Cell. Mol. Life Sci. 65 (2008) 24 – 32
1420-682X/08/010024-9
DOI 10.1007/s00018-007-7427-x

Cellular and Molecular Life Sciences

Skin stem and progenitor cells: using regeneration as a tissue-engineering strategy

A. D. Metcalfe* **and M. W. J. Ferguson**

UK Centre for Tissue Engineering, Faculty of Life Sciences, University of Manchester,
3.239 Stopford Building, Oxford Road, Manchester, M13 9PT (United Kingdom), Fax: +44 161 606 7333,
e-mail: anthony.metcalfe@renovo.com

Online First 20 November 2007

Abstract. Cell plasticity and mesenchymal-epithelial interactions are regarded as a hallmark of embryonic development and are not believed to occur extensively in the adult. Recently, adult mesenchymal stem cells were reported to differentiate in culture into a variety of mature cell types, including epithelial cells. Progress in stem and progenitor cell biology and recognition of the unique properties of such cells may enable intelligent bioengineering design of replacement skin which allows regeneration to occur *in vivo*. Ideally, a scaffold-free environment which stimulates skin stem cells *in situ* to initiate cell signals that result in regeneration rather than scar formation is required. Various skin progenitor cell types are considered along with the signalling cascades that they affect. We also discuss a mammalian model of scar-free regeneration. Many of these mechanisms, if fully understood, could be harnessed after injury to perfectly restore the skin. (Part of a Multi-author Review)

Keywords. Skin, fibrocyte, regeneration, MRL, stem cells, tissue engineering, scarring, TGF-β.

Engineering new tissues for replacement therapies

Patients with a variety of diseases may be treated with transplanted tissues or organs. However, there is a shortage of donor tissues and organs, which worsens annually due to an aging population [1]. Tissue engineers aim to apply the principles of cell transplantation, development, materials science and bioengineering to construct biological substitutes in attempt to restore and maintain function in diseased and injured tissues [2]. The list of tissues with the potential to be engineered is growing steadily. Translation of this technology into clinical reality has only been reached in a few areas, notably where there is a long-standing insight into stem cell biology [3]. One such area where there have been relative successes has been in the development of skin substitutes used to repair wounds or burn injury. There are, however, many problems associated with such replacements, including lack of vascularisation and scarring at the wound margins to name but two [2]. Here we will consider another of the problems, namely a source of relevant cells mobilised during skin repair that could be utilised in skin regeneration. Most current strategies for engineering tissues depend on a sample of autologous cells from the damaged host organ [1]. Access to such autologous cells is not always possible, especially if the damage or disease to an organ or tissue is extensive. Alternative cell sources are the focus of debate and scientific interest in the emerging field of regenerative medicine.

For tissues and organs to maintain a balance between cell loss and replacement, tissues must possess cells capable of self-renewal as well as differentiation. It has long intrigued researchers why some but not all organisms can regenerate different body parts. At the heart of this process lie the functional units of regeneration – stem and progenitor cells. Tissue-specific stem cells were first described by Lajtha et al. [4]. They introduced the concept of a multipotent stem cell capable of both self-renewal and of giving

* Corresponding author.

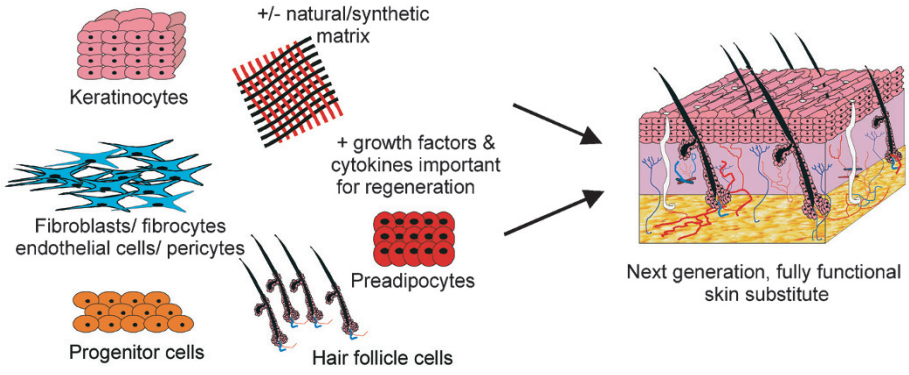

Figure 1. A simple schematic representation of skin tissue engineering. The different skin stem and progenitor cells are mixed with or without a natural or synthetic matrix to produce a fully functional skin substitute.

rise to more differentiated progeny. From this initial discovery has risen the concept of 'cellular hierarchy' within tissues [5]. At the apex of the hierarchy is a resident tissue stem or progenitor cell. Such cells are capable of asymmetric mitosis, producing a replica cell and a slightly more committed progeny cell. The latter is destined for differentiation and tissue regeneration. Such a hierarchy exists in skin cells, specifically in keratinocyte cell culture, which under clonogenic conditions form different types of colonies: holoclones, meroclones and paraclones [6]. Holoclones are the product of a true stem cell, as defined by their ability to self-replicate, whereas meroclones and paraclones are populations of transient amplifying cells and senescent or differentiating progenitors, respectively. Plasticity of adult cells may provide new insights towards understanding cellular differentiation and has promising therapeutic implications [7]. Bone marrow is an obvious source of such cells, but there are various alternative sources for multipotent cells, including lipoaspirates, which contain adipose derived stem cells; peripheral blood, which contains progenitor-like cells such as fibrocytes; and various skin-derived precursor cells [8]. This review aims to focus on some of these cell types, discussing their role in skin repair and regeneration, with a view to utilising them in engineering novel skin replacements (Fig. 1).

Types of progenitor or stem cells and their possible uses in skin engineering

It was originally thought that adult mammalian stem cells were only present in organs such as blood, skin, gut, testis and the respiratory tract which have high cell turnover rates. It now appears, however, that most, if not all, adult organs contain stem cells, or at least can produce stem cells in culture [9]. Adult mesenchymal stem cells (MSCs) are believed to be restricted in their ability to produce a wide range of replacement cells but function well as a renewable source of cells for tissue repair and homeostasis. There are, however, other non-adult sources of stem cells which we will briefly consider. Embryonic stem (ES) cells represent an attractive and viable proposition for cell replacement therapy. They are totipotent and are able to differentiate into many different cell types. ES cells are derived from the inner cell mass (ICM) of the embryonic blastocyst. These cells can be maintained indefinitely *in vitro* without loss of differentiation potential. To date, many of the studies on ES cells have been limited to mouse models, since human cell lines have only recently become available [10]. There are, however, many controversial ethical and technical problems that need to be overcome before the full potential of this type of cell can be realised [11–13].

Skin – a niche for reparative and regenerative cells

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 ES 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 [14]. 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.

Keratinocytes

From a bioengineering viewpoint, skin is a good candidate because keratinocytes can be maintained and propagated in the laboratory [15, 16]. The cells that are contained in these primary cultures have a remarkable ability to proliferate because they are capable of being passaged for many hundreds of generations without undergoing senescence [14]. From the work of Morris and Potten [17] it is known that slowly cycling cells in vivo are more clonogenic than actively dividing cells when placed into culture, suggesting that the less proliferative cells may be stem cells. The ability to maintain and grow such cells has led to major advances in skin-grafting technologies such that burn patients routinely have cultured skin keratinocytes engrafted [18–20]. Unfortunately, at present the skin substitutes that are used are not fully functional in that they lack differentiated structures as well as nerve and blood supplies. Whether long-term keratinocyte cultures contain multipotent stem cells which could produce hair follicles remains a subject of debate [14]. Similarly, sweat glands, like other differentiated structures, do not regenerate. The regulatory mechanisms involved in regeneration of cutaneous appendages is of great interest to tissue engineers – by mastering this, injured skin could be perfectly restored. One theory why differentiated structures do not regenerate after major trauma is that the epidermis cannot receive the correct inducing signals from the underlying wound dermis [21]. Other theories centre around the fact that the pluripotent progenitor cells, which migrate downward from embryonic epidermis to replicate and differentiate into structures like the mature exocrine sweat gland, do not exist in adult skin [22]. These theories have been challenged by the hypothesis that progenitor cell populations like bone marrow stromal cells might be able to differentiate into non-haematopoietic tissues [23], a theory that will be further discussed later in this review.

The problem for bioengineers is that although the existence of skin stem cells is highly likely within primary cell cultures, their isolation and characterisation is proving to be challenging. Fractionation by fluorescence-activated cell sorting (FACS) has provided some insights. Jones and Watt [24] found that some cells within the primary keratinocyte culture showed great proliferative capacity *in vitro* and are enriched for β1 integrins, and other studies by Li et al. [25] and Kaur and Li [26] show that similar cells have upregulated α6 integrin. Further characterisation of these cells has shown that they adhere preferentially to the ECM (extracellular matrix) components fibronectin (α5β1 receptor) and collagen IV (α2β1 receptor), suggesting that the reason stem cells are kept in their niche is the very strong interaction with and adherence to basement membrane components [27, 28].

Hair follicle stem cells

Multipotent skin stem cells also reside in the hair follicle bulge [29]. This niche resides at the base of the permanent epithelial portion of the hair follicle, which is the deepest, most protected place within the contiguous epithelial compartment [14]. Kobayashi and Nishimura [30] dissected dermal papillae cells and reconstituted them with fragments of hair follicle containing the hair bulge region. On transplantation under the kidney capsule of an athymic mouse, viable hair follicles formed. Other studies have shown that when the bulge cells of a normal mouse are surgically replaced with cells from a lac-Z mouse, the new chimeric hair follicles ubiquitously express lac-Z within all lineages of the hair follicle [31]. Several studies suggest that this compartment provides a source of multipotent stem cells which could be used for bioengineering hair follicles [29]. When the skin suffers trauma such as burn injury or wounding, it is thought that the bulge cells migrate to the surface to aid re-epithelialization [32]. Thus, the regenerative role of bulge cells (or dissociated cells) from the skin is multiple; not only is it thought that they contribute to the production of sebaceous glands and epidermis, but they are key to the formation of hair follicles [33]. Probably one of the most remarkable examples of true organogenesis from adult tissue in culture is described by these authors. Zheng et al. [33] 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. Such approaches hold great hope for the future of incorporating differentiated structures in a new generation of skin substitutes.

Skin pigmentation and the role of melanocytes

Skin wounds often display pigmentation problems and sometimes suffer from a lack of sensation. Bioengineers are interested in this since currently available skin substitutes often lack melanocytes and thus skin pigmentation; they also do not have nerve supplies and so suffer from a lack of sensation or control of temperature. Critically, skin substitutes have no resident Langerhans cells, which play an important

function in immune regulation in the skin. Novel skin replacement therapies need to develop strategies to incorporate or induce not only differentiated structures but pigmentation in skin constructs. Extensive studies have been undertaken that incorporate melanocytes and Langerhans cells in skin substitutes for testing purposes. Melanocytes have also been incorporated in therapeutic products for the treatment of vitiligo. A recent study by Hachiya et al. used mixed cell slurries containing keratinocytes and fibroblasts with melanocytes on the backs of severely immunodeficient mice to produce a skin substitute with spontaneously sorted melanocytes [34]. Further developments to this technology may offer a means of treating both structural and cosmetic aspects of skin conditions.

Recruiting circulating stem cells to a wound during repair and regeneration

Injury to the skin results in the physical disruption of the normal cellular architecture and triggers wound healing: a process involving inflammation, cell proliferation and migration, cell recruitment, angiogenesis and extracellular matrix deposition. Growth factors and cytokines released from inflammatory cells dictate the function of those cells present within the wound [35, 36]. Wound fibroblasts produce new extracellular matrix proteins, including collagen types I and III, fibronectin and proteoglycans, and are ultimately responsible for tissue remodelling, scar formation and repair [37]. Hence, fibroblasts recruited to the site of tissue injury are considered essential for successful wound healing; yet their precise origin is uncertain.

Most of what is known about adult mammalian stem cells has come from studies of hematopoietic stem cells (HSCs). They were the first stem cells to be characterized and isolated, and they were the first to be used clinically [9]. In fact, most adult stem cell plasticity experiments have used either HSCs or bone marrow, which contains most of the HSCs in an adult, but also contains other types of stem cells. The literature on HSCs is vast and outside the scope of this review (reviewed in [9]), but bone marrow is a logical candidate for sourcing cells to seed in a skin substitute [38]. In some wounds, e.g. chronic ulcers or non-healing burns, it is thought that the mesenchymal cells that fill the dermis become phenotypically altered or senescent [39–41]. The plasticity of bone marrow-derived stem cells means they would have the inherent capacity to produce new skin cells if the conditions for growth were correct. Since bone marrow is key to the process of haematopoiesis, it is also likely to contain hormones such as granulocyte monocyte colony- stimulating factor (GMCSF), which is thought to accelerate wound repair [42, 43]. A potentially important source of MSCs for skin repair and regeneration, derived from bone marrow, is found in the circulating peripheral blood [10, 44]. Bucala et al. described a distinct population of blood-borne fibroblast-like cells that rapidly entered sites of tissue injury in a wound chamber model [45]. The presence of fibroblasts in wound chambers had mainly been thought to be attributable to recruitment from surrounding subcutaneous tissue [46]. However, the presence of such large numbers of fibroblast-like cells coinciding with the entry of circulating inflammatory cells suggested that the cell population was arising from peripheral blood and not exclusively by slow migration from adjacent connective tissue [45]. This new cell type was termed a fibrocyte with a distinct phenotype originally described as collagen$^+$/vimentin$^+$/CD34$^+$ [45]. More recently, leukocyte-specific protein-1 (lsp-1) has been added to this profile as a fibrocyte marker in hypertrophic scars [47]. Fibrocytes are unusual and are possibly unique in that they are matrix-producing cells of the peripheral blood [48]. In structural and supportive tissue as well as when grown in culture, these cells typically have a stellate or fusiform appearance (Fig. 2). Additionally, when grown in culture they are characterised initially by long spindle-like cytoplasmic extensions that eventually form into a cobblestone phenotype (Fig. 3a, b). Although these cells only make up 0.5 % of peripheral blood leukocytes, they constitute 10 % of the cells infiltrating subcutaneously implanted wound chambers in mice [45]. They are thought to have diverse roles in the wound-healing process, inducing angiogenesis *in vivo* and *in vitro* [49]; they produce chemoattractants to recruit CD4+ lymphocytes [50]; and they express the chemokine receptor CCR7, involved in the process of cell migration into the wound [51]. They also have the ability to form tubelike structures in culture [A. D. Metcalfe, H. Willis and M. W. J. Ferguson, unpublished data] (Fig. 3c). It has been suggested that with a relatively minor wound, fibroblasts may be able simply to migrate in from surrounding undamaged tissues. With deeper wounds or areas of extensive tissue loss (e.g. burn injuries), it may be circulating fibrocytes recruited from the blood which are responsible for wound remodelling, as the migratory distance from uninjured tissues would be too great [52, 53]. 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 TGF (transforming growth factor)-β1 [52].

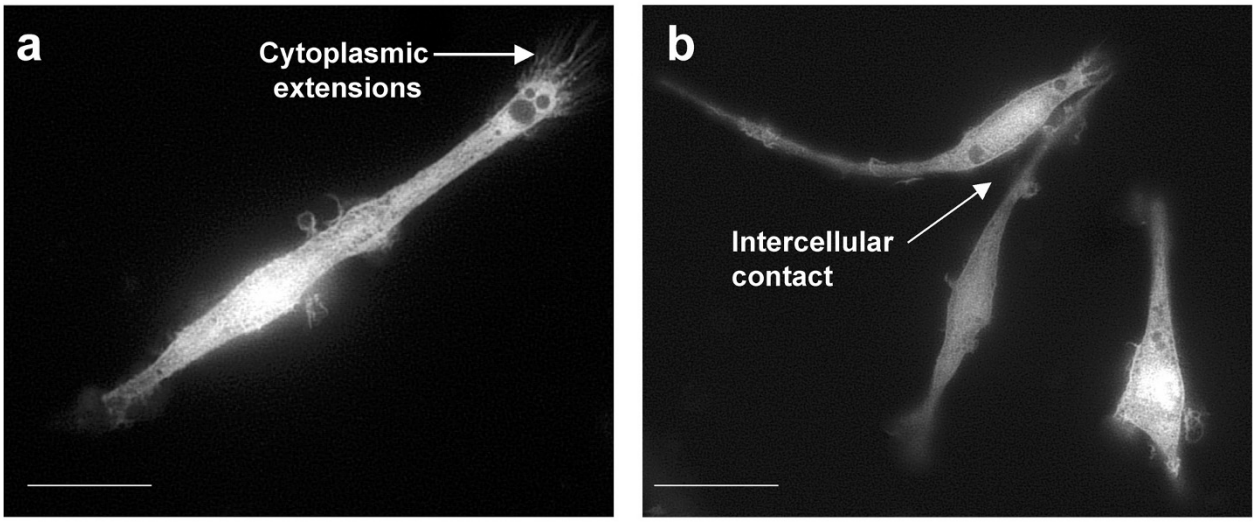

Figure 2. Peripheral blood-derived green fluorescent protein-labelled fibrocyte structure grown for 7 days in culture. (*a*) Note the elongated cell type with vacuoles and cytoplasmic extensions. (*b*) Note the close intercellular contact when fibrocytes are in proximity to one another, and the variation in cell shape. Scale bar, 5 μm.

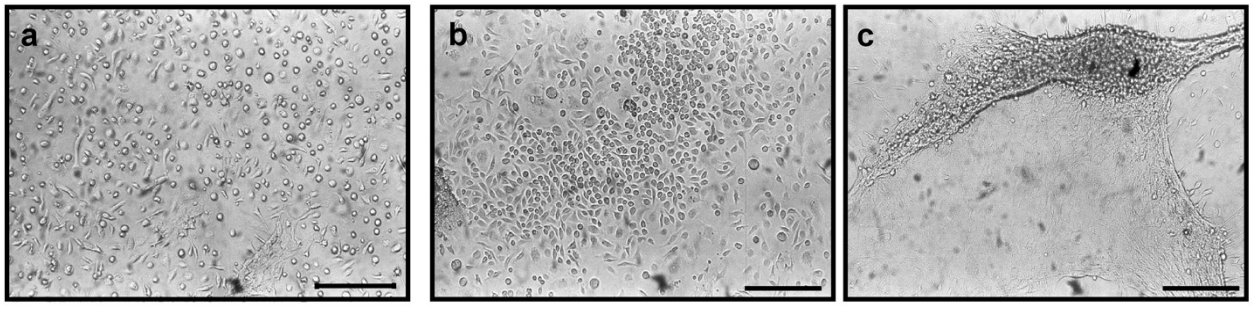

Figure 3. Peripheral blood-derived fibrocytes. (*a*) 3 days *in vitro* culture; note the appearance of both round and spindle-like cells. (*b*) 10 days *in vitro* culture; a distinctive cobblestone cell phenotype was observed. (*c*) 18–25 days *in vitro* culture; cells aggregate to form tubelike projections. Scale bar, 100 μm.

In cutaneous wounds, especially in the expanding margins of keloids or inflammatory scars, CD34+ expression decreases with time, whilst expression of proline-4-hydroxylase, an enzyme required to produce collagen, increases [54]. These authors also propose that the reversion of CD34 expression in scars may represent a decrease in the inflammatory profile of the wound and that overall CD34 positivity is inversely correlated to collagen synthesis. Locally produced interleukin-1 (IL-1), a major inflammatory cytokine in tissue injury, is thought to regulate the phenotypic transition in function from an inflammatory to a remodelling phase [55]. IL-1 also promotes fibrocyte expression of the cytokines TNF (tumour necrosis factor) α, IL-6, IL-8 and IL-10 and chemokines, and the secretion of matrix metalloproteases (MMP-9), whilst reducing collagen production [55]. As inflammatory levels of IL-1 decrease, this phenotypic transition is enhanced by elevated levels of TGFβ1 present in the lesion, known to be important in collagen synthesis, scarring and the expression of α-smooth muscle actin (α-SMA) [51]. When added to cultured fibrocytes, TGFβ1 decreases the expression of cell surface CD34 and increases α-SMA production [55]. This increase in α-SMA is considered to be a differentiating characteristic of contractile myofibroblasts. Quan et al. [48] suggest that transition of fibrocytes to a myofibroblast phenotype may contribute importantly to the progression of many types of pathologic fibroses. Scleroderma and nephrogenic fibrosing dermopathy (NFD) are two such examples thought to be associated with enhanced fibrocyte activation and recruitment to the skin [48]. The intercellular signals that modulate fibrocyte trafficking, proliferation and differentiation are only partially defined, but a better understanding of these signals is likely to enable new therapies to prevent pathologic fibrosis or to improve tissue repair response [48]. Additionally, the precise role of fibrocytes in antigen presentation, adaptive immunity and their capacity to

support angiogenesis are also important parameters for skin bioengineers to research further and understand.

Understanding regenerative mechanisms: a key to developing novel skin substitutes

Medical interest in regeneration has often focussed on the repair of damaged adult tissues. The challenge faced here would be to incorporate molecules associated with the regeneration process into a smart matrix [2]. Only a few examples exist in vertebrate species of tissues where the initial phase of repair is followed by perfect functional and structural restoration of the organ. Classically, when we consider regeneration in mammals, we think of liver regeneration [56], rabbit ear regeneration [57], regeneration of the digit tip in a child [58, 59], axolotl and *Xenopus* limb regeneration [60, 61], antler regeneration [62] and interdental papilla regeneration [63].

Important links between regeneration and development may exist where undifferentiated stem cells are the source from which differentiated cell types arise in order to create (or recreate) functionally organised adult tissues. A murine model for mammalian wound repair and regeneration was discovered by Clark et al. [64]. Two mm through-and-through punch wounds made into the ears of MRL/MpJ mice closed with regeneration after 30 days, whereas they did not close in the control strain C57BL/6 mice. A further study in our laboratory by Rajnoch et al. detailed more specifically how the MRL/MpJ mouse ear wound closed in response to different traumas [65]. Here, the effects of two different types of punches were compared (a crude thumb punch and a clinical biopsy punch) on three strains of mice. MRL/MpJ ear wounds healed faster than either C57BL/6 or Balb/c mice. The MRL/MpJ mouse ears healed with enhanced blastema formation and markedly thickened tip epithelium. Rajnoch et al. postulated that the speed by which a punched ear hole closes might be an important factor before the onset of differentiation [65]. The histological organization of the regenerating MRL/MpJ ear edges closely resembles the blastema of a regenerating limb.

Furthermore, the regenerative ability of this strain may be restricted to the ear because of its structure: a thin tissue covered on both sides by epithelium [65]. The small dimensions of the ear were thought to allow for the diffusion of growth factors and the establishment of appropriate gradients similar to embryonic development, whereas in large thick tissues, e.g. skin, this may not be possible [65]. This was confirmed in studies involving wounding both the ear and the back skin of the MRL/MpJ mouse where the ear wounds completely regenerate, whereas the back skin wounds heal with a scar [66–69]. In keeping with studies of scar-free embryonic skin wound healing [70–74] and the therapeutic manipulation of adult dermal healing to reduce scarring [75], the reduced inflammatory infiltrate seen with the biopsy as opposed to the crude thumb punch correlated with a faster, more regenerative repair process with reduced scarring. Other studies on athymic Nude-*nu* mice have also shown that wound healing of the ear resembles regeneration [76]. In this instance, it is thought that the absence of T lymphocytes in wounded ears provides a microenvironment conducive to regeneration of mesenchymal tissues [76, 77].

The process of regeneration involves the complex tissue remodelling brought about by the formation of a blastema during healing, after which time cartilage, sebaceous glands, hair follicles and blood networks reform. Thus, scarless healing accompanies blastema formation and regrowth of various tissues such as cartilage and other differentiated structures [64–66]. The tissue restoration process in MRL/MpJ and Nude-*nu* mice resembles foetal-like healing and is a rare example of regeneration amongst adult mammals. 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 from an adult-wound [35, 36, 70, 74]. 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. Application of neutralizing antibodies to TGFβ1 and or TGFβ2 (preferably both) to healing adult rodent wounds results in markedly improved scarring [75, 78]. Interestingly, pan-neutralization of all three TGFβ isoforms (TGFβ1, TGFβ2 and TGFβ3) does not improve scarring, suggesting that neutralization of TGFβ3 may be detrimental [75,78]. 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 [75]. From these studies it can be seen that subtle alterations of the TGFβ isoform profile result in either scarring repair or scar-free healing. This is an important consideration in tissue-engineering skin, and the role of various growth factors would be a key consideration in intelligent design [2].

In summary, regeneration is characterised by a constantly changing environment in which cells are exposed to a complex pattern of molecular cues and

signals which impart positional information necessary for correct development. These cell signals trigger a series of events that in combination control cell proliferation, differentiation and cell death, such that a specific tissue can be delineated and its edges specifically defined. Since these molecules are often major components of early developmental pathways for cell specification, incorporating them into a tissue-engineered product could produce major advances in regenerating adult structures such as skin.

Future developments

Cell plasticity is a hallmark of embryonic development, characterised by epithelial-mesenchymal transitions and ultimately the formation of new tissues and organs. Although such transitions are not thought to occur extensively in the adult, the fact that MSCs can differentiate in culture into a variety of mature cell types holds promise for the bioengineer. The question remains whether MSCs transdifferentiate *in vivo*, and this must be the focus of attention for bioscientists. Tissue damage itself may create a special extra-haematopoietic tissue milieu where apoptosis or other forms of cell death lead to the initiation of cytokine cascades and changes to the ECM [79, 80]. Many of the studies documenting progenitor cell plasticity have used models of tissue injury to induce homing and differentiation of progenitor cells in damaged tissues such as skin. This has led to speculation about several possible candidate cell types that are recruited during tissue injury, some of which we have considered here. From studies of the rare examples of mammalian regeneration that also exist, these may yield some additionally important insights for future regenerative approaches. During regeneration, as seen during foetal wound repair mechanisms, changes brought about by cell-signalling cascades allow progenitor cells access for efficient engraftment and subsequent differentiation. Perhaps the greatest challenge in stem cell biology is to uncover the extracellular and intracellular mechanisms that determine whether a daughter cell of a stem cell division self-renews or commits to a particular pathway of differentiation [9]. Biomedical scientists need to understand the subtleties involved in these processes to create an environment that will permit successful engineering of a new generation of skin substitute.

Acknowledgements. The authors are grateful to the BBSRC, MRC and EPSRC for their support of the UK Centre for Tissue Engineering.

1 Eberli, D. and Atala, A. (2006) Tissue engineering using adult stem cells. Methods Enzymol. 420, 287–302.
2 Metcalfe, A. D. and Ferguson, M. W. J. (2007) Tissue engineering of replacement skin: the crossroads of biomaterials, wound healing, embryonic development, stem cells and regeneration. J. Roy. Soc. Int. 4, 413–437.
3 Bianco, P. and Robey, P. G. (2001) Stem cells in tissue engineering. Nature 414, 118–121.
4 Lajtha, L. G., Gilbert, C. W., Porteous, D. D. and Alexanian, R. (1964) Kinetics of a bone-marrow stem-cell population. Ann. N. Y. Acad. Sci. 113, 742–752.
5 Perryman, S. V. and Sylvester, K. G. (2006) Repair and regeneration: opportunities for carcinogenesis from tissue stem cells. J. Cell. Mol. Med. 10, 292–308.
6 Barrandon, Y. and Green, H. (1987) Three clonal types of keratinocyte with different capacities for multiplication. Proc. Natl. Acad. Sci. USA 84, 2302–2306.
7 Chunmeng, S. and Tianmin, C. (2004) Skin: a promising reservoir for adult stem cell populations. Med. Hypoth. 62, 683–688.
8 Freitas, C. S. and Dalmau, S. R. (2006) Multiple sources of non-embryonic multipotent stem cells: processed lipoaspirates and dermis as promising alternatives to bone-marrow-derived cell therapies. Cell Tissue Res. 325, 403–411.
9 Raff, M. (2003) Adult stem cell plasticity:fact or artefact? Annu. Rev. Cell Dev. Biol. 19, 1–22.
10 Tuan, R. S., Boland, G. and Tuli, R. (2002) Adult mesenchymal stem cells and cell-based tissue engineering. Arthritis Res. Ther. 5, 32–45.
11 Ferrari, G., Cusella-De Angelis, G., Colletta, M., Paolucci, E., Stornaiuolo, A., Cossu, G. and Mavilio, F. (1998) Muscle regeneration by bone marrow derived myogenic progenitors. Science 279, 514–519.
12 Gussoni, E., Soneka, Y., Strickland, C. D., Buzney, E. A., Khan, M. K., Flint, A. F., Kumkel, L.M and Mulligan, R. C. (1999) Dystrophin expression in the mdx mouse restored by stem cell transplantation. Nature 401, 390–394.
13 Peterson, D. A. (2002) Stem cells in brain plasticity and repair. Curr. Opin. Pharmacol. 2, 34–42.
14 Alonso, L. and Fuchs, E. (2003) Stem cells in the skin: waste not, Wnt not. Genes Dev. 17, 1189–1200.
15 Rheinwald, J. G. and Green, H. (1975) Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinising colonies from single cells. Cell 6, 331–343.
16 Rheinwald, J. G. and Green, H. (1977) Epidermal growth factor and the multiplication of cultured human epidermal keratinocytes. Nature 265, 421–424.
17 Morris, R. J. and Potten, C. S. (1994) Slowly cycling (label-retaining) epidermal cells behave like clonogenic stem cells in vitro. Cell Prolif. 27, 279–289.
18 Ronfard, V., Rives, J. M., Neveux, Y., Carsin, H. and Barrandon, Y. (2000) Long-term regeneration of human epidermis on third degree burns transplanted with autologous cultured epithelium grown on a fibrin matrix. Transplantation 70, 1588–1598.
19 Brouard, M. and Barrandon, Y. (2003) Controlling skin morphogenesis: hope and despair. Curr. Opin. Biotechnol. 14, 520–525.
20 Gambardella, L. and Barrandon, Y. (2003) The multifaceted adult epidermal stem cell. Curr. Opin. Cell Biol. 15, 771–777.
21 Martin, P. (1997) Wound healing – aiming for perfect skin regeneration. Science 276, 75.
22 Schon, M., Benwood, J., O'Connell-Willstaedt, T. and Rheinwald, J. G. (1999) Human sweat gland myoepithelial cells express a unique set of cytokeratins and reveal the potential for alternative epithelial and mesenchymal differentiation states in culture. J. Cell Sci. 112, 1925.
23 Prockop, D. J. (1997) Marrow stromal cells as stem cells for non hematopoietic tissues. Science 276, 71–74.
24 Jones, P. H. and Watt, F. M. (1993) Separation of human epidermal stem cells from transit amplifying cells on the basis

of differences in integrin function and expression. Cell 73, 713–24.
25 Li, A., Simmons, P. J. and Kaur, P. (1998) Identification and isolation of candidate human keratinocyte stem cells based on cell surface phenotype. Proc. Natl. Acad. Sci. USA 95, 3902–3907.
26 Kaur, P. and Li, A. (2000) Adhesive properties of human basal epidermal cells: an analysis of keratinocyte stem cells, transit amplifying cells, and postmitotic differentiating cells. J. Invest. Dermatol. 114, 413–420.
27 Watt, F. M. (1998) Epidermal stem cells: markers, patterning and the control of stem cell fate. Philos. Trans. R. Soc. Lond. B Biol. Sci. 353, 831–837.
28 Segre, J. A., Bauer, C. and Fuchs, E. (1999) Klf4 is a transcription factor required for establishing the barrier function of the skin. Nat. Genet. 22, 356–360.
29 Stenn, K. S. and Cotsarelis, G. (2005) Bioengineering the hair follicle: fringe benefits of stem cell technology. Curr. Opin. Biotechnol. 16, 493–497.
30 Kobayashi, K. and Nishimura, E. (1989) Ectopic growth of mouse whiskers from implanted lengths of plucked vibrissae follicles. J. Invest. Dermatol. 92, 278–282.
31 Oshima, H., Rochat, A., Kedzia, C., Kobayashi, K. and Barrandon, Y. (2001) Morphogenesis and renewal of hair follicles from adult multipotent stem cells. Cell 104, 233–245.
32 Taylor, G., Lehrer, M. S., Jensen, P. J., Sun, T. T. and Lavker, R. M. (2000) Involvement of follicular stem cells in forming not only the follicle but the epidermis. Cell 102, 451–461.
33 Zheng, Y., Du, X., Wang, W., Boucher, M., Parimoo, S. and Stenn, K. (2005) Organogenesis from dissociated cells: generation of mature cycling hair follicles from skin-derived cells. J. Invest. Dermatol. 124, 867–876.
34 Hachiya, A., Sriwiriyanont, P., Kaiho, E., Kitahara, T., Takema, Y. and Tsuboi, R. (2005) An in vivo mouse model of human skin substitute containing spontaneously sorted melanocytes demonstrates physiological changes after UVB irradiation. J. Invest. Dermatol. 125, 364–372.
35 O'Kane, S. and Ferguson, M. W. (1997) Transforming growth factor beta's and wound healing. Int. J. Biochem. Cell Biol. 29, 63–78.
36 Ferguson, M. W. J. and O'Kane, S. (2004) Scar-free healing: from embryonic mechanisms to adult therapeutic intervention. Philos. Trans. R. Soc. Lond. B Biol. Sci. 29; 359, 839–50.
37 Singer, A. J. and Clarke, R. A. (1999) Cutaneous wound healing. N. Engl. J. Med. 341, 738–746.
38 Badiavas, E. V. and Falanga, V. (2003) Treatment of chronic wounds with bone marrow-derived cells. Arch. Dermatol. 139, 510–6.
39 Mendez, M. V., Stanley, A., Park, H. Y., Shon, K., Phillips, T. and Menzoian, J. O. (1998) Fibroblasts cultured from venous ulcers display cellular characteristics of senescence. J. Vasc. Surg. 28, 876–883.
40 Raffetto, J. D., Mendez, M. V., Phillips, T. J., Park, H. Y. and Menzoian, J. O. (1999) The effect of passage number on fibroblast cellular senescence in patients with chronic venous insufficiency with and without ulcer. Am. J. Surg. 178, 107–112.
41 Vande Berg, J. S., Rudolph, R., Hollan, C. and Haywood-Reid, P. L. (1998) Fibroblast senescence in pressure ulcers. Wound Repair Regen. 6, 38–49.
42 Lingen, M. W. (2001) Role of leukocytes and endothelial cells in the development of angiogenesis in inflammation and wound healing. Arch. Pathol. Lab. Med. 125, 67–71.
43 Gillitzer, R. and Goebeler, M. (2001) Chemokines in cutaneous wound healing. J. Leukoc. Biol. 69, 513–521.
44 Mori, L., Bellini, A., Stacey, M. A., Schmidt, M. and Mattoli, S. (2005) Fibrocytes contribute to the myofibroblast population in wounded skin and originate from the bone marrow. Exp. Cell Res. 304, 81–90.
45 45. Bucala, R., Spiegel, L. A., Chesney, J., Hogan, M. and Cerami, A. (1994) Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. Mol. Med. 1, 71–81.
46 Dunphy, J. E. (1967) The healing of wounds. Can. J. Surg. 10, 281–287.
47 Yang, L., Scott, P. G., Dodd, C., Medina, A., Jiao, H., Shankowsky, H. A., Ghahary, A. and Tredget, E. E. (2005) Identification of fibrocytes in postburn hypertrophic scar. Wound Repair Regen. 13, 398–404.
48 Quan, T. E., Cowper, S. E. and Bucala, R. (2006) The role of circulating fibrocytes in fibrosis. Curr. Rheumatol. Rep. 8, 145–150.
49 Hartlapp, I., Abe, R., Saeed, R. W., Peng, T., Voelter, W., Bucala, R. and Metz, C. N. (2001) Fibrocytes induce an angiogenic phenotype in cultured endothelial cells and promote angiogenesis in vivo. FASEB J. 15, 2215–2224.
50 Chesney, J., Bacher, M., Bender, A. and Bucala, R. (1997) The peripheral blood fibrocyte is a potent antigen-presenting cell capable of priming naive T cells in situ. Proc. Natl. Acad. Sci. USA 94, 6307–6312.
51 Abe, R., Donnelly, S. C., Peng, T., Bucala, R. and Metz, C. N. (2001) Peripheral blood fibrocytes: differentiation pathway and migration to wound sites. J. Immunol. 166, 7556–7562.
52 Metz, C. N. (2003) Fibrocytes: a unique cell population implicated in wound healing. Cell. Mol. Life Sci. 60, 1342–1350.
53 Yang, L., Scott, P. G., Giuffre, J., Shankowsky, H. A., Ghahary, A. and Tredget, E. E. (2002) Peripheral blood fibrocytes from burn patients: identification and quantification of fibrocytes in adherent cells cultured from peripheral blood mononuclear cells. Lab. Invest. 82, 1183–1192.
54 Aiba, S. and Tagami, H. (1997) Inverse correlation between CD34 expression and proline-4-hydroxylase immunoreactivity on spindle cells noted in hypertrophic scars and keloids. J. Cutan. Pathol. 24, 65–69.
55 Schmidt, M., Sun, G., Stacey, M. A., Mori, L. and Mattoli, S. (2003) Identification of circulating fibrocytes as precursors of bronchial myofibroblasts in asthma. J. Immunol. 171, 380–389.
56 Fausto, N. (2000) Liver regeneration. Hepatology 32 (Suppl. 1), 19–31.
57 Goss, R. J. and Grimes, L. N. (1975) Epidermal downgrowths in regenerating rabbit ear holes. J. Morphol. 146, 533–542.
58 Douglas, B. S. (1972) Conservative management of guillotine amputation of the finger of children. Aust. Paediatr. J. 8, 86–89.
59 Illingworth, C. M. (1974) Trapped fingers and amputated finger tips in children. J. Pediatr. Surg. 9, 853–858.
60 Gardiner, D. M. and Bryant, S. V. (1996) Molecular mechanisms in the control of limb regeneration: the role of homeobox genes. Int. J. Dev. Biol. 40, 797–805.
61 Goss, R. J. and Holt, R. (1992) Epimorphic vs. tissue regeneration in Xenopus forelimbs. J. Exp. Zool. 261, 451–457.
62 Allen, S. P., Maden, M. and Price, J. S. (2002) A role for retinoic acid in regulating the regeneration of deer antlers. Dev. Biol. 251, 409–423.
63 Chai, Y. and Slavkin, H. C. (2003) Prospects for tooth regeneration in the 21st century: a perspective. Microsc. Res. Tech. 60, 469–79.
64 Clark, L. D., Clark, R. K. and Heber-Katz, E. (1998) A new murine model for mammalian wound repair and regeneration. Clin. Immunol. Immunopathol. 88, 35–45.
65 Rajnoch, C., Ferguson, S., Metcalfe, A. D., Herrick, S. E., Willis, H. S. and Ferguson, M. W. (2003) Regeneration of the ear following wounding in different mouse strains is dependent on the severity of wound trauma. Dev. Dyn. 226, 388–397.
66 Metcalfe, A. D. and Ferguson, M. W. J. (2005) Harnessing wound healing and regeneration for tissue engineering. Biochem. Soc. Trans. 33, 413–417.
67 Metcalfe, A. D., Willis, H., Beare, A. and Ferguson, M. W. (2006) Characterizing regeneration in the vertebrate ear. J. Anat. 209, 439–446.
68 Beare, A. H., Metcalfe, A. D. and Ferguson, M. W. (2006) Location of injury influences the mechanisms of both regeneration and repair within the MRL/MpJ mouse. J. Anat. 209, 547–559.

69 Colwell, A. S., Krummel, T. M., Kong, W., Longaker, M. T. and Lorenz, H. P. (2006) Skin wounds in the MRL/MPJ mouse heal with a scar. Wound Repair Regen. 14, 81–90.
70 Cowin, A. J., Holmes, T. M., Brosnan, M. P. and Ferguson, M. W. (2001) Expression of TGF-beta and its receptors in murine fetal and adult dermal wounds. Eur. J. Dermatol. 11, 424–431.
71 Cowin, A. J., Brosnan, M. P., Holmes, T. M. and Ferguson, M. W. (1998) Endogenous inflammatory response to dermal wound healing in the fetal and adult mouse. Dev. Dyn. 212, 385–393.
72 Ferguson, M. W. J., Whitby, D. J., Shah, M., Armstrong, J., Siebert, J. W. and Longaker, M. T. (1996) Scar formation: the spectral nature of fetal and adult wound repair. Plast. Reconstr. Surg. 97, 854–60.
73 Whitby, D. J. and Ferguson, M. W. (1991a) Immunohistochemical localization of growth factors in fetal wound healing. Dev. Biol. 147, 207–215.
74 Whitby, D. J. and Ferguson, M. W. (1991b) The extracellular matrix of lip wounds in fetal, neonatal and adult mice. Development 112, 651–668.
75 Shah, M., Foreman, D. M. and Ferguson, M. W. (1995) Neutralisation of TGF-beta 1 and TGF-beta 2 or exogenous addition of TGF-beta 3 to cutaneous rat wounds reduces scarring. J. Cell. Sci. 108, 985–1002.
76 Gawronska-Kozak, B. (2004) Regeneration in the ears of immunodeficient mice: identification and lineage analysis of mesenchymal stem cells. Tissue Eng. 10, 1251–1265.
77 Gawronska-Kozak, B., Bogacki, M., Rim, J. S., Monroe, W. T. and Manuel, J. A. (2006) Scarless skin repair in immunodeficient mice. Wound Repair Regen. 14, 265–276.
78 Shah, M., Foreman, D. M. and Ferguson, M. W. (1994) Neutralising antibody to TGF-beta 1,2 reduces cutaneous scarring in adult rodents. J. Cell Sci. 107, 1137–1157.
79 Habecker, B. A., Symes, A. J., Stahl, N., Francis, N. J., Economides, A., Fink, J. S., Yancopolous, G. D. and Landis, S. C. (1997) A sweat gland-derived differentiation activity acts through known cytokine signalling pathways. J. Biol. Chem. 272, 30421–30428.
80 Fu, X., Qu, Z. and Sheng, Z. (2006) Potentiality of mesenchymal stem cells in regeneration of sweat glands. J. Surg. Res. 136, 204–208.