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Current Advancements and Strategies in Tissue Engineering for Wound Healing: A Comprehensive Review

J Ho*[1], C Walsh*[1], D Yue[2], A Dardik,[3] ,U Cheema[1]

[1] University College London, UCL Institute for Orthopaedics and Musculoskeletal Sciences, UCL division of Surgery and Interventional Sciences. Stanmore Campus, London, HA7 4LP, UK

[2] Department of Plastic and Reconstructive Surgery, Royal Stoke University Hospital, Newcastle Rd, Stoke-on-Trent, ST4 6QG, UK

[3] The Vascular Biology and Therapeutics Program and the Department of Surgery, Yale University School of Medicine, New Haven, Connecticut, USA

* These authors have made equal contributions

Corresponding Author:

Dr Umber Cheema

University College London

UCL Institute for Orthopaedics and Musculoskeletal Sciences,

UCL division of Surgery and Interventional Sciences,

Stanmore Campus, Brockley Hill, London HA7 4LP, UK

Tel: +44 (0) 20 8909 5116

Fax: +44 (0) 20 8954 8560

E-mail: u.cheema@ucl.ac.uk

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Abstract

Significance

With an ageing population leading to an increase in diabetes and associated cutaneous wounds, there is pressing clinical need improve wound healing therapies.

Recent Advances

Tissue engineering approaches for wound healing and skin regeneration have been developed over the last few decades. A review of current literature has identified common themes and strategies that are proving successful within the field: The delivery of cells, mainly mesenchymal stem cells, within scaffolds of native matrix, is one such strategy. We overview these approaches and give insights into mechanisms which aid wound healing in different clinical scenarios.

Critical Issues

We discuss the importance of the biomimetic niche, and how recapitulating elements of the native microenvironment of cells can help direct cell behaviour and fate.

Future Directions

It is crucial that during the continued development of tissue engineering in wound repair there is close collaboration between tissue engineers and clinicians to maintain the translational efficacy of this approach.

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1.0 **Scope and Significance**

Cutaneous wound healing is a major burden for healthcare systems worldwide. Here, we review the key tissue engineering strategies in cutaneous wound healing, including scaffolds, growth factors and cellular therapies to create biological skin equivalents. We also address the current challenges and future implications to the ever-evolving scientific research and technology.

2.0 **Translational Relevance**

Normal wound healing is commonly described as four, overlapping and co-ordinated stages: Haemostasis, inflammation, proliferation and remodelling. The role of endogenous stem cells is crucial to the process. Tissue engineered solutions that combine stem cells, growth factors and a supporting matrix are being used to create products for clinical wound care applications. There has been a recent research focus on systems of stem cell delivery to wound sites, which ensure cell viability and efficacy in promoting wound healing and the regeneration of its appendages.

3.0 **Clinical Relevance**

Management of wounds is a routine part of medical practice worldwide and delays in healing represent a significant clinical and economic burden. From national data in the United Kingdom, the National Health Service manages 2.2 million patients, costing an estimated £5.3 billion. These numbers are ever increasing, especially with an aging population[1]. They also have a higher mortality, prolonged hospital stays, poorer quality of life and increased rate of being in a long-term care facility when discharged[1–3].

4.0 Background

Skin is the largest organ in the body consisting of the epidermis, dermis, subcutaneous tissue layers as well as skin appendages such as hairs and glands, which expands from deep in the dermis to the superficial epidermal layers (Figure 1). It is very vascular, highly innervated and is functionally responsible for maintenance of homeostasis of the living body by regulation of temperature, hydration, vitamin D synthesis as well as the all-important protective barrier against external chemicals and pathogens. Damage to any part of this organ from the development of a skin wound will inevitably compromise the functional properties mentioned above, exposing individuals to the risk of other health complications.

Normal wound healing is commonly described as four, overlapping and precisely co-ordinated stages: Haemostasis, inflammation, proliferation and remodelling[4]. During the first stage, when the epidermal barrier is violated, keratinocytes react to cell damage. Haemostasis is achieved by endothelial activated vasoconstriction and the clotting cascade. Platelets degranulate alpha granules, leading to secretion of growth factors and pro-inflammatory cytokines[5]. The inflammation phase also begins early with one of the predominant cell types at this stage being neutrophils acting to debride the wound. Another important group of cells are monocytes, which, regulated by TGF-β, transform into macrophages. These further amplify the inflammatory response and the formation of granulation tissue as the proliferative phase is entered, lasting up to 14 days[6,7]. This phase encompasses the multiple processes of angiogenesis, epithelialisation, granulation tissue and collagen deposition. As part of the formation of granulation tissue and laying down of extracellular matrix (ECM), endothelial cell proliferation and angiogenesis has to occur. This is stimulated by vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF)[2,8]. Fibroblasts are the predominant cell type in the early stages. Some of this population transform into myofibroblasts, which are responsible for wound contraction. Fibroblasts secrete components of ECM which form the foundations for the healing skin[9]. The re-epithelialisation process with epithelial cell proliferation and migration starts early after injury and continues into the remodelling phase which can last from months to years. The initial increased fibroblast activity results in laying down of type III collagen which initially may account for 30% of the healing wound collagen. Gradually this is replaced by type I collagen and by the second week,

type I production is predominant again. Both type I and III are produced during wound healing, but it is the ratio of their production that determines the proportion of collagen type. Net collagen accumulation peaks at around the third week after injury. Throughout the rest of the remodelling stage, collagen is produced at elevated rates without an overall net increase. This is due to collagen production being balanced by degradation[10,11].

Chronic wounds have decreased levels of growth factors, display abnormal ECM function and poor blood supply, in addition they show increased levels of the inflammatory interleukins and TNF which which prevents the start of the proliferative stage of healing and can hinder the remodelling process. An overview of the key contributing cells and factors involved in wound healing are presented in Table 1.

4.1 Wounds in the clinical setting

There have been significant advancements to the manufacturing of wound care products over the last few decades. To be able to maximize healing potential through the choice of management options, it is important to have an understanding of different wound types and the pathophysiology of wound environments. Figure 2 broadly defines the different types of wounds seen in a clinical setting[14]. The most common types of chronic wounds being treated include leg ulcers of vasculopathic and diabetic origin, pressure ulcers and surgical or traumatic wounds [1]. It is well established that some patient characteristics predispose them to delayed wound healing. Local factors include oxygenation, infection, foreign bodies or venous disease. Important systemic factors are age, stress, ischaemic factors, obesity, immunosuppression, smoking and nutrition [15]. Certain co-morbidities have also been shown to be independent risk factors for developing open wounds or ulcers.
We have overviewed wound type by severity, and listed specific challenges each type of wound exhibits and strategies used to overcome these (Figure 2)[14].

There are several approaches to wound management in the clinical setting and to consider each one in detail would go beyond the scope of this review. As this article is most concerned about current tissue engineered strategies for wound healing, the use of skin substitutes will be discussed. Skin substitutes can be used alone or as an adjunct to skin grafting for wound coverage, depending on which layers of the skin the product is designed to support. Horch *et al*. have described three types of skin substitutes which have been classified according to the relevant biological action in patients [16]. These were historically developed from how surgeons treated wounds in clinical practice and is summarized in Table 2.

The relevance of this table is seen in how the different commercially available skin products are aimed at different types of wounds treatments (Figure 2 and Table 3). This distinction is particularly helpful for researchers in this field to be able to tailor tissue engineered products to the required patient groups.

The challenge of generating tissue repair is therefore the ability to regenerate native tissue in a manner that allows for the restoration of function to the lost tissue in both acute and chronic wound settings. Tissue engineering can provide the necessary ingredients to replicate tissue via the use of three central components: scaffolds, cells and growth factors, to develop three-dimensional structural units which aim to restore the function to cutaneous tissue [17]. Strategies mainly involve covering wounds with native matrix and/or polymer scaffold dressings, injection of cells directly to the wound site, or indeed cell encapsulation within materials that can then be implanted.

In the following sections we review the key strategies in the use of tissue engineering in cutaneous wound healing, including scaffolds, cellular therapies and growth factors to create biological skin equivalents. We also aim to address the current challenges and future implications to the ever-evolving scientific research and technology.

5.0 Systematic literature review methodology

In order to summarise the key research avenues currently being explored for addressing cutaneous wound healing we have performed a systemic search of the available literature with particular focus on the use of cells and scaffolds in animal wound healing models. The final search term was chosen based on the raw number of hits and the proportion of experimental verses reviews or opinion papers. The search term used was (Scaffold OR "Mesenchymal stem cell" OR Biomaterial OR “cell based therapy”) AND ("Diabetic wound" OR "skin wound") AND ("ischemia" OR "hypoxia"). The search was performed in Google scholar with restrictions on date from 2006-2016, and on English language papers only. The first exclusion pass was based on the title only. Papers were excluded if they were found to meet any of the following criteria:

- Review papers.
- Abstract only.
- Unable to retrieve a full copy of the paper.
- Non-cutaneous wounding.
- Non-wound healing
- Non-English.

The initial search returned 3901 papers. After the first pass this was reduced to 238 papers. Following the first pass, remaining papers were reviewed for scientific quality and variables of interest.

A set of variables relating to methodology and results were created, any papers for which two or more of the chosen variables could not be extracted were excluded. After the second pass 121 papers remained for comparative review.

The variables assessed in this review were chosen with an initial aim to perform a meta-analysis of the effectiveness of various scaffold/cell therapy based treatments and highlight promising research avenues. Despite many high quality individual papers reporting significant results on the basis of well

constructed experimental procedures, the heterogeneity in approaches and protocols prevents meta-analysis of the data.

Despite being unable to perform meta-analysis of wound healing efficacy many of the individual works still report findings of crucial importance to the progression of the wound healing field. Some of these are highlighted in the relevant sections of this review.

6.0 Discussion

6.1 Experimental approaches

The heterogeneity and lack of standardised protocol/approach to reporting of results frustrated any meta-analysis of the systematic review data. This problem is well-recognised and reported in other literature surveys involving *in vivo* animal studies [18–20]. One such instance found was the lack of standardized method or calculation for measuring the size of the wound and reporting the rate of healing. Another discrepancy of approach was the use of splints to prevent wound closure by contraction. This is of particular importance in rodent studies in which wound healing occurs predominantly via contraction rather than epithelial migration as it does in human wound healing [21]. Great heterogeneity can also be found in the type of animal model used and in use of diabetic or immunocompromised strains. Faster research progress in the field could be achieved by a more standardised approach to animal model use, which would allow for large meta-analyses. We report data gathered from the literature review to allow future researchers to standardise their methodology against the consensus in the field where appropriate. Data are shown in Figure 3 for: the proportions different animal models; the use of splints with and without immunocompromised animals; the initial wound sizes created and length of study.

6.2 Role of cells in wound healing

Endogenous stem cells feature prodominatly in the complex and co-ordinated signalling cascades of wound healing. The most abundant skin stem cells are the adnexal structures, particularly the hair follicle bulge stem cells which represents the best characterised epidermal stem cell population. There are other stem cell populations described in the interfollicular epidermis and sebaceous glands [22] (Figure 1). Hair follicle bulge stem cells are most commonly characterised by expression of Keratin 15, although other markers including Lgr6 and MTS24 have been more recently identified [23]. The seminal work of Ito *et al*. demonstrated that new skin cells arose from hair follicle bulge stem cells that had migrated to the epidermis after damage [24]. Since this work many other researchers have used a wider variety of markers to demonstrate the presence of these cells in the epidermis long after wound healing[25]. However, recent controversy has emerged over the time course of the hair follicle bulge stem cells' involvement in wound healing. Whilst Langton *et al*. have shown that in the absence of these stem cells, the initial wound healing rate (4 days) is significantly reduced[26]; more recently Garcin *et al.* showed that these cells may in fact be excluded from the early stages of wound healing for excisional wounds[23].. In the case of burns, the hair bulge's regenerative function has been particularly noted: Superficial burns, which leave the structures intact (see Figure 2) heal rapidly and regenerate epidermal appendages. With more severe burns in which the hair bulge is affected, the regenerated skin shows scarring and lacks adnexal structures[27].

The use of cultured epithelial autografts (CEA) for treatment of burns has been used to treat cutaneous defects since it was described by Green *et al*. in 1979 [28]. This was based on the hypothesis that delivery of the CEA would deliver the inherent skin stem cell population and would enhance wound healing. However, researchers were quick to realise that applying CEA alone into wounds did not achieve good clinical results [29]. Clinically, they were cumbersome to use and patients experienced poor quality of healing with frequent blistering and wound contractures months after grafting[30,31]. Since then, studies have shown that providing matrix support and a delivery system for CEA improves its *in vivo* success, leading to the development of bio-engineered cultured skin substitutes [32,33]. There is still a role for cellular therapies, such as the commercially available Epicel® (Genzyme),

through the instant replacement of lost cell mass in difficult-to-treat wounds, although its efficacy and economical benefits compared to other advanced therapies has yet to be determined[34].

From our analysis of the literature, cellular therapies utilised a variety of cell sources. The predominant cell were stem cells (81.9%) which included bone marrow, adipose derived stem cells, as well as umbilical cord, and Wharton's jelly MSCs. Fibroblasts were the next most common cell type (7.1%). Table 4 lists all cell sources found in the reviewed literature. In order to understand the progression of various cellular therapies currently used for wound healing, the difference between stem cell versus differentiated cells needs to be first appreciated. Differentiated cells, such as fibroblast and keratinocytes, form the basis of commercially available autologous and allogeneic cell-based products. Some products have been on the market since the late 1990s. Their role in skin substitute products, such as Dermagraft® (Organogenesis Inc.) and Apligraf® (Organogenesis Inc and Novartis), is to provide the necessary materials for wound closure via the laying down of matrix proteins and production of growth factors [35,36]. This stimulates healing by promoting host cell migration and infiltration, as well as neoangiogenesis into the wound bed, thereby enhancing rapid re-epithelialisation and closure of the wound. However, there are inherent disadvantages with using allogeneic products. All though the risk is very low, there is a possibility of disease transmission and graft rejection [37]. Conversely, it has been shown in several studies that allogeneic differentiated cells delivered via the biological skin substitute Alipgraf® do not persist in the wound site beyond six weeks, which may explain why rejection is not commonly reported in literature [38,39]. Despite such theoretical benefits, researchers and clinicians still experience limited success with its use. Reported clinical trial studies, using differentiated cell-based products, have shown a collective success rate in wound closure of 35%– 56%, leaving approximately half of the wounds ineffectively treated and vulnerable to the risk of infection and other complications [40]. This has prompted researchers to consider stem cells based therapies as a possible solution to further improve wound healing.

Stems cells' capacity for self-renewal and their inherent clonogenicity and potency make them fundamental in the healing and regeneration of bodily tissues. From the wound healing perspective, stems cells have the potential of correcting the biological deficiencies in chronic wounds, thereby offering the potential of complete skin regeneration, including the restoration of the skin appendages. Although embryonic and induced pluripotent stem cells have the most valuable potency of all cells to differentiate and regenerate, the ongoing issues around the ethics and safety of its use have prompted researchers to focus more on the other stem cell populations such as mesenchymal stem cells (MSCs) instead.

The delivery of MSCs to wounds is gaining popularity in this field. There are distinct advantages of the use of MSCs over differentiated cells. They are known to possess beneficial immunomodulatory effects, such as immune suppressive and immune privilege functions, theoretically making its allographic uses more suitable from that respect [107]. They also have a strong trophic capability of releasing the necessary pro-regenerative cytokines and growth factors for regeneration [108,109] and their ability to differentiate provides a potential cell source for native tissue restoration (See Figure 4). All these help to provide further building blocks to the healing process [110].

Using a porcine model, Mansilla *et al.* investigated the use of bone marrow derived mesenchymal stem cells (BMMSCs) seeded on an 'intelligent' acellular dermal matrix in burns and found total regeneration of wounds with little scarring, including the regrowth of hair follicles as well as burned muscle and even ribs [111]. Li *et al.* and Kataoka *et al.* also reported similar skin appendage formations on addition of BMMSC in a rat and mouse models respectively. Promisingly in both studies, the labeled MSCs were found within regenerated hair follicles, sebaceous glands and dermis, demonstrating MSCs innate ability to contribute functionally to the wound healing process [112,113]. Conversely, there is also a growing body of evidence showing that the therapeutic effect of implanted MSCs comes from the release of necessary secretomes rather than long-term contribution to the structure and transdifferentiation [114–116]. What is clear, however, is the fact that MSCs have the right regenerative characteristics and potential to improve wound healing where differentiated cells are not

implanted. A direct comparison of the role of stem cells to commercially available differentiated cells would be helpful to both researchers and clinicians, but studies in this field are currently lacking. Interestingly, stem cells have been shown to display cellular cross-talk with differentiated cells when co-cultured together, improving and enhancing the therapeutic potential in wound healing. Aoki *et al*. demonstrated how bone-marrow derived MSCs interacts with keratinocytes such that rete ridge-like structures were created in regenerated epidermis in its presence indicating the benefits of cellular diversity within the wound healing environment [117]. However, there exist conflicting evidence on the importance of these cellular interactions as shown in a study by Rodriguez-Menocal *et al.* [118]. 'Dose-dependent' effects were reported in their *in vitro* study, where it was shown that higher levels of co-cultured MSCs inhibited fibroblast migration whereas lower doses of MSCs actually enhanced fibroblast migration patterns. Therefore better understanding of co-culture cellular dynamics , especially with the use of stem cells is needed, and further research will be needed to address this void.

6.2.1 Delivery systems

There are 4 main mechanisms through which cells are delivered to wounds *in vivo*. These include delivery through topical spray, direct injection, systemic delivery and cell-seeded scaffolds. Each delivery mode has advantages. The topical spray and direct injection are easy to administer but difficult to localise cells long term as cells can 'escape' the delivery site. As cells are not encapsulated within a material or matrix, these cells are also considered non-protective [110]. With a systemic delivery of cells, there is reason to believe that stem cells in particular may 'home-in' on the wound site, however this is not certain and the localisation of other cells at the wound is unlikely. Generally, it is accepted that 3D scaffolds (either native matrix rich or polymer), afford cells within them a protective environment and enable one to localise cells to a wound site [110]. Modification of scaffolds' material properties, including degradation times, stiffness, porosity and incorporation of growth factors and/or drugs, is also possible.

There is an emerging appreciation of supporting the stem cell niche in its 3D state, as it is known that maintenance of stem cell pluripotency or multipotency and differentiation is associated with specific micro-environmental cues. These niches provide a set of unique and specific features that help to maintain multipotency proliferation and differentiation an regulate stem cell maintenance . The specific microenvironmental features include chemical signals (including growth factors), cell-cell adhesion and interactions, cell-matrix attachment, mechanical features, stiffness and oxygen environment [119,120]. There is thus, a need to understand which features can influence specific stem cell behaviours we may wish to manipulate, in this case related to the wound healing process. The systematic review data reflects the growing awareness of the importance of the biomimetic niche over the past 10 years. Figure 5 shows the relative proportions of studies which utilize scaffolds, cells and growth factors and the combination thereof.

6.3 The importance of scaffolds in wound healing

As discussed above, cells *in vivo* reside within distinct microenvironments which help to direct cell function, state and signalling. Such microenvironments are critical to wound healing, therefore it is crucial that any cell based therapies, support new or host cell populations by providing a suitable microenvironment. Only in such cases will cells contribute maximally to tissue regeneration and repair. Culturing cells within a 3D environment such as a scaffold, is a method that aids in the creation and maintenance of specific microenvironments or biomimetic niches.. In the case of delivering cell-seeded scaffolds into wound defects, this can allow for new tissue genesis[110]. This greater appreciation of the native 3D microenvironment has brought about the emergence of biomimetic scaffolds that aim to create a biomimetic cell niche, with particular attention to the stem cell niche. Biomimetic tissue engineered scaffolds make use of biomaterials that mimic one or multiple characteristics of the native ECM [121]. This can be in the form of its biodegradability, mechanical properties, matrix composition and/or architecture. There are two main types of tissue-engineered scaffolds, biological and synthetic.

Biological scaffolds can exist as organic molecular polymer or as matrix protein, such as collagen and hyaluronic acid. These scaffolds tend to contain a maximum of 3 matrix components, so are relatively simple in terms of composition. There is also the use of decellularized (acellular) allogeneic or xenographic derived dermal matrices (ADM), which are complex in terms of matrix composition and architecture. Using an ADM as a biological modulator is specifically thought to interrupt the continuous inflammatory process characteristic of chronic wounds and in so doing lead to angiogenesis, cell infiltration and re-epithelialization[122]. However, only small numbers of good quality clinical trials exist for supporting usage of ADM's in chronic wounds and the exact mechanism of action is not fully understood[122–124]. The main processes used to decellularise tissues include detergents, hypo- and –hypertonic solutions, enzymes and chelating agents [124]. The process by which tissues are decellularized is critical as it determines (i) retention of matrix proteins, (ii) maintenance of matrix architecture and (iii) retention of growth factors sequestered within the matrix. Overall, it seems that decellularized acellular dermis potentially provides the most biomimetic matrix to host the all-important cellular niche for tissue engineering purposes.

Commercial wound dressings products commonly use molecular polymers and matrix proteins. Research on tissue engineered wound healing products have mostly moved away from the use of single biomolecular agents on wounds, favouring more sophisticated biologically processed acellular matrix to provide the necessary ECM template for wound healing (Table 1). They can now be found in combination with other biomaterials, such as Apligraf® (Organogenesis Inc.), or as carriers for delivery of cellular products, such as Laserskin®/Vivoderm® (Fidia Advanced Biopolymers/ER Squibb & Sons Inc), to create more complex tissue engineered products for wound healing.

An advantage of synthetic scaffolds, is that they can be customised for purpose in a controlled environment to mimic the tissue architecture of interest. Another major advantage with synthetic scaffolds is the possibility of mass-production,, facilitating the key goal of providing a point-of-care

product in tissue engineered wound care. First described by Yannas and Burke in 1980, the design and use of artificial skin has been an evolving science [125].

Currently opinions in such research are turning towards the use of both organic and inorganic composite materials combined together to create a hybrid scaffold for skin tissue engineering[126]. Organic scaffold components address the need for a biological proteins to provide a favourable environment for cells to proliferate and differentiate; whist inorganic components may facilitate manufacture process and quality control.

It is important to emphasise that scaffolds used alone without the addition of any other cellular or bioactive molecules only promote healing via secondary intention. Therefore, the scaffold influence is to mainly assist the *in vivo* host response in wound repair by provision of the right environment for cell and tissue adherence. Hence, these products are typically used in combination with the conventional gold-standard split thickness skin grafts.

In addition, there is increasing use of scaffold-based delivery systems for stem cell transplantation over other delivery modalities. The driving hypothesis here is that the 3D environment provides cells with the necessary protection and matrix spatial cues for the seeded cells during the delivery process[110]. There are also key advantages in the use of cell-seeded scaffolds over cell-only therapies in wound healing, especially for larger wounds. 3D scaffolds provide greater coverage as well as help to maintain integrity of tissue architecture during wound healing.

Scaffolds may be engineered in a variety of ways. Despite decellularized skin being the most biomimetic of biological scaffolds, limited availability of donor skin and shelf life of such products would deny it from being a solution to the growing demand for tissue engineered skin products. More recently, researchers have been turning to the use of 3D bioprinting technologies as another viable option for skin replacement [127–131]. Bioprinting allows for precise and predefined positioning of living cells and other biological material, enabling the manufacture of customizable tissue constructs based on computer generated designs[132]. Bioprinting is a layer-by-layer process, hence it allows for the

creation of smaller functional tissue units made up of cells which can subsequently be assembled together like building blocks into larger, more complex organs[132]. The skin as an organ, having a layered structure, is highly suited to this printing technology. Mini-tissue blocks with functional units, such as adenexal structures, can technically be recreated using 3D bioprinting using the relevant skin cells types[133]. Amongst all the successful *in vivo* models, Cubo *et al*. have successfully bioprinted and transplanted human skin made up of cells from skin biopsy and have shown the resultant regenerated skin to be histologically similar to that of normal human skin[130]. Hence it is no surprise that the feasibility of this technology has caught the attention of the commercial industry, with bioprinting company Organovo collaborating with cosmetic giant L'Oreal US to invest and research into the bioprinting of skin in 2015[134]. Despite its great potential, this field is still very much in its infancy and will require a few more years of fine tuning of it technology before it finds it way to the bedside [132].

For all scaffolds, the manufactory process must take into account the specific tissue microenvironment that the scaffold aims to mimic. Mechanical, physical and biochemical modifications, are often introduced applied to scaffolds to enhance their wound healing potential[16].. Figure 6 depicts seven features of the cellular microenvironment that should be considered when targeting a biomimetic cell niche[135].

6.3.1 **Material properties:** Recent studies have shown that strength and type of intermolecular bonds within scaffolds may affect cell proliferation and differentiation [136]. Work has also been done to show that the stiffness of the matrix in which cells are seeded can direct lineage specification[137]. Where these cells are seeded in a 'soft matrix' (E_{brain} ~ 0.1-1 kPa) they commit to a neurogenic lineage, compared to being seeded on a stiffer matrix (E_{muscle} ~8-17kPa) where MSCs commit to a myogenic lineage [137]. It is imperative that where stem cells are being delivered to wounds within matrices, this aspect is considered, as MSC fate is influenced by a host of micro-environmental features and triggers.

6.3.2 **Matrix composition.** There has been much work done on the importance of integrin-mediated adhesion for stem cell maintenance. Although functional roles for β1 integrin is not completely understood in terms of stem cell maintenance, it is expressed across human MSCs of three different tissue origins including bone marrow [138]. α6 β1 is an integrin associated with attachment to laminin and addition of laminin to scaffolds can enhance the ability of endothelial cells to fuse to form tube-like structures [139]. Collagen/laminin scaffolds were used to deliver MSCs to an in vivo diabetic wound model, with significantly enhanced wound healing compared to collagen I only [140]. This suggests that variation in MSCs' surface integrin expression and the ability to change integrin expression in 3D matrices can direct their behavior. More so, this highlights the importance of an appropriate choice of ECMs for a given cellular population [138] . Within the literature reviewed here a wide variety of scaffold materials were found with 47.9% of studies used a natural scaffold material, 9.2% a synthetic and 11.6% a hybrid of synthetic and natural materials. The three most widely used materials were Collagen or Collagen:Chitosan, Fibrin, and Alginate.

6.3.3 **Topographic cues.** There is an increasing body of work which shows that the topographic pattern onto which cells are seeded in vitro, can direct stem cell behavior, including differentiation and commitment to different lineages [141]. It has been found that cells attach to different nano-topographical features on material surfaces with specific integrin receptors and focal adhesions which can contribute to cell fate through changes in both cell morphology and biochemistry [142].

6.3.4 **Hypoxia.** Cells cultured *in vitro* are, in the main, exposed to atmospheric O_2 which is far higher than physiological hypoxia, which ranges between 1-9%, and in some cases can be as low as 0% [120,143]. It is known that oxygen tension can directly affect cell proliferation and differentiation, especially for bone marrow derived mesenchymal stem cells [144]. It is also appreciated that a hypoxic

environment, even less than 1% oxygen, is critical to maintenance of stem cell pluripotency and quiescence[145].

6.3.5 **Growth factors.** Scaffolds have also been shown to be good vectors for delivery of growth factors and other necessary biomolecules beneficial to wound healing such as anti-inflammatory and antioxidant substances. Growth factors are indicated in all tissue healing cascades. They are key in coordinating the biological signalling component for cell function and tissue regeneration. They represent biological material that can potentially be used to target distinct wound-healing phases. A variety of growth factors which attempt to target one or several of these phases have been investigated as found through the literature review conducted here and summarised in Table 5. VEGF, SDF-1-α and FGF are the three most widely used factors.

However, given the complexity of the wound healing process, the exogenous use of growth factors does not usually produce results of satisfactory wound healing due to difficulties with mimicking the specific endogenous growth factor production. One effort to replicate this complexity is to control the release of the growth factor to create a more biomimetic environment and a longer lasting therapeutic effect[135,178].

There are two primary ways in which the release from scaffolds can be modulated, either by altering the porosity or surface area of the scaffold to control the rate of diffusion of the factors through and out of the scaffold or via chemical binding of the factor to the scaffold. In the former case the rate of release of the factor depends on a factors diffusivity through the scaffold, the relative concentration of the factor within the native tissue, and the rate of uptake or utilisation of the factor by cells.

Control of the scaffold porosity, is often done by altering the concentration of the scaffold's fibrous components. This effect is consistent across many types of scaffold and growth factor, for example for release of SDF-1 from a Poly (polyethylene glycol citrate-co-N-isopropylacrylamide) (PPCN) scaffold [147], increasing the concentration of the polymer slows the release of SDF-1-α into the

surrounding media. Similarly increasing the chitosan proportion in a collagen:chitosan sponge, produces slower release of Thymosin-β-4 [172]. In addition to controlling the initial scaffold density controlling the scaffolds surface area will affect not only the diffusion of the factors through the scaffold surface, but has also been shown to affect the scaffold degradation rate. As the scaffold degrades, factors will be released at a greater rate. By controlling the degradation rate, the release of the scaffold may also be controlled. Fibrin microspheres within a fibrin scaffold described by Kulkarni et a [179] , show some spatio-temporal control over the release of two factors through exploiting the differing degradation rates of microspheres as compared to larger fibrin gels. Although such approaches represent an highly interesting avenue of research, the temporal control over the release of such factors with these methods is highly simplified by comparison to those released via cellular mechanisms.

The alternative method to controlling factor release is to bind the growth factor chemically or electrostatically to the scaffold. Two such systems were found in the literature reviewed. Fibrin binding bFGF was shown to increase angiogenesis of implanted scaffolds [156], as was fibrin binding VEGF [151]. Another approach is to bind cells into the scaffolds through similar techniques, Wang *et al*. [174] utilised a collagen scaffold with a collagen binding peptide with an affinity for MSCs. Using a porcine model the authors report an increase in wound closure rate for the binding scaffold as compared to a non-binding scaffold as well as increased cell retention [174].

6.3.6 **Architecture.** Tissue architecture dictates physical properties of tissues. For example, the orthogonal pattern of collagen fibrils in cornea confer transparency to that tissue, whilst the parallel array of a bi-modally distributed collagen diameter size confers mechanical strength to tendon in one plane[180]. Skin is a meshwork of interwoven ECM proteins which give skin anisotropic properties, which helps to give skin its flexibility. In regeneration of skin, scarring can limit the flexibility of new skin and therefore strategies to reproduce the normal architectural woven structure of skin are desirable. This biomimetic feature may be introduced into a scar by grafting de-cellularized skin, with the hope that cells would use the biomimetic cues to align themselves and deposit matrix using the

scaffold cues[181]. There is also an emerging view that whereas tissue architecture is seen as a consequence of cell behaviour, mainly deposition of matrix protein and application of strain and tension applied to tissues, it is likely that tissue architecture itself may direct cell behaviour and fate[182]. So cells within an aligned tissue are likely to stress shield and align along principal axis of strain. They are also more likely to deposit matrix along this alignment.

The interactions of cells with the native scaffold within tissues can direct and influence cell behaviour. It is critical to decipher the specific components of microenvironment which can successfully deliver cells to an injury site and direct them to aid tissue repair and re-growth.

7.0 Future of tissue engineering in wound repair

The end-goal for tissue engineering in wound repair is to be able to provide patients with high quality, universal 'off the shelf' skin substitutes which can regenerate skin in wounds as quickly as possible, with minimum scarring. There are still significant challenges in this rapidly evolving research field to be able to achieve this goal. Skin substitute products currently available to patients can only go so far as to partially replace the skin as a protective barrier. However, functional restoration such as its innervation, thermoregulation, perspiration, melanin production as well as aesthetic appearances have yet to be achieved by current bioengineering techniques. It seems the next steps for tissue engineered skin products is the marriage between the use of stem cells within a tissue engineered scaffold to be able to achieve such a full regeneration of skin. Although there are several pre-clinical animal research incorporating such techniques, there only been a handful of clinical studies looking at the usage of MSCs in combination with a biological scaffold to aid skin wound healing in humans. This is summarized in Table 6.

A 2005 study in Japan experimented in a murine model with a bovine-derived collagen sponge (*Terudermis*) impregnated with a suspension of cells derived from bone marrow [183]. They showed that the rate of angiogenesis in a healing wound was greater in the mice population implanted with

collagen matrix containing bone marrow suspension compared with the control group. Their paper also reported a case report of a chronic leg ulcer that was treated with Terudermis impregnated with autologous bone marrow cell suspension. Two weeks following application, healthy granulation tissue had formed and a split thickness skin graft was performed, with successful outcome at long-term follow-up. Although the paper did not specifically mention MSCs, the BM derived cell suspension would have contained some. Yoshikawa *et al*. in 2008 reported the use of artificial dermis (*Pelnac*) soaked with marrow mesenchymal stem cell suspension on 20 subjects with chronic lower limb wounds [184]. In nine cases, this composite graft was placed on the wound and allowed to heal secondarily. In five cases, the composite graft was followed by a split thickness skin graft. Finally, in six cases, diced full thickness skin graft pieces were placed on the wound before the composite graft was applied. 16 of the 20 cases demonstrated complete healing of the chronic defect. The remaining four partially healed, of which 2 subjects died prior to study conclusion. Most recently, Ravari *et al*. in 2011 published data on eight patients who had chronic diabetic foot ulcers [185]. The authors used a very different approach to the studies in Japan and utilised an intensive combined technique. Autologous BM aspirate were injected into a debrided wound bed. A mixture of more suspension, platelet growth factors and fibrin glue was then applied and allowed to form a clot. Finally, BM aspirate impregnated collagen matrix (*Surgicoll*) was placed on top. Three patients had complete wound closure and the others significantly decreased in size (average wound area decrease was 57%, range 24-79%). Again, the paper did not specifically mention mesenchymal stem cells in the aspirate, but some would have been in the suspension.

Table 6 summarises the key features of each study. There is a paucity of cutaneous wound healing clinical studies that utilize both BMMSC's and dermal matrices. Preliminary data are from case reports and series, but nonetheless seem to reveal real clinical potential. Further evidence from good quality, well-powered, double-blinded RCT's are needed before its place can be established in the armament of options for chronic wound healing and reconstruction.

However, it is worth noting that there has been some degree of success of MSCs delivery to patients with wounds resulting from peripheral vascular disease, which have been demonstrated in several published clinical trials. In double blind randomised placebo-controlled trial by Powell *et al*., they found that intramuscular injection of patient-specific, expanded bone marrow cells (CD90+ MSCs and CD14+ monocyte/macrophage subset of CD45+ hematopoietic cells) may have resulted in the prevention of wound area doubling, delayed time to treatment failure as well as prolonged amputation-free survival in the leg of patients with baseline wound from critical limb ischaemia [186]. Clinical trials have also shown there is an advantage over using bone marrow derived stem cells compared to bone marrow derived mononuclear cells at significantly enhancing limb perfusion and ulcer healing rates in patients with diabetes and peripheral vascular disease [187]. These studies have found a potential for therapeutic angiogenesis through the use of MSC therapy. Due to the underlying pathology of chronic non-healing wounds, these findings can be easily translatable to the treatment of these wounds and beyond.

Despite such clinical possibilities of therapeutic success, there are still important questions which need addressing with regards to the use of stem cell-based therapies, such as which type of stem cell population would be best served for wound healing therapies and what are the safety issues that could potentially arise from the autologous compared to allogeneic stem cell use in a clinical setting. There are very few studies addressing these specific issues, thus making it difficult for researchers and clinicians to have absolute confidence in its future clinical applications [40]. It is very likely that potential complications and safety issues will surface in literature with time, an example seen in a paper published in 2004, almost three decades since the introduction of the use of CEA was advocated. It reported the first case of graft site malignancy in a patient who received CEA to his burns injury more thirteen years ago [188]. Five separate localised skin cancer lesions were diagnosed and completely excised in different anatomical distributions of the body previously exposed to CEA, raising the safety concerns of the use of cellular therapies clinically. Although we must take into account that burns injuries itself carries an innate risk of malignant transformations into squamous cell carcinomas, the author also noted the use of mitogenic stimulators and other chemicals during *in vitro*

expansion which may contribute to an increased risk of cancer [188]. Therefore, such issues must be borne in mind when advocating cellular therapies to patients.

8.0 Summary

The field of tissue engineering has come through leaps and bounds over the past decade. There are still many challenges and limitations in the translation of cell therapies for wound healing such as safety, cost and efficacy of treatment. The delivery of stem cells in three-dimensional scaffolds to wounds seems to be the most promising approach. It is safe to predict that as our understanding of stem cell biology improves along with technological advancements in bio-scaffold fabrication; the near future will see tissue engineered techniques become a standard practice for wound regeneration.

Take home messages

1. Experimental measurements of animal model in wound healing should be standardized to facilitate study comparisons.

2. It is important to take into account clinically different wound types when designing and applying tissue-engineering strategies to the management of these wounds.

3. The most widely used cell type for wound healing application are bone marrow derived stem cells.

4. The future of tissue engineering in wound regeneration lies with the use of scaffolds that provide a suitable stem cell environment by mimicking the biological architecture of skin.

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About the Authors

Jasmine Ho (MBBS BSc MSc MRCS) is a MRC and Rosetrees Trust funded Clinical research fellow at University College London, UK. Her research interests include vascularization of tissue engineered constructs

Claire Walsh (MSci MRes PhD) is a Post-doctoral researcher at University College London, UK. Her research interest include computational modelling of tissue engineered constructs and bubble dynamics.

Dominic Yue (MBBS BSc MRCS) is a Specialist Registrar in Plastic and Reconstructive Surgery at the Royal Stoke University Hospital, Stoke-on-Trent, UK.

Alan Dardik MD/PhD is a Professor of Surgery and Vice-Chairman of the Department of Surgery at Yale University as well as Chief of Vascular Surgery at VA Connecticut. He is a surgeon-scientist who seeks to understand the molecular mechanisms by which blood vessels, fistulae and patches that are used to treat patients, successfully adapt to their new environment, yet often proceed to failure.

Umber Cheema (BSc (Hon) PhD) is a Senior lecturer at University College London, UCL Institute for Orthopaedics and Musculoskeletal Sciences, UCL Division of Surgery and Interventional Sciences, UK. Her research interests include engineering vascular networks in 3D engineered tissues to further understand the role native matrix density and composition have on this biological process.

Abbreviations and Acronyms

ADM- Artificial dermal matrix

ADMSCs- Adipose derived mesenchymal stem cells

bFGF- Basic fibroblast growth factor

BM- Bone marrow

BMMSCs- Bone marrow derived mesenchymal stem cells

CEA- Cultured epithelial autografts

ECM- Extracellular matrix

EGF- Epidermal growth factor

FGF- Fibroblast growth factor

FTSG – Full thickness skin graft

GF- Growth factor

hEGF- Human epidermal growth factor

IFN-α – Interferon

IGF - insulin-like growth factor

IL-1- Interleukin 1

MSCs – Mesenchymal stem cells

PDGF- Platelet deriver growth factor

PPCN- Poly (polyethylene glycol citrate-co-N-isopropylacrylamide)

RCT- Randomised control trial

SDF-1-α – Stromal derived growth factor 1- α

STSG- split thickness skin graft

TE – Tissue engineered

TGF - Transforming growth factor

TNF – Tumor necrosis factor

TXA2- thromboxane

VEGF- Vascular endothelial derived growth factor

References

1. Guest JF, Ayoub N, McIlwraith T, et al. Health economic burden that wounds impose on the National Health Service in the UK. BMJ Open 2015;5:e009283.

2. Barrientos S, Brem H, Stojadinovic O, et al. Clinical application of growth factors and cytokines in wound healing. Wound Repair Regen 2014;22:569–78.

3. Brem H, Maggi J, Nierman D, et al. High cost of stage IV pressure ulcers. Am J Surg 2010;200:473–477.

4. Ziegler TR, Pierce GF, Herndon DN. Growth Factors and Wound Healing: Basic Science and Potential Clinical Applications. Springer New York; 2012.

5. Broughton G, Janis JE, Attinger CE. The basic science of wound healing. Plast Reconstr Surg 2006;117:12S–34S.

6. Minutti CM, Knipper JA, Allen JE, et al. Tissue-specific contribution of macrophages to wound healing. Semin Cell Dev Biol 2017;61:3–11.

7. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nat Rev Immunol 2008;8:958–969.

8. Barrientos S, Stojadinovic O, Golinko MS, et al. PERSPECTIVE ARTICLE: Growth factors and cytokines in wound healing. Wound Repair Regen 2008;16:585–601.

9. Tracy LE, Minasian RA, Caterson EJ. Extracellular Matrix and Dermal Fibroblast Function in the Healing Wound. Adv wound care 2016;5:119–136.

10. Campos ACL, Groth AK, Branco AB. Assessment and nutritional aspects of wound healing. Curr Opin Clin Nutr Metab Care 2008;11:281–288.

11. Madden JW, Peacock Jr EE. Studies on the biology of collagen during wound healing. I. Rate of collagen synthesis and deposition in cutaneous wounds of the rat. Surgery 1968;64:288.

12. Delavary BM, van der Veer WM, van Egmond M, et al. Macrophages in skin injury and repair. Immunobiology 2011;216:753–762.

13. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. Physiol Rev 2003;83:835–870.

14. Sussman C, Bates-Jensen BM. Wound Care: A Collaborative Practice Manual. Wolters Kluwer Health / Lippincott Williams & Wilkins; 2007.

15. Guo S, Dipietro LA. Factors affecting wound healing. J Dent Res 2010;89:219–229.

16. Horch RE, Kopp J, Kneser U, et al. Tissue engineering of cultured skin substitutes. J Cell Mol Med 2005;9:592–608.

17. Lee KH. Tissue-engineered human living skin substitutes: development and clinical application. Yonsei Med J 2000;41:774–779.

18. Isakson M, de Blacam C, Whelan D, et al. Mesenchymal stem cells and cutaneous wound healing: current evidence and future potential. Stem Cells Int 2015;2015.

19. Kilkenny C, Parsons N, Kadyszewski E, et al. Survey of the Quality of Experimental Design, Statistical Analysis and Reporting of Research Using Animals. McLeod M, ed. PLoS One 2009;4:e7824.

20. Hooijmans CR, Leenaars M, Ritskes-Hoitinga M. A gold standard publication checklist to improve the quality of animal studies, to fully integrate the Three Rs, and to make systematic reviews more feasible. Altern Lab Anim 2010;38:167–82.

21. Falanga V, Iwamoto S, Chartier M, et al. Autologous bone marrow-derived cultured mesenchymal stem cells delivered in a fibrin spray accelerate healing in murine and human cutaneous wounds. Tissue Eng 2007;13:1299–1312.

22. Taylor G, Lehrer MS, Jensen PJ, et al. Involvement of follicular stem cells in forming not only the follicle but also the epidermis. Cell 2000;102:451–461.

23. Garcin CL, Ansell DM, Headon DJ, et al. Hair Follicle Bulge Stem Cells Appear Dispensable for the Acute Phase of Wound Re-epithelialization. Stem Cells 2016;34:1377–1385.

24. Ito M, Liu Y, Yang Z, et al. Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis. Nat Med 2005;11:1351–1354.

25. Pastar I, Stojadinovic O, Yin NC, et al. Epithelialization in Wound Healing: A Comprehensive Review. Adv wound care 2014;3:445–464.

26. Langton AK, Herrick SE, Headon DJ. An Extended Epidermal Response Heals Cutaneous Wounds in the Absence of a Hair Follicle Stem Cell Contribution. J Invest Dermatol 2008;128:1311–1318.

27. Zhang CP, Fu XB. Therapeutic potential of stem cells in skin repair and regeneration. Chin J Traumatol 2008;11:209–221.

28. Green H, Kehinde O, Thomas J. Growth of cultured human epidermal cells into multiple epithelia suitable for grafting. Proc Natl Acad Sci 1979;76:5665–5668.

29. Jones I, Currie L, Martin R. A guide to biological skin substitutes. Br J Plast Surg 2002;55:185–193.

30. Hafemann B, Hettich R, Ensslen S, et al. Treatment of skin defects using suspensions of in vitro cultured keratinocytes. Burns 1994;20:168–172.

31. Woodley DT, Peterson HD, Herzog SR, et al. Burn wounds resurfaced by cultured epidermal autografts show abnormal reconstitution of anchoring fibrils. JAMA 1988;259:2566–2571.

32. Fang T, Lineaweaver WC, Sailes FC, et al. Clinical Application of Cultured Epithelial Autografts on Acellular Dermal Matrices in the Treatment of Extended Burn Injuries. Ann Plast Surg 2014;73:509–515.

33. Raghunath M, Meuli M. Cultured epithelial autografts: diving from surgery into matrix biology. Pediatr Surg Int 1997;12:478–483.

34. Rennert RC, Rodrigues M, Wong VW, et al. Biological therapies for the treatment of cutaneous wounds: phase III and launched therapies. Expert Opin Biol Ther 2013;13:1523–1541.

35. Phillips TJ, Gilchrest BA. Cultured epidermal allografts as biological wound dressings. Prog Clin Biol Res 1991;365:77–94.

36. Hansbrough JF, Morgan J, Greenleaf G, et al. Development of a temporary living skin replacement composed of human neonatal fibroblasts cultured in Biobrane, a synthetic dressing material. Surgery 1994;115:633–644.

37. Hart CE, Loewen-Rodriguez A, Lessem J. Dermagraft: Use in the Treatment of Chronic Wounds. Adv Wound Care (New Rochelle) 2012;1:138–141.

38. Hu S, Kirsner RS, Falanga V, et al. Evaluation of Apligraf persistence and basement membrane restoration in donor site wounds: a pilot study. Wound Repair Regen 2006;14:427–433.

39. Griffiths M, Ojeh N, Livingstone R, et al. Survival of Apligraf in acute human wounds. Tissue Eng 2004;10:1180–1195.

40. Sorice S, Rustad KC, Li AY, et al. The Role of Stem Cell Therapeutics in Wound Healing: Current Understanding and Future Directions. Plast Reconstr Surg 2016;138:31S–41S.

41. Javazon EH, Keswani SG, Badillo AT, et al. Enhanced epithelial gap closure and increased angiogenesis in wounds of diabetic mice treated with adult murine bone marrow stromal progenitor cells. Wound Repair Regen 2007;15:350–359.

42. Nakamura Y, Ishikawa H, Kawai K, et al. Enhanced wound healing by topical administration of mesenchymal stem cells transfected with stromal cell-derived factor-1. Biomaterials 2013;34:9393–9400.

43. Li Y, Zheng L, Xu X, et al. Mesenchymal stem cells modified with angiopoietin-1 gene promote wound healing. Stem Cell Res Ther 2013;4:1.

44. Chehelcheraghi F, Eimani H, Homayoonsadraie S, et al. Effects of Acellular Amniotic Membrane Matrix and Bone Marrow-Derived Mesenchymal Stem Cells in Improving Random Skin Flap Survival in Rats. Iran Red Crescent Med J 2016.

45. Hou C, Shen L, Huang Q, et al. The effect of heme oxygenase-1 complexed with collagen on MSC performance in the treatment of diabetic ischemic ulcer. Biomaterials 2013;34:112–120.

46. Lian Z, Yin X, Li H, et al. Synergistic effect of bone marrow-derived mesenchymal stem cells and platelet-rich plasma in streptozotocin-induced diabetic rats. Ann Dermatol 2014;26:1–10.

47. Inoue H, Murakami T, Ajiki T, et al. Bioimaging assessment and effect of skin wound healing using bone-marrow-derived mesenchymal stromal cells with the artificial dermis in diabetic rats. J Biomed Opt 2008;13:64036.

48. Lu F, Mizuno H, Uysal CA, et al. Improved viability of random pattern skin flaps through the use of adipose-derived stem cells. Plast Reconstr Surg 2008;121:50–58.

49. Chehelcheraghi F, Eimani H, Sadraie SH, et al. Improved viability of random pattern skin flaps with the use of bone marrow mesenchymal-derived stem cells and chicken embryo extract. Iran J Basic Med Sci 2015;18:764.

50. Castilla DM, Liu Z-J, Tian R, et al. A novel autologous cell based therapy to promote diabetic wound healing. Ann Surg 2012;256:560.

51. Liu Z-J, Tian R, An W, et al. Identification of E-Selectin as a Novel Target for the Regulation of Post-Natal Neovascularization: Implications for Diabetic Wound Healing. Ann Surg 2010;252:625.

52. Kuo Y-R, Wang C-T, Cheng J-T, et al. Bone marrow--derived mesenchymal stem cells enhanced diabetic wound healing through recruitment of tissue regeneration in a rat model of streptozotocin-induced diabetes. Plast Reconstr Surg 2011;128:872–880.

53. Sukpat S, Isarasena N, Wongphoom J, et al. Vasculoprotective effects of combined endothelial progenitor cells and mesenchymal stem cells in diabetic wound care: their potential role in decreasing wound-oxidative stress. Biomed Res Int 2013;2013.

54. Cerqueira MT, Pirraco RP, Santos TC, et al. Human adipose stem cells cell sheet constructs impact epidermal morphogenesis in full-thickness excisional wounds. Biomacromolecules 2013;14:3997–4008.

55. Lee EJ, Park H-W, Jeon H-J, et al. Potentiated therapeutic angiogenesis by primed human mesenchymal stem cells in a mouse model of hindlimb ischemia. Regen Med 2013;8:283–293.

56. Li M, Xu J, Shi T, et al. Epigallocatechin-3-gallate augments therapeutic effects of mesenchymal stem cells in skin wound healing. Clin Exp Pharmacol Physiol 2016;43:1115–1124.

57. Takeda K, Fukumoto S, Motoyama K, et al. Injectable cell scaffold restores impaired cell-based therapeutic angiogenesis in diabetic mice with hindlimb ischemia. Biochem Biophys Res Commun 2014;454:119–124.

58. Raheja LF, Genetos DC, Wong A, et al. Hypoxic regulation of mesenchymal stem cell migration: the role of RhoA and HIF-1α. Cell Biol Int 2011;35:981–989.

59. Assi R, Foster TR, He H, et al. Delivery of mesenchymal stem cells in biomimetic engineered scaffolds promotes healing of diabetic ulcers. Regen Med 2016;11:245–260.

60. Xu K, Cantu DA, Fu Y, et al. Thiol-ene Michael-type formation of gelatin/poly (ethylene glycol) biomatrices for three-dimensional mesenchymal stromal/stem cell administration to cutaneous wounds. Acta Biomater 2013;9:8802–8814.

61. Rustad KC, Wong VW, Sorkin M, et al. Enhancement of mesenchymal stem cell angiogenic capacity and stemness by a biomimetic hydrogel scaffold. Biomaterials 2012;33:80–90.

62. Xu J, Zgheib C, Hu J, et al. The role of microRNA-15b in the impaired angiogenesis in diabetic wounds. Wound Repair Regen 2014;22:671–677.

63. Öksüz S, Ülkür E, Öncül O, et al. The effect of subcutaneous mesenchymal stem cell injection on statis zone and apoptosis in an experimental burn model. Plast Reconstr Surg 2013;131:463–471.

64. Yeum CE, Park EY, Lee S-B, et al. Quantification of MSCs involved in wound healing: use of SIS to transfer MSCs to wound site and quantification of MSCs involved in skin wound healing. J Tissue Eng Regen Med 2013;7:279–291.

65. Naaldijk Y, Johnson AA, Ishak S, et al. Migrational changes of mesenchymal stem cells in response to cytokines, growth factors, hypoxia, and aging. Exp Cell Res 2015;338:97–104.

66. Rettinger CL, Fourcaudot AB, Hong SJ, et al. In vitro characterization of scaffold-free three-dimensional mesenchymal stem cell aggregates. Cell Tissue Res 2014;358:395–405.

67. Tong C, Hao H, Xia L, et al. Hypoxia pretreatment of bone marrow---derived mesenchymal stem cells seeded in a collagen-chitosan sponge scaffold promotes skin wound healing in diabetic rats with hindlimb ischemia. Wound Repair Regen 2015.

68. Guo X, Xia B, Lu X-B, et al. Grafting of mesenchymal stem cell-seeded small intestinal submucosa to repair the deep partial-thickness burns. Connect Tissue Res 2016;57:388–397.

69. Peng Y, Xuan M, Zou J, et al. Freeze-dried rat bone marrow mesenchymal stem cell paracrine factors: a simplified novel material for skin wound therapy. Tissue Eng Part A 2014;21:1036–1046.

70. Zonari A, Martins TMM, Paula ACC, et al. Polyhydroxybutyrate-co-hydroxyvalerate structures loaded with adipose stem cells promote skin healing with reduced scarring. Acta Biomater 2015;17:170–181.

71. Gao W, Qiao X, Ma S, et al. Adipose-derived stem cells accelerate neovascularization in ischaemic diabetic skin flap via expression of hypoxia-inducible factor-1α. J Cell Mol Med 2011;15:2575–2585.

72. Trinh NT, Yamashita T, Tu TC, et al. Microvesicles enhance the mobility of human diabetic adipose tissue-derived mesenchymal stem cells in vitro and improve wound healing in vivo. Biochem Biophys Res Commun 2016;473:1111–1118.

73. Nie C, Zhang G, Yang D, et al. Targeted delivery of adipose-derived stem cells via acellular dermal matrix enhances wound repair in diabetic rats. J Tissue Eng Regen Med 2015;9:224–235.

74. Li Q, Guo Y, Chen F, et al. Stromal cell-derived factor-1 promotes human adipose tissue-derived stem cell survival and chronic wound healing. Exp Ther Med 2016;12:45–50.

75. Verseijden F, Posthumus-van Sluijs SJ, van Neck JW, et al. Vascularization of prevascularized and non-prevascularized fibrin-based human adipose tissue constructs after implantation in nude mice. J Tissue Eng Regen Med 2012;6:169–178.

76. Feng J, Doi K, Kuno S, et al. Micronized cellular adipose matrix as a therapeutic injectable for diabetic ulcer. Regen Med 2015;10:699–708.

77. Sun B, Guo S, Xu F, et al. Concentrated hypoxia-preconditioned adipose mesenchymal stem cell-conditioned medium improves wounds healing in full-thickness skin defect model. Int Sch Res Not 2014;2014.

78. Kuo Y-R, Wang C-T, Cheng J-T, et al. Adipose-derived stem cells accelerate diabetic wound healing through the induction of autocrine and paracrine effects. Cell Transplant 2016;25:71–81.

79. Zeng Y, Zhu L, Han Q, et al. Preformed gelatin microcryogels as injectable cell carriers for enhanced skin wound healing. Acta Biomater 2015;25:291–303.

80. Park I-S, Chung P-S, Ahn JC. Enhancement of ischemic wound Healing by spheroid grafting of human adipose-derived stem cells treated with low-level light irradiation. PLoS One 2015;10:e0122776.

81. Park I-S, Chung P-S, Ahn JC. Angiogenic Synergistic Effect of Adipose-Derived Stromal Cell Spheroids with Low-Level Light Therapy in a Model of Acute Skin Flap Ischemia. Cells Tissues Organs 2016;202:307–318.

82. Caiado F, Carvalho T, Silva F, et al. The role of fibrin E on the modulation of endothelial progenitors adhesion, differentiation and angiogenic growth factor production and the promotion of wound healing. Biomaterials 2011;32:7096–7105.

83. Ackermann M, Pabst AM, Houdek JP, et al. Priming with proangiogenic growth factors and endothelial progenitor cells improves revascularization in linear diabetic wounds. Int J Mol Med 2014;33:833–839.

84. Tellechea A, Silva EA, Min J, et al. Alginate and DNA gels are suitable delivery systems for diabetic wound healing. Int J Low Extrem Wounds 2015:1534734615580018.

85. Shrestha C, Zhao L, Chen K, et al. Enhanced healing of diabetic wounds by subcutaneous administration of human umbilical cord derived stem cells and their conditioned media. Int J Endocrinol 2013;2013.

86. Han K-H, Kim A-K, Kim M-H, et al. Enhancement of angiogenic effects by hypoxia-preconditioned human umbilical cord-derived mesenchymal stem cells in a mouse model of hindlimb ischemia. Cell Biol Int 2016;40:27–35.

87. Wang S, Yang H, Tang Z, et al. Wound Dressing Model of Human Umbilical Cord Mesenchymal Stem Cells-Alginates Complex Promotes Skin Wound Healing by Paracrine Signaling. Stem Cells Int 2015;2016.

88. Edwards SS, Zavala G, Prieto CP, et al. Functional analysis reveals angiogenic potential of human mesenchymal stem cells from Wharton's jelly in dermal regeneration. Angiogenesis 2014;17:851–866.

89. Tam K, Cheyyatraviendran S, Venugopal J, et al. A nanoscaffold impregnated with human wharton's jelly stem cells or its secretions improves healing of wounds. J Cell Biochem 2014;115:794–803.

90. Arno AI, Amini-Nik S, Blit PH, et al. Human Wharton's jelly mesenchymal stem cells promote skin wound healing through paracrine signaling. Stem Cell Res Ther 2014;5:1.

91. Fong C-Y, Tam K, Cheyyatraivendran S, et al. Human Wharton's jelly stem cells and its conditioned medium enhance healing of excisional and diabetic wounds. J Cell Biochem 2014;115:290–302.

92. Kazemi-Darabadi S, Sarrafzadeh-Rezaei F, Farshid A-A, et al. Allogenous skin fibroblast transplantation enhances excisional wound healing following alloxan diabetes in sheep, a randomized controlled trial. Int J Surg 2014;12:751–756.

93. Hu G-L, Ma Y-G, Li Y-M. Transplantation of artificial gelatin-co-bletillastriata gelatin/Salvia miltiorrhiza Corium promotes dermal repair in rats. Trop J Pharm Res 2016;15:735–741.

94. Kairuz E, Upton Z, Dawson RA, et al. Hyperbaric oxygen stimulates epidermal reconstruction in human skin equivalents. Wound repair Regen 2007;15:266–274.

95. Velander P, Theopold C, Bleiziffer O, et al. Cell suspensions of autologous keratinocytes or autologous fibroblasts accelerate the healing of full thickness skin wounds in a diabetic porcine wound healing model. J Surg Res 2009;157:14–20.

96. De Angelis B, Gentile P, Orlandi F, et al. Limb rescue: a new autologous-peripheral blood mononuclear cells technology in critical limb ischemia and chronic ulcers. Tissue Eng Part C Methods 2015;21:423–435.

97. O'Loughlin A, Kulkarni M, Vaughan EE, et al. Autologous circulating angiogenic cells treated with osteopontin and delivered via a collagen scaffold enhance wound healing in the alloxan-induced diabetic rabbit ear ulcer model. Stem Cell Res Ther 2013;4:1.

98. Quan R, Zheng X, Xu S, et al. Gelatin-chondroitin-6-sulfate-hyaluronic acid scaffold seeded with vascular endothelial growth factor 165 modified hair follicle stem cells as a three-dimensional skin substitute. Stem Cell Res Ther 2014;5:1.

99. Ke T, Yang M, Mao D, et al. Co-Transplantation of Skin-Derived Precursors and Collagen Sponge Facilitates Diabetic Wound Healing by Promoting Local Vascular Regeneration. Cell Physiol Biochem 2015;37:1725–1737.

100. Jiang Y, Chen B, Liu Y, et al. Effect of collagen scaffold with adipose-derived stromal vascular fraction cells on diabetic wound healing: A study in a diabetic porcine model. Tissue Eng Regen Med 2013;10:192–199.

101. Jun EK, Zhang Q, Yoon BS, et al. Hypoxic conditioned medium from human amniotic fluid-derived mesenchymal stem cells accelerates skin wound healing through TGF-β/SMAD2 and PI3K/Akt pathways. Int J Mol Sci 2014;15:605–628.

102. Shen Y-I, Cho H, Papa AE, et al. Engineered human vascularized constructs accelerate diabetic wound healing. Biomaterials 2016.

103. Yuan H, Guan J, Zhang J, et al. Exosomes secreted by human urine-derived stem cells accelerate skin wound healing by promoting angiogenesis in rat. Cell Biol Int 2016.

104. Tong X, Lv G, Huang J, et al. Gr-1+ CD11b+ myeloid cells efficiently home to site of injury after intravenous administration and enhance diabetic wound healing by neoangiogenesis. J Cell Mol Med 2014;18:1194–1202.

105. Egaña JT, Danner S, Kremer M, et al. The use of glandular-derived stem cells to improve vascularization in scaffold-mediated dermal regeneration. Biomaterials 2009;30:5918–5926.

106. Murphy MP, Wang H, Patel AN, et al. Allogeneic endometrial regenerative cells: An" Off the shelf solution" for critical limb ischemia? J Transl Med 2008;6:1.

107. Dazzi F, Lopes L, Weng L. Mesenchymal stromal cells: a key player in "innate tolerance"? Immunology 2012;137:206–213.

108. Caplan AI, Correa D. The MSC: an injury drugstore. Cell Stem Cell 2011;9:11–15.

109. Caplan AI, Dennis JE, Department of Biology SRCCWRUARMSCCO- . Mesenchymal stem cells as trophic mediators. J Cell Biochem 2016;98:1076–1084.

110. Duscher D, Barrera J, Wong VW, et al. Stem cells in wound healing: the future of regenerative medicine? A Mini-Review. Gerontology 2015;62:216–225.

111. Mansilla E, Spretz R, Larsen G, et al. Outstanding survival and regeneration process by the use of intelligent acellular dermal matrices and mesenchymal stem cells in a burn pig model. Transpl Proc 2010;42:4275–4278.

112. Li H, Fu X, Ouyang Y, et al. Adult bone-marrow-derived mesenchymal stem cells contribute to wound healing of skin appendages. Cell Tissue Res 2006;326:725–736.

113. Kataoka K, Medina RJ, Kageyama T, et al. Participation of adult mouse bone marrow cells in reconstitution of skin. Am J Pathol 2003;163:1227–1231.

114. Kurtz A. Mesenchymal stem cell delivery routes and fate. Int J stem cells 2008;1:1.

115. Badiavas E V, Falanga V. Treatment of chronic wounds with bone marrow--derived cells. Arch Dermatol 2003;139:510–516.

116. Hocking AM, Gibran NS. Mesenchymal stem cells: paracrine signaling and differentiation during cutaneous wound repair. Exp Cell Res 2010;316:2213–2219.

117. Aoki S, Toda S, Ando T, et al. Bone marrow stromal cells, preadipocytes, and dermal fibroblasts promote epidermal regeneration in their distinctive fashions. Mol Biol Cell 2004;15:4647–4657.

118. Rodriguez-Menocal L, Salgado M, Ford D, et al. Stimulation of skin and wound fibroblast migration by mesenchymal stem cells derived from normal donors and chronic wound patients. Stem Cells Transl Med 2012;1:221–229.

119. Jones DL, Wagers AJ. No place like home: anatomy and function of the stem cell niche. Nat Rev Mol Cell Biol 2008;9:11–21.

120. Stamati K, Mudera V, Cheema U. Evolution of oxygen utilization in multicellular organisms and implications for cell signalling in tissue engineering. J Tissue Eng 2011;2.

121. Ma PX. Biomimetic materials for tissue engineering. Adv Drug Deliv Rev 2008;60:184–198.

122. Harding K, Kirsner R, Lee D, et al. International Consensus. Acellular matrices for the treatment of wounds. London Wounds Int 2010:1–15.

123. Climov M, Bayer LR, Moscoso A V, et al. The Role of Dermal Matrices in Treating Inflammatory and Diabetic Wounds. Plast Reconstr Surg 2016;138:148S–57S.

124. Crapo PM, Gilbert TW, Badylak SF. An overview of tissue and whole organ decellularization processes. Biomaterials 2011;32:3233–3243.

125. Yannas I V., Burke JF. Design of an artificial skin. I. Basic design principles. J Biomed Mater Res 1980;14:65–81.

126. Ninan N, Muthiah M, Park I-K, et al. Natural Polymer/Inorganic Material Based Hybrid Scaffolds for Skin Wound Healing. Polym Rev 2015;55:453–490.

127. Skardal A, Mack D, Kapetanovic E, et al. Bioprinted Amniotic Fluid-Derived Stem Cells Accelerate Healing of Large Skin Wounds. Stem Cells Transl Med 2012;1:792–802.

128. Koch L, Deiwick A, Schlie S, et al. Skin tissue generation by laser cell printing. Biotechnol Bioeng 2012;109:1855–1863.

129. Binder KW, Zhao W, Aboushwareb T, et al. In situ bioprinting of the skin for burns. J Am Coll Surg 2010;211.

130. Cubo N, Garcia M, del Cañizo JF, et al. 3D bioprinting of functional human skin: production and *in vivo* analysis. Biofabrication 2016;9:015006.

131. Michael S, Sorg H, Peck C-T, et al. Tissue Engineered Skin Substitutes Created by Laser-Assisted Bioprinting Form Skin-Like Structures in the Dorsal Skin Fold Chamber in Mice. Slominski AT, ed. PLoS One 2013;8:e57741.

132. Murphy S V, Atala A. 3D bioprinting of tissues and organs. Nat Biotechnol 2014;32:773–785.

133. Ng WL, Wang S, Yeong WY, et al. Skin Bioprinting: Impending Reality or Fantasy? Trends Biotechnol 2016;34:689–699.

134. Organovo Holdings I. L'Oreal USA Announces Research Partnership with Organovo to Develop 3-D Bioprinted Skin Tissue. Available at: http://ir.organovo.com/phoenix.zhtml?c=254194&p=irol-newsArticle&ID=2129344. Accessed February 3, 2017.

135. Hadjipanayi E, Brown RA, Mudera V, et al. Controlling physiological angiogenesis by hypoxia-induced signaling. J Control Release 2010;146:309–317.

136. Jeon O, Alsberg E. Regulation of Stem Cell Fate in a Three-Dimensional Micropatterned Dual-Crosslinked Hydrogel System. Adv Funct Mater 2013;23:4765–4775.

137. Engler AJ, Sen S, Sweeney HL, et al. Matrix Elasticity Directs Stem Cell Lineage Specification. Cell 2006;126:677–689.

138. Prowse ABJ, Chong F, Gray PP, et al. Stem cell integrins: Implications for ex-vivo culture and cellular therapies. Stem Cell Res 2011;6:1–12.

139. Stamati K, Priestley J V, Mudera V, et al. Laminin promotes vascular network formation in 3D in vitro collagen scaffolds by regulating VEGF uptake. Exp Cell Res 2014;327:68–77.

140. Assi R, Foster TR, He H, et al. Delivery of mesenchymal stem cells in biomimetic engineered scaffolds promotes healing of diabetic ulcers. Regen Med 2016;11:245–260.

141. Dalby MJ, Gadegaard N, Oreffo ROC. Harnessing nanotopography and integrin–matrix interactions to influence stem cell fate. Nat Mater 2014;13:558–569.

142. Dalby MJ, Gadegaard N, Oreffo ROC. Harnessing nanotopography and integrin–matrix interactions to influence stem cell fate. Nat Mater 2014;13:558–569.

143. Simon MC, Keith B. The role of oxygen availability in embryonic development and stem cell function. Nat Rev Mol Cell Biol 2008;9:285–296.

144. Ivanovic, Bartolozzi, Bernabei, et al. Incubation of murine bone marrow cells in hypoxia ensures the maintenance of marrow-repopulating ability together with the expansion of committed progenitors. Br J Haematol 2000;108:424–429.

145. Ezashi T, Das P, Roberts RM. Low O2 tensions and the prevention of differentiation of hES cells. Proc Natl Acad Sci U S A 2005;102:4783–8.

146. Rabbany SY, Pastore J, Yamamoto M, et al. Continuous delivery of stromal cell-derived factor-1 from alginate scaffolds accelerates wound healing. Cell Transplant 2010;19:399–408.

147. Zhu Y, Hoshi R, Chen S, et al. Sustained release of stromal cell derived factor-1 from an antioxidant thermoresponsive hydrogel enhances dermal wound healing in diabetes. J Control Release 2016;238:114–122.

148. Tan Q, Chen B, Yan X, et al. Promotion of diabetic wound healing by collagen scaffold with collagen-binding vascular endothelial growth factor in a diabetic rat model. J Tissue Eng Regen Med 2014;8:195–201.

149. Losi P, Briganti E, Errico C, et al. Fibrin-based scaffold incorporating VEGF-and bFGF-loaded nanoparticles stimulates wound healing in diabetic mice. Acta Biomater 2013;9:7814–7821.

150. Murali R, Thanikaivelan P. Bionic, porous, functionalized hybrid scaffolds with vascular endothelial growth factor promote rapid wound healing in Wistar albino rats. RSC Adv 2016;6:19252–19264.

151. Ehrbar M, Zeisberger SM, Raeber GP, et al. The role of actively released fibrin-conjugated VEGF for VEGF receptor 2 gene activation and the enhancement of angiogenesis. Biomaterials 2008;29:1720–1729.

152. Chen F, Wan H, Xia T, et al. Promoted regeneration of mature blood vessels by electrospun fibers with loaded multiple pDNA-calcium phosphate nanoparticles. Eur J Pharm Biopharm 2013;85:699–710.

153. Huang C, Orbay H, Tobita M, et al. Proapoptotic effect of control-released basic fibroblast growth factor on skin wound healing in a diabetic mouse model. Wound Repair Regen 2015.

154. Liem PH, Morimoto N, Ito R, et al. Treating a collagen scaffold with a low concentration of nicotine promoted angiogenesis and wound healing. J Surg Res 2013;182:353–361.

155. Park CJ, Clark SG, Lichtensteiger CA, et al. Accelerated wound closure of pressure ulcers in aged mice by chitosan scaffolds with and without bFGF. Acta Biomater 2009;5:1926–1936.

156. Zhao W, Han Q, Lin H, et al. Improved neovascularization and wound repair by targeting human basic fibroblast growth factor (bFGF) to fibrin. J Mol Med 2008;86:1127–1138.

157. Gérard C, Bordeleau L-J, Barralet J, et al. The stimulation of angiogenesis and collagen deposition by copper. Biomaterials 2010;31:824–831.

158. Wang W, Lin S, Xiao Y, et al. Acceleration of diabetic wound healing with chitosan-crosslinked collagen sponge containing recombinant human acidic fibroblast growth factor in healing-impaired STZ diabetic rats. Life Sci 2008;82:190–204.

159. Chu Y, Yu D, Wang P, et al. Nanotechnology promotes the full-thickness diabetic wound healing effect of recombinant human epidermal growth factor in diabetic rats. Wound repair Regen 2010;18:499–505.

160. Hajimiri M, Shahverdi S, Esfandiari MA, et al. Preparation of hydrogel embedded polymer-growth factor conjugated nanoparticles as a diabetic wound dressing. Drug Dev Ind Pharm 2016;42:707–719.

161. Goh M, Hwang Y, Tae G. Epidermal growth factor loaded heparin-based hydrogel sheet for skin wound healing. Carbohydr Polym 2016;147:251–260.

162. Kim HS, Yoo HS. In vitro and in vivo epidermal growth factor gene therapy for diabetic ulcers with electrospun fibrous meshes. Acta Biomater 2013;9:7371–7380.

163. Shen L, Zeng W, Wu Y-X, et al. Neurotrophin-3 accelerates wound healing in diabetic mice by promoting a paracrine response in mesenchymal stem cells. Cell Transplant 2013;22:1011–1021.

164. Kwon SH, Bhang SH, Jang H-K, et al. Conditioned medium of adipose-derived stromal cell culture in three-dimensional bioreactors for enhanced wound healing. J Surg Res 2015;194:8–17.

165. Arul V, Masilamoni JG, Jesudason EP, et al. Glucose oxidase incorporated collagen matrices for dermal wound repair in diabetic rat models: a biochemical study. J Biomater Appl 2011:0885328210390402.

166. Ito R, Morimoto N, Pham LH, et al. Efficacy of the controlled release of concentrated platelet lysate from a collagen/gelatin scaffold for dermis-like tissue regeneration. Tissue Eng Part A 2013;19:1398–1405.

167. Losi P, Briganti E, Sanguinetti E, et al. Healing effect of a fibrin-based scaffold loaded with platelet lysate in full-thickness skin wounds. J Bioact Compat Polym Biomed Appl 2015;30:222–237.

168. Bhang SH, Park J, Yang HS, et al. Platelet-rich plasma enhances the dermal regeneration efficacy of human adipose-derived stromal cells administered to skin wounds. Cell Transplant 2013;22:437–445.

169. Sun W, Sun W, Lin H, et al. Collagen membranes loaded with collagen-binding human PDGF-BB accelerate wound healing in a rabbit dermal ischemic ulcer model. Growth Factors 2007;25:309–318.

170. Kulkarni M, O'Loughlin A, Vazquez R, et al. Use of a fibrin-based system for enhancing angiogenesis and modulating inflammation in the treatment of hyperglycemic wounds. Biomaterials 2014;35:2001–2010.

171. Park JH, Kim S, Hong HS, et al. Substance P promotes diabetic wound healing by modulating inflammation and restoring cellular activity of mesenchymal stem cells. Wound Repair Regen 2016.

172. Ti D, Hao H, Xia L, et al. Controlled release of thymosin beta 4 using a collagen--chitosan sponge scaffold augments cutaneous wound healing and increases angiogenesis in diabetic rats with hindlimb ischemia. Tissue Eng Part A 2014;21:541–549.

173. Chen H, Jia P, Kang H, et al. Upregulating Hif-1α by Hydrogel Nanofibrous Scaffolds for Rapidly Recruiting Angiogenesis Relative Cells in Diabetic Wound. Adv Healthc Mater 2016.

174. Wang H, Yan X, Shen L, et al. Acceleration of wound healing in acute full-thickness skin wounds using a collagen-binding peptide with an affinity for MSCs. Burn Trauma 2015;2:181.

175. Kobayashi H, Katakura O, Morimoto N, et al. Effects of cholesterol-bearing pullulan (CHP)-nanogels in combination with prostaglandin E1 on wound healing. J Biomed Mater Res Part B Appl Biomater 2009;91:55–60.

176. Romana-Souza B, Porto LC, Monte-Alto-Costa A. Cutaneous wound healing of chronically stressed mice is improved through catecholamines blockade. Exp Dermatol 2010;19:821–829.

177. Nguyen PD, Tutela JP, Thanik VD, et al. Improved diabetic wound healing through topical silencing of p53 is associated with augmented vasculogenic mediators. Wound repair Regen 2010;18:553–559.

178. Cheema U, Alekseeva T, Abou-Neel E a., et al. Switching off angiogenic signalling: Creating channelled constructs for adequate oxygen delivery in tissue engineered constructs. Eur Cells Mater 2010;20:274–281.

179. Kulkarni MM, Greiser U, O'Brien T, et al. A temporal gene delivery system based on fibrin microspheres. Mol Pharm 2011;8:439–446.

180. Cicchi R, Vogler N, Kapsokalyvas D, et al. From molecular structure to tissue architecture: collagen organization probed by SHG microscopy. J Biophotonics 2013;6:129–142.

181. Zhang Q, Johnson JA, Dunne LW, et al. Decellularized skin/adipose tissue flap matrix for engineering vascularized composite soft tissue flaps. Acta Biomater 2016;35:166–184.

182. Xu R, Boudreau A, Bissell MJ. Tissue architecture and function: dynamic reciprocity via extra- and intra-cellular matrices. Cancer Metastasis Rev 2009;28:167–176.

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

184. Yoshikawa T, Mitsuno H, Nonaka I, et al. Wound Therapy by Marrow Mesenchymal Cell Transplantation. Plast Reconstr Surg 2008;121:860–877.

185. Ravari H, Hamidi-Almadari D, Salimifar M, et al. Treatment of non-healing wounds with autologous bone marrow cells, platelets, fibrin glue and collagen matrix. Cytotherapy 2011;13:705–711.

186. Powell RJ, Marston W a, Berceli S a, et al. Cellular Therapy With Ixmyelocel-T to Treat Critical Limb Ischemia: The Randomized, Double-blind, Placebo-controlled RESTORE-CLI Trial. Mol Ther 2012;20:1280–1286.

187. Lu D, Chen B, Liang Z, et al. Comparison of bone marrow mesenchymal stem cells with bone marrow-derived mononuclear cells for treatment of diabetic critical limb ischemia and foot ulcer: A double-blind, randomized, controlled trial. Diabetes Res Clin Pract 2011;92:26–36.

188. Theopold C, Hoeller D, Velander P, et al. Graft site malignancy following treatment of full-thickness burn with cultured epidermal autograft. Plast Reconstr Surg 2004;114:1215–1219.

Tables, and Figure legends

Table 1. Key contributing cells and factors involved in the phases of wound healing.

Table 2. The classification of skin substitutes according to their biological actions. Adapted from Horch et al.[16]

Table 3. Current available commercial tissue engineered therapies for wound healing.

Table 4. Cell types used in the studies systematically reviewed.

Table 5. Summary of all growth factors used in the studies systematically reviewed.

Table 6. Summary of the clinical trial data, that uses MSCs and ECM scaffolds.

Figure 1. Adult skin consists of epidermis, dermis and appendages. Skin stem cells have been described in the hair follicles, sebaceous glands as well as the interfollicular epidermis. Adapted from Servier Medical Art.

Figure 2. A basic diagrammatic representation of different wound types, the treatment challenges and possible tissue engineering solutions in relation to the varying wound severities[14]. The hatched shading over diagrammatic skin layers represents tissue loss.

Figure 3. A Pie chart showing the number of studies performed in the various animal models. B showing the percentage of studies employing splints and either diabetic strains or inducing diabetes in the animal prior to wounding. C the number of days animal wound healing is measured over, modal value found to be 14 days. D Top panel shows the diameter of the circular wound for various animal models bottom panel shows the area in the case of rectangular wounds.

Figure 4. Mesenchymal stem cells possess the right characteristics for use in tissue regeneration of skin.

Figure 5. Representation of the number of studies which adopt various approaches to wound healing. Percentage of studies applying either: cells directly to the wound, growth factors directly or a scaffold as well as those which combine these approaches, i.e. scaffold with encapsulated cells, scaffolds with

encapsulated growth factors, all three combine or cells with additional growth factors.

Figure 6. Overview of a number of cell-scaffold interactions to re-create elements of the biomimetic niche. Re-capitulating elements of the biomimetic niche helps to direct cell behavior, response and fate[135].