Comparison of equine amnion and a nonadherent wound dressing material for bandaging pinch-grafted wounds in ponies

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Objective—To evaluate healing of pinch-grafted wounds on the distal aspect of the limbs of ponies bandaged with equine amnion or a standard nonadherent wound dressing material.

Animals—6 ponies.

Procedure—A 2.5 × 2.5-cm full-thickness section of skin was removed from the dorsal aspect of each limb at the midpoint of the metacarpus or metatarsus. Six days later, wounds were grafted with partial-thickness pinch grafts. Half the wounds were bandaged with amnion, and the other half were bandaged with a nonadherent dressing. Bandages were changed every 3 days until wound healing was complete. At each bandage change, numbers of grafts lost were recorded, and wounds were measured.

Results—Percentage of grafts lost from wounds bandaged with amnion was not significantly different from percentage lost from wounds bandaged with the nonadherent dressing. Median healing time for wounds bandaged with amnion (30 days) was significantly less than median healing time for wounds bandaged with the nonadherent dressing (39 days). All wounds were healed by day 45.

Conclusions and Clinical Relevance—Results suggest that amnion can be used for bandaging pinch-grafted wounds on the distal aspect of the limbs of ponies. (Am J Vet Res 2000;61:326–329)

In horses, large wounds involving the distal portions of the limbs are frequently allowed to heal by second intention, either because the size of the wound prohibits primary closure or because of risk of infection associated with closure of a contaminated wound. However, this method of healing is slow and subject to complications, particularly formation of excessive granulation tissue. In addition, the epithelium that forms during healing is fragile and easily traumatized.1

Skin grafting is often performed to accelerate healing of granulating wounds,2 as grafting results in faster healing and more cosmetic results than second intention healing alone. Of the various methods of skin grafting available, pinch grafting is the most applicable in field settings because of the minimal time and equipment needed. Furthermore, pinch grafts have a better survival rate than large grafts, such as split-thickness meshed grafts.

After skin grafts are placed, a bandage is routinely secured over the wound to protect them. However, grafts may be displaced from the wound bed during bandage changes or engulfed by excessive granulation tissue. Therefore, using bandage materials that do not adhere to healing skin grafts and discourage formation of excessive granulation tissue should improve graft survival rate and decrease healing time. In a previous study,3 wounds bandaged with amnion produced less granulation tissue and healed significantly faster than wounds bandaged with a conventional wound dressing. To our knowledge, however, what effect amnion may have on healing of skin grafts has not been determined. Therefore, the purpose of the study reported here was to evaluate healing of pinch-grafted wounds on the distal aspect of the limbs of ponies bandaged with equine amnion or a standard nonadherent wound dressing material.

Materials and Methods

Amnion procurement and storage—Placentas were collected within 2 hours after parturition from foals born to mares in the veterinary college’s teaching herd. The amnion was separated with scissors from the chorionic portion of the placenta near the convergence of umbilical vessels. Visible contamination was removed by means of copious lavage with tap water and debridement. The amnion was then spread on a sterile impervious drape and cut into 5 × 5-cm segments. Segments were washed in a 0.05% solution of chlorhexidine diacetate (1:40 dilution of the 2% concentrate) and rinsed in tap water. They were then placed in plastic containers containing 0.05% chlorhexidine diacetate solution and refrigerated at 4°C until used. Amnion was stored between 3 and 6 months before being used in this study.

Creation and bandaging of wounds—Six ponies (mean age, 9.2 years; range, 8 to 11 years) were used in the study. Ponies were determined to be healthy on the basis of results of physical examination, a CBC, and serum biochemical analyses and had been dewormed with ivermectin (0.2 mg/kg of body weight) and vaccinated against tetanus, eastern and western encephalitis, and influenza.4 Prior to the study, ponies were maintained at pasture for 4 weeks.

Food and water were withheld from the ponies for 12 hours prior to surgery. Anesthesia was induced with xylazine hydrochloride (1.1 mg/kg, IV), butorphanol tartrate (0.03 mg/kg, IV), and ketamine hydrochloride (2.2 mg/kg, IV). The metacarpus and metatarsus were prepared for surgery, and a 2.5 × 2.5-cm full-thickness section of skin was removed from the dorsal aspect of each limb at the midpoint of the metacarpus or metatarsus. A template constructed of radiographic film was used to ensure uniformity of the wounds. All wounds were bandaged with a commercial rayon-polyethyl-
ene nonadherent dressing that was covered with stretch gauze and elastic adhesive tape. Ponies were treated with phenylbutazone (2.2 mg/kg, PO) twice daily for 2 days after surgery. For the remainder of the study, ponies were housed in 3 X 3-m box stalls and fed alfalfa or grass hay free choice; water was provided ad libitum.

Skin grafting—Six days after wounds were created, a healthy bed of granulation tissue had formed, and all wounds were traced on polyethylene sheets, using a fine-tipped permanent marker. Wounds were then grafted with partial-thickness pinch grafts (approx 4 mm in diameter) harvested from the pectoral region. Pinch grafts were harvested with a #10 scalpel blade and were visually examined to ensure that they were partial thickness. Grafts were placed in tissue pockets created in the granulation tissue bed with a #15 scalpel blade. Pockets were approximately 1 cm deep and 0.7 cm apart and opened in an upward direction. Three rows of grafts with 3 grafts per row were implanted in each wound. Phenylbutazone (2.2 mg/kg, PO) was administered before and after surgery.

Wounds on the left metacarpus and metatarsus of ponies 1, 2, and 3 and the right metacarpus and metatarsus of ponies 4, 5, and 6 were bandaged with equine amnion covered by an absorbent layer of gauze and elastic adhesive tape. Amnion was rinsed with tap water to remove residual storage solution prior to placement on the wound. Wounds on the right metacarpus and metatarsus of ponies 1, 2, and 3 and the left metacarpus and metatarsus of ponies 4, 5, and 6 were bandaged with a rayon-polyethylene nonadherent dressing covered with gauze and elastic adhesive wrap. The study protocol was approved by the Virginia-Maryland Regional College of Veterinary Medicine Animal Care Committee.

Assessment of wound healing—Bandages were changed every 3 days following pinch grafting, and number of grafts lost on the bandage was recorded. At every other bandage change until wound healing was complete, amount of granulation tissue was graded as follows: grade 1, granulation tissue below the skin edges; grade 2, granulation tissue at the level of the skin edges; grade 3, granulation tissue above the skin edges; and grade 4, granulation tissue above the skin edges and projecting over the advancing border of epithelium. For this study, grade-3 and grade-4 granulation tissue was considered exuberant.

Also at every other bandage change, wounds were traced on sterile transparent polyethylene sheets, using a fine-tipped permanent marker, and wound areas were calculated, using a computerized digitizing table. Briefly, transparent polyethylene sheets containing the wound tracings were fixed to the digitizing table, and the edges were followed with an electronic probe. Two wound areas were then calculated by the computer. The first (L1) represented total wound area (ie, the area bounded by the contracting wound edge). The second (L2) represented granulation tissue that had not yet been epithelialized (ie, the area bounded by advancing epithelium). Skin grafts were included as part of the advancing epithelium when the grafts coalesced with epithelium advancing from the wound edges. Wound tracings obtained immediately prior to skin grafting were also digitized, and surface area of the wound on the day skin grafting was performed (L0) was also calculated. These wound area measurements were then used to calculate percentage of wound contraction (100 X [L0 – L1]/L0), percentage of wound epithelialization (100 X [L1 – L2]/L1), and percentage of the wound that had healed (100 X [L0 – L2]/L0).

Healing was considered complete when the wound surface was completely covered with epithelium. Bandaging was discontinued when wound healing was complete, and number of days for wounds to heal completely was recorded.

Statistical analyses—Repeated-measures ANOVA was used to determine whether percentage of wound contraction, percentage of wound epithelialization, and percentage of the wound that had healed was significantly different between wounds bandaged with amnion and wounds bandaged with the nonadherent dressing. The Wilcoxon signed-rank test was used to compare number of days for wounds to heal between groups. Two-sample Z tests were used to compare percentages of grafts lost and percentages of wounds with exuberant granulation tissue between groups. For all analyses, a value of P < 0.05 was considered significant.

Results

Subjectively, the nonadherent dressing was often adhered to the wound surface, and removal of the dressing resulted in wound irritation (eg, hemorrhage and redness) and scab removal. Amnion appeared dry on its external surface, but the surface facing the wound remained moist. Application of tap water to the amnion allowed it to be removed easily with little evidence of wound irritation. Application of tap water to the nonadherent dressing did not seem to make removal any easier.

Graft loss—Percentage of grafts lost from wounds bandaged with amnion (10/144; 7%) was not significantly different from percentage lost from wounds bandaged with the nonadherent dressing (16/144; 11%). Most grafts were lost on day 9 (with the day wounds were created considered day 0). None of the grafts were lost after day 15.

Exuberant granulation tissue—From day 12 through day 27, the percentage of wounds bandaged with amnion that had exuberant granulation tissue was significantly less than the percentage of wounds bandaged with the nonadherent dressing. On day 27, 6 of 12 wounds bandaged with the nonadherent dressing had exuberant granulation tissue, whereas only 1 of 12 wounds bandaged with amnion did. Throughout the study, only 1 wound had grade-4 granulation tissue. This was a wound that was bandaged with the nonadherent dressing, and grade-4 granulation tissue was noticed on day 18. The wound was treated topically twice with an antibiotic-steroid ointment. After day 27, all wounds in the study had grade-1 or grade-2 granulation tissue.

Wound contraction—Wounds bandaged with the nonadherent dressing expanded initially, whereas wounds bandaged with amnion did not. On day 12, the wounds bandaged with nonadherent dressing had increased in size by a mean (± SD) of 12 ± 32% compared with a mean contraction (± SD) of 1 ± 30% for wounds bandaged with amnion. This difference was not significant. On days 30 and 36, percentage of wound contraction was significantly higher for wounds bandaged with amnion than for wounds bandaged with the nonadherent dressing (Fig 1); however, on days 42 and 48, significant differences between groups were not detected.

Wound epithelialization—Mean percentage of wound epithelialization for wounds bandaged with
Amnion was not significantly different from mean percentage for wounds bandaged with the nonadherent dressing (328 AJVR, Vol 61, No. 3, March 2000)

Wound healing—On day 18, mean (± SD) percentage of the wound that had healed was significantly (P = 0.04) lower for wounds bandaged with amnion (39 ± 19%) than for wounds bandaged with the nonadherent dressing (44 ± 19%; Fig 3). By day 24, however, percentage of the wound that had healed was not significantly different between groups, and on day 30, percentage of the wound that had healed was significantly (P = 0.04) higher for wounds bandaged with amnion (94 ± 8%;) than for wounds bandaged with the nonadherent dressing (86 ± 13%).

Healing time—Median healing time for wounds bandaged with amnion (30 days; range, 27 to 36 days) was significantly (P = 0.006) less than median healing time for wounds bandaged with the nonadherent dressing (39 days; range, 33 to 45 days). On day 36, 10 of 12 wounds bandaged with amnion were healed, and only 6 of 12 wounds bandaged with the nonadherent dressing were healed. On day 42, all 12 wounds bandaged with amnion were healed and only 8 of 12 wounds bandaged with the nonadherent dressing were healed. All wounds were healed by day 45.

Discussion

Results of the present study suggest that amnion is an effective biological bandage when used on pinch-grafted wounds of the distal portion of the limbs of ponies. Compared with a standard nonadherent bandage material, use of amnion was not associated with a significant decrease in graft survival. In addition, wounds in this study that were bandaged with amnion healed an average of 9 days faster than wounds bandaged with the nonadherent dressing.

In the study reported here, we elected to use wound area on day 6 as the baseline measurement (L0) for determining percentage of wound contraction, percentage of wound epithelialization, and percentage of the wound that had healed. Use of day-6 wound measurements probably did not affect the outcome of the study, because all wounds were treated similarly between day 1 (ie, the day of wound creation) and day 6 (ie, the day of grafting) and because there were not any significant differences in regard to day-6 measurements between wounds bandaged with amnion and wounds bandaged with the nonadherent dressing. Individual epithelial islands created by the grafts were not included as part of the epithelialized portion of the wound until they were continuous with epithelium advancing from the outer edges of the wound. We elected to calculate epithelialized wound area in this way, because even with the use of finetipped markers, we thought there was a possibility of too much error in measurement of individual graft areas. Had we included this epithelialized portion of grafts there is a possibility a significant difference in rate of epithelialization would have been detected.

In wound healing, contraction is preferred over epithelialization, because there is minimal hair growth following healing of wounds by epithelialization. Other authors have suggested that amnion, by drying to the periphery of wounds, may act as a splint to keep wounds from expanding. In the present study, wounds bandaged with the nonadherent dressing underwent an initial period of expansion, whereas wounds bandaged with amnion did not. However, by the end of the study, there was no significant difference in percentage of wound contraction between treatment groups, so the shorter healing time of wounds bandaged with amnion may not be a result of splinting by the amnion.

From day 12 through day 27, the percentage of wounds bandaged with amnion that had exuberantly healed was not significantly different from mean percentage for wounds bandaged with the nonadherent dressing (Fig 2).
granulation tissue was significantly less than the percentage of wounds bandaged with the nonadherent dressing. Other authors have theorized that because amnion conforms to the wound surface, causing less wound irritation at the time of bandage removal, it is less likely to promote formation of granulation tissue. Amnion also remains moist and pliable; therefore, it may be less abrasive than other bandage materials. In addition, because amnion conforms to the wound, fewer environmental bacteria may contaminate the wound, and this may also aid in healing. Any or all of these factors may help explain the lower percentage of wounds with excessive granulation tissue in the present study.

In a previous study, it was suggested that ponies may not be an adequate model for wound healing in horses. In that study, larger horses had longer wound healing times, and 2 of 3 ponies had faster wound healing rates than did larger horses. The ponies also did not develop exuberant granulation tissue. In the present study, 7 of the 24 wounds had exuberant granulation tissue (grade 3 or 4) on day 27, indicating that ponies are capable of producing exuberant granulation tissue. Nevertheless, given the variation in wound healing among equids, results of the present study should be extrapolated to horses only with caution.

References