

**An overview of the therapeutic potential of regenerative medicine in  
cutaneous wound healing**

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## **Abstract**

The global burden of disease associated with wounds is an increasingly significant public health concern. Current treatments are often expensive, time-consuming and limited in their efficacy in chronic wounds. The challenge of overcoming current barriers associated with wound care requires innovative management techniques.

Regenerative medicine is an emerging field of research that focuses on the repair, replacement or regeneration of cells, tissues or organs to restore impaired function. This article provides an overview of the pathophysiology of wound healing and reviews the latest evidence on the application of the principal components of regenerative medicine (growth factors, stem cell transplantation, biomaterials and tissue engineering) as therapeutic targets. Improved knowledge and understanding of the pathophysiology of wound healing has pointed at new therapeutic targets. Regenerative medicine has the potential to underpin design of specific target therapies in acute and chronic wound healing. This personalised approach could eventually reduce the burden of disease associated with wound healing. Further evidence is required in the form of large animal studies and clinical trials to assess long-term efficacy and safety of these new treatments.

**Keywords:** Biomaterials; Growth Factors; Regenerative Medicine; Stem Cells; Tissue Engineering; Wound Healing.

**Key messages:**

- Wound healing is a complex process which if disrupted has significant implications for patients and health-care services.
- This article provides an overview of the progress and limitations of how growth factors, stem cells, biomaterials and tissue engineering can be used to stimulate and accelerate wound healing.
- Regenerative medicine has the potential to revolutionise wound healing by providing therapies that modulate the wound repair cascade, modify the wound environment or replace defective tissue.

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## Introduction

Loss of continuity of the epithelium of the skin results in a wound, which may also include damage to the underlying soft tissue structures. Wounds may be caused by a number of insults including mechanical, chemical, biological or thermal injuries. Additionally, wounds can be crudely classified as either acute or chronic depending on their duration. The burden of disease associated with wounds is great with diabetic wounds alone possessing a prevalence of 5-7% and cost an estimated €4-6 billion to treat annually in the European Union (1). Acute wounds, often as a result of surgery or trauma, can be superficial, involving the epidermis and dermis, or full thickness, where the subcutaneous layer is damaged. Typically acute wound healing is a well-organized process that heals within three weeks. Acute wounds are a common health problem with an estimated 11 million people affected and approximately 300,000 hospital admissions every year in the United States (2, 3). Chronic wounds are generally classified as vascular, diabetic or pressure ulcers and usually occur as a complication of a disease process. These wounds typically persist for a minimum of three months because of an interruption in the healing process. This may include a prolonged or excessive inflammatory process, persistent infections and inability of dermal and/or epidermal cells to respond to regenerative stimuli (4, 5). Chronic wounds are a global epidemic with the number of cases dramatically increasing due to an ageing population and increased incidence of diabetes and obesity. Hence, the disease burden of non-healing chronic wounds continues to increase (6). Management of these wounds is expensive as repetitive treatments are

required with an estimation of over \$20 billion spent each year in the United States alone (6).

Conventional treatment of wounds primarily focuses on identifying and removing any precipitating or aggravating factors then allowing the healing cascade to proceed. Infection control, wound bed preparation, dressings and surgery are the keystones to wound management and maybe used alone or in combination to achieve wound healing indirectly (optimisation of the wound to allow it to heal by secondary intention) or directly (closure of the wound and primary healing). Wound bed preparation allows optimisation of the wound by producing a well-vascularized bed with minimal exudate through inflammation and infection control, moisture balance and epithelial (edge) advancement. Infection control involves the removal of necrotic, devitalized tissue via debridement with the use of antimicrobials as required. The use of appropriate dressings allows regulation of the wound-healing environment to maintain temperature and moisture. Surgical closure of a wound allows direct closure of the wound through advancement of the epithelial edges or if this is not possible, bridging this closure with skin grafting or a free flap. These wound management techniques have been used safely and effectively for many years, however, there are limitations such as failure of healing due to systemic or local factors, tissue availability and donor-site morbidity where tissue transfer is required and antimicrobial resistance. Faced with an ageing population and a rise in smoking, obesity and diabetes, the epidemic of chronic wounds requires management protocols that can overcome the current barriers associated with wound care.

Regenerative medicine is an emerging field of research that focuses on the repair, replacement or regeneration of cells, tissues or organs to restore impaired function. This involves various strategies that include, but are not limited to, tissue engineering, stem cell transplantation, biomaterials and growth factor therapy. Several reviews have been previously published on the topic of regenerative medicine as relevant to wound healing. However, these reviews have so far either primarily addressed each of these regenerative medicine approaches in isolation (7-9) or focused on chronic wounds (10). In this review, we discuss the pathophysiology of wounds and present an overview of the latest studies in regenerative medicine and how they may be applied to stimulate and promote healing in the management of both acute and chronic wounds.

### **The Pathophysiology of Wound Healing**

Wound healing is a complex and dynamic process whereby the skin attempts to repair itself after injury (Figure 1). The wound repair process can be broadly divided into three phases; inflammatory, proliferative and maturation (11). During the inflammatory phase, cytokine and chemokine release causes neutrophils, macrophages and lymphocytes to migrate to the wound. These inflammatory cells then secrete growth factors and provisional matrices which promote recruitment of neighbouring epidermal and dermal cells to the wound bed (11). The proliferative phase is characterized by the formation of granulation tissue, depicted by the increased levels of keratinocyte and fibroblast proliferation, epidermal cell migration and extracellular matrix synthesis thus resulting in reepithelialisation and angiogenesis (12). The final

phase of wound healing entails maturation of the wound and remodelling of the extracellular matrix. Differentiation of fibroblasts to myofibroblasts results in smooth muscle actin deposition leading to wound contraction and replacement of Collagen III by Collagen I in the extracellular matrix. Cells and blood vessels that are no longer required are removed via metalloproteinase mediated remodelling eventually leading to the formation of an acellular scar (13).

The delicate co-ordinated wound repair process is however susceptible to interruption or failure by multiple factors which can be related to the characteristics of the wound itself (e.g. contamination or size), specific abnormalities in the healing cascade (such as signalling pathway or gene expression abnormalities) or the overall physiology of the patient (e.g. systemic disease or immune deficiency). These factors may occur in isolation or in combination to affect any or all of the phases of the wound healing process thus giving rise to impaired healing and a chronic wound. One of the best-studied and proposed therapeutic targets is the transition phase between inflammation and proliferation of the wound healing process. Whilst the inflammatory phase of wound healing is necessary in microbial control and clearing of cellular debris, it is critical that this stage is not prolonged and there is swift transition to the proliferative stage which allows neovascularisation and fibroblast recruitment (14). Prolonged inflammation impairs wound healing through leucocyte and matrix metalloprotease dysfunction and inflammatory cell over activity (15, 16). Similarly, absence or inadequate inflammatory response is responsible for delayed wound healing (17, 18). There is increasing evidence of the wide-ranging roles that

inflammatory cells play in this complex process and that their function maybe dependent on the subset of cells within a population and the stage of the healing cascade in which cells are recruited (19-21).

Another important consideration in wound healing is the role played by the fibroblasts and stromal cells recruited during the proliferative phase. The latter modulate the immune response through paracrine signalling and promote angiogenesis and epidermal cell migration through release of chemokines such as Stromal cell-derived factor-1 (22). Fibroblasts directly contribute to wound repair by producing extracellular matrix and indirectly through chemokine release to perform immune modulation and promote cell migration (14).

Impairment of wound healing due to disruption of the inflammatory or the cellular (proliferative response) as described may occur due to a specific problem with that part of the healing process such an interleukin deficiency (23) or can occur as part of a wider systemic illness such as diabetes mellitus (24). Additionally, impaired healing might be due to senescence (25).

### **Therapeutic Potential of Regenerative Medicine In Wound Healing**

Regenerative medicine encompasses a wide-variety of potential therapies, which ultimately aim to promote healing and tissue repair. These therapies can be broadly classified as based on growth factors/modulation of signalling pathways, stem cells, biomaterials, and tissue engineering, although there is usually a great deal of overlap (Figure 2). In this review we describe the potential applications of regenerative medicine in wound healing and discuss the progress and limitations of the most recent studies relating to this.

## **Growth Factors involved in stimulating wound healing**

Growth factors are biologically active polypeptides that interact with specific cell surface receptors in controlling the process of tissue repair. These factors primarily promote cell migration into the wound, promote epithelialization, initiate angiogenesis and stimulate matrix formation and remodelling of the affected area (26). The growth factor families that have been most studied and of particular interest in wound healing are epidermal growth factor (EGF), transforming growth factor beta (TGF $\beta$ ), fibroblast growth factor (FGF), and platelet derived growth factor (PDGF) (Table 1). There is also emerging evidence for the role of Stromal cell-derived factor 1 (SDF-1) in regulating epidermal cell migration and proliferation during wound repair.

EGF is secreted by platelets, macrophages and fibroblasts and plays an important role in re-epithelialization. In addition to its role in stimulating growth of keratinocytes in vitro, Brown et al showed that topical application of EGF can accelerate epidermal repair in partial thickness wounds in a clinical study (27). This was further supported by a double blind clinical trial by the same group, which demonstrated that application of EGF to skin graft donor sites accelerated the rate of dermal regeneration (28).

Platelets, keratinocytes, macrophages, lymphocytes and fibroblasts produce TGF $\beta$ , which is essential in inflammation, granulation tissue formation, re-epithelialization, matrix formation and remodelling. Addition of TGF $\beta$  to incisional wounds in rats were shown to accelerate wound healing through increased mononuclear cell infiltration, fibroblast migration and collagen deposition by Mustoe et al (29). The mechanism by which this occurs is unclear although a study by Pierce et al suggested that TGF $\beta$  may be

responsible for transient migration of fibroblasts into the wound and direct stimulation of collagen production (29).

PDGF is produced by platelets, keratinocytes, macrophages, endothelial cells and fibroblasts and also plays a role in each stage of wound healing (30). In a study of both PDGF and TGF $\beta$ , Pierce et al showed that although both growth factors accelerated in vivo wound repair, this was through different mechanisms of action (31). PDGF was involved in chemoattraction of macrophages and fibroblasts thus promoted wound healing through stimulating these cells to express growth factors including TGF $\beta$  (31). A more recent double-blind randomised control trial by Steed et al demonstrated that topical application of PDGF to chronic full-thickness diabetic ulcers, to safely and effectively stimulate healing (32).

FGF is produced by keratinocytes, mast cells, fibroblasts, endothelial cells, smooth muscle cells and chondrocytes, which was shown to promote granulation tissue formation, re-epithelialization, matrix formation and remodelling in acute rat wounds (33). This had been previously described by McGee et al who showed that application of recombinant FGF promoted faster healing in an acute wound model in rats (34). The effect of FGF on wound healing was also investigated in a randomised control trial that showed FGF could be used to safely and effectively accelerate healing of chronic wounds (35, 36).

Whilst the role of specific growth factors in wound repair have been demonstrated by various studies, a few groups have presented evidence for the use of combinations of growth factors to optimise wound healing. Of these, platelet-derived wound healing factor (PDWHF) has received attention

due to its ease of derivation from autologous sources, evidence of promoting healing in chronic wounds without adverse effects and cost efficiency. The topical application of PDWHF to promote chronic wound regeneration was first shown by Knighton et al who achieved increased closure of chronic cutaneous wounds treated with autologous PDWHF (37). This was further validated in a blind randomised control trial which also showed that autologous PDWHF stimulated reepithelialisation of chronic non healing wounds when applied locally (38). The value of using a combination of growth factors and the importance of the mode of delivery was reinforced by Yang et al who showed that delivery of growth factors (contained in platelet rich plasma) accelerated full thickness wound regeneration in mice when using a heparin conjugated fibrin carrier (39).

Recruitment of epidermal stem cells to the wound site from the neighbouring uninjured tissue has been shown to induce reepithelialisation (40). Guo et al induced full-thickness excisional skin wound model in rats to study the in vitro and in vivo role of SDF-1 on epidermal stem cell mediated wound healing (22). Skin wounds showed immediate upregulation of SDF-1 peaking at day 7 after injury with weak expression by day 9 with a similar pattern of expression for its cellular receptor (CXCR4). In vitro culture of isolated rat epidermal stem cells revealed enhanced migration after addition of SDF-1. Rat wounds treated with SDF-1 exhibited accelerated closure compared with controls. Additionally this study used an inhibitor of SDF-1 (ADM3100) to demonstrate that in vitro cell migration and in vivo wound healing were significantly reduced compared with controls and SDF-1 treated groups thus reinforcing their findings.

Although topical application of growth factors have been shown to accelerate wound healing in vitro as well as in a number of animal and human studies (Table 1), a number of barriers limit therapeutic application. A major consideration is that these factors must be resistant to rapid degradation from the wound's proteolytic environment and have controlled release (26). As such, the focus of many studies is now combination of biomaterial research with growth factor studies to find a suitable carrier or in combination with stem cells to induce differentiation. Since wound repair is a dynamic process, it remains to be answered whether delivery of growth factors should be sustained or transient and how long they are required. Furthermore, there is much interplay between the different cells and components of the wound-healing cascade. The limitation of many of the studies that have shown the usefulness of growth factor application to wounds is that often they study one or two of these in isolation. Future studies are required to identify whether this is the best approach or if dynamic environment such as that occurs should be recreated whereby combinations of growth factors at different time points would be more effective.

### **Stem cells in aiding skin repair**

Stem cells are characterized by their self-renewal capacity, multilineage differentiation potential (41) and can be derived from various tissues including embryonic, foetal and adult sources. Of these, mesenchymal stem cells (MSC) have been most widely studied in wound regeneration research due to their safe and relatively easy isolation from tissues like fat and skin. MSC derived from skin, fat and bone marrow have shown promising results in

induction and acceleration of healing in both acute and chronic wounds. Here, we discuss the key outcomes from research into the therapeutic potential of epidermal, adipose derived and bone marrow derived stem cells (Table 2). Epidermal stem cells (ESC) are an attractive target for developing wound therapies because they already reside within the skin, more specifically the terminal hair follicle, and are part of the healing response in mammals (40). Ma et al isolated these cells from human hair follicles and using in vitro co-culture assays, showed that they increase proliferation and migration of fibroblasts and keratinocytes as well as enhancing angiogenesis by human umbilical vascular endothelial cells (HUVEC) (42). Additionally, application of these cells to acute full-thickness wounds significantly reduced the time for closure in a type 2 diabetic nude mouse model. In a clinical study, Jimenez et al attempted to implant autologous scalp end terminal hair follicular grafts to non-healing leg wounds of ten patients in order to introduce epidermal stem cells to these wounds (43). At 18 weeks there was a significant reduction in wound size with increased reepithelialisation and vascularisation on histology. The conclusion that this healing was mediated by the terminal hair follicles was supported in a later randomized controlled trial, which compared implantation of grafts containing scalp hair follicles with non-hairy skin grafts on chronic wound healing in 12 patients (44). There was a significant reduction in the terminal hair follicle treated group.

Several studies have highlighted the role that adipose derived stem cells and adipocytes play in wound healing with immune modulation and paracrine signalling shown to be the mode of action. Kim et al investigated the wound healing effect of human adipose derived stem cells both in vitro and in vivo on

acute wounds (45). Results suggested that ADSCs promoted human dermal fibroblast (HDF) proliferation by cell-to-cell direct contact and also by paracrine activation through secretory factors. In vitro wound healing models also demonstrated ADSC conditioned medium stimulatory effects on migration of HDFs. Furthermore, in vivo nude mouse work confirmed wound healing effect of ADSCs by reducing wound size and accelerating re-epithelialization from the edge of the wound after a week. This study provides an important insight into the roles played by ADSC in wound healing to reveal that they directly promote repair through enhancing the wound healing effect of HDF. The work of Schmidt and Horlsey demonstrated that adipocyte lineage cells are activated and function during acute skin wound healing in mouse models (46). These authors showed that the proliferative phase involves the repopulation of adipocytes within skin wounds. An in vivo mouse study indicated that immature adipocytes are activated during the proliferative phase in parallel with mature adipocytes and fibroblast migration. Furthermore, lipoatrophic mice demonstrated impaired wound healing when compared with controls suggesting that adipocytes are required for wound repair. The findings of this study further support the role of adipocytes and their precursors in promoting fibroblast activity in wound healing.

Bone marrow derived stem cells (BMSC) have also been proposed as a potential therapy in wound healing. Their role in acute wound healing was explored by Han et al who compared proliferation, collagen synthesis and growth factor production of bone marrow stromal cells with those of dermal fibroblasts in vitro (47). They found that the amount of collagen synthesis and the levels of FGF and the VEGF were much higher in the BMSC group than

the fibroblast group suggesting that the BMSC may have superior potential to accelerate wound healing than the fibroblasts. In vivo, Uysal et al demonstrated that the addition of ADSC or BMSC to acute wounds in rats resulted in reduced healing time, increasing angiogenesis and reduced wound contraction (48). The mechanisms by which these cells do so maybe related to down regulation of  $\alpha$ -smooth muscle actin and enhanced FGF expression. This is also supported by the work of Wu et al who examined the benefit of BMSC in wound healing using an excisional wound splinting model in both diabetic and non-diabetic mice (49). They showed that injection of BMSC around the wound significantly promoted the healing process in normal and diabetic mouse mice possibly through the release of proangiogenic factors such as VEGF and angiopoietin.

The role of BMSC in chronic wound repair was investigated by Kwon et al who showed that local or systemic delivery of BMSC to a diabetic wound in rats increased wound breaking strength which was associated with increased collagen and growth factor expression (50). The BMSC subpopulation that originate from the haemopoietic cells pool, increases during the early inflammatory phase of wound healing whereas those BMSC from the mesenchymal cells pool are predominant within the healed wound. The effect of different bone marrow preparations in wound healing was investigated by Rodriguez-Menocal et al who demonstrated that whole bone marrow enhanced healing in both in vitro wound assays and in mouse models of radiation induced delayed wound healing (51). These results suggest that different populations of cells in the bone marrow may be responsible for the various effects on wound healing observed upon application of BMSCs to the

injured skin, such as stimulation of angiogenesis, induction of fibroblast migration and reduction of the wound size.

A small number of clinical studies have supported the therapeutic potential of MSC in human wounds. Falanga et al successfully topically delivered autologous BMSC to acute surgical wounds and chronic lower extremity wounds using fibrin spray (52). They showed accelerated wound healing in the acute wounds and significant reduction in size or complete healing in chronic wounds by 20 weeks post treatment. The efficacy of autologous BMSC in the treatment of chronic non-healing ulcers of the lower extremities was compared with standard wound care in a clinical study by Dash et al (53). This study demonstrated a significant decrease in ulcer size in the BMSC treatment group.

Whilst the above studies provide evidence of the contribution of MSC to wound healing and illustrate that this maybe due to immune modulation, paracrine effect on dermal cells and proangiogenic properties, the key limitation has been the use of animal models from which it is not always possible to directly extrapolate findings to the human wound physiology. Additionally, a number of studies use a nude mouse model that may have an abnormal response to wound healing due to its immunosuppressed state. Furthermore, the short duration of the in vivo experiments again does not allow for long-term effect of systemic and local delivery of MSC. Clinical studies Whilst the results of clinical studies are promising, they are limited by a small sample size, short follow up period and lack of randomised control trials.

## **Biomaterials for wound dressing**

Currently, the clinical application of biomaterials in wound healing has been in the form of wound dressings which maintain a moist environment and protect the wound bed (54). Increasingly biomaterial research has sought to use these dressings to actively stimulate wound healing through immunomodulation, cell infiltration, generation of extracellular matrix (ECM) and vascularization (55). A number of natural and synthetic biomaterials have shown promise in acute and chronic wound healing (Table 3).

Natural polymers such as polysaccharides (e.g. alginates, chitosan), proteoglycans and proteins (e.g. collagen, keratin, fibrin) are widely used in wound dressings due to their biocompatibility, biodegradation and similarity to the ECM. In the acute wound, Rho et al demonstrated increased adhesion and spreading of human keratinocytes when cultured on an electrospun collagen matrix (56). Natural derived biomaterials such as chitosan have shown promise in use as a biological dressing because of inherent properties such as haemostatic control, biocompatibility and that they can be modified to allow drug delivery. Chitosan alone was shown to promote wound closure of pressure ulcers in mouse models in an in vivo study by Park et al (57).

Additionally, the same in vivo study showed that wound closure was further accelerated by using chitosan to deliver FGF and as such was an effective drug delivery agent. However, the main limitations of natural polymers are their immunogenicity and potential to inhibit cell function in the long term as a result of their degradation not being easily controlled (58).

The use of animal derived acellular matrices allows for the use of a dressing with similar properties to the ECM but with low immunogenicity due to

decellularisation protocols. This sort of biomaterial has been shown to induce closure of chronic diabetic wounds in humans by Yonehiro et al whose cohort exhibited increased cell infiltration, vascularisation and integration (59). The usefulness of the ECM components of decellularized matrix was again demonstrated by Brigido et al who used a synthetic skin substitute matrix as a wound dressing which again accelerated wound closure in diabetic patients (60).

Synthetic polymers bypass the immunogenic effects of natural materials and are increasingly used to design bioactive dressings. These materials can also be easily functionalised to incorporate drugs to create bioactive dressings. These capabilities were recently demonstrated by Oh et al who created a composite of poly( $\epsilon$ -caprolactone) and chitosan which was then conjugated with caffeic acid to generate biodegradable electrospun mats which promoted dermal fibroblast cell proliferation and displayed anti-microbial effects in vitro (61). Pawar et al loaded electrospun nanofibres with an antimicrobial (Gati), which demonstrated controlled-drug delivery, and low cytotoxicity in vitro as well as accelerated acute full thickness wound healing in rats (62).

Biomaterials with broad-spectrum anti-microbial activity were also created by Pascual et al who synthesised a polycarbonate hydrogel functionalised with methyl iodide (63). These materials exhibited rapid degradation and effective killing of gram positive and negative bacteria as well as fungi in vitro. Xie et al fabricated a composite chitosan poly(ethylene oxide) nanofibrous scaffold embedded with nanoparticles containing vascular endothelial derived growth factor (VEGF) and platelet derived growth factor (PDGF) in an attempt to mimic the extracellular matrix and promote wound healing (64). These

biomaterials exhibited controlled growth factor release, enhanced dermal fibroblast proliferation and antimicrobial action in vitro. There was also a significant increase in reepithelialisation, angiogenesis, collagen deposition and earlier remodelling in acute full thickness wounds dressed with these constructs in a rat model. Further demonstration of the versatility of synthetic materials was provided by Volkova et al who combined gold nanoparticles with cryopreserved fibroblasts in a methylcellulose gel to accelerate wound repair in a rat third degree burn model (65). This resulted in accelerated wound repair with complete reepithelialisation, organisation of collagen and elastic fibres as well as microvessel formation.

The therapeutic potential for synthetic biomaterial dressings in chronic wounds was most recently described by Wang et al who created a composite of copper containing bioactive glass and nanofibrillated cellulose aerogel (66). This biomaterial supported survival and proliferation of fibroblasts, had a proangiogenic effect on HUVEC sprouting assay and was effective in inhibiting gram-negative bacteria. They modelled the hypoxic wound environment using a 3D spheroid culture system, which showed that this biomaterial enhanced HUVEC sprouting and promoted endothelial-fibroblast interactions. Chen et al created a self assembling nanofibre gel which was conjugated with a human placental DNA sequence (polydeoxyribonucleotide) used to treat ischemia and hypoxia (67). This combination promoted embryonic fibroblast cell proliferation and increased expression of cytokines and proangiogenic growth factors in vitro and induced reepithelialisation and granular tissue formation of chronic ulcers in a diabetic mouse model (67).

So far studies have shown great potential for biomaterials in enhancing wound healing with nanotechnology permitting the modification and customisation of material properties to suit the wound repair environment. A number of limitations exist, which has made it difficult to identify which materials would be best for wide spread clinical translation. As previously mentioned, natural materials provide the structural properties required to mimic the extracellular matrix but are limited with regards to immunogenic potential, expensive fabrication protocols (in the case of non cellular matrices) and limited modification potential. Nanomaterials on the other hand are extremely versatile with regards to fabrication and design methodology. They can be generated as nanofibres or particles depending on whether scaffold, dressing or carrier functions are required. An example of this is that whilst nanoparticles enable the targeted delivery of active drugs that may not be bioavailable in vivo due to poor solubility, short half-life and/or leakage from the site of the wound. Further long-term studies are clearly required to also assess their safety and bioactivity in the long term.

### **Skin Tissue Engineering**

Tissue engineering combines many of the key components of regenerative medicine such as biomaterial design, stem cell biology and differentiation protocol often containing growth factors to replace or repair damaged or diseased tissues using biological substitutes. Whilst the previous sections have focused on how endogenous wound repair maybe accelerated by the application of exogenous substances, this portion of the article will focus on

the application of tissue engineering to reconstruct wound defects with functional replacement tissue.

A number of studies have attempted to mimic the extracellular matrix environment in order to direct stem cell differentiation and bioengineer skin tissue. Decellularized animal matrices preserve native skin architecture and have shown promise as suitable scaffolds for skin tissue engineering.

Nakagawa et al investigated the wound healing effects of human mesenchymal stem cells in porcine skin substitute using a nude rat model (68). They found that the wound size was considerably smaller using this construct and that additionally, this could be used to deliver FGF and further accelerate wound healing. In a clinical study, Yoshikawa et al et al cultured BMSC on a collagen scaffold to generate an artificial dermis which induced skin regeneration in 18 out of 20 patients with intractable dermatopathies (69).

Nanotechnology can be used to influence cell behaviour and survival. This capability was demonstrated by Mashinchian et al who used nanotechnology to generate scaffolds with keratinocyte imprints which mediated ADSC differentiation into keratinocytes (70). Seeding of human keratinocytes onto a hybrid gelatin/nanofiber scaffold by Huan et al provided an engineered epidermis which was found to repair skin wounds in a nude mouse model (71). This is further supported by another study by Ma et al where the combination of BMSC and nanofiber promoted complete and accelerated closure of full thickness wounds in a rat model (72). Importantly, the wounds demonstrated an intact epithelium with hair follicles and sebaceous glands as well as normal collagen deposition.

In order to recreate the complexity of normal tissue, it is important to consider that skin is comprised of different cell types with distinct functions that work together to maintain haemostasis and co-ordinate the response to injury (Figure 1). This interdependence was demonstrated through co-culture of human ESC with dermal papilla cells (DPC) on a porcine acellular matrix (73). ESC/DPC constructs were shown to produce a more structured multi-layered stratified epidermis when compared with culture of either of these cells or dermal fibroblasts alone. Engraftment of constructs in a full thickness defect in nude mice demonstrated improved vascularisation and architecture closer to normal skin including the development of hair-bud like structure.

There is increasing evidence that tissue engineering of skin substitutes may eventually provide autologous solutions for wound repair. Protocols that mimic the extracellular environment and reproduce the complex cellular arrangements have succeeded in bioengineering tissue with similar structure to immature skin. It is however still unclear which cell type, scaffold and differentiation protocol are optimal. Additionally, most studies have so far been limited to regenerating the superficial layers of the skin whereby any attempt at skin tissue engineering is likely to require inclusion of the subcutaneous tissues, which provide structure and vascularisation.

## **Conclusion**

Acute and chronic wounds are an increasing public health burden, which is only likely to increase due to the rise in diabetic and ageing populations. Greater understanding of the pathophysiology of wound healing allows the design of more targeted therapies. Regenerative medicine provides a number

of opportunities for accelerating and promoting wound healing. Growth factors, stem cells and biomaterials can be used to induce repair or indirectly to modify the wound environment and stimulate healing. Harnessing the power of tissue engineering by combining stem cells and biomaterials also has huge potential benefits for improving both function and form for patients.

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**Table 1:** Outcomes of growth factor therapy in wound repair.

<b>Growth Factor</b>	<b>Wound type</b>	<b>Study</b>	<b>Summary of outcomes</b>
EGF	Acute	Clinical study	Accelerates epidermal repair in partial thickness wounds (27) and epidermal regeneration in burns (28).
TGFβ	Acute	In vivo	Direct application to rat wounds increases wound strength, collagen deposition and fibroblast influx (29).
FGF	Acute Chronic	In vivo Clinical study	Accelerates rat wound healing (34) Topical application increases closure of traumatic ulcers (35) and pressure sores (36).
PDGF	Acute Chronic	In vivo Clinical study	Impaired wound healing associated with reduced PDGF expression in diabetic mouse wounds (74) whilst addition of PDGF accelerated wound repair (75). Topical PDGF stimulated healing of diabetic lower extremity ulcers (32).
SDF-1	Acute	In vitro and in vivo	Increases epidermal cell migration in vitro and accelerates closure of full thickness wounds in rats (22).

PDWHF	Acute	In vivo	Combination of growth factors (contained in platelet rich plasma) accelerated full thickness wound regeneration in mice (39).
	Chronic	Clinical study	Stimulated reepithelialisation of chronic non healing wounds in blind randomised control trial (38).

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**Table 2:** Mesenchymal stem cell applications in wound healing.

Cell type	Wound type	Study	Summary of outcomes
Epidermal stem cells	Acute	In vitro	Increases proliferation/migration of fibroblasts and keratinocytes and angiogenesis (42).
		In vivo	Accelerates full-thickness wound closure in diabetic mice (42).
	Chronic	Clinical study	Engraftment of terminal hair follicles in chronic leg ulcers increased reepithelialisation, vascularisation and closure (44).
Adipose derived stem cells and Adipocytes	Acute	In vitro and in vivo	Promote fibroblast migration (46), (45), up-regulate collagen I production and down regulate matrix metalloprotease (45).
Bone Marrow derived Stem cells	Acute	In vitro	Increase collagen synthesis and growth factor production (47).
		In vivo	Accelerate healing, increase epithelialisation and angiogenesis in normal (48) and diabetic wounds (49). Optimize wound healing properties of porcine skin substitute (68) and nanofiber scaffolds (72).
		Clinical	Accelerate resurfacing of acute

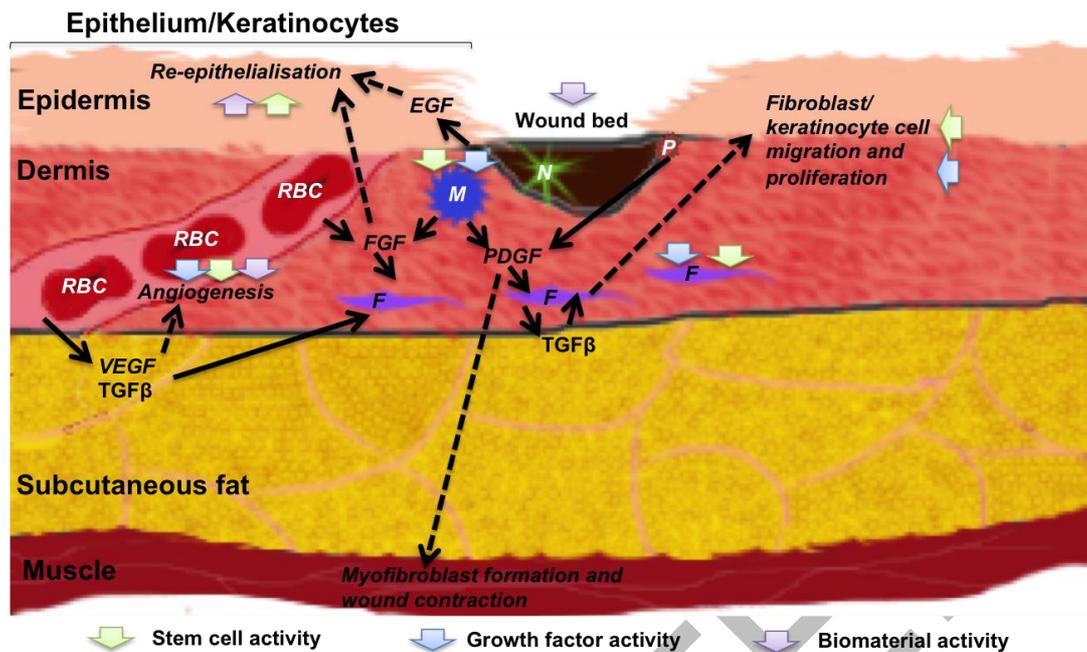
		study	surgical wounds (52)
	Chronic	In vivo	Improve wound strength, collagen I-V and growth factor production in diabetic rat wounds (50)
		Clinical study	Reduce lower extremity ulcer size (53) and cause closure of non healing chronic wounds (69), (76)

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**Table 3:** Biomaterials as bioactive dressings for wound repair.

<b>Biomaterial</b>	<b>Wound type</b>	<b>Study</b>	<b>Summary of outcomes</b>
Natural	Acute	In vitro and in vivo	Electrospun collagen nanofibrous matrix promotes keratinocyte adhesion and spreading (56).
	Chronic	In vivo          Clinical study	Chitosan scaffolds deliver growth factors and accelerate pressure ulcer healing (57). Gelatin mineral composite scaffolds confer antibacterial properties and promote wound healing in burn wounds (77).  Decellularised matrices promote closure of diabetic wounds (59) (60) through increasing cell infiltration and vascularization (59)
Synthetic	Acute	In vitro       In vivo	Biodegradable electrospun mats conjugated with antimicrobial promoted fibroblast and displayed anti-microbial effects (61).  Nanofibre mats allow control antimicrobial drug release and promotes full thickness wound healing (62) whilst nanofibrous

			scaffolds embedded with nanoparticle containing growth factors induce proliferation in vitro and healing in acute full thickness wounds in vivo (64). Gold nanoparticles with cryopreserved fibroblasts mediated repair in a rat third degree burn model (65).
	Chronic	In vitro	Composite copper containing bioactive glass and nanofibrillated cellulose aerogel stimulate angiogenesis and endothelial-fibroblast interaction in a hypoxic wound model (66).
		In vivo	Self assembling nanofibre gels promote reepithelialisation of mouse diabetic chronic ulcers (67).



**Figure 1: An overview of acute wound healing and therapeutic targets for stem cells, growth factors and biomaterials.** Injury to skin triggers an immediate haemostatic response, which results in fibrin clot formation and growth factor release. Acute inflammatory cells, platelets and endothelial cells are active during the inflammatory and proliferative phases of healing whereby they secrete growth factors to promote collagen deposition, vascularization and chemotaxis either directly or through paracrine effects on other cells such as dermal fibroblasts. In the mature stages of wound healing, Dermal fibroblast and myofibroblasts cause wound contraction and scar maturation. Stem cells and growth factors have been shown to promote wound healing through activity on immune cells, promoting angiogenesis and extracellular matrix deposition as well as re-epithelialisation. Biomaterials have shown value in accelerating angiogenesis, regulating the wound environment as a dressing or used alone or with stem cells to promote re-epithelialisation. M; macrophage, N; neutrophil, F; Fibroblast, P; platelet, RBC; red blood cells, EGF; epidermal growth factor, FGF; fibroblast growth factor, PDGF; platelet derived growth factor, VEGF; vascular endothelial growth factor, TGFβ; transforming growth factor beta.



**Figure 2: Therapeutic applications of regenerative medicine in wound healing.** The key components of regenerative medicine (stem cells, biomaterials and growth factors) can be used to target different stages of wound healing such as angiogenesis, immunomodulation, cell proliferation and extracellular matrix (ECM) deposition in order to induce repair. Tissue engineering may combine the use of stem cells, biomaterials and growth factors to generate replacement tissue for repairing non-healing chronic wounds.