Accelerated Healing of Full-Thickness Skin Wounds in a Wet Environment

Tor Svensjö, Bohdan Pomahac, M.D., Feng Yao, Ph.D., Jaromir Slama, M.D., and Elof Eriksson, M.D., Ph.D.

Boston, Mass., and Malmö, Sweden

Full-thickness skin wounds are preferably allowed to heal under controlled hydration dressings such as hydrocolloids. It was hypothesized that a wet (liquid) environment rather than a dry or moist one would accelerate the wound healing process. We compared skin repair by secondary intention in full-thickness skin wounds in wet (saline), moist (hydrocolloid), and dry (gauze) conditions in an established porcine wound healing model. The study included three animals with a total of 70 wounds layered in a standardized fashion on the back of young Yorkshire pigs. Twelve days after wounding, 0 percent of dry, 20 percent of moist, and 86 percent of saline-treated wounds were completely reepithelialized (p values = 0.0046 and 0.027 for saline wounds compared with dry and moist wounds, respectively). The accelerated healing was caused at least in part by faster contraction in wet wounds (p value < 0.005 compared with that of other groups 9 and 12 days after wounding). Development of granulation tissue was faster in moist conditions than it was for dry and wet wounds. The thickness and number of cell layers of the newly formed epidermis were greater in dry and wet wounds than in moist ones. It was concluded that these full-thickness porcine skin wounds healed faster in a wet rather than in moist or dry environments.

From the Laboratory of Tissue Repair and Gene Transfer, Division of Plastic Surgery, Brigham and Women’s Hospital, Harvard Medical School; and the Department of Plastic and Reconstructive Surgery, Malmö University Hospital. Received for publication November 12, 1998; revised December 1, 1999.

Presented at the Annual Clinical Congress of the American College of Surgeons, in Chicago, Illinois, October 12 through 17, 1997.

602
wound surface. Previous studies of partial-thickness wounds have shown reduced inflammation and acceleration of healing in a wet environment. This study investigated the healing of full-thickness wounds that, unlike partial-thickness wounds, also heal by contraction and substantial neodermal formation. Most wounds presenting as clinical problems are full-thickness, and a direct comparison among dry, moist, and wet conditions has not yet been performed. The results presented here demonstrate the significance of wound hydration in all three basic aspects of full-thickness skin wound repair by secondary intention: fibrovascular tissue formation, contraction, and reepithelialization.

**Materials and Methods**

**Animals**

Porcine and human skin are very similar in structure and cell turnover time. Like humans, pigs are relatively hairless. Because their skin correlates well with the thickness and overall size of human skin, pigs were chosen for our wound healing studies. All animal procedures were approved by the Harvard Medical Area Standing Committee on animals. Female Yorkshire pigs, about 3 months old and weighing 30 to 40 kg, were fed a standard porcine diet with free access to water and housed at 20 to 23°C in an atmosphere of about 65 percent humidity with a light cycle of 12 hours on and 12 hours off. They were kept in separate, custom-made, smooth-sided, stainless steel cages to minimize wound trauma and disruption of applied chambers and dressings. Intramuscular injections of buprenorphine (0.075 mg/kg) before awakening provided postoperative analgesia, and the pigs were killed by intravenous injection of 5 g thioental administered during anesthesia.

**Wounding**

Fasted pigs were placed in a Panepinto sling (Charles River Breeding Laboratories, Portage, Canada) and anesthetized with 1.0% to 2.5% halothane (Wyeth-Ayerst Laboratories, Philadelphia, Pa.) delivered in conjunction with a 3:5 mixture of oxygen and nitrous oxide by means of a facial mask. Heart rate and blood oxygen saturation were monitored throughout all procedures. The dorsal skin hair was clipped and the stubble was carefully shaved using shaving cream and disposable razors. The skin surface was washed with mild soap and water and decontaminated by consecutive applications of 7.5% and 10% povidone-iodine solutions (Clinipad, Rocky Hill, Conn.) and 70% isopropanol (Diamond Drug, Westhaven, Conn.), then cleansed with trichloroethane (Sigma, St. Louis, Mo.) and wetted gauze; the two latter steps were performed to achieve optimal conditions for chamber and dressing adhesion. After sterile draping, wound sites measuring 1.5 cm × 1.5 cm and separated by at least 4 cm of unwounded skin were outlined along four parallel paraspinal stripes between the crest of the shoulders and the buttocks and then tattooed with black tattoo ink using an electric tattoo marker (Spaulding and Rogers, Voorheesville, N.Y.) and template. Full-thickness excisional wounds (1.5 cm × 1.5 cm, approximately 0.8 cm deep) were created by vertically excising the skin area within the tattooed borders down to the level of, but not including, the panniculus. Complete hemostasis was achieved by direct pressure and cautery.

**Treatment**

A thin layer of medical adhesive (Hollister, Inc., Libertyville, Ill.) was carefully brushed onto the skin surrounding each wound and the wounds were covered with gauze (7.6 cm × 7.6 cm, Taylor Medical, Indianapolis, Ind.), chambers (P.A. Medical Corp., Columbia, Tenn.), or hydrocolloids (Duoderm CGF 6 × 6; Squibb & Sons, Irvine, Calif.), firmly pressed into place to avoid leakage. Experimental groups were layered in a standardized fashion (A, B, C; B, C, A; and so forth) along and across the longitudinal rows of wounds in a zigzag fashion to minimize any effects in healing rates that would depend on wound location. Seventy-eight wounds on three animals were prepared for this study. Chambers and gauze were changed daily, and hydrocolloids were changed if leaking (according to manufacturer’s instructions) or every 3 days, when photographing the wounds. The skin surface around the wounds was cleared of old adhesive with trichloroethane and shaved as necessary, and medical adhesive was reapplied to ensure chamber and hydrocolloid adhesion and skin cleanliness. Gauze that adhered to the wound surface was left in place after carefully removing unadhered sections with scissors. Saline (1.2 ml, 0.9% NaCl injection; USP, Baxter, Ill.) was added to each chamber with a syringe and 22-gauge needle, air bubbles were aspirated,
and the injection sites were sealed with clear tape. The resulting wound fluids were aspirated, collected into syringes, and replaced with saline together with chambers every 24 hours. The pigs received daily intramuscular injections of erythromycin (8 mg/kg, Erythro-200; Elf Sanofi, Shawnee Mission, Kan.) to minimize any bacterial growth in the wounds.

**Histologic Examination**

Cross-sectional, full-thickness biopsies were taken from the wounds at 4, 6, 8, 12, 14, and 16 days after wounding (dry, hydrocolloid, and saline treatment). Samples were divided in half (through the center of the wound), fixed in 10% neutral buffered formaldehyde solution, and paraffin-embedded. Medial samples were sectioned (6 µm) perpendicular to the surface, starting from the center of the wound, and stained with hematoxylin and eosin. The first complete section was examined and judged in a blinded fashion as either healed or non-healed, with the definition of a healed wound being the presence of a noninterrupted epithelium at least one cell layer thick covering the entire distance between the margins of the wound. Fibrovascular tissue (granulation tissue) thickness was recorded at five standardized coordinates, from which means were calculated. Average thickness and number of epidermal cell layers (not including stratum corneum) overlying the granulation tissue and the unwounded dermis (3 to 5 mm from the wound edge) were recorded for each wound site based on at least 10 standardized measurements.

**Contraction Studies**

The outer margins of the tattooed lines, in standardized wound photographs taken at a fixed distance (37.5 cm) every 3 days, were traced onto a digitizing tablet (Summasketch; Summagraphics, Huntington Beach, Calif.) by using a scanning software (Sigmascan; Jandel Scientific, San Rafael, Calif.) that had been calibrated to a photographed ruler. The wound area for each wound was then expressed as a percent of its original size on day 0.

**Statistics**

The numbers of healed versus nonhealed wounds on day 12 were compared by using Fisher’s exact test (two-tailed). Area, tissue thickness, and protein concentrations were compared using the Mann-Whitney U non-parametrical test. No correction was made for multiple testing. Differences were considered significant if the $p$ value was less than 0.05.

**RESULTS**

**Macroscopic and Histologic Appearance of Healing Wounds**

After 3 days, a fibrinous clot had developed in moist and wet (saline) wounds, but such a clot was not apparent in dry wounds (Fig. 1, second row, left to right). At 6 days after wounding, reddish-pink granulation tissue was observed in moist wounds and, to a lesser degree, in dry and wet wounds. Nine days after wounding, a crust that had formed over the dry wounds (Fig. 1, third row, left) was thick and hard, as judged from excisions made on days 8 to 16. Moist and wet wounds (Fig. 1, third row, center and right) were completely filled with granulation tissue on day 9. No crust formed in these wounds, but they were covered with a thin film or slough of fibrinous appearance that gradually detached during the process of reepithelialization. The exposed dermis of dry wounds became necrotic and was dissected away during epidermal healing (Fig. 2, above left). This did not occur in corresponding moist and wet wounds (Fig. 2, above right and below). A multilayered and stratified epidermis covered the majority of the wet wounds on day 12 (Fig. 3, below), but not until day 16 in dry wounds (Fig. 4, above). The epidermis of all wounds was, to some degree, hyperplastic and thicker than the surrounding unwounded skin (Fig. 4 and Table I). This was most prominent in dry wounds, followed by wet and moist conditions, and correlated with a greater number of cell layers (Table I).

**Reepithelialization of Dry, Moist, and Wet Wounds**

The wound sections taken 12 days after wounding showed that 0 percent ($n = 7$) of dry wounds, 20 percent of moist wounds ($n = 10$), and 86 percent ($n = 7$) of wet wounds were completely healed (Fig. 5). The difference between dry and wet treatment was significant ($p = 0.0046$), as was the difference between moist and wet wounds ($p = 0.027$). No wounds were healed at day 8 or earlier. At day 14, all moist ($n = 4$) and wet ($n = 4$) wounds were healed, as compared with 25 percent ($n = 4$) of
FIG. 1. Gross appearance of dry (left column, above to below), moist (center column, above to below), and wet (right column, above to below) neighboring wounds on days 0 (above, left to right), 3 (second row, left to right), 9 (third row, left to right), and 12 (below, left to right) after wounding. At 3 days after injury (second row), note the gelatinous clot in moist and wet wounds compared with the dry wound. Nine days after wounding, moist (third row, center) and wet (right) wounds had granulation tissue extending to the upper skin level covered by gelatinous exudate and dry wounds (left) were covered with a crust. Nonhealed dry (below, left) and moist (center) wounds and healed wet (right) wounds are shown 12 days after wounding.
dry wounds. Sixteen days after wounding, all wounds were healed (n = 2 to 3). Eight wounds were excluded from the study because of chamber leakage (n = 5), traumatization caused by pig movement (n = 2), and too deep excision of the wound (n = 1).

**Wet Wounds Exhibit Accelerated Contraction**

The area for each wound, as defined by the tattooed outer margins, was expressed as a percent of the area on day 0. The mean areas for each treatment group were then plotted against the number of days after wounding (Fig. 6). Dry wounds contracted steadily to 70.0 ± 2.0 percent of original size on day 12. Moist wounds behaved somewhat differently compared with those of other groups. There was a slight increase (not significant compared with day 0) in wound area from day 0 to day 3 (to 103.4 ± 1.8 percent), but from day 3 to 6 the wound size rapidly decreased to 80.8 ± 1.8 percent, similar to levels observed in wet wounds (82.1 ± 3.5 percent on day 6). Similar speed of contraction was, however, not seen on the subsequent days, and at day 12 the moist wound area had decreased to 60.5 ± 2.2 percent. In wet wounds, contraction was already evident at day 3 (94.3 ± 2.2 percent) and it proceeded steadily, reaching 49.3 ± 1.9 percent on day 12. The differences between groups on days 9 and 12 were all significant (p <0.005). The absolute areas on day 0 were not significantly different between experimental groups (dry 366.1 ± 7.8 mm², moist 347.4 ± 5.3 mm², and wet 368.6 ± 7.7 mm²).

**Granulation Tissue Formation**

Granulation tissue formation was fastest in moist tissue (Fig. 7), where the thickness exceeded that of dry and wet on days 4 to 6 by at least twofold (p <0.05 dry versus moist). On day 8, granulation tissue in wet wounds had...
reached the levels of that in moist but, on average, dry wounds still exhibited thinner granulation tissue. At days 12 and 16 after wounding, the thickness of granulation tissue in dry wounds exceeded that of the moist and wet wounds \((p < 0.05\) dry versus moist).}

**DISCUSSION**

Wound repair is influenced by many factors. This study investigated only the effect of hydration on contraction, reepithelialization, granulation tissue thickness, and epithelial thickness. These parameters were influenced by the different conditions achieved by the respective treatment. In this experimental setup, the wet environment, composed of isotonic saline and wound fluid, was the most favorable for fast wound healing.

It is quite possible that events that are secondary to hydration per se, such as preservation of nutrients and growth factors at the wound surface, play a major role. Wet treatment in a wound chamber completely eliminates—and moist treatment reduces—desiccation that are associated with evaporation. In a dry wound, the development of a necrotic surface that must be removed by debridement also delays healing. In addition, necrosis enlarges the wound, thereby necessitating additional regenerative processes. The dry environment may also delay healing by interference with cell communication because the process relies on diffusion of soluble factors. When observing the increased contraction in wet healing in particular, it is tempting to speculate that micrometabolism in the wound environment is a rate-limiting factor in tissue repair. In the wet wound, and to some extent in the moist wound, an increase in available nutrients and a reduction in evaporative loss make the micrometabolic balance more favorable for repair.

In this study, wet and moist wounds reepithelialized faster than dry wounds. This is in agreement with other studies that have demonstrated accelerated reepithelialization of partial thickness wounds in moist conditions \(^3,^4\) and in saline-immersed conditions \(^11\) compared with dry treatment. The full-thickness wounds studied here are, however, much different from the relatively superficial partial-thickness skin wounds studied earlier. In addition to reepithelialization, full-thickness skin defects also heal by extensive fibrovascular ingrowth and contraction. Several factors may explain the accelerated epidermal repair seen in moist and wet conditions. First, the presence of growth factors and proteinases in the fluid exudate under occlusive dressings is likely to be important. \(^8\) For example, it has been shown that chamber fluids collected from porcine \(^13\) and human \(^14\) wounds contain several growth factors essential to wound repair. In addition, wound fluids contain antimicrobial peptides, which may function as natural antibiotics, \(^15\) and a protein, which induces synthesis of certain cell surface proteoglycans important to growth factor and receptor interaction. \(^16\) Second, epidermal regeneration may also be enhanced in the clot-inducing environment of occlusive dressings, \(^11\) possibly because of increased precipitation of fibrinogen and fibronecin. \(^10\) In our study, both hydrocolloid-covered and fluid-treated wounds, but not dry wounds, developed a clot. Third, easier migration of epidermal cells over the moist wound surface instead of under the scab and necrotic dermis has been suggested to facilitate reepithelialization. \(^8\) Furthermore, the dry environment is far
from ideal compared with the wet, incubator-like milieu created by the saline-containing chamber. Wound fluid collected from acute wounds is similar to diluted serum with regard to total protein concentration, osmolality, and glucose and electrolyte concentration. It also contains several soluble growth factors, such as transforming growth factor-beta, platelet-derived growth factor, and fibroblast growth factor, which are known to stimulate the repair process. The fluid-treated wound environment therefore has many similarities to the conditions established in cell culture. Consequently, it has been demonstrated that single-
cell suspensions of keratinocytes transplanted into this environment adhere, proliferate, differentiate, and regenerate the epidermis.\textsuperscript{17}

Reepithelialization was also faster in wet wounds compared with moist wounds. This is in contrast to a study performed on porcine partial-thickness wounds that employed similar treatment modalities but resulted in no significant difference in wound reepithelialization.\textsuperscript{11}

This could be explained by the difference in contraction observed between hydrocolloid and saline-immersed wounds in this study, whereas the superficial wounds used in the former study\textsuperscript{11} displayed no significant contraction. The saline environment also led to a significantly thicker epithelium consisting of significantly more cell layers than hydrocolloid-treated wounds. This indicates that epidermal proliferation was greater in wet wounds, which also may accelerate reepithelialization. Unfortunately, it was not possible to determine the

\begin{table}
\centering
\footnotesize
\begin{tabular}{|c|c|c|c|c|c|}
\hline
 & Dry & & Moist & & Wet & Unwounded Skin \\
 & Mean ± SD & n & Mean ± SD & n & Mean ± SD & n \\
\hline
Epidermal thickness (\(\mu\)m) & & & & & & \\
Day 12 & 228.4 ± 65.0 & 7\textsuperscript{***} & 99.4 ± 18.2 & 10\textsuperscript{***} & 167.5 ± 52.4 & 7\textsuperscript{i} & 66.9 ± 15.2 & 24 \\
Day 14 & 186.8 ± 33.5 & 4\textsuperscript{†} & 70.1 ± 7.5 & 4\textsuperscript{*} & 115.2 ± 38.8 & 4\textsuperscript{†} & 66.7 ± 11.4 & 12 \\
Day 16 & 211.1 ± 21.2 & 3\textsuperscript{*} & 112.2 ± 12.4 & 3\textsuperscript{*} & 163.8 ± 1.8 & 2 & 58.8 ± 14.3 & 8 \\
Epidermal cell layers & & & & & & \\
Day 12 & 11.6 ± 4.5 & 7\textsuperscript{*} & 7.8 ± 1.2 & 10\textsuperscript{††} & 11.4 ± 1.7 & 7\textsuperscript{††} & 6.5 ± 0.9 & 24 \\
Day 14 & 12.4 ± 1.3 & 4\textsuperscript{*} & 7.2 ± 1.1 & 4\textsuperscript{*} & 10.0 ± 1.4 & 4\textsuperscript{*} & 5.7 ± 0.6 & 12 \\
Day 16 & 13.9 ± 1.3 & 3\textsuperscript{*} & 8.2 ± 0.7 & 3\textsuperscript{*} & 11.3 ± 0.1 & 2 & 5.6 ± 0.7 & 8 \\
Distance (\(\mu\)m) across the wound separating epidermis & & & & & & \\
Day 12 & 3614 ± 1678 & 7\textsuperscript{††} & 1680 ± 1333 & 10\textsuperscript{††} & 357 ± 944 & 7\textsuperscript{††} & – & – \\
Day 14 & 2375 ± 2584 & 4\textsuperscript{†} & 0 ± 0 & 4\textsuperscript{*} & 0 ± 0 & 4\textsuperscript{†} & – & – \\
Day 16 & 0 ± 0 & 3 & 0 ± 0 & 3 & 0 ± 0 & 2 & – & – \\
\hline
\end{tabular}
\caption{Thickness of Epidermis and Distance across the Wound Separating Epidermis}
\end{table}

\textsuperscript{a}p values <0.05 (*), <0.01 (**), or <0.001 (***)) comparing dry and moist by Mann-Whitney U test.
\textsuperscript{†}p values <0.05 (†) or <0.01 (††) comparing dry and wet by Mann-Whitney U test.
\textsuperscript{‡}p values <0.05 (‡) or <0.01 (‡‡) comparing moist and wet by Mann-Whitney U test.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig5.png}
\caption{Reepithelialization of wounds. Bars represent percentages of wounds that were completely reepithelialized on the day of biopsy. Numbers in parentheses indicate the number of observations. *p = 0.0046 (wet versus dry) or 0.027 (wet versus moist) as determined by Fisher’s exact test.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig6.png}
\caption{Contraction was faster in wet wounds than in moist ones, which in turn contracted faster than dry wounds. Error bars indicate standard error of the mean (\(n = 13\) to 17 on days 0 to 12; \(n = 2\) to 3 on day 15). *p<0.005 compared with other groups (Mann-Whitney U test).}
\end{figure}
extent to which enhanced contraction and faster epidermal regeneration may have contributed to achieve the observed acceleration of reepithelialization. The slough and crust that covered the wounds prevented accurate identification of the epithelium in photographs, and it was not possible to extrapolate data from the histologic sections regarding the surface area covered with epithelium.

Interestingly, the development of granulation tissue, which was more rapid in moist wounds than in dry or wet wounds, was not accompanied by accelerated reepithelialization. The slough and crust that covered the wounds prevented accurate identification of the epithelium in photographs, and it was not possible to extrapolate data from the histologic sections regarding the surface area covered with epithelium.

Interestingly, the development of granulation tissue, which was more rapid in moist wounds than in dry or wet wounds, was not accompanied by accelerated reepithelialization. In a study of rats, hydrocolloid-treated full-thickness wounds healed faster and with significantly more granulation tissue formation than wounds covered with saline-soaked dressings, which are functionally similar to dry dressings. The thicker granulation tissue formation in hydrocolloid-treated wounds in that study was combined with a more pronounced inflammatory response. Previous studies have also noted greater granulation tissue formation in hydrocolloid-treated full-thickness wounds and greater inflammation in moist hydrocolloid-treated partial-thickness wounds than in saline-treated wounds. It is likely that moist and dry wounds, which also exhibit increased inflammation, demonstrate thicker granulation tissue than that exhibited in wet wounds because of a greater inflammatory response.

Saline-immersed and hydrocolloid-covered wounds contracted more than dry wounds. In dry wounds, a crust developed over time, which may have physically impeded contraction. The crust was, however, not fully developed until 9 days after wounding, at which stage slower contraction in dry wounds was already observed. Other factors, such as less evaporative loss and a more favorable nutritional balance in wet and moist wounds, may also explain the greater contraction in moist and wet conditions. Interestingly, the fluid environment also resulted in significantly greater contraction than that observed in hydrocolloid-treated wounds. Fibroblasts and myofibroblasts in granulation tissue are believed to be important for wound contraction. When observing the curves of contraction and granulation tissue formation, it appears as if the rate of contraction, to some extent, correlates with the speed of granulation tissue formation. This may explain the initial fast rate of contraction observed in moist wounds on days 3 to 6, when fibrovascular tissue formation was most rapid. In this study only the thickness of the granulation tissue was measured, and based on the observations that moist wounds exhibited greater wound areas than wet wounds, it is reasonable to believe that the amount of fibrovascular tissue was greater in moist wounds compared with wet wounds. This may seem contradictory considering the greater contraction that was observed in the fluid-treated wounds compared with the wounds covered with a hydrocolloid dressing. However, studies on contraction have revealed that a great deal of the fibrovascular tissue most likely does not participate in the contractile process, based on the fact that wounds from which the center of the granulation tissue is excised still contract at a normal rate. It is believed that the contractile activity is located in the wound margins rather than in the center of the fibrovascular tissue.

This study demonstrates the beneficial influence of a liquid environment on wound contraction and reepithelialization. Neither these nor previous experiments have suggested any complications associated with the use of a liquid environment, such as tissue maceration or wound infection. On the contrary, the chamber system has been success-
fully used as a delivery system for growth factors in an animal model, as well as in a clinical setting, for the treatment of a chronic infected wound with antibiotics. A fluid wound environment has many similarities with the conditions established in an in vitro cell culture system. In this environment, it has been possible to transplant both fibroblasts and keratinocytes as single cell suspensions to the wound. Future applications may lie in the treatment of human burns and chronic ulcers. Enclosing wounds in a sealed chamber may be laborious and may require methodologic precision to achieve firm adhesion. In our experience, it has been important to consider location of the wound, patient compliance, and quality of skin before attempting to apply chambers. Some areas, especially the knee, ankle, and elbow, may be difficult to enclose with the current technique. These locations could, for example, be immersed in fluid using watertight, transparent envelopes, as previously described.

In conclusion, there was faster healing of wet full-thickness wounds compared with moist wounds, which in turn healed faster than dry wounds. Contraction was more pronounced in wet wounds than in moist or dry ones, and the granulation tissue developed faster in moist wounds than in wet or dry wounds. The regenerated epidermis was thicker in dry and wet wounds compared with moist wounds.

Elow Eriksson, M.D., Ph.D.
Division of Plastic Surgery
Brigham and Women's Hospital
75 Francis Street
Boston, Mass. 02115
eriksson@partners.org

ACKNOWLEDGMENTS

Supported by Public Health Service Grant RO1GM-5144904 from the National Institutes of Health to Dr. Elof Eriksson. Tor Svensjö was supported by the Sweden-America Foundation. We thank Dr. Bernard Rosner (Channing Laboratory, Brigham and Women's Hospital, Boston, Mass.) for the statistical calculations in Figure 3, and Dr. Sverker Svensjö (Falun Hospital, Falun, Sweden) for helpful statistical advice. In addition, we greatly appreciate Stærk Johnson’s (Brigham and Women's Hospital) critical reading of the manuscript.

REFERENCES


