An In Vivo Comparison of Commonly Used Topical Antimicrobials on Skin Graft Healing After Full-Thickness Burn Injury

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Topical antimicrobials are frequently used for local control of infections in burn patients. It has been postulated that these agents retard wound healing. There are limited data about the effects of topical antimicrobial agents on skin graft healing. In this study, we aimed to evaluate the effects of nitrofurazone, 1% silver sulfadiazine, and povidone-iodine on skin graft healing. Forty male rats were used in this study. A meshed skin graft, placed on an excised burn wound, was used as a model to compare topical agents with a control group. Skin graft survival rates, closure of meshed graft interstices (based on physical parameters, namely epithelialization and wound contraction), and histological changes were analyzed. Graft take was more than 85% in all groups. There was no difference between the mean values of the percent graft survival for each group ($P > .05$). Epithelialization occurred significantly earlier in animals in the nitrofurazone group ($P < .05$). There was no significant difference between groups in wound contraction rates ($P > .05$). There was no histological difference between the biopsy specimens of skin grafts. In specimens obtained from the interstices of the meshed graft, no significant differences were found among the groups regarding the wound healing parameters ($P > .05$). We found that nitrofurazone, silver sulfadiazine, and povidone-iodine had no negative effect on graft healing and take in noncontaminated burn wounds. (J Burn Care Res 2015;36:e47–e54)

Based on our improved understanding of altered physiology that accompanies thermal injury, conventional treatment for major full-thickness burns now consists of early staged excision and wound closure.[1] Autologous skin remains the standard of care for definitive coverage of burn wounds.[2–6] The excised bed on which a skin graft is placed must be kept moist, warm, and bacteria free until the graft attaches itself and becomes vascularized.[7] When meshed grafts are used, the bed must also be protected until the interstices of the mesh epithelialize. The intention when using topical antimicrobials is to provide a moist environment and minimize bacterial colonization until grafts heal.[8] In the absence of evidence-based clinical practice guidelines, the use of topical antimicrobials continues to promote a great deal of debate among clinicians. The main consideration here is the possible retarding effect on wound healing. Several studies have shown that topical antimicrobials have negative effect on wound healing.[9–17] Despite common use, there are limited data about the effects of topical antimicrobial agents on skin graft healing. In this study, we investigated the effect of commonly used topical antimicrobial agents on graft healing using a model of early excision and grafting after full-thickness burn injury.

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METHODS

This study was approved by Baskent University Ethical Committee for Experimental Research on Animals. Forty male Sprague-Dawley rats, weighing 250 ± 15 g, were used in this study. The National

Research Council's guidelines for the care and use of laboratory animals were followed. The experiments were performed under general anesthesia using a mixture of 100 mg/kg ketamine and 10 mg/kg xylazine. Fentanyl hydrochloride (0.02 mg/kg) was used for analgesia after the procedures.

After induction of anesthesia, full-thickness burns were induced on the dorsum of each rat by a 2×2 cm brass plate with a burn size covering approximately 30% of the TBSA. For full-thickness burn induction, the brass plate was held for 2 minutes in the flame of a Bunsen burner and was, subsequently, pressed against the prepared skin for 10 seconds in the same way as described by Cambier et al.[18] The burn eschar was debrided 3 days after the burn, and the defect was covered with a 2×2 full-thickness skin autograft. The graft was harvested from the abdominal area. The donor site was sutured with 4-0 silk primarily. The incision was covered with petroleum-impregnated gauze. The graft was meshed by creating four 4×4 mm interstices (Figure 1). Tie-over dressings were accomplished using 1% silver sulfadiazine in group 1 (n = 10), nitrofurazone in group 2 (n = 10), povidone-iodine in group 3 (n = 10), and liquid petroleum jelly in group 4 (n = 10). Tie-over dressings were opened on the third day, and the antimicrobials were applied daily.

Graft Survival Analysis

Twelve days after grafting, the grafted area of each rat was photographed with a digital camera. These photographs were processed by appropriate software, and viability of each graft was assessed based on the appearance, texture, and color of the graft. Graft survival in each rat was calculated as a percentage of the total graft using the following formula:

$$\text{Graft survival}(\%) = \frac{\text{Area of intact graft} \quad 100}{\text{Area of total graft}}$$

Closure of Meshed Graft Interstices

After tie-over dressings were opened, the interstices of meshed grafts were inspected daily and photographs were obtained using a digital camera. Closure of these interstices was assessed based on physical parameters, namely epithelialization and wound contraction. To evaluate epithelialization, daily photographs were analyzed using a digital planimetry program to determine the days required for full epithelialization across the interstices. Wound contraction was calculated by measuring the change in the raw wound area. Using the planimetry program, the wound surface area was measured on photographs obtained 12 days after grafting. The evaluated surface

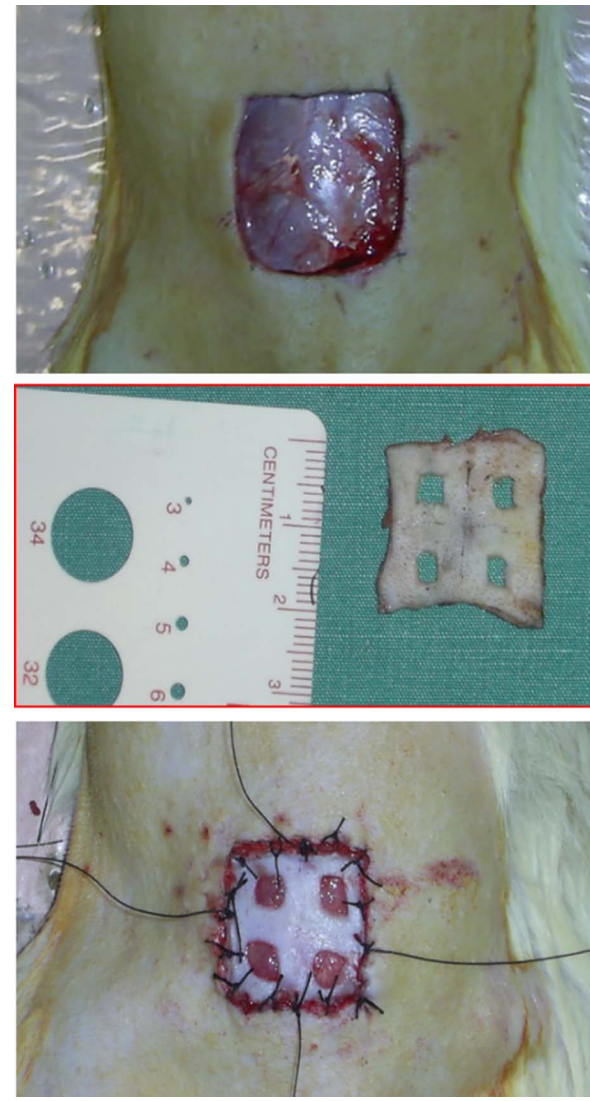

Figure 1. Full-thickness burn eschar was debrided 3 days after the burn, and the defect was covered with a 2×2 full-thickness skin autograft. The graft was meshed by creating four 4×4 mm interstices.

area was used to calculate the percentage of wound contraction, taking the initial size of the wound as %100 by using the following formula[19]:

$$\text{Wound contraction}(\%) = \frac{\text{Initial wound size} - \text{wound size on day 12 after grafting}}{\text{Initial wound size}} \times 100$$

Histological Assessment

After survival measurements, the rats were killed with an overdose of anesthetic. For each animal, two punch biopsies were performed. The first biopsy

Table 1. Histological grading scale

0	No evidence
1+	Occasional evidence
2+	Light scattering
3+	Abundant evidence
4+	Confluence of cells or fibers

The following parameters were each assessed individually: inflammatory cell infiltration, angiogenesis, fibroblast proliferation, and collagen deposition.

specimen was taken from the skin autograft tissue to evaluate graft healing histologically. The second biopsy specimen was taken from the interstices of the meshed graft, and wound healing parameters were evaluated using the Ehrlich/Hunt numerical scale (Table1).[20,21]

The tissue samples were fixed in 10% formalin and embedded in paraffin. Prepared sections were stained with hematoxylin and eosin for histological examination. Furthermore, Masson's trichrome stain was performed for observation of the collagen framework.

Statistical Analysis

Statistical analyses were performed with SPSS software (SPSS, an IBM company, version 11.0, IBM Corporation, Armonk, NY). The results are expressed as the mean ± SD of the mean. The Mann–Whitney *U* test was used to compare graft survival rates. A Dunnett's *t*-test was used to analyze the rate of epithelialization because this test allows for comparisons at multiple time periods. Histological scores were compared using a one-way ANOVA, and multiple comparisons between the groups were performed using the least significant difference post hoc test. Results were considered statistically significant at $P < .05$.

RESULTS

Graft Survival Analysis

At 12 days after grafting, the regions of survival and necrosis of the skin graft were certainly demarcated. The surviving skin appeared pink-white, soft, and normal in its texture, whereas the necrotic skin was black and rigid. There was no statistically significant difference between the mean values of the percent graft survival for each group ($P > .05$). The results are shown in Figure 2.

Closure of Meshed Graft Interstices

Planimetric evaluations showed that epithelialization occurred significantly earlier in animals in the nitrofurazone group (6.2 ± 1.135 days) followed by animals in the 1% silver sulfadiazine group (7.3 ± 0.948 days), the petroleum jelly group (8.8 ± 0.918 days), and the povidone-iodine group (10.6 ± 0.843 days), respectively (Figures 3 and 4; $P < .05$). At day 12 after grafting, there was no significant difference between groups in wound contraction rates (Figure 5; $P > .05$).

Histological assessment

There was no histological difference between the biopsy specimens of skin grafts, and it revealed epidermal hyperplasia, acanthosis, decrease in adnexal structures, subepithelial fibrosis, and increase in connective tissue, dermoepidermal disjunction, and edema. In biopsy specimens obtained from the interstices of the meshed graft, no significant differences were found among the groups regarding the wound healing parameters (Figure 6; $P > .05$). The results of histological grading are shown in Figures 7 to 10.

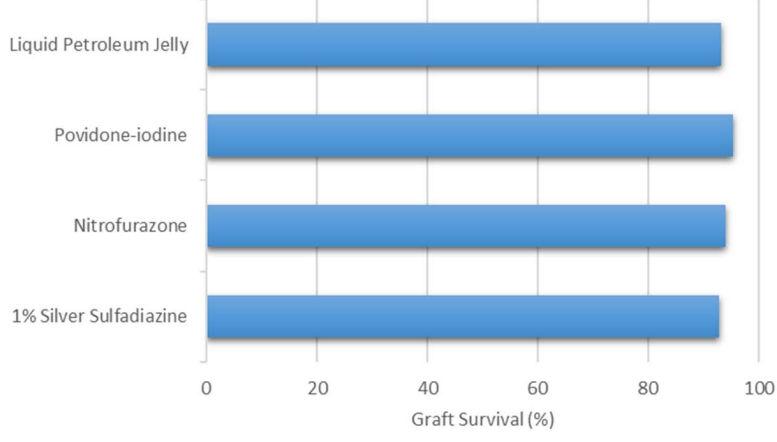

Figure 2. Mean percentage of the surviving area of the four groups. There was no statistically significant difference between the mean values of the percent graft survival for each group ($P > .05$).

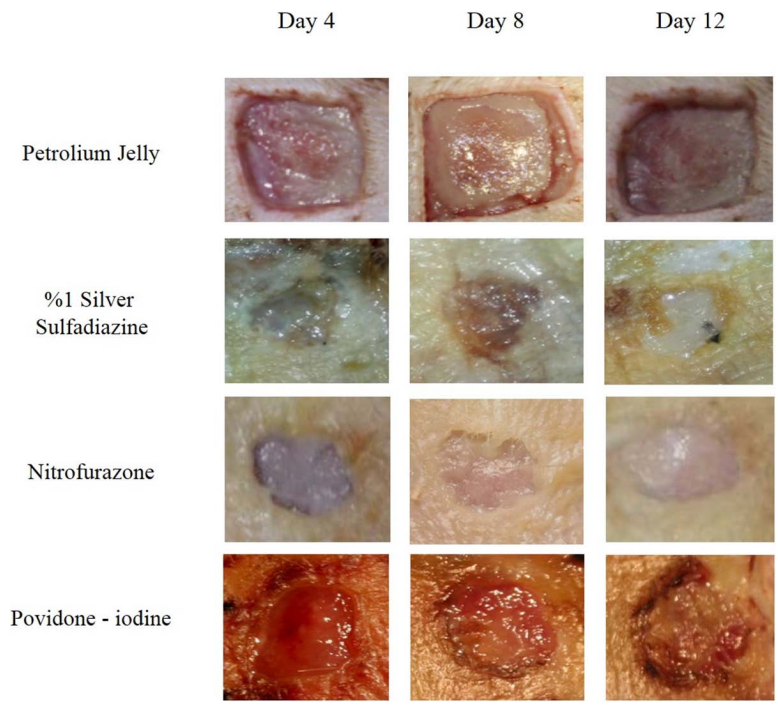

Figure 3. Planimetric evaluations showed that epithelialization occurred significantly faster in the nitrofurazone group on days 4 and 8, and the nitrofurazone group was followed by the silver sulfadiazine group, the petroleum jelly group, and the povidone-iodine group, respectively ($P < .05$).

DISCUSSION

The aim of burn management and therapy is wound healing and epithelialization as soon as possible to prevent infection and reduce functional and aesthetic after-effects.[22] A number of factors, such as disruption of the skin barrier, availability of bacterial nutrients in the wound, destruction of the vascular supply to the burned skin, and systemic immunosuppression, combined together to make burns particularly susceptible to infection.[23,24] A considerable proportion of skin grafts are lost because of infection. Colonization of the open wound in the meshed graft may also lead to formation of infected granulation tissue, which may impair the outgrowth of epithelium from the small dermoepidermal elements in the meshed grafts.[25] In addition, infection of the wound may lead to the formation of burn scar hypertrophy.[26] Therefore, there is a need for frequent application of topical antimicrobial products.[27,28]

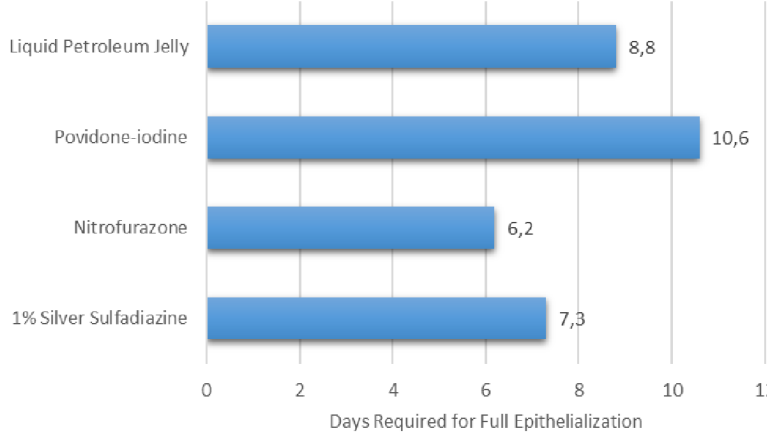

Figure 4. Planimetric evaluations showed that epithelialization occurred significantly earlier in animals in the nitrofurazone group (6.2 ± 1.135 days) followed by animals in the 1% silver sulfadiazine group (7.3 ± 0.948 days), the petroleum jelly group (8.8 ± 0.918 days), and the povidone-iodine group (10.6 ± 0.843 days), respectively ($P < .05$).

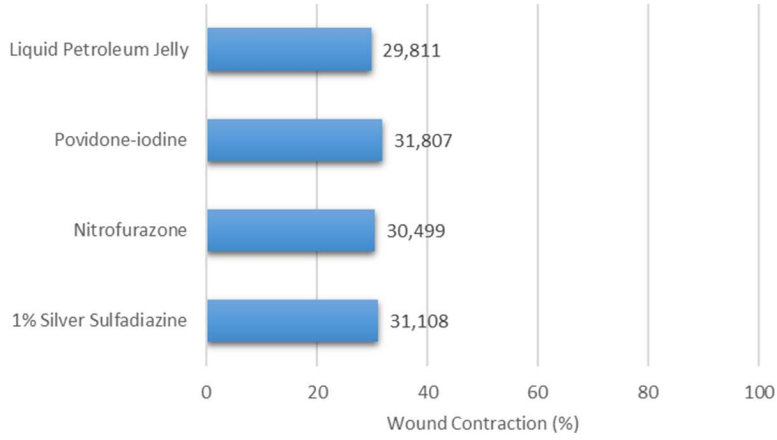

Figure 5 There was no significant difference between groups in wound contraction rates at 12 days after grafting ($P > .05$).

This study represents a comparison of commonly used topical agents, with variations in antimicrobial efficacy. We used a full-thickness burn wound, excised after 3 days and covered with a meshed skin graft, as our wound model. Early excision resulted in a wound bed that was minimally colonized before grafting. This allows for the study of graft healing parameters without the concern of bacterial colonization or infection.[29] The meshed skin graft provides a standardized model for the study of graft healing and epithelialization across the interstices of the meshed graft.[30,31]

We have shown that nitrofurazone, silver sulfadiazine, and povidone-iodine had no negative effect on skin graft healing and take. Graft take was more than 85% in all groups, and there was no statistically significant difference between groups. Topical agents are thought to promote graft healing by minimizing bacterial colonization at the graft area while providing a moist environment for better healing. This may help in reducing graft losses because of infection.

There was a significant increase in epithelialization across the mesh graft in the nitrofurazone-treated wound compared with silver sulfadiazine and povidone-iodine. In addition, epithelialization was complete in the presence of silver sulfadiazine and povidone-iodine at 10 days after grafting. In contrast to in vitro testing, application of topical

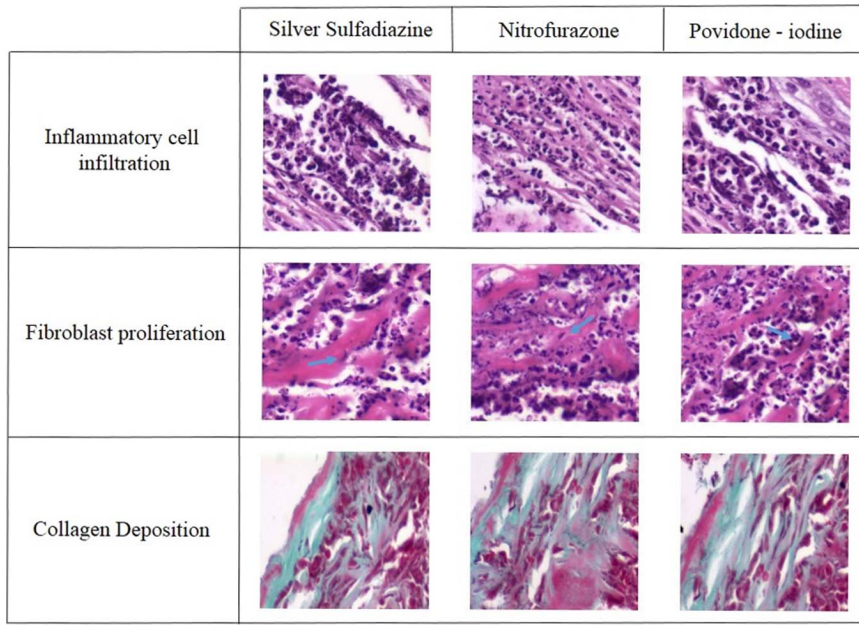

Figure 6. Inflammatory cell infiltration, fibroblast proliferation, and collagen deposition were assessed individually using the Ehrlich/Hunt numerical scale. No significant differences were found among the groups regarding these parameters ($P > .05$).

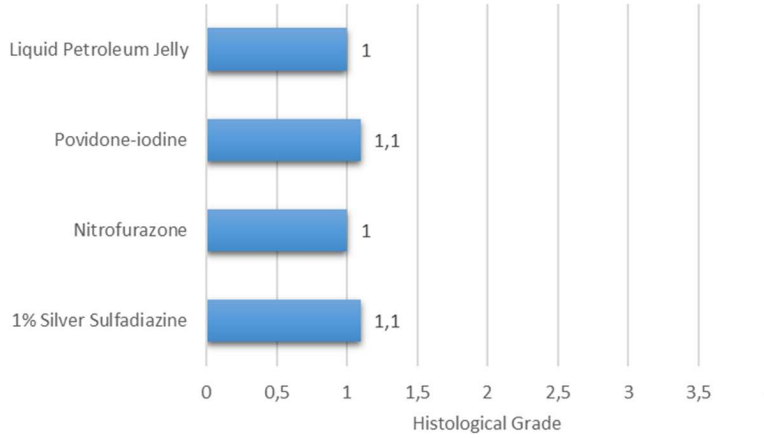

Figure 7. Histological scoring of inflammatory cell infiltration using the Ehrlich/Hunt numerical scale.

antimicrobials did not prolong the reepithelialization time. The difference can be explained by the fact that in vitro tissue culture protocols do not adequately replicate the complexities and extensive interactions that are present within the milieu of the wound bed. Isolated cells without the protective extracellular environment are much more vulnerable than cells embedded in their own tissue.

Wound contraction is a process that requires intact functioning fibroblasts and collagen production. It has been found that wound contraction is significantly impeded by silver sulfadiazine application in an acute rat wound model.[32] The underlying mechanism is thought to be the disruption of fibroblasts. In this study, there were no significant differences in means of contraction rates between groups. The percentage decrease in wound surface area was found to increase in a time-dependent manner in all the groups.

Nitrofurazone as a topical cream contains 0.2% nitrofurazone in a water-soluble cream base. In recent years, nitrofurazone seems to have been abandoned, principally for fear of contact allergy and hypersensitivity reactions. In the largest review of this topic, the rate of hypersensitivity among patients treated with topical nitrofurazone was 1.2%.[33] In 1979, Geronemus et al[34] evaluated the effect of nitrofurazone on the rate of epithelialization of clean wounds in white domestic pigs. He found that nitrofurazone retarded the healing rate by 24%. Boyce et al[35] examined nitrofurazone on cultured human keratinocytes and fibroblasts. There was a dose-dependent toxicity to human cells. However, in this study we found that nitrofurazone application increased the rate of mesh closure compared with silver sulfadiazine and povidone-iodine. We think that the prohealing effect was unrelated to antimicrobial properties of nitrofurazone. The mechanism requires additional study.

Povidone-iodine is a complex of iodine with povidone and is the most widely used form of iodine as an antiseptic. This complex provides a reservoir of iodine, with a gradual release of iodine to the target

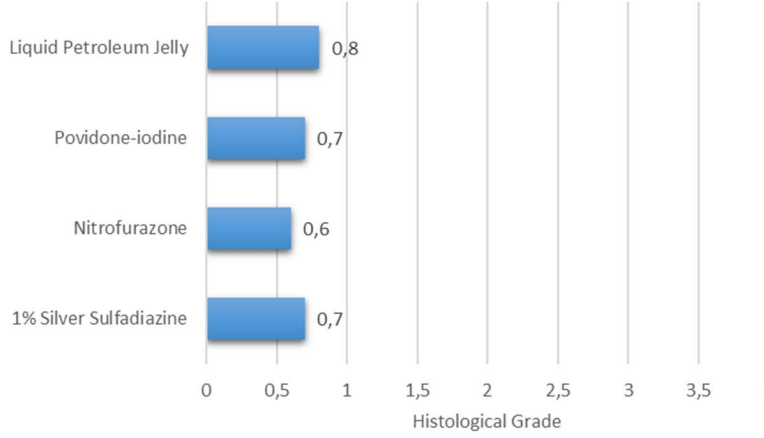

Figure 8. Histological scoring of fibroblast proliferation using the Ehrlich/Hunt numerical scale.

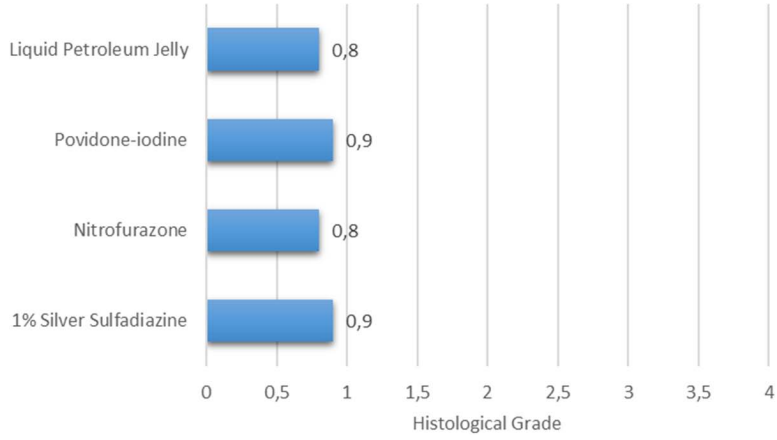

Figure 9. Histological scoring of vascular proliferation using the Ehrlich/Hunt numerical scale.

tissue. As illustrated in the animal and in vitro models, povidone-iodine impairs collagen synthesis, has a toxic effect on fibroblasts and keratinocytes, and impairs epithelial cell migration, therefore potentially having a detrimental effect on the process of wound healing.[14,36,37] A recent clinical study found that the application of povidone-iodine ointments on split-thickness skin grafts did not delay wound healing.[38] In the current study, povidone-iodine–treated epithelialization seemed to be slower at the beginning but resulted in good epithelialization at the end of the study. In contrast to previous in vitro studies, treatment with povidone-iodine ointment did not dramatically prolong the total wound healing time.

Silver sulfadiazine is the most popular topical agent for the treatment of burn wounds.[39] It contains 1% insoluble silver sulfadiazine in micronized form. The active component is a mixture of silver nitrate and sodium sulfadiazine. Its silver ions bind to nucleic acids of individual microorganisms and release sulfadiazine, which poisons the metabolism of microbe.[40] Extensive treatment of acute burn wounds with silver sulfadiazine, however, has recently raised concern about potential silver toxicity.[41] Laboratory studies confirm that both keratinocytes and fibroblasts are susceptible to lethal damage when exposed to silver.[42–45] However, several studies suggest that silver may play a role in compressing the inflammatory events in wounds and facilitating the early phases of wound healing. These benefits are associated with reduced local matrix metalloproteinase levels and enhanced cellular apoptosis.[46,47]

CONCLUSION

The current study found that nitrofurazone, silver sulfadiazine, and povidone-iodine had no negative effect on graft healing and take in noncontaminated burn wounds. When meshed graft is used, we recommend avoiding povidone-iodine application because it resulted in a slower epithelialization. However, epithelialization was good at the end of the study.

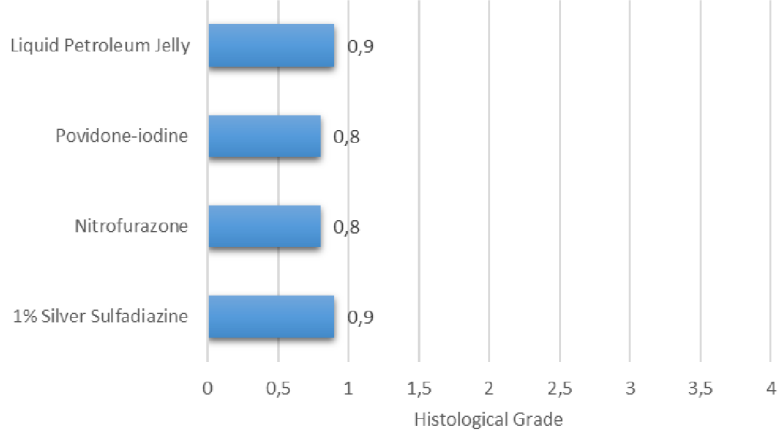

Figure 10. Histological scoring of Collagen deposition using the Ehrlich/Hunt numerical scale.

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