Published in final edited form as:
Ann Biomed Eng. 2014 July ; 42(7): 1494–1507. doi:10.1007/s10439-014-1010-z.

Tissue Engineering and Regenerative Repair in Wound Healing

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Abstract

Wound healing is a highly evolved defense mechanism against infection and further injury. It is a complex process involving multiple cell types and biological pathways. Mammalian adult cutaneous wound healing is mediated by a fibroproliferative response leading to scar formation. In contrast, early to mid-gestational fetal cutaneous wound healing is more akin to regeneration and occurs without scar formation. This early observation has led to extensive research seeking to unlock the mechanism underlying fetal scarless regenerative repair. Building upon recent advances in biomaterials and stem cell applications, tissue engineering approaches are working towards a recapitulation of this phenomenon. In this review, we describe the elements that distinguish fetal scarless and adult scarring wound healing, and discuss current trends in tissue engineering aimed at achieving scarless tissue regeneration.

Keywords

Biomaterials; Cell-based therapeutics; Fetal wound healing; Stem cells

INTRODUCTION

Wound healing is an elaborate process that occurs in three distinct yet overlapping phases—inflammation, cell proliferation, and remodeling.[74,124] The final outcome of the healing process varies based on the developmental stage of the injured organism and the tissue type

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CONFLICTS OF INTEREST
The authors declare no conflicts of interest.

damaged. In 1979, Rowlatt reported that human fetal wound healing did not cause scar formation.[115] Since then, numerous studies have validated this finding by demonstrating that mammalian cutaneous wounds in early to mid-gestational fetal skin, unlike similar wounds in adult skin, heal by regeneration without scarring (Fig. 1).

Adult cutaneous wound repair is characterized by the fibroproliferative response, a highly evolved process that quickly restores the skin barrier, thereby reducing the risk of infection and further injury. However, this response does have drawbacks as it is accomplished through incomplete regeneration of the original tissue and an overproduction of poorly organized collagen. The resulting scar tissue has less than 80% of the tensile strength of the original tissue and is further characterized by a flattened epidermis and loss of dermal appendages, including hair follicles and sebaceous glands.[52,124] Additionally, aberrations in the fibroproliferative response can lead to pathological scarring. Pathologic scars, most commonly either hypertrophic or keloid in nature, are characterized by an excessive deposition of collagen, sometimes beyond the boundary of the original wound. The additional deposit of collagen may result in the formation of a prominent scar that is further complicated by pain and itching.[25]

This fibrotic response has significant clinical implications, as it can restrict growth in the pediatric population[24] and even impair movement when occurring across joints. In addition, there are psychosocial ramifications associated with scar formation, as even small changes in scar appearance can significantly impact patient quality of life.[1,7,40,80] Burn injuries, in particular, illustrate the debilitating effects of fibrotic tissue and the limitations of currently available treatments. Extensive damage from burns and resultant scarring necessitate painful and costly surgeries, sometimes over many years, to restore limited functionality.[7,40]

In contrast, early to mid-gestational mammalian fetuses are able to heal without scar formation. Due in part to greater cellular mobility, fetal wound healing proceeds at a faster rate than adult healing and results in complete regeneration of the dermis, including epidermal appendages, without fibrosis. This mechanism is preserved across a number of mammalian species, including humans.[1,2,80] Moreover, the transition from fetal scarless wound healing to post-natal fibrotic healing occurs at a specific gestational age,[25] typically at around 18 days gestation (E18.5) in mice (term = E22)[32] and 24 weeks gestation in humans.

Although the factors responsible for the fetal wound healing phenotype were originally attributed to the intrauterine environment, this has since been shown to be neither necessary nor sufficient for scarless healing.[79] The capacity for regeneration is now believed to be intrinsic to the tissue itself, and is closely associated with fetal dermal constitution. Differences in extracellular matrix (ECM) components, functional gene expression, cytokine and growth factor release, signal transduction and the inflammatory response have all been implicated in the differential healing outcomes between fetuses and adults (Table 1). Herein, we review the fundamental differences between scarless fetal wound regeneration and scarring adult wound repair, with a focus on the inflammatory response and ECM synthesis, deposition and remodeling. We also discuss recent advances in tissue engineering that seek to replicate regeneration by utilizing bioengineered scaffolds and stem cell applications.

FETAL VS. ADULT WOUND HEALING

Inflammatory Response

Inflammatory Cells—The inflammatory response heralds the initiation of the wound healing process. After injury, inflammation is instigated by the recruitment of several circulating cell types. Inflammation in both the fetus and adult entail the release of transforming growth factor β1 (TGF-β1), platelet-derived growth factor (PDGF), tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) by activated platelets. These cytokines act to recruit neutrophils into the wound to initiate debridement and to upregulate adhesion molecule expression.[102]

Interestingly, fetal wound healing involves a reduced expression of neutrophil adhesion molecules compared to adult wound healing,[96] with subsequently decreased neutrophil migration into the fetal wound.[102,119] Furthermore, neutrophil infiltration in adult wounds is typically followed by the arrival of macrophages[119] responsible for: further debridement of the wound; secretion of cytokines such as colony stimulating factor-1 (CSF-1), TNF-α, and PDGF; perpetuation of the inflammatory phase[119]; and an increase in the fibrotic response.[112] In contrast, macrophage recruitment is almost nonexistent in murine fetal wounds before E14.5. This phenomenon is potentially mediated by a markedly reduced expression of TGF-β1[125] in combination with increased expression of TGF-β3[12,19] in fetal wounds. Transgenic neonatal mice lacking macrophages demonstrate minimal fibrosis during wound healing, supporting the argument that macrophages play an important role in scar formation.[119]

Finally, mast cells enter the adult wound environment in response to IL-33[83] and release TNF-α. TNF-α acts to recruit additional neutrophils, promote the fibroblast to myofibroblast transition, and enhance the activity of matrix metalloproteinases (MMPs). Mast cells therefore govern tissue remodeling, the final phase of wound healing. It is worth noting that mast cells are deficient in fetal wounds. This is believed to further contribute to the overall decreased inflammatory response seen in fetal wounds, and corresponds with a reduction in the secretion of inflammatory factors such as histamine, TGF-β, TNF-α, and vascular endothelial growth factor (VEGF).[145]

Inflammatory Cytokines—Inflammatory cytokines play an important role throughout the different phases of wound healing. These cytokines predominantly consist of members of the TGF-β family, interleukins, and VEGF. In parallel with the previously described observation that adult wounds attract greater numbers of inflammatory cells, adult wounds have also been found to express higher levels of inflammatory factors in comparison to fetal wounds.

For instance, adult wounds have been demonstrated to express higher levels of TGF-β1 and 2, and lower levels of TGF-β3 than fetal wounds. TGF-β1 acts as a chemotactic agent for neutrophils, monocytes, and fibroblasts, which in turn express additional TGF-β1 in a positive feedback mechanism. TGF-β3 expression is linked to hypoxia[121] and is thought to keep fetal cells in a relatively undifferentiated state with a blunted inflammatory response, promoting scarless regeneration.[12]

Interleukins, another important component of the signaling cascade for wound repair, are also present at different levels in fetuses and adults. Interleukin-6 and -8 (IL-6 and -8) are both pro-inflammatory cytokines that are increased in adult wound healing.[71,119] Conversely, interleukin-10 (IL-10), an anti-inflammatory cytokine that allows for organized collagen deposition, is almost absent in adult wounds but highly expressed in fetal wounds.[105]

Lastly, VEGF, a growth factor and one of the major regulators of neovascularization through promotion of endothelial cell proliferation and angiogenesis, has been associated with the development of hypertrophic and keloid scarring.[6,87,94,134,141] Correspondingly, fetal wounds express lower levels of VEGF. In summary, fetal wounds have a muted vascular response[119] in addition to reduced inflammation and subsequently display decreased scar formation.[142]

Extracellular Matrix

Once thought to be an inert substance, the ECM, has been identified as a dynamic network of fibrous adhesion proteins, proteoglycans, and glycosaminoglycans (GAGs) critical to wound repair after injury. For instance, the rate at which wound matrix proteins are deposited is a key feature that distinguishes fetal wound regeneration from adult wound repair. Additionally, differential expression and production of collagen and hyaluronic acid (HA) as well as several other major elements of the ECM have been shown to contribute to the scarless healing ability of fetal skin. An in-depth knowledge of the synthesis and remodeling of the ECM by fibroblasts and other modulators is critical to our understanding of the biological mechanisms underlying fetal wound regeneration. Here we describe several key components of ECM in fetal and adult wounds, including the role of collagen, fibroblasts, HA, proteoglycans, adhesion proteins and metalloproteinases in fetal and adult wound healing.

Collagen—Collagen is the dominant structural component of mammalian connective tissue. There are over 20 different types of collagen in humans, with types I–IV being the most abundant. Type I collagen is the primary collagen found in fetal and adult skin, and together with type III collagen provide tensile strength to skin. Studies have demonstrated that there are distinct variations in the collagen-type profile and cross-linking patterns of collagen in fetal skin and wounds compared to their adult counterparts. These findings have many implications for the role of collagen in wound regeneration.

Fetal skin contains a greater proportion of type III collagen, whereas adult skin consists of predominantly type I collagen. As the fetus develops, the collagen profile of the skin transitions to the post-natal adult phenotype with an increased type I to type III ratio. This transition correlates with the shift from scarless repair to scar formation.[17,76,93]

The patterns of collagen deposition in fetal and adult wounds undergoing repair also differ markedly. Fetal skin, with higher concentrations of the smaller and finer type III collagen, regenerates collagen fibers in a fine, well-organized reticular pattern that closely resembles the collagen architecture of uninjured skin. In contrast, skin wounds that have acquired the adult phenotype contain densely arranged collagen bundles that are oriented parallel to the

wound surface. Additionally, collagen cross-linking increases with gestational age, leading to a parallel increase in matrix rigidity and scar formation.[82] Studies in fetal lambs suggest that these differences in collagen deposition contribute significantly to scarless repair.[76]

However, variations in fetal and adult collagen production may be secondary to interactions between specific cell surface receptors on the fibroblasts. Collagen fibers bind to discoid domain receptors (DDRs), a family of tyrosine kinases that regulate cell proliferation, differentiation, and wound healing. DDR-1 is increasingly expressed on cell surfaces of early gestational fetal fibroblasts but decreases with gestational age.[30] The variable expression of cell surface receptor DDR-1 may modulate collagen production and contribute to the remarkable properties of scarless fetal wound regeneration.[16]

Fibroblasts—Fibroblasts are an additional vital factor to successful wound repair. They perform many key roles, including deposition of ECM and generation of appropriate response to endogenous stimuli such as growth factors. Alterations in fibroblast performance of any of those roles could significantly impact wound healing outcomes.

Fetal fibroblasts *in vitro* have been found to synthesize more total collagen. Not surprisingly, they also synthesize a higher proportion of type III and IV collagen in comparison to adult fibroblasts. As previously mentioned, experimental evidence has indicated that there are different type I to III collagen ratios at different stages of fetal development. During early gestation, fetal fibroblasts exhibit a higher rate of prolyl hydroxylase (the rate-limiting enzyme in collagen synthesis) activity than in late-gestational fetal or adult fibroblasts.[16] Fibroblasts from mid-gestation (E15) fetal mice also demonstrate decreased expression of pro-collagen 1α1, indicating lower rates of type I collagen synthesis. In contrast, fibroblasts from late gestation (E18) showed increased pro-collagen 1α1 expression with decreased pro-collagen 3 expression,[23] demonstrating upregulation of type I collagen synthesis and decreased type III collagen synthesis with advancing gestational age.

Fetal fibroblasts have also been observed to migrate to the wound site at a faster rate than adult fibroblasts. Fetal fibroblasts have the ability to both proliferate and synthesize collagen simultaneously at the wound site. In contrast, adult fibroblasts need to divide prior to laying down collagen, leading to delayed wound healing.[67] As a whole, delayed migration velocity and differing rates of collagen deposition in adult wound healing are believed to be major contributing factors to scar formation.

Adult fibroblast lineage now has a better understood impact on repair outcome. Myofibroblasts, a specialized group of contractile fibroblasts that have acquired smooth muscle α-actin, are present in adult wounds early in the post-injury period. Scarless wounds have virtually no myofibroblasts, whereas in adult wounds the number of active myofibroblasts correlate directly with contraction and the degree of scarring.[41] Mechanistically, the contractile forces generated by myofibroblasts are thought to contribute to scar formation by altering the orientation of collagen fibrils.

Distant fibroblast lineages within the dermis are now known to impact scar formation and hair follicle formation. In contrast, reticular dermal fibroblasts are responsible for much of

the scar deposition found during repair.[37] During adult wound repair, the early deposition of collagen and ECM is performed by reticular fibroblasts, which are thought to be responsible for scarring.[37]

Hyaluronic Acid and Wnt—The abundance of GAGs, particularly HA, is another distinguishing feature of the fetal dermis and wound matrix.[66,91] HA is a linear GAG containing disaccharide units of alpha-1,4-D-glucuronic acid and beta-1,3-*N*-acetyl-D-glucosamine. The molecular weight of HA ranges from 10^{-5} to 10^{7} Da.[122] It plays an important role in the structure of ECM and is present in high concentrations and thought to exert influence during rapid cell proliferation, motility, and de-differentiation.

HA is deposited early in the course of both fetal and adult wound repair, but a sustained deposition occurs more rapidly during fetal wound regeneration. In adult wound repair, HA increases much more slowly.[77] Consistent with this observation, fetal wounds have also been found to contain a larger amount of HA-stimulating activity (HASA) than adult wounds, withHAand HASA in fetal lamb wound fluid shown to decrease significantly during the transition period from scarless to scarring wound repair.[75] The balance between HA synthesis and degradation governs its levels in the dermis. Hyaluronan synthases (HASs) are responsible for HA production in response to inflammatory cytokines[64] while the degradation of HA is controlled by hyaluronidases, in particular hyaluronidase-2 (Hyal2). Interestingly, the wingless proteins (Wnts), which are involved in both dermal and epidermal development[8] and are differentially regulated post-injury in fetal and adult dermis,[33,101] appear to regulate HA levels in the dermis. Fetal and adult wounds differentially express the hyaluronan synthase genes (HAS1-3), each of which transcribes an enzyme with unique characteristics in their production of HA.[59,126,127] Under the influence of either Wnt3a or TGF-β1, fetal fibroblasts increase expression of HAS2 and 3 while post-natal fibroblasts down-regulate both HAS2 and 3. Hyal2 expression, however, is increased byWnt3a in post-natal fibroblasts but not fetal fibroblasts.[22] The higher level of HAS expression and decreased Hyal expression in fetal dermis compared to adult, under the influence of Wnt signaling, underscores the role ofHAdeposition in scarless healing.

HA also plays a role in collagen synthesis. Fetal fibroblasts have 2 to 4-fold more surface HA receptors than their adult counterparts.[4] The higher concentration of HA receptors serve to enhance fibroblast migration without compromising fibroblast HA production. In fetal wounds this results in increased type III collagen deposition along with non-collagen ECM proteins,[76,90] and subsequently accelerated wound repair.

Proteoglycans—Another source of investigatory interest is the heterogeneous group of polyanionic macromolecules, specifically small leucine-rich proteoglycans (SLRPs), which covalently bind to linear sulfated GAG chains. SLRPs such as decorin, chondroitin sulfate and fibromodulin are abundantly expressed in connective tissues and ECM. Decorin production increases by 72% during the transitional period from scarless to scarring healing in fetal rat skin and continues to increase into the post-natal period, achieving a level that is 300 times that of early gestational fibroblasts.[13] In contrast, chondroitin sulfate proteoglycan is present within fetal mouse wounds during collagen fibril formation, but is absent at that time in adult mouse wounds.[139] Thus, chondroitin sulfate is likely to play a role in

regeneration, whereas other sulfated GAGs that are more prevalent in adult wounds, such as decorin, may be important for scar formation. Fibromodulin, another proteoglycan abundantly present in fetal ECM, inhibits collagen fibrillogenesis. Fibromodulin binds and inactivates TGF-β and has an anti-scarring effect during wound healing.[125] Fibromodulin production decreases with maturation and its facilitated cellular migration is decreased in adult wounds but remains unchanged in fetal wounds.

Adhesion Proteins—The amount of ECM adhesion proteins and cellular surface receptors differ between fetal and adult wound healing. Production of cell adhesion proteins, fibronectin and tenascin, increases dramatically early in the fetal wound regenerative process and is present in greater amounts in fetal wounds. These proteins mediate cellular attachment to the ECM and attract fibroblasts, keratinocytes, and endothelial cells to sites of injury.

In early gestational fetal wounds, fibronectin is synthesized within 4 h after wounding, whereas in adult wounds fibronectin expression does not occur until 12 h after injury.[78,139] Similarly, tenascin is deposited more rapidly in fetal wounds than in adult wounds.[140] This rapid deposition of fibronectin and tenascin stimulates early cell attachment and migration, respectively. Together, this promotes the formation of an organized wound matrix that results in skin regeneration.

Early gestational fetal wounds are also characterized by rapid increases in epidermal integrin receptors that bind fibronectin and tenascin in the provisional matrix. A greater proportion of activated fetal keratinocytes near the wound edge express these receptors and higher per cell receptor expression is present in fetal wounds within the first 4 h of injury. This rapid integrin up-regulation is believed to facilitate fetal keratinocyte proliferation and migration resulting in prompt epidermal repair.[26]

Metalloproteinases—The composition and organization of ECM is modulated by the balance between matrix metalloproteinases (MMPs) and tissue-derived inhibitors of metalloproteinases (TIMPs). During wound repair, their expression is tightly regulated. MMPs are a family of zinc-dependent proteinases that have been implicated in ECM remodeling and wound healing, specifically, MMP-1 (collagenase-1), MMP-2 (gelatinase A), MMP-3 (stromelysin-1), MMP-7 (matrilysin), MMP-8 (collagenase-2), MMP-9 (gelatinase B), and MMP-10 (stromelysin-2).[86,104] The proteolytic activity of MMPs is inhibited by 4 distinct TIMPs.

Different expression and temporal changes of specific MMPs and TIMPs have been found in fetal wound regeneration and adult wound repair. Scarless fetal wounds have a higher ratio of MMP to TIMP expression in comparison to adult wounds. The greater presence of activatedMMPs indicates that the fetal healing process favors remodeling over accumulation of collagen.[34] The relative activity of MMP and TIMP is thus crucial not only to the determination of the composition of the wound matrix but also the healing outcome.

Tissue Engineering in Wound Healing

Biomaterials and Scaffolds—The ECM, as discussed in detail above, is a critical determinant in regulating regeneration vs. repair. With growing awareness of the importance of ECM in wound healing, engineering approaches using ECMmimetic materials to construct scaffolding for the delivery of therapeutic cells have become increasingly popular. Modeled after the ECM, scaffolds provide a suitable environment for cell delivery, wound support, and guidance for tissue regeneration. In this section, we discuss the current trends in materials utilized for scaffold production (Table 2). The ideal matrix is biocompatible without toxicity, promotes regeneration and cellular incorporation, and fully incorporates into the recipient tissue site. The following materials each have distinct advantages and disadvantages that are being explored through experimentation.

Collagen—Collagen has great biocompatibility and is absorbable. Hence, it is the gold standard for wound healing. Collagens are the most abundant proteins in the body and are the major components in bone, cartilage, skin, ligament, and tendon. Since collagen has similar properties and structure as the natural ECM, it is a reasonable scaffold material for regenerative therapies.[81] Collagen-based scaffolds have been tested in the repair of cartilage,[45,128,129] skin,[3,108] and bone.[63,117,146,147] There are currently several collagen-based devices on the market. Promogram, BICOL, and Puraply are all collagen-based applications for wound protection, wound site support, ulcer protection, and tissue regeneration.[29,81,116] Unfortunately, the majority of these scaffolds are not comprised of the fetal Collagen I/III ratio and therefore are not ideally suited to reciprocate the fetal dermis and promote scarless wound healing.

Hyaluronic Acid—Hyaluronic acid is an important polysaccharide present in connective tissues such as the skin and tendon. HAs have good biocompatibility and can be readily digested by hyaluronidases in the body.[122] As a major component of ECM and described above as particularly prominent in fetal dermis, HA scaffolds represent an attractive option for regenerative healing and are being developed for cartilage regeneration and wound healing.[54,73] Hyaff, Hycoat, and Laser skin are current HA-based commercial devices.

Fibrin—Fibrin is a glycoprotein present in the blood. It is the product of the thrombin enzymatic cleavage of fibrinogen during coagulation. Fibrin is a major component in blood clots, which are crucial for reparative wound healing and limiting blood loss. Fibrin gels can be made from a patient's own blood and used as an autologous scaffold. This allows the avoidance of potential problems such as immune response and transmission of blood-borne diseases. Fibrin is currently used as a medical sealant and glue. For example, Tisseel, Beriplast, and Biocol, all fibrin-based products, are used extensively in surgery. Due to its excellent biocompatibility, fibrin scaffolds have been fabricated for use in mesenchymal stem cell transplantation,[14] preserving cardiac function,[31] bone tissue regeneration,[103,106] and neural fiber sprouting.[62] From the perspective of achieving scarless, regenerative repair, though, fibrin is associated with adult, reparative healing and does not represent an appropriate scaffold to reciprocate fetal healing.

Chitosan—Chitosan is a natural linear aminopolysaccharide with a unique chemical structure and composition. Chitosan is produced from N-deacetylation of chitin, which is found in the exoskeleton of insects, shellfishes, and fungus.[109,111] In biomedical application, chitosan usually contains less than 50% D-acetyl-D-glucosamine in its structure.[111] Chitosan can be degraded by lysozyme.[132] Chitosan has been used in tissue engineering and drug delivery because of its biocompatibility and biodegradability. In regenerative medicine, porous chitosan has been tested for tissue engineering,[85] cartilage tissue engineering,[44,51,99] skin tissue engineering,[84] and orthopedic tissue engineering.[35] Chitosan may represent a useful addition to the ideal regenerative scaffold when combined with HA and the appropriate ratio of collagen types.

Acellular Matrix—Acellular matrix is derived from animal tissue through the removal of living cells.[143] It has been developed and applied in surgery for reconstruction.[72] There are many advantages to this matrix. It is biocompatible and biodegradable since it originates from living tissue. Also, it remains morphologically identical to natural tissue structure, which provides a preferred environment or blueprint for cellular migration and proliferation. Furthermore, the mechanical property of this matrix is similar to the adjacent environment.[143] The acellular dermal matrix (ADM) allograft has been used for treating burns,[136] urethral repair,[29] and dermis reconstruction.[72,137,150] One of the better characterized and validated ADMs is OASIS Wound Matrix, derived from submucosal layers of porcine jejunum.[11] It has demonstrated significant wound healing advantages in clinical trials compared to conventional therapy[95,113] and alternative regenerative scaffolds.[100,114] Its efficacy may be related to its growth factor content, which includes basic fibroblast growth factor (bFGF)[53] and transforming growth factor-beta 1 (TGF-β1).[92] AlloDerm, DermaMatrix, and Flex HD are other current commercially available acellular matrices, but are all from adult tissue. Available acellular fetal dermal matrices are bovine in origin, including PriMatrix and SurgiMend, though their clinical efficacy is uncertain[48,138] and their ability to promote scarless healing has not been investigated.

Synthetic Biodegradable Polymers—The most common synthetic biodegradable material used for tissue engineering includes polyesters such as polycaprolactone, polyglycolide, polylactic acid, poly(lactic-co-glycolic acid)[18] and their copolymers. Compared to natural materials, synthetic polymers have the advantage of mechanical strength, processability, controllable degradation rate, and adjustable physical properties. These polyesters can be degraded by hydrolysis under physiological conditions. The degraded products can then be safely metabolized or reabsorbed by the body. These polymers can be fabricated into various scaffolds including fibers by electrospinning,[36,50,70,148,149] porous scaffolds by salt-leaching,[56,110] and 3D scaffolds by soft lithography stamping[47] or solid free-form fabrication.[58,130] Numerous studies have demonstrated the feasibility of biodegradable polyester scaffolds.[55,69,110,135] Derma-graft is a knitted mesh medical device comprised of polyglycolic and polylactic acid that is currently in use for treatment of chronic diabetic foot ulcers.[88] While not biodegradable, poly(butyl methacrylate-co-methacrylic acid) is of interest when considering materials for scaffold design, promoting angiogenic ingrowth[18] and accelerating diabetic wound healing,[89] presumably by promoting *Shh* signaling.[43]

Regenerative Scaffolds—Our understanding of fetal ECM composition should inform the development of scaffolds capable of regenerative, scarless healing. McDevitt's group described an approach involving the use of acellularized ECM produced by embryonic stem cells (ESCs) as regenerative matrix.[97] Interestingly, murine ESCs synthesized hyaluronan and expressed HAS2 as part of their ECM,[123] consistent with the fetal dermis. An alternative approach described by Uijtdewilligen *et al.* involves the incorporation of molecules identified to be upregulated during embryonic skin development, including hyaluronan, into a type I collagen-heparin scaffold.[133] Though McDevitt's group have not yet assessed the regenerative potential of ESC produced ECM and Uijtdewilligen *et al.* employed only type I collagen and should have prioritized type III collagen, in the authors opinions, these approaches represents a significant step towards recapitulation of the fetal dermal environment.

Stem Cells in Wound Healing

Advances in tissue engineering have enhanced feasibility and efficacy of cell-based therapies as improved cell delivery methods increase the effectiveness of therapeutic cell transplants. The ultimate goal of such therapy would be the utilization of a cell-seeded scaffold to recapitulate fetal wound regeneration (Fig. 2). Stem cells are a cell source of particular interest as they play a key role in tissue regeneration during wound healing.[68] Stem cells provide daughter cells that replenish lost or injured tissue directly through differentiation and/or recruit inflammatory and tissue progenitor cells through paracrine signaling.[10,28] For many years, mesenchymal stem cells derived from bone marrow (BM-MSCs) were the main donor cells used in tissue engineering research.[20,21,107] Thus, the majority of cell-based regenerative studies have focused on BM-MSCs. More recently, however, adipose- derived stem cells (ASCs) have been increasingly utilized due to the ease with which adipose tissue can be harvested and the abundance of therapeutic stem cells found in adipose tissue.[120]

Mesenchymal Stem Cells

Although MSCs can be found in a variety of tissue, much of the wound healing literature to date has focused on BM-MSCs. These cells are considered attractive for regenerative therapy as endogenous MSCs have the ability to migrate to the site of injury and play an active role in wound healing.[60]

Several preclinical studies have demonstrated the ability of MSCs to accelerate wound closure. Using the excisional wound healing model[46] with a variety of delivery methods (topical, local and systemic injection, and collagen scaffold), numerous studies have demonstrated enhanced cutaneous wound repair with MSCs in both wild-type and diabetic mice.[27,28,39,61,118,144] Falanga *et al.*[42] was able to prevent ulceration and accelerate wound closure in mice by spraying autologous BM-MSCs with a mixture of fibrin and thrombin into wounds. The same method applied in a small human clinical trial was found to accelerate wound closure and resurfacing without adverse effects. Additionally, in chronic wounds there was a strong correlation between reduction in ulcer area and number of BM-MSCs applied to the wound.[42] Another small clinical trial examined the topical application of culture expanded autologous BM-MSCs on chronic ulcers unresponsive to prior

treatment. Investigators were able to achieve wound closure with increased cellularity and dermal rebuilding[9] with application of BM-MSCs.

Although these studies show great promise, confirmation of initial observations in larger clinical trials as well as the development of less invasive harvesting techniques are necessary for the full transition of BMMSCs from bench to bedside.

Adipose-Derived Stem Cells

In recent years, ASCs have become an increasingly attractive donor cell source for cell-based therapy because they can be readily obtained in large numbers through minimally invasive techniques with low donor- site morbidity[131] and are abundantly present in stroma tissue, negating the need for expansion in culture. Moreover, ASCs secrete a wide range of growth factors involved in wound healing in a regulated manner. Studies have also shown that ASCs remain viable at the wound site over significant periods of time[15,38,65] and have the ability to differentiate into epidermal and endothelial cells as well as dermal fibroblasts.[5]

In a recent study utilizing the excisional wound healing model in mice, Kim *et al.*[65] delivered a collagen gel solution with ASCs to cutaneous wounds. These results revealed that application of ASCs led to a significant reduction of wound size with accelerated re-epithelialization. A separate study in which acellular dermal matrix was seeded with ASCs, then applied to the same model similarly demonstrated accelerated wound closure.[57] Yet another study found that the application of ASCs overexpressing VEGF accelerated wound healing in comparison to unmodified ASCs.[98] The results from these studies all attest to the potential for the use of ASCs in cell-based regeneration.

CONCLUSION

Fetal skin possesses the unique ability to regenerate after wounding, with emerging research linking this ability to multiple aspects of the wound environment. Specific differences in the fetal and adult inflammatory and cell response, cytokine and growth factor expression profiles, and ECM composition have been all identified. Our increased understanding of the fetal tissue regeneration process has led to a variety of promising translational approaches attempting to recapitulate this environment. Less well understood are the cell populations, particularly fibroblasts, involved in fetal vs. adult healing and their relevance to the scarless to scarring transition. Exciting developments, such as new transgenic mouse models and high throughput single cell analysis[49] should facilitate an interrogation of these cell populations. Future work should be aimed at improving our understanding of the fetal wound environment and the cell populations involved in addition to leveraging our current understanding to develop biomaterials that more closely recapitulate this environment.

The approach described by Uijtdewilligen *et al.*[133] highlights the potential to incorporate ECM proteins and growth factors reflective of the fetal milieu into tissue engineering scaffolds. As previously mentioned, increased fibroblast proliferation and migration with rapid wound healing is characteristic of the fetal regenerative response and the increased cellularity and rate of wound closure achieved through stem cell based therapies, whether BM-MSCs or ASCs, partially recapitulates the fetal response to injury. This enhanced

healing is largely linked to secretion of growth factors and cytokines, which can be overexpressed. Tailoring the expression profile of these cells would allow their use as a vehicle for the delivery of fetal regenerative factors to injured tissue and combined with fetal-like scaffolds, represents an exciting area of therapeutic development for regenerative healing.

Acknowledgments

The authors thank Ingrid Lai and William Shu for their generous gift support of this work. This work was supported in part by a grant from NIH grant R01 GM087609 (to H.P.L.).

REFERENCES

1. Adzick NS, Longaker MT. Animal models for the study of fetal tissue repair. J. Surg. Res. 1991; 51:216–222. [PubMed: 1908924]
2. Adzick NS, Longaker MT. Scarless fetal healing. Therapeutic implications. Ann. Surg. 1992; 215:3–7. [PubMed: 1731647]
3. Ahn S, et al. Designed three-dimensional collagen scaffolds for skin tissue regeneration. Tissue Eng. Part C. 2009; 16:813–820.
4. Alaish SM, Yager D, Diegelmann RF, Cohen IK. Biology of fetal wound healing: hyaluronate receptor expression in fetal fibroblasts. J. Pediatr. Surg. 1994; 29:1040–1043. [PubMed: 7525917]
5. Altman AM, et al. Dermal matrix as a carrier for in vivo delivery of human adipose-derived stem cells. Biomaterials. 2008; 29:1431–1442. [PubMed: 18191190]
6. Amadeu T, et al. Vascularization pattern in hypertrophic scars and keloids: a stereological analysis. Pathol. Res. Pract. 2003; 199:469–473. [PubMed: 14521263]
7. Asuku ME, Ibrahim A, Ijekeye FO. Post-burn axillary contractures in pediatric patients: a retrospective survey of management and outcome. Burns. 2008; 34:1190–1195. [PubMed: 18692966]
8. Atit R, et al. Beta-catenin activation is necessary and sufficient to specify the dorsal dermal fate in the mouse. Dev. Biol. 2006; 296:164–176. [PubMed: 16730693]
9. Badiavas EV, Falanga V. Treatment of chronic wounds with bone marrow-derived cells. Arch. Dermatol. 2003; 139:510–516. [PubMed: 12707099]
10. Badillo AT, Zhang L, Liechty KW. Stromal progenitor cells promote leukocyte migration through production of stromal-derived growth factor 1alpha: a potential mechanism for stromal progenitor cell-mediated enhancement of cellular recruitment to wounds. J. Pediatr. Surg. 2008; 43:1128–1133. [PubMed: 18558195]
11. Badylak SF, Lantz GC, Coffey A, Geddes LA. Small intestinal submucosa as a large diameter vascular graft in the dog. J. Surg. Res. 1989; 47:74–80. [PubMed: 2739401]
12. Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M. Growth factors and cytokines in wound healing. Wound Repair Regen. 2008; 16:585–601. [PubMed: 19128254]
13. Beanes SR, et al. Down-regulation of decorin, a transforming growth factor-beta modulator, is associated with scarless fetal wound healing. J. Pediatr. Surg. 2001; 36:1666–1671. [PubMed: 11685698]
14. Bensaid W, et al. A biodegradable fibrin scaffold for mesenchymal stem cell transplantation. Biomaterials. 2003; 24:2497–2502. [PubMed: 12695076]
15. Blanton MW, et al. Adipose stromal cells and platelet-rich plasma therapies synergistically increase revascularization during wound healing. Plast. Reconstr. Surg. 2009; 123:56S–64S. [PubMed: 19182664]
16. Bullard KM, Longaker MT, Lorenz HP. Fetal wound healing: current biology. World J. Surg. 2003; 27:54–61. [PubMed: 12557038]
17. Burd DA, Longaker MT, Adzick NS, Harrison MR, Ehrlich HP. Foetal wound healing in a large animal model: the deposition of collagen is confirmed. Br. J. Plast. Surg. 1990; 43:571–577. [PubMed: 2224353]

18. Butler MJ, Sefton MV. Poly(butyl methacrylateco-methacrylic acid) tissue engineering scaffold with proangiogenic potential in vivo. J. Biomed. Mater. Res. 2007; A82:265–273.
19. Caniggia I, et al. Hypoxia-inducible factor-1 mediates the biological effects of oxygen on human trophoblast differentiation through TGFbeta(3). J. Clin. Investig. 2000; 105:577–587. [PubMed: 10712429]
20. Caplan AI. Mesenchymal stem cells. J. Orthop. Res. 1991; 9:641–650. [PubMed: 1870029]
21. Caplan AI. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. J. Cell. Physiol. 2007; 213:341–347. [PubMed: 17620285]
22. Carre AL, et al. Interaction of wingless protein (Wnt), transforming growth factor-beta1, and hyaluronan production in fetal and postnatal fibroblasts. Plast. Reconstr. Surg. 2010; 125:74–88. [PubMed: 20048602]
23. Carter R, Jain K, Sykes V, Lanning D. Differential expression of procollagen genes between mid- and late-gestational fetal fibroblasts. J. Surg. Res. 2009; 156:90–94. [PubMed: 19560165]
24. Cass DL, Meuli M, Adzick NS. Scar wars: implications of fetal wound healing for the pediatric burn patient. Pediatr. Surg. Int. 1997; 12:484–489. [PubMed: 9238112]
25. Cass DL, et al. Wound size and gestational age modulate scar formation in fetal wound repair. J. Pediatr. Surg. 1997; 32:411–415. [PubMed: 9094005]
26. Cass DL, et al. Epidermal integrin expression is upregulated rapidly in human fetal wound repair. J. Pediatr. Surg. 1998; 33:312–316. [PubMed: 9498408]
27. Chen L, Tredget EE, Liu C, Wu Y. Analysis of allogenicity of mesenchymal stem cells in engraftment and wound healing in mice. PLoS ONE. 2009; 4:e7119. [PubMed: 19771171]
28. Chen L, Tredget EE, Wu PY, Wu Y. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. PLoS ONE. 2008; 3:e1886. [PubMed: 18382669]
29. Chen F, Yoo JJ, Atala A. Acellular collagen matrix as a possible “off the shelf” biomaterial for urethral repair. Urology. 1999; 54:407–410. [PubMed: 10475343]
30. Chin GS, et al. Discoidin domain receptors and their ligand, collagen, are temporally regulated in fetal rat fibroblasts in vitro. Plast. Reconstr. Surg. 2001; 107:769–776. [PubMed: 11304604]
31. Christman KL, Fok HH, Sievers RE, Fang Q, Lee RJ. Fibrin glue alone and skeletal myoblasts in a fibrin scaffold preserve cardiac function after myocardial infarction. Tissue Eng. 2004; 10:403–409. [PubMed: 15165457]
32. Colwell AS, Krummel TM, Longaker MT, Lorenz HP. An in vivo mouse excisional wound model of scarless healing. Plast. Reconstr. Surg. 2006; 117:2292–2296. [PubMed: 16772931]
33. Colwell AS, Krummel TM, Longaker MT, Lorenz HP. Wnt-4 expression is increased in fibroblasts after TGF-beta1 stimulation and during fetal and postnatal wound repair. Plast. Reconstr. Surg. 2006; 117:2297–2301. [PubMed: 16772932]
34. Dang CM, et al. Scarless fetal wounds are associated with an increased matrix metalloproteinase-to-tissue-derived inhibitor of metalloproteinase ratio. Plast. Reconstr. Surg. 2003; 111:2273–2285. [PubMed: 12794470]
35. Di Martino A, Sittinger M, Risbud MV. Chitosan: a versatile biopolymer for orthopaedic tissue-engineering. Biomaterials. 2005; 26:5983–5990. [PubMed: 15894370]
36. Doshi J, Reneker DH. Electrospinning process and applications of electrospun fibers. J. Electrostat. 1995; 35:151–160.
37. Driskell RR, et al. Distinct fibroblast lineages determine dermal architecture in skin development and repair. Nature. 2013; 504:277–281. [PubMed: 24336287]
38. Ebrahimian TG, et al. Cell therapy based on adipose tissue-derived stromal cells promotes physiological and pathological wound healing. Arterioscler. Thromb. Vasc. Biol. 2009; 29:503–510. [PubMed: 19201690]
39. Egana JT, et al. Use of human mesenchymal cells to improve vascularization in a mouse model for scaffold-based dermal regeneration. Tissue Eng. 2009; A15:1191–1200.
40. Egeland B, More S, Buchman SR, Cederna PS. Management of difficult pediatric facial burns: reconstruction of burn-related lower eyelid ectropion and perioral contractures. J. Craniofac. Surg. 2008; 19:960–969. [PubMed: 18650718]

41. Estes JM, et al. Phenotypic and functional features of myofibroblasts in sheep fetal wounds. Differentiation. 1994; 56:173–181. [PubMed: 8034132]
42. Falanga V, et al. Autologous bone marrow-derived cultured mesenchymal stem cells delivered in a fibrin spray accelerate healing in murine and human cutaneous wounds. Tissue Eng. 2007; 13:1299–1312. [PubMed: 17518741]
43. Fitzpatrick LE, Lisovsky A, Sefton MV. The expression of sonic hedgehog in diabetic wounds following treatment with poly(methacrylic acid-co-methyl methacrylate) beads. Biomaterials. 2012; 33:5297–5307. [PubMed: 22541537]
44. Francis Suh JK, Matthew HWT. Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: a review. Biomaterials. 2000; 21:2589–2598. [PubMed: 11071608]
45. Fujisato T, Sajiki T, Liu Q, Ikada Y. Effect of basic fibroblast growth factor on cartilage regeneration in chondrocyte-seeded collagen sponge scaffold. Biomaterials. 1996; 17:155–162. [PubMed: 8624392]
46. Galiano RD, Michaels Jt, Dobryansky M, Levine JP, Gurtner GC. Quantitative and reproducible murine model of excisional wound healing. Wound Repair Regen. 2004; 12:485–492. [PubMed: 15260814]
47. Gallego D, Ferrell N, Sun Y, Hansford DJ. Multilayer micromolding of degradable polymer tissue engineering scaffolds. Mater. Sci. Eng. 2008; C28:353–358.
48. Gaster RS, et al. Histologic analysis of fetal bovine derived acellular dermal matrix in tissue expander breast reconstruction. Ann. Plast. Surg. 2013
49. Glotzbach JP, et al. An information theoretic, microfluidic-based single cell analysis permits identification of subpopulations among putatively homogeneous stem cells. PLoS ONE. 2011; 6:e21211. [PubMed: 21731674]
50. Greiner A, Wendorff JH. Electrospinning: a fascinating method for the preparation of ultrathin fibers. Angew. Chem. Int. Ed. 2007; 46:5670–5703.
51. Griffon DJ, Sedighi MR, Schaeffer DV, Eurell JA, Johnson AL. Chitosan scaffolds: interconnective pore size and cartilage engineering. Acta Biomater. 2006; 2:313–320. [PubMed: 16701890]
52. Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. Nature. 2008; 453:314–321. [PubMed: 18480812]
53. Hodde JP, Ernst DM, Hiles MC. An investigation of the long-term bioactivity of endogenous growth factor in OASIS Wound Matrix. J. Wound Care. 2005; 14:23–25. [PubMed: 15656461]
54. Hollander AP, Kon E. Hyaluronan-based scaffolds (Hyalograft1 C) in the treatment of knee cartilage defects: preliminary clinical findings. Tissue Eng. Cartil. Bone. 2003; 249:203.
55. Hollister SJ. Porous scaffold design for tissue engineering. Nat. Mater. 2005; 4:518–524. [PubMed: 16003400]
56. Hou Q, Grijpma DW, Feijen J. Porous polymeric structures for tissue engineering prepared by a coagulation, compression moulding and salt leaching technique. Biomaterials. 2003; 24:1937–1947. [PubMed: 12615484]
57. Huang SP, et al. Adipose-derived stem cells seeded on acellular dermal matrix grafts enhance wound healing in a murine model of a full-thickness defect. Ann. Plast. Surg. 2012; 69:656–662. [PubMed: 23154338]
58. Hutmacher DW, Sittinger M, Risbud MV. Scaffold-based tissue engineering: rationale for computer-aided design and solid free-form fabrication systems. Trends Biotechnol. 2004; 22:354–362. [PubMed: 15245908]
59. Itano N, et al. Three isoforms of mammalian hyaluronan synthases have distinct enzymatic properties. J. Biol. Chem. 1999; 274:25085–25092. [PubMed: 10455188]
60. Jackson WM, Nesti LJ, Tuan RS. Concise review: clinical translation of wound healing therapies based on mesenchymal stem cells. Stem Cells Transl. Med. 2012; 1:44–50. [PubMed: 23197639]
61. Javazon EH, et al. Enhanced epithelial gap closure and increased angiogenesis in wounds of diabetic mice treated with adult murine bone marrow stromal progenitor cells. Wound Repair Regen. 2007; 15:350–359. [PubMed: 17537122]

62. Johnson PJ, Parker SR, Sakiyama-Elbert SE. Fibrin-based tissue engineering scaffolds enhance neural fiber sprouting and delay the accumulation of reactive astrocytes at the lesion in a subacute model of spinal cord injury. J. Biomed. Mater. Res. 2010; A92:152–163.

63. Kakudo N, Shimotsuma A, Miyake S, Kushida S, Kusumoto K. Bone tissue engineering using human adipose- derived stem cells and honeycomb collagen scaffold. J. Biomed. Mater. Res. 2008; A84:191–197.

64. Kennedy CI, Diegelmann RF, Haynes JH, Yager DR. Proinflammatory cytokines differentially regulate hyaluronan synthase isoforms in fetal and adult fibroblasts. J. Pediatr. Surg. 2000; 35:874–879. [PubMed: 10873029]

65. Kim WS, et al. Wound healing effect of adipose-derived stem cells: a critical role of secretory factors on human dermal fibroblasts. J. Dermatol. Sci. 2007; 48:15–24. [PubMed: 17643966]

66. Krummel TM, et al. Fetal response to injury in the rabbit. J. Pediatr. Surg. 1987; 22:640–644. [PubMed: 3302202]

67. Larson BJ, Longaker MT, Lorenz HP. Scarless fetal wound healing: a basic science review. Plast. Reconstr. Surg. 2010; 126:1172–1180. [PubMed: 20885241]

68. Lau K, Paus R, Tiede S, Day P, Bayat A. Exploring the role of stem cells in cutaneous wound healing. Exp. Dermatol. 2009; 18:921–933. [PubMed: 19719838]

69. Li W, Danielson KG, Alexander PG, Tuan RS. Biological response of chondrocytes cultured in three-dimensional nanofibrous poly (caprolactone) scaffolds. J. Biomed. Mater. Res. 2003; A67:1105–1114.

70. Li, Wu; Laurencin, CT.; Caterson, EJ.; Tuan, RS.; Ko, FK. Electrospun nanofibrous structure: a novel scaffold for tissue engineering. J. Biomed. Mater. Res. 2002; 60:613–621. [PubMed: 11948520]

71. Liechty KW, Adzick NS, Crombleholme TM. Diminished interleukin 6 (IL-6) production during scarless human fetal wound repair. Cytokine. 2000; 12:671–676. [PubMed: 10843743]

72. Livesey SA, Herndon DN, Hollyoak MA, Atkinson YH, Nag A. Transplanted acellular allograft dermal matrix: Potential as a template for the reconstruction of viable dermis. Transplantation. 1995; 60:1–9. [PubMed: 7542811]

73. Loken S, et al. Bone marrow mesenchymal stem cells in a hyaluronan scaffold for treatment of an osteochondral defect in a rabbit model. Knee Surg. Sports Traumatol. Arthrosc. 2008; 16:896–903. [PubMed: 18592218]

74. Longaker MT, Gurtner GC. Introduction: wound repair. Semin. Cell Dev. Biol. 2012; 23:945. [PubMed: 23059792]

75. Longaker MT, et al. Studies in fetal wound healing. IV. Hyaluronic acid-stimulating activity distinguishes fetal wound fluid from adult wound fluid. Ann. Surg. 1989; 210:667–672. [PubMed: 2818035]

76. Longaker MT, 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; 25:63–68. discussion 68–69. [PubMed: 2299547]

77. Longaker MT, et al. Studies in fetal wound healing. V. A prolonged presence of hyaluronic acid characterizes fetal wound fluid. Ann. Surg. 1991; 213:292–296. [PubMed: 2009010]

78. Longaker MT, et al. Fetal diaphragmatic wounds heal with scar formation. J. Surg. Res. 1991; 50:375–385. [PubMed: 2020189]

79. Longaker MT, et al. Adult skin wounds in the fetal environment heal with scar formation. Ann. Surg. 1994; 219:65–72. [PubMed: 8297179]

80. Lorenz HP, et al. Scarless wound repair: a human fetal skin model. Development. 1992; 114:253–259. [PubMed: 1576963]

81. Lorenzetti, OJ.; Fortenberry, B.; Busby, E.; Uberman, R. Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine. Vol. 140. New York: Royal Society of Medicine; 1972. Influence of microcrystalline collagen on wound healing I. Wound closure of normal excised and burn excised wounds of rats, rabbits, and pigs; p. 896-900.

82. Lovvorn HN, et al. Relative distribution and crosslinking of collagen distinguish fetal from adult sheep wound repair. J. Pediatr. Surg. 1999; 34:218–223. [PubMed: 10022176]

83. Lunderius-Andersson C, Enoksson M, Nilsson G. Mast cells respond to cell injury through the recognition of IL-33. Front. Immunol. 2012; 3:82. [PubMed: 22566963]
84. Ma L, et al. Collagen/chitosan porous scaffolds with improved biostability for skin tissue engineering. Biomaterials. 2003; 24:4833–4841. [PubMed: 14530080]
85. Madihally SV, Matthew HWT. Porous chitosan scaffolds for tissue engineering. Biomaterials. 1999; 20:1133–1142. [PubMed: 10382829]
86. Madlener M. Differential expression of matrix metalloproteinases and their physiological inhibitors in acute murine skin wounds. Arch. Dermatol. Res. 1998; 290(Suppl):S24–S29. [PubMed: 9710380]
87. Mak K, et al. Scarless healing of oral mucosa is characterized by faster resolution of inflammation and control of myofibroblast action compared to skin wounds in the red Duroc pig model. J. Dermatol. Sci. 2009; 56:168–180. [PubMed: 19854029]
88. Marston WA, Hanft J, Norwood P, Pollak R. The Efficacy and Safety of Dermagraft in Improving the Healing of Chronic Diabetic Foot Ulcers Results of a prospective randomized trial. Diabetes Care. 2003; 26:1701–1705. [PubMed: 12766097]
89. Martin DC, Semple JL, Sefton MV. Poly(methacrylic acid-co-methyl methacrylate) beads promote vascularization and wound repair in diabetic mice. J. Biomed. Mater. Res. 2010; A93:484–492.
90. Mast BA, Diegelmann RF, Krummel TM, Cohen IK. Hyaluronic acid modulates proliferation, collagen and protein synthesis of cultured fetal fibroblasts. Matrix. 1993; 13:441–446. [PubMed: 8309423]
91. Mast BA, et al. Hyaluronic acid is a major component of the matrix of fetal rabbit skin and wounds: implications for healing by regeneration. Matrix. 1991; 11:63–68. [PubMed: 2027330]
92. McDevitt CA, Wildey GM, Cutrone RM. Transforming growth factor-beta1 in a sterilized tissue derived from the pig small intestine submucosa. J. Biomed. Mater. Res. 2003; A67:637–640.
93. Merkel JR, DiPaolo BR, Hallock GG, Rice DC. Type I and type III collagen content of healing wounds in fetal and adult rats. Proc. Soc. Exp. Biol. Med. 1988; 187:493–497. [PubMed: 3353398]
94. Mogili NS, et al. Altered angiogenic balance in keloids: a key to therapeutic intervention. Translational research. J. Lab. Clin. Med. 2012; 159:182–189.
95. Mostow EN, Haraway GD, Dalsing M, Hodde JP, King D. Effectiveness of an extracellular matrix graft (OASIS Wound Matrix) in the treatment of chronic leg ulcers: a randomized clinical trial. J. Vasc. Surg. 2005; 41:837–843. [PubMed: 15886669]
96. Naik-Mathuria B, et al. Age-dependent recruitment of neutrophils by fetal endothelial cells: implications in scarless wound healing. J. Pediatr. Surg. 2007; 42:166–171. [PubMed: 17208559]
97. Nair R, Shukla S, McDevitt TC. Acellular matrices derived from differentiating embryonic stem cells. J. Biomed. Mater. Res. 2008; A87:1075–1085.
98. Nauta A, et al. Adipose-derived stromal cells over-expressing vascular endothelial growth factor accelerate mouse excisional wound healing. Mol. Ther. 2013; 21:445–455. [PubMed: 23164936]
99. Nettles DL, Elder SH, Gilbert JA. Potential use of chitosan as a cell scaffold material for cartilage tissue engineering. Tissue Eng. 2002; 8:1009–1016. [PubMed: 12542946]
100. Niezgoda JA, Van Gils CC, Frykberg RG, Hodde JP. Randomized clinical trial comparing OASIS Wound Matrix to Regranex Gel for diabetic ulcers. Adv. Skin Wound Care. 2005; 18:258–266. [PubMed: 15942317]
101. Okuse T, Chiba T, Katsuumi I, Imai K. Differential expression and localization of WNTs in an animal model of skin wound healing. Wound Repair Regen. 2005; 13:491–497. [PubMed: 16176457]
102. Olutoye OO, Zhu X, Cass DL, Smith CW. Neutrophil recruitment by fetal porcine endothelial cells: implications in scarless fetal wound healing. Pediatr. Res. 2005; 58:1290–1294. [PubMed: 16306210]
103. Osathanon T, et al. Microporous nanofibrous fibrin-based scaffolds for bone tissue engineering. Biomaterials. 2008; 29:4091–4099. [PubMed: 18640716]
104. Parks WC. Matrix metalloproteinases in repair. Wound Repair Regen. 1999; 7:423–432. [PubMed: 10633001]

105. Peranteau WH, et al. IL-10 overexpression decreases inflammatory mediators and promotes regenerative healing in an adult model of scar formation. J. Invest. Dermatol. 2008; 128:1852–1860. [PubMed: 18200061]
106. Perka C, et al. Segmental bone repair by tissue-engineered periosteal cell transplants with bioresorbable fleece and fibrin scaffolds in rabbits. Biomaterials. 2000; 21:1145–1153. [PubMed: 10817267]
107. Pittenger MF, et al. Multilineage potential of adult human mesenchymal stem cells. Science. 1999; 284:143–147. [PubMed: 10102814]
108. Powell HM, Boyce ST. EDC cross-linking improves skin substitute strength and stability. Biomaterials. 2006; 27:5821–5827. [PubMed: 16919327]
109. Ravi Kumar MNV. A review of chitin and chitosan applications. React. Funct. Polym. 2000; 46:1–27.
110. Reignier J, Huneault MA. Preparation of interconnected poly (epsilon-caprolactone) porous scaffolds by a combination of polymer and salt particulate leaching. Polymer. 2006; 47:4703–4717.
111. Rinaudo M. Chitin and chitosan: properties and applications. Prog. Polym. Sci. 2006; 31:603–632.
112. Robson MC, Barnett RA, Leitch IO, Hayward PG. Prevention and treatment of postburn scars and contracture. World J. Surg. 1992; 16:87–96. [PubMed: 1290271]
113. Romanelli M, Dini V, Bertone MS. Randomized comparison of OASIS wound matrix versus moist wound dressing in the treatment of difficult-to-heal wounds of mixed arterial/venous etiology. Adv. Skin Wound Care. 2010; 23:34–38. [PubMed: 20101114]
114. Romanelli M, Dini V, Bertone M, Barbanera S, Brilli C. OASIS wound matrix versus Hyaloskin in the treatment of difficult-to-heal wounds of mixed arterial/venous aetiology. Int. Wound J. 2007; 4:3–7. [PubMed: 17425543]
115. Rowlatt U. Intrauterine wound healing in a 20 week human fetus. Virchows Arch. A Pathol. Anat. Histol. 1979; 381:353–361. [PubMed: 155931]
116. Ruszczak Z. Effect of collagen matrices on dermal wound healing. Adv. Drug Deliv. Rev. 2003; 55:1595–1611. [PubMed: 14623403]
117. Sarkar MR, et al. Bone formation in a long bone defect model using a platelet-rich plasma-loaded collagen scaffold. Biomaterials. 2006; 27:1817–1823. [PubMed: 16307796]
118. Sasaki M, et al. Mesenchymal stem cells are recruited into wounded skin and contribute to wound repair by transdifferentiation into multiple skin cell type. J. Immunol. 2008; 180:2581–2587. [PubMed: 18250469]
119. Satish L, Kathju S. Cellular and molecular characteristics of scarless versus fibrotic wound healing. Dermatol. Res. Pract. 2010; 2010:790234. [PubMed: 21253544]
120. Schaffler A, Buchler C. Concise review: adipose tissue-derived stromal cells–basic and clinical implications for novel cell-based therapies. Stem Cells. 2007; 25:818–827. [PubMed: 17420225]
121. Scheid A, et al. Physiologically low oxygen concentrations in fetal skin regulate hypoxia-inducible factor 1 and transforming growth factor-beta3. FASEB J. 2002; 16:411–413. [PubMed: 11790723]
122. Shimada E, Matsumura G. Viscosity and molecular weight of hyaluronic acids. J. Biochem. 1975; 78:513–517. [PubMed: 1225913]
123. Shukla S, et al. Synthesis and organization of hyaluronan and versican by embryonic stem cells undergoing embryoid body differentiation. J. Histochem. Cytochem. 2010; 58:345–358. [PubMed: 20026669]
124. Singer AJ, Clark RA. Cutaneous wound healing. N. Engl. J. Med. 1999; 341:738–746. [PubMed: 10471461]
125. Soo C, et al. Differential expression of fibromodulin, a transforming growth factor-beta modulator, in fetal skin development and scarless repair. Am. J. Pathol. 2000; 157:423–433. [PubMed: 10934147]
126. Spicer AP, Augustine ML, McDonald JA. Molecular cloning and characterization of a putative mouse hyaluronan synthase. J. Biol. Chem. 1996; 271:23400–23406. [PubMed: 8798545]

127. Spicer AP, McDonald JA. Characterization and molecular evolution of a vertebrate hyaluronan synthase gene family. J. Biol. Chem. 1998; 273:1923–1932. [PubMed: 9442026]
128. Stone KR, Rodkey WG, Webber R, McKinney L, Steadman JR. Meniscal regeneration with copolymeric collagen scaffolds In vitro and in vivo studies evaluated clinically, histologically, and biochemically. Am. J. Sports Med. 1992; 20:104–111. [PubMed: 1558234]
129. Stone KR, Steadman JR, Rodkey WG, Li ST. Regeneration of meniscal cartilage with use of a collagen scaffold. Analysis of preliminary data. J. Bone Joint Surg. 1997; 79:1770–1777. [PubMed: 9409790]
130. Taboas JM, Maddox RD, Krebsbach PH, Hollister SJ. Indirect solid free form fabrication of local and global porous, biomimetic and composite 3D polymer-ceramic scaffolds. Biomaterials. 2003; 24:181–194. [PubMed: 12417192]
131. Tobita M, Orbay H, Mizuno H. Adipose-derived stem cells: current findings and future perspectives. Discov. Med. 2011; 11:160–170. [PubMed: 21356171]
132. Tomihata K, Ikada Y. In vitro and in vivo degradation of films of chitin and its deacetylated derivatives. Biomaterials. 1997; 18:567–575. [PubMed: 9105597]
133. Uijtdewilligen PJ, et al. Towards embryonic-like scaffolds for skin tissue engineering: identification of effector molecules and construction of scaffolds. J. Tissue Eng. Regen. Med. 2013
134. van der Veer WM, et al. Time course of the angiogenic response during normotrophic and hypertrophic scar formation in humans. Wound Repair Regen. 2011; 19:292–301. [PubMed: 21539647]
135. Vaz CM, Van Tuijl S, Bouten CVC, Baaijens FPT. Design of scaffolds for blood vessel tissue engineering using a multi-layering electrospinning technique. Acta Biomater. 2005; 1:575–582. [PubMed: 16701837]
136. Wainwright DJ. Use of an acellular allograft dermal matrix (AlloDerm) in the management of full-thickness burns. Burns. 1995; 21:243–248. [PubMed: 7662122]
137. Wainwright D, et al. Clinical evaluation of an acellular allograft dermal matrix in full-thickness burns. J. Burn Care Res. 1996; 17:124–136.
138. Wanitphakdeedecha R, Chen TM, Nguyen TH. The use of acellular, fetal bovine dermal matrix for acute, full-thickness wounds. J. Drugs Dermatol. 2008; JDD7:781–784. [PubMed: 18720696]
139. Whitby DJ, Ferguson MW. The extracellular matrix of lip wounds in fetal, neonatal and adult mice. Development. 1991; 112:651–668. [PubMed: 1724421]
140. Whitby DJ, Longaker MT, Harrison MR, Adzick NS, Ferguson MW. Rapid epithelialisation of fetal wounds is associated with the early deposition of tenascin. J. Cell Sci. 1991; 99(Pt 3):583–586. [PubMed: 1719005]
141. Wilgus TA. Immune cells in the healing skin wound: influential players at each stage of repair. Pharmacol. Res. 2008; 58:112–116. [PubMed: 18723091]
142. Wilgus TA, Ferreira AM, Oberyszyn TM, Bergdall VK, Dipietro LA. Regulation of scar formation by vascular endothelial growth factor. Laboratory investigation. J. Tech. Methods Pathol. 2008; 88:579–590.
143. Wilson GJ, Courtman DW, Klement P, Michael Lee J, Yeger H. Acellular matrix: a biomaterials approach for coronary artery bypass and heart valve replacement. Ann. Thorac. Surg. 1995; 60:S353–S358. [PubMed: 7646187]
144. Wu Y, Chen L, Scott PG, Tredget EE. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. Stem Cells. 2007; 25:2648–2659. [PubMed: 17615264]
145. Wulff BC, et al. Mast cells contribute to scar formation during fetal wound healing. J. Invest. Dermatol. 2012; 132:458–465. [PubMed: 21993557]
146. Xiao Y, Qian H, Young WG, Bartold PM. Tissue engineering for bone regeneration using differentiated alveolar bone cells in collagen scaffolds. Tissue Eng. 2003; 9:1167–1177. [PubMed: 14670104]
147. Yang XB, Bhatnagar RS, Li S, Oreffo ROC. Biomimetic collagen scaffolds for human bone cell growth and differentiation. Tissue Eng. 2004; 10:1148–1159. [PubMed: 15363171]

148. Yoshimoto H, Shin YM, Terai H, Vacanti JP. A biodegradable nanofiber scaffold by electrospinning and its potential for bone tissue engineering. Biomaterials. 2003; 24:2077–2082. [PubMed: 12628828]

149. Zong X, et al. Electrospun fine-textured scaffolds for heart tissue constructs. Biomaterials. 2005; 26:5330–5338. [PubMed: 15814131]

150. Zhang X, et al. Expansion and delivery of human fibroblasts on micronized acellular dermal matrix for skin regeneration. Biomaterials. 2009; 30:2666–2674. [PubMed: 19171377]

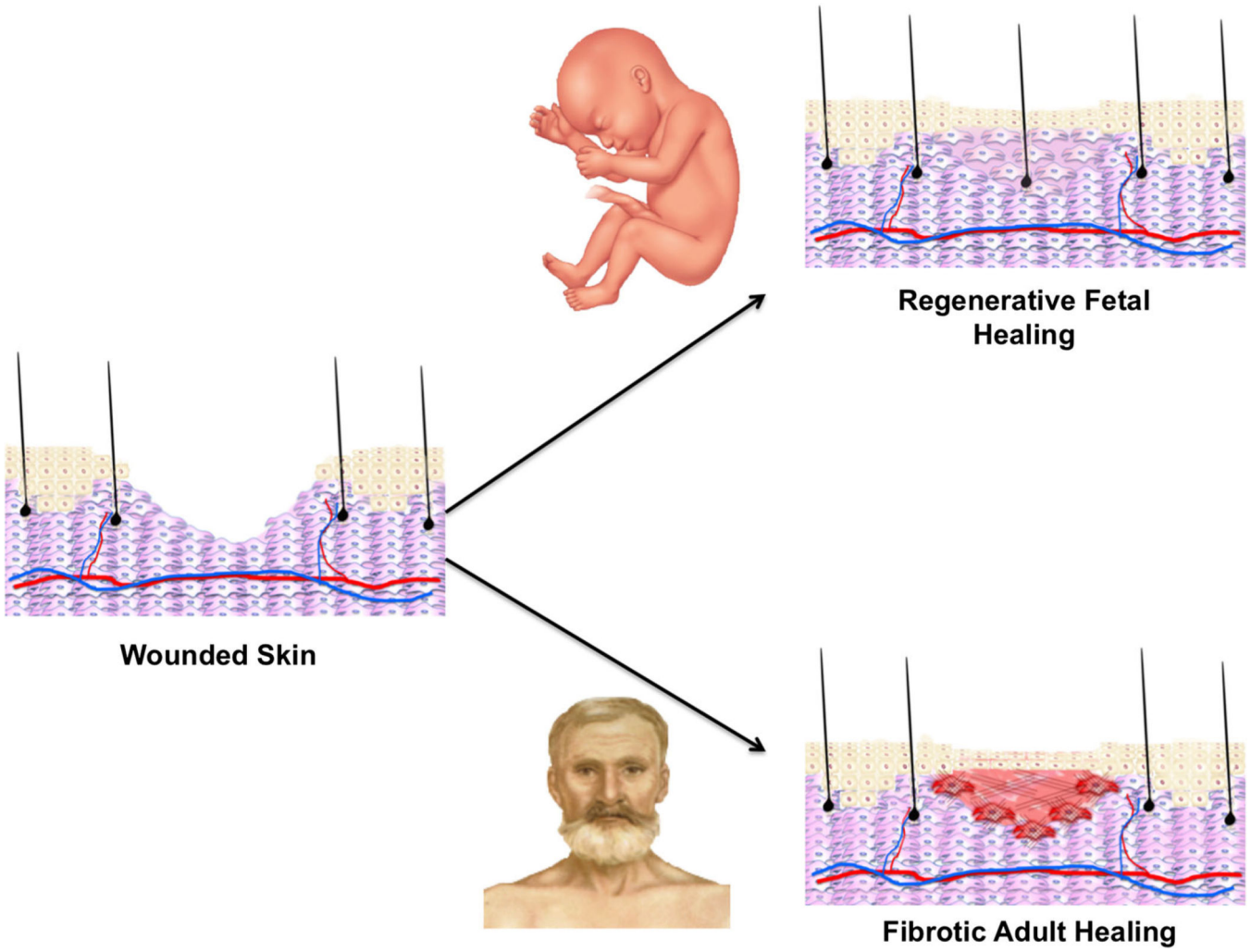

FIGURE 1.
Fetal vs. adult wound healing.

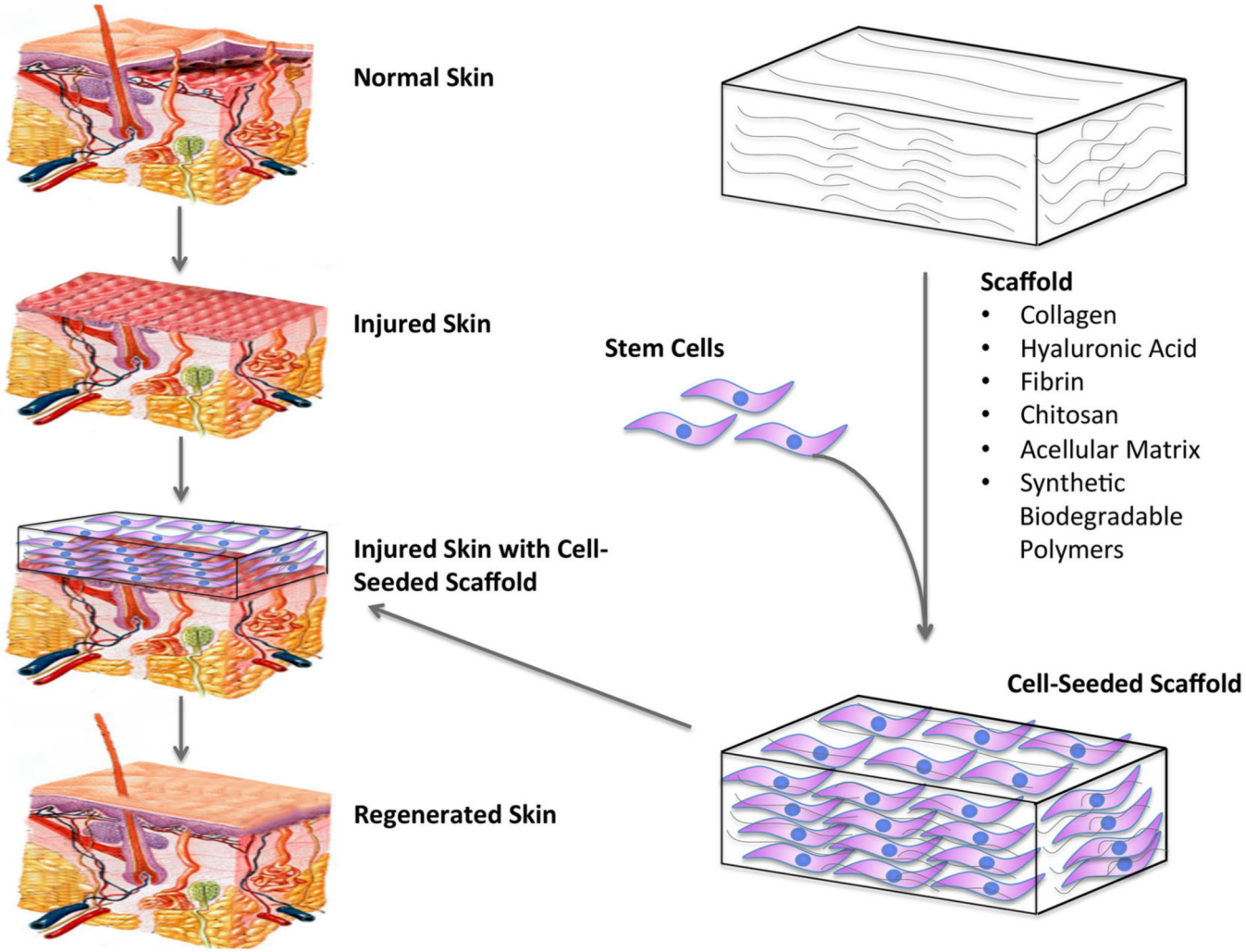

FIGURE 2.
Cell-based regenerative therapy.

TABLE 1

Fetal vs. adult wound healing.

Healing	Fetal healing	Adult healing
Inflammatory response		
Inflammatory cells	Low	High
Cytokines		
IL-6, IL-8	Low	High
IL-10	High	Low
Growth factors		
VEGF	Low	High
TGF-β		
TGF-β1/2	Low	High
TGF-β3	High	Low
Fibroblasts		
Rate of ECM synthesis	High	Low
Myofibroblasts	Absent	Present
Extracellular matrix		
Collagen		
Rate of deposition	Rapid	Delayed
Histological pattern	Fine, reticular, large fibers	Dense, parallel, small fibers
Collagen type III:I ratio	High	Low
Cross-linking	Low	High
Hyaluronic acid		
Expression	High	Low
Receptors	High	Low
HASA	High	Low
ECM modulators		
Fibromodulin	Increased	Decreased
Decorin	Decreased	Increased
Adhesion proteins	Rapid increase	Diminished increase
MMP:TIMP ratio	High	Low

TABLE 2

Biomaterials and scaffolds.

Scaffold type	Description	Merits of scaffold	Clinical application	Commercial products
Collagen	Most abundant protein in the body; major component of bone, cartilage, skin, tendons, and ligaments	High biocompatibility and absorb-ability; similar properties and structure to ECM	Repair of cartilage, skin, and bone	Promogram, BICOL, Puraply
Hyaluronic acid	Polysaccharide in connective, tissues such as skin and tendons; major component of ECM	Good biocompatibility; simulates environment of ECM for healing	Cartilage regeneration and wound healing	Hyaff, Hycoat, Laserskin
Fibrin	Glycoprotein present in blood; major component in blood clots to prevent blood loss and promote healing	Excellent biocompatibility; synthesis of autologous fibrin gels prevents immune response and transmission of blood- borne diseases	Used in mesenchymal stem cell transplantation, preservation of cardiac, function, bone regener-ation, and neural fiber sprouting	Tisseel, Biocol, Beriplast
Chitosan	Aminopolysaccharide found in exoskeleton of insects, shellfish and fungi	Good biocompatibility and biodegradability	Potential for cartilage, skin, and orthopedic tissue regeneration	N/A
Acellular matrix	Animal tissue-derived matrix absent of living cells; identical to natural tissue structure	Biocompatible and biodegradable since organically derived; optimal conditions for cell migration and proliferation	Allografts used for burn treatment, urethral repair, and dermal reconstruction	OASIS Wound Matrix, AlloDerm, DermaMa-trix, Flex HD, PriMa-trix, SurgiMend
Synthetic biodegradable polymers	Most common are polyesters, such as polyglycolide, polylactic acid, polycaprolactone, etc.	Good mechanical strength; easily processed; adjustable degradation rate and physical properties	Fabrication into fibrous, porous, or 3D scaffolds; treatment of chronic diabetic foot ulcers	Dermagraft