$ ). The wounds treated with OAPM+H+SSD and OAPM+H+OFSE+SSD revealed considerable inflammation reduction in comparison with the wounds treated with OAPM+SSD and SSD and control group, by day 14 ( $p < 0.01$ ,  $p < 0.05$ , and  $p < 0.01$ , respectively). Fibroplasia was significantly increased in these groups when compared to the OAPM+SSD, SSD, and control groups by day 14 ( $p < 0.05$ ,  $p < 0.001$ , and  $p < 0.001$  respectively). On day 28, fibroplasia was considerably reduced in comparison with the OAPM+SSD treated and control groups ( $p < 0.05$ ,  $p < 0.01$ ). Also, the epidermal cells proliferation was significantly higher in these groups in comparison with

the OAPM+SSD ( $p < 0.01$ ), SSD ( $p < 0.001$ ) and control ( $p < 0.001$ ) groups, by day 28. In the OAPM+H+SSD treated group, a complete re-epithelialization has occurred after 28 days of treatment. Although, the new epidermal layer showed an augmented thickness compared to the normal skin layer. In the OAPM+H+OFSE+SSD treated wounds, epithelialization was completed with the formation of rete ridges in the lesion area on day 28. This group demonstrated more similarity to normal skin, with a thin epidermal layer, presence of normal rete ridges, and rejuvenation of the hair follicles and skin appendages. In general, the results indicated that the treatment strategy using OAPM+H+SSD and OAPM+H+OFSE+SSD could reduce inflammation and promote fibroplasia and re-epithelialization. Representative histological images and histomorphometric analysis are shown in Figures 3 and 4.



**Fig. 3.** Photomicrographs of the healing wounds on days 14 and 28 after wounding in different experimental groups. Treatment with OAPM+H+OFSE+SSD revealed more similarity to normal skin, on day 28. A complete re-epithelialization was occurred in this group by the formation of a thin epidermal layer with the presence of normal rete ridges and rejuvenation of the hair follicles and skin appendages. Thick black arrows: Crusty scab, thin black arrows: Infiltration of inflammatory cells, white arrows: Re-epithelialization, red arrows: Rejuvenation of skin appendages, (H & E), SSD: Silver-sulfadiazine, OAPM: Ovine acellular peritoneal matrix, H: Honey, OFSE: Ovine fetal skin extract).



**Fig. 4.** Histomorphometric analysis of the healing wounds on days 14 and 28 after wounding in different experimental groups.

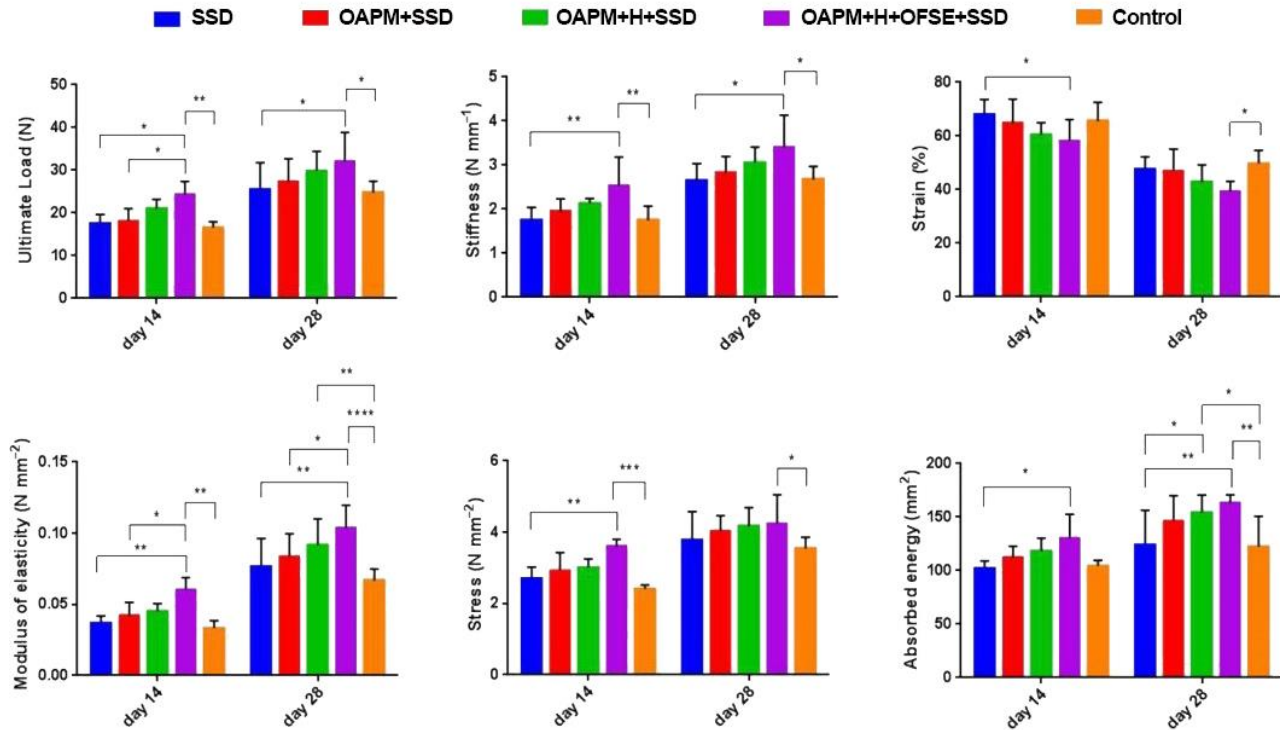
**Biomechanical assessment.** Biomechanical evaluation of the healing wounds on day 14 after surgery indicated that the ultimate load of healing wounds was significantly higher in OAPM+H+OFSE+SSD treated group compared to the groups treated with SSD ( $p < 0.05$ ), OAPM+SSD ( $p < 0.05$ ) and control group ( $p < 0.01$ ). The stiffness of wounds in the OAPM+H+OFSE+SSD treated group was significantly higher compared to the SSD treated ( $p < 0.01$ ) and control ( $p < 0.01$ ) groups at this stage. The strain was significantly decreased in the OAPM+H+OFSE+SSD treated wounds compared to the SSD treated group ( $p < 0.05$ ). The skin modulus of elasticity of the OAPM+H+OFSE+SSD treated group was significantly higher than the wounds treated with SSD ( $p < 0.01$ ), OAPM+SSD ( $p < 0.05$ ), and control group ( $p < 0.01$ ). The stress of the skin of the OAPM+H+OFSE+SSD treated group was increased compared to the SSD treated ( $p < 0.01$ ) and control ( $p < 0.001$ ) groups. Also, the OAPM+H+OFSE+SSD treated group showed higher absorbed energy when compared to the SSD treated group ( $p < 0.05$ ), (Fig. 5).

On day 28, the ultimate load of skin samples was significantly higher in the OAPM+H+OFSE+SSD treated group when compared to the SSD treated ( $p < 0.05$ ) and control ( $p < 0.05$ ) groups. There was a significant increase in stiffness of the wounds treated with OAPM+H+OFSE+SSD in comparison with SSD treated and control groups ( $p < 0.05$ ). The strain in samples treated with OAPM+H+OFSE+SSD was significantly reduced in comparison with the control group ( $p < 0.05$ ). The modulus of elasticity was significantly higher in OAPM+H+OFSE+SSD treated group compared to the SSD, OAPM+SSD and control group ( $p < 0.01$ ,  $p < 0.05$  and  $p < 0.0001$  respectively).

Biomechanical evaluation of skin samples revealed increased stress in the OAPM+H+OFSE+SSD treated group compared to the control group ( $p < 0.05$ ) at this stage. The absorbed energy was significantly higher in the OAPM+H+OFSE+SSD treated group compared to the SSD treated ( $p < 0.01$ ) and control ( $p < 0.01$ ) groups (Fig. 5).

## Discussion

In the current study, we used a combined application of OAPM with honey and OFSE on the healing of the experimentally induced infected burn wounds. This treatment regimen was found as an effective strategy in improving the healing process and eliminating the infection in such wounds. In the present study, we infected the burn wounds with *S. aureus* and *P. aeruginosa* which are the most common pathogens found in burn infections.<sup>2,6,45,46</sup> We observed that treatment with SSD was very effective in eliminating the bacterial loads by days 3 and 7 of therapy. Despite the standard approach of excisional debridement, considerable levels of *S. aureus* were remained on the wound bed in the control group, by days 3 and 7, indicating that debridement alone was not sufficient for reducing the bacterial burden and adjunct topical antibiotics were essential. However, on day 14, the *S. aureus* counts were decreased to the baseline level of  $0.00 \pm 0.00$  in the control group. We believed that this data could be contributed to the inflammatory cell infiltration and high vascular supply of granulation tissue, as could be noted in histological images (Fig. 3). Further work focused on further details of the inflammatory response would disclose more on bacterial clearance in untreated wounds.



**Fig. 5.** Biomechanical assessment of skin samples on days 14 and 28 after treatment. The wounds treated with OAPM+H+SSD demonstrated a significant increase in modulus of elasticity and absorbed energy, by day 28. The OAPM+H+OFSE+SSD treated wounds revealed superior biomechanical properties by increasing the ultimate load, stiffness, modulus of elasticity, stress, absorbed energy, and reducing the strain in both time points. (SSD: Silver-sulfadiazine, OAPM: Ovine acellular peritoneal matrix, H: Honey, OFSE: Ovine fetal skin extract; \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ ). Error bars represent SD.

Acellular matrices when placed on the wound bed can isolate the wound from contaminants, maintain a moist environment for optimal healing, and improve epithelialization and wound contraction.<sup>13,17,47,48</sup> Burn wounds treated with porcine acellular dermal matrix showed significant recellularization, revascularization, and reduced scar tissue formation.<sup>10</sup> Several studies indicated the successful application of animal-derived acellular dermal matrices in soft tissue reconstruction procedures of the breast.<sup>21,23,48</sup> It was also demonstrated that an acellularized peritoneal scaffold combined with HA and bFGF significantly promoted wound healing and increased regeneration of epidermal and dermal layers in rabbits skin wounds.<sup>16</sup> In our study, OAPM was used as a dressing for infected burn wounds. Our results provided significant improvement in wound contraction, fibroplasia, and re-epithelialization on wound bed when OAPM was applied.

Many clinical trials have documented the healing properties of honey and suggested its use in managing various wounds.<sup>24-31</sup> The wounds treated with topical application of honey showed less edema, fewer inflammatory cell infiltrations, improved wound contraction and epithelialization.<sup>25</sup> Using honey as a wound dressing can rapidly clear infection, reduce inflammation and exudation and increase the rate of healing by improving angiogenesis, granulation tissue

formation, and re-epithelialization.<sup>27</sup> The effects of honey compared to SSD was evaluated on burn wounds and suggested that honey promoted wound healing when compared to SSD.<sup>28</sup> The results of two individual studies showed that pasture and manuka honey prevented the growth of *P. aeruginosa* and *S. aureus* and suggested that honey could be a potentially effective treatment option for burn wounds infected with these pathogens.<sup>29,30</sup> Our experiment indicated that using honey as a hydrogel could be very effective in eliminating bacterial loads, improving wound contraction, reducing inflammatory cells, and enhancing fibroplasia and re-epithelialization. The results of this experiment also revealed that the wound retarding effect of SSD could be efficiently reversed when honey was added to SSD. Besides, treatment with OAPM+H+SSD resulted in improved modulus of elasticity and absorbed energy in the healing wounds.

Fetal wound healing is characterized by the regeneration of an organized dermis with normal appendages and a lack of inflammation.<sup>32,33</sup> Experimental findings supported the intrinsic properties of fetal skin, independent of the intra-uterine environment, that are responsible for scarless wound healing.<sup>32-40</sup> Several experiments also have shown that fetal or progenitor cells would be efficient in repairing of 2<sup>nd</sup> and 3<sup>rd</sup>-degree burns, acute and chronic wounds.<sup>36-38</sup> In our study, the group

treated with OFSE showed the best results in reducing the wound area and inflammatory cells and achieving complete re-epithelialization by the rejuvenation of the hair follicles and skin appendages. This treatment modality improved the biomechanical profiles of healing wounds by increasing the ultimate load, stiffness, modulus of elasticity, stress, absorbed energy, and reducing the strain parameters. Moreover, our results indicated that OFSE could accelerate the rate of wound healing and promote scarless healing which resembled the most cosmetic appearance in the healed wounds. Further molecular and cellular studies are needed to more completely comprehend the mechanisms of fetal regenerative wound healing.

In conclusion, our study demonstrated an efficient treatment approach to optimize the treatment of infected full-thickness burn wounds, using OAPM+H+OFSE+SSD in the rat model.

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### Conflict of interest

The authors declare that there are no conflicts of interest related to this article.

### References

- Atalay S, Coruh A, Deniz K. Stromal vascular fraction improves deep partial thickness burn wound healing. *Burns* 2014;40(7):1375-1383.
- Tsai DM, Tracy LE, Lee CCY, et al. Full-thickness porcine burns infected with *Staphylococcus aureus* or *Pseudomonas aeruginosa* can be effectively treated with topical antibiotics. *Wound Repair Regen* 2016;24(2):356-365.
- Bowler PG, Duerden BI, Armstrong DG. Wound microbiology and associated approaches to wound management. *Clin Microbiol Rev* 2001;14(2):244-269.
- Alp E, Coruh A, Gunay GK, et al. Risk factors for nosocomial infection and mortality in burn patients: 10 years of experience at a university hospital. *J Burn Care Res* 2012;33(3):379-385.
- Church D, Elsayed S, Reid O, et al. Burn wound infections. *Clin Microbiol Rev*. 2006;19(2):403-434.
- Gallagher JJ, Williams-Bouyer N, Villarreal C, et al. Treatment of infection in burns: Total Burn Care. Philadelphia, USA: Elsevier 2007:136-176.
- Tiwari VK. Burn wound: How it differs from other wounds? *Indian J Plast Surg* 2012;45(2):364-373.
- Muller MJ, Hollyoak MA, Moaveni Z, et al. Retardation of wound healing by silver sulfadiazine is reversed by Aloe vera and nystatin. *Burns* 2003;29(8):834-836.
- Shahzad MN, Ahmed N. Effectiveness of Aloe Vera gel compared with 1% silver sulphadiazine cream as burn wound dressing in second degree burns. *J Pak Med Assoc* 2013;63(2):225-230.
- Chen X, Shi Y, Shu B, et al. The effect of porcine ADM to improve the burn wound healing. *Int J Clin Exp Pathol* 2013;6(11):2280-2291.
- Reinke JM, Sorg H. Wound repair and regeneration. *Eur Surg Res* 2012;49(1):35-43.
- Schultz GS, Wsocki A. Interactions between extracellular matrix and growth factors in wound healing. *Wound Repair Regen* 2009;17(2):153-162.
- Zhong S, Zhang YZ, Lim CT. Tissue scaffolds for skin wound healing and dermal reconstruction. *Wiley Interdiscip Rev Nanomed Nanobiotechnol* 2010;2(5):510-525.
- Chen RN, Ho HO, Tsai YT, et al. Process development of an acellular dermal matrix (ADM) for biomedical applications. *Biomaterials* 2004;25(13):2679-2686.
- Shukla AK, Dey N, Nandi P et al. Acellular dermis as a dermal matrix of tissue engineered skin substitute for burns treatment. *Ann Public Health Res* 2015;2(3):1023. 1-17.
- Wu Z, Fan L, Xu B, et al. Use of decellularized scaffolds combined with hyaluronic acid and basic fibroblast growth factor for skin tissue engineering. *Tissue Eng Part A* 2015;21(1-2):390-402.
- Meimandi-Parizi A, Oryan A, Moshiri A. Tendon tissue engineering and its role on healing of the experimentally induced large tendon defect model in rabbits: a comprehensive *in vivo* study. *PLoS ONE* 2013;8(9):e73016. doi:10.1371/journal.pone.0073016.
- Rizzi SC, Upton Z, Bott K, et al. Recent advances in dermal wound healing: biomedical device approaches. *Expert Rev Med Devices* 2010;7(1):143-154.
- Crapo PM, Gilbert TW, Badylak SF. An overview of tissue and whole organ decellularization processes. *Biomaterials* 2011;32(12):3233-3243.
- Hasan A, Kumar N, Gopinathan A, et al. Bovine reticulum derived extracellular matrix (b-REM) for reconstruction of full thickness skin wounds in rats. *Wound Medicine* 2016;12:19-31.
- Headon H, Kasem A, Manson A, et al. Clinical outcome and patient satisfaction with the use of bovine-derived acellular dermal matrix (SurgiMend™) in implant based immediate reconstruction following skin sparing mastectomy: A prospective observational study in a single centre. *Surg Oncol* 2016;25(2):104-110.
- Sahin I, Ozturk S, Deveci M, et al. Experimental assessment of the neo-vascularisation of acellular



- dermal matrix in the wound bed pretreated with mesenchymal stem cell under subatmospheric pressure. *J Plast Reconstr Aesthet Surg* 2014; 67(1):107-114.
23. Salzberg CA, Dunavant C, Nocera N. Immediate breast reconstruction using porcine acellular dermal matrix (Strattice™): Long-term outcomes and complications. *J Plast Reconstr Aesthet Surg* 2013;66(3):323-328.
  24. Al-Waili N, Salom K, Al-Ghamdi AA. Honey for wound healing, ulcers, and burns; data supporting its use in clinical practice. *Sci World J* 2011;11:766-787.
  25. Oryan A, Zaker S. Effects of topical application of honey on cutaneous wound healing in rabbits. *J Vet Med* 1998;45(1-10):181-188.
  26. Oryan A, Alemzadeh E, Moshiri A. Biological properties and therapeutic activities of honey in wound healing: a narrative review and meta-analysis. *J Tissue Viability* 2016;25(2):98-118.
  27. Mogoşanu GD, Grumezescu AM. Natural and synthetic polymers for wounds and burns dressing. *Int J Pharm* 2014;463(2):127-136.
  28. Aziz Z, Hassan BAR. The effects of honey compared to silver sulfadiazine for the treatment of burns: A systematic review of randomized controlled trials. *Burns* 2017;43(1):50-57.
  29. Cooper RA, Halas E, Molan PC. The efficacy of honey in inhibiting strains of *Pseudomonas aeruginosa* from infected burns. *J Burn Care Rehabil* 2002;23(6):366-370.
  30. Cooper RA, Molan PC, Harding KG. Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds. *J R Soc Med* 1999;92(6):283-285.
  31. Mohamed SH, Attia GM. A comparison between the effects of topical application of honey and that of amino acids on improving wound healing in aged rats: A histological and immunohistochemical study. *Egypt J Histol* 2013;36(2):354-564.
  32. Lapp A, Furrer P, Ramelet AA, et al. Cellular derivatives and efficacy in wound and scar management. *J Chem Dermatol Sci Appl* 2013;03(01):36-45.
  33. Leung A, Crombleholme TM, Keswani SG. Fetal wound healing: implications for minimal scar formation. *Curr Opin Pediatr* 2012;24(3):371-378.
  34. Dublet B, van der Rest M. Type XIV collagen, a new homotrimeric molecule extracted from fetal bovine skin and tendon, with a triple helical disulfide-bonded domain homologous to type IX and type XII collagens. *J Biol Chem* 1991;266(11):6853-6858.
  35. Habuchi H, Kimata K, Suzuki S. Changes in proteoglycan composition during development of rat skin. The occurrence in fetal skin of a chondroitin sulfate proteoglycan with high turnover rate. *J Biol Chem* 1986;261(3):1031-1040.
  36. Hohlfield J, De Buys Roessingh AS, Hirt-Burri N, et al. Tissue engineered fetal skin constructs for paediatric burns. *Lancet* 2005;366(9488):840-842.
  37. De Buys Roessingh AS, Hohlfield J, Scaletta C et al. Development, characterization, and use of a fetal skin cell bank for tissue engineering in wound healing. *Cell Transplant* 2006;15(8-9):823-834.
  38. Ramelet AA, Hirt-Burri N, Raffoul W, et al. Chronic wound healing by fetal cell therapy may be explained by differential gene profiling observed in fetal versus old skin cells. *Exp Gerontol* 2009;44(3):208-218.
  39. Quintin A, Hirt-Burri N, Scaletta C, et al. Consistency and safety of cell banks for research and clinical use: preliminary analysis of fetal skin banks. *Cell Transplant* 2007;16(7):675-684.
  40. Skehel JM. Preparation of extracts from animal tissues. In: Cutler P (Ed). *Protein purification protocols*. California, USA: Springer 2004; 15-20.
  41. Cai EZ, Ang CH, Raju A, et al. Creation of consistent burn wounds: a rat model. *Arch Plast Surg* 2014;41(4):317-324.
  42. Oryan A, Jalili M, Kamali A, et al. The concurrent use of probiotic microorganism and collagen hydrogel/scaffold enhances burn wound healing: An *in vivo* evaluation. *Burns* 2018; 44(7):1775-1786.
  43. Edwards C, Marks R. Evaluation of biomechanical properties of human skin. *Clin Dermatol* 1995;13(4):375-380.
  44. Lu WW, Ip WY, Jing WM, et al. Biomechanical properties of thin skin flap after basic fibroblast growth factor (bFGF) administration. *Br J Plast Surg* 2000; 53(3):225-229.
  45. Atiyeh BS, Costagliola M, Hayek SN, et al. Effect of silver on burn wound infection control and healing: review of the literature. *Burns* 2007;33(2):139-148.
  46. Vingsbo Lundberg C, Frimodt-Møller N. Efficacy of topical and systemic antibiotic treatment of methicillin-resistant *Staphylococcus aureus* in a murine superficial skin wound infection model. *Int J Antimicrob Agents* 2013;42(3):272-275.
  47. Clark RAF, Ghosh K, Tonnesen MG. Tissue engineering for cutaneous wounds. *J Invest Dermatol* 2007;127(5): 1018-1029.
  48. Salzberg CA, Ashikari AY, Berry C, et al. Acellular dermal matrix-assisted direct-to-implant breast reconstruction and capsular contracture: a 13-year experience. *Plast Reconstr Surg* 2016;138(2):329-337.