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A comparative burn wound model in the New Yorkshire pig for the histopathological evaluation of local therapeutic regimens: silver sulfadiazine cream as a standard

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SUMMARY. **A standard burn wound model was developed in the pig to enable evaluation of histopathological parameters of wound healing under different circumstances. Wounds on one flank were treated with silver sulfadiazine 1% cream (SSD, Flammazine-Duphar), which is a standard treatment. On identical places of the contralateral side different topical agents were applied. From the SSD treated burns a typical histopathologic picture of wound healing under SSD could be derived: SSD has the potential to preserve viable dermal tissue, epidermal regeneration is rather slow and irritated, while the formation of granulation tissue is pronounced, with an abundance of myofibroblasts.**

In order to assess the influence of local therapeutic regimens on the healing pattern of burn wounds, differently treated burns have to be comparable in depth and extent. Profound insight into the healing pattern of burn wounds cannot be acquired solely by the evaluation of macroscopic parameters like crust formation, epithelialisation, contraction and scar formation. In addition to the macroscopic observation, histopathological evaluation is crucial.

In the clinical situation these demands can hardly be met. Therefore a burn wound model in the New Yorkshire pig has been developed.

The aim of our research is to describe the histomorphology of burn wound healing with respect to the application of a local therapeutic agent. A comparison was made between burn wounds treated in different ways and burn wounds treated with a standard topical agent, silver sulfadiazine 1% cream (SSD, Flammazine-Duphar, Weesp, The Netherlands).

The antibacterial activity of SSD has been investigated thoroughly.[1,2] Details are known about the distribution and penetration of SSD in scalds in the pig.[3]

With respect to macroscopic aspects and rate of wound healing comparative evaluation of SSD and other topical agents has been described.[4,5]

The histomorphological healing pattern of untreated burns in the pig has also been described.[6–8] Very little knowledge exists, however, about the influence of SSD on the histomorphological healing pattern of burn wounds during the course of treatment.

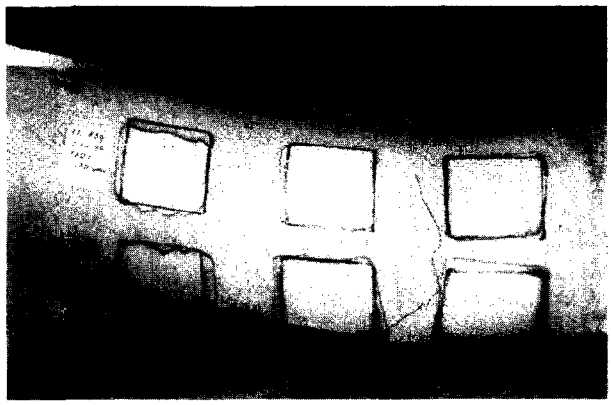

Fig. 1

Figure 1—View of the left flank of a New Yorkshire pig; 6 deep dermal burns just after infliction.

Fig. 2

Figure 2—Brass block used to inflict a standardised burn wound with a temperature of exactly 170°C.

Choice of the animal model

The New Yorkshire pig has been chosen as the experimental animal because of all animal species the domestic pig seems to be the one with morphological and functional skin characteristics nearest to human skin and so best fulfils the requirements of a model for human skin.[9] In basic architecture it resembles human skin in the relative thicknesses of the epidermis and dermis, the presence of epidermal ridges, a distinct dermal papillary layer and a deep layer of subdermal fat.[10]

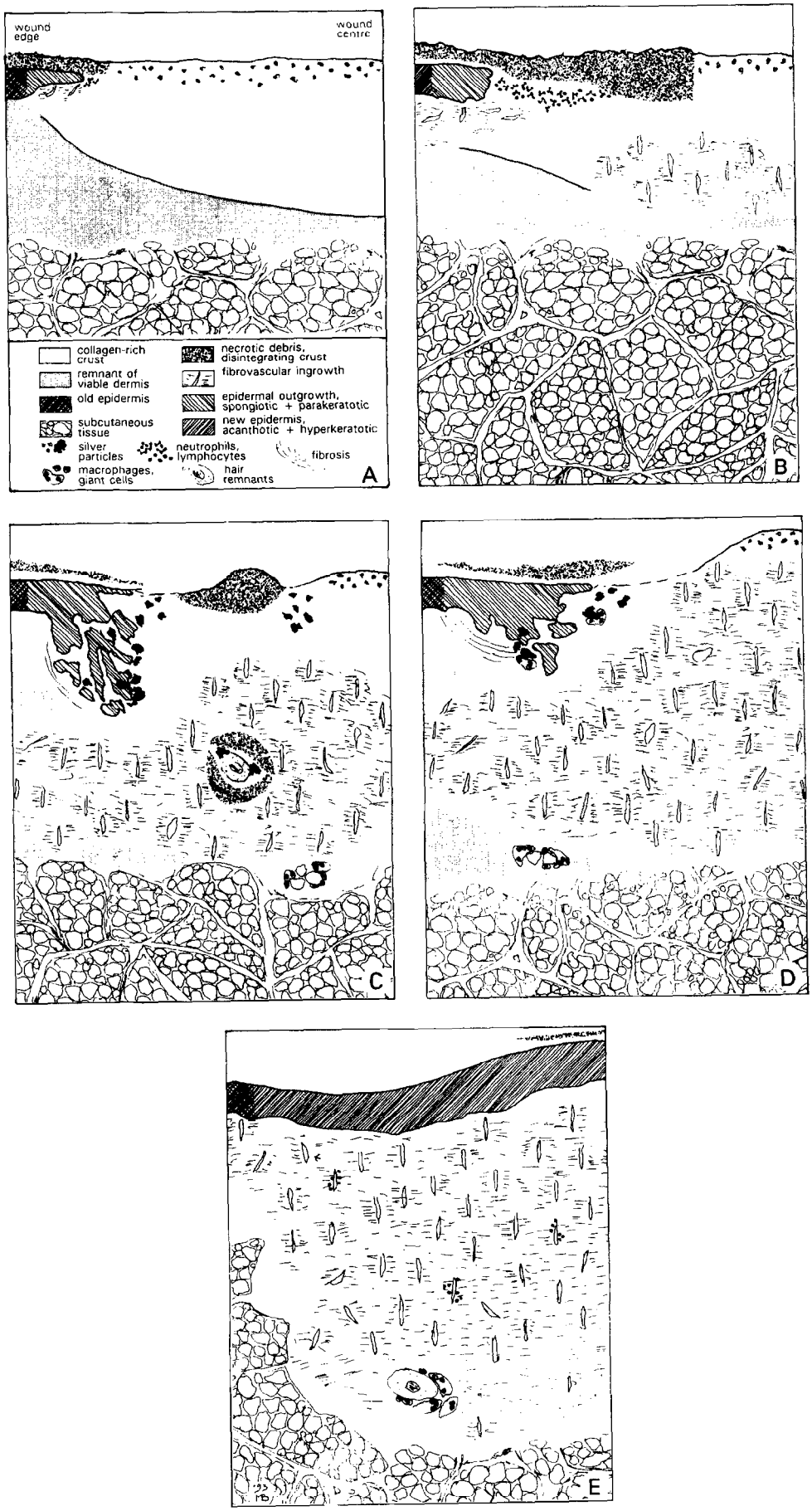
wound edge
wound centre
collagen-rich crust
remnant of viable dermis
old epidermis
subcutaneous tissue
silver particles
macrophages, giant cells
neutrophils, lymphocytes
hair remnants
necrotic debris, disintegrating crust
fibrovascular ingrowth
epidermal outgrowth, spongiotic + parakeratotic
new epidermis, acanthotic + hyperkeratotic
fibrosis
A
B
C
D
E

Fig. 3

Compared to humans the elastic fibre content of porcine dermis is relatively low, but higher than in any other species.[11,12]

Comparison of human and porcine epidermis and its appendages also suggests common traits. Studies on the proliferation rate of porcine epidermis show parallels with those of humans. The keratinous proteins are similar.[13] Unlike the skin of rodents, the follicular pattern in pigs and humans is relatively sparse and arranged as single hairs or in groups of two or three follicles.[11,14] Pigs do not sweat. The regulation of body temperature by the skin is more evident in humans than in the pig. In the skin of the pig no eccrine glands are found. It does have apocrine glands, but their role in thermoregulation remains debatable.[15]

The vascular anatomy of pig skin consists of a three-layered network; lower, mid-dermal and subepidermal.[9] The size, orientation and distribution of the vessels are strikingly similar to human skin[16,17] but it does differ from humans in that the subepidermal network is less dense.[18]

The vascularisation of the lower region of the follicle corresponds to that in humans.[16] The healing of deep dermal burns, which depends on this phenomenon,[19] might also be analogous.

Studies on the thermal properties of porcine skin as a function of depth have been performed by measuring the tissue water content. With the use of a mathematical model the heat capacity and thermal conductivity could be calculated and results for pig skin were found to be consistent with those for human skin.[20]

Choice of the burn wound model

The quality of a model for the infliction of standard burns depends on its reproducibility. The consecutive burn wounds should ideally be identical in depth and extent.

For this purpose a standardisation of the method practised is imperative. This can be achieved by exactly defining the size and location of the burn wound,[8] the temperature gradient, duration of exposure[21] and method of applying the burn.[22] For the infliction of a contact burn the determination of the application pressure is essential.[8] Depth and extent of the burn wound in relation to the total body surface area have to be determined in advance.[23] A contact burn by means of a heated brass block can fulfil these criteria.[24–27] The brass block has a high thermal capacity and causes efficient heat transfer primarily by conduction.[22]

The wound surface area has to be of sufficient size so that healing cannot occur by contraction alone. On the other hand, the total wound surface area should be too small to cause major systemic problems. The latter can be concluded from undisturbed weight gain of the animals.[28] On the left and right flank of the animal burns of identical localisation, depth and extent can be inflicted. By this method differences in skin thickness, water content and other variation can be avoided as much as possible.

Temperature, humidity, pH and blood flow at the moment of burn infliction are identical for the paired wounds.[29] By means of these mirror image wounds, each treated burn has its control on the contralateral side of the animal.[30] The severity of the burn was such that sufficient observation time (4–6 weeks) was achieved, while total healing occurred.

Thus, classification of wound healing characteristics with respect to different topical agents and wound covering materials is feasible.

Materials and methods

The experimental material consisted of 6 New Yorkshire pigs. At the beginning of the study the age was approximately 14 weeks and the weight was around 35 kg. During the treatment the pigs were housed according to G.L.P. standards of Dutch Veterinary Law.

As premedication, 4 mg Stressnil and 0,5 mg atropine i.m. were given at least 30 min before the procedure.

Infliction of burns and daily wound treatment were performed under general anaesthesia using a halothane, N_2O, O_2 mixture, without intubation.

Prior to the experiment both flanks of the pigs were shaved electrically and cleaned with chlorhexidine 0,5 % in ethanol 70 % solution.

Twelve areas of 7 × 7 cm (6 on each flank) were marked in a symmetrical way, using the processus spinosi as an anatomical landmark (Fig. 1).

Deep dermal burns were inflicted by applying a brass block of 6.7 × 6.7 cm, weighing 450 g (Fig. 2). The block was heated up to 170°C and was applied during 20 s, without exerting pressure. The outer remnants of skin were removed after infliction of burns. The total burned surface area amounted to not more than 10 % of the total body surface area.

Treatment, observation and biopsy

All six pigs were treated on one flank with SSD cream that was applied daily after removal of cream remnants. On the contralateral side different topical agents were applied, again after removal of remnants, or the wound was left untreated. The wounds on both flanks were covered with a woven cotton material (Kings Cotton).

Figure 3—Histopathology of burn wounds treated with silver sulfadiazine 1 % cream (SSD). Evaluation at successive post burn days (PBD) of crust, re-epithelialisation, granulation tissue and dermis, inflammation and silver particles. (A) 7th PBD. The crust is mainly intact, only at the wound edges some disintegration with a neutrophilic infiltrate. Beginning outgrowth of the epithelium. There is a remnant of viable dermis, no fibrovascular ingrowth yet. Penetration of silver particles only in the upper layers of the crust. (B) 14th PBD. Disintegration of the paracentral crust, an infiltrate demarcates viable and necrotic tissue. Broadening of the epidermal outgrowth. Start of fibrovascular ingrowth from the remnant of viable dermis and under the new epidermis. (C) 21st PBD. Only the central part of the crust is intact. Where the crust is missing silver particles can penetrate, also around hair remnants. Around the hairs suppuration or macrophages with giant cells. Macrophages and giant cells are also found around superficial fat cells. The epidermis shows wild pseudocarcinomatous outgrowth, accompanied by fibrosis. In the wound centre a strong fibrovascular reaction. (D) 28th PBD. Intact crust only in the central part. Penetrated silver particles are surrounded by giant cells. Pseudocarcinomatosis diminishes. The granulation tissue is thicker than the unburned dermis. (E) 35th–42nd PBD. Further disintegration of the crust. The re-epithelialisation is completed. Further thickening of a more or less uneventful granulation tissue with only small perivascular infiltrates and reaction around hairs and fat cells. Sometimes there is no remnant of viable dermis.

As a control for the animals' wellbeing, body weight and temperature were measured at the same occasion. The treatment and observation were continued daily until at least a complete epithelialisation of the wound surface had occurred.

Each week (on post burn days 7, 14, 21 *etc*) biopsies were taken symmetrically from the left and right flank. Biopsies included the wound bed as well as the healthy skin of the wound margins. The wounds left by the biopsy were dressed with collagen fleece (Bioplex Medical BV, Vaals, The Netherlands).

The biopsies were fixed in Kryofix (E. Merck, Darmstadt, Prod. Nr. 5211), cut into a central and two peripheral parts, and four stainings were routinely performed: a modified Papanicoulaou staining for the general microscopic evaluation, the Elastica Van Gieson staining for a better visualisation of the crust and the regenerating dermis, the Von Kossa staining to distinguish the silver particles of SSD, and the alpha-smooth muscle actin to identify the myofibroblasts.

During the microscopic observation four wound healing parameters were evaluated: crust formation, re-epithelialisation, formation of granulation tissue and inflammatory reaction.

Results

Wound healing with SSD

The wound healing pattern for the SSD treatment was largely the same in the six pigs.

Macroscopically the wound showed a typical mixed aspect of eschar within granulation tissue and outgrowing epithelium. A time-dependent change was seen in the microscopic observation of the four parameters (see Fig. 3).

The crust is mainly formed by a broad layer of necrotic collagen. Disintegration of the crust starts on the seventh post burn day (7th PBD) at the wound edges, reaches the paracentral region at 14th PBD, at 21st PBD penetration of silver particles takes place where the crust is missing, and total disintegration is seen at 35th/42nd PBD. Re-epithelialisation, starting from the edges, can be seen at 7th PBD, at 14th PBD the epidermis broadens and by 21st PBD it has a maximal pseudocarcinomatous outgrowth (Fig. 3C and Fig. 4). From 7th PBD till 28th PBD the new epithelium shows spongiosis and parakeratosis. When re-epithelialisation is completed at 35th/42nd PBD there is still some acanthosis, while parakeratosis has been replaced by hyperkeratosis.

Figure 4—Pseudocarcinomatous outgrowth of the epithelium at the 21st post burn day under SSD treatment. Keratin staining, × 25.

The first fibrovascular reaction is seen at 14th PBD. It starts from the small layer of surviving dermis, is strong at 21st PBD, when myofibroblasts begin to spread, and the resulting granulation tissue is thicker than the unburned dermis by 28th PBD. The thickening continues until the end of the experiment (42nd PBD), and sometimes the remnant of viable dermis is lost by this time.

An inflammatory reaction over the full width of the wound appears only at 14th PBD, when neutrophils and lymphocytes have demarcated the border between viable and necrotic tissue.

Giant cells appear at 21st PBD around remnants of hair follicles, while some of the follicle fragments are surrounded by heavy suppuration. Macrophages attack fat cells, and by 28th PBD also silver particles. At the end of the experiment small perivascular infiltrates are seen, sometimes admixed with eosinophils. The reactions around hair remnants and fat cells still exist.

Other wounds not treated with SSD

For the other treatments we can indicate the most striking differences, which have to be reaffirmed in further experiments.

In untreated wounds, the wound deepened and fibrovascular ingrowth was first seen in the septa of the subcutaneous fat. The crust was thicker, remained longer and the outgrowth of epithelium was faster and less irritated.

In wounds treated with a newly developed non toxic formulation of tannic acid the crust not only survived longer but in addition its clinical appearance was very supple. This resulted in an uneventful and rapid re-epithelialisation, while inflammation and fibrosis were reduced (details will be published later).[32]

When SSD was applied in a liposome formulation, silver particles seemed to penetrate through the whole crust, which remained intact during the treatment period (details to be published later).[33]

Discussion

Comparison of different test animals in the literature showed the pig to be a reliable model for burn wounds when these are inflicted in a reproducible and standardised way. To be able to compare different topical agents it is necessary to know the histopathology of a standard burn wound treatment such as SSD.

SSD is used in burn wounds because of its superior antibacterial action. Its influence on wound healing has been noted macroscopically;[34] the histopathologic events, however, were unknown till now.

The microscopic evaluation of SSD treated wounds shows that SSD has the potential to preserve viable dermal tissue. Epidermal regeneration takes place at a rather slow rate under an easily disintegrating crust. The newly formed epidermis has temporarily an 'irritated' aspect with spongiosis, parakeratosis and pseudocarcinomatosis. This dermatitis-like reaction is

probably caused by direct exposure to SSD. Eventually a normal epidermis develops. In the underlying dermis the presence of myofibroblasts is noted, which is positively correlated with wound contraction.[31] At the same time there is an abundance of granulation tissue which is known to be associated with the extent of fibrosis. Given these facts we now have the opportunity to test other well known or newly developed topical agents which can suppress inflammation and granulation tissue formation.

References

1. Fox CL Jr. Topical therapy and the development of silver sulfadiazine. Surg Gynecol Obst 1983; 157: 82–8.
2. Nangia AK, Hung CT, Lim JCK. Silver sulfadiazine in the management of burns: an update. Drugs of Today 1987; 23: 21–30.
3. Lazare R, Watson PA, Winter GD. Distribution and excretion of silver sulphadiazine applied to scalds in the pig. Burns 1974; 1: 57–64.
4. Kaufman T, Levin M, Hurwitz DJ. The effect of topical hyperalimentation on wound healing rate and granulation tissue formation of experimental deep second degree burns in guinea-pigs. Burns 1984; 10: 252–6.
5. Kaiser W, Von der Lieth H, Potel J, Heymann H. Experimental study of the local application of silver sulfadiazine, cefsulodin and povidone-iodine in burns. Infection 1984; 12: 31–5.
6. Winter GD. Histological aspects of burn wound healing. Burns 1975; 1: 191–6.
7. Behounkova-Houskova E, Moserova J. Histology of healing of deep dermal burns in pigs. Scand J Plast Reconstr Surg 1979; 13: 57–60.
8. Moserva J, Houskova E, eds. The healing and treatment of skin defects. Basel: Karger, 1989: 39–46.
9. Meyer W, Schwarz R, Neurand K. The skin of domestic mammals as a model for the human skin with special reference to the domestic pig. Curr Probl Dermatol 1978; 7: 39–52.
10. Winter GD, ed. Epidermal regeneration studied in the domestic pig. Chicago: Medical Yearbook, 1972: 4, 71–112.
11. Marcarian H, Calhoun ML. Microscopic anatomy of the integument of adult swine. Am J Vet Res 1966; 27: 765–72.
12. Montagna W, Yun JS. The skin of the domestic pig. J Invest Derm 1964; 43: 11–21.
13. Weinstein GD. Comparison of turnover time and of keratinous protein fractions in swine and human epidermis. In: Bustad LK, McClellan RD, eds. Swine in biomedical research. Seattle: Frayn, 1966: 287–9.
14. Carter HB. Variation in the hair follicle population of the mammalian skin. In: Lyne AG, Short BF, eds. Biology of skin and hair growth. New York: American Elsevier Publishing Company, 1965: 25–33.
15. Mount LE, ed. Adaptation to thermal environment. Baltimore: University Park Press, 1979: 182–207.
16. Forbes PD. Vascular supply of the skin and hair in swine. In: Montagna W, Dobson RL, eds. Hair Growth—advances in biology of skin. Oxford: Pergamon, 1967: 419–32.
17. Bloch BF, Pinkus H, Spalteholtz W, eds. Anatomie der Haut. Berlin: Springer Verlag, 1927: 32–47.
18. Ingram DL, Weaver ME. A quantitative study of the blood vessels of the pig's skin and the influence of environmental temperature. Anat Rec 1969; 163: 517–24.
19. Levenson SM. Pathophysiology of burns—an overview of some aspects of the metabolic and nutritional responses to burns. Symposium on the treatment of burns. St Louis: The C.V. Mosby Co, 1973.
20. Knox FS, Wachtel TL, McCahan GR, Knapp SC. Thermal properties calculated from measured water content as a function of depth in porcine skin. Burns 1986; 12: 556–62.
21. Moritz AR, Henriquez FC. Studies of thermal injury. II. The relative importance of time and surface temperature in the causation of cutaneous burns. Am J Path 1947; 23: 695–720.
22. Davies JWL, ed. Physiological responses to burning injury. London: Academic Press, 1982: 9–44.
23. Bourrinet P, Queavauviller A. Les brûlures expérimentales. Therapie 1971; 26: 259–90.
24. Price PB, Call DE, Hansen FL, Zerwick CJ. Penetration of heat in thermal burns. Surg Forum 1953; 4: 433–8.
25. Chvapil M, Speer DP, Owen JA, Chvapil T. Identification of the depth of burn injury by collagen stainability. Plast Reconstr Surg 1984; 74: 438–41.
26. Güler Gürsu K. An experimental study for diagnosis of burn depth. Burns 1984; 4: 97–102.
27. Brown GL, Curtsinger L, Brightwell JR, et al. Enhancement of epidermal regeneration by biosynthetic epidermal growth factor. J Exp Med 1986; 163: 1319–24.
28. Kistler D, Hafemann B, Schmidt K. A model to reproduce predictable full-thickness burns in an experimental animal. Burns 1988; 14: 297–302.
29. Kaufman T, Hirshowitz B. The influence of various microclimate conditions on the burn wound: a review. Burns 1982; 9: 84–8.
30. Kaufman T, Lusthaus SN, Sagher U, Wexler MR. Deep partial skin thickness burns: a reproducible animal model to study burn wound healing. Burns 1990; 16: 13–6.
31. McGrath MH, Hundahl SA. The spatial and temporal quantification of myofibroblasts. Plast Reconstr Surg 1982; 69: 975–82.
32. Dutrieux RP, Hoekstra MJ, Kreis RW. Tanning wounds: new prospects? In: Proceedings of the 18th world congress of dermatology. New York, 1992 (in press).
33. Hoekstra MJ. Rogmans MP. Experimental burns in pigs for the evaluation of topical wound therapy. In: Harding KG, Leaper DL, Turner TD, eds. Proceedings of the 1st European conference on advances in wound management. London: Macmillan Magazines Ltd, 1992: 11–3.
34. Geronemus RG, Mertz PM, Eaglstein WH. Wound healing, the effects of topical antimicrobial agents. Arch Dermatol 1979; 115: 1311–4.

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