Use of Novel Biomaterial Design and Stem Cell Therapy in Cutaneous Wound Healing

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3.1 Introduction

The spontaneous regenerative capacity of skin is dependent on the depth and area of cutaneous damage. This is the case as it dictates the extent of destruction of reparative basal and stem cell populations. Minor epidermal injury, where basement membrane and basal keratinocyte populations remain intact, results in rapid and complete cutaneous regeneration. However, partial-thickness (papillary dermal damage) and particularly full-thickness (papillary and reticular dermal damage) defects often heal through debilitating scar formation and contraction. Indeed, without surgical intervention the damage to physiological homeostasis resulting from large full-thickness wounds can be so acute that death may result. The rapid closure of such wounds is essential to restore the barrier functions of the skin and reduce scar formation (Cubison et al. 2006).

The clinical gold standard for the treatment of full-thickness wounds is the application of autologous split-thickness skin grafts, the well-known side effects of which include lack of donor site availability in severely injured patients and resulting

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donor site morbidity (MacNeil 2007; Groeber et al. 2011). Clearly this solution, although it remains an important life-saving tool, is far from ideal. This fact has resulted in the development and increasing implementation of engineered artificial skin substitutes. These substitutes exist in a diverse array of materials and designs ranging from inert synthetic polymer lattices (e.g. Biobrane™, Dow B. Hickam, Inc., USA) to biological matrices containing live cellular material (e.g. Orcel® (Ortec International, USA)). These products, although often successful in achieving wound closure, fall short in terms of resulting wound functionality, mechanical strength, aesthetics and cellular complexity and organisation (Kemp 2006). This is due in part to the top-down approach that arises when applying a model 'mature' tissue rather than constructing tissue de novo from progenitor cells, as in nature. Accordingly, recent research has focused on the local application, direction and manipulation of stem and progenitor cell populations to enhance the natural wound-healing response and direct cutaneous regeneration. This, it has been proposed, can be achieved in a number of ways (Discher et al. 2009). Acellular scaffolds could mobilise and attract resident adult stem cell populations through the release of growth factors/cytokines and presentation of cell attachment motifs in artificial niche environments (Discher et al. 2009). Once stem cells have been recruited to the wound site, cell fate decisions could be manipulated by presentation of dermis-specific cell attachment sites, further growth factor presentation and mechano-physical matrix properties (Discher et al. 2009). Alternatively, stem cells could be directly delivered to the wound. It is hypothesised that stem cells can augment cutaneous healing via (1) the enhancement of endogenous cell regenerative capacity through paracrine signalling, (2) direct cellular contribution through transdifferentiation and/or (3) cell fusion (Prockop et al. 2003; Spees et al. 2003; Stoff et al. 2009). Aside from safety concerns including tumour formation and disease transmission, the direct transplantation of stem cells is currently limited by the poor survival rates and low level of persistence of viable cells at transplant sites (Kolokol'chikova et al. 2001; Navsaria et al. 2004). At present, less than 5 % of cells survive initial engraftment (Discher et al. 2009), which is almost certainly due to harsh necrotic environment of the cutaneous wound milieu and the destruction of suitable stem cell niches. It is clear for stem cell therapy to be effective; the mode of cell delivery must confer some level of protection to maintain viability whilst allowing direct contact with, and migration into, the wound environment.

3.2   Stem Cell Populations for Cutaneous Wound Healing

Stem cells are defined by their ability to self-replicate and produce more specialised progeny (Schofield 1978; Lajtha 1979). Several populations of stem cells have been identified that hold potential for therapeutic use in cutaneous wound healing including embryonic stem cells, adult stem cells (in particular bone marrow-derived mesenchymal stem cells (BM-MSCs), bone marrow-derived hematopoietic stem cells (BM-HSCs), adipose-derived stem cells (Ad-SCs) and epidermis and hair follicle-derived stem cells (HF-SCs)) and induced pluripotent stem cells (iPSCs).

Each source has specific characteristics, markers and advantages, although all must overcome similar obstacles to become therapeutically successful, including safety concerns such as tumour formation and disease transmission as well as technical issues such as controlling cell fate decisions (Table 3.1).

3.3   Embryonic Stem Cells

Human embryonic stem cells can be derived from the inner cell mass of blastocysts, 5-day-old embryos or 4–8-day-old morula and are pluripotent (Thomson et al. 1998a). Uniquely, embryonic stem cells are capable of indefinite undifferentiated in vitro propagation (Beddington and Robertson 1989). Due to their source, the use of ESCs is surrounded by well-known public controversy and governed by stringent, complex legislation and regulations. Additionally, teratoma formation when using this stem cell source remains a serious safety concern (Yao et al. 2006). As such, stem cells alternatively sourced stem cell populations are favoured by researchers for regenerative medicine and wound-healing applications.

3.4   Adult Stem Cell Populations

Adult stem cells populations have been identified in almost all tissues in the human body, where their function is to maintain cell turnover and replace damaged cells (Hodgkinson et al. 2009). Within these tissues, stem cells reside in the basal layers of tissue in protective microenvironments termed 'stem cell niches'. The stem cell population within a tissue niche is maintained in a stable naïve state by cell–cell and cell–matrix binding, cell–matrix mechanical interaction and soluble factor gradients (Watt 2000). It is the recreation of these niche environments, and the signals, which establish and maintain them, which currently is a main research focus of tissue engineering and regenerative medicine (Fig. 3.1).

3.5   Bone Marrow-Derived Stem Cells

Bone marrow is a well-characterised reservoir of multipotent mesenchymal and haematopoietic stem cells (Crisan et al. 2008; Kränkel et al. 2011). Under physiological conditions, small numbers of stem cells migrate from the bone marrow, enter tissues and their niches maintaining a dynamic equilibrium (Kränkel et al. 2011). Bone marrow collection is an invasive procedure and the collected cells contain less than 0.05 % stem cells (Kita et al. 2010; Pittenger et al. 1999). Haematopoietic stem cell therapy was the first available stem cell therapy (Wagner and Gluckman 2010). The local and systemic delivery of BM-MSCs are potentially effective treatments to heal acute and particularly chronic wounds (Fu et al. 2006; Badiavas and Falanga 2003; Falanga et al. 2007). Proof of principle applications of BM-MSCs to cutaneous defects have demonstrated significant wound closure acceleration in

Table 3.1 Selected stem cell sources, selected markers and advantages/disadvantages for therapeutic use in cutaneous wound healing

Stem cell population location	Selected cell markers	Advantages	Disadvantages	References
Embryonic	Positive for SSEA-4, TRA-1-60, TRA-1-82, AC133, c-kit (CD117), flt3 (CD135), CD9, Oct3/4, Sox2, Nanog	Pluripotent Can be propagated indefinitely Some demonstrated immune-privileged properties Open to genetic manipulation	Teratoma formation Legal/ethical restrictions Currently allogeneic only – immunosuppressive therapy required Difficult to isolate	Thomson et al. (1998b), Knoepfler (2009), Schlüter and Kaur (2009), Carpenter et al. (2004, 2009), Andrews et al. (1984), Kaufman et al. (2001), Hoffman and Carpenter (2005)
Bone marrow				
Mesenchymal	Positive for CD73, CD90, CD105, CD106, Stro-1, CD29, CD166, CD146, CD44 Negative for CD34, CD45, CD14	Multipotent Demonstrate immune-privileged properties – no immunosuppressive therapy required	Careful control of differentiation needed Extraction can be painful and low yield	Togel and Westenfelder (2007), Arthur et al. (2009), Dennis et al. (2002), Osawa et al. (1996), Larochelle et al. (1996), Kawashima et al. (1996), Michallet et al. (2000), Goodell (1996), Uchida et al. (2001)
Hematopoietic	Positive for CD133, Bcrp-1, CD34 (variable) Negative for CD38, Lin	Can be autologous Recruited in natural wound healing	Allogeneic transplantation risks disease transmission	

Adipose

	Markers	Advantages	Disadvantages	References
Mesenchymal	Positive for CD13, CD29, CD44, CD49e, CD54, CD55, CD63, CD73, CD90, CD105, CD144, CD146, CD166. Negative for: CD11b, CD14, CD19, CD31 (variable), CD34 (variable), CD45, Stro-1 (variable), CD3, CD117, CD62L, CD95L	Available in large quantities and easily harvested. Most abundant tissue source of adult stem cells. Demonstrate immune-privileged properties – no immunosuppressive therapy required. Can be autologous	Body site source and age-dependent properties. Allogeneic transplantation risks disease transmission	Pittenger et al. (1999), Deans and Moseley (2000), Gronthos et al. (2001)

Hair follicle

	Markers	Advantages	Disadvantages	References
Epithelial (bulge)	Positive for CK15, CK19, CD200, PHLDA1, follistatin, Frizzled homolog 1. Negative for CD34, CD24, CD71, CD146, connexin43 and nestin	Physiologically involved in the repair of damaged dermis and epidermis. Several populations identified	May be destroyed in patients with large % of TBSA injuries. May require in vitro expansion to obtain sufficient cell quantities	Kloepper et al. (2008), Hoogduijn et al. (2006), Tiede et al. (2007)
Mesenchymal	Positive for CD44, CD73, CD90, CD34, Lef-1	Easily extracted		

Induced pluripotent stem cells

	Markers	Advantages	Disadvantages	References
	Positive for SSEA-4, TRA-1-60, TRA-1-82, AC133, c-kit (CD117), flt3 (CD135), CD9, Oct3/4, Sox2, Nanog	Autologous pluripotent cells generated from somatic cells. In vitro expansion required	Long-term safety unknown. No standard practices or procedures in place	Takahashi and Yamanaka (2006), Takahashi et al. (2007), Nakagawa et al. (2008)
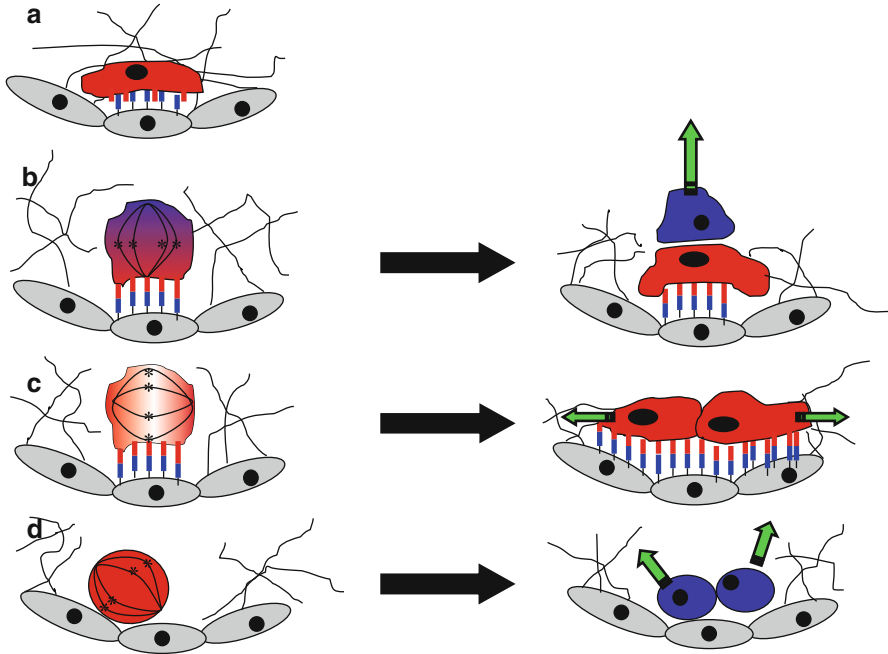
Fig, 3.1 Schematic mechanisms of stem cell niche maintenance. (**a**) Stem cells maintained in relative quiescence. (**b**) Asymmetric cell division can be controlled by cell–cell and cell–matrix binding, which can segregate cell fate factors in the cytoplasm. The orientation of the spindle results in only one of the progenitor cells remaining in contact with the niche cells, whilst the other daughter cell is differentiated and moves out of the niche. (**c**) When the niche is in need of repopulation, both progenitor cells are able to maintain contact with the niche cells and are exposed to the same microenvironment. In this case both cells retain stemness and the stem cell pool is expanded. (**d**) When the niche signalling environment is disrupted or when contact with niche cells is lost, symmetric cell division resulting in two differentiated cells can occur (Modified from Lutolf and Blau 2009)

mice/rats (Falanga et al. 2007; Wu et al. 2007; Amann et al. 2009; Chen et al. 2008; McFarlin et al. 2006) and in humans (Badiavas and Falanga 2003; Falanga et al. 2007; Ichioka et al. 2005) increased epithelialisation and angiogenesis (Wu et al. 2007; Sasaki et al. 2008; Javazon et al. 2007). Despite the lack of blinded studies in the literature, therapeutic potential is evident. Whilst others have identified limitations, for example, in the treatment of severe burns where sulphadiazine toxicity (Gamelli et al. 1993) and sepsis (Gamelli et al. 1995) significantly suppress response to BM-MSCs.

3.6   Adipose-Derived Stem Cells

Adipose-derived stem cells that possess characteristics which are similar to MSCs represent the most abundant adult tissue population (Kim et al. 2007). Liposuction surgery offers opportunity to obtain volumes of anywhere from 100 mL to >3 L

of adipose tissue in the form of lipoaspirate, which is normally discarded (Katz et al. 1999; Bunnell et al. 2008). The stem cell yield from such a procedure is typically around 400,000 viable cells per ml extracted (Zuk et al. 2002; Guilak et al. 2006). Importantly, it is thought that in vitro expansion of Ad-SCs can yield 100–1,000 times more progenitor cells than isolation from bone marrow (Utsunomiya et al. 2011). Recently, Ad-SCs have been reported to promote human dermal fibroblast proliferation through both direct cell–cell contact signalling and secretory paracrine activation, which in turn accelerated wound re-epithelialisation (Kim et al. 2007). It is likely that physiologically Ad-SCs play a crucial role in the healing of full-thickness skin damage through direct migration from subcutaneous adipose tissue. Additionally, adipose tissue acts as an endocrine organ, secreting numerous hormones, growth factors and cytokines such as leptin; epidermal growth factor (EGF); tumour necrosis factor-α (TNF-α); basic fibroblast growth factor (bFGF); keratinocyte growth factor (KGF); transforming growth factor $\beta1$ (TGF-$\beta1$); vascular endothelial growth factor (VEGF); hepatocyte growth factor (HGF); interleukins (IL)-6, IL-7, IL-8, IL-11 and IL-12; macrophage-colony stimulation factor; and platelet-derived growth factor (PDGF) (Utsunomiya et al. 2011; Witkowska-Zimny and Walenko 2011).

## 3.7   Hair Follicle-Derived Stem Cells

Due to their ease of access and demonstrated multi-(and even possibly pluri-) potency, HF-SCs could be a promising population for application in cutaneous wound healing. Utilising HF-SCs, located mainly in the hair follicle bulge, has the added advantage of being naturally involved in healing both epidermal and dermal injuries (Ito et al. 2005; Jahoda and Reynolds 2001). Following cutaneous injury HF-SCs are involved in the secretion of HGF, EGF and in particular heparin-binding (HB)-EGF, FGF-7 and FGF10, which activate the STAT3 and AP1 signalling pathways, promoting re-epithelialisation (Gurtner et al. 2008).

3.8   Induced Pluripotent Stem Cells

The research interest in iPSCs since the original breakthrough by Takahashi and Yamanaka has been intensive (Takahashi and Yamanaka 2006). Their initial reprogramming of mouse embryonic fibroblasts through the enforced expression of four transcription factor genes (*OCT3/4, Sox2, Klf4, and c-Myc* (Takahashi and Yamanaka 2006; Müller et al. 2009)) has since led to the generation of human iPSCs epigenetically and developmentally indistinguishable from ES cells (Maherali et al. 2007; Okita et al. 2007; Park et al. 2007; Wernig et al. 2007; Liu et al. 2008). The generation method has been refined by the dispensing of *c-Myc* which has led to a reduction in malignant transformation of iPSC derivatives (Müller et al. 2009). To address safety fears of retroviral gene transfer-induced mutagenesis, alternative viral-free methods for the induction of pluripotency have been developed. This has been achieved through the repeated addition of
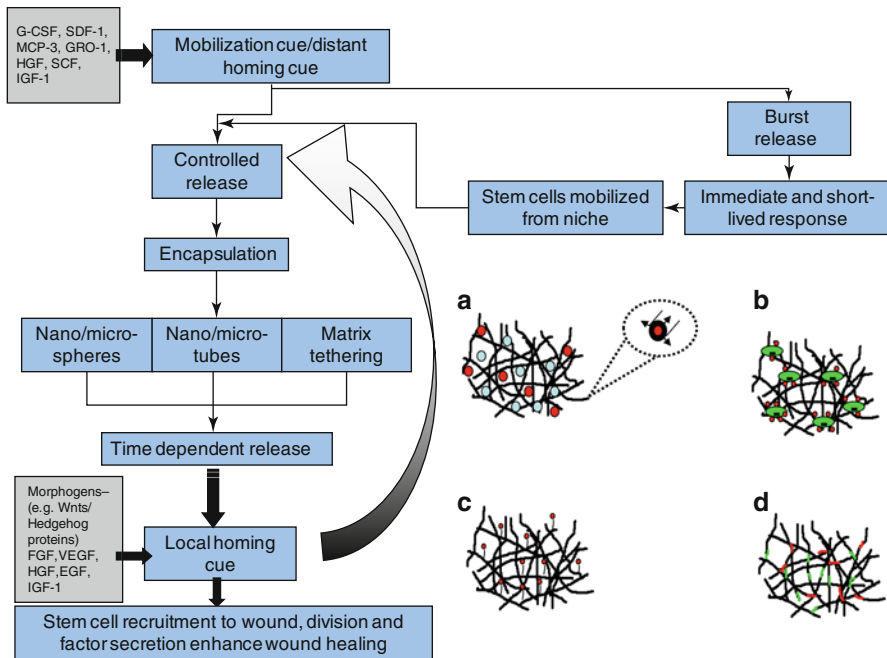
Fig. 3.2 Flow chart showing series of events to recruit endogenous stem cells to wound. (**a**) Protective encapsulation allows controlled release through diffusion and/or enzymatic degradation (*inset*) nanotube encapsulation. (**b**) Ionic binding and sequestering of factors, localising factors to the matrix. (**c**) Covalently tethered factors, released through enzymatic cleavage. (**d**) Cell adhesion sites and niche-specific matrix molecules (e.g. laminin, heparan sulphate)

polyarginine protein transduction-tagged recombinant proteins (Zhou et al. 2009; Kim et al. 2009) and with highly basic HIV-TAT-derived basic peptide sequences (Kim et al. 2009). Several serious safety concerns, such as tumour formation potential, remain to be addressed prior to clinical application of IPSCs. Despite this, it is likely that stem cells obtained through pluripotent induction in some form are likely to be therapeutically important in the future.

3.9 Biomaterial Transplantation-Induced Homing of Endogenous Stem Cell Populations for Cutaneous Repair

The implantation of an acellular biomaterial that possesses the ability to mobilise, attract and manipulate endogenous stem cell populations offers significant potential benefits over in vivo delivery of viable stem cells. Such cell-homing devices would be less costly and complex to produce, have longer shelf life and increased safety whilst avoiding the need for allogeneic transplant or autologous tissue harvest (Fig. 3.2).

3.10   Mobilisation and Navigation Cues

On implantation into the wound site, the scaffold must release a stimulatory mobilisation signal which when received by ASCs causes them to exit their niche, whether this is in the uninjured surrounding tissue or nonlocal niches, e.g. bone marrow. Physiologically, following wound healing, such stem cell mobilisation naturally occurs both locally and from bone marrow-derived stem cells, which then go on to contribute to the healing wound (Wu et al. 2007; Badiavas et al. 2003). This relies on growth factor, cytokine release and cellular expression of the relevant receptors; however, the precise regulatory mechanisms that co-ordinate this response are incompletely understood (Yoshikawa et al. 2008; Wang et al. 2007; Sordi 2009). In an effort to enhance this natural signal, a plethora of growth factors and cytokines have been trialled in preclinical and clinical studies. At present, granulocyte colony-stimulating factor (G-CSF) is the most widely utilised stem cell mobilisation agent in the clinic (Chen et al. 2011). This growth factor also serves as a good example of the cautionary approach that must accompany the administration of growth factors as it is also linked with inflammatory cell mobilisation and promotion of atherosclerosis (Kränkel et al. 2011; Chen et al. 2011). Aside from G-CSF, it appears that a degree of specificity in recruitment targets can be conferred by the choice of growth factors. Stromal-derived factor-1 (SDF-1) recruits cells expressing its receptor CXCR4 (cytokine receptor type 4) such as haematopoietic stem cells (HSCs), endothelial progenitor cells (EPCs), cardiac stem cells (CSCs) and MSCs (Askari et al. 2003; Tang et al. 2005; Schenk et al. 2007; Hohensinner et al. 2009; Haider et al. 2008; Unzek et al. 2007; Lapidot 2001). Monocyte chemoattractant protein-3 (MCP-3) mobilises MSCs (Schenk et al. 2007), growth-related oncogene-1 (GRO-1) attracts bone marrow-derived EPCs (Kocher et al. 2006), hepatocyte growth factor (HGF) targets myoblasts (O'Blenes et al. 2010), and stem cell factor (SCF) and insulin growth factor-1 (IGF-1) activate cardiac stem cells (CSCs) (Kuang et al. 2008; Guo et al. 2009; Hohenstein et al. 2010). SDF-1α/CXCL12 appears to be pivotal in the mobilisation following injury in a number of tissues.

3.11   Local Navigation Cues and Cell Binding: Designing the Artificial Stem Cell Niche

Locally to the wound site, further signalling molecules are required to direct the recruited stem cells through the implanted scaffold and within the wound. This could be created through localised cytokine/growth factor gradients, matrix-tethered factors or incorporation of specific cell attachment motifs. The list of soluble local navigation candidate molecules is long and includes morphogens like Wnts and hedgehog proteins alongside growth factors like FGF (Lutolf and Blau 2009). Importantly for biomaterial design, soluble growth factors in the physiological niche are electrostatically bound to heparan sulphate proteoglycans (e.g. heparin), localising their action to the niche and serving as a protective reservoir, preventing proteolytic degradation (Lutolf and Blau 2009; Ramirez and Rifkin 2003).
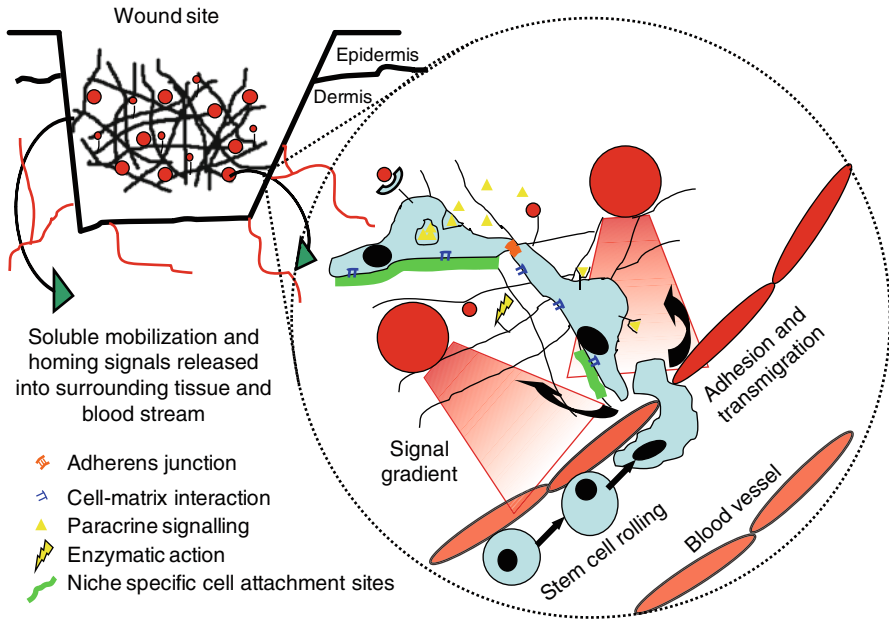
Fig. 3.3 Schematic representation of stem cell migration stimulated by biomaterial factor release. Stem cells recruited migrate through vascular system adjacent to implanted factor releasing biomaterial. Upon signal-receptor activation cell adhere and transmigrate into biomaterial along signalling gradients. Cells adhere to matrix to niche-specific matrix and to each other through adherens junctions. Cells secrete paracrine and autocrine factors, enzymatically release tethered matrix factors and directly contribute to wound healing through asymmetric cell division

Mimicking such associations through incorporation of relevant proteoglycans and glycosaminoglycans (GAGs) into biomaterials may provide a simple and effective way of delivering tissue-specific localised stem cell cues (Fig. 3.3).

Problematically, the required signalling factors have short half-lives in vivo and consequently they must be protected from degradation and released in a controlled way to sustain effect. To achieve this several strategies have been proposed. The majority of those involve the protective encapsulation of growth factors, which are then released either passively through diffusion or polymer breakdown or through active enzymatic degradation. Modes of encapsulation are mainly in the form of polymer spheres or polymer fibres on the micro-nano scale. By controlling the encapsulated 'payload' of bioactive factor and the degradation profile/diffusion properties of the encapsulating polymer, the delivery of the factor can be controlled. Delivering a cocktail of growth factors/cytokines with differing release profiles and concentrations could make the effective local control of recruited cells possible.

In the case of micro-nanofibre encapsulation, the bioactive soluble factors are contained within the supporting matrix itself. One promising method of fibre production on this scale is electrospinning. This process involves the application of a high-voltage electrical current to a droplet of polymer solution or melt. When the repulsive

charge generated inside the droplet is sufficient to overcome the surface tension of the fluid, a liquid jet is expelled from the tip of the droplet towards a grounded collector plate. In transit to the ground electrode, the fluid jet is stretched by inertial and electrostatic forces and the solvent evaporates. A dry uniform fibre, typically in the nano-micrometre range, can be collected (Ayres et al. 2010). This scale corresponds to that of natural ECM fibres and has been reported to increase cell–cell and cell–matrix interactions (Li et al. 2002; He et al. 2007). Bioactive molecules can be blended with spinning solutions prior to deposition, depending on spinning parameters and solvent systems used, and have been shown to maintain their action both in vitro and in vivo (Fu et al. 2008). Alternatively, core–shell fibres can be obtained by the use of a concentric spinneret and two-fluid spinning system (Jiang et al. 2005) allowing greater control of encapsulation and release of desired bioactive factors.

The electrospinning technique could be easily adapted for the creation of a sequentially layered scaffold (Ayres et al. 2010), mimicking the layered structure of the skin. This could be achieved by the adjustment of spinning process parameters, the spinning solution used and directing fibre alignment. Interestingly, Yang and co-workers electrospun viable human dermal fibroblasts and keratinocytes (Yang et al. 2009), although further investigation is needed to confirm cells were unaltered by the process.

3.12 Cell Adhesion Sites and Mimicking the Spatial Complexity of Signalling in the Niche

Establishment of signalling gradient polarity in a complementary but spatially discrete manner is essential for the maintenance of engineered niche environments. To date, attempts to engineer these structures have centred on 2D systems or simplified simulated 3D environments. Biomaterial technologies such as atomic force dip-pen lithography (Piner et al. 1999), stencil lithography surface patterning (Kim et al. 2003) and inkjet and microcontact printers (Roth et al. 2004; Théry et al. 2007) hold promise for the precise spatial patterning of factors. Inkjet printing has successfully been used to create 2D artificial niches by the patterning of immobilised BMP-2 which inducted mesenchymal progenitors to differentiate osteogenically, where in off-pattern sections myogenic differentiation was observed (Phillippi et al. 2008). In particular, inkjet systems have received particular attention but several limitations in the current methodology exist. Importantly, the concentrations of ECM-based 'bioinks' is limited by constraints on solution viscosities, surface tensions and densities required by printing set-ups (Mironov et al. 2006). In turn, this restricts the mechanical properties of 3D printed materials where bioinks must provide support to the layers deposited above them. It has been suggested that to improve the structural support of 3D bioprinted scaffolds, a combined materials approach is required where bioink is deposited onto a bio-paper and sequentially layered (Nakamura et al. 2005; Mironov et al. 2009). This bio-paper could, for example, be in the form of a nanofibrous electrospun polymer mat, which itself could be engineered to provide important biological cues.

Conclusions and Future Perspectives

The activation, homing and control of endogenous stem cells through novel biomaterial design have become significant goals of tissue engineering and regenerative medicine. Several material processing techniques now exist that allow the biomimicry of important biological structures. Electrospinning, most prominently amongst other fibre formation techniques, allows the creation of fibres on the same scale as natural ECM (50–500 nm). Micro-nanosphere factor encapsulation allows the controlled and sustained release of soluble factors, which can be used for local and systemic recruitment and homing of stem cells. The identification of exactly which factors should be delivered and when remains to be seen and could be variable depending on the type of cutaneous wound that requires treatment, e.g. a chronic wound environment may be different from a burn. High-throughput techniques will allow the screening of the identified factors, and others, in different combinations to maximise their effect. The ability to engineer biomaterials with increasingly accurate cell signalling capabilities will continue, with a feasible endpoint being the precise delivery of only the required factors only where they are needed, only when they needed.

References

Amann B et al (2009) Autologous bone marrow cell transplantation increases leg perfusion and reduces amputations in patients with advanced critical limb ischemia due to peripheral artery disease. Cell Transplant 18(3):371–380

Andrews PW et al (1984) Pluripotent embryonal carcinoma clones derived from the human teratocarcinoma cell line Tera-2. Differentiation in vivo and in vitro. Lab Invest 50(2):147

Arthur A, Zannettino A, Gronthos S (2009) The therapeutic applications of multipotential mesenchymal/stromal stem cells in skeletal tissue repair. J Cell Physiol 218(2):237–245

Askari AT et al (2003) Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. Lancet 362(9385):697–703

Ayres CE et al (2010) Nanotechnology in the design of soft tissue scaffolds: innovations in structure and function. Wiley Interdiscip Rev Nanomed Nanobiotechnol 2(1):20–34

Badiavas EV, Falanga V (2003) Treatment of chronic wounds with bone marrow-derived cells. Arch Dermatol 139(4):510

Badiavas EV et al (2003) Participation of bone marrow derived cells in cutaneous wound healing. J Cell Physiol 196(2):245–250

Beddington R, Robertson E (1989) An assessment of the developmental potential of embryonic stem cells in the midgestation mouse embryo. Development 105(4):733–737

Bunnell BA et al (2008) Adipose-derived stem cells: isolation, expansion and differentiation. Methods 45(2):115–120

Carpenter MK et al (2004) Properties of four human embryonic stem cell lines maintained in a feeder-free culture system. Dev Dyn 229(2):243–258

Carpenter MK, Frey-Vasconcells J, Rao MS (2009) Developing safe therapies from human pluripotent stem cells. Nat Biotechnol 27(7):606–613

Chen L et al (2008) Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. PLoS One 3(4):e1886

Chen FM et al (2011) Homing of endogenous stem/progenitor cells for in situ tissue regeneration: promises, strategies, and translational perspectives. Biomaterials 32:3189–3209

Crisan M et al (2008) A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 3(3):301–313

Cubison T, Pape SA, Parkhouse N (2006) Evidence for the link between healing time and the development of hypertrophic scars (HTS) in paediatric burns due to scald injury. Burns 32(8):992–999

Deans RJ, Moseley AB (2000) Mesenchymal stem cells biology and potential clinical uses. Exp Hematol 28(8):875–884

Dennis JE et al (2002) The STRO-1+ marrow cell population is multipotential. Cells Tissues Organs 170:73–82

Discher DE, Mooney DJ, Zandstra PW (2009) Growth factors, matrices, and forces combine and control stem cells. Science 324(5935):1673

Falanga V et al (2007) Autologous bone marrow-derived cultured mesenchymal stem cells delivered in a fibrin spray accelerate healing in murine and human cutaneous wounds. Tissue Eng 13(6):1299–1312

Fu X et al (2006) Enhanced wound-healing quality with bone marrow mesenchymal stem cells autografting after skin injury. Wound Repair Regen 14(3):325–335

Fu YC et al (2008) Optimized bone regeneration based on sustained release from three-dimensional fibrous PLGA/HAp composite scaffolds loaded with BMP-2. Biotechnol Bioeng 99(4):996–1006

Gamelli RL, Paxton TP, O'Reilly M (1993) Bone marrow toxicity by silver sulfadiazine. Surg Gynecol Obstet 177(2):115

Gamelli RL, He LK, Liu H (1995) Recombinant human granulocyte colony-stimulating factor treatment improves macrophage suppression of granulocyte and macrophage growth after burn and burn wound infection. J Trauma 39(6):1141

Goodell MA (1996) Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. J Exp Med 183(4):1797–1806

Groeber F et al (2011) Skin tissue engineering-in vivo and in vitro applications. Adv Drug Deliv Rev 63(4–5):352–366

Gronthos S et al (2001) Surface protein characterization of human adipose tissue-derived stromal cells. J Cell Physiol 189(1):54–63

Guilak F et al (2006) Clonal analysis of the differentiation potential of human adipose-derived adult stem cells. J Cell Physiol 206(1):229–237

Guo J et al (2009) Ischaemia/reperfusion induced cardiac stem cell homing to the injured myocardium by stimulating stem cell factor expression via NF-κB pathway. Int J Exp Pathol 90(3):355–364

Gurtner GC et al (2008) Wound repair and regeneration. Nature 453(7193):314–321

Haider HK et al (2008) IGF-1–overexpressing mesenchymal stem cells accelerate bone marrow stem cell mobilization via paracrine activation of SDF-1α/CXCR4 signaling to promote myocardial repair. Circ Res 103(11):1300–1308

He W, Horn SW, Hussain MD (2007) Improved bioavailability of orally administered mifepristone from PLGA nanoparticles. Int J Pharm 334(1–2):173–178

Hodgkinson T, Yuan XF, Bayat A (2009) Adult stem cells in tissue engineering. Expert Rev Med Devices 6(6):621–640

Hoffman LM, Carpenter MK (2005) Characterization and culture of human embryonic stem cells. Nat Biotechnol 23(6):699–708

Hohensinner P et al (2009) The inflammatory mediator oncostatin M induces stromal derived factor-1 in human adult cardiac cells. FASEB J 23(3):774

Hohenstein B et al (2010) Enhanced progenitor cell recruitment and endothelial repair after selective endothelial injury of the mouse kidney. Am J Physiol Renal Physiol 298(6):F1504–F1514

Hoogduijn MJ, Gorjup E, Genever PG (2006) Comparative characterization of hair follicle dermal stem cells and bone marrow mesenchymal stem cells. Stem Cells Dev 15(1):49–60

Ichioka S et al (2005) Bone marrow-impregnated collagen matrix for wound healing: experimental evaluation in a microcirculatory model of angiogenesis, and clinical experience. Br J Plast Surg 58(8):1124–1130

Ito M et al (2005) Stem cells in the hair follicle bulge contribute to wound repair but not to homeo-stasis of the epidermis. Nat Med 11(12):1351–1354

Jahoda CAB, Reynolds AJ (2001) Hair follicle dermal sheath cells: unsung participants in wound healing. Lancet 358(9291):1445–1448

Javazon EH et al (2007) Enhanced epithelial gap closure and increased angiogenesis in wounds of diabetic mice treated with adult murine bone marrow stromal progenitor cells. Wound Repair Regen 15(3):350–359

Jiang H et al (2005) A facile technique to prepare biodegradable coaxial electrospun nanofibers for controlled release of bioactive agents. J Control Release 108(2–3):237–243

Katz AJ et al (1999) Emerging approaches to the tissue engineering of fat. Clin Plast Surg 26(4):587

Kaufman DS et al (2001) Hematopoietic colony-forming cells derived from human embryonic stem cells. Proc Natl Acad Sci 98(19):10716

Kawashima I et al (1996) CD34+ human marrow cells that express low levels of Kit protein are enriched for long-term marrow-engrafting cells. Blood 87(10):4136

Kemp P (2006) History of regenerative medicine: looking backwards to move forwards. Regen Med 1(5):653–669

Kim GM, Van Den Boogaart MAF, Brugger J (2003) Fabrication and application of a full wafer size micro/nanostencil for multiple length-scale surface patterning. Microelectron Eng 67:609–614

Kim WS et al (2007) Wound healing effect of adipose-derived stem cells: a critical role of secre-tory factors on human dermal fibroblasts. J Dermatol Sci 48(1):15–24

Kim D et al (2009) Generation of human induced pluripotent stem cells by direct delivery of repro-gramming proteins. Cell Stem Cell 4:236–257

Kita K et al (2010) Isolation and characterization of mesenchymal stem cells from the sub-amniotic human umbilical cord lining membrane. Stem Cells Dev 19(4):491–502

Kloepper JE et al (2008) Immunophenotyping of the human bulge region: the quest to define useful in situ markers for human epithelial hair follicle stem cells and their niche. Exp Dermatol 17(7):592

Knoepfler PS (2009) Deconstructing stem cell tumorigenicity: a roadmap to safe regenerative medicine. Stem Cells 27(5):1050–1056

Kocher A et al (2006) Myocardial homing and neovascularization by human bone marrow angio-blasts is regulated by IL-8/Gro CXC Chemokines. J Mol Cell Cardiol 40(4):455–464

Kolokol'chikova E et al (2001) Morphological changes in burn wounds after transplantation of allogenic fibroblasts. Bull Exp Biol Med 131(1):89–93

Kränkel N et al (2011) Targeting stem cell niches and trafficking for cardiovascular therapy. Pharmacol Ther 129(1):62–81

Kuang D et al (2008) Stem cell factor/c-kit signaling mediated cardiac stem cell migration via activation of p38 MAPK. Basic Res Cardiol 103(3):265–273

Lajtha L (1979) Stem cell concepts. Differentiation 14(1–3):23–33

Lapidot T (2001) Mechanism of human stem cell migration and repopulation of NOD/SCID and B2mnull NOD/SCID mice. Ann N Y Acad Sci 938(1):83–95

Larochelle A et al (1996) Identification of primitive human hematopoietic cells capable of repopu-lating NOD/SCID mouse bone marrow: implications for gene therapy. Nat Med 2(12):1329–1337

Li WJ et al (2002) Electrospun nanofibrous structure: a novel scaffold for tissue engineering. J Biomed Mater Res A 60(4):613–621

Liu H et al (2008) Generation of induced pluripotent stem cells from adult rhesus monkey fibroblasts. Cell Stem Cell 3(6):587–590

Lutolf MP, Blau HM (2009) Artificial stem cell niches. Adv Mater 21(32–33):3255–3268

MacNeil S (2007) Progress and opportunities for tissue-engineered skin. Nature 445(7130):874–880

Maherali N et al (2007) Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. Cell Stem Cell 1(1):55–70

McFarlin K et al (2006) Bone marrow-derived mesenchymal stromal cells accelerate wound healing in the rat. Wound Repair Regen 14(4):471–478

Michallet M et al (2000) Transplantation with selected autologous peripheral blood CD34+ Thy1+ hematopoietic stem cells (HSCs) in multiple myeloma impact of HSC dose on engraftment, safety, and immune reconstitution. Exp Hematol 28(7):858–870

Mironov V, Reis N, Derby B (2006) Review: bioprinting: a beginning. Tissue Eng 12(4):631–634

Mironov V et al (2009) Organ printing: tissue spheroids as building blocks. Biomaterials 30(12):2164–2174

Müller LUW, Daley GQ, Williams DA (2009) Upping the ante: recent advances in direct reprogramming. Mol Ther 17(6):947–953

Nakagawa M et al (2008) Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. Nat Biotechnol 26(1):101–106

Nakamura M et al (2005) Biocompatible inkjet printing technique for designed seeding of individual living cells. Tissue Eng 11(11–12):1658–1666

Navsaria HA et al (2004) The role of allogenic fibroblasts in an acute wound healing model. Plast Reconstr Surg 113(6):1719

O'Blenes SB et al (2010) Engraftment is optimal when myoblasts are transplanted early: the role of hepatocyte growth factor. Ann Thorac Surg 89(3):829

Okita K, Ichisaka T, Yamanaka S (2007) Generation of germline-competent induced pluripotent stem cells. Nature 448(7151):313–317

Osawa M et al (1996) Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. Science 273(5272):242

Park IH et al (2007) Reprogramming of human somatic cells to pluripotency with defined factors. Nature 451(7175):141–146

Phillippi JA et al (2008) Microenvironments engineered by inkjet bioprinting spatially direct adult stem cells toward muscle- and bone-like subpopulations. Stem Cells 26(1):127–134

Piner RD et al (1999) " Dip-pen" nanolithography. Science 283(5402):661

Pittenger MF et al (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284(5411):143

Prockop DJ, Gregory CA, Spees JL (2003) One strategy for cell and gene therapy: harnessing the power of adult stem cells to repair tissues. Proc Natl Acad Sci U S A 100(Suppl 1):11917

Ramirez F, Rifkin DB (2003) Cell signaling events: a view from the matrix. Matrix Biol 22(2):101–107

Roth EA et al (2004) Inkjet printing for high-throughput cell patterning. Biomaterials 25(17):3707–3715

Sasaki M et al (2008) Mesenchymal stem cells are recruited into wounded skin and contribute to wound repair by transdifferentiation into multiple skin cell type. J Immunol 180(4):2581

Schenk S et al (2007) Monocyte chemotactic protein 3 is a myocardial mesenchymal stem cell homing factor. Stem Cells 25(1):245–251

Schlüter H, Kaur P (2009) Bioengineered human skin from embryonic stem cells. Lancet 374(9703):1725–1726

Schofield R (1978) The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells 4(1–2):7

Sordi V (2009) Mesenchymal stem cell homing capacity. Transplantation 87(9S):S42

Spees JL et al (2003) Differentiation, cell fusion, and nuclear fusion during ex vivo repair of epithelium by human adult stem cells from bone marrow stroma. Proc Natl Acad Sci 100(5):2397

Stoff A et al (2009) Promotion of incisional wound repair by human mesenchymal stem cell transplantation. Exp Dermatol 18(4):362–369

Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126(4):663–676

Takahashi K et al (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131(5):861–872

Tang YL et al (2005) Mobilizing of haematopoietic stem cells to ischemic myocardium by plasmid-mediated stromal-cell-derived factor-1 [alpha] treatment. Regul Pept 125(1–3):1–8

Théry M et al (2007) Experimental and theoretical study of mitotic spindle orientation. Nature 447(7143):493–496

Thomson JA et al (1998) Embryonic stem cell lines derived from human blastocysts. Science 282(5391):1145

Tiede S et al (2007) Hair follicle stem cells: walking the maze. Eur J Cell Biol 86(7):355–376

Togel F, Westenfelder C (2007) Adult bone marrow-derived stem cells for organ regeneration and repair. Dev Dyn 236(12):3321–3331

Uchida N et al (2001) Transplantable hematopoietic stem cells in human fetal liver have a CD34+ side population (SP) phenotype. J Clin Invest 108(7):1071–1077

Unzek S et al (2007) SDF-1 recruits cardiac stem cell-like cells that depolarize in vivo. Cell Transplant 16(9):879–886

Utsunomiya T et al (2011) Human adipose-derived stem cells: potential clinical applications in surgery. Surg Today 41(1):18–23

Wagner JE, Gluckman E (2010) Umbilical cord blood transplantation: the first 20 years. Semin Hematol 47(1):3–12

Wang Y et al (2007) Stem cell transplantation: a promising therapy for Parkinson's disease. J Neuroimmune Pharmacol 2(3):243–250

Watt FM (2000) Out of Eden: stem cells and their niches. Science 287(5457):1427

Wernig M et al (2007) In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. Nature 448(7151):318–324

Witkowska-Zimny M, Walenko K (2011) Stem cells from adipose tissue. Cell Mol Biol Lett 16:236–257

Wu Y et al (2007) Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. Stem Cells 25(10):2648–2659

Yang X, Shah JD, Wang H (2009) Nanofiber enabled layer-by-layer approach toward three-dimensional tissue formation. Tissue Eng Part A 15(4):945

Yao S et al (2006) Long-term self-renewal and directed differentiation of human embryonic stem cells in chemically defined conditions. Natl Acad Sci U S A 103:6907–6912

Yoshikawa T et al (2008) Wound therapy by marrow mesenchymal cell transplantation. Plast Reconstr Surg 121(3):860

Zhou H et al (2009) Generation of induced pluripotent stem cells using recombinant proteins. Cell Stem Cell 4:236–257

Zuk PA et al (2002) Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 13(12):4279–4295