An adult-fetal skin interface heals without scar formation in sheep

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Background. *Fetal skin heals by regeneration rather than by adult-type scarring. Prior studies indicate that scarless healing is an intrinsic property of fetal tissue.*
Methods. *To answer the question of whether fetal tissue could influence the healing of adjacent adult tissue, adult sheepskin was transplanted on the backs of 60-days' gestation fetal lambs (term, 145 days). At time points 1, 3, 7, 14, 21, 28, 42, 56, and 63 days after transplantation, the grafts were harvested and the interfaces between adult and fetal skin were analyzed.*
Results. *The adult-fetal interface healed without scar formation. Control fetal sheep autograft (fetal-fetal) interfaces healed without scar, and adult sheep autograft (adult-adult) interfaces healed with scar.*
Conclusions. *These results suggest that the juxtaposed fetal tissue prevented scar formation at the adult-fetal interface. We hypothesize that fetal cellular and matrix factors permit adjacent wounded adult tissue to heal without scar. These factors, once characterized, might be used clinically to induce scarless healing in children and adults. (SURGERY 1995;118:82-6.)*

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THE FIBROSIS AND SCARRING that follow operation, disease, and trauma can be devastating. For this reason we seek a way to produce scarless healing. The fetus is uniquely capable of healing skin wounds without scar formation and provides a model of ideal tissue repair. If we can determine the factors that allow this scarless regeneration in the fetus and identify the factors that prevent scarless regeneration in the adult, we may be able to modulate wound healing in children and adults to become more fetal-like. When adult sheepskin was grafted on the backs of fetal sheep and the adult graft was subsequently wounded in its midportion, it healed with scar.[1] Thus two factors initially thought to promote scarless repair, the amniotic fluid environment and perfusion by fetal blood, were not sufficient to induce scarless adult skin healing. Because fetal serum and amniotic fluid do not produce fetal healing in this transplanted adult skin, fetal healing must be due to factors in the tissue itself.

To determine whether fetal tissue could promote scarless healing in adjacent adult skin, we transplanted adult sheepskin on the backs of fetal sheep and studied the healing of the interface between the cut edge of the adult graft and the adjacent cut edge of the fetal recipient skin.

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MATERIAL AND METHODS

Animals. Sixteen time-dated pregnant ewes at 60-days' gestation (term, 145 days) were transported from Torrel Farms (Ukiah, Calif.) to the University of California, San Francisco animal care facility and fed food and water ad libitum. The animals were fasted for 48 hours before operation. Animal management was in accordance with the policies of the University of California, San Francisco Animal Care Committee and the National Institutes of Health guidelines for the care of experimental animals.

Skin graft transfer. Ewes underwent general anesthesia as previously described.[1] The abdomen was shaved and sterilely prepared, and a 1 × 2 cm full-thickness maternal skin graft was harvested from the abdomen, defatted, and kept moist. A second 1 × 2 cm maternal graft was harvested, rotated 180 degrees, and resecured on the ewe's abdomen with 6-0 Surgilene suture (Davis & Geck, Danbury, Conn.) as a control for the effect of transplantation on adult wound healing.

After a midline laparotomy to expose the uterus, a 5 cm hysterotomy was made, the fetal back was delivered into the operative field, and a 1 × 2 cm area of fetal skin was excised to create the recipient graft bed. The maternal graft was sutured on the fetal graft bed with multiple interrupted 6-0 Surgilene sutures to provide accurate approximation of the fetal and adult skin

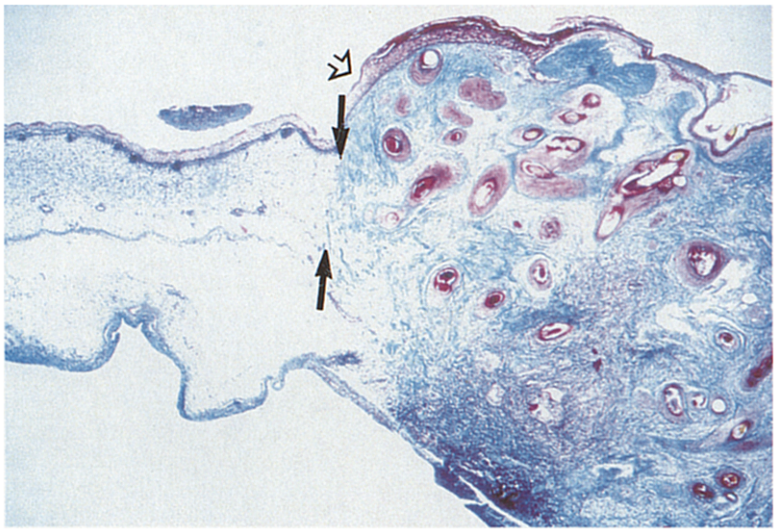

Fig. 1. Fourteen-day interface section of maternal-fetal skin graft stained with Mallory's trichrome (original magnification ×40). Smooth transition from fetal to adult epidermis *(open arrow)* and dermal junction *(solid arrows)* are noted.

edges. A 1 × 2 cm piece of full-thickness fetal skin was also harvested, rotated, and auto transplanted on the fetal back as a fetal autograft control for the effect of transplantation on fetal wound healing. Amniotic fluid was restored with warm saline solution to which was added 1 million units of penicillin G, and the uterus was closed by using a TA-90 stapler (U.S. Surgical, Norwalk, Conn.). The laparotomy was closed in layers, the maternal graft site was closed primarily, and the ewe was returned to her stall.

Graft harvest. At 1, 3, 7, 14, 21, 28, 54, and 63 days after transplantation ewes again underwent general anesthesia. After laparotomy and hysterotomy the fetus was delivered. Grafts were excised with a 1 cm margin of surrounding fetal tissue (skin and muscle) so as not to disturb the graft-host interface. Maternal autografts were excised with a 1 cm margin of surrounding adult tissue. The fetuses and ewes were killed by intracardiac Beuthanasia-D (Schering, Kenilworth, N.J.) overdose.

Graft interface analysis. Interfaces were formalin fixed and paraffin embedded for histologic examination. Ten micron sections from the paraffin-embedded tissue were stained with either hematoxylin-eosin or Mallory's trichrome.

RESULTS

Twelve fetuses survived to harvest and four fetuses aborted. Two maternal control autografts were lost to desiccation and one was lost to infection.

Adult-fetal grafts. All of the adult grafts on surviving fetuses were viable. Grossly, the surface of the adult skin grafts was covered by matted wool and debris. Beneath this layer the grafts were supple and adherent to the fetal graft bed. At the interface the wool of the adult skin met the finely haired skin of the fetus without a gap or ridge of scar. In the later time points as the surrounding fetal skin matured it also produced wool. All grafts bled well when nicked with a scalpel. Trichrome and hematoxylin-eosin staining revealed scarless healing between the adult skin graft and the adjacent fetal skin at the interface. A smooth convergence of adult and fetal epidermis was noted at the interface at all eight postwounding time points (Fig. 1). On the adult side of the interface normal adult dermiswas present with its coarse collagen pattern and multiple large follicles. On the fetal side of the interface the fetal dermis was present with its fine collagen pattern and fine hair follicles. The transition from adult to fetal dermis was subtle. No evidence was found of a scar or a band of tissue that lacked follicles at the interface (Fig. 2). The tissue at the interface under the grafts was normal muscle, vessels, and fat; no scarring was present at the base of the grafts. On histologic examination three grafts were irregular: the 1-day sample's delicate interface was disrupted during processing, and overgrowth of the fetal skin on the adult skin was noted at the interface in two grafts.

Fetal autografts. Fetal autografts were indistinguishable from surrounding fetal skin and were identified only by the presence of the sutures. Trichrome and hematoxylin-eosin stains revealed that normal fetal dermal and epidermal architecture continued across the interface between graft and adjacent skin. No scarring was present at the interface or at the base of the graft (Fig. 3).

Adult autografts. Grossly, the viable adult autografts produced wool at the same rate as their surrounding

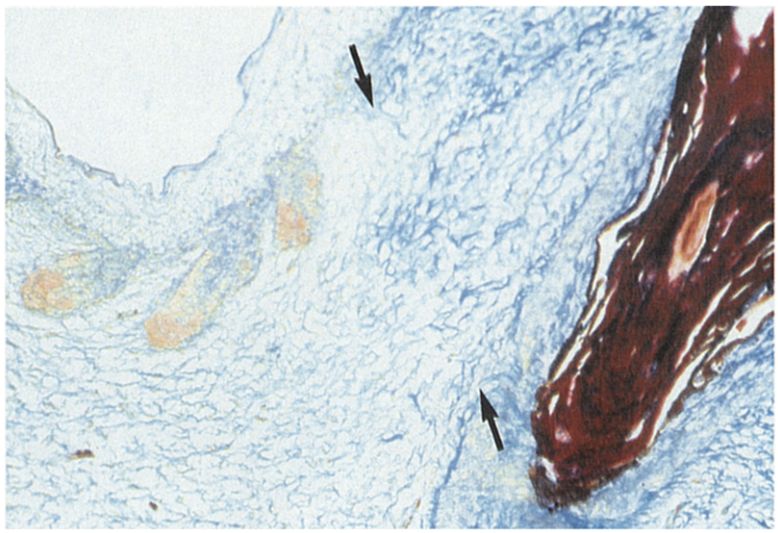

Fig. 2. High power (×100) view of 21-day interface section of maternal-fetal skin graft stained with Mallory's trichrome. Subtle fetal to adult dermal transition zone *(arrows)* is present.

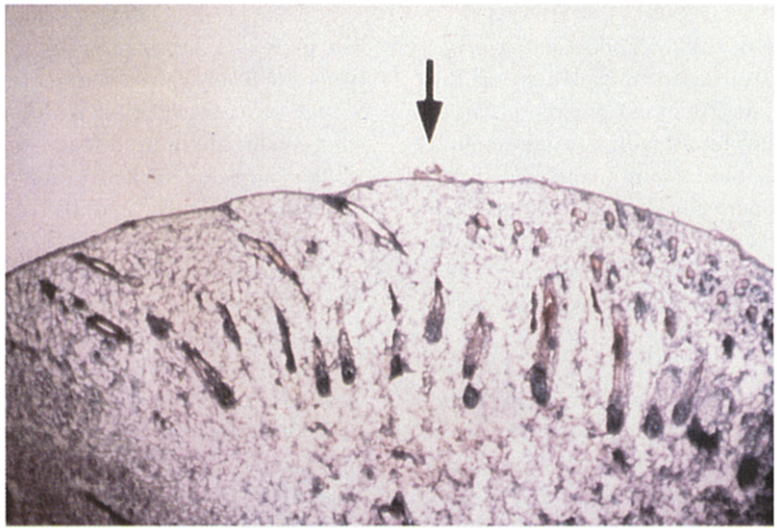

Fig. 3. Fourteen-day interface section of fetal autograft stained with Mallory's trichrome. *Arrow* indicates interface. Note restoration of tissue architecture without gap in hair follicles (original magnification ×40).

adult skin, but graft edges were delineated by a woolless ridge of scar. Final graft size was 1.0 × 0.75 cm, and final graft shape was rectangular with all sides contracted inward. This contraction was not seen in the adult-fetal grafts. Trichrome and hematoxylin-eosin stains revealed dense blue bands of collagen scar formation at the graft-recipient interfaces and a dense band of scar at the base of the graft (Fig. 4).

DISCUSSION

Although the adult skin grafted on the fetus retained its adult phenotype, no scarring was present at the adult-fetal interface. In keeping with prior studies, no scarring was noted at the fetal autograft interface and scarring was present at the maternal autograft interface. Possible explanations for the scarless healing at the adult-fetal interface are that the juxtaposed wounded fetal skin rapidly "out-healed" the adult skin, closing the gap in its scarless fashion, or that the fetal skin induced the adult skin to heal scarlessly.

The scarring of previously studied midgraft wounds in adult-to-fetal grafts showed that the more differentiated cells of adult skin cannot be modulated to heal in a scarless manner simply by perfusion with fetal blood or bathing in amniotic fluid.[1] Newborn marsupials are physiologically fetal-like, and they heal without scarring

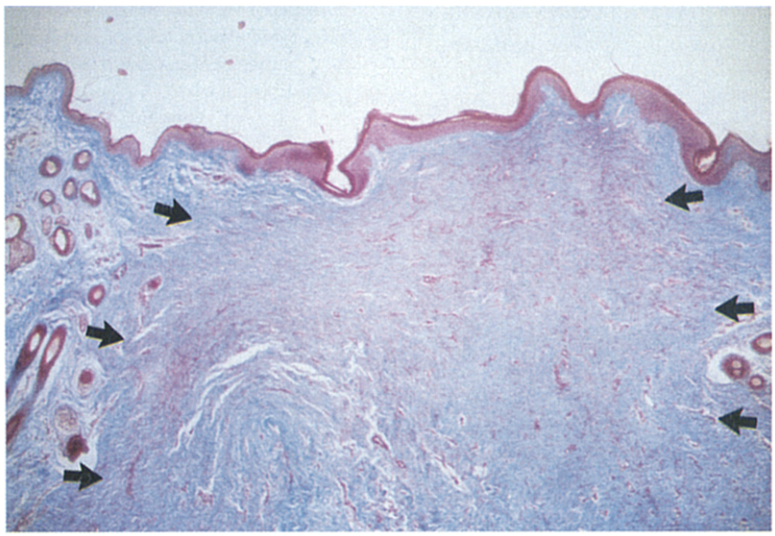

Fig. 4. Fourteen-day interface section of maternal autograft stained with Mallory's trichrome. Large gap in hair follicles and disorganized band of collagen *(arrows)* represent scar (original magnification ×40).

despite their location out in the maternal pouch where no amniotic fluid is present.[2] In rats, sheep, and monkeys fetal skin heals scarlessly in midgestation and begins to scar later in gestation, without a significant change in hormonal or physical environment.[3] These phenomena suggest that gestational changes in the skin itself are primarily responsible for scarring. In our study adult skin edges did not produce scar when in apposition with fetal skin. The adjacent fetal tissue cells, extracellular matrix (ECM), cell surface receptors, or local growth factor milieu may play critical roles in the scarless healing at this interface.

Fetal cells may be responsible for scarless healing at the adult-fetal interface. Fetal cells divide faster and have higher biosynthetic activity than adult cells. Embryonic and fetal cells often undergo an isoform transition during development (e.g., in their migratory behavior[4] or in secretion of motility factors).[5] After wounding, fetal fibroblasts deposit dermal collagen rapidly in an organized pattern, whereas adult fibroblasts deposit collagen in a disorganized pattern.[6] As a result the healed fetal dermis is composed of a fine network of thin collagenous fibrils in a glycosaminoglycan-rich matrix,[7] whereas adult dermis is denser, consists of collagen fibrils of larger diameter, and shows less remodeling after wounding. In our adult-fetal interface model the fetal fibroblasts may have healed the wound scarlessly by vastly outmaneuvering the adult fibroblasts and then outstripping the collagen and matrix deposition of these adult cells.

The fetal ECM may be important for scarless healing at the adult-fetal interface. The fetal ECM is different from adult ECM in that it often consists of fetal isoforms of matrix molecules such as laminin[8] and fibronectin.[9] Generous hyaluronate[10] and rapid tenascin[11] deposition in fetal wounds appears to provide a path for fast and orderly cell migration, so the timing of the ECM deposition may be critical. The use of cross-species grafts and species-specific antibodies[12] may identify the key matrix components at the scarfree adult-fetal interface.

Scarless healing at the interface may also reflect a difference in receptor population between fetal and adult fibroblasts. Cell surface receptors for integrins and ECM molecules regulate the cell-cell interactions of wound healing. There is a developmental change in integrin receptor expression in maturing epithelium,[13] and a higher density of the cell surface hyaluronic acid receptors is present in fetal fibroblasts compared with adult fibroblasts.[14] These receptor differences between the fetus and adult may be important in modulating the healing response, because various adult cell receptors may be up- or down-regulated by paracrine influences from the adjacent fetal tissue.

The fact that a wound in the middle of an adult graft perfused by fetal blood heals with scar makes it unlikely that a bloodborne fetal peptide growth factor prevents scarring at the interface in this model.[1] However, differences exist between the profile of growth factors produced locally in fetal and adult skin,[15, 16] and at this interface the adult tissue may heal differently when exposed to the adjacent fetal growth factor environment. For example, the cytokine transforming growth factor–beta (TGF-β) is associated with adult tissue scarring, but TGF-β is deficient in scarless fetal wounds.[17] If the adult skin in our model is exposed to an abnormally low level of TGF-β, it may heal scarlessly. Indeed, neutralizing antibodies to TGF-β markedly decrease scar in adult rat wounds.[18] The fetal tissue may block the synthesis,

binding, or activation of TGF-β produced by the adult tissue. Thus the growth factor milieu at the adult-fetal interface, whether by selective production of fetal or blocking of adult growth factors, may be quite fetal-like and thereby promote scarless healing by both types of skin at the interface.

Multiple complex differences are noted between the fetal and the adult response to wounding, and we have discussed their possible roles in the production of scarless healing at an adult-fetal skin interface. This study has shown that adult skin does appear to heal scarlessly when in apposition with fetal skin in a fetal environment. Additional work is needed to detect paracrine factor(s) produced by the fetal skin that might cause the adult skin to heal scarlessly or blocking strategies used by the fetal skin that prevent scarring in the adjacent adult skin. By selectively crippling the fetal skin's healing, we may be able to determine whether the rapidly healing fetal tissue closes the wound by healing onto a slowly healing "template" of adult tissue or whether some other mechanism permits scarless healing at the adult side of this interface. In much the same way as transplanted fetal neural tissue or adrenal tissue might soon be used as a source for dopamine in adults with Parkinson's disease[19] and fetal islet cell transplants may someday provide insulin for adults with diabetes,[20] components of fetal skin may be used to induce scarless healing in wounded or burned adult skin. The challenge is to identify the factors at work in this system of fetal-induced scarfree adult healing and to use those factors, their precursors, or the cells that make them to promote scarless healing in children and adults.

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