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Stem Cells for Skin Tissue Engineering and Wound Healing

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Abstract

The tremendous ability of the skin epidermis to regenerate is due to the presence of epidermal stem cells that continuously produce keratinocytes which undergo terminal differentiation to a keratinized layer that provides the skin's barrier properties. The ability to control this process in vitro has made it possible to develop various types of tissue engineered skin grafts, some of which being among the first tissue engineered products to ever reach the marketplace. In the past 30 years these products have been applied with some success to the treatment of chronic skin wounds, such as diabetic and venous ulcers, as well as deep acute wounds, such as burns. Current technologies remain partially effective in their ability to restore other skin structures, for example the dermis, which is critical to the overall long-term appearance and function of the skin. Furthermore, to this day none of these approaches regenerate skin appendages (e.g. hair follicles, sweat glands). The use of earlier progenitor and stem cells, including embryonic stem cells is gaining interest to overcome such limitations. Furthermore, recent evidence suggests that "adult" stem cells, which are present in the circulation of the patient, home into areas of injury and likely participate in the wound healing process. In this paper, we start with an overview of the wound healing process and current methods used for wound treatment, both conventional and tissue engineered-based. We then review current research on the various types of stem cells used for skin tissue engineering and wound healing, and provide future directions.

Keywords

stem cells; wound healing; skin tissue engineering; skin appendages

I. INTRODUCTION TO WOUND HEALING

I.A. Cost and Prevalence of Wounds

A wound is defined as a disruption of normal anatomic structure and function.[1] Skin wound treatment is a very diverse part of the health care system, encompassing surgical and accidental lacerations, burns, pressure ulcers, diabetic and venous ulcers. The treatment of wounds and associated complications exceeds $20 billion annually in the US.[2] Chronic and nonhealing wounds are especially costly because they require repetitive treatments; for example, a diabetic foot ulcer typically costs $50,000 to treat.[3] Chronic wounds affect 1% of the population at any given time.[4]

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I.B. Anatomy of Skin

The largest organ in the body, skin consists of three layers: epidermis, dermis, and hypodermis. The epidermis is in fact a multi-layered epithelium extending from the basement membrane that separates it from the dermis to the air. Except for the basement membrane, it is virtually devoid of extracellular matrix (ECM). Progenitor cells are located on the basement membrane and undergo continuous self-renewal and differentiation to keratinocytes. The keratinocytes migrate towards the surface of the skin where they eventually get sloughed off. While this process occurs, keratinocytes undergo terminal differentiation and maturation.[5] As they approach the skin surface, they form a keratinized layer of dead cells which confers the main barrier properties of the skin.[6]

Below the epidermis is the dermis, the thickest of the three layers of skin which accounts for most of the skin's mechanical properties and resilience. The dermis is a connective tissue comprised of ECM, fibroblasts, vascular endothelial cells, and skin appendages (hair follicles, sweat glands, etc.).[6] Fibroblasts secrete collagen and elastin, providing mechanical strength and elasticity to the skin, respectively.

The hypodermis underneath the dermis is composed of adipose tissue, which functions as insulation and cushioning between the skin and other skeletal structures, like bone and muscle.[6]

I.C. Acute and Chronic Wounds

Cutaneous wound healing requires a well-orchestrated integration of the complex biological and molecular events of cell migration and proliferation, as well as ECM deposition, angiogenesis, and remodeling.[7] Acute skin wounds result from some form of trauma, and undergo a repair process that, when it is orderly and timely, lead to a benign scar.[1] Failure of this process, because the wound area and/or depth exceed the patient's ability to heal, may lead to an undesirable scar or a chronic or nonhealing wound. The ability to heal seems to diminish with age, for various reasons, including age-related decreased strength and elasticity of skin,[6] decreased blood flow to the extremities due to sedentary lifestyle, smoking, etc. Several studies, both in humans and in experimental animals, also suggest that psychological stress negatively impacts on wound healing.[8, 9] In many instances, patients with chronic wounds (most notably diabetic foot ulcers) have other underlying conditions, such as diabetes, that impair wound healing.

II. PHYSIOLOGY OF SKIN WOUND HEALING

The skin wound healing process can be divided into 3 phases (shown in Figure 1):

II.A. Inflammatory Phase

The earliest phase of wound healing starts with blood coagulation leading to the formation of a blood clot covering the wound, which acts as a temporary shield against pathogens as well as fluid loss. Although blood flow within the wound is often impaired due to destruction of the blood vessels, it is elevated in the areas immediately adjacent to the wound, and local inflammatory agents (activated complement, histamine, etc.) increase vascular permeability leading to plasma extravasation and the generation of a fibrin matrix, also causing swelling and redness.[6] This matrix is rapidly invaded by neutrophils, followed by monocytes and other immunocompetent cells to remove dead tissue and control infection.[5] This inflammatory phase typically lasts for the first 4 days.[10]

II.B. Proliferation Phase

Inflammatory cells secrete a host of factors which promote the recruitment and proliferation of vascular endothelial cells and fibroblasts. Fibroblasts begin to secrete collagen, gradually replacing the fibrin matrix. As more collagen is deposited and undergoes cross-linking, the mechanical strength of the wound increases. Fibroblasts may also differentiate into myofibroblasts that express α-smooth muscle actin, which causes the wound to contract, thus reducing the wound area that needs to be closed by cell proliferation. Vascular endothelial cells and capillaries invade through a process of angiogenesis extending from nearby healthy tissue, as well as from the recruitment of endothelial progenitors, which are present in low levels in the circulation. This "granulation tissue" is observed between days 5–20.[6] Keratinocytes also start to migrate from the wound edges and proliferate on the surface of the granulation tissue, below the blood clot.[10] The base of hair follicles (not shown on figure), which is located fairly deep into the dermis, is also an important source of keratinocytes for large area wounds. If these structures are destroyed (as is the case in deep second degree and third degree burns), reepithelialization is very slow and medical interventions, such as skin grafting (described further below) become necessary.

II.C. Maturation Phase

In this last phase, the wound has reepithelialized and the dermis has regained most of its tensile strength, although it is no longer as elastic as normal skin, and may be susceptible to re-opening. The scar will continue to undergo further remodeling over a time scale of months to years.[6]

III. CURRENT TREATMENT STRATEGIES FOR WOUND HEALING

III.A. Traditional Strategies

1. Skin Grafting with Autografts—Wounds that extend deep into the dermis tend to heal very poorly and slowly because no keratinocytes remain to reform the epithelium. For such wounds, skin grafting with an "autograft" is the treatment of choice; since the patient donates its own tissue, there is no risk of rejection.[5] The technique is extremely well established and evolved from use in the back alleys of India in pre-Christian times.[11] A dermatome, which is a surgical instrument that holds a razor-sharp blade parallel to the skin surface, is used to "shave" a thin layer of skin from the donor site (most commonly a conspicuous area such as inner thighs and buttocks) that includes the full epidermis and portion of the dermis, or what is commonly known a split-thickness graft.[12] The skin graft is then placed on the wound site. If large areas need to be covered, such as in cases of extensive burns, the graft is meshed to enable stretching it over the larger area (typically using an expansion ratio of 1:3). The appearance of the healed wound is best if the graft is thicker (thus including more dermis) and unmeshed, and those factors are taken into consideration depending on the site of grafting. Conversely, healing of the donor site will be more compromised if a thicker graft is harvested. In general, the thicker the underlying dermis, the better the graft take, the faster the healing, and the better the long-term appearance of the healed wound. Donor sites will heal and can be reharvested, albeit a limited number of times because the dermis does not regenerate and becomes thinner each time.

2. Skin Allografts and Xenografts—Skin grafting with an autograft may not be immediately possible because of limited availability of donor tissue. In this instance, wounds may be covered with allografts, which will serve as temporary covering since they typically get rejected by the host's immune system after a week.[5] Allografts are harvested from consenting donors after death and stored frozen in skin banks where they can be used whenever needed.[13] Allografts provide a barrier function and it is thought that growth

factors released from these grafts have a positive effect on wound healing until an autograft can be placed onto the wound. Xenografts made of pig skin have also been used for the same purpose.[14]

III.B. Tissue Engineered Skin Substitutes

Skin was the first tissue application to be successfully engineered in the laboratory, first with the development of biodegradable matrix materials that can emulate the dermis, and subsequently the development of keratinocyte culture techniques leading to live cultured skin products.

1. Matrix-based products—The first engineered skin substitutes, which are still in use today, consist of porous matrices which function as templates for dermal regeneration. The matrices are placed on the wound bed and allowed to integrate and vascularize. After sufficient revascularization of the matrix, these products must be covered with autografts.[15] Integra® is the first commercially available engineered skin substitute and consists of a matrix of cross-linked collagen and chondroitin-6-sulfate copolymer are mixed together to form the dermal matrix. A silicon sheet is attached to one side that functions as a temporary epidermal layer.[13] Integra® is primarily used for the treatment of deep burn wounds, which are prone to forming undesirable scars. The matrix undergoes degradation while the host's cells invade and proliferate within it, thus promoting dermal regeneration while inhibiting wound contraction, leading to a better function and appearance of the healed wound.[16] Another skin substitute, Alloderm®, is made from decellularized donor skin. Removing all the cells and keeping only the protein component, prevents an allogeneic immunological response and also reduces the risk of disease transmission. [13, 17] It is used both for wound repair and reconstructive surgery. As is the case with Integra, an autograft must be eventually applied to reepithelialize the wound.

2. Cell-based products—Cultured skin began when methods for harvesting keratinocytes from patients and proliferating the cells in vitro became available. This pioneering work led to Epicel™, a cultured autologous epidermis which was first produced in 1988. It takes several weeks for a skin biopsy to be expanded into sufficient cultured epidermis that can be applied onto the patient, and the product is very costly, on the order of $800 per a 50 cm^2 area.[13] The product does not have a dermis, and is only a few cells thick, therefore it is very fragile and difficult to use. For these reasons, its use is limited to catastrophic burns where very little autologous viable skin remains.

Another tissue engineered skin product consists of allogeneic neonatal dermal fibroblasts cultured on a polyglactin mesh. The cells produce ECM matrix proteins as the mesh degrades, producing a matrix usable on the wound.[13] This product is a dermal analog called Dermagraft®, which has been used to cover diabetic foot ulcers. Although this product is eventually rejected, it appears to help restore the dermis and promote keratinocyte migration to close the wound.[18]

Another allogeneic skin product is Apligraf®, a bilayered construct using fibroblasts and keratinocytes to create a dermis and epidermis, respectively.[13] Both sets of cells are taken from neonatal foreskin, and the fibroblasts are mixed with type 1 collagen to form a strong network of cells and matrix proteins. The keratinocytes are then seeded onto the construct and stratified into layers. One potential negative consequence to using this product is that some wounds have contracted more than using skin grafts.[5] As any other allogeneic skin, Apligraf® ultimately get rejected.[19]

A new approach is to distribute a "minced micrograft" over the wound area. This technique excises a small area (~ 2 cm^2) of full thickness skin from the patient and minces it. The

mixture, which contains both the dermal and epidermal components of skin, is mixed with a hydrogel and applied onto the wound. The distributed cells proliferate and participate to the wound healing. This may be a future alternative to traditional skin grafts, since only a small area is needed at the donor site, and it is also cheaper and simpler than products that attempt to emulate the skin geometry.[20]

IV. STEM CELLS & WOUND HEALING

While engineered skin substitutes represent significant advances in wound care, their use is not routine because of their high cost, limited effectiveness, and their inability to reconstitute skin appendages.[21] Stem cells, defined based on the findings of Ernest A.McCulloch and James E. Till,[22, 23] are characterized by (1) a prolonged self-renewal capacity and (2) the ability to differentiate into mature stages and different tissue types by asymmetric replication.[24, 25] Stem cells, due to their ability to differentiate into various tissue types by asymmetric replication, may help create those skin components that are not found in the tissue engineered skin substitutes. Stem cells have two distinguishing properties: i) They are undifferentiated cells that renew themselves for the entire life span of an organism through cell division and ii) they have a remarkable capacity to develop from a common precursor into multiple cell types with specialized functions. Among the main sources of cells that might be used for repair and regeneration of injured skin are adult stem cells, embryonic stem cells (ESCs), and induced pluripotent stem cells (iPS) cells.

IV.A. The Involvement of Stem Cells in the Wound Healing Process

While it is well-known that during the inflammatory phase of wound healing, blood-borne immunocompetent cells invade the wound area, recent evidence suggests that bone marrow-derived stem cells are also recruited into the wound site.[26, 27] This is not completely surprising, since a small number of hematopoeitic and mesenchymal stem cells is always present in peripheral blood. Furthermore, severe injury has been shown to increase the number of circulating stem cells.[28] Evidence of this phenomenon has been shown in various models of tissue injury. For example, Badiavas et al. used a skin wound model in mice that had been transplanted with green fluorescent protein (GFP) tagged bone marrow. They found GFP-labeled cells in the wound site that had differentiated into various lineages.[29] In similar experiments, Fathke et al. reported that distant bone marrow-derived stem cells contribute to the reconstitution of the dermal fibroblast population in cutaneous wounds.[30] These findings suggest a potential important contribution of stem cell homing to the wound healing process, which is currently not well understood and warrants further study.

IV.B. Adult stem cells from bone marrow

The development of therapies using stem cells in the context on injury and wound healing has primarily relied on adult stem cells, and especially mesenchymal stromal cells, also known as mesenchymal stem cells (MSCs). MSCs are self-renewing and capable of differentiating into various tissues and cells, including skin cells.[31, 32] MSCs can be isolated from the patient's bone marrow and other tissues such as adipose tissue, nerve tissue, umbilical cord blood, and dermis.[33–37] The other important benefit of MSCs is that even allogeneic MSCs induce little immunoreactivity in the host after local transplantation or systemic administration.[38, 39] Hence, MSCs have received considerable attention for modulating wound repair.[40] MSCs have been examined in skin repair and regeneration after various acute and chronic skin injuries, such as acute incisional and excisional wounds, diabetic skin ulcers, radiation burns, and thermal burns.[7, 41, 42]

Bone marrow-derived MSCs appear to synthesize higher amounts of collagen and several growth and angiogenic factors, when compared to native dermal fibroblasts, indicating a

potential use in accelerating wound healing.[43] The effects of bone marrow impregnated collagen on wound healing were studied in a microcirculatory mouse model, showing significant increases in angiogenesis.[44] Patients with chronic leg ulcers demonstrated successful wound closure after a treatment with these impregnated collagen matrices. A variation of this approach described by Falaga et al.[45] utilized a fibrin polymer spray to apply cultured autologous MSCs obtained from bone marrow aspirates to accelerate the rate of healing of acute and non-healing cutaneous wounds in both humans and mice. This approach may represent a feasible method for introducing cells into wounds. Badiavas and Falanga published clinical results using autologous bone marrow cells directly applied on chronic cutaneous ulcerations from 3 patients with wounds resistant to standard conventional treatment for more than 1 year.[46] All patients showed improvement of their wounds within days following administration, characterized by a steady overall decrease in wound size and an increase in the vascularity of the dermis and the dermal thickness of the wound bed. Another study of chronic diabetic foot ulcers involved a 29-day treatment with an autologous graft composed of autologous skin fibroblasts on biodegradable collagen membranes combined with autologous MSCs applied directly to the wound and injected into the edges of the wound on days 1, 7, and 17; the wound size decreased, and the vascularity of the dermis and dermal thickness of the wound increased.[42] A case report of a severe buttock radiation burn has described treatment combining physical techniques, surgery, and autologous bone marrow-MSCs therapy; clinical evolution (radiation pain and healing progression) during the 11-month follow-up was favorable, with no recurrence of radiation inflammatory waves seen on radiography.[41]

While these results with MSCs are impressive, the treatment approach generally requires that the MSCs must be cultured in sufficient numbers for topical application; this may not be a major issue for small chronic wounds, but may become very impractical when treating large wounds. Relevant to the last point is that severe burns and trauma tend to cause bone marrow suppression with concomitant decrease in MSCs either as a result of silver sulphadiazine toxicity[47] or sepsis.[48, 49] Another consideration is that bone marrow MSCs significantly decrease with age, which may reduce the applicability of using autologous MSCs for chronic wounds.[50]

IV.C. Embryonic stem cells and induced pluripotent stem cells

The embryo with its developmental plasticity and high proliferative capacity is thought to be the ultimate source for pluripotent stem cells.[51, 52] Aside from ethical concerns regarding the use of human embryos for cell harvesting which can now be circumvented to a large extent,[53] a major limitation of using embryonic stem cells (ESCs) for skin therapies is that ESC-derived skin is allogeneic and therefore cannot be used as a permanent wound coverage. Since allogeneic and xenogeneic skin are already available at reasonable cost, there is no clear advantage of using ESC-derived skin from the clinical standpoint. However, reports have shown successful differentiation of ESC-derived skin in vitro,[54] and such studies may yield useful and important information about skin development.

Induced pluripotent stem cells (iPS cells) are a type of pluripotent stem cell artificially derived from a non-pluripotent cell, typically an adult somatic cell, by inducing a “forced” expression of certain genes. iPS cells were first produced in 2006 from mouse cells[55] and in 2007 from human cells.[56] This was a potentially important advancement in stem cell research, as it may overcome two important obstacles associated with human ESCs: immune rejection after transplantation and ethical concerns regarding the use of human embryos.

The technique was first described by Yamanaka,[57] who, in 2007, showed that the introduction of four genes (Oct-3/4, Sox2, c- Myc, and KLF4) into an adult human skin cell could reprogram the cell back to an embryonic state. These induced pluripotent stem cells

were shown to be remarkably similar to ESCs in morphology, proliferation potential, gene expression pattern, pluripotency, and telomerase activity. Like ESCs, iPS cells could be coaxed into becoming other types of cells—from skin to nerve to muscle. Since one of the four genes used (namely, c-Myc) is oncogenic, and 20% of the chimeric mice developed cancer, safety issues were a concern. Subsequently, Takahashi et al. successfully reprogrammed dermal fibroblasts into iPS cells without using the c-Myc retrovirus.[56] Finally, Dimo et al. derived iPS cells from two octogenarians with Lou Gehrig's disease, aged 82 and 89, suggesting that iPS cells can be successfully derived from sick and/or older patients, who are more likely to need iPS cell-based therapies than young healthy individuals.[58] Further developments with iPS cell technology have focused on switching from viruses to using plasmids to deliver the time-reversing genes into adult cells in order to eliminate safety concerns associated with viral vectors.[59] Recent studies also suggest that the generation of iPS cells may be possible without any genetic alterations as described by Zhou et al., who used recombinant proteins channeled into the cells via poly-arginine anchors.[60]

IV.D. Inducing skin lineage commitment in stem cells

1. Keratinocytes—There are two approaches to commit ES cells and adult stem cells (of non-epidermal origin) to the keratinocyte lineage in vitro. One approach would be to expose the cells to a cocktail of exogenous cytokines, growth factors, chemicals, and extracellular matrix (ECM) substrata over a prolonged duration of in vitro culture. Only a fraction of the stem cells would be expected to undergo commitment to the keratinocyte lineage, because many of these cytokines, growth factors, chemicals, and ECM substrata would exert non-specific pleitropic effects on stem cell differentiation into multiple lineages. At best, the cocktail combination of various cytokines, growth factors, chemicals, and ECM substrata can be 'optimized' by trial and error, to maximize the proportion of stem cells committing to the keratinocyte lineage, while at the same time yielding a large number of other undesired lineages. Hence, extensive selection/purification and proliferation of the commited keratinocyte progenitors is likely to be required.

By using such an approach, Coraux et al.[54] managed to achieve commitment and subsequent differentiation of murine ES cells into the keratinocyte lineage, in the presence of a cocktail combination of bone morphogenetic protein-4 (BMP-4), ascorbate, and ECM derived from human normal fibroblasts (HNFs) and murine NIH-3T3 fibroblasts. Nevertheless, it must be noted that the study of Coraux et al.[54] also reported a high degree (approximately 80%) of non-specific differentiation into multiple uncharacterized lineages, and no attempt was made to purify differentiated keratinocytes or keratinocyte progenitors from the mixture of lineages derived from murine ES cells. Bagutti et al.[61] reported that coculture with human dermal fibroblasts (HDFs) as well as HDF-conditioned media could induce beta integrin-deficient murine ES cells to commit and differentiate into the keratinocyte lineage. However, as with the study of Coraux et al.,[54] the keratinocytes were interspersed with differentiated cells of other lineages. Recently, differentiation of human ES cells into the keratinocyte lineage was also reported by Green et al.[62] However, this study was based on in vivo teratoma formation within a SCID mouse model, and to date, there are no parallel in vitro studies that have been reported.

With adult stem cells of non-epidermal origin, there are also few studies [63, 64] which have successfully achieved re-commitment and trans-differentiation to the keratinocyte lineage. Even so, these studies were based primarily on the transplantation of undifferentiated stem cells in vivo, with the observed trans-differentiation occurring sporadically and at extremely low frequencies. Moreover, the validity of the experimental data may be clouded by controversy over the artifact of stem cell fusion in vivo.[65] To date, there are no parallel in

vitro studies that have achieved recommitment and trans-differentiation of non-epidermal adult stem cells to the keratinocyte lineage. It can therefore be surmised that the use of exogenous cytokines, growth factors, chemicals, and ECM substrata to induce ES cell and nonepidermal adult stem cell commitment to the keratinocyte lineage is a relatively inefficient, time-consuming, and labor-intensive process that would require extensive selection and purification of the committed keratinocyte progenitors. Hence, it would be technically challenging to apply this to the clinical situation.

The other approach for inducing ES cell and non-epidermal adult stem cell commitment to the keratinocyte lineage is through genetic modulation. This may be achieved by transfecting stem cells with recombinant DNA constructs encoding for the expression of signaling proteins that promote commitment to the keratinocyte lineage. Of particular interest are the Lef-1/Tcf family of Wnt regulated transcription factors that act in concert with b-catenin,[66, 67] c-myc which is a downstream target of the Wnt-signaling pathway,[68, 69] and the transactivation domain containing isoform of transcription factor p63 (Tap63).[70, 71] Interestingly, the transcription factor GATA-3, which is well known to be a key regulator of T-cell lineage determination, has also been shown to be essential for stem cell lineage determination in skin, where it is expressed at the onset of epidermal stratification and Inner Root Sheath (IRS) specification in follicles.[72] Recombinant overexpression of p63[73] and c-Myc[74] has been reported to promote commitment and differentiation to the keratinocyte lineage.

The disadvantage of directing differentiation through genetic modulation is the potential risks associated with utilizing recombinant DNA technology in human clinical therapy. For example, the overexpression of any one particular protein within transfected stem cells would certainly have unpredictable physiological effects upon transplantation in vivo. This problem may be overcome by placing the recombinant expression of the particular protein under the control of ‘switchable’ promoters, several of which have been developed for expression in eukaryotic systems. Such ‘switchable’ promoters could be responsive to exogenous chemicals,[75] heat shock,[76] or even light.[77] Genetically modified stem cells may also run the risk of becoming malignant within the transplanted recipient. Moreover, there are overriding safety concerns with regard to the use of recombinant viral based vectors in the genetic manipulation of stem cells.[78] It remains uncertain as to whether legislation would ultimately permit the use of genetically modified stem cells for human clinical therapy. At present, the potential detrimental effects of transplanting genetically modified stem cells in vivo are not well studied. More research needs to be carried out on animal models to address the safety aspects of such an approach.

More recently, there is emerging evidence that some transcription factors (which are commonly thought of as cytosolic proteins) have the ability to function as paracrine cell to cell signaling molecules.[79] This is based on intercellular transfer of transcription factors through atypical secretion and internalization pathways.[79] Hence, there is an exciting possibility that transcription factors implicated in commitment to the keratinocyte lineage may in the future be genetically engineered to incorporate domains that enable them to participate in novel paracrine signaling mechanisms. This in turn would have tremendous potential for inducing the commitment of ES cells and non-epidermal adult stem cells to the keratinocyte lineage.

2. Skin appendages—Skin appendages, including hair follicles, sebaceous glands and sweat glands, are linked to the epidermis but project deep into the dermal layer. The skin epidermis and its appendages provide a protective barrier that is impermeable to harmful microbes and also prevents dehydration. To perform their functions while being confronted with the physicochemical traumas of the environment, these tissues undergo continual

rejuvenation through homeostasis, and, in addition, they must be primed to undergo wound repair in response to injury. The skin's elixir for maintaining tissue homeostasis, regenerating hair, and repairing the epidermis after injury is its stem cells.

The hair follicle is composed of an outer root sheath that is contiguous with the epidermis, an inner root sheath and the hair shaft. The matrix surrounding the dermal papilla, in the hair root, contains actively dividing, relatively undifferentiated cells and is therefore a pocket of MSCs that are essential for follicle formation. The lower segment of each hair follicle cycles through periods of active growth (anagen), destruction (catagen) and quiescence (telogen).[80] A specialized region of the outer root sheath of the hair follicle, known as the bulge, is located below the sebaceous gland, which is also the attachment site of the arrector pili muscle, receiving inputs from sensory nerve endings and blood vessels. Furthermore, the hair follicle bulge is a reservoir of slow-cycling multipotent stem cells.[81, 82] Subsets of these follicle-derived multipotent stem cells can be activated and migrate out of hair follicles to the site of a wound to repair the damaged epithelium; however, they contribute little to the intact epidermis. These hair follicle stem cells can also contribute to the growth of follicles themselves and the sebaceous gland. For example, in the absence of hair follicle stem cells, hair follicle and sebaceous gland morphogenesis is blocked, and epidermal wound repair is compromised.[83] In addition to containing follicle epidermal stem cells, the bulge contains melanocyte stem cells.[84] Recent studies show that nestin, a marker for neural progenitor cells, is selectively expressed in cells of the hair follicle bulge and that these stem cells can differentiate into neurons,[85] glia, keratinocytes, smooth muscle cells, melanocytes and even blood vessels.[86, 87] Examination of close developmental and anatomical parallels between epithelial tissue and dermal tissue in skin and hair follicles has revealed dermal tissue to have stem cells. Paus et al. indicated that hair follicle dermal sheath cells might represent a source of dermal stem cells that not only incorporate into the hair-supporting papilla, low down in the follicle, but also move up and out from the follicle dermal sheath into the dermis of adjoining skin.[88] Hair follicle dermal sheath cells taken from the human scalp can form new dermal papilla, induce the formation of hair follicles, and produce hair shafts when transplanted onto skin.[89] There is also a clear transition from dermal sheath to dermal papilla cells.[90] When the follicle dermal cells are implanted into skin wounds, they can be incorporated into the new dermis in a manner similar to that of skin wound-healing fibroblasts.[91] However, these cell populations still lack specific markers for purifying and distinguishing the stem cells from their progeny. Furthermore, of prime importance is improving our understanding of the relation between bulge cells and interfollicular epidermal stem cells and between bulge cells and other stem cells inhabiting the skin and the mechanisms of hair growth.

Recently, cell replacement therapy has offered a novel and powerful medical technology for skin repair and regeneration: a new population of stem cell, called a neural crest stem cell, from adult hair follicles, was discovered to have the ability to differentiate in vitro to keratinocytes, neurons, cartilage/bone cells, smooth muscle cells, melanocytes, glial cells, and adipocytes.[92–96] In mammalian skin, skin-derived neural progenitors were isolated and expanded from the dermis of rodent skin and adult human scalp and could differentiate into both neural and mesodermal progeny.[97, 98] Skin-derived neural progenitor cells were isolated based on the sphere formation of floating cells after 3–7 days of culture in uncoated flasks with epidermal growth factor and fibroblast growth factor, and characterized by the production of nestin and fibronectin, markers of neural precursors. In addition, skin-derived neural progenitor cells were identified as neural crest derived by the use of Wnt1 promoter driving LacZ expression in the mouse. Some of the LacZ-positive cells were found in the skin of the face, as well as in the dermis and dermal papilla of murine whisker.[99] These skin derived neural crest cells have already shown promising results in regenerative medicine such as the promotion of regenerative axonal growth after transplantation into injured adult

mouse sciatic nerves [95] or spinal cord repair,[100] resulting in the recovery of peripheral nerve function. This new study marks an important first step in the development of real stem-cell-based therapies and skin tissue regeneration.

V. SUMMARY AND FUTURE DIRECTIONS

Skin tissue engineering technologies have been available for the past 3 decades, and provide a number of alternatives to traditional skin grafting. There is nevertheless much room for improvement given the many practical and therapeutic limitations of tissue engineered skin. The “holy grail” of skin tissue engineering and skin wound regeneration remains the inability to reliably reconstitute skin appendages, most notably hair follicles and sweat glands. The availability of adult stem cells and iPS cells from the patient provide opportunities for eventually generating these structures without the risk of immune rejection. Recent studies with skin-derived progenitors provide clues as to the mechanisms that generate and maintain skin appendages, and this information will eventually form the basis of new therapies that address these limitations.

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ABBREVATIONS

ECM	extracellular matrix
ESC	embryonic stem cell
iPS	induced pluripotent stem cells
GFP	green fluorescent protein
MSCs	mesenchymal stem cells
BMP-4	bone morphogenetic protein-4
HNFs	human normal fibroblasts
HDFs	human dermal fibroblasts
IRS	inner root sheath

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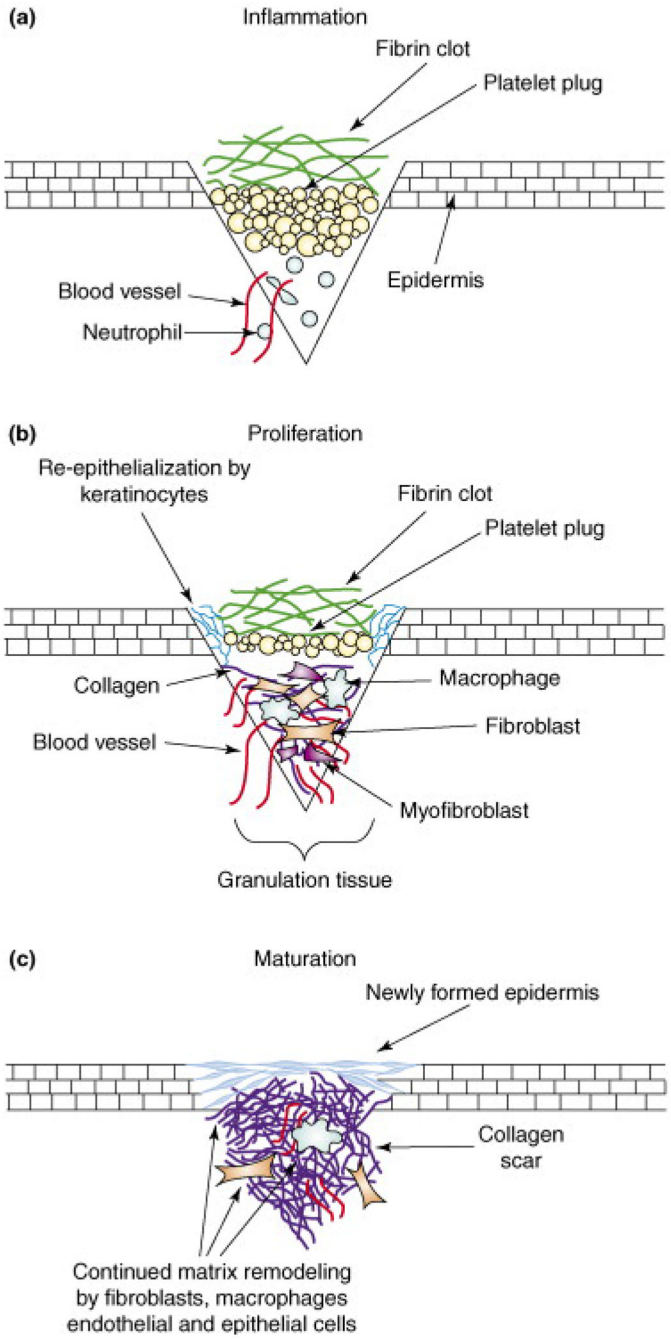

Figure 1.
Phases of Wound Healing. Reproduced by permission from ref 10.