# Regeneration of Mammalian Skin

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*Based in part on the previous version of this Encyclopedia of Life Sciences (ELS) article, Regeneration of Mammalian Skin by Bruce A Mast.*

**Advanced article**

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**Online posting date: 17th January 2011**

**Regeneration and repair of tissue injury in mammalian skin is an intricate process in which cellular, biochemical and molecular interactions occur. These interactions are the foundations of new therapeutics and approaches designed to facilitate tissue repair. The ultimate goal is to regenerate skin such that the complete structural and functional properties of the wounded area are restored to the levels before injury without a scar. Novel pharmaceutical approaches to scar reduction are under development, with the furthest progressed being avotermin (Juvista; transforming growth factor beta 3 (TGFβ3)). In addition, new synthetic biomaterials are constantly being developed that may in future enable some control over the capacity for tissues to regenerate by manipulating stem cells, cell adhesion, growth and differentiation for optimal tissue development. The success of these new approaches to skin regeneration is likely to be underpinned by the manipulation of the mechanisms responsible for wound repair and regeneration.**

**ELS subject area:** Developmental Biology

**How to cite:**
Metcalfe, Anthony D; and Ferguson, Mark WJ (January 2011) Regeneration of Mammalian Skin. In: Encyclopedia of Life Sciences (ELS). John Wiley & Sons, Ltd: Chichester.
DOI: 10.1002/9780470015902.a0001109.pub2

## Introduction

The primary functions of the skin include host defence against microorganisms, by provision of barrier function and balancing thermoregulation with fluid preservation. The skin can potentially be subjected to different traumas, and as a consequence, tissue repair has evolved as a way to restore integrity and barrier function. It has long intrigued researchers why some organisms respond to injury using repair mechanisms but others can regenerate different tissues and structures. Regeneration can either be epimorphic; where replacement cells arise from undifferentiated cells that form a blastema, or morphallactic regeneration; where new cells are derived from existing tissues by cell differentiation or migration. Both forms of regeneration have largely been superseded in favour of repair mechanisms as mammals have evolved. Typically, limb regeneration in the salamander proceeds by the local formation of a blastema; a growth zone of mesenchymal stem cells on the stump (Brockes and Kumar, 2005). Such appendage regeneration in adult vertebrates has implications for regenerative medicine (Brockes and Kumar, 2005) and there are a few examples of mammalian tissues and organs that can regenerate. These are exemplified by liver regeneration (Fausto, 2000), rabbit and mouse ear regeneration (Goss and Grimes, 1975; Metcalfe and Ferguson, 2007), antler regeneration, regeneration of the digit tip in a child (reviewed in Han *et al*., 2005) or interdental papilla regeneration. Shedding cycles of the epidermis or the epithelial cells lining the gut are also forms of regeneration, as is the renewal of the endometrium after a menstrual period. Mammalian tissue repair in comparison is largely a process of fibrosis, in which scar formation restores the integrity of injured tissue. Scar formation,

although not ideal, tends to be significantly faster than complete regeneration, yielding the evolutionary advantage of rapid wound closure, prevention of infection, sepsis and ultimately death.

The challenge facing biotechnologists is to pharmaceutically manipulate the wound environment to trigger the processes of regeneration. Pharmaceutically, this involves developing a drug which is capable of impacting upon critical signalling pathways associated with the induction of regeneration. Alternatively, the next best approach involves bioengineering skin replacement therapies which combine novel materials with living cells to produce a functional and durable skin equivalent. For both disciplines there are difficult challenges to overcome. For tissue engineers and stem cell scientists the challenges are even greater than the pharmaceutical approach, involving the development of a fully functional skin substitute that can regenerate all the differentiated structures of the skin, as well as integrating seamlessly into the host without forming a scar (MacNeil, 2007; Metcalfe and Ferguson, 2007; Dudas *et al*., 2008). In addition, from a commercial standpoint, the skin replacement will require a reasonable shelf life; a major challenge in itself facing the development of such live cell products. Importantly, both approaches should allow the integration and orchestration of the cell biology of the host cells and the multitude of signals that control their behaviour to produce an end result that matches the original area of skin before injury. To understand tissue repair, and perhaps how to activate the processes of regeneration in mammals, it is important to understand the composition of the skin and the specific processes responsible for healing to occur. **See also**: Regeneration: Growth Factors in Limb Regeneration; Regeneration of the Urodele Limb; Regeneration of the Vertebrate Tail; Regeneration of Vertebrate Tissues: Model Systems; Skin: Immunological Defence Mechanisms

## Mammalian Skin

The skin is the largest organ of the body in vertebrates, and is composed of the epidermis, dermis and hypodermis with a complex nerve and blood supply. These three layers play an important role in protecting the body from any mechanical damage, trauma or injury. The epidermis is thin and completely cellular, composed mainly of keratinocytes but has sufficient thickness to provide vital barrier function to the outside environment. Langerhans cells and dendritic cells also reside in the epidermis and contribute to the immune function of the skin. Mammalian epidermis and its appendages (hair, nail, sweat and sebaceous glands) maintain homeostasis by constant recycling of the basal cell layer. The basal layer of the epidermis also contains pigment producing cells called melanocytes; their activity being the major determinant of the colour of mammalian hair and skin. Melanocytes, via their dendrites can supply melanin to numerous keratinocytes within their vicinity. In the epidermis, there are two forms of melanin produced; eumelanin and pheomelanin. Eumelanin contributes to the brown to black pigmentation, whereas pheomelanin contributes to the yellow to red pigmentation of skin and hair.

The dermis situated directly below the epidermis, constitutes the bulk of the skin and is composed of collagen with some elastin and glycosaminoglycans (GAG's). Collagens, present in the skin, are mainly synthesised by fibroblasts and myofibroblasts. Type I collagen is present in the dermis and fasciae and is a major component of scar tissue. Of the 20 different types of collagen, collagens I, II, III, V and XI assemble into fibrils. Other collagens form networks, for example collagen IV, the major component of basement membrane which helps to define barrier function in the skin at the epidermal-dermal junction. Collagens can also form transmembrane proteins, beaded structures or associate with fibril surfaces. The arrangement of collagen within the dermis of a healing wound is an important determinant of the ultimate severity of the resultant scar. In uninjured skin, collagen forms a regular basketweave arrangement of fibrils. On wounding, a parallel arrangement of the collagen matrix generally leads to an abnormal scar appearance that can be wide, raised or depressed but ultimately very noticeable and often problematic physically, mechanically and psychologically. Fibroblasts are also capable of producing remodelling enzymes such as elastases, metalloproteases and collagenases, which play an important role in the wound repair process.

The hypodermis is the layer located beneath the dermis, and contains a considerable amount of adipose and loose connective tissue that is well vascularised and contributes to both the mechanical and thermoregulatory properties of the skin. **See also**: Epithelial Cells: Immunological Aspects; Evolution of Skin Pigmentation Differences in Humans; Glycosaminoglycans: Structure and Biological Functions

## Normal Wound Healing of the Skin in Adult mammals

Tissue repair is normally a rapid process that has been devised through evolution to allow animals to escape danger and rapidly recover tissue integrity using scarring to join the wound edges or to fill tissue voids (Caplan, 2003). The differences between tissue repair and regeneration are likely to be subtle divergences of related (or potentially identical) signalling pathways. In order to fully understand these differences, it is useful to briefly consider the processes of normal wound repair and the scarring process, and to cite some examples of mammalian tissues which are capable of scar-free healing.

Wound healing has often been described as a sequential mechanism; it is more specifically an event-driven process, whereby signals from one cell type set off cascades in other cell types, driving the wound through the phases of healing (Sweitzer *et al*., 2006). Wound healing although complex,

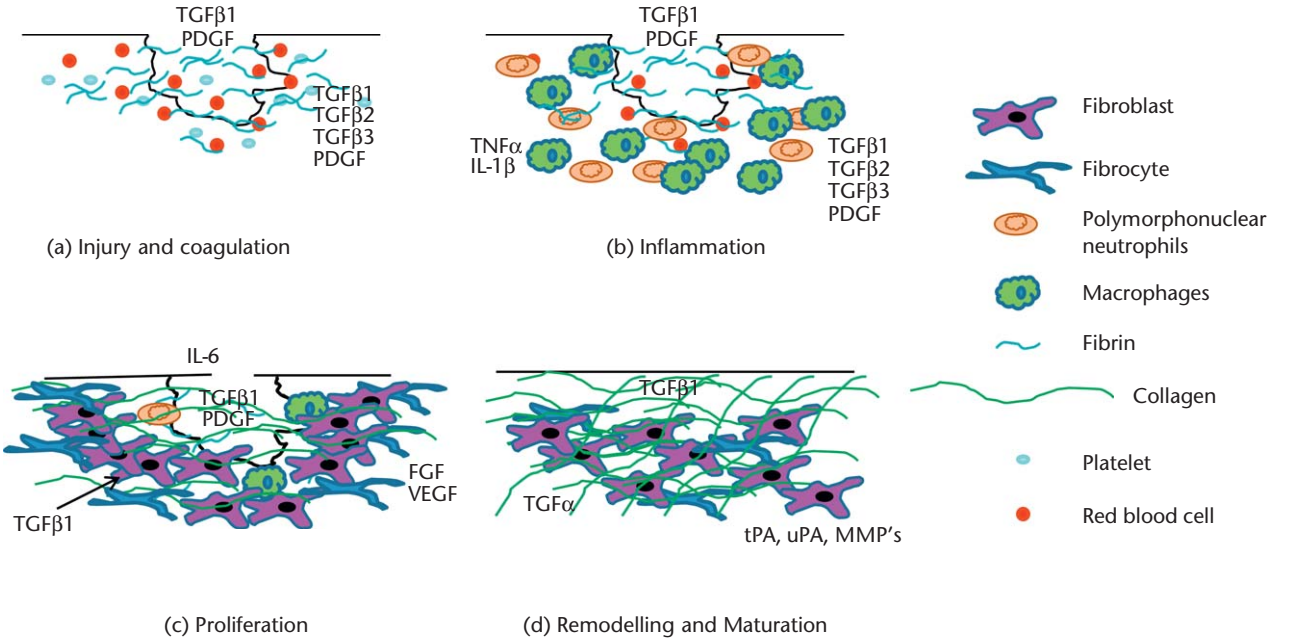


**Figure 1** *The phases of wound healing in the skin involve* a number of overlapping phases, including injury and coagulation (a), inflammation (b), proliferation and epithelialisation (c), angiogenesis and matrix deposition during the remodelling and maturation phases (d). Various growth factors and cytokines are expressed during these phases such as transforming growth factor beta (TGFβ1,-2, -3), TGF alpha (TGFα), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), tumour necrosis factor alpha (TNFα) and interleukin-1 beta (il-1β), interleukin 6, matrix metalloproteinases (MMP's), urokinase (uPA) and tissue type plasminogen activator (tPA).

can be considered to occur in four overlapping phases; injury and haemostasis, inflammation, proliferation and remodelling (**Figure 1**).

## Haemostasis

Tissue repair begins immediately with fibrin clot deposition at the site of injury, preventing haemorrhage from damaged blood vessels. Circulating platelets then aggregate at the site of injury and various inflammatory mediators, such as platelet-derived growth factor (PDGF), transforming growth factors (TGFα and TGFβ) epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1) and fibroblast growth factors (FGF's) are released. These molecules are also believed to play pivotal roles downstream in the wound repair process (Chettibi and Ferguson, 1999; Werner and Grose, 2003; Barrientos *et al*., 2008; Buchanan *et al*., 2009) and have variously been investigated as potential scar reduction therapies. **See also**: Fibrinogen and Fibrin; Platelets; Thrombin; Transforming Growth Factor Beta: Role in Cell Growth and Differentiation

## Inflammation

The inflammatory response of adult tissues to wounding is characterised by an early influx of neutrophils whose numbers steadily increase and reach a maximum 24–48 h post wounding (Chettibi and Ferguson, 1999). Neutrophils release proteases (such as elastase and collagenase) that remove damaged and denatured extracellular matrix components and aid in debridement of devitalised tissue. Because of their bactericidal and phagocytic mechanisms, neutrophils additionally control local bacterial contamination and prevent infection. When the neutrophil numbers begin to decline, macrophages and monocytes take over and repopulate the wound site. Macrophages are the most important source of growth factors due to their prolonged presence within the wound (several days). Other innate receptors of the skin (langerhans and dendritic cells) are also activated on wounding. They are located throughout the epithelium of the skin, where in their immature form they are attached by long cytoplasmic processes. The primary function of dendritic cells is to capture and present protein antigens to naive T lymphocytes. Dendritic cells engulf microorganisms and other materials and degrade them with their lysosomes. Peptides from microbial proteins are then bound to a groove of major histocompatability complex-II (MHC-II) molecules produced by macrophages, dendritic cells and B lymphocytes. The peptide epitopes bound to the MHC-II molecules are then put on the surface of the dendritic cell where they can be recognised by complementary shaped T-cell receptors (TCR) and CD4 molecules on naive T4 lymphocytes. Re-epithelialisation also occurs alongside the inflammatory response, with keratinocytes migrating across the granulation tissue from deep within the dermis and the basal cells of the wound edge. As soon as the keratinocytes have redefined the barrier property of the skin, they resume a

basal cell phenotype upon contact inhibition and differentiate into a stratified squamous keratinising epidermis (Schaffer and Nanney, 1996). **See also**: Inflammation: Acute; Inflammatory Mediators; Macrophages; Mononuclear Phagocytic System; Neutrophils; Skin: Immunological Defence Mechanisms

The involvement of the immune system in the response to tissue injury has also raised the possibility that it might influence the outcome not only of tissue repair but tissue regeneration. A common hypothesis is that the process of inflammation may preclude the ability of a structure to regenerate. There is, however, evidence of the immune system playing a more positive role in the regeneration of immune privileged sites such as the lens (Godwin and Brockes, 2006). In various newt species, the ocular tissues such as the lens are regenerative and it has been recently shown that the response to local injury of the lens involves activation of antigen-presenting cells which traffic to the spleen and return to displace and engulf the lens, inducing regeneration from the dorsal iris (Godwin and Brockes, 2006). Mammals have a very highly developed adaptive immunity and a relatively poor capacity to regenerate, whereas urodeles (such as the newt) regenerate structures more easily but have a less robust immune system (Godwin and Brockes, 2006). **See also**: Regeneration: Growth Factors in Limb Regeneration; Regeneration of the Vertebrate Lens and Other Eye Structures; Regeneration of the Vertebrate Tail; Regeneration of Vertebrate Tissues: Model Systems

## Cell Proliferation, Remodelling and Scarring

The proliferative or repair phase often lasts several weeks. As the number of macrophages in the wound begins to decrease, growth factor synthesis and secretion is chiefly produced by other cells in the wound such as fibroblasts, endothelial cells and keratinocytes. The final phases of the inflammatory response and epithelialisation coincide with the migration of fibrocytes, fibroblasts and endothelial cells and the formation of granulation tissue. Angiogenesis and fibroplasia then take place, with fibroblasts becoming the dominant cell type, laying down collagen and extracellular matrix (ECM).

The last and longest phase of wound healing is remodelling, taking months to years to complete, resulting in the formation of a mature scar. Remodelling of collagen occurs using matrix metalloproteinases (MMP's) produced by the fibroblasts and macrophages and this is a phase that can last several months and in the adult is characterised by scar formation (Chettibi and Ferguson, 1999). The balance of newly formed collagen with the destruction of old collagen, assists in the final physical characteristics of the scar. Among the many growth factors that affect collagen flux, TGFβ is the most influential. It not only induces production of type I collagen by fibroblasts, but effectively diminishes collagen degradation by inhibiting collagenase gene transcription and stimulating the production of tissue inhibitors of metalloproteinases (TIMPs) (Mast and Schultz, 1996). This process is conserved across species from mouse to man (**Figure 2**).

Clinically, a 12 month endpoint is often chosen to represent mature scar formation. There are four different types of scars; atrophic, normotrophic, hypertrophic and keloidal. Atrophic scars cause a depression in the skin. Normotrophic scars are level with the skin. Hypertrophic scars are elevated and may subside with time. Keloidal scars are elevated, exuberant and continue to grow beyond the margin of the original wound. In addition to appearance, location and orientation are also important considerations in determining scar type. All types of scarring can occur on all areas of the body, but some areas such as the chest, and deltoid regions are more susceptible to excessive scarring. **See also**: Extracellular Matrix; Fibrinogen and Fibrin

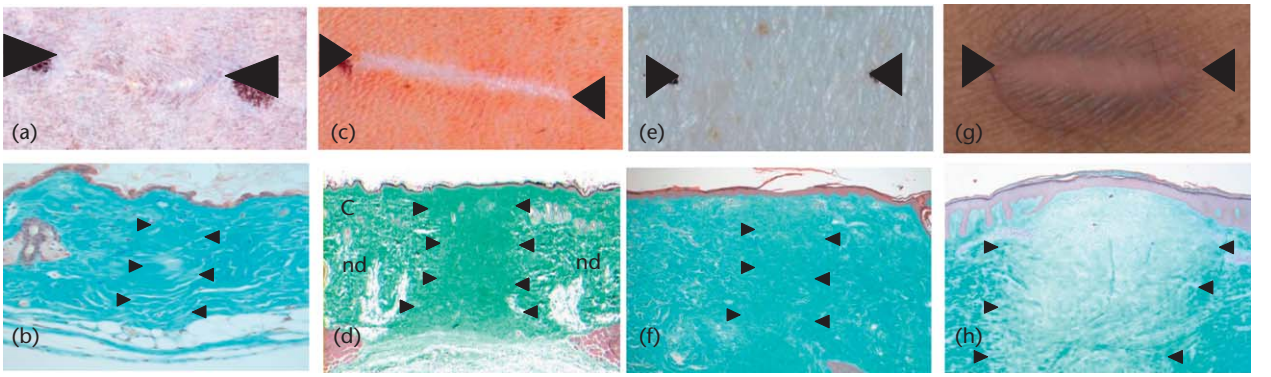


**Figure 2** Scarring is conserved across species. Scarring response in mice 70 days following a 1 cm fullthickness incisional wound to the dorsum at the macroscopic (a) and microscopic (b) levels. Scarring response in rats 70 days following a 1 cm full thickness incisional wound to the dorsum at the macroscopic (c) and microscopic (d) levels. Scarring response in pigs 168 days following a 1 cm full thickness incisonal wound to the dorsum at the macroscopic (e) and microscopic (f) levels. Scarring response in humans 365 days following a 1 cm full thickness incisional wound to the inner aspect of the upper arm at the macroscopic (g) and microscopic (h) levels. Arrows on both the macroscopic and microscopic images define the scar edges.

## Normal Wound Healing of the Skin in the Fetus: A Regenerative Process

Fetal wound repair is essentially a regenerative process, characterised by an absence of scarring and fibrosis (see reviews by Ferguson *et al*., 1996; McCallion and Ferguson, 1996; Garg and Longaker, 2000; Wilgus, 2007; Colwell *et al*., 2003; Ferguson and O'Kane, 2004; Buchanan *et al*., 2009). These differences in the healing processes have sparked great interest and have lead to the development of several animal models in which scarless fetal healing has been described. These include the sheep, pig, rabbit, mouse, rat, guinea pig, chicken, opossum and monkey (Adzick and Longaker, 1991; reviewed in Chettibi and Ferguson, 1999). Wounds made in fetal tissues heal via different mechanisms and result in much less scarring than an equivalent wound in adult tissues (Whitby and Ferguson, 1991).

The characteristics of scar-free healing after incisional wounding have been shown in various studies (Ferguson and O'Kane, 2004). They include minimal inflammation and complete restoration of normal skin structure, with normal collagen deposition and regularly distributed hair follicles, capillaries and glands. Such healing is believed only to occur through a gestational age equivalent to the first third of human development; after that time, normal scarring as evident in the adult occurs (extensively reviewed in Ferguson and O'Kane, 2004). As a consequence of an altered inflammatory response and skin morphogenesis, the growth factor profile of a healing embryonic wound is very different qualitatively, quantitatively and temporally compared with an adult wound (Ferguson and O'Kane, 2004). Embryonic wounds express very high levels of TGFβ3, and very low levels of TGFβ1 and TGFβ2. By contrast, adult wounds contain predominantly TGFβ1 and TGFβ2, which is derived initially from degranulating platelets and subsequently from inflammatory cells such as monocytes and macrophages.

Neutralising antibodies to TGFβ1 or TGFβ2 when applied to healing adult rodent wounds results in markedly improved scarring (Ferguson and O'Kane, 2004). Interestingly, pan-neutralisation of all three TGFβ isoforms (TGFβ1, TGFβ2 and TGFβ3) does not improve scarring, suggesting that neutralisation of TGFβ3 may be detrimental (Ferguson and O'Kane, 2004). By contrast, exogenous addition of TGFβ3 to healing adult wounds (to elevate levels similar to those seen in scar-free embryonic wounds) results in markedly improved or absent scarring during adult wound healing. From these studies, it can be seen that TGFβ isoform protein expression plays a major part in both processes of repair and regeneration. **See also**: Transforming Growth Factor Beta: Role in Cell Growth and Differentiation

Additional mechanisms underlying embryonic wound repair have also been elucidated. In the adult wound, keratinocytes migrate across the exposed substratum during wound closure, in the embryonic epidermis a wound is closed by the purse-string contraction of a rapidly assembled actin network (Redd *et al*., 2004). These authors discuss how this re-epithelialisation can be hindered by blocking assembly of this actin cable network in chick and mouse embryos, using drugs or by inactivation of the small GTPase Rho (Redd *et al*., 2004). *In vivo* studies of epithelial repair in *Drosophila* embryos that express GFP-actin revealed actin-rich filopodia associated with the cable that are essential for final closure of the wound edges (Redd *et al*., 2004). This wound re-epithelialisation mechanism demonstrates parallels to the morphogenetic events of dorsal closure seen in *Drosophila*.

In order to identify genes involved in purse-string wound closure Campos *et al*. (2010) developed a wounding strategy that allowed them to screen large numbers of *Drosophila* embryos. From this screening these authors identified wound healing defects in Jun-related antigen (encoding DJUN) and scab (encoding *Drosophila* alphaPS3 integrin) mutant flies. They carried out a forward genetics screen that led to the discovery of 30 lethal insertional mutants with defects in embryonic epithelia repair (Campos *et al*., 2010). One of the mutants identified was an insertion in the karst locus (encoding *Drosophila* beta (Heavy)-spectrin). Campos *et al*. (2010) further demonstrated beta (Heavy)-spectrin localised to the edges of the wounds where it is thought to be an important molecule required for wound closure.

Other model systems have been used to investigate the mechanisms of scarring in the skin. The PU.1 null mouse is genetically incapable of raising the standard inflammatory response due to the fact that it lacks macrophages and functioning neutrophils (Martin *et al*., 2003). These authors demonstrated that PU.1 null mice can repair skin wounds in a scar-free manner without raising an inflammatory response (Martin *et al*., 2003).

Oxidants released by activated inflammatory cells during wound healing may also be involved in scarring (Wilgus *et al*., 2005). These authors used a murine fetal wound repair model, to demonstrate that hydrogen peroxide influences healing, inducing fetal fibroblast proliferation and fibrosis, potentially by the induction of transforming growth factor-beta1. Wilgus *et al*. (2005) suggest that identifying the factors produced during the inflammatory response in a wound leading to scar formation could be important for the development of new therapies designed to minimise scarring.

Wound healing and scarring may be incompatible with regeneration in most organisms but in the lizard this is not the case. Lizards can lose substantial portions of their tails by autotomy, initiate wound healing and wound epithelial formation but it is quickly followed by blastema formation and subsequent regeneration of the tail (Alibardi and Toni, 2005). It is as yet unclear how this switch from wound healing and fibrosis leads to the initiation of blastema formation. The outcomes of wound repair and regeneration are profoundly different but may be subtly linked by many different signalling pathways. Immunomodulation is likely to be a key determinant, but the context of tissue and species ultimately governs the outcome of the injury response. Understanding these subtle alterations in the

various pathways, using model systems may begin to unravel the complexities of the regenerative process. The likelihood is that the permissive environment/signalling cascades necessary for regeneration to occur is similar to that observed during embryonic development. A more comprehensive appreciation of these permissive conditions has major implications for further advances in regenerating the skin (Metcalfe and Ferguson, 2007). **See also**: Regeneration: Growth Factors in Limb Regeneration; Regeneration of the Urodele Limb; Regeneration of the Vertebrate Tail

## Therapeutic Advances for Skin Regeneration/Healing

Multiple factors exist that can delay or prevent successful wound closure and healing. Such factors result in problematic or chronic wounds, estimated to cost running into billions of dollars and pounds yearly in the United States and the United Kingdom. Until recently, the best treatment available for chronic wounds included elimination of tissue trauma, wound debridement, eradication of infection, pressure bandaging, water jet therapy and providing wound dressings that support healing.

## Growth Factor Therapy

Considering the key role of growth factors in the process of wound repair and regeneration, researchers have been investigating effective means of applying exogenous growth factors to both acute and chronic wounds in order to stimulate healing. The major growth factors involved in wound healing include PDGF, TGFβ, fibroblast growth factors (FGF), vascular endothelial growth factor (VEGF), insulin-like growth factors (IGF-1 and IGF-2) and epidermal growth factors (EGF). Of these growth factors, PDGF is the only one to date approved for wound care by the Food and Drug Administration and European Authorities. Given their role in wound repair, surprisingly little is known clinically regarding the effects of many of these growth factors. The TGF-β family of proteins are perhaps the only other growth factors that have been extensively studied and advanced clinically. We will consider this latter example a little further.

One of the most important growth factor families implicated in wound healing is the TGFβ. As previously discussed, a complex interplay of the three isoforms of this family helps determine whether skin repairs or regenerates. Current treatments for scarring in the skin have variable or limited effectiveness and have typically not been evaluated in randomised, controlled, double-blind clinical trials (Ferguson *et al*., 2009; Occleston *et al*., 2009). Until recently, the field of scar assessment has lacked a standard methodology. Various scar scoring scales existed that use a variety of scar assessment scales which may evaluate different scar characteristics. They include the Manchester Scar Scale (MSS), the Vancouver Scar Scale (VSS), the Patient and Observer Scar Assessment Scale (POSAS) and the Visual Analogue Scale (VAS). Duncan *et al*. (2006) have shown the VAS scoring and scar ranking methods to be consistent, reliable, valid and feasible. These methods for scar assessment are highly sensitive and capable of reliably measuring differences in scar quality, making them valuable techniques, reaching an unmet clinical need and enabling investigation of changes in scar quality. In addition to these scar scoring scales, there is a lack of rigorously validated patient-based outcome measures of scarring. Durani *et al*. (2009) set up a study to construct such a scale and demonstrate reliability and validity by applying the scale in a wide range of scarring samples. The Patient Scar Assessment Questionnaire with five subscales (i.e. Appearance, Symptoms, Consciousness, Satisfaction with Appearance and Satisfaction with Symptoms) was constructed using multiple categorical response items. The results of this study demonstrated that the Patient Scar Assessment Questionnaire is a reliable and valid measure of the patient's perception of scarring, although the Symptoms subscale requires further refinement (Durani *et al*., 2009).

The prophylactic improvement in scar appearance, through administration of agents at or around the time of injury, represents a new therapeutic approach for which there are currently no registered pharmaceuticals. As described earlier, extensive research into the mechanisms of scar-free and scar-forming healing has provided a robust scientific rationale for the development of TGFβ3 as a potential therapeutic for the improvement of scar appearance in humans (**Figure 2**; reviewed in Ferguson and O'Kane, 2004; Ferguson *et al*., 2009; Occleston *et al*., 2009).

In three double-blind, placebo-controlled human clinical trials, intradermal avotermin (human recombinant TGFβ3) was shown to significantly improve subsequent scar appearance (Ferguson *et al*., 2009).

These studies, which show a clear translation from preclinical efficacy models to the clinical environment, have shown that prophylactic scar improvement is pharmaceutically achievable. It is anticipated that therapeutics such as avotermin represent a new class of prophylactic medicines capable of promoting the regeneration of normal skin and improving scar appearance. **See also**: Transforming Growth Factor Beta: Role in Cell Growth and Differentiation

## Future Areas for Regenerative Therapy

Skin regeneration in the future may come from advances made in the areas of molecular pathway analysis, stem cell biology and tissue engineering. It is useful to briefly consider these areas and some of the research that has been carried out to date.

## Molecular Pathway Analysis

Further to the work of Martin *et al*. (2003), subsequent microarray studies of wound tissues from wild-type mice compared to PU.1-null litter mates revealed a series of genes that were expressed only in the presence of a robust inflammatory response (Cooper *et al*., 2005). One such gene was osteopontin (OPN), known to expressed by wound granulation tissue fibroblasts during the wound healing process (Cooper *et al*., 2005). Mori *et al*. (2008) conducted further *in vitro* and *in vivo* studies, where they analysed the effects of blocking OPN expression at the wound, and determined which inflammatory cells, and paracrine factors from these cells were able to stimulate OPN expression in wound fibroblasts. Finally Mori *et al*. (2008) delivered OPN antisense oligodeoxynucleotides via a pluronic gel into mouse skin wounds which resulted in accelerated healing and reduced granulation tissue formation and scarring.

Several studies have linked Wnt proteins with skin morphogenesis. Accumulating evidence also suggests that Wnt signalling and its effector beta-catenin also play important roles in wound healing. Sato (2006) studied Wnt/beta-catenin signalling in hypertrophic scars and keloids. Using fibroblast cell lines established from normal skin and hypertrophic scar, transcriptional assays and western blotting were performed in the presence of TGFβ1. Sato (2006) demonstrated that TGFβ1 induces activation of beta-catenin-mediated transcription in human dermal fibroblasts via the Smad3 and p38 MAPK pathways. Additional immunohistochemical studies performed by this author demonstrated that beta-catenin protein levels were also elevated in hypertrophic scar and keloid tissues. In another study, Ono *et al*. (2009) cultured normal dermal fibroblasts from Fisher 344 rats and transfected them with adenovirus vector (ad)-bone morphogenetic protein 2 and ad-wingless int 3 genes in addition to fibroblast growth factor-2 protein. The transfected fibroblasts were then grown on hydroxyapatite beads, before transferring them to the surface of a collagen sponge. This entire construct was transplanted into a full-thickness skin defect prepared on the backs of rats (Ono *et al*., 2009). At 4 weeks transplantation, follicle or primitive hair germs were observed only in the ad-bone morphogenetic protein 2 + ad-wnt 3 combined with the fibroblast growth factor-2 protein group. By Week 16 transplantation, hair follicles that contained mature pilosebaceous systems with equally spaced localisation had formed. Carre *et al*. (2010) demonstrated that increased canonical Wnt signalling during postnatal but not fetal cutaneous wound repair. These authors also showed that fetal and postnatal fibroblasts have a disparate response to recombinant murine (rm) Wnt3a *in vitro* and that rmWnt3a affects postnatal fibroblasts in a similar pro fibrotic way to TGFβ1.

In other studies by Fathke *et al*. (2006) forced expression of Wnt5a in a wound was shown to induce changes in the interfollicular epithelium mimicking regeneration. These authors describe rudimentary hair follicles and sebaceous glands, without formation of tumours. Taken together these findings suggest that adult interfollicular epithelium is capable of responding to morphogenic signals to restore epithelial tissue patterning in the skin during wound repair.

## Utilising Resident Stem Cells to Regenerate the skin

Skin represents an ideal model system in which to investigate the use of stem cells as a source of cell replacement therapy because it contains one of the few well characterised adult stem cell types; the keratinocyte. Adult somatic stem cells could resolve the problems that embryonic stem cells potentially have, namely if adult stem cells are transplanted back into the same individual, then there should not be any inherent problems with rejection. Treating patients with their own cells also avoids ethical and moral objections. Unless they are responding to trauma, adult stem cells typically divide infrequently to maintain homeostasis within their resident tissues (Alonso and Fuchs, 2003). Since they are responsible for all cell replacement within a tissue, they are essential for tissue repair, wound healing and regeneration. Adult stem cells reside in specific niches and the niche exposes the stem cells to different differentiation cues – important in maintaining the stem cell state. One such niche where multipotent skin stem cells reside is in the hair follicle bulge and a remarkable example of true organogenesis from adult tissue in culture is described by Zheng *et al*. (2005). These authors injected a mixture of isolated neonatal dermal cells with epidermal aggregates into the dermis of nude mice. These aggregates were then able to interact and undergo relatively normal hair morphogenesis to give rise to cycling hair follicles within 8–12 days.

Cell therapy is an emerging therapeutic strategy aimed at replacing or repairing severely damaged tissues with cultured cells (Pellegrini *et al*., 1999; Pellegrini and De Luca, 2010). Epidermal regeneration using autologous cultured keratinocytes (cultured autografts) can potentially be lifesaving for patients suffering from massive full-thickness burns. However, the widespread use of cultured autografts has been hampered by variable clinical results, even when cells were applied on properly prepared wound beds (Pellegrini *et al*., 1998, 1999). This variable clinical outcome might arise from the depletion of epidermal stem cells (holoclones) when grown in culture. Pellegrini *et al*. (1999) describe cultured autografts containing keratinocyte holoclones that can rapidly and permanently cover a large body surface using fibrin as a suitable substrate for keratinocyte cultivation and transplantation. The data from these and other studies strengthen the concept that the success of cell therapy at a clinical level requires careful evaluation and rigorous cultivation methods before the transplantation of stem cells.

Another potentially important source of mesenchymal stem cells for skin repair and regeneration, derived from

bone marrow, is found in the circulating peripheral blood. Bucala *et al*. (1994) described a distinct population of blood-borne fibroblast-like cells that rapidly entered sites of tissue injury in a wound chamber model. These cells have since been called fibrocytes, and are possibly unique in that they are matrix producing cells of the peripheral blood (Quan *et al*., 2006). In wounds that are deep or have areas of extensive tissue loss (e.g. burn injuries) it is thought that circulating fibrocytes recruited from the blood may play a role in wound remodelling, as the migratory distance from uninjured tissues would be too great. It has also been proposed that circulating 'progenitor fibrocytes' interact with T cells before migrating to the wound site, where they differentiate into mature fibrocytes, following exposure to TGFβ1 (Yang *et al*., 2002).

Cells derived from these niches hold some hope for the future of tissue-engineering approaches to regenerating skin. Further understanding and characterisation of these cell types is obviously required regarding how to manipulate and appropriately control these cells. In time, however, given sufficient advances in technology, it may be possible to incorporate them into a new generation of tissue engineered skin substitute. **See also**: Bone Marrow

## Skin Substitutes and the Potential for Skin Regeneration using Tissue Engineering

Conventionally, tissue engineered skin exists as cells grown *in vitro* and subsequently seeded onto an artificial scaffold or some porous material which is then placed *in vivo* at the site of injury. Artificial, bioengineered skin for treating acute and chronic wounds has, over the past 30 years, advanced from a scientific concept to a series of commercially viable products. The number of artificial skin substitutes licensed for clinical use is growing with more than 20 products being commercially available, but they have yet to replace the current 'gold standard' of an autologous skin graft (Supp and Boyce, 2005; Auger *et al*., 2009).

Currently available skin substitutes often suffer from a range of problems that include poor integration into the host (which in many cases is a direct result of inadequate vascularisation), scarring at the graft margins, a complete lack of differentiated structures and because they are live cell products, having a reasonable shelf life. Modifications and improvements are currently aimed at improving the healing potential of those products through the use of recombinant growth factors and additional features such as microvascularisation. Collagen has been used for some time in the design of skin substitutes (Supp and Boyce, 2005) and has been used to create a model of endothelialised, reconstructed dermis that promotes the spontaneous formation of a human capillary-like network (Hudon *et al*., 2003; Tremblay *et al*., 2005). Butler and Orgill (2005) describe a tissue-engineering technique that combines disaggregated autologous keratinocytes and a highly porous, acellular collagen-glycosaminoglycan matrix that has been shown in a porcine model to regenerate dermis and epidermis *in vivo*. Fujimori *et al*. (2006) have evaluated a novel treatment of burn scar contracture in children which involves the application of an autologous cultured dermal substitute, followed by a graft of superthin split-thickness skin. Such skin substitute technologies, although exploratory and preliminary, may have useful applications if they can be proven to work repeatedly in a clinical setting.

The repeated failure of fabricated skin replacements to adequately vascularise has led to renewed efforts to understand autologous skin graft revascularisation (O'Ceallaigh *et al*., 2006). This inability for substitutes to 'take' leads to cells in the substitute dying and ultimately the construct sloughs away from the host. Until recently revascularisation of skin autografts was thought to occur either by direct anastomosis between graft vessels and bed vessels and by ingrowth of bed vessels (angiogenesis) into the graft (O'Ceallaigh *et al*., 2006). These authors have recently shown that the initial onset of revascularisation is attributable to early anastomoses between graft and bed vessels, mainly within the central area of the graft. One outcome of this research is that bioengineered skin substitutes incorporating prefabricated vessels may vascularise more rapidly in a fashion similar to autologous skin grafts.

New synthetic biomaterials are constantly being developed that may enable control over wound repair and regeneration mechanisms by manipulating cell adhesion, growth and differentiation and biomechanics for optimal tissue development. One potential hope for the future of skin replacement therapies maybe that therapeutics such as Avotermin could also be combined with an intelligently designed, living skin substitute that may allow integration into the host seamlessly, without scarring.

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