Scarless Skin Wound Repair in the Fetus

H. PETER LORENZ, MD, *and* N. SCOTT ADZICK, MD, *San Francisco, California*

The ability of a fetus to heal without scar formation depends on its gestational age at the time of injury and the size of the wound defect. In general, linear incisions heal without scar until late in gestation whereas excisional wounds heal with scar at an earlier gestational age. The profiles of fetal proteoglycans, collagens, and growth factors are different from those in adult wounds. The less-differentiated state of fetal skin is probably an important characteristic responsible for scarless repair. There is minimal inflammation in fetal wounds. Fetal wounds are characterized by high levels of hyaluronic acid and its stimulator(s) with more rapid, highly organized collagen deposition. The roles of peptide growth factors such as transforming growth factor-β and basic fibroblast growth factor are less prominent in fetal than in adult wound healing. Platelet-derived growth factor has been detected in scarless fetal skin wounds, but its role is unknown. An understanding of scarless tissue repair has possible clinical application in the modulation of adult fibrotic diseases and abnormal scar-forming conditions.

(Lorenz HP, Adzick NS: Scarless skin wound repair in the fetus, *In* Fetal Medicine [Special Issue]. West J Med 1993; 159:350-355)

Wounds in fetuses heal rapidly and generally without scar formation until late in gestation.[1] Relative to that in adults, fetal wound repair is characterized by more rapid epithelialization, fibroblast migration, extracellular matrix (ECM) deposition, and the ultimate restoration of normal tissue architecture. Major differences between fetal and adult wound healing are summarized in Table 1. This unique phenomenon of fetal skin wounds healing with a regeneration of normal tissue structure has stimulated research interest in the process of fetal wound healing. An understanding of this phenomenon has clinical implications in possibly modulating adult fibrotic diseases and abnormal scar-forming conditions such as burn contractures, keloids, strictures, and intra-abdominal adhesions.

Fetal Skin

The less-developed state of fetal skin at the time of wounding may be important for scar-free tissue repair. The human fetal epidermis begins with two cell layers, the basal cell layer and the periderm, at about 4 weeks' gestation. The periderm is the outermost single-cell layer of the fetal skin. Its cells have microvilli and blebs projecting off the surface of the skin into the amniotic fluid. Although the function of the periderm has not been determined, a secretive or absorptive process has been hypothesized.[2] As development continues, an intermediate epidermal cell layer develops. Keratinization begins at 9 to 16 weeks' gestation. During this period, primordial hair follicles and sebaceous glands become apparent. By 24 weeks' gestation, the epidermis has completed keratinization and stratifies into adult morphologic layers.[3]

The dermis, which is the location of scar in adult wounds, is similarly undergoing morphologic and biochemical changes. Early in gestation, the fetal dermis is thin and cellular, owing to the paucity of ECM. As development progresses, dermal collagen is deposited and sulfated glycosaminoglycans replace nonsulfated gly-

TABLE 1.—*Comparison of Adult and Fetal Skin Wound Healing Characteristics*

Wound Healing Characteristic	*Adult*	*Fetus*
Scar	Present	Absent
Underlying cell growth and development	Absent	Present
Speed to closure	Slower	Faster
Scab	Present	Absent
Oxygen tension	Greater	Lesser
Fluid environment	Absent	Present
Sterile environment	Absent	Present
Skin temperature	Cooler	Warmer, more uniform
Acute inflammation	Present	Reduced
Matrix deposition	Slower	Faster
Angiogenesis	Greater	Reduced
Epithelialization	Slower	Faster
Keratinization	Present	Immature, periderm

From the Departments of Surgery and Pediatrics, University of California, San Francisco, School of Medicine.
Reprint requests to N. Scott Adzick, MD, Fetal Treatment Center, Dept of Surgery, UCSF School of Medicine, San Francisco, CA 94143-0570.

ABBREVIATIONS USED IN TEXT

ECM = extracellular matrix
FPCL = fibroblast-populated collagen lattice
PVA = polyvinyl alcohol
TGF-β = transforming growth factor-β

cosaminoglycans of which hyaluronic acid is predominant.[4] The rapid growth and the relatively undifferentiated state of fetal skin set the stage for the unique response of fetal skin to injury.

Fetal Environment

Several differences that exist between the fetal and adult environments may affect wound repair. Fetal skin wounds are continuously bathed in warm, sterile amniotic fluid that is rich in growth factors. Fibronectin and hyaluronic acid, which are predominant ECM components in fetal wounds, are also present in amniotic fluid. The continuous presence of amniotic fluid may play a role in the deposition of the scarless wound matrix by providing a growth factor profile not found in the environment of adult skin.

Fetal tissue oxygenation is much less than that of adult tissue. The fetal arterial Po_2 is 20 to 25 mm of mercury, making the fetus much more hypoxemic than the adult.[5] We have measured fetal tissue Po_2 of sheep in midgestation, which is when scarless repair occurs, and found a fetal tissue Po_2 of 16 mm of mercury. Adult wound studies have traditionally emphasized that wound hypoxia may result in impaired leukocyte function, delayed healing, and an increased incidence of infection.[6] These studies in adult animals have shown that supplemental oxygen helps stimulate collagen deposition by adult fibroblasts at the wound edge. The ability of the fetus to heal rapidly and without scar in a relatively hypoxic environment is intriguing and requires further study.

Fetal serum also differs from adult serum. Levels of insulinlike growth factor II and hyaluronic acid stimulating factor have been higher in fetal serum than in adult serum.[7,8] The profile of other growth factors in fetal serum is probably different from that in adult serum, considering that the fetus is continuously growing and developing. Thus, the presence or absence of specific growth factors perfusing the fetal wound may also play a role in scarless repair.

Wounded adult tissue in the fetal environment has been shown not to heal free of scar. We transplanted adult sheep skin onto fetal lambs at 60 days' gestation (term = 145 days), before the fetal immune system has developed sufficiently to reject allografts. The adult grafts were wounded 40 days later (100 days' gestation), at a time when scarless repair occurs in the fetal sheep model. The wounds were analyzed by immunostaining for specific collagen types and were shown to heal with scar formation.[9] Neither an amniotic fluid environment nor perfusion by fetal blood prevented scar formation in wounded adult skin grafts. This suggests that the fetal environment is not crucial for scarless wound healing.

To determine the effect of an adult environment on fetal repair, we transplanted human fetal skin grafts to either a cutaneous or a subcutaneous location on athymic mice. Wounds made in human fetal skin grafts at an identical gestational age (15 to 22 weeks) healed with scar in cutaneous grafts and without scar in the subcutaneous grafts (Figure 1).[10] Immunohistochemical analysis of the wounds with species-specific antibodies against either mouse or human collagen types revealed that the cutaneous graft wounds healed with scar composed of mouse collagens. The subcutaneous graft wounds healed without scar and regenerated with human collagens.[11] Thus, the adult (mouse) fibroblasts deposited collagen with scar formation whereas fetal (human) fibroblasts deposited collagen in a scarless pattern. Scarless repair thus appears to be intrinsic to fetal tissue, and the major fetal cell type responsible for scarless repair may be the fetal fibroblast.

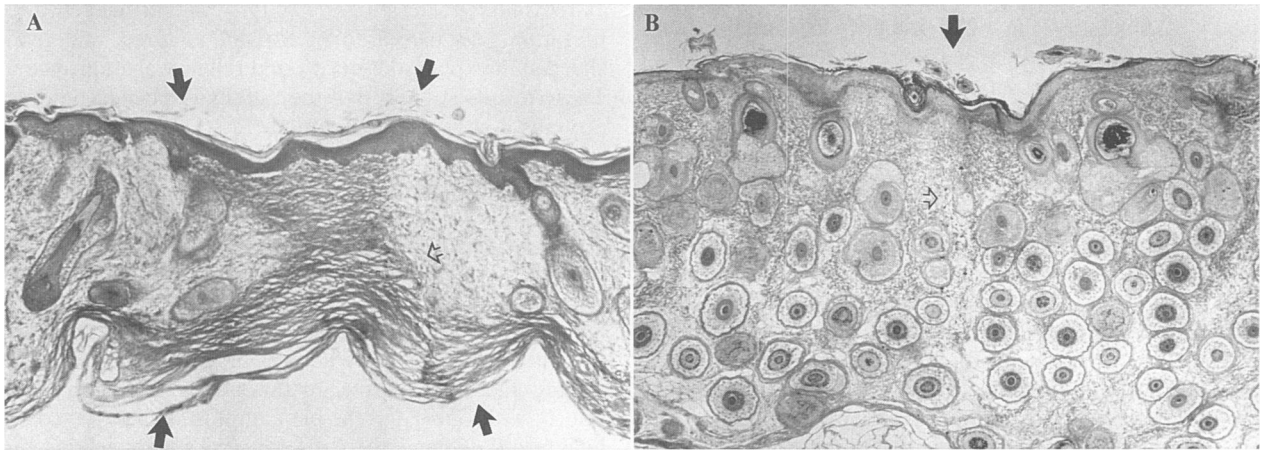

Figure 1.—Human fetal skin at 19 weeks' gestational age was transplanted onto athymic mice, incisionally wounded and tattooed with india ink **(open arrows)** 7 days after transplantation, then harvested 14 days after wounding and stained with Mallory's trichrome. Cutaneous graft **(A)** healed with scar formation **(closed arrows)**. Subcutaneous graft **(B)** healed without scar **(closed arrow)**. The hair follicle and collagen patterns are unchanged from the surrounding unwounded dermis, demonstrating scarless human fetal skin repair (original magnification × 50) (from Lorenz et al[10]).

The ability of fetal skin to heal without scar in an adult environment gives hope that adult skin can be modulated to heal without scar.

Fetal Inflammatory Cells

Although inflammation is an integral component of adult wound repair, evidence is mounting that it plays a much less prominent role in fetal wound healing. The fetal immune system is functionally immature relative to that in adults. Immunohistochemical studies in both fetal lamb[12] and mouse[13] have shown an absence of immunoglobulin deposition in fetal wounds. Fetal polymorphonuclear leukocytes are conspicuously absent in fetal rabbit wounds. Fetal polymorphonuclear leukocytes, however, will migrate into polyvinyl alcohol (PVA) sponges placed subcutaneously into fetal rabbits if the sponges are first implanted and then removed from adult rabbits.[14] This suggests that adult wound chemoattractants can elicit fetal leukocyte migration to the wound site. The lack of acute inflammation in fetal wounds is well documented, but a direct effect on scarless repair remains to be proved.

Wound macrophages coordinate repair in adults, and preliminary studies suggest a similar role in fetuses. Macrophages have been shown to be present in abundance in fetal rabbit wounds by enzyme histochemistry,[15] suggesting site-specific recruitment. Macrophages from adult wounds express many growth factors, and fetal wound macrophages also express growth factor transcripts.[16] In addition, adult wound macrophages are intimately involved in matrix remodeling because these cells express metalloproteinases and their inhibitors, thus coordinating ECM turnover. Because fetal skin repair is characterized by the reformation of a normal ECM, a regulatory role for fetal wound macrophages seems likely.

Fetal Extracellular Matrix

The extracellular matrix comprises several varieties of macromolecules that are reformed in a unique way after injury in the fetus: the ECM integrity is reestablished without scar formation. Hyaluronic acid, collagen, elastin, and adhesion glycoproteins are the major components of the matrix. Their synthesis and organization after injury differs between fetal and adult wound healing.

Hyaluronic Acid

Hyaluronic acid is a high-molecular-weight glycosaminoglycan composed of alternating units of glucuronic acid and *N*-acetylglucosamine. Hyaluronic acid is present in high concentrations during embryogenesis and during periods of rapid tissue proliferation and regeneration.[17] It has been shown to inhibit cell differentiation and can create an environment that promotes cell proliferation. It has a large volume of hydration due to its high charge, which facilitates cellular movement by opening up tissue spaces. These properties have led several investigators to study its role in fetal wound repair.

The fetal sheep model permits fetal surgical manipulation in both the second and third trimesters as well as wound analysis several weeks after injury. We have shown that hyaluronic acid levels in both fetal and adult sheep wounds rapidly increase until three days after wounding. These elevated levels persist at least 21 days after wounding in the fetus, whereas they rapidly return to baseline in the adult.[18] This prolonged presence of hyaluronic acid in fetal wounds may provide an environment conducive to scarless repair.

The prolonged presence of hyaluronic acid in fetal wounds may be due to either its increased synthesis or its decreased degradation. Despite its obvious importance in growth and development, little is known regarding the regulation of its synthesis. Several growth factors have been shown to stimulate hyaluronic acid synthesis. A stimulating activity has been described in fetal bovine sera as a 55-kilodalton glycoprotein. Studies by our group have shown that fetal lamb and bovine sera each contain hyaluronic acid stimulating factor, levels of which peak in midgestation.[19]

To investigate the role of this stimulating factor in fetal wound repair, we measured its levels in fetal sera, wound fluid, and amniotic fluid.[20,21] Analysis of wound fluid from wire-mesh cylinders implanted in midgestational fetal lambs showed elevated levels of hyaluronic acid stimulating activity that was absent in adult wound fluid. Thus, increased stimulating activity in fetal wounds may result in a hyaluronic acid-rich ECM promoting rapid cell mobility and proliferation. High levels of hyaluronic acid and its stimulating factor are also present in amniotic fluid, which bathes fetal skin wounds. This provides another mechanism by which the glycosaminoglycan is made available at the skin wound site.

Mammalian hyaluronic acid also has associated binding proteins that have been implicated in its biologic activity. By comparing purified hyaluronic acid extracts from bacteria that have much less associated binding proteins and human umbilical cord extracts, Burd and colleagues demonstrated that human hyaluronic acid-protein extracts had greater biologic activity: epidermal cell proliferation was increased in explant cultures, and fetal sheep skin explant dermal wound repair was improved.[22] These results suggest that the tightly associated binding proteins confer on hyaluronic acid some of its biologic activity. Their in vivo function in fetal repair remains to be determined.

As more studies are completed, hyaluronic acid becomes ever more implicated in fetal wound healing. Its stimulating activity is present in fetal wounds for a prolonged period, and hyaluronic acid is deposited quickly and persists throughout the period of repair. This mechanism is not present in the adult wound in which the stimulating factor is absent, hyaluronic acid is deposited briefly with fibrin and the platelet plug, it is removed by hyaluronidase, and then this provisional matrix is replaced by collagen and sulfated glycosaminoglycans. The prolonged hyaluronic acid-rich ECM in fetal wounds provides an environment that fosters cell motility and proliferation, which may contribute to scarless repair.

Collagen

The deposition of collagen in fetal wounds is in a highly organized pattern that is indistinguishable from unwounded fetal dermis. The regulation of collagen synthesis and fibrillogenesis is arguably the single most important mechanism underlying scarless repair and remains to be elucidated. Collagen is rapidly deposited in fetal wounds. Whitby and Ferguson, by performing immunohistochemical studies using antibodies to collagen types I, III, IV, and VI, showed that the speed of collagen deposition in mouse lip wounds is inversely related to age (fetal > newborn > adult).[13] In both fetal lambs and fetal rhesus monkeys, a transition from scarless fetal skin repair to healing with collagen scar formation in the middle of the third trimester has been demonstrated.[21,23] This transition shows that there is a spectrum to fetal repair, suggesting that as the fetus differentiates it develops an adult-like wound repair response before birth. A unique "transition" wound during the early third trimester has been described in fetal rhesus monkeys: the wound heals without hair follicles or sweat glands but with a normal reticular collagen pattern.

Quantitative analysis of fetal wound collagen has been done by various methods and in many animal models. There is a similar ratio of type I and III collagen content in fetal rat wounds and unwounded dermis, in which collagen type III is present in greater proportion than in adults. To study collagen content and wound-breaking strength, Burd and associates implanted PVA sponges into 75- and 100-day gestation fetal lambs and nonpregnant adult sheep.[24] Before implantation, the PVA sponges were bisected and the halves stapled together to study wound-breaking strength. Interestingly, the hydroxyproline content was greater in the fetal wounds, but the breaking strength was comparable in the two groups. Recent studies investigating fetal rabbit skin-bursting strength suggest that fetal wounds regain tensile strength more rapidly than adult wounds when compared with normal fetal and adult skin, respectively.[25] The mechanisms underlying this phenomenon have yet to be determined but probably relate to collagen fibrillogenesis and degrees of collagen cross-linking.

In vitro fetal fibroblast studies suggest that these cells are more proficient at collagen production than are adult fibroblasts. The relative collagen synthesis of first- and second-passage fetal rabbit fibroblasts is greater than in adults. Though both fetal and adult fibroblast collagen production drops during the second passage, the fetal fibroblasts produce more type III and V collagen than do the adult fibroblasts.[26] This is not unexpected as fetal skin has a higher ratio of type III to type I collagen. In human fetal skin, type III collagen comprises 30% to 60% of the total whereas in adults it is 10% to 20% of total dermal collagen.[26] In vitro studies of human fetal skin fibroblasts show that the ratio of type I to type III collagen synthesis parallels the ratio of their corresponding messenger RNAs, which suggests coordinated control at the transcriptional level.[27] The relative abundance of type III collagen in fetal skin, and presumably in fetal wounds, may be an important factor in scarless repair.

Differences in the regulation of overall collagen synthesis have been found between fetal and adult fibroblasts. Prolyl hydroxylase controls an important rate-limiting step in collagen production. Studies comparing early-passage human fetal and adult fibroblasts show that prolyl hydroxylase activity in fetal fibroblasts is much greater until about 20 weeks' gestation, after which it begins to fall toward adult levels.[28] Unlike in the adult, fetal prolyl hydroxylase is regulated by polyadenosine diphosphate-ribose synthetase, an enzyme implicated in cell repair and tumorigenesis. Thus, fetal skin collagen type ratios are different, overall collagen synthesis is increased, and a different enzymatic regulation is used in the fetus compared with adults. By investigating these differences, more insight will be gained into the ability of fetal fibroblasts to form highly organized collagen.

An abundance of collagen laid down in the wound matrix in parallel fibers constitutes scar in postnatal wounds, whereas a more organized, reticular collagen deposition pattern characterizes fetal wound repair. As more data accumulate, the effect of specific matrix components takes on greater importance. A hyaluronic acid-rich matrix present early and persisting throughout the period of fetal repair may provide the environment necessary for the orderly deposition of collagen fibrils. Collagen type III fibrils of a narrow diameter have been shown to be present at times when hyaluronic acid is abundant. Collagen fibril diameters increase in midgestation, concomitantly with decreasing hyaluronic acid content.[29] Topically applied tissue-extracted hyaluronic acid improves matrix organization in adult tympanic membrane perforations and results in less scar formation.[30] These studies suggest that adult wound repair can be modified by the exogenous application of specific ECM components found in large quantities in fetal wounds.

Adhesion Glycoproteins

As cells become mobile during embryogenesis and repair, specific interactions occur between them and the ECM that allow cells to detach and migrate. The matrix provides the scaffolding for cell attachment and migration through various glycoprotein components such as fibronectin and tenascin. Cells bind to these adhesion glycoproteins with integrins. Cell motility direction may be determined by the relative integrin-ligand binding affinities of the various adhesion glycoproteins bound to a particular cell.

Some of the major differences between fetal and adult wound repair are the temporal patterns of adhesion glycoproteins present in the wound, which are seen at the earliest stages of repair. These differences may lead to differences in cell mobility, migration, adhesion, and proliferation. Fibronectin and laminin have been demonstrated in fetal and adult rabbit wounds using indirect immunofluorescence techniques.[31] Fibronectin is deposited earlier in fetal than in adult wounds whereas there are no differences in laminin deposition.

Tenascin is an ECM glycoprotein that inhibits the cell adhesion effect of fibronectin by interfering with the integrin-mediated fibroblast attachment to fibronectin. In addition, the appearance of tenascin in the matrix correlates with the initiation of cell migration. Using immunohistochemical staining techniques for fibronectin, laminin, and tenascin in fetal mice and fetal sheep, Whitby and co-workers found that tenascin was deposited rapidly in fetal wounds and that its temporal appearance parallels the rate of wound healing, being most rapid in the fetus and slowest in adults.[32] Tenascin appears before cell migration in each group. Thus, tenascin may initiate cell migration, and its early appearance in the fetal wound may lead to rapid wound closure. The remarkable ability of fetal wounds to rapidly reform dermis and reepithelialize may be due in part to the rapid deposition of specialized ECM glycoproteins during wound repair.

Fetal Wound Contraction

Discrepancies between the various fetal animal models of wound repair exist with regard to the presence or absence of wound contraction. For example, excisional wounds in fetal rabbit skin gape open and increase in size as the fetus grows.[33] Examination of open fetal rabbit wounds for myofibroblasts, the cells thought responsible for wound contraction, by immunohistochemistry and by electron microscopy have failed to show their presence.[34] In contrast, excisional wounds in the fetal lamb show rapid healing with wound contraction. Immunohistochemical staining for α-smooth muscle actin, a marker for myofibroblasts, in excisional wounds of fetal lambs at 100 days' gestation shows myofibroblasts present in the wounds that heal with scar formation.[35] This association of fetal myofibroblasts with wound contraction in fetal wounds that do heal with scar requires further investigation.

An in vitro method, consisting mainly of the fibroblast-populated collagen lattice (FPCL) technique, has been used to further study fetal wound contraction. In this technique, fibroblasts populate and actively contract a collagen lattice. The contractile ability of different fibroblasts on different collagen types and under different media conditions can be assessed quantitatively. Fetal rabbit fibroblasts contract both adult and fetal collagen lattices to a greater extent than do adult fibroblasts. This shows that the absence of fetal rabbit excisional wound contraction is not due to an intrinsic contractile defect in the fetal rabbit fibroblasts. When rabbit amniotic fluid is added to the FPCL medium, contraction is inhibited in a dose-dependent manner, which suggests that rabbit amniotic fluid contains an inhibitor of wound contraction. Similarly, human amniotic fluid has been shown to inhibit both fetal and adult human fibroblast contraction in FPCL.[36] Conversely, sheep amniotic fluid has been shown to stimulate fetal and adult fibroblast contraction in FPCL.[37] Fractionation of amniotic fluid may allow for the identification of such putative factor(s) involved in inhibiting (rabbit and human) or potentiating (sheep) contraction. The treatment of pathologic wound contractures or the enhancement of large open wound contraction may then be possible.

Growth Factors

Polypeptide growth factors are intimately associated with development and adult tissue repair, which implies they have a prominent regulatory function in fetal tissue repair. The application of several growth factors, either alone or in combination, on adult wounds accelerates repair. With the critical effects growth factors have on adult repair, attention has recently turned to the important functions of growth factors in fetal skin repair.

Transforming growth factor-β (TGF-β) induces fibroplasia and increases wound tensile strength in adult wounds, and similar effects have been recorded in fetal wounds. Exogenous TGF-β impregnated in PVA sponges that are then implanted into fetal rabbits results in an acute inflammatory cell infiltrate and fibroplasia.[38] Thus, fetal fibroblasts can respond to TGF-β with increased collagen deposition. Although exogenous TGF-β application results in fetal wound scarring, recent studies show that TGF-β is present in fetal wound fluid and may function as a modulator during scarless fetal repair as the isoforms present are in different ratios between the fetus and adult. These findings suggest that TGF-β functions in a regulatory capacity during scarless fetal repair as well as during adult repair.[16]

Although Whitby and Ferguson used immunohistochemical staining techniques in the fetal mouse model and demonstrated that platelet-derived growth factor polypeptide is present in fetal wounds, the authors could not demonstrate basic fibroblast growth factor or TGF-β in fetal wounds, whereas these growth factors were present in neonatal and adult wounds.[39] The lack of TGF-β staining in the fetal mouse may be due to its actual absence in fetal mouse wounds, its presence in low and nondetectable levels, its presence in different isoforms for which the investigator's antibody was not specific, or its presence in some masked or cryptic form. Investigators in the same laboratory blocked the effects of TGF-β isoforms 1 and 2 with neutralizing antibody in adult rat wounds and found healing with much less scar formation, but with normal wound tensile strength and collagen deposition by 14 days after the wound occurred.[40] This is the first use of knowledge gained from the study of fetal repair applied specifically to enhance adult wound repair.

Wound Healing in Other Fetal Tissues

Most studies have focused on the unique ability of fetal skin to regenerate after injury. Recent work has shown that this does not necessarily extrapolate to other tissue types. To investigate the healing characteristics of mesothelial-lined fetal tissue, we made incisional wounds in the diaphragm muscle of fetal lambs at 100 days' gestation.[41] The wounds were either excluded from or exposed to amniotic fluid to examine the effects of amniotic fluid on the healing process. The fetal diaphragm wounds healed with scarring, independent of amniotic fluid exposure, at a gestational age when skin healed without scar.

The healing of fetal long bone fractures has also been examined in fetal lambs by our group.[42] Both incisional and excisional fetal bone wounds heal rapidly and with

minimal callus formation. Incisional fetal bone wounds appear to heal by endochondral ossification in which no cartilage intermediate is present in the callus, unlike adult long bones that heal by endochondral new bone formation wherein a cartilaginous intermediate is present in the callus. Even more remarkable is that excisional wound lengths of three times the bone diameter, including periosteum, completely healed in the fetus. A defect of that size in adult long bone invariably results in nonunion. Fetal hard tissue repair is only now beginning to be studied and may lead to better postnatal bone repair.

Future Implications

There are multiple mechanistic differences between fetal and adult wound repair that are just beginning to be elucidated and that ultimately result in dramatically different end points: scar-free fetal repair versus scar formation in adults. These differences include the wound environment, the differentiation of wound repair effector cells, the deposition of early and late ECM components, and the modulation of type-specific collagen fibrillogenesis. As we gain understanding of these differences and new knowledge of fetal cell-cell, cell-matrix, and cell-growth factor interactions, the possibility of the modulation of adult wound scar formation may be realized. Although the possibility of in utero surgical procedures to take advantage of the unique fetal scarless repair process and correct craniofacial anomalies exists, the greatest clinical benefit of studying and understanding the biology of fetal repair may be in developing therapeutic strategies to avert postnatal scarring and fibrosis.

REFERENCES

1. Adzick NS, Longaker MT (Eds): Fetal Wound Healing. New York, NY, Elsevier Scientific Press, 1992

2. Holbrook KA, Odland GF: The fine structure of developing human epidermis: Light, scanning and transmission electron microscopy of the periderm. J Invest Dermatol 1975; 65:16-38

3. Lane AL: Human fetal skin development. Pediatr Dermatol 1986; 3:487-491

4. Smith LT, Holbrook KA, Byers PH: Structure of the dermal matrix during development and in the adult. J Invest Dermatol 1982; 79:935-1045

5. Nelson NM: Respiration and circulatory changes before birth, *In* Smith CA, Nelson NM (Eds): The Physiology of the Newborn Infant, 4th edition. Springfield, Mass, CC Thomas, 1976, pp 75-121

6. Hunt TK, Zederfeldt B, Goldstick TK: Oxygen and healing. Am J Surg 1969; 118:521-525

7. Decker M, Chiu ES, Dollbaum C, et al: Hyaluronic acid-stimulating activity in sera from the bovine fetus and from breast cancer patients. Cancer Res 1989; 49:3499-3505

8. Estes JM, Spencer EM, Longaker MT, Adzick NS: Insulin-like growth factor-II in ovine wound fluid: Evidence for developmental regulation. Surg Forum 1991; 42:659-661

9. Longaker MT, Whitby DJ, Jennings RW, et al: Adult skin in the fetal environment heals with scar formation. Surg Forum 1990; 41:639-641

10. Lorenz HP, Longaker MT, Perkocha LA, Jennings RW, Harrison MR, Adzick NS: Scarless wound repair: A human fetal skin model. Development 1992; 114:253-259

11. Lorenz HP, Longaker MT, Whitby DJ, Harrison MR, Adzick NS: Scarless fetal skin repair is intrinsic to the fetal fibroblast. Surg Forum 1992; 43:694-696

12. Longaker MT, Whitby DJ, Adzick NS, et al: Studies in fetal wound healing—VI. Second and early third trimester fetal wounds demonstrate rapid collagen deposition without scar formation. J Pediatr Surg 1990; 25:63-69

13. Whitby DJ, Ferguson MWJ: The extracellular matrix of lip wounds in fetal, neonatal and adult mice. Development 1991; 112:651-668

14. Mast BA, Krummel TM: Acute inflammation in fetal wound healing, chap 15, *In* Adzick NS, Longaker MT (Eds): Fetal Wound Healing. New York, NY, Elsevier Scientific Press, 1992, pp 227-240

15. Adzick NS, Harrison MR, Glick PL, et al: Comparison of fetal, newborn, and adult wound healing by histologic, enzyme-histochemical, and hydroxyproline determinations. J Pediatr Surg 1985; 20:315-319

16. Longaker MT, Bouhana KS, Roberts AB, Harrison MR, Adzick NS, Banda MJ: Regulation of fetal wound healing. Surg Forum 1991; 42:575-577

17. Toole BP: Glycosaminoglycans in morphogenesis, *In* Hay ED (Ed): Cell Biology of the Extracellular Matrix. New York, NY, Plenum Press, 1982, pp 259-294

18. Longaker MT, Chiu ES, Adzick NS, Stern M, Harrison MR: Studies in fetal wound healing: V. A prolonged presence of hyaluronic acid characterizes fetal wound fluid. Ann Surg 1991; 213:292-296

19. Longaker MT, Harrison MR, Crombleholme TM, et al: Studies in fetal wound healing: I. A factor in fetal serum that stimulates deposition of hyaluronic acid. J Pediatr Surg 1989; 24:789-792

20. Longaker MT, Chiu ES, Harrison MR, et al: Studies in fetal wound healing: IV. Hyaluronic acid stimulating activity distinguishes fetal from adult wound fluid. Ann Surg 1989; 210:667-672

21. Longaker MT, Adzick NS, Hall JL, et al: Studies in fetal wound healing: VII. Fetal wound healing may be modulated by hyaluronic acid stimulating activity in amniotic fluid. J Pediatr Surg 1990; 25:430-433

22. Burd DAR, Longaker MT, Adzick NS, et al: Fetal wound healing: An in vitro explant model. J Pediatr Surg 1990; 25:898-901

23. Lorenz HP, Whitby DJ, Longaker MT, Adzick NS: The ontogeny of scar formation in the non-human primate. Ann Surg 1993; 217:391-396

24. Burd DAR, Longaker MT, Adzick NS, Harrison MR, Ehrlich HP: Fetal wound healing in a large animal model: The deposition of collagen is confirmed. Br J Plast Surg 1990; 43:571-577

25. Franz FW, Mast BA, Diegelmann RF, Cohen IK: Rapid restoration of breaking strength in healing fetal rabbit wounds. Surg Forum 1991; 42:661-663

26. Smith LT, Holbrook KA, Madri JA: Collagen types I, III, and V in human embryonic and fetal skin. Am J Anat 1986; 175:507-521

27. Sandberg M, Mäkelä JK, Multimäki P, Vuorio L, Vuorio E: Construction of a human pro α1(III) collagen cDNA clone and localization of type III collagen expression in human fetal tissues. Matrix 1989; 9:82-91

28. Duncan BW, Qian J, Liu X, Bhatnagar RS: Regulation of prolyl hydroxylase activity in fetal and adult fibroblasts, chap 20, *In* Adzick NS, Longaker MT (Eds): Fetal Wound Healing. New York, NY, Elsevier Scientific Press, 1992, pp 303-323

29. Scott JE, Hughes EW: Proteoglycan-rich relationships in developing chick and bovine tendons: Influence of the physiologic environment. Conn Tissue Res 1986; 14:267-278

30. Rivas Lacarte MP, Casasin T, Pumarola F, Alonso A: An alternative treatment for the reduction of tympanic membrane perforations: Sodium hyaluronate. Acta Otolaryngol (Stockh) 1990; 110:110-114

31. Longaker MT, Whitby DJ, Ferguson MWJ, et al: Studies in fetal wound healing: III. Early deposition of fibronectin distinguishes fetal from adult wound healing. J Pediatr Surg 1989; 24:799-805

32. Whitby DJ, Longaker MT, Harrison MR, Adzick NS, Ferguson MW: Rapid epithelialisation of fetal wounds is associated with early deposition of tenascin. J Cell Sci 1991; 99:583-586

33. Burrington JD: Wound healing in the fetal lamb. J Pediatr Surg 1971; 6:523-528

34. Krummel TM, Ehrlich HP, Nelson JM, et al: Fetal wounds do not contract in utero. Surg Forum 1989; 40:613-615

35. Longaker MT, Burd DAR, Gown AM, et al: Midgestational fetal lamb excisional wounds contract in utero. J Pediatr Surg 1991; 26:942-947

36. Chang B, Siebert JW, Longaker MT, et al: Do human fetal wounds contract? Ann Plast Surg, in press

37. Rittenberg T, Longaker MT, Adzick NS, Ehrlich HP: Sheep amniotic fluid has a protein which stimulates human fibroblast populated collagen lattice contraction. J Cell Physiol 1991; 149:444-450

38. Krummel TM, Nelson JM, Diegelmann RF, et al: Fetal response to injury in the rabbit. J Pediatr Surg 1987; 22:640-644

39. Whitby DJ, Ferguson MWJ: Immunohistochemical localisation of growth factors in fetal wound healing. Dev Biol 1991; 147:207-215

40. Shah M, Foreman DM, Ferguson MW: Control of scarring in adult wounds by neutralising antibody to transforming growth factor beta. Lancet 1992; 339:213-214

41. Longaker MT, Whitby DJ, Jennings RW, et al: Fetal diaphragmatic wounds heal with scar formation. J Surg Res 1991; 50:375-385

42. Longaker MT, Moelleken BRW, Cheng JC, et al: Fetal fracture healing in a lamb model. Plast Reconstr Surg 1992; 90:161-171