MAYO CLINIC

Wound Healing: A Paradigm for Regeneration

Victor W. Wong, MD; Geoffrey C. Gurtner, MD; and Michael T. Longaker, MD, MBA

From the Hagey Laboratory for Pediatric Regenerative Medicine, Department of Surgery, Stanford University, Stanford, CA.

CME Activity

Target Audience: The target audience for *Mayo Clinic Proceedings* is primarily internal medicine physicians and other clinicians who wish to advance their current knowledge of clinical medicine and who wish to stay abreast of advances in medical research.

Statement of Need: General internists and primary care physicians must maintain an extensive knowledge base on a wide variety of topics covering all body systems as well as common and uncommon disorders. *Mayo Clinic Proceedings* aims to leverage the expertise of its authors to help physicians understand best practices in diagnosis and management of conditions encountered in the clinical setting.

Accreditation: College of Medicine, Mayo Clinic is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians.

Credit Statement: College of Medicine, Mayo Clinic designates this journal-based CME activity for a maximum of 1.0 *AMA PRA Category 1 Credit(s)*.™ Physicians should claim only the credit commensurate with the extent of their participation in the activity.

Learning Objectives: On completion of this article, you should be able to (1) describe noncellular components of the wound environment that contribute to cutaneous tissue repair, (2) distinguish between different types of skin stem cells and their respective niches, and (3) describe how mechanical forces influence wound repair on the tissue, cellular, and molecular levels.

Disclosures: As a provider accredited by ACCME, College of Medicine, Mayo Clinic (Mayo School of Continuous Professional Development) must ensure balance, independence, objectivity, and scientific rigor in its educational activities. Course Director(s), Planning Committee members, faculty, and all others who are in a position to control the content of this educational activity are required to disclose all relevant financial relationships with any commercial interest related to the subject matter of the educational activity. Safeguards against commercial bias have been put in place. Faculty also will disclose any off-label and/or investigational use of pharmaceuticals or instruments discussed in their presentation. Disclosure of this information will be published in course materials so that those participants in the activity may formulate their own judgments regarding the presentation.

In their editorial and administrative roles, William L. Lanier, Jr, MD, Terry L. Jopke, Kimberly D. Sankey, and Nicki M. Smith, MPA, have control of the content of this program but have no relevant financial relationship(s) with industry.

The authors report no competing interests.

Method of Participation: In order to claim credit, participants must complete the following:

1. Read the activity.
2. Complete the online CME Test and Evaluation. Participants must achieve a score of 80% on the CME Test. One retake is allowed.

Participants should locate the link to the activity desired at http://bit.ly/18YvD0B. Upon successful completion of the online test and evaluation, you can instantly download and print your certificate of credit.

Estimated Time: The estimated time to complete each article is approximately 1 hour.

Hardware/Software: PC or MAC with Internet access.

Date of Release: 08/01/2013

Expiration Date: 07/31/2015 (Credit can no longer be offered after it has passed the expiration date.)

Privacy Policy: http://www.mayoclinic.org/global/privacy.html

Questions? Contact dletcsupport@mayo.edu.

Abstract

Human skin is a remarkably plastic organ that sustains insult and injury throughout life. Its ability to expeditiously repair wounds is paramount to survival and is thought to be regulated by wound components such as differentiated cells, stem cells, cytokine networks, extracellular matrix, and mechanical forces. These intrinsic regenerative pathways are integrated across different skin compartments and are being elucidated on the cellular and molecular levels. Recent advances in bioengineering and nanotechnology have allowed researchers to manipulate these microenvironments in increasingly precise spatial and temporal scales, recapitulating key homeostatic cues that may drive regeneration. The ultimate goal is to translate these bench achievements into viable bedside therapies that address the growing global burden of acute and chronic wounds. In this review, we highlight current concepts in cutaneous wound repair and propose that many of these evolving paradigms may underlie regenerative processes across diverse organ systems.

 ■ Mayo Clin Proc. 2013;88(9):1022-1031

Skin is the largest organ in the human body and serves key functions, including physical protection, sensation, temperature regulation, and insulation. Multiple cell populations and matrix components form distinct yet interdependent compartments that regulate skin behavior during development and throughout life.[1] Despite the constant exposure to physical, biochemical, and radiation injury, a functional integumentary system is able to counteract these forces and maintain a relative state of homeostasis. This dynamic balance underlies the remarkable plasticity of skin and has been effectively exploited in reconstructive settings, including tissue expansion, scar revision surgery, and skin grafting.

The current understanding of skin biology and its response to injury provides insight into intrinsic restorative pathways in complex organs.[2] For example, the epithelial layer of

skin is continuously renewed throughout life, and autologous skin grafts can be transplanted and survive long-term without major adverse effects. Success in hair follicle transfer supports the concept that skin appendages themselves are also capable of promoting regenerative pathways.[3] Basic science research continues to identify stem cell populations that may play a central role in skin regeneration.[4-7] Thus, human skin represents a unique paradigm for organ homeostasis that enables researchers to study putative repair mechanisms for regenerative medicine.

Wound healing has traditionally been viewed as the sequential activation of local and systemic cells that function in concert to restore skin integrity via scar formation. Accordingly, *abnormal* or *pathologic* wound healing has been attributed to any disruption of these cellular events, such as prolonged inflammation, a primary characteristic of nonhealing wounds and fibroproliferation. Research during the past decade has elucidated a more complex understanding of wound biology that acknowledges the importance of noncellular components, including the extracellular matrix (ECM) and mechanical force.[8,9] Furthermore, skin stem cells are recognized to contribute to wound repair and may play a prominent role in the future of regenerative medicine.[10,11] In this article, we review current and emerging themes in cutaneous wound healing and highlight concepts in skin regeneration that are potentially applicable to diverse biological systems.

FETAL WOUND HEALING

Although all human wounds heal with some degree of scar formation, the wide diversity of wound outcomes (eg, "normal" scar vs pathologic scar or keloid formation) suggests that tissue repair may be modulated by multiple factors after injury. A better understanding of these influences may allow researchers to ultimately promote wound regeneration. For example, early-gestation human fetuses repair cutaneous wounds without scar formation.[12] Investigation of the fetal wound environment has elucidated specific biological attributes that may be responsible for the scarless phenotype.[13] For example, fetal wounds exhibit a diminished inflammatory response, with reduced leukocyte counts and cytokine levels. There are greater levels of antifibrotic cytokines compared with adult wounds, and, specifically, the balance of transforming growth factor (TGF) β isoforms has been highly implicated in scar-free fetal healing vs scar formation in postnatal wounds.[14]

Another component of the fetal wound microenvironment that has been well studied is the ECM.[15] The fetal matrix contains higher levels of specific glycosaminoglycans (eg, hyaluronic acid and chondroitin sulfate) and a unique structural organization of proteoglycans and glycoproteins compared with adult wounds that may differentially regulate cell activity and wound remodeling. Furthermore, fetal wounds demonstrate altered collagen biosynthesis pathways, including higher ratios of collagen III to collagen I, a more reticular organization of deposited collagen, and less stiff mechanical properties.[1] Taken together, studies in fetal wound healing have provided important insight into potential cellular, biochemical, and mechanical pathways that might play critical roles in modulating postnatal wounds to heal with less scarring and become more "fetal-like."

SKIN STEM CELLS

Stem cells are capable of self-renewal and differentiation into specialized daughter cells (Figure 1). They have been identified in almost all adult tissues and play critical roles in maintaining homeostasis in health and disease states. In the epidermis, 3 distinct stem cell populations have been described based on their location in the interfollicular epithelium, hair follicle bulge, or sebaceous gland.[10] Recent studies have documented that 2 distinct proliferative cell types contribute to epithelial homeostasis but that slow-cycling stem cells (as opposed to committed progenitors) seem to predominate during wound repair.[16] Signaling pathways involving sonic hedgehog/shh, wingless-type/wnt, TGF, and bone morphogenic proteins have been implicated in epithelial stem cell activities, including cell stratification, hair folliculogenesis, and cutaneous repair.[17]

In dermal tissues, progenitor cells from the dermal papilla of hair follicles have been isolated and classified based on the type of hair produced and the relative expression of the transcription factor Sox2.[17] Another cell population recently identified from the dermal papilla is the skin-derived precursor cell.

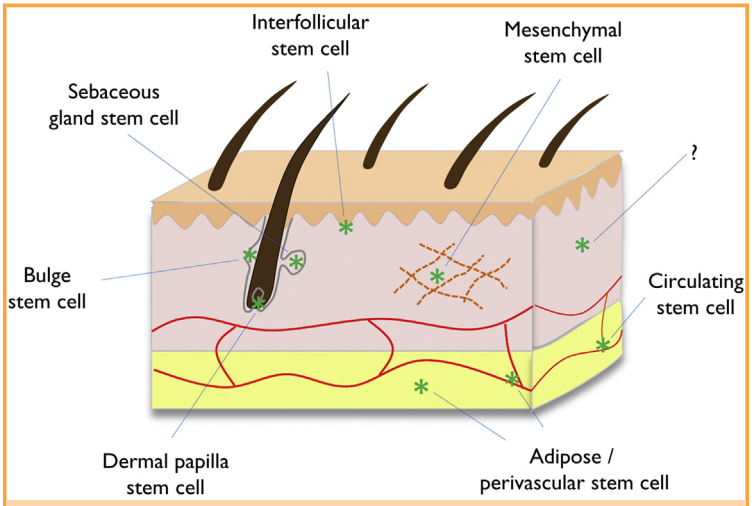

FIGURE 1. Putative skin stem cells. Multiple stem cell populations have been identified in different skin compartments and contribute to skin homeostasis and repair. Epithelial stem cells seem to derive from the interfollicular epithelium, sebaceous glands, and bulge area of hair follicles. Dermal stem cells may originate from the dermal papilla of hair follicles or from perivascular regions. Adipose tissue harbors multipotent cell populations that may originate from the perivascular space. The role of circulating stem cells remains controversial, and other as yet unidentified populations may facilitate skin repair and renewal throughout life. Asterisks indicate potential locations of various stem cell populations.

These cells can be differentiated into mesenchymal cell types in vitro and are thought to promote wound repair.[17] Perivascular sites in the dermis and hypodermis are also thought to harbor stem cells. For example, a perifollicular cell population has been identified in the human scalp and expresses markers for pericytes (NG2) and mesenchymal stem cells (CD34).[17] In addition, even fibroblasts are capable of differentiating into specialized cell types in vitro, but the relevance of this phenomenon in vivo remains unknown.[18]

Stem cells have also been identified in adipose tissue and may play an important role in skin repair. These adipose-derived stem cells (ADSCs) are known to secrete multiple paracrine factors that can potentially regulate fibroblast and keratinocyte activity.[19] Furthermore, ADSCs are intimately associated with blood vessels and may actually be pericytes or vascular stem cells at various stages of differentiation.[20] Their ability to differentiate into multiple tissue types in vitro and relative ease of harvest via liposuction have prompted much excitement for regenerative medicine.[21]

The fundamental mechanisms by which these myriad stem cells restore skin may provide insight into how regenerative processes occur in other organs, such as intestine, heart, and liver. Traditional paradigms for the regeneration of injured limbs assumed that the regrowth of complex tissues proceeded via dedifferentiation of mature cells into a blastema, a population of undifferentiated multipotent cells.[22] However, a digit tip amputation model was recently described that allowed researchers to investigate the regeneration of complex mammalian tissues.[23] Using bone marrow transplantation studies of labeled progenitor cells and parabiosis models (surgically combining the circulation between two mice), researchers discovered that progenitor cells responsible for digit regeneration are tissue specific and reside at the injury site. These findings suggest that intrinsic regenerative programs remain latent in adult mammals but can potentially be activated under specific (as yet unknown) conditions.

STEM CELL NICHE

Another important concept in stem cell biology is the niche, which describes the dynamic cellular and noncellular microenvironment of stem cells that regulates their "stemness."[24-27] This includes neighboring cells, soluble signaling molecules, ECM, mechanical forces, oxygen tension, and other factors that enable a stem cell to maintain its regenerative potential.[17] Multiple stem cell populations are known to exist throughout skin, and the unique milieu that enables each stem cell compartment to function is actively being studied.

The epidermal stem cell niche may be regulated by matrix components from the basement membrane and by interactions among transmembrane integrins, laminin, and cadherins.[28,29] Soluble cues from the dermis, such as from the family of bone morphogenic proteins, may also mediate keratinocyte function, indicating a role for epithelial-mesenchymal crosstalk in skin homeostasis.[30] Differentiated progeny of epithelial stem cells may even be capable of "recycling" back into the niche, further underscoring the complexity of epithelial regeneration.[31]

The dermal stem cell niche is less well defined, but soluble mediators, including insulinlike growth factor and fibroblast growth

Mayo Clin Proc. ■ September 2013;88(9):1022-1031 ■ http://dx.doi.org/10.1016/j.mayocp.2013.04.012
www.mayoclinicproceedings.org

factor, may be important in maintaining regenerative signaling networks.[32-34] A population of dermal perivascular cells has been identified and found to be intimately involved in remodeling via regulation of matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs.[35] The dermal fibroblast has long been assumed to be terminally differentiated, but recent in vitro studies suggest that some degree of differentiation potential is maintained.[36,37] Whether fibroblasts truly have regenerative potential and whether specific matrix cues can influence their multipotency in vivo remains to be seen.

The adipose stem cell niche is also poorly understood, in part because the adipose stem cell itself remains a controversial entity. Its relationship to a putative vascular stem cell has been hypothesized by several studies, suggesting that it is not necessarily the adipocyte that underlies the multipotency of ADSCs.[19,20] However, it is clear that adipose tissue is highly vascular and that numerous cytokines are elaborated by ADSCs, many of which are involved in wound repair.[38] Endothelial cell—ADSC interactions may also serve a key role during wound repair by regulating tissue neovascularization and hair follicle regeneration.[39,40] Collectively, these studies suggest that the niche concept may be relevant to regenerative pathways throughout the body.

ECM AND WOUND REMODELING

The ECM is composed of a noncellular scaffold of proteins, glycosaminoglycans, polysaccharides, and water that facilitates bidirectional communication between cells and their biochemical/biophysical microenviroment (Figure 2).[41] It provides physical support to the skin and actively regulates cell function by controlling biochemical gradients, cell density and spatial organization, and attachment ligands.[42] Dysfunction of the ECM has been linked to diseases such as fibrosis, cancer, and various genetic diseases.[43] In contrast to traditional concepts of the ECM as static scaffolding, it is now clear that the ECM is a dynamic regulator of cellular activity and an important blueprint for tissue repair.

After injury, biological programs are activated to restore skin integrity, including the formation of a provisional matrix that provides scaffolding for migrating and invading cells.[2] Although this matrix is continuously modified and remodeled for more than a year, the resultant wound (whether a "normal" fine scar or a pathologically thick hypertrophic scar) is dissimilar from unwounded skin in terms of appearance, structure, and strength. Initially, a collagen III—dominant environment (thinner, weaker fibrils) is produced by fibroblasts, but within weeks, a collagen I—dominant environment (thicker, stronger fibrils) predominates and is maintained.[1] However, this simplistic

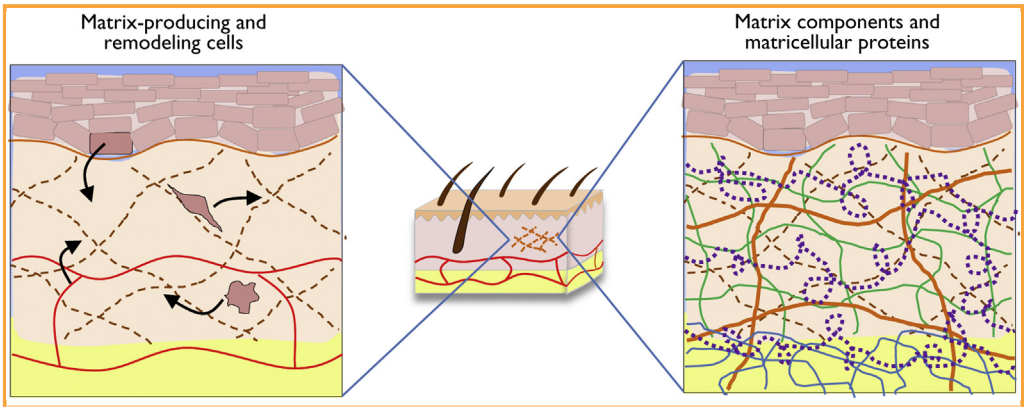

FIGURE 2. The extracellular matrix (ECM). The ECM is remodeled throughout life and must respond to constant physical and chemical insults. Fibroblasts, keratinocytes, macrophages, and endothelial cells are just some of the cell types that are known to regulate matrix architecture and function. In addition, numerous matrix components, including collagens, elastins, proteoglycans, and glycosaminoglycans, form a dynamic structural network that provides physical protection to skin cells and the human body. Matricellular proteins comprise a class of nonstructural matrix components that modulate cell behavior and are increasingly implicated in wound repair.

description of wound remodeling does not account for the multitude of other cell types and matrix components involved.

Macrophages were initially studied as phagocytes and immune cells, but modern molecular tools have elucidated additional functions in tissue development and remodeling.[44] Multiple subsets have been identified with overlapping roles in wound healing, immune regulation, and host defense.[45] Macrophages seem to work closely with fibroblasts during matrix remodeling, and conditional depletion experiments in mice have revealed discrete roles for macrophages (including cytokine production, matrix elaboration, and matrix breakdown) during different stages of skin repair.[46,47] Keratinocytes are also known to regulate fibroblast activity and can secrete growth factors and remodeling enzymes that affect dermal remodeling.[48] In addition, defects in these reciprocal interactions have been proposed to drive hypertrophic scar formation.[49] The role of endothelial cells in matrix remodeling is less appreciated, but recent studies suggest that sprouting neovessels actively remodel surrounding collagen fibrils and that endothelial-matrix interactions are likely important in tissue regeneration.[50,51]

Many structural elements contribute to the ECM architecture. For example, 28 types of collagen have been identified in vertebrates, and although collagens I and III are predominant in the dermis, other collagens may have roles in wound repair that remain undiscovered.[52] Additional fibrous proteins in the ECM include elastin and fibronectin, which are intimately associated with wound repair.[41,53] Another component of the ECM is proteoglycans (chondroitins, heparans, keratans, and hyaluronans), which are extremely hydrophilic and impart viscoelastic properties that modulate skin flexibility and strength. Moreover, newly discovered classes of proteoglycans that function as signal transduction molecules highlight the growing diversity of matrix components implicated in wound repair.[54]

A class of ECM proteins that function as cell modulators rather than as structural elements is the family of matricellular proteins.[55] These regulatory proteins have been implicated in processes such as tissue development, cancer metastasis, fibrosis, and matrix remodeling.[56,57] For example, proteins of the CCN (CYR61: cysteine-rich, angiogenic inducer 61/CTGF: connective tissue growth factor/NOV: nephroblastoma overexpressed) family directly bind cell surface integrin receptors and heparan sulfate proteoglycans to activate intracellular pathways linked to a broad range of developmental and repair programs.[58] The expression of tenascin proteins is highly restricted during embryogenesis, and re-expression occurs during adult wound healing. Specifically, tenascin-C has been linked to inflammation, reepithelialization, fibroblast activity, and ECM remodeling.[59]

Another group of matricellular proteins includes thrombospondins 1 and 2, extracellular glycoproteins that regulate cell-matrix interactions, collagen fibril formation, and angiogenesis, potentially via modulation of MMPs.[60,61] Several other matricellular proteins, including SPARC (secreted protein, acidic and rich in cysteine), periostin, and fibulin, have been implicated in tissue repair and represent promising therapeutic targets for regeneration.[62-64] Although many questions remain about the dynamic pathways maintaining matrix health, these novel regulatory matrix-associated proteins suggest possible mechanisms by which the ECM is regulated and likewise how the ECM modulates cell activity.

MECHANICAL FORCES

Human skin is the largest mechanoresponsive organ, and it contains diverse cell types with a range of mechanosensory functions.[9] Accordingly, wounds of various etiologies and in different anatomical locations have intrinsic mechanical properties that influence repair outcomes.[65,66] Researchers have begun to elucidate the cellular and subcellular mechanisms that enable physical forces to regulate biochemical pathways (in a process known as mechanotransduction), many of which are conserved across different organ systems.[67,68] An emerging concept of tensional homeostasis suggests that structural perturbations (such as injury) activate biological responses to restore the mechanical equilibrium of skin.[69]

The mechanical activation of integrin—focal adhesion complexes, stretch ion channels, cell surface receptors, and direct transmission of physical force can profoundly alter intracellular pathways. Mechanical forces are highly implicated in fibrosis, and fibroblast mechanotransduction has been extensively linked to tissue

Mayo Clin Proc. ■ September 2013;88(9):1022-1031 ■ http://dx.doi.org/10.1016/j.mayocp.2013.04.012
www.mayoclinicproceedings.org

inflammation and remodeling.[70] For example, molecular manipulation of the fibroblast mechanosensor focal adhesion kinase effectively blocked fibrosis in a mouse model.[71] Keratinocyte mechanotransduction signaling has also been linked to proliferation, remodeling, and epithelial morphogenesis.[72,73] Even stem cell fate can be regulated through physical cues, suggesting that mechanomodulatory therapies targeting progenitor populations may have a role in wound repair.[9,74]

Therapeutic approaches to wound healing that specifically focus on mechanical forces have become increasingly widespread. The success of negative pressure wound therapy for acute and chronic wounds demonstrates the ability of micromechanical forces to augment tissue repair in a clinical setting.[75] Treatments for scar reduction after injury, including compression garments, silicone sheeting, and paper tape, are thought to act in part through mechanotransduction.[9] In human studies, an elastomeric dressing to off-load profibrotic forces in human wounds significantly reduced hypertrophic scar formation for up to a year.[76] Taken together, these results demonstrate the importance of the physical environment in tissue repair and indicate that mechanotransduction pathways are viable targets to promote wound regeneration.

CELL PLASTICITY

Recent studies have highlighted the plasticity of adult skin cell populations (Figure 3). For example, researchers have reported that specialized adult cells can be induced to transdifferentiate into cells from another lineage, suggesting that cell fate is highly convertible.[77] In addition, induced pluripotent stem cell technology has revealed that mature skin cells, including fibroblasts and keratinocytes, can be reprogrammed into myriad cell types by activating/introducing a specific set of transcription factors.[78,79] These advances provide researchers with a powerful new tool to potentially regenerate complex tissues using available autologous adult cells.

Another process that reveals the plasticity of human tissues is transdifferentiation, which may occur naturally during repair processes throughout the body. Epithelial-mesenchymal transition describes the process by which polarized epithelial cells assume a mesenchymal cell phenotype characterized by invasiveness, resistance to apoptosis, and matrix production.[80] It seems to occur during 3 major processes (embryogenesis, tissue repair, and cancer progression) that affect many organ types. In the context of wound healing, epithelial-mesenchymal transition may regulate remodeling and activate contractile myofibroblast populations via TGF-β signaling.[81,82]

A related process involving endothelial cells and mesenchymal-like transdifferentiation is called endothelial-mesenchymal transition. Endothelial-mesenchymal transition pathways are activated during cardiac development and postischemic tissue repair and may also have a role in cutaneous wound healing.[80,83] Another putative cell population recently implicated in skin repair is the fibrocyte, a circulating hematopoietic cell thought to migrate to wounds and to function as a fibroblast precursor and/or regulator.[84,85] These studies highlight the plasticity of cells involved in wound healing and suggest novel strategies to exploit for regenerative applications.

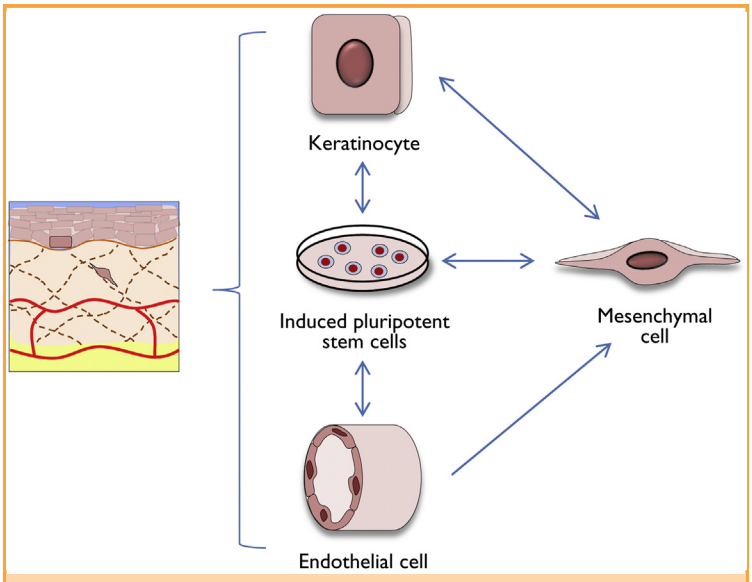

FIGURE 3. Skin cell plasticity. Skin cells demonstrate remarkable plasticity, and numerous in vitro studies have shown that epithelial and endothelial cells are capable of undergoing transition or transdifferentiation into mesenchymal-like cells. Mesenchymal cells have also been reported to differentiate into epithelial-like cells during development. Recent advances in induced pluripotent stem cell technology have documented that differentiated adult skin cells can be reprogrammed to an embryonic stem cell–like state. Given the ease of access and the ability to safely remove small amounts of skin, this technology offers exciting potential for regenerative medicine.

SKIN ENGINEERING

The bioengineering of skin presents several challenges that may be relevant to the fabrication of solid organs and other complex tissues (Figure 4). A common multilayered design consists of a highly cellular keratinocyte layer overlying a fibroblast-incorporated dermal matrix to mimic the epidermis and dermis, respectively.[86,87] The dermal matrix can be derived from natural sources (eg, decellularized human or pig skin), created from natural proteins (eg, collagens, fibronectin, or chitosan), or engineered from synthetic molecules (eg, glycolic acid or polycaprolactone).[88,89] The use of decellularized scaffolds has also been extended to heart, liver, and lung engineering, providing a 3-dimensional prepatterned scaffold onto which delivered cells can organize and mature.[90]

Stem cells have also proved highly promising for regenerating skin based on tissue-engineering strategies.[91] For example, the clinical use of cultured epithelial autografts (sheets of patient-derived keratinocytes fabricated ex vivo) for massive burn injuries is based on the ability of progenitor cells to expand keratinocyte populations.[92] Skin grafts are the gold standard for severe burn injuries, and their restorative abilities may also rely on stem cell–mediated processes.[93] Moreover, hair follicle stem cells have been shown to regulate wound repair and may play a critical role in regenerating functional skin.[6,94] The concept of integrating different populations of stem cells to create complex tissue structures may prove more applicable than using individual stem cell populations in isolation.

Engineered skin constructs have also benefitted from advances in nanotechnology and biomechanics.[87] Nanofabrication techniques allow researchers to design complex scaffolds that mimic microenvironment domains that facilitate skin regeneration.[95] Topographical modifications to biomaterial surfaces can regulate cell behavior and potentially guide stem cell differentiation.[96] The mechanical properties of engineered matrices also play an important role in how incorporated cells behave. These factors are especially critical for the engineering of skin, a flexible, pliable, and resilient structure that has viscoelastic properties similar to biomaterial hydrogels.[97] These mechanical properties have been characterized on a microscopic scale and may influence future designs of engineered skin grafts.[98]

Cytokines play an instrumental role in facilitating cellular communication within and across different skin compartments. These signaling pathways are centrally involved in skin development, homeostasis, and disease, but the ability of bioengineers to recapitulate these biochemical networks remains limited.[99] Progress has been made in developing biomaterial substrates that contain various growth factors and that can be activated to release stored contents under controlled conditions, hence the term *controlled-release* or *control-release* systems. These delivery systems can be regulated by factors such as temperature, pH, time, and solubility, providing an important means of recreating the complex biochemical milieu during tissue regeneration.[89] Advances in microfluidics technology (interconnected microchannel networks capable of precise delivery of biomolecules) may enable researchers to reproduce morphogenic gradients in temporospatial scales never before achieved.[100] Ultimately, successful skin engineering will rely on characterizing and recapitulating the optimal cellular, matrix, biochemical, and biophysical cues that drive tissue regeneration.

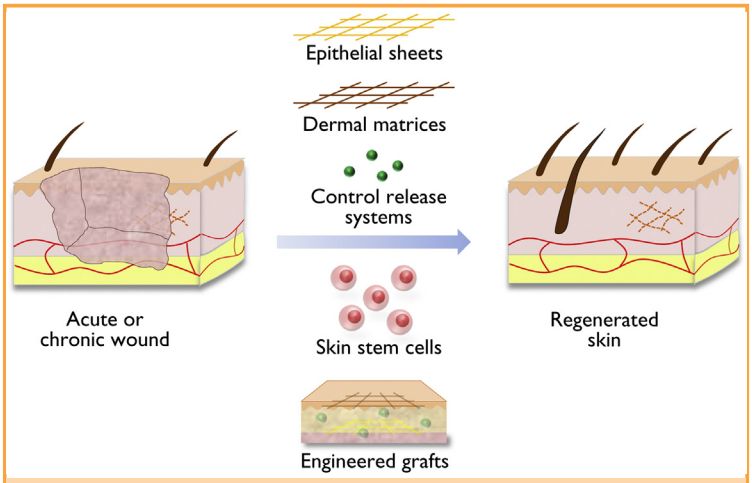

FIGURE 4. Skin engineering. Skin engineering has traditionally relied on multilayered construction of epithelial sheets overlying a dermal-type matrix. Advances in control-release systems, nanotopography, biomechanics, materials science, and stem cell biology will enable researchers to design increasingly sophisticated engineered skin grafts with the potential to treat acute or chronic wounds. A multidisciplinary approach will be needed to integrate these novel technologies and implement effective skin regeneration strategies.

Mayo Clin Proc. ■ September 2013;88(9):1022-1031 ■ http://dx.doi.org/10.1016/j.mayocp.2013.04.012
www.mayoclinicproceedings.org

CONCLUSION

As researchers continue to unlock the mysteries of skin regeneration after injury, novel pathways may be elucidated that drive tissue regeneration across the body. Human skin is known to contain multiple progenitor and differentiated cell types that remain active throughout life and demonstrate tremendous plasticity in response to injury. Moreover, extracellular biophysical and biochemical cues have been characterized that modulate distinct intracellular pathways during tissue restoration. Ultimately, a dynamic homeostasis that exists between cells and their ECM must be reestablished after cutaneous injury. The ability to recapitulate these biological programs may define the success of novel technologies that are establishing new frontiers in bioengineering. In summary, evolving concepts in cutaneous wound healing may shed light on fundamental regenerative processes in other organ systems and enable researchers to develop innovative therapies that revolutionize wound repair.

Abbreviations and Acronyms: **ADSC** = adipose-derived stem cell; **ECM** = extracellular matrix; **MMP** = matrix metalloproteinase; **TGF** = transforming growth factor

Correspondence: Address to Michael T. Longaker, MD, MBA, Hagey Laboratory for Pediatric Regenerative Medicine, 257 Campus Dr, GK 106, Stanford, CA 94305 (longaker@stanford.edu). Individual reprints of this article and a bound reprint of the entire Symposium on Regenerative Medicine will be available for purchase from our website www.mayoclinicproceedings.org.

The Symposium on Regenerative Medicine will continue in an upcoming issue.

REFERENCES

1. Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. *Nature*. 2008;453(7193):314-321.
2. Singer AJ, Clark RA. Cutaneous wound healing. *N Engl J Med*. 1999;341(10):738-746.
3. Yang L, Peng R. Unveiling hair follicle stem cells. *Stem Cell Rev*. 2010;6(4):658-664.
4. Blanpain C, Fuchs E. Epidermal stem cells of the skin. *Annu Rev Cell Dev Biol*. 2006;22:339-373.
5. Cha J, Falanga V. Stem cells in cutaneous wound healing. *Clin Dermatol*. 2007;25(1):73-78.
6. Ito M, Liu Y, Yang Z, et al. Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis. *Nat Med*. 2005;11(12):1351-1354.
7. Morasso MI, Tomic-Canic M. Epidermal stem cells: the cradle of epidermal determination, differentiation and wound healing. *Biol Cell*. 2005;97(3):173-183.
8. Schultz GS, Davidson JM, Kirsner RS, Bornstein P, Herman IM. Dynamic reciprocity in the wound microenvironment. *Wound Repair Regen*. 2011;19(2):134-148.
9. Wong VW, Akaishi S, Longaker MT, Gurtner GC. Pushing back: wound mechanotransduction in repair and regeneration. *J Invest Dermatol*. 2011;131(11):2186-2196.
10. Fuchs E. Skin stem cells: rising to the surface. *J Cell Biol*. 2008;180(2):273-284.
11. Fuchs E, Nowak JA. Building epithelial tissues from skin stem cells. *Cold Spring Harb Symp Quant Biol*. 2008;73:333-350.
12. Larson BJ, Longaker MT, Lorenz HP. Scarless fetal wound healing: a basic science review. *Plast Reconstr Surg*. 2010;126(4):1172-1180.
13. Bullard KM, Longaker MT, Lorenz HP. Fetal wound healing: current biology. *World J Surg*. 2003;27(1):54-61.
14. Colwell AS, Longaker MT, Lorenz HP. Fetal wound healing. *Front Biosci*. 2003;8:s1240-s1248.
15. Wilgus TA. Regenerative healing in fetal skin: a review of the literature. *Ostomy Wound Manage*. 2007;53(6):16-31.
16. Mascre G, Dekoninck S, Drogat B, et al. Distinct contribution of stem and progenitor cells to epidermal maintenance. *Nature*. 2012;489(7415):257-262.
17. Wong VW, Levi B, Rajadas J, Longaker MT, Gurtner GC. Stem cell niches for skin regeneration. *Int J Biomater*. 2012;2012:926059.
18. Glotzbach JP, Wong VW, Gurtner GC, Longaker MT. Regenerative medicine. *Curr Probl Surg*. 2011;48(3):148-212.
19. Lin CS, Xin ZC, Deng CH, Ning H, Lin G, Lue TF. Defining adipose tissue-derived stem cells in tissue and in culture. *Histol Histopathol*. 2010;25(6):807-815.
20. Lin G, Garcia M, Ning H, et al. Defining stem and progenitor cells within adipose tissue. *Stem Cells Dev*. 2008;17(6):1053-1063.
21. Cherubino M, Rubin JP, Miljkovic N, Kelmendi-Doko A, Marra KG. Adipose-derived stem cells for wound healing applications. *Ann Plast Surg*. 2011;66(2):210-215.
22. Hyun JS, Chung MT, Wong VW, Montoro D, Longaker MT, Wan DC. Rethinking the blastema. *Plast Reconstr Surg*. 2012;129(5):1097-1103.
23. Rinkevich Y, Lindau P, Ueno H, Longaker MT, Weissman IL. Germ-layer and lineage-restricted stem/progenitors regenerate the mouse digit tip. *Nature*. 2011;476(7361):409-413.
24. Fuchs E. Finding one's niche in the skin. *Cell Stem Cell*. 2009;4(6):499-502.
25. Li L, Xie T. Stem cell niche: structure and function. *Ann Rev Cell Dev Biol*. 2005;21(1):605-631.
26. Scadden DT. The stem-cell niche as an entity of action. *Nature*. 2006;441(7097):1075-1079.
27. Voog J, Jones DL. Stem cells and the niche: a dynamic duo. *Cell Stem Cell*. 2010;6(2):103-115.
28. Jamora C, DasGupta R, Kocieniewski P, Fuchs E. Links between signal transduction, transcription and adhesion in epithelial bud development. *Nature*. 2003;422(6929):317-322.
29. Marthiens V, Kazanis I, Moss L, Long K, Ffrench-Constant C. Adhesion molecules in the stem cell niche—more than just staying in shape? *J Cell Sci*. 2010;123(pt 10):1613-1622.
30. Plikus MV, Mayer JA, de la Cruz D, et al. Cyclic dermal BMP signalling regulates stem cell activation during hair regeneration. *Nature*. 2008;451(7176):340-344.
31. Hsu YC, Pasolli HA, Fuchs E. Dynamics between stem cells, niche, and progeny in the hair follicle. *Cell*. 2011;144(1):92-105.
32. Kellner JC, Coulombe PA. SKPing a hurdle: Sox2 and adult dermal stem cells. *Cell Stem Cell*. 2009;5(6):569-570.
33. Biernaskie J, Paris M, Morozova O, et al. SKPs derive from hair follicle precursors and exhibit properties of adult dermal stem cells. *Cell Stem Cell*. 2009;5(6):610-623.
34. Driskell RR, Clavel C, Rendl M, Watt FM. Hair follicle dermal papilla cells at a glance. *J Cell Sci*. 2011;124(pt 8):1179-1182.
35. Lozito TP, Tuan RS. Mesenchymal stem cells inhibit both endogenous and exogenous MMPs via secreted TIMPs. *J Cell Physiol*. 2011;226(2):385-396.
36. Huang HI, Chen SK, Ling QD, Chien CC, Liu HT, Chan SH. Multilineage differentiation potential of fibroblast-like stromal cells derived from human skin. *Tissue Eng Part A*. 2010;16(5):1491-1501.

37. Lorenz K, Sicker M, Schmelzer E, et al. Multilineage differentiation potential of human dermal skin-derived fibroblasts. *Exp Dermatol*. 2008;17(11):925-932.
38. Jeong JH. Adipose stem cells and skin repair. *Curr Stem Cell Res Ther*. 2010;5(2):137-140.
39. Festa E, Fretz J, Berry R, et al. Adipocyte lineage cells contribute to the skin stem cell niche to drive hair cycling. *Cell*. 2011;146(5):761-771.
40. Traktuev DO, Prater DN, Merfeld-Clauss S, et al. Robust functional vascular network formation in vivo by cooperation of adipose progenitor and endothelial cells. *Circ Res*. 2009;104(12):1410-1420.
41. Frantz C, Stewart KM, Weaver VM. The extracellular matrix at a glance. *J Cell Sci*. 2010;123(pt 24):4195-4200.
42. Hynes RO. The extracellular matrix: not just pretty fibrils. *Science*. 2009;326(5957):1216-1219.
43. Bateman JF, Boot-Handford RP, Lamande SR. Genetic diseases of connective tissues: cellular and extracellular effects of ECM mutations. *Nat Rev Genet*. 2009;10(3):173-183.
44. Stefater JA III, Ren S, Lang RA, Duffield JS. Metchnikoff's policemen: macrophages in development, homeostasis and regeneration. *Trends Mol Med*. 2011;17(12):743-752.
45. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol*. 2008;8(12):958-969.
46. Glaros T, Larsen M, Li L. Macrophages and fibroblasts during inflammation, tissue damage and organ injury. *Front Biosci*. 2009;14:3988-3993.
47. Lucas T, Waisman A, Ranjan R, et al. Differential roles of macrophages in diverse phases of skin repair. *J Immunol*. 2010;184(7):3964-3977.
48. Werner S, Krieg T, Smola H. Keratinocyte-fibroblast interactions in wound healing. *J Invest Dermatol*. 2007;127(5):998-1008.
49. Ghahary A, Ghaffari A. Role of keratinocyte-fibroblast crosstalk in development of hypertrophic scar. *Wound Repair Regen*. 2007;15(suppl, 1):S46-S53.
50. Kirkpatrick ND, Andreou S, Hoying JB, Utzinger U. Live imaging of collagen remodeling during angiogenesis. *Am J Physiol Heart Circ Physiol*. 2007;292(6):H3198-H3206.
51. Vorotnikova E, McIntosh D, Dewilde A, et al. Extracellular matrix-derived products modulate endothelial and progenitor cell migration and proliferation in vitro and stimulate regenerative healing in vivo. *Matrix Biol*. 2010;29(8):690-700.
52. Gordon MK, Hahn RA. Collagens. *Cell Tissue Res*. 2010;339(1):247-257.
53. Rnjak J, Wise SG, Mithieux SM, Weiss AS. Severe burn injuries and the role of elastin in the design of dermal substitutes. *Tissue Eng Part B Rev*. 2011;17(2):81-91.
54. Schaefer L, Schaefer RM. Proteoglycans: from structural compounds to signaling molecules. *Cell Tissue Res*. 2010;339(1):237-246.
55. Bornstein P, Sage EH. Matricellular proteins: extracellular modulators of cell function. *Curr Opin Cell Biol*. 2002;14(5):608-616.
56. Chong HC, Tan CK, Huang RL, Tan NS. Matricellular proteins: a sticky affair with cancers. *J Oncol*. 2012;2012:351089.
57. Matsui Y, Morimoto J, Uede T. Role of matricellular proteins in cardiac tissue remodeling after myocardial infarction. *World J Biol Chem*. 2010;1(5):69-80.
58. Chen CC, Lau LF. Functions and mechanisms of action of CCN matricellular proteins. *Int J Biochem Cell Biol*. 2009;41(4):771-783.
59. Midwood KS, Orend G. The role of tenascin-C in tissue injury and tumorigenesis. *J Cell Commun Signal*. 2009;3(3-4):287-310.
60. Bornstein P, Agah A, Kyriakides TR. The role of thrombospondins 1 and 2 in the regulation of cell-matrix interactions, collagen fibril formation, and the response to injury. *Int J Biochem Cell Biol*. 2004;36(6):1115-1125.
61. Maclauchlan S, Skokos EA, Agah A, et al. Enhanced angiogenesis and reduced contraction in thrombospondin-2-null wounds is associated with increased levels of matrix metalloproteinases-2 and -9, and soluble VEGF. *J Histochem Cytochem*. 2009;57(4):301-313.
62. Jun JI, Lau LF. Taking aim at the extracellular matrix: CCN proteins as emerging therapeutic targets. *Nat Rev Drug Discov*. 2011;10(12):945-963.
63. Zhou HM, Wang J, Elliott C, Wen W, Hamilton DW, Conway SJ. Spatiotemporal expression of periostin during skin development and incisional wound healing: lessons for human fibrotic scar formation. *J Cell Commun Signal*. 2010;4(2):99-107.
64. Timpl R, Sasaki T, Kostka G, Chu ML. Fibulins: a versatile family of extracellular matrix proteins. *Nat Rev Mol Cell Biol*. 2003;4(6):479-489.
65. Wong VW, Longaker MT, Gurtner GC. Soft tissue mechanotransduction in wound healing and fibrosis. *Semin Cell Dev Biol*. 2012;23(9):981-986.
66. Agha R, Ogawa R, Pietramaggiori G, Orgill DP. A review of the role of mechanical forces in cutaneous wound healing. *J Surg Res*. 2011;171(2):700-708.
67. DuFort CC, Paszek MJ, Weaver VM. Balancing forces: architectural control of mechanotransduction. *Nat Rev Mol Cell Biol*. 2011;12(5):308-319.
68. Wang N, Tytell JD, Ingber DE. Mechanotransduction at a distance: mechanically coupling the extracellular matrix with the nucleus. *Nat Rev Mol Cell Biol*. 2009;10(1):75-82.
69. Cox TR, Erler JT. Remodeling and homeostasis of the extracellular matrix: implications for fibrotic diseases and cancer. *Dis Model Mech*. 2011;4(2):165-178.
70. Chiquet M, Gelman L, Lutz R, Maier S. From mechanotransduction to extracellular matrix gene expression in fibroblasts. *Biochim Biophys Acta*. 2009;1793(5):911-920.
71. Wong VW, Rustad KC, Akaishi S, et al. Focal adhesion kinase links mechanical force to skin fibrosis via inflammatory signaling. *Nat Med*. 2012;18(1):148-152.
72. Reichelt J. Mechanotransduction of keratinocytes in culture and in the epidermis. *Eur J Cell Biol*. 2007;86(11-12):807-816.
73. Zhang H, Landmann F, Zahreddine H, Rodriguez D, Koch M, Labouesse M. A tension-induced mechanotransduction pathway promotes epithelial morphogenesis. *Nature*. 2011;471(7336):99-103.
74. Wang JH, Thampatty BP. Mechanobiology of adult and stem cells. *Int Rev Cell Mol Biol*. 2008;271:301-346.
75. Orgill DP, Manders EK, Sumpio BE, et al. The mechanisms of action of vacuum assisted closure: more to learn. *Surgery*. 2009;146(1):40-51.
76. Gurtner GC, Dauskardt RH, Wong VW, et al. Improving cutaneous scar by controlling the mechanical environment: large animal and phase I studies. *Ann Surg*. 2011;254(2):217-225.
77. Vierbuchen T, Wernig M. Direct lineage conversions: unnatural but useful? *Nat Biotechnol*. 2011;29(10):892-907.
78. Stadtfeld M, Maherali N, Breault DT, Hochedlinger K. Defining molecular cornerstones during fibroblast to iPS cell reprogramming in mouse. *Cell Stem Cell*. 2008;2(3):230-240.
79. Aasen T, Raya A, Barrero MJ, et al. Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. *Nat Biotechnol*. 2008;26(11):1276-1284.
80. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest*. 2009;119(6):1420-1428.
81. Nistico P, Bissell MJ, Radisky DC. Epithelial-mesenchymal transition: general principles and pathological relevance with special emphasis on the role of matrix metalloproteinases. *Cold Spring Harb Perspect Biol*. 2012;4(2). http://dx.doi.org/10.1101/cshperspect.a011908.
82. Jarvinen PM, Laiho M. LIM-domain proteins in transforming growth factor beta-induced epithelial-to-mesenchymal transition and myofibroblast differentiation. *Cell Signal*. 2012;24(4):819-825.
83. Lin F, Wang N, Zhang TC. The role of endothelial-mesenchymal transition in development and pathological process. *IUBMB Life*. 2012;64(9):717-723.

84. Kao HK, Chen B, Murphy GF, Li Q, Orgill DP, Guo L. Peripheral blood fibrocytes: enhancement of wound healing by cell proliferation, re-epithelialization, contraction, and angiogenesis. *Ann Surg*. 2011;254(6):1066-1074.
85. Wang JF, Jiao H, Stewart TL, Shankowsky HA, Scott PG, Tredget EE. Fibrocytes from burn patients regulate the activities of fibroblasts. *Wound Repair Regen*. 2007;15(1):113-121.
86. Wong VW, Rustad KC, Longaker MT, Gurtner GC. Tissue engineering in plastic surgery: a review. *Plast Reconstr Surg*. 2010; 126(3):858-868.
87. Metcalfe AD, Ferguson MW. Bioengineering skin using mechanisms of regeneration and repair. *Biomaterials*. 2007;28(34): 5100-5113.
88. Zhong SP, Zhang YZ, Lim CT. Tissue scaffolds for skin wound healing and dermal reconstruction. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*. 2010;2(5):510-525.
89. Wong VW, Gurtner GC. Tissue engineering for the management of chronic wounds: current concepts and future perspectives. *Exp Dermatol*. 2012;21(10):729-734.
90. Badylak SF, Taylor D, Uygun K. Whole-organ tissue engineering: decellularization and recellularization of three-dimensional matrix scaffolds. *Annu Rev Biomed Eng*. 2011;13:27-53.
91. Cerqueira MT, Marques AP, Reis RL. Using stem cells in skin regeneration: possibilities and reality. *Stem Cells Dev*. 2012; 21(8):1201-1214.
92. Atiyeh BS, Costagliola M. Cultured epithelial autograft (CEA) in burn treatment: three decades later. *Burns*. 2007;33(4): 405-413.
93. Zakine G, Mimoun M, Pham J, Chaouat M. Reepithelialization from stem cells of hair follicles of dermal graft of the scalp in acute treatment of third-degree burns: first clinical and histologic study. *Plast Reconstr Surg*. 2012;130(1):42e-50e.
94. Asakawa K, Toyoshima KE, Ishibashi N, et al. Hair organ regeneration via the bioengineered hair follicular unit transplantation. *Sci Rep*. 2012;2:424.
95. Mohamed A, Xing MM. Nanomaterials and nanotechnology for skin tissue engineering. *Int J Burns Trauma*. 2012;2(1):29-41.
96. Kim DH, Provenzano PP, Smith CL, Levchenko A. Matrix nanotopography as a regulator of cell function. *J Cell Biol*. 2012;197(3):351-360.
97. Ghosh K, Pan Z, Guan E, et al. Cell adaptation to a physiologically relevant ECM mimic with different viscoelastic properties. *Biomaterials*. 2007;28(4):671-679.
98. Crichton ML, Chen X, Huang H, Kendall MA. Elastic modulus and viscoelastic properties of full thickness skin characterised at micro scales. *Biomaterials*. 2013;34(8):2087-2097.
99. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. *Physiol Rev*. 2003;83(3):835-870.
100. Huang GY, Zhou LH, Zhang QC, et al. Microfluidic hydrogels for tissue engineering. *Biofabrication*. 2011;3(1):012001.