Topical Stem and Progenitor Cell Therapy for Diabetic Foot Ulcers

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1. Introduction

The prevalence of diabetes mellitus is increasing to epidemic proportions worldwide. Diabetic foot ulceration can affect up to 25 percent of people with diabetes mellitus throughout their lives. The most significant complication of foot ulceration is lower limb amputation, which arises from pre-existing ulcers in the majority of cases. Despite current clinical care protocols for ulcer treatment, there exists a high amputation rate. This presents a major burden for individual patients' health and well-being in addition to significant financial cost for health care systems. There is an urgent need for new medicinal products to treat diabetic ulcers. Cell-based therapies offer a novel treatment strategy to augment diabetic wound healing, increase ulcer healing rate and prevent amputation. The field of tissue engineering has developed commercially available skin substitutes for diabetic cutaneous wound repair. These products have incorporated somatic cells delivered in a bioengineered scaffold. However, having been available for the last decade, the majority have demonstrated only moderate clinical benefit in small clinical trials. In comparison, stem and progenitor cell therapy offer the potential for accelerated wound repair in addition to structural skin regeneration with functional recovery.

Stem cells have the ability to self-renew and differentiate into other cell types and are classified into adult stem and progenitor cells, embryonic stem cells and induced pluripotent stem cells. The mechanisms of action of stem and progenitor cells are not fully elucidated but include 1) differentiation to specialised cells e.g. skin cells of the dermis and epidermis 2) acting by paracrine or autocrine effects through the secretion of trophic factors e.g. the production of soluble mediators for neo-angiogenesis and 3) immuno-modulatory functions. Much research endeavour is determining the benefit of stem cell treatment on diabetic cutaneous wound healing with encouraging results in animal models. Regenerative medicine and tissue engineering specialties are rapidly elucidating the mechanisms of action of stem cells and translating the results of in-vitro and in-vivo experiments to human clinical trials. The requirements for success will be patient safety, clinical efficacy and convenience of use.

The focus of this chapter is to review the area of topical stem and progenitor cell therapy as a treatment for non-healing diabetic foot ulcers. It will focus on adult stem cells as these are nearer to use in human trials and do not pose the ethical constraints associated with the use of embryonic stem cells. Topical treatment with endothelial progenitor cell (EPC) and mesenchymal stem cell (MSC) therapy is presented in this review, and more specifically the delivery of these cells using biomaterial scaffolds. The currently available cell therapy

products for wound repair will be presented. The case for adopting stem and progenitor cell therapy in research and treatment of diabetic foot ulcers will be discussed. The benefits of biomaterials and functionalised scaffolds for mediating cell therapy to a wound will be described. For both endothelial progenitor cells and mesenchymal stem cells, the potential mechanisms of action will be discussed with reference to key pre-clinical and clinical studies. The chapter will also describe strategies to enhance the therapeutic potential of stem and progenitor cells for wound healing. These will include the employment of matri-cellular proteins i.e. proteins associated with the extracellular matrix that mediate diverse biological functions, gene therapy, conditioned media experiments and the delivery of several cell types. A section of the chapter will focus on translational of these advanced biological medicines to clinical trials. This includes issues regarding pre-clinical animal models, optimal cell source, safety and regulatory approval. Finally the chapter will highlight the potential of cell based therapies in other conditions causing cutaneous wounding, i.e burns, decubitus ulcers and other rare blistering conditions e.g. epidermolysis bullosa.

2. The biology of cutaneous wounds

The repair of cutaneous wounds is a highly complex biological process.   After injury, multiple biological pathways immediately become activated and are synchronised to respond.(Gurtner et al., 2008) Adult wound healing occurs by tissue repair with consequent scarring. The goal of adult wound healing is to repair a skin defect, to ensure the restoration of a barrier and to regain tensile strength. There is involvement of several cell types, cytokines and extra-cellular matrix components. The physiological overlapping pathways that are required for optimal wound healing include haemostasis (which occurs immediately on wounding), inflammation with cell migration and proliferation (neutrophils initially and subsequently macrophages). The proliferation of fibroblasts results in extra-cellular matrix deposition. Remodeling and wound contraction occur once closure of the wound takes place. Angiogenesis (growth of new blood vessels from pre-existing blood vessels) and re-epithelialisation are central processes in wound healing. This is a superficial description of wound healing and conveys the complexity of the process, but highlights the potential for disruption in a difficult to heal wound. (Breen et al., 2008 ;Harding er al., 2002) The physiological response to acute cutaneous wounds usually takes 3-14 days to complete. (Liu et al., 2008) Wound healing involves activation of keratinocytes, fibroblasts, endothelial cells, macrophages and platelets.(Brem et al., 2007) Figure I details the stages of normal cutaneous wound healing.

2.1 Diabetic wound healing

Delayed wound healing as occurs in diabetes mellitus results from dysregulation of the normal healings pathways. The diabetic wound is complex with contribution from infection, neuropathy and impaired vascular supply. There are many physiological defects in diabetic wounds. These include decreased or impaired growth factor production, angiogenic response, macrophage function, collagen accumulation, epidermal barrier function, quantity of granulation tissue, keratinocyte, fibroblast migration and proliferation and bone healing. There is an imbalance between the accumulation of extra-cellular matrix components and their re-modeling by matrix metallo-proteinases.(Brem et al. 2007) In addition fibroblasts from diabetic wounds become senescent and show a decreased proliferative response to growth factors.(Falanga et al., 2005) There is a chronic inflammatory environment associated

with diabetic wounds. This is associated with a persistent increase in pro-inflammatory cytokines by various immune and non-immune cells and it is hypothesized that this blunts the acute, focused cytokine response needed to progress through the normal phases of wound healing.(Pradhan et al., 2009)
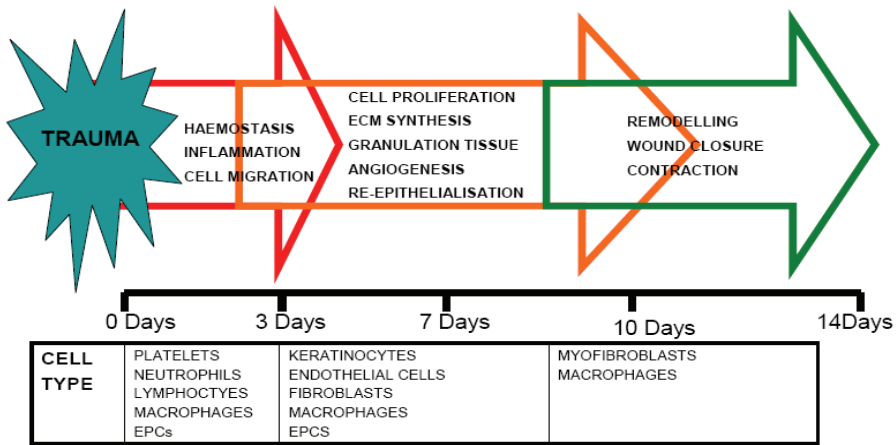
Stages of Normal Cutaneous Wound Healing

Fig. I. Stages of normal wound healing with predominant cell types involved at each stage of process. The wound healing spectrum is a continuum with overlapping phases.

2.2 Angiogenesis and wound Healing

The impaired vascular supply associated with diabetes leads to poor blood flow at the wound site impeding the optimal endogenous reparative response (Jeffcoate & Harding 2003) Impaired angiogenesis is a feature of diabetic wounds. In addition neovascularisation, or the de novo formation of new blood vessels is critical for granulation tissue formation and tissue regeneration in wound healing. (Gurtner et al., 2008) The impaired angiogenic response that occurs in diabetes mellitus leads to hypoxia at the wound site. Temporary hypoxia is requisite for normal wound healing. In the non-diabetic situation, hypoxia leads to activation of the transcription factor complex HIF-1α (Hypoxia inducible factor-1α), which leads to transcription of multiple genes required for successful wound healing. With diabetes, hyperglycaemia affects the stability and activation of HIF-1α. This suppresses platelet-derived growth factor, vascular endothelial growth factor and transforming growth factor-β, which are required for angiogenesis, in vitro and in vivo wound healing.(Botusan et al., 2008)

2.3 Wound repair versus regeneration

Adult wound healing occurs by repair. Wound repair leads to scarring and results in decreased tensile strength of wounds. Skin regeneration is the regeneration of wounds with restoration of the normal function and anatomy of skin. In biology, foetal wound repair is a regenerative process, and some vertebrate species demonstrate successful tissue

regeneration where the initial phase of wound repair is followed by perfect structural and functional regeneration of the organ. An example of this is Xenopus limb regeneration. The challenge for scientists is to produce tissue engineered products that exhibit extra-cellular matrix re-modeling characteristics seen in embryonic wound repair to produce functional and durable skin. (Metcalfe & Ferguson 2007)

3. The case for novel topically applied stem and progenitor cell therapies

3.1 Burden of diabetic ulceration

There exists a growing global epidemic of diabetes mellitus. It is predicted that the prevalence of diabetes mellitus will be 4.4% of the global population or 366 million people by the year 2030.(Wild et al., 2004) In 2010, the prevalence of diabetes in China was reported as 9.7%.(Yang et al., 2010) This will likely continue to increase based on the prevalence of obesity in populations. Foot ulcers can affect 12 to 25 percent of persons with diabetes mellitus throughout their lives.(Brem et al., 2006) Lower limb disease is the most common source of complications and hospitalisation in the diabetic population. (Boyko et al., 2006) Major lower limb amputations in patients with diabetes arise from preceding ulcers in 85% of cases. (Frykberg et al., 2006) The cost of treating diabetic foot ulcers creates a burden on healthcare resources. Boulton et al. reviewed the epidemiology and cost of treating foot ulceration globally and one report estimated the cost of diabetic foot ulceration treatment including amputation at €10.9 billion in the United States of America for the year 2001.(Boulton et al., 2005) In addition to the cost to healthcare system budgets, for individual patients, the parameters of pain, social isolation, physical morbidity, restrictions in work capacity, and psychological well-being are negatively affected by leg ulceration.(Herber et al., 2007)

3.2 Classification of diabetic ulcers

Diabetic foot ulcers can be classified as ischaemic, neuropathic or neuro-ischaemic. The ability to heal ulcers is predicated on the restoration of an adequate blood supply. The typical angiographic pattern of ischaemic diabetic vasculopathy is occluded distal blood vessels. The optimal treatment of ischemic lower extremity ulcers is the restoration of blood flow. This review paper focuses on treatment of neuropathic ulcers. Neuropathic ulcers develop due to distal sensory loss and consequent foot deformity. Ulceration develops at sites of excessive pressure predominantly under the first metatarsalphalangeal joint, in the majority due to unperceived trauma. Neuroischaemic ulcers are a combination of ischaemic and neuropathic ulcers.

3.3 Current treatment strategies

The management of the diabetic foot is complex requiring a multidisciplinary approach. A non-healing ulcer is an ulcer which has been present for > 8 weeks. Our group has reviewed the current standards of care required to investigate, treat and prevent diabetic foot ulceration and consequent amputation.(O'Loughlin et al.,2010) This manuscript highlights the benefit of routine examination and evaluation of the diabetic foot with identification of risk factors for ulceration. There are published risk stratification guidelines for diabetic foot ulceration based on the presence or absence of sensory loss, foot deformity and vascular insufficiency.(Boulton et al.,2008) The current standard care involves removal of pressure from the ulcer, restoration of blood flow if peripheral vascular disease is present, debridement of the ulcer and institution of antibiotic therapy to control infection. Topical

dressings, patient education, podiatry review, and orthotics are beneficial. A systematic review of the control arms of trials investigating novel treatments reported that for standard treatment of neuropathic diabetic ulcers, where blood supply had been adequate (as defined by a transcutaneous oxygen pressure of > 30 mmHg or an ankle-brachial index > 0.7), after 20 weeks 31% of diabetic neuropathic ulcers were healed and at 12 weeks, 24% of neuropathic ulcers were completely healed.(Margolis et al., 1999)A protocol for the management of diabetic foot ulcers suggested treatment with growth factors and/or cellular therapy if wound healing is not is not observed after 2 weeks of standard therapy and a new epithelial layer has not formed.(Brem et al., 2004)

3.4 Benefit of a cell-based therapy for non-healing diabetic ulcers

It is evident that there is a critical clinical need to develop novel therapies for treatment of non-healing diabetic ulcers in order to prevent amputation and reduce the significant financial drain on healthcare budgets and burden on individuals health. The understanding of the patho-physiology of diabetic wound healing is important in the development of advanced wound healing treatments. It allows therapeutic targeting of the different phases of wound healing. Cell therapy may reverse the biological defects in diabetic wounds by acting as reservoirs for cell and growth factor production. Gurtner *et al*. states that the ultimate solution to both under-healing and over-healing is likely to be administration of cells that retain the ability to elaborate the full complexity of biological signaling, together with the environmental cues that are needed to regulate the differentiation and proliferation of these cells (Gurtner et al., 2008)

3.5 Limitations with current cell-based therapy

To date clinical trials of topical cell based therapy for non-healing diabetic foot ulcers have yielded limited results. There are several reasons for this. One reason is methodological flaws in the clinical trials which have raised concerns over the validity of the results. Systematic reviews on skin replacement therapy have reported statistical benefit in wound healing endpoints. However there was a lack of information reported on safety, method of recruitment, randomization methods and blinding strategy for outcome assessments. There is a lack of power size calculations in some of the trials and little mention of dropouts in trial. The interventions did appear as safe as standard treatments. (Barber et al., 2008) It is felt that the deficiencies in clinical trials investigating skin replacement therapies for diabetic foot ulcers affect the conclusions of systematic reviews.(Blozik et al., 2008; Barber et al., 2008;Teng et al., 2010) Further larger scale trials are required.

However the lack of clinical success with these advanced medicinal products is most likely not solely due to the aforementioned flaws in trial design. The current somatic cell therapies do not address the underlying pathology in the diabetic wound i.e. chronic inflammation and impaired angiogenesis. An efficient blood supply is central to normal wound healing, and delayed or inefficient angiogenesis will prolong ulceration and increase the probability of amputation. The current cell treatments do not target angiogenesis (blood vessel formation from pre-existing blood vessels) or neo-vasculogenesis (de novo blood vessel formation). Somatic cells do not differentiate into other cell types of the dermis and epidermis. The most frequently studied somatic cells include fibroblasts and keratinocytes. The employment of these cell treatments result in wound healing by repair and not by regeneration.

3.6 Potential superiority of treatment with stem and progenitor cells

Endothelial progenitor cells are a newly described cell type involved in angiogenesis. They can migrate to a site of injury/ischaemia and play a central role in vascular maintenance, angiogenesis and neo-vascularisation. (Marrotte et al., 2010). Adult mesenchymal stem cell treatment holds promise as this cell type addresses the key wound impairments seen in non-healing diabetic ulcers. They are immuno-modulatory and may create a more favourable inflammatory environment of the diabetic wound. They also promote angiogenesis by paracrine effects. Adult mesenchymal stem cells in diabetic wounds may in addition to beneficial paracrine activity, differentiate into other cell types e.g. epidermal keratincocytes, endothelial cells and pericytes in vivo.(Wu et al., 2007) In fact there is a growing body of evidence that the use of stem cells in wound healing in addition to augmenting wound repair, also promote skin regeneration and scarless wound healing.(Fu et al., 2009)

4. Endothelial Progenitor Cells (EPCs)

4.1 Background

The discovery of putative EPCs by Ashara et al in 1997 (Asahara, et al., 1997) has illuminated the fields of vascular biology and diabetes related vascular dysfunction. For the first time, vasculogenesis or de novo blood vessel formation was determined to occur post-natally, as previously it was assumed to occur only during embryogenesis. The delivery of EPCs to ischaemic sites in the body offers the possibility of successful treatment of diabetic vascular disease. Worldwide, research groups are testing the hypothesis that EPC therapy may treat peripheral vascular disease and prevent the progression of non-healing diabetic foot ulcers to amputation. These cells are suitable for autologous therapy without immunological rejection but this approach may be hindered due to disease associated cell dysfunction.

EPC research is complicated by several issues. These include a lack of a standardised definition of the cell-type. The reports in the literature describe different identities, sources of isolation, culture methodologies and function. The cells maybe isolated from the peripheral blood, umbilical cord blood or bone marrow. They are referred to as progenitor cells or stem cells. In a comprehensive review, Hirschi *et al.* describe three different EPC types isolated from mononuclear cells.(Hirschi et al., 2008) This classification reflects the different cell types reported as EPCs.

All three cell types are cultured in endothelial based media. The first cell type is named colony forming unit-Hill cells which arise from peripheral blood mononuclear cells which are non-adherent and give rise to a colony after 5 days in culture. The second cell type is a heterogenous collection of cells termed circulating angiogenic cells or early EPCs. These arise from mononuclear cells which are adherent to fibronectin or other matrix adhesion proteins after 4-7 days. They do not form colonies and have a low proliferative potential. They retain monocytic properties, secrete angiogenic factors and die after approximately 4 weeks in culture.(Liew et al.,2008) The third cell type is the endothelial colony forming cell or late EPC. These cells are derived from mononuclear cells that adhere to fibronectin and appear after 6-21 days. They display cobblestone morphology and from blood vessels in vitro. They are highly proliferative. (Hirschi et al.,2008) The cells maybe further characterised by their ability to ingest acetylated low density lipoprotein and bind *Ulex europaeusagglutinin 1* plant lectin. The different cell types may also be characterized by flow cytometry for surface immunophenotype. Late EPCs display markers CD 34, CD 133, VEGFR2, CD 31 and are negative for CD 45.

4.2 Benefit in wound healing

Topical and systemic EPC therapy is beneficial in wound healing. The predominant mechanism is the augmentation of angiogenesis and neo-vascularisation. Suh *et al.* reported that EPC therapy increased recruitment of monocytes and macrophages in addition to augmenting angiogenesis. (Suh et al., 2005) This highlights the benefit in early stages of wound healing. It is known that EPCs in wounds result in increased granulation tissue and wound closure. (Asai et al., 2006)It is intuitive that this is the case as a multitude of in vitro studies have shown the production of growth factors and cytokines from EPCs which are closely involved in wound healing. Table 1 presents the in vivo studies of EPC treatment for diabetic ulcers. These studies support the benefit of topical EPC therapy in diabetic wound healing. The mechanism is reported as via paracrine effect, direct incorporation in blood vessels and differentiation into endothelial cells. The field of topical EPC therapy is in the early stages with benefit demonstrated in these studies. Intramuscular EPC therapy has shown benefit in critical limb ischaemia. (Huang et al., 2005) Further research is required to determine the benefit of EPCs delivered in a biomaterial. In addition the standardisation of cell dose, definition of cell type and animal model is required. The use of human cells in immunocompromised animals are required to further elucidate therapeutic efficacy

4.3 Mechanisms of actions
4.3.1 Paracrine effect

Early EPCs and Late EPCs may contribute to post-natal neovascularisation by secretion of angiogenic cytokines and growth factors. The secretome of EPCs contains cytokines and growth factors which stimulate wound healing by increasing proliferation, migration and cell survival of the different cell types required for wound healing i.e. keratinocytes, endothelial cells and fibroblasts. The conditioned media from EPC cultures revealed production of interleukin-8, Stromal-derived factor-1α, vascular endothelial growth factor, platelet-derived growth factor and monocyte chemo-attractant protein-1(Di Santo et al.,2009;Barcelos et al., 2009;Zhang et al.,2009) These cytokines are central to cutaneous wound healing. Extensive secretome analysis can be undertaken using mass spectrometry to determine novel factors involved in EPC biology.(Pula et al., 2009)

4.3.2 Direct incorporation in blood vessels

The second mechanism of action is the direct incorporation of EPCs into the growing blood vessel wall or the differentiation of these cells into mature endothelial cells. This mechanism is associated with late EPCs This mechanism has been shown in animal models and may not be as significant as the paracrine effect of cell therapy. (Di Santo et al., 2009) The comparison of EPC conditioned media as compared to EPC therapy alone for wound healing is important. The transplantation of conditioned media or identified therapeutic factors would allow for protein-based therapy. One study compared conditioned media from EPCs to EPC treatment alone in an animal model of cutaneous wound healing. Injection of EPC conditioned media alone into the same diabetic wound in mice promoted wound healing and increased neovascularization to a similar extent as achieved with EPC transplantation alone.(Kim et al., 2010) However Marrote et al. did not find similar therapeutic efficacy with less wound healing effect from EPC conditioned media.(Marrotte et al., 2010)

4.4 Impaired angiogenesis in diabetes due to EPC dysfunction

It is known that EPCs are decreased in number and dysfunctional in people suffering from diabetes mellitus. The decrease in number of circulating EPCs in people with diabetes is still under investigation but defects in the SDF-1α/CXCR-4 pathway are becoming evident. (Tepper et al., 2010)There are defects in EPC recruitment to wound sites. This is due to decreased mobilisation from the bone marrow and decreased homing to cutaneous wounds. (Brem et al., 2007) With diabetes there is decreased EPC participation in neoangiogenesis and neovsacularisation. Studies show that there are defects in cell migration, adhesion and tube formation. (Tepper et al., 2002) There is also an increase in reactive oxygen species in EPCs isolated from diabetes patients leading to cellular dysfunction. There is a body of evidence indicating that diabetes mellitus related EPC cell dysfunction represents a mechanism for impaired angiogenesis and impaired wound healing seen in diabetic patients.(Marrotte et al.,2010) The obstacle with autologus EPC therapy for diabetic complications is that there is a decreased number of cells available for transplantation. In addition, these autologous cells are dysfunctional.

4.5 Strategies to increase EPC efficacy

4.5.1 Topical delivery

In normal healing EPCs are released into the circulation from the bone marrow in response to ischaemia and travel to sites of tissue injury and participate in angiogenesis. (Takahashi et al., 1999) Diabetes-related vascular dysfunction arises from impairments in EPC mobilisation and homing to sites of ischaemia and cutaneous wounds. This has been shown in animal models of diabetic wound healing. In mice with cutaneous wounds and 4 weeks of streptozocin induced hyperglycaemia, the levels of circulating EPCs were unchanged but the levels of bone marrow derived EPCs within the wound granulation tissue were decreased as compared to non-diabetic controls. The bone marrow derived EPCs from diabetic mice showed increased apoptosis and decreased proliferation in diabetic wound tissue as compared to non-diabetic controls. (Albiero et al., 2011) The topical delivery of cells to a wound would overcome this homing defect and in addition would allow for ex-vivo manipulation during the cell isolation process. This ex-vivo manipulation may restore the EPC functional defect and succeed in restoring diabetic wound healing to the non-diabetic phenotype. Systemic delivery of stem cell results in cells being taken from the circulation in the lungs, spleen and liver and not reaching the wound. (Sorrell & Caplan 2010) The high prevalence of peripheral vascular disease in people with disease also inhibits the intravascular delivery of cell to the affect foot ulcer. The topical delivery of cells allows for concentrated doses of cells to be delivered to a skin wound and not become trapped in other sites in the body.

4.5.2 Matricellular proteins: Osteopontin

Osteopontin (OPN) is a matricellular protein and is involved in tissue repair and angiogenesis. These proteins modulate cell function by interacting with cell-surface receptors, proteases, hormones, and other bioeffector molecules, as well as with structural matrix proteins such as collagens (Bornstein, 2009) Decreased OPN is found in EPCs in diabetes mellitus. Dysfunction is reversed by exposure of EPCs to Osteopontin. (Vaughan EE, Liew A et al. 2011 In Press) Osteopontin is involved in angiogenesis. Osteopontin knockout mice have decreased myocardial angiogenesis in response to ischaemia and delayed recovery after hindlimb ischaemia. OPN is involved in wound healing. Wound

healing studies in osteopontin knockout mice show more residual debris and less matrix organisation than wildtype mice. (Scatena et al., 2007) OPN expression is associated with enhanced angiogenesis and collagenisation of the wound bed. Delay in diabetic wound healing may arise in part because of the low expression of OPN early in the wound bed after wounding, resulting in the reduced migration of immune cells to the site of injury leading to the accumulation of cell debris, decreased recruitment of endothelial cells, delayed angiogenesis and poor matrix organization. (Sharma et al., 2006)

4.5.3 Biomaterials and encapsulated cells
Adhesion to a substrate allows transplanted cell survival over even short time frames, and manipulation of major cellular processes (e.g., migration, proliferation, and differentiation) over longer time scales.(Mooney & Vanderburg 2008) Sufficient numbers of cells do not remain in place when applied to the wound surface.(Falanga, 2007). The use of biomaterials allows for more control in mediating delivery of cells to a wound. Current delivery options include injection of cells, delivery in extra-cellular matrix, delivery on a scaffold and delivery as part of a tissue engineering skin equivalents. (Sorrell & Caplan 2010) Silva *et al.* reported that delivery of EPCs using an alginate scaffold created a depot of endothelial progenitor cells which ensured sustained viability and function of cells in a mouse model of hind-limb ischaemia. This method was more successful than direct injection of cells alone. The vascular progenitor cells exit the biomaterial over time and repopulate damaged tissue and participate in the vascular network. (Silva et al., 2008) Cell encapsulation using biomaterials holds promise for both autologous and allogeneic cell therapy. The potential benefit of cell encapsulation with biomaterials includes sustained viability , the ability of the cell to avoid immune rejection, secrete therapeutic proteins and protect against mechanical stress(Orive et al., 2003;Freimark et al., 2010) Encapsulation of adult mesenchymal stem cells permits cell survival, proliferation and differentiation.(Anderson et al., 2011)

4.5.4 Co-culture, gene therapy and hyperoxia
It is hypothesised that endothelial progenitor cells act as angiogenic support cells by their paracrine activity. Co-administration of EPCs with smooth muscle progenitor cells increased vessel density in a mouse model of hind-limb ischaemia to a greater degree than administration of either cell alone. (Foubert et al., 2008) Endothelial cells increase mesenchymal stem cell proliferation. (Saleh et al. 2010) Gene therapy may rescue diabetic EPC dysfunction. Using an ex vivo gene transfer strategy, EPC cell cultures can serve as gene carriers and function as a temporal local production unit of de novo synthesized growth factors within the wound or skin replacement. (Dickens et al., 2010)Increased reactive oxygen species and oxidative stress has been shown to give rise to the dysfunction of diabetic EPCs, leading to inhibition of cell proliferation, nitric oxide production, matrix metalloproteinase-9 activity and migration. Manganese superoxide dismutase gene therapy reverses this dysfunction restoring the cells ability to mediate angiogenesis and wound repair.(Marrotte et al., 2010) Hyperoxia increases nitric oxide mediated EPC activity. (Gallagher et al., 2007) The diabetes related dysfunction in hypoxia inducible factor -1α which reduces vascular endothelial growth factor production (required for EPC activity) can be reversed by topical wound administration of the iron chelating agent desferoxamine. (Thangarajah et al., 2010)

4.5.5 Increase number of EPCs

Increasing EPC number for topical treatment increases the wound healing benefit of EPCs. (Marrotte et al., 2010) Granulocyte macrophage-colony stimulating factor (GM-CSF) increases monocyte derived peripheral blood EPCs. In-vitro animal studies reveal that proliferation of EPCs derived from the bone marrow can be accelerated by GM-CSF. (Wang et al., 2009) GM-CSF is routinely used in the patients receiving chemotherapy. It has been used in human clinical trials for investigation of autologous therapy in critical limb ischaemia. (Huang et al., 2005) In diabetic patients medications such as statins and angiotensin-converting enzyme inhibitor therapy can increase EPC number. (Liew et al., 2008)

Wound Model	EPC type	Delivery	Results	Mechanism	Ref.
Diabetic immuno-deficient mouse Ischemic ulcer	Human fetal CD133+ progenitor cells	Topical type 1 collagen seeded with EPCs	↑ wound closure ↑ angiogenesis	Paracrine signalling	{Barcelos, et al. 2009}
Diabetic Mouse Full thickness ulcer	CD34+ EPCs	Intradermal injection	↑ wound closure. ↑epithelial coverage ↑ vascularisation	Not addressed	{Sivan-Loukianova et al., 2003 }
Diabetic Mouse full thickness ulcer	bone marrow derived CD34+ EPCs	Intradermal injection	↑vascularisation ↑wound closure	Paracrine signalling	(Stepanovic et al., 2003)
Diabetic immuno-deficient mouse Full thickness ulcer	Human umbilical cord blood EPCs	Intradermal injection of EPCs and Topical EPC-CM	↑angiogenesis ↑ wound closure. Conditioned media showed therapeutically equivalent effect	Paracrine signalling	{Kim et al., 2010}
Genetically Diabetic mouse full thickness ulcer	Early EPCs	Topical delivery of genetically modified EPCs	↑wound closure ↑angiogenesis ↑ benefit with gene therapy and ↑ cell dose	Paracrine signaling EPCs present in capillaries	(Marrotte, et al.)
Diabetic mice full thickness cutaneous wounds	Lineage Negative progenitor cells (EPCs)	topically applied in a collagen scaffold	↑Wound Closure ↑ Vascular density	Differentiate into endothelial cells	(Lin et al., 2008)
Human diabetic critical limb ischaemia and foot ulceration	Autologous GM-CSF mobilized EPCs	Intra-muscular injections	Ulcer healing,	↑vessel density	(Huang et al., 2005)

Table I. Animal and human trials of EPC therapy for diabetic wounds

5. Mesenchymal Stem Cells (MSCs)

MSCs are adult fibroblast-like cells that differentiate along multiple mesenchymal pathways when exposed to appropriate stimuli. They adhere to tissue culture plastic and express cell surface markers for CD 105, CD 73, CD 90, and fail to express cell surface markers for CD 45, CD 34, CD 14, CD 11b, CD 79a and CD 19.(Sorrell & Caplan 2010) MSCs were originally isolated from bone marrow by Friedenstein *et al.* in 1968.(Friedenstein et al., 1968) They may also be known as fibroblast colony forming units, marrow stromal cells, multipotent adult progenitor cells, connective tissue progenitor cells or multipotent mesenchymal stromal cells. MSCs may be found in almost all postnatal organs and tissues, including adipose, periosteum, synovial membrane, synovial fluid, muscle, dermis, deciduous teeth, pericytes, trabecular bone, infrapatellar fat pad, articular cartilage and umbilical cord blood. (Si, et al., 2011) Stem cells located outside of the bone marrow are generally referred to as "tissue stem cells". Tissue stem cells are located in sites called niches, which differ among various tissues e.g. a stem cell niche in the bulge area of hair follicles. (Cha & Falanga 2007)

5.1 MSC treatment and wound healing

The complex pathology of diabetic foot ulceration requires that novel treatments are developed. The factors which are central to ongoing ulceration include poor blood supply, inflammation and decreased functioning of resident wound healing cells. MSC treatment has been shown to augment angiogenesis, suppress inflammation and augment wound healing cell functions. The focus of this review is the topical application of MSCs directly to the wound. There have been animal and human studies showing benefit of MSC therapy in the treatment of cutaneous wounds. (Fu & Li 2009) Table 2 details the animal and human trials investigating topical MSC therapy in diabetic wounds. Topical MSC therapy is further advanced than EPC therapy. The in vivo studies in table 2 demonstrate that topical delivery of MSCs result in benefit in diabetic animal cutaneous wounds. It is clear that augmented wound repair occurs by differentiation of MSCs to cells with keratinocyte markers and paracrine mediated increases in angiogenesis and vessel density. Human studies although with a small number of patients have shown benefit with several treatments. Further evidence is required from human cells in immunocompromised animal models to assess wound healing response. Standardisation in wound healing endpoints in both human and animal studies will allow comparison of effect between MSCs and modified MSCs. More research is required on the benefit of cells delivered using biomaterials.

Previous reports have investigated the benefit of topically applied fresh autologous bone marrow to wounds and have not been included in the table. In response to wounding and ischaemic conditions there is a mobilisation and homing of bone marrow MSCs to the wound. MSCs can undergo differentiation and act in a paracrine manner to reduce inflammation, stimulate angiogenesis and cause proliferation and migration of other cell types involved in wound healing. The MSC secretome is of central importance in realising the beneficial paracrine effects of the cells.

5.2 MSC: Mechanisms of action
5.2.1 Differentiation

MSCs may differentiate into mesodermal tissue including osteocytes, chondrocytes and adipocytes. They can differentiate into several cell types including cardiomyocytes, vascular endothelial cells, neurons, hepatocytes and epithelial cells, making them a potential cell

based treatment for human disease.(Volarevic et al. 2011) Allogeneic green fluorescent protein labelled bone marrow-derived MSCs have been applied directly to and injected around a cutaneous wound. MSC treatment accelerated wound closure, with increased re-epithelialisation, cellularity and angiogenesis. In the wound the MSCs expressed keratinocyte-specific protein keratin and formed glandular structures suggesting MSCs contribute to tissue regeneration by differentiating into keratinocytes.(Wu et al., 2007) MSCs differentiate into epidermal keratinocytes in vivo and in-vitro and also into skin appendages{Sasaki et al., 2008;Li et al., 2006).

5.2.2 Migration/Homing of MSCs

Bone marrow-derived MSCs contribute to cutaneous wound healing. The homing mechanisms are complex. Potential mechanisms include specific receptors or ligands undergoing up-regulation in response to injury. This not only facilitates trafficking, adhesion and infiltration of MScs but also provide MSCs with a specialised niche to support self-renewal and maintain pluripotency.(Si et al., 2011) MSCs become arrested in blood vessels of injured or ischaemic tissues and secrete a variety of growth factors and cytokines beneficial for wound healing.(Karp & Leng Teo 2009)

5.2.3 Paracrine effects of MSCs

MSCs act in a paracrine fashion to exert their beneficial effects. MSC-conditioned media medium augments wound repair with accelerated epithelialisation. (Wu et al, 2007) The analysis of MSC conditioned media revealed cytokines and growth factors required for wound healing. Vascular endothelial growth factor-a, Insulin like growth factor-1, epidermal growth factor, keratinocyte growth factor, angiopoietin-1, stromal derived factor-1, macrophage inflammatory protein-1, alpha and beta erythropoietin were increased in MSC conditioned media when compared to dermal fibroblast conditioned media. Bone marrow-derived MSC conditioned medium attracts macrophages and endothelial progenitor cells to wounds.(Chen et al., 2008) MSC paracrine signaling has potential beneficial effects on angiogenesis, epithelialisation and fibro-proliferation during wound repair (Hocking & Gibran 2010) Wu *et al.* reported that BM-MSC treated diabetic wounds had increased capillary density, but the bone marrow-derived MSCs were not found in the new capillary structures. This paracrine effect was supported by analysis of the conditioned media which revealed high levels of VEGF-α and angiopoeitin-1 with increased endothelial tube formation. (Wu et al., 2007)

5.2.4 Immunomodulation

An important characteristic of MSCs is that they express low levels of major histocompatibility complex-I (MHC-I) molecules and do not express MHC-II molecules, CD 80, CD 40 or CD 86 on their cell surface.(Zhang et al., 2010) This allows for allogeneic transplantation as MSCs. Human clinical trials have been conducted using allogeneic MSCs for the treatment of many conditions including graft-versus-host disease, type-1 diabetes, ischaemic heart disease, and neurological disorders e.g. stroke. MSCs possess immunosuppressive and anti-inflammatory properties in vitro and in vivo. They may suppress the proliferation and function of the innate and adaptive immune response and the immunomodulatory functions may occur by direct cell-cell contact or by paracrine means.(Zhang et al., 2010) Macrophages are a fundamental cell type in wound healing and

immunity. They can be classified as having a pro-inflammatory M1 phenotype or polarisation and an anti-inflammatory M2 or wound healing phenotype. MSCs are capable of eliciting M2 polarisation of macrophages which contributes to marked acceleration of wound healing (Zhang et al., 2010)

5.3 Optimising MSC therapeutic effect

The high proliferation capacity of MSCs mean that there is less dose limiting obstacles with MSC therapy. The allogeneic treatment allows for an "off-the-shelf" product. This is possible as the cells maybe cryopreserved for use in the future. MSCs are amenable to ex-vivo manipulation by gene therapy to provide cellular protection in an ischaemic environment. (McGinley et al., 2011). Highly concentrated cell doses can be directly applied to the wound surface or adjacent to the wound and delivery can be mediated using biomaterials. (Sorrell & Caplan 2010) As is the case with EPCs, biomaterials ensure sustained viability of cells and cell encapsulation technology may protect cells from mechanical stress common in diabetic foot ulceration. (Anderson et al., 2011;Orive, et al. 2003) Table 2 summarises the published research, and includes studies showing the benefit of MSCs on wound healing. There is also a need to better understand the stem cell niche involved in diabetic cutaneous wounds. This is required as this niche is the necessary microenvironment for controlling stem cell fate. Tissue engineering should provide both cells and adequately functionalised biomaterials in order to restore the elements of the stem cell niche. (Becerra et al., 2010)

Wound	MSC type	Delivery	Results	Mechanism	Ref.
Diabetic Mouse ulcers Human chronic ulcers DFU, n=1	Autologous Bone Marrow-Derived MSCs (BM-MSCs)	Topical Fibrin spray	↑ Wound Closure in mice and humans. No adverse events	↑ elastin fibres in MSC treated wound	(Falanga et al 2007)
Human chronic ulcers DFU, n=2	Autologous BM-MSCs	Collagen sponge with silicone film	Healing of wounds in 18 of 20 patients	↑ fibrous, fat and vascular tissue	(Yoshikawa et al.,2008)
Human DFU, n=1	Autologous BM-MSC	Fresh Bone marrow isolate applied to wound then covered with collagen seeded with MSCs	↓wound size with closing and healing of ulcer.	N/A	(Vojtassak et al., 2006)
Human chronic wounds DFU, n=6	Autologous BM-MSCs + standard wound dressing	MSCs injected in and around ulcer, and ulcer covered by dressing	↓ ulcer size at 12 weeks	Increased inflammatory cells and capillary proliferation	(Dash et al., 2009)

Wound	MSC type	Delivery	Results	Mechanism	Ref.
Diabetic rats Full thickness wounds	BM-MSCs transfected with hepatocyte growth factor	Direct injection to wound dermis	Decreased wound healing time with adHGF MSCs	↑blood vessels ↓ collagen formation, ↓ AGEs with AdHGF MSCs	(Ha et al., 2010)
Diabetic mouse with full thickness ulcer	Allogeneic BM-MSCs	Topical application and injection around wound edge	↑wound closure ↑epithelia ↑cellularity ↑angiogenesis	Differentiate MSCs to keratinocytes Paracrine ↑angiogenesis	(Wu et al., 2007)
Diabetic mouse Full thickness ulcer	ATSC over-expressing SDF-1	Topical cell application to wound	↑ % wound closure ↓epithelial gap, ↑ cellularity	Differentiation and paracrine effect on wound cells	(Di Rocco, et al. 2010)
Diabetic Mouse Full thickness ulcer	Diabetic MSCs co-applied with14S,21R -diHDHA	Topical MSCs applied to wound bed and injected intra-dermally	↑ epithelium ↑ GT	↑angiogenesis due to paracrine effect	(Tian et al., 2011)
Diabetic mouse Full thickness Ulcer	Umbilical cord-MSCs	Topical MSCs or systemic MSCs injection	↓ wound size with topically applied MSCs	TGF-β Paracrine effect	(Tark et al., 2010)
Diabetic mouse Full thickness ulcer	Autologous ATSC	Topical delivery using collagen scaffold	↑GT ↑epithelium ↑ no, capillary	Paracrine	(Nambu et al., 2009)
Diabetic Mouse ulcer	Allogeneic BM-MSCs	Topical Delivery	↑epithelium ↑ GT ↑ blood vessels	Paracrine	(Javazon et al., 2007)

DFU = Diabetic Foot Ulcer, BM = Bone Marrow, AGE = Advanced Glycation Endproducts
ATSC = Adispose Tissue-derived stromal cells, GT = Granulation Tissue

Table II. Animal and human trial on Topical MSC treatment of diabetic wounds

6. Biomaterial scaffolds for cell therapy in diabetic wound healing

6.1 Benefit of cell delivery using scaffolds for cell therapy

As explained above, a limitation of systemic delivery of stem cells is the poor engraftment efficiency to the target site, specifically to the wound. It is known that cell infusions e.g. into ischaemic muscle, typically result in > 90% of cells rapidly dying. (Silva et al., 2008) Therefore some of the failures experienced in clinical cell transplantation may directly arise from the manner of administration of the cells rather than a lack of intrinsic bioactivity of the cells. (Silva et al., 2008) The use of a matrix is vital to the integrity of cell maintenance and growth because cells are anchorage dependent and require an appropriate milieu of

mechanical strength, material support, controlled porosity and interconnected channelling (Yang et al., 2002)

6.2 Determining the optimal biomaterial for topical treatment of diabetic wounds

The goal of developing novel wound healing treatments is to reduce the time to complete wound closure and restore the barrier function of the skin. The ideal qualities of a skin substitute for diabetic ulcer wound repair is that it will be clinically effective, safe to the patient, inexpensive, easy to use, readily available, durable and encourage cell-matrix interactions. The ideal biomaterial should support reconstruction of new tissues without inflammation. (Huang & Fu 2010)

There is a multitude of biomaterials for wound treatments commercially available and undergoing research. They may have different physicochemical profiles with differing mechanical and degradation properties. They may be synthetic or natural. Natural biomaterials are generally considered more biocompatible and similar to the host extra-cellular matrix. The drawback of synthetic biomaterials is their lack of cellular recognition signals. (Huang & Fu 2010) Skin substitutes can be classified based on **1.** anatomical structure (dermal, epidermal, dermo-epidermal), **2.** duration of cover (permanent, semi-permenant, temporary), **3.** type of biomaterial (biological: autologous, allogeneic, xenogeneic or synthetic: biodegradable, non-biodegradable), **4.** skin substitute composition (cellular, acellluar) and **5.** Where primary biomaterial loading with cellular components occurs(in vitro, in-vivo).(Shevchenko et al., 2010) There are techniques used for development of tissue engineered ulcer healing products. These include 1. Transplantation of cells without matrix or scaffold, 2. Transplantation of biomaterials alone or with the addition of proteins e.g. cytokines and 3. Transplantation of cells in a 3-D scaffold. (Jimenez and Jimenez 2004)

6.3 Currently available cell-based biomaterial dressings for wound healing

The focus of this chapter is on cell-based treatments using a 3-D scaffold. There are several terms that encompass such skin substitutes i.e. tissue-engineered skin, tissue engineered skin constructs, skin substitute bioconstructs, bioengineered skin, living skin replacements and living skin equivilants. (Shevchenko et al., 2010) The gold standard skin replacement treatment for many conditions has been full-thickness skin grafting. There are inherent risks associated with autologous grafts e.g. donor site pain, scarring and infection or delayed healing and failure of graft at recipient site. The risks with non-autologous skin grafts include immune rejection and infection transmission. (Wu et al., 2010) A disadvantage of the currently available cell-based topical therapies is that they do not address the lack of angiogenic properties of the skin substitute. This is important as the successful ability of a skin graft to take to an ulcer is an adequate vascular supply. Table III summarises some of the commercially available skin substitutes and the clinical indications for their use. Apligraf and Dermagraf are temporary treatments for non-healing diabetic ulcers. These skin substitutes are biomaterials seeded with keratinocytes and/or fibroblasts. They are indicated as a topical treatment for non-healing diabetic ulcers in the USA.

6.4 Collagen as a biomaterial

Collagen is the major extra-cellular matrix protein of the dermal layer of the skin. It forms an intrinsic part of blood vessels and supports angiogenesis. It is a commonly used biomaterial

for topical cell based wound dressings e.g Apligraf (Organogenesis). It displays low antigenicity with purification techniques available to eliminate the immunogenic telo-peptides.(Huang & Fu 2010) Collagen is appropriate for temporary dressings as it is mechanically weak and undergoes degradation on implantation.(Huang & Fu 2010) It is possible to manipulate collagen by cross-linking and enhance its physico-chemical properties. There are widely used commercial collagen based dressings for diabetic foot ulcers (e.g Promogram, which contains oxidised regenerated cellulose by Johnson & Johnson). (Zhong et al., 2010) Integra (LifeSciences) is a wound healing product consisting of bovine type 1 collagen cross-linked with chondroitin-6-sulphate which is bonded to a silicone membrane. It acts as a template for fibroblast migration and capillary growth in vivo. (Zhong et al., 2010) We have successfully seeded stem and progenitor cells in a collagen scaffold. Figure 2 is a scanning electron microscope image of EPCs and MSCs seeded in a collagen scaffold for 24 hours.
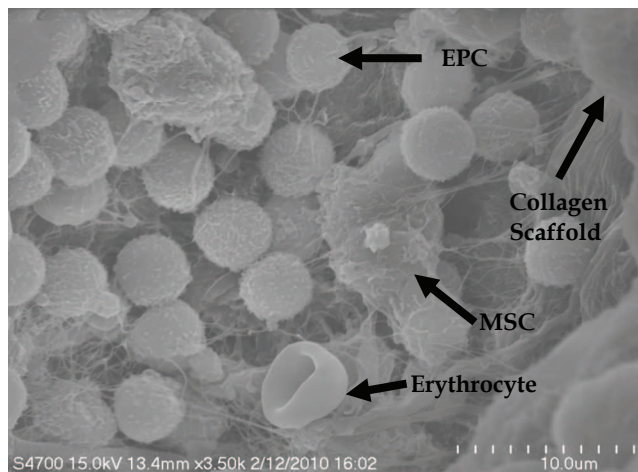

Fig. II. Scanning electron microscope of co-culture of mesenchymal stem cells and early endothelial progenitor cells in a type 1 bovine collagen scaffold.

Product	Description	Indication
Apligraft /Graftskin Organogenesis Canton, MA, USA	Allogeneic neonatal foreskin keratinocytes and fibroblasts seeded in a type 1 bovine collagen	Diabetic foot ulcers venous leg ulcers Partial thickness burns Epidermolysis Bullosa
Dermagraft Advanced Biohealing Inc Lojalla, Ca, USA.	Allogeneic neonatal fibroblasts seeded in a polyglycolic acid (Dexon) or polyglactin-9-10Vicryl scaffold.	Full thickness DFU Epidermolysis Bullosa
TissueTech Autograft system. Laserskin and Hyalograft Fidia Farmaceutical Abano Terme Italy	Autologous fibroblasts and keratinocytes cultured on a hyaluronic acid laser perforated membrane	DFU and Chronic wounds
Epicel Genzyme Biosurgery Cambridge, MA, USA	Autologous keratinocytes and xenogenic proliferation-arrested mouse fibroblasts in petroleum gauze dressing	Full thickness burns burns taking >30% of body area
Transcyte Advanced Biohealing Inc, Lojolla California	Human allogeneic fibroblasts cultured on a nylon mesh pre-coated with collagen	Burns Transparent dressing
Orcel Ortec International New York ,NY USA	Type 1 Bovine collagen seeded with allogeneic neonatal fibroblasts and keratinocytes	donor sites for autografting, DFU Epidermolysis Bullosa
Epidex Modex Therapeutics Luzanne Switzerland	Cultured epidermal skin equivalent derived from keratinocyte precursors of human hair follicles	Chronic Leg uclers
Myskin Altrika Sheffield UK	Autologous keratinocytes grown on a silicone layer with irradiated murine fibroblasts	Non-healing wounds DFU, Burns, Pressure ulcers
Bioseed-S BioTissue Technologies Freiburg, Germany	Autologous keratinocytes resuspended in a fibrin sealant	Venous leg ulcers
Permaderm Regenicin www.regenicin.com	Autologous keratinocytes and fibroblasts seeded on collagen biomaterial	Burns Chronic Wounds
DFU = Diabetic foot ulcers		

Table III. Sample of currently available Cell-Scaffold skin replacement therapies and their indications

7. Translation to human therapy

7.1 Safety and regulatory approval

With any new cell-based therapy, it is mandatory to ensure safety for the patient. Any negative toxic side-effect of cell-based therapies would be a set back for the field of tissue engineering and regenerative medicine. In Europe, the European Medicines Agency (EMA) controls regulation and clinical trials of new cell based products. In North America, this process is under the remit of the Food and Drugs Administration (FDA). The EMA also advises on the development of stem cell products which are an example of an advanced therapy medicinal product (ATMP). In February 2011, the EMA published a document entitled "Reflection paper on stem cell-based medicinal products", highlighting the current situation in the field of stem cell therapy. (EMA 2011)   Safety and clinical efficacy is first proven by scientifically robust methodology in pre-clinical studies. It is required that the product is produced and clinical trials carried out according to international standards. These standards include GLP (good lab practice), GMP (good manufacturing practice), and GCP (Good clinical Practice). There is a requirement for quality checks in the manufacturing process. This includes analysis of cell treatment batches to ensure cell quality, identity, viability and traceability of cells. The goal is a robust, stringently controlled production and manufacturing process.

7.2 Preclinical animal models: choice of model and regulatory issues

It is necessary to prove treatment efficacy in an animal model. An in vitro wound healing model is not sufficient to confirm treatment efficacy. The complexity of diabetic foot ulceration with its multi-factorial pathology cannot be realised in an animal model. There are over 10 different animal models of diabetic ulceration in the reported literature. There are inherent differences between animals and humans. These include cutaneous anatomy, vascular supply, duration of diabetes and the presence of other cardiovascular risk factors e.g. smoking.

In addition there are a myriad of endpoints reported in animal wound healing studies. The most robust clinically relevant wound healing endpoints are percentage wound closure and time to complete healing. The myriad of new treatment modalities under investigation have effects on different phases of the wound healing spectrum. The pig has skin felt to be the most close to humans but these are large expensive animals. The genetically modified, leptin receptor deficient diabetic mouse is widely used as a model of type 2 diabetes, but wound healing occurs by contraction in this model and does not reflect the human situation. The rabbit ear dermal ulcer model is a powerful model for examining re-epithelialisation and granulation tissue formation in an excisional wound. (Breen et al., 2008) A comprehensive review by Lammers et al. recommends a more systematic evaluation of tissue-engineered constructs in animal models to enhance the comparison of different constructs, accelerating the trajectory to application in human patients. (Lammers et al., 2010)

The EMA provides advice on the animal models to use for translation of cell-based therapy to humans. The choice of the most relevant animal model should be determined by the specific safety aspect to be evaluated. It advises the use of human cells to be tested in proof of concept and safety studies. This methodology requires the use of immuno-compromised models either genetically immuno-suppressed or treated with immuno-suppressants.(EMA 2011) The persistence of cells and the functionality of the cells should be assessed. The potential of undifferentiated pluripotent stem cells to form tumours and

be genetically unstable due to ex-vivo manipulation requires this to be assessed in animal models. This is more likely with embryonic stem cells and pluripotent stem cells. Bio-distribution of cells to other organs and ectopic tissue formation need to be investigated. Prior to first-in-man studies, there are guidelines published by the EMA to identify and mitigate risks. Dose finding studies, immunological, pharmacokinetic, pharmco-dynamic and long term pharmaco-vigilant studies should be undertaken and planned. (EMA 2011)

The use of biomaterials in conjunction with stem and progenitor cells is defined by the EMA as a 'tissue-engineered product' and falls under the term ATMP.(EU 2007) The experience with the development of allogeneic bi-layered skin has provided valuable information on the development of skin replacement therapy. Apligraf (Organogenesis), a living bi-layered skin substitute has received approval from FDA. It is described as a Class III medical device via premarket approval and meets requirements for a human cell, tissue, cellular and tissue-based product. As the product is made from viable human skin cells, it cannot be terminally sterilized, but safety concerns have been addressed. These include risk of transmission of infection, immunogenicity, immunological graft rejection and tumour formation. As cells are derived from neonatal foreskin, maternal blood of the neonatal donor and the cell banks are thoroughly screened for infectious agents, pathogens and other contaminants.(EU 2007;Wu et al., 2010)

7.3 Structured diabetic foot care

Stem and progenitor cell-based topical treatments will not be used in isolation to treat diabetic foot ulceration. Ideally, these advanced biological treatments will be part of a treatment algorithm, which would see the implementation of standard care prior to use of cell therapy. If the restoration of vascular supply, removal of pressure, control of infection and debridement of the wound does not succeed in ulcer healing, then the indication for cell based therapy would apply. There are analyses of factors associated with lack of healing with fibroblast dermal substitutes. An episode of infection during 12 weeks of treatment was associated with a 3.4 times increased risk of non-closure of a wound. (Wu et al., 2010) High bacterial load in the wound negatively affects wound healing with Dermagraft and Browne et al. recommend reducing the bacterial load with combination antibiotics prior to the application of skin substitutes. (Browne et al., 2001)   New treatment modalities are under investigation which may augment wound healing and reduce bacterial load. Plasma therapy may reduce bacterial burden and enhance wound healing. (Heinlin et al., 2010)

7.4 Cost: Benefit analysis

To ensure development of a successful topical cell based therapy, the product must have potential widespread use in the clinical arena. It must demonstrate clinical efficacy in clinical trials. In randomised controlled clinical trials the new product must show superiority both in comparison to standard care and to other market leaders in the field. It is expensive to conduct human clinical trials, therefore the product must demonstrate favourable health economics so as to be attractive to health care providers and industrial partners. To gain market access, manufacturers have to establish not only the efficacy of the product but also whether the product provides a cure at an acceptable cost per unit of health gain. (Langer et al., 2009) Several studies have investigated the cost-effectiveness of these

products. The results feature favourable cost-effectiveness ratios in selected patient groups with chronic wounds. The cost of the product and product development should be offset against the total cost of care of the patient with a non-healing diabetic foot ulcer. (Langer et al., 2009) There is a need for high quality clinical trials in this area.

8. Cell-based therapies in other dermatological conditions

MSCs and EPCs have the potential to treat other dermatological conditions apart from diabetic foot ulceration. As seen in table III there are several conditions which may be suitable for these therapies including chronic venous and pressure ulcers, burns and epidermolysis bullosa. The economic burden of chronic wounds is potentially the largest burden on healthcare systems. Stem and progenitor cells may be used as orphan medications for life-threatening or extremely rare debilitating conditions. These drugs are not developed by large pharmaceuticals and are not subject to the same regulatory process. An example of this is the blistering disorder epidermolysis bullosa. In addition research into basic stem cell biology will elucidate mechanisms of action of stem cells which may guide the development of future therapies. The development of successful skin regeneration and elucidation of key molecules and biological systems will allow for scar free repair and increased strength of healed wounds. There are further exciting developments in the field of stem and progenitor cell therapy for tissue regeneration. Hair follicle biology is important for skin biology and epidermal haemostasis. There are resident stem cells in the bulge area of the hair follicle which are required for re-epithelialisation during wound healing. (Wu et al., 2011) They are a readily isolatable source of adult stem cell suitable for autologous therapy.(Amoh et al., 2010)

9. Conclusions

This book chapter has reviewed the current state of Stem and Progenitor cell therapy for non-healing diabetic foot ulceration. The urgent clinical need for developing improved novel cell treatments is stressed. The scientific basis for potential success with topical stem and progenitor therapy is reviewed. The advantage of using biomaterials to mediate cell delivery is discussed. Further developments in tissue engineering will provide more intelligent biomaterials which ensure better viability and control of stem cell fate and function. The logistical hurdles to translation of bench-side discoveries are reviewed and information provided on accelerated development of these advanced medicinal products.
The importance of translational science is being recognised as a key driver to the realisation of basic science discoveries for humans. There are strategic efforts to translate basic science to clinical benefit. This bench-to-bedside approach is the focus of government policies throughout the world with collaborations developing between pharmaceutical and biotechnology industries, academia and clinicians. The success of treatments will rely on clinical efficacy, safety, ease of use and cost-effectiveness. The potential to translate this technology to a variety of clinical dermatological disorders increases the attractiveness for industrial investment for further research and development of these products. A central component to the successful translation of this treatment will be the performance of robust randomised controlled trials. Stem cell therapy is a new field encompassing both tissue engineering and regenerative medicine science and holds promise for the improved treatment of diseases which are suboptimally managed with current therapies.

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11. References

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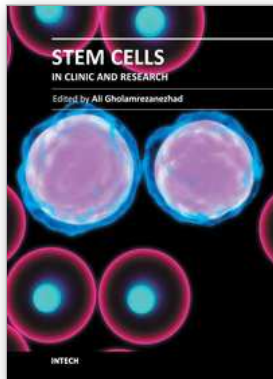
Stem Cells in Clinic and Research

Edited by Dr. Ali Gholamrezanezhad

Based on our current understanding of cell biology and strong supporting evidence from previous experiences, different types of human stem cell populations are capable of undergoing differentiation or trans-differentiation into functionally and biologically active cells for use in therapeutic purposes. So far, progress regarding the use of both in vitro and in vivo regenerative medicine models already offers hope for the application of different types of stem cells as a powerful new therapeutic option to treat different diseases that were previously considered to be untreatable. Remarkable achievements in cell biology resulting in the isolation and characterization of various stem cells and progenitor cells has increased the expectation for the development of a new approach to the treatment of genetic and developmental human diseases. Due to the fact that currently stem cells and umbilical cord banks are so strictly defined and available, it seems that this mission is investigationally more practical than in the past. On the other hand, studies performed on stem cells, targeting their conversion into functionally mature tissue, are not necessarily seeking to result in the clinical application of the differentiated cells; In fact, still one of the important goals of these studies is to get acquainted with the natural process of development of mature cells from their immature progenitors during the embryonic period onwards, which can produce valuable results as knowledge of the developmental processes during embryogenesis. For example, the cellular and molecular mechanisms leading to mature and adult cells developmental abnormalities are relatively unknown. This lack of understanding stems from the lack of a good model system to study cell development and differentiation. Hence, the knowledge reached through these studies can prove to be a breakthrough in preventing developmental disorders. Meanwhile, many researchers conduct these studies to understand the molecular and cellular basis of cancer development. The fact that cancer is one of the leading causes of death throughout the world, highlights the importance of these researches in the fields of biology and medicine.

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