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Advanced drug delivery systems and artificial skin grafts for skin wound healing☆

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ABSTRACT

Cutaneous injuries, especially chronic wounds, burns, and skin wound infection, require painstakingly long-term treatment with an immense financial burden to healthcare systems worldwide. However, clinical management of chronic wounds remains unsatisfactory in many cases. Various strategies including growth factor and gene delivery as well as cell therapy have been used to enhance the healing of non-healing wounds. Drug delivery systems across the nano, micro, and macroscales can extend half-life, improve bioavailability, optimize pharmacokinetics, and decrease dosing frequency of drugs and genes. Replacement of the damaged skin tissue with substitutes comprising cell-laden scaffold can also restore the barrier and regulatory functions of skin at the wound site. This review covers comprehensively the advanced treatment strategies to improve the quality of wound healing.

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1. Introduction

As the largest organ of the human body, skin plays a pivotal role in maintaining homeostasis as well as protecting the internal organs from the external environment. Cutaneous injuries, especially chronic wounds, burns, and skin wound infection, require painstakingly long-term treatment with an immense financial burden to healthcare systems worldwide. An aging population coupled with escalating rates of diabetes and obesity continue to increase the prevalence of chronic wounds. It has been estimated that 1–2% of the population in developed countries will experience a chronic wound in their lifetime [1]. In the US, chronic wounds affect 6.5 million patients, with about 18% of diabetic patients over the age of 65 suffer from non-healing foot ulcers [2]. Non-healing wounds such as large-area burns, full-thickness wounds, infected wounds, and chronic wounds not only impair the physiological functions of the skin barrier but can also inflict morbidity and even death [3]. Those wounds require intensive and long-term care with costly wound care products [4,5]. Unfortunately, despite of intense investigation to improve cutaneous wound care, clinical management of chronic wounds remains unsatisfactory in many cases.

Various strategies including growth factor and gene delivery as well as cell therapy have been used to enhance the healing of non-healing wounds. Drug delivery systems across the nano, micro, and macroscales can extend half-life, improve bioavailability, optimize pharmacokinetics, and decrease dosing frequency of drugs and genes. Nanoparticle-mediated delivery would be needed for protein and nucleic acid therapeutics to access the intracellular targets. On the other hand, microparticle-mediated delivery would offer a more sustained therapeutic effect if only extracellular delivery is required because the lower surface-to-volume ratio would slow the release kinetics [6]. Microcapsules or microgels may also be used for cell delivery [7,8]. Among various drug delivery systems, macro-system delivery through tissue-engineered scaffolds is particularly relevant to wound healing as they can serve as a depot for incorporating therapeutics. Furthermore, they can physically protect the wounds as wound dressings [9–11]. Therefore, drug-incorporated scaffolds are particularly promising for synergistically accelerating the healing process of chronic wounds.

Although many bioactive approaches based on bioengineered acellular scaffolds have been explored to improve non-healing wound care, many have proved ineffective in clinical trials [12–15]. The innate wound healing ability of skin is largely depended on the type of wound, patient's accompanying diseases or injury state [12–18]. For advanced wound healing, replacement of the damaged skin tissue with cellular skin substitutes is particularly effective for restoration of the barrier and regulatory functions of the skin at the wound site. Cellular scaffolds incorporating fibroblasts, keratinocytes, stem/progenitor cells, or reprogrammed cells have shown promising results for accelerating in vivo wound healing and reducing scar formation [19–23]. In particular, stem cell-based approaches show increasing promise by acting through immune modulation, paracrine effects, and differentiation into epidermal and dermal cells to replace the damaged skin [24]. Bone marrow-derived mesenchymal stem cells (BM-MSC) as well as MSCs isolated from adipose tissues, skin tissues (epidermal stem cells), umbilical cord, and blood, have been explored in wound healing delivery systems [19,25–27]. In addition, induced pluripotent stem cells (iPSCs) from somatic cells can be an attractive alternative as autologous cell sources [22,23,28,29]. However, the poor viability of stem cells in wound beds characterized by a harsh inflammatory environment often decreases the therapeutic potential of the cells [30–32]. Thus, cell engineering and reprogramming via genetic modification are exciting strategies for advanced wound healing by improving the survival of the transplanted cells, controlling the secretion of therapeutic factors and enhancing the biological functions in vivo [21,33,34]. Recently, genome editing/correction of cells from patients with chronic wounds or skin disorders have also drawn attention for the potential of patient-specific wound therapy [35–39].

Cell-laden bioengineered scaffolds have been produced as skin substitutes mimicking the morphological and biological features of the skin tissue. Conventional approaches to skin tissue engineering have focused on imitating the layer-by-layer structure of skin, but simplifying the complexity of the skin tissue to two major compartments, epidermis and dermis [40–43]. Although the first few generations of skin constructs have produced acceptable results in many cases, there remains ample room for improvement with advanced skin constructs possessing control over cellular composition and spatial distribution to recapitulate the complicated architecture and functions of native skin tissues.

This review covers comprehensively the advanced treatment strategies to improve the quality of cutaneous wound healing and further explores techniques for cell engineering and skin tissue engineering to develop cellular skin substitutes.

2. Complexity of wound healing process – an overview

2.1. Phases of normal wound healing

Cutaneous wound healing consists of three partially overlapping phases: hemostasis and inflammation, new tissue formation, and tissue remodeling (Fig. 1). Within these broad phases, there exist a series of tightly regulated event involving chemotaxis, phagocytosis, neocollagenesis, collagen degradation, and collagen remodeling [44]. In addition, wound healing in human is a complicated biological process requiring the coordinated migration and proliferation of both keratinocytes and fibroblasts, as well as other cell types to mount an inflammatory response, synthesize granulation tissue, and restore the epithelial layer. Immediately following skin injury, a platelet plug and a blood clot result in a temporary sealing of the wound, which prevents blood loss and initiates cascading molecular and cellular events leading to formation of an early, make-shift extracellular matrix that provides a scaffold for cellular attachment and subsequent proliferation. The blood clot and damaged epithelial and endothelial cells also release a variety of chemotactic factors to recruit inflammatory cells from the surrounding tissues and the circulation to the site of tissue damage. In response to inflammatory cues, neutrophils arrive first, followed by circulating monocytes that subsequently differentiate into mature tissue macrophages [45]. Mast cell (MC) infiltration into the wound bed also increases, with most of which originating in the adjacent tissue [46]. A recent series of depletion studies using knockout and other approaches have delineated the different roles of immune cell lineages at the wound site. These studies support the concept that neutrophil recruitment is essential for killing invasive microorganisms in early wounds, whereas cells from the monocyte/macrophage lineage are needed for clearing apoptotic neutrophils and orchestrating tissue-specific functions during the different stages of wound healing [47,48]. Another key leukocyte lineage, mast cells, appear to fine-tune many aspects of wound healing with contrasting actions depending on the timing and amount of MC-derived cytokines/mediators released [49,50]. Although the recruited inflammatory cells at the site of tissue injury are crucial for defense functions and produce cocktails of growth factors and cytokines that are required for the later phase of new tissue formation [51], these cells also secrete numerous toxic mediators, including proteases and reactive oxygen species (ROS) that are harmful to the surrounding tissues. Therefore, convergent data from mouse and human studies have shown that the excessive infiltration of inflammatory cells (especially neutrophils and macrophages) to the wound bed might inhibit rather than enhance the healing process [1,2]. This is particularly apparent in chronic wound situations, and might well underlie the persistent tissue-destroying nature of such wounds [52,53].

As the inflammatory phase subsides, accompanied by resolution of the inflammatory response, the proliferative phase of tissue repair begins by the migration and hyperproliferation of dermal and epidermal cells within the wound bed. Following the endothelial activation and degradation of endothelial basement membrane, blood-vessel sprouting occurs at the wound edge, and new vasculature develops (angiogenesis) [54–56]. Formation of new blood vessels is crucial for the supply of nutrients, oxygen and metabolite exchange. In parallel to angiogenesis, fibroblasts migrate into the wound in response to platelet-derived growth factor (PDGF) [12,57,58], transforming growth factor

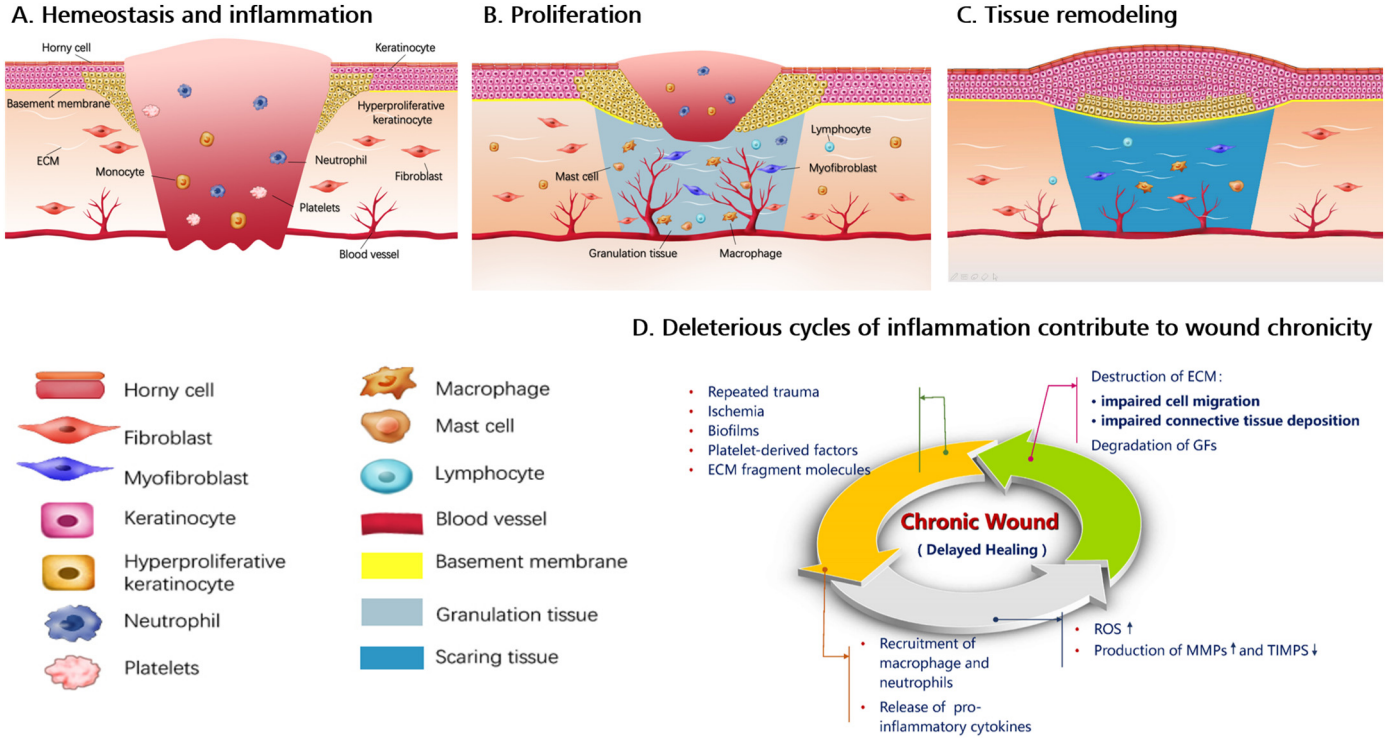

Fig. 1. (A-C) Different phases of normal wound healing. Normal wound healing is a complicated biological process, which can be divided into: inflammatory phase (A), proliferation phase (B), and tissue remodeling phase (C). The inflammatory phase occurs shortly after injury, and is characterized by the influx of inflammatory cells. In response to inflammatory cues, neutrophils migrate to the wound first, followed by monocyte/macrophage lineages, as well as mast cells. As the inflammatory phase subsides, the proliferative phase of tissue repair begins by the migration and hyperproliferation of dermal and epidermal cells within the wound bed. This phase is marked by epithelialization, collagen deposition, angiogenesis, and formation of granulation tissue. The tissue remodeling phase is characterized by matrix remodeling and declined cellularity. During this phase, the wound undergoes contraction, resulting in the formation of a scar with reduced tensile strength. (D) Schematic to show the deleterious cycles of inflammation that contribute to wound chronicity. It is believed that persistent inflammation is a hallmark of chronic non-healing wounds. Due to repeated tissue injury, microorganisms (e.g. biofilms), and platelet-derived factors stimulate the influx of inflammatory cells and the prolonged release of pro-inflammatory cytokines (e.g. IL-1β and TNFα), leading to elevated levels of ROS and proteases (e.g. MMPs) in the wound bed. Particularly, the protease levels in chronic wounds exceed that of their respective inhibitors. High levels of ROS together with the imbalances between *MMPs* and *TIMPs result in the* destruction of ECM components and the degradation of growth factors. The proteolytic destruction of ECM further in turn attracts more inflammatory cells to the wound, thus promoting the inflammation into a *detrimental* vicious *cycle and contributing to* wound chronicity.

β1 (TGF-β1) [59] and fibroblast growth factor (FGF), where they proliferate and produce large amounts of extracellular matrix (ECM). Some fibroblasts differentiate into myofibroblasts, which are responsible for wound contraction and the deposition of additional matrix. The new tissue that forms at the wound site is called granulation tissue because of the granular appearance composed of numerous capillaries, fibroblasts, inflammatory cells, endothelial cells, myofibroblasts, and the components of a new, provisional ECM. The formation of granulation tissue allows the re-epithelialization to take place, as keratinocytes from the wound edge and dermal appendages migrate into the wound bed to cover it with a new epidermis. To migrate efficiently, keratinocytes involved in re-epithelialization rely on an epithelial-mesenchymal transition (EMT)-like process to impart migratory ability to the epithelial cells [60], which allows them to shed their cell-cell adhesions, lose their apical-basal polarity, dissolve the basement membrane, and rearrange their cytoskeletal structure to generate cytoplasmic extensions such as lamellipodia. Following the completion of wound re-epithelialization, keratinocytes gradually stop migrating, revert from their mesenchymal-like phenotype to the epithelial phenotype, and re-differentiate to restore the epidermal integrity [60]. Concomitantly, myofibroblasts undergo apoptosis, angiogenesis is inhibited, and a transition from granulation tissue to acellular scar occurs progressively. It has been shown that a prolonged presence of myofibroblasts at the wound site leads to the formation of hypertrophic scars or even keloids [60]. The tissue remodeling phase is characterized by matrix remodeling and declined cellularity. During this phase, the initial collagen type III of the granulation tissue is gradually dominated by collagen type I, which is the main structural component of the dermis. Originally disorganized collagen fibers are rearranged, cross-linked, and aligned in parallel bundles [61]. These processes result in the formation of a scar with reduced tensile strength and a lack of appendages [60]. Different phases of normal wound healing are shown in Fig. 1A-C.

2.2. Acute and chronic wound healing: classification and mechanisms

Acute wounds, which typically are traumatic or surgical in origin, progress through the normal stages of wound healing and result in a time-dependent but predictable and orderly pattern of tissue repair [62]. Unlike acute wounds, which generally heal without significant interventions, chronic wounds are frequently caused by an underlying pathologic process, and fail to heal in a timely manner. Chronic wounds can be mainly classified into vascular ulcers (e.g., venous and arterial ulcers), diabetic ulcers, and pressure ulcers. Despite differences in etiology at the molecular level, chronic wounds share certain common features, including excessive levels of pro-inflammatory cytokines, proteases, ROS, and senescent cells, as well as the existence of persistent infection, and the inability of dermal and/or epidermal cells to respond to reparative stimuli [52,63].

Persistent inflammation is a hallmark of chronic non-healing wounds. These nonhealing wounds might be trapped in a chronic inflammatory state that fails to progress [44]. Due to repeated tissue injury, microorganisms, particularly in the form of biofilms, and platelet-derived factors, such as TGF-β or ECM fragment molecules, stimulate the excessive recruitment of inflammatory cells to the wound bed. The pro-inflammatory cytokine cascade, e.g. interleukin-1β (IL-1β) and tumor necrosis factor–α (TNFα), therefore becomes amplified and persists for a prolonged period of time, leading to elevated levels of proteases in the lesion. In acute wounds, proteases are tightly regulated by their inhibitors. In chronic wounds, protease levels exceed that of their respective inhibitors, leading to destruction of ECM and degradation of growth factors and their receptors. The proteolytic destruction of ECM not only prevents the wound from moving forward into the proliferative phase but also attracts more inflammatory cells, thus amplifying the inflammation cycle [64]. Meanwhile, inflammatory cells accumulated inside the chronic wound produce high levels of ROS that damage ECM proteins and cause premature cell senescence [65–69]. The toxicity of ROS is reflected by the severe endothelial cell damage and hemorrhage that is observed in wounds of mice lacking the ROS-detoxifying enzyme peroxiredoxin-6 [70]. Conversely, enhancing the expression of ROS-detoxifying enzymes, for example, by the activation of cytoprotective transcription factor nuclear factor erythroidderived-2-like-2 (NRF2), might limit inflammation and improve skin wound healing [71]. In addition to these direct negative effects, ROS together with pro-inflammatory cytokines induce an enhanced stimulation of proteases (including matrix metalloproteinases (MMP)), which degrade components of the ECM and growth factors necessary for normal cell function [52,63]. Inactivation of protease inhibitors by proteolytic degradation augments this process [64]. In a clinical study on measurement of MMPs and their natural inhibitors, tissue inhibitor of metalloproteinases (TIMPs), in fluid from chronic pressure ulcers showed there was a close correlation between high ratios of TIMP/MMP-9 and poorly healing outcomes [72]. Therefore, although the production of growth factors is often increased in chronic compared with acute wounds, their quantity and bio-availability are often compromised [52]. Deleterious cycles of inflammation contributing to wound chronicity are shown in Fig. 1D.

Chronic wounds are also characterized by the phenotypic abnormalities of epidermis- and dermis-derived cells in the wound bed. These abnormalities include lower density of growth factor receptors and reduced mitogenic potential, preventing the resident cells from responding properly to wound healing signals [73]. For example, Loot et al., investigated the mitogenic responses of dermal fibroblasts isolated from diabetic ulcers to various growth factors including recombinant human PDGF-AB, epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and insulin-like growth factor (IGF—I). The growth factors were applied separately or in combination in vitro. As compared with the mitogenic responses of dermal fibroblasts isolated from non-lesional or non-chronic wounds of age-matched control patient, those of diabetic ulcer fibroblasts significantly diminished to the single application of PDGF-AB, EGF, or IGF-I [74]. Vasquez et al., separated dermal fibroblasts from the patients with venous ulcer wounds to investigate the effects of PDGF on fibroblast proliferation. Administration of PDGF-AB, however, had no effect on the growth rate of venous ulcer fibroblasts in vitro due to the decrease in receptor density on these cells [75]. Likewise, fibroblasts associated with chronic nonhealing wounds displayed decreased proliferative ability, early senescence and altered patterns of cytokine release [76], as well as abnormal MMP and TIMP activity [77]. Additionally, further investigations indicate that keratinocytes, endothelial cells, mesenchymal stem cells (MSCs), and macrophages derived from chronic ulcers display a “chronic wound–associated” phenotype. For instance, mesenchymal stem cells in animals and patients with diabetes or chronic wounds are both deficient and defective [78–80]. In addition, chronic wound–derived keratinocytes exhibit defects in differentiation in vivo [81] and impaired migratory potential both in vitro [81] and in vivo [81,82]. Since the abnormal phenotype of the cells residing in chronic wounds is thought to be a cause of impaired healing, investigators suggest gene-modified therapeutic approaches to induce the phenotypic changes and transformation of these cells [83–86], which subsequently improve their responsiveness to wound healing signals and modify the wounds toward the characteristics of an acute healing wound.

ECM also serves as a porous and pliable scaffold for supporting the movement of cells, nutrients, and growth factor through the wound environment. Studies on chemical composition of ECM during wound healing indicate that deposition of a number of matrix components is different in chronic as compared with acute wounds [87,88]. Meanwhile, excessive or insufficient production [82] and post-translational modification [89–91] of the ECM structural components can also negatively influence the cellular responses to injury, resulting in matrix instability [89–91] and impaired re-epithelialization [82]. Since inhibition of matrix degradation, addition of exogenous matrices, and induction of matrix synthesis by resident cells provide therapeutic

opportunities for nonhealing wounds, many collagen dressings (e.g. Promogran®) have been developed to enhance wound repair, particularly for non-infected, chronic, indolent skin ulcers [92]. In summary, an inappropriate inflammatory response in combination with the impaired cellular and extracellular response to the healing environment leads to the deleterious consequence of wound pathogenesis.

3. Chronic wound care treatment & management

3.1. Guidelines for wound assessment and management

As mentioned above, chronic wounds are commonly caused by an underlying pathologic process, such as infection or vascular insufficiency, that produces repeated and prolonged insults to the tissues. Failure to correct or control the underlying pathology can result in a persistent cycle of injury that causes repetitive tissue damage. Consequently, the care for chronic wounds aims at not only removing or ameliorating the etiologic causes, but also addressing the underlying systemic and metabolic perturbations that contribute to wound chronicity [63]. Therefore, wound assessment is a vital first step in precision management of the wound before devising a clinical treatment plan. It includes inspecting the wound, surveying the patient, as well as identifying relevant clinical data from physical examination and patient's health history. Several wound assessment tools, such as Bates-Jensen Wound Assessment Tool (BWAT), Photographic Wound Assessment Tool (PWAT), have been proposed to help monitor and score the wound systematically.

To aid decision-making by linking assessment findings to clinical actions, a multistep approach based on the current understanding of wound healing mechanisms and known by the acronym TIME was developed a decade ago [93]. Now, the TIME framework has been widely used as an organized approach for the optimal preparation of a recipient wound bed. The TIME acronym stands for: (1) Tissue (T), assessment and debridement of nonviable or foreign material (such as necrotic, nonviable tissue, adherent dressing material, multiple organism-related biofilm or slough, exudate and debris) on the surface of the wound; (2) Infection/inflammation (I), assessment of the etiology of each wound, need for topical antiseptic and/or systemic antibiotic application to control infection and management of inappropriate inflammation unrelated to infection; (3) Moisture balance (M), establishing a moisture balance and management of wound exudate in the wound bed, generally with carefully selected dressings; and (4) Edge (E) of wound observation and management [52,63]. Although the basic principles of the TIME concept have not changed greatly since its first inception, the application of these principles has expanded, and led to the development of new wound assessment tools [93]. A key finding in recent studies highlights the importance of the skin beyond the edge, considering the peri-wound skin as an integral part of the wound healing paradigm [94]. Hence, the Triangle of Wound Assessment (TWA), which examines the wound bed, the wound edge, and the peri-wound skin, provides a new approach for wound assessment [95]. It is important to point out that chronic wounds require a systematic approach to both assessment and treatment, no matter what is the underlying etiology of the wound. TIME or TWA framework only provides the basis for chronic wound care. To optimize patient well-being, these principles could be redefined from merely assessing the status of the wound to managing the whole patient by understanding their underlying molecular and cellular abnormalities that prevent the wound from healing.

3.2. Current clinical approaches and advanced strategies

3.2.1. Wound bed preparation: Debridement

Debridement has long been recognized as a critical component for wound care. The aim of debridement is to remove non-viable tissue from the wound bed with the objective to promote wound healing. Chronic wounds are likely to require ongoing maintenance debridement rather than a single intervention. Current methods of debridement used in clinical practice include surgical, autolytic, enzymatic, and biological or mechanical. Surgical, also known as sharp debridement, is the fastest method for large-scale removal of necrotic/septic tissues, and has the benefit of converting a non-healing chronic wound to that of an acute wound within a chronic wound environment [73]. Surgical debridement is normally indicated in life- or limb-threatening infections with necrotic eschar or gangrene. However, this method can be limited by the bleeding tendency and pain tolerance of the patient, and can damage normal healthy tissue and nerves [96]. In this regard, hydrosurgical or low-frequency ultrasound debridement can be used for improving the treatment outcome along with decreasing the number of surgeries required for the wound bed preparation in acute and chronic wounds [97].

Autolytic debridement is a highly selective process involving macrophage and endogenous proteolytic enzymes that digest and break down necrotic tissue and eschar from healthy tissues [73]. Autolytic debridement is indicated for non-infected wounds or used in infected wounds as an adjunctive therapy. It can be also used in conjunction with other debridement techniques such as mechanical debridement in infected wounds. This natural process is further enhanced by use of moisture-retaining dressings (e.g. occlusive and semi-occlusive dressings). Autolytic debridement is usually painless and leaves the wound bed at the correct moisture balance to promote tissue granulation and growth of epithelial cells. However, it is time consuming and carries the risk of invasive infection and wound edge maceration [96,98].

Enzymatic debridement involves topical application of exogenous enzymes to the wound bed where they work synergistically with endogenous enzymes to break down the devitalized tissues. These enzymatic agents include collagenase, varidase, papain (from papaya) and bromelain (from pineapple). Enzymatic debridement can be used alone or in conjunction with other debridement methods, such as sharp or surgical debridement. Although this method appears to be useful for wounds with a large amount of necrotic debris or eschar formation, it is expensive, and the effectiveness of these enzymes has not yet been fully evaluated [96]. In particular, most enzymatic agents typically require prolonged and repeated exposures to achieve sufficient debridement [99]. To allow maximum enzymatic activity, more work remains to be done to develop an efficient system for the delivery of enzymatic debriding agents.

Larval therapy, also known as maggot debridement therapy (MDT) or biosurgery, has been used for many years in patients not suitable for surgical debridement. In this method, sterile larvae are introduced to a wound in order to remove slough and necrotic tissues. Maggots secrete a mixture of proteolytic enzymes that lyse non-viable tissue and bacteria into a form that can be ingested. Larval therapy is proved to be a cost-effective treatment in rapid debridement of chronic wounds with little or no side effects. Particularly, there has been a renewed interest in MDT and its roles on the antimicrobial activity for infected wounds [100,101]. In addition to the impact of MDT on antimicrobial treatment, MDT also promotes wound healing by stimulating tissue regeneration [102]. There is some evidence that maggot excretions could stimulate fibroblast proliferation in culture [103], hepatocyte growth factor (HGF) synthesis in 3 T3 cells [104], as well as increased HGF levels in femoral vein blood of patient during MDT [104]. The presence of maggot may also be associated with increased secretion of anti-inflammatory cytokines (e.g., interleukin-10) [105], while inhibiting the production of pro-inflammatory cytokines (e.g., TNFα) [106], which ultimately lead to the alterations of local wound environment during MDT. Given these findings, Linger et al. developed a tetracycline-repressible system for inducible production of human PDGF-BB protein in the genetically modified L. *sericata* larvae [107]. With the emergence of antimicrobial resistance in the treatment of complicated non-healing wounds, the authors' results highlight the future development in MDT technology that involves genetic engineering-mediated delivery of growth factors and anti-microbial peptides to the wound bed with the aim of improving patient outcome in a cost-effective manner.

Mechanical debridement is done with force to remove devitalized tissue and/or foreign materials from a wound bed. As a non-selective technique, it is usually carried using wet-to-dry dressings, pulsatile lavage, or wound irrigation. Its most common form is wet-to-dry dressings by leaving wet gauze in direct contact with wound surfaces and removing it when dry together with any adhering slough tissue. However, this method is discouraged in the clinic setting, as it causes excessive pain as well as bleeding and can damage newly formed granulation tissue when the dressing is changed.

The clinical significance of wound debridement is to remove necrotic tissue, biofilm, and bioburden along with senescent cells from the wound bed to promote wound healing. It also provides the benefits of stimulating activity of growth factors and promoting wound reepithelization. It becomes clear that the mode of debridement should be tailored not only to the wound presentation and evaluation, but also to the patient's co-morbidities and physical examination.

3.2.2. Infection prevention strategies

A myriad of factors can result in non-healing wounds, for example chronic disease, vascular deficiency, diabetes, malnutrition, aging, and local factors such as inappropriate pressure, infection, and edema [108,109]. Although the etiology varies, all chronic wounds are susceptible to contamination by microorganisms. In general, wounds that show no sign of improved healing within 30 days are deemed chronic and are contaminated with bacteria. Identifying infections and determination of its severity are critical for appropriate wound management and classification [110]. However, the presence of bacteria in a chronic wound does not necessarily indicate that infection has occurred or that it will lead to impairment of wound healing [73]. It has been suggested that low levels of bacteria can actually stimulate wound repair. Traditionally, wound microbiology has been described in four phases: contamination (attachment of nonreplicating microorganisms), colonization (multiplication of the microorganisms, but without clinical host reaction, i.e. no inflammation), critical colonization (release of toxins causes a delay in wound healing without manifest signs of inflammation), and invasive infection (local and/or systemic host reaction with inhibition or even stagnation of wound healing). A critical threshold of 10^5 or 10^6 bacteria per gram of wound tissue is generally proposed as the delineation between colonization and a clinically relevant infection that may have a deleterious effect on healing in various wounds as well as skin grafts [111–113] In a study of the bacteriology of chronic leg ulcers, Trengove et al., showed that the presence of any one specific bacterial group did not appear to be detrimental to wound healing, whereas the presence of four or more bacterial groups (inclusive of *S. aureus*, *P. aeruginosa*, beta-hemolytic streptococci, anaerobes, and coliform bacteria) was associated with delayed healing [111]. Similarly, Bowler and Davies reported a greater diversity of microorganisms in infected leg ulcers than in noninfected leg ulcers [114], indicating that microbial interactions may have induced an enhanced pathogenic effect in the damaged tissues. Besides direct damage to the host, bacteria in wound bed can lead to either the development or maintenance of a chronic wound, due to the release of toxins (such as IL-1, TNF-α, and MMPs), competitive metabolism and prolonged inflammation. The prolonged inflammation, as mentioned above, can further contribute to abundant levels of proteases and decreased levels of protease inhibitors, that degrade ECM and growth factors as well as angiogenic factors and ultimately influence the whole cellular behaviors in the wound [64]. Meanwhile, similar to other infective processes, the microorganisms in chronic wounds often form polymicrobial biofilms, which are complex communities consisting of aggregated bacteria embedded in a protective extracellular polymeric substance (EPS, mainly consists of polysaccharides, proteins, and glycoproteins) [115]. The specific composition and structure of EPS have an effect on the physico-chemical characteristics of the biofilm and determines its tolerance to the host's immune system, antimicrobial agents, and environmental stresses.

Although the treatment and prevention of wound infections is still an area of debate, wound infection control can be achieved using topical antiseptics, topical antibacterials, and systemic antibiotics. Based on the site of tissue damaged and clinical symptoms received, wound sampling technique in combination with antibiotic susceptibility assay is an important aspect of obtaining the information required to guide appropriate antibiotic treatment [116]. In addition to antibiotic therapy, wound debridement, cleansing and pulsating jet lavage for removal of the devitalized tissue may assist antibiotic treatment by reducing the microbial load and enabling better penetration of antibiotics to where they are needed. Hyperbaric oxygen (HBO) therapy has also been used to treat a variety of oxygen compromised acute and chronic wound types as a valuable adjunct to surgical debridement and antibiotic therapy. HBO therapy directly impairs the growth of some anaerobic bacteria (e.g., *C. perfringens*), encourages the growth of new blood vessels in ischemic tissues, and enhances the potency of the oxygen-dependent antimicrobial mechanisms in polymorphonuclear leukocytes (PMNs) [113]. As antibiotic resistance of skin wound flora has emerged as a significant problem, topical antiseptics offer alternative solutions to antimicrobial wound management. It has been shown that antiseptics may present advantages over topical antibiotics in treatment of certain infections, particularly in open and chronic wounds, since they do not cause a risk of developing clinically relevant drug resistance and have broader antimicrobial spectrum, as well as lower skin sensitization rates [117]. In particular, the effectiveness of antiseptic agents in preventing the growth of biofilm-forming bacteria has been demonstrated in several in-vitro and in-vivo studies [118]. Chen and Schluesener have shown that treatment with high concentrations of silver nanoparticles (up to 100– 150gml^{-1}) enabled eradication of biofilms associated with *P. aeruginosa* and S. proteamaculans [119]. Phillips et al., indicated that the silver dressing was the most effective in reducing mature biofilm among differential types of antimicrobial agents (iodine, silver, polyhexamethylene biguanide, honey and ethanol), and the efficacy was influenced by time of exposure, number of applications, moisture level and agent formulation [120]. Alternative methods, such as negative pressure wound therapy with instillation (NPWTi) using antimicrobial solutions and povidone iodine (PVP-I)-containing dressings, were increasingly used as an adjunct therapy for treatment of multidrug-resistant and biofilm-associated bacteria [118]. Currently, there is no conclusive evidence that one antibiotic or antiseptic is superior to any other in wound management. With increased understanding of the complex interplay between the physical and physiological environments of a wound, continued research is needed to address treatment-resistant bacteria and biofilms. Modern antimicrobial therapies synergizing antibiotics or antiseptics, as well as their appropriate combined usage, need to be explored for efficient elimination of infection and decreasing time to healing.

3.2.3. Biological therapy: growth factors

Biological therapies involving tissue-based treatments (acellular and cellular), autologous platelet-rich plasma (PRP), as well as recombinant human growth factor application have received significant attention in the field of wound healing in the past few decades. Given that wound healing is a complex, evolutionarily conserved and multi-cellular process, growth factors and cytokines released by autocrine and/or paracrine means influence many processes of the healing, including proliferation and migration of resident cells, chemotaxis of inflammatory cells and fibroblasts [121]. In vivo and in vitro studies analyzing non-healing chronic wounds have demonstrated dysregulation of the biological activity of growth factors or cytokines, e.g. PDGF [122], vascular endothelial growth factor (VEGF) [123] and bFGF [124]. Thus, modifying their levels in chronic wounds to match those in an acute wound can extinguish inflammation and provide an optimal environment for granulation formation. PDGF, for instance, is released from the alpha granules of activated platelets and functions as a key mediator in the inflammation and repair phases of the healing. PDGF has been

shown to have important biological effects on wound healing including: recruitment of vascular smooth muscle cells and pericytes to increase the structural integrity of newly formed vessels [125,126]; upregulaiton of insulin growth factor 1 and thrombospondin-1 to mediate the motility and proliferation of keratinocytes [127,128]; simulation of fibroblast proliferation and differentiation to induce the production of ECM consequently [129,130]. Its importance is also highlighted by being the first US Food and Drug Administration (FDA) approved growth factor (Becaplermin) for topical application to accelerate wound closure [116,131]. In a multicenter, randomized, double-blind placebo-controlled trial in patients with lower-extremity diabetic neurotrophic ulcers of at least 8 weeks' duration, significant overall improvement was seen in wound closure during the course of 20-week treatment with recombinant human PDGF-BB compared with placebo [132]. Certain evidence further suggests that improved healing outcomes are obtained with PDGF-BB as adjuncts to aggressive sharp debridement of necrotic tissue and pathogens [133], or in combination with HBO therapy [134]. PDGF-BB also shows promise for treatment of advanced pressure ulcers (PUs) based on anecdotal data from several clinical trials [135,136].

Other growth factors, such as bFGF, have yielded mixed clinical results despite promising preclinical studies. FGFs are produced by a large number of cell types, including keratinocytes, fibroblasts, endothelial cells, smooth muscle cells, chondrocytes, and mast cells, with important implications in granulation tissue formation, re-epithelialization and tissue remodeling [137]. Although clinical studies have proved the effectiveness of recombinant human-bFGF (rh-bFGF) topical application in the management of PUs [138,139] and second-degree burns [140], topical treatment of bFGF has no advantage over placebo for healing diabetic neuropathic foot ulcers [141]. In a randomized placebo-controlled clinical trial, however, patients' diabetic ulcers treated with 0.01% (*w*/*v*) rh-bFGF showed a 75% or greater reduction in the area of the ulcer compared to placebo [142]. In a double-blinded controlled study, Yao et al., administered rh-bFGF-adsorbed collagen sponges to chronic traumatic ulcers of patients. It significantly increased the incidence of complete wound closure, shortened the healing time, and improved the healing quality of chronic traumatic ulcers compared with placebo control group [143]. Similar to bFGF in its biological properties, keratinocyte growth factors (KGFs), EGFs, and VEGFs have been also shown to enhance ECM formation, cellular proliferation, and angiogenesis [121,137]. By using randomized, double-blinded, placebo-controlled trials, patients with diabetic foot ulcer (DFU) were recruited to evaluate the efficacy and safety of recombinant human EGF (rh-EGF), and the results indicated that either intralesional application [144] or topical spray treatment [145] with rh-EGF accelerated healing of diabetic ulcers. Randomized controlled trials have been also conducted on the efficacy of topical application of rh-VEGF (Telbermin) in patients with neuropathic DFUs. The data revealed positive trends suggestive of potential signals of biological activity observed for incidence of complete ulcer healing (41.4% treatment vs. 26.9% placebo) and time to complete ulcer healing (25th percentile of 32.5 days treatment vs. 43.0 days placebo) [146]. In addition, platelet-rich plasma (PRP) contains high levels of platelets and a full complement of clotting and growth factors. PRP is, therefore, widely used throughout diverse fields of medicine for improving tissue regeneration [147]. An advantage of PRP over the use of single recombinant human growth factor delivery is the release of multiple growth factors and differentiation factors upon platelet activation [148]. Despite several small trials and retrospective studies having affirmed the potential efficacy of topically applied, activated, autologous platelet supernatants in the treatment of chronic lower extremity wounds [149–151], further controlled, prospective clinical trials are needed to support their usage in wound healing.

In summary, administration of exogenous growth factors and cytokines has shown promise in improving healing results in wounds. However, many unanswered questions remain regarding the use of topical growth factors in the treatment of chronic ulcers. Larger randomized controlled trials are required to support the efficacy, safety, and long-term outcomes of these products for the management of non-healing wounds. Future studies including growth factor-based gene therapy and biomaterial-based drug delivery systems might revolutionize treatment of chronic wounds.

3.2.4. Advanced approaches to wound care

The choice on treatment strategies depends on the type of wound of individual patients. Although successful wound debridement helps to convert a poorly healing wound microenvironment into a microenvironment more closely resembling an acute wound, additional approaches including complex surgical procedures, well-timed revascularization, infection control, off-loading or complete pressure relief for diabetic foot and pressure ulcers, and suitable compression for venous ulcers are required to match patient's satisfaction and restore the quality of life [52,63,152]. Particularly, the relevance of excessive or insufficient wound exudate and its molecular components has led to the development and use of a wide range of dressings to substitute for lost native epithelium, to regulate moisture balance and restrict liquid and microbial penetration, to provide appropriate pressure for hemostasis and allow for air exchange, to protect peri-wound skin, and promote re-epithelialization during the reparative phase [52,63,152]. Based on the cause and type of wound, there are >3000 types of wound dressings available on the market today. These dressings are usually based on natural and synthetic materials and are classified into traditional, artificial, and biological dressing products [153,154]. In addition, adjunctive treatment with advanced therapies, such as NPWT, HBO, bioengineered skin substitutes and cell-based therapies, is also encouraged when the wound is not responding well to standard wound care after a 4-week therapy [63]. As chronic wound healing is multifactorial, it is clear that future treatment paradigm for chronic wounds must move toward precision medicine strategies that tailor personalized therapy for individual patient. Next generation products for wound management should promote a wound to heal by regeneration rather than repair, allowing the functional recovery of injured skin and reconstruction of its appendages in a site- and time-specific pattern.

4. Advanced delivery systems for wound healing

This section introduces the most recent and advanced drug delivery systems based on nanoparticles, microcarriers and tissue-engineered scaffolds for wound healing (Fig. 2). Nano-sized carriers are required for delivery of biologics that act intracellularly, such as peptides or nucleic acids. Small size would favor an improved drug penetration into wound beds and an increased intracellular uptake. On the other hand, micro-sized carriers offer a slower extracellular drug release due to a low surface-to-volume ratio. Microcarriers can encapsulate macromolecules and extend their half-life in wound beds by physically protecting them against a hostile environment. Among various drug delivery systems, scaffold-mediated drug delivery is advantageous for wound healing because the scaffolds can serve the dual purpose of a drug depot and a physical barrier as a wound dressing material. Table 1 summarizes the drug delivery systems based on nanoparticles or microcarriers for wound healing, and highlights their advantages and drawbacks. Additionally, various strategies of substrate-mediated drug delivery are summarized in Table 2 and Fig. 3.

4.1. Nanoparticles

Nanoparticulate drug delivery systems provide controlled or sustained release of drugs at therapeutic concentrations. Nanocarriers could extend drug half-life, improve bioavailability, optimize pharmacokinetics profiles, and decrease frequency of drug administration. Nanocarriers ranging from polymeric to lipid-based and to inorganic

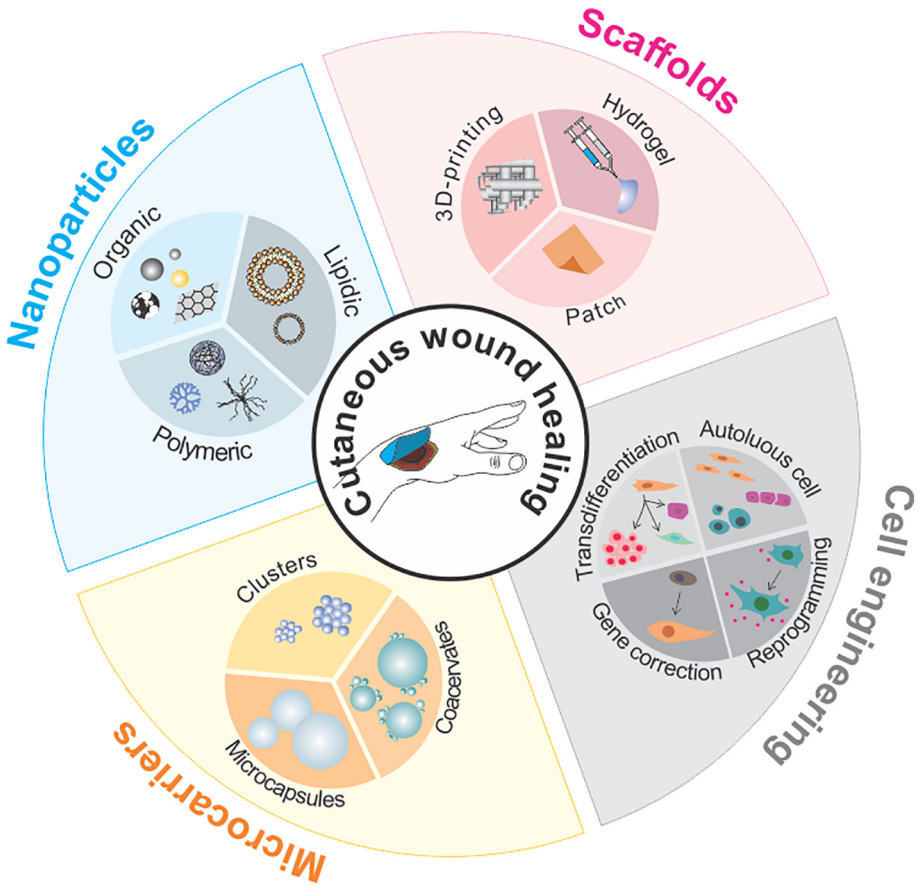

Fig. 2. Schematic overview of drug and cell delivery systems for cutaneous wound healing.

nanoparticles have been investigated for drug delivery in wound healing.

4.2. Polymeric nanoparticles

Polymeric nanocarriers have been popular for drug delivery because of their biocompatibility, biodegradability, injectability and intracellular delivery capability. The physico-chemical properties of polymer nanocarriers can be tailored precisely at a molecular level to enhance drug loading and control drug release. Poly (lactic-*co*-glycolic acid) (PLGA)-based nanoparticles have been widely investigated due to its versatile degradation kinetics and controlled drug release properties. Generally, PLGA nanoparticles are prepared by using the water/oil/water (W/O/W) double emulsion method and can result in a high drug encapsulation efficiency and relatively uniform size distribution. For example, LL37, an antibacterial peptide can be encapsulated in PLGA nanoparticles (PLGA-LL37 NPs), with a ~70% efficiency and a particle size around 300 nm [155]. Similarly, PLGA nanoparticles incorporating recombinant human epidermal growth factor (rhEGF) could be prepared with a uniform size of 193.1 nm and an encapsulation efficiency of 86% [156]. Sustained delivery of rhEGF from these nanoparticles significantly improved the therapeutic outcome of a full thickness wound in diabetic mice, in comparison with a bolus delivery of free rhEGF [156]. Interestingly, PLGA can also perform as a wound healing agent itself by supplying lactate byproducts upon degradation because exogenous lactate can accelerate angiogenesis, activation of procollagen factors and recruitment of endothelial progenitor cells in wounds [157].

Cationic polymers have been extensively used as carriers for anionic drugs, in particular for nucleic acids, due to their ability to condense drugs into nano-sized particles via electrostatic interactions. Moreover, the positive zeta potential of nanoparticles possess an intrinsic bioadhesiveness to the negatively charged mucosal surface of wound beds [158,159]. Liu et al. fabricated cationic self-assembled nanofibers as a carrier for anionic antibiotics, piperacillin-tazobactam (PT) [160]. The cationic multivalency of the nanofiber is particularly useful for anionic PT incorporation through ionic interaction. These nanostructures exhibited strong antifungal activity against drug-sensitive and drug–resistant fungal strains with high selectivity. Therefore, the PT-loaded nanofibers exhibited higher in vivo anti-bacterial efficacy than free PT and significantly improved re-epithelization in a *P. aeruginosa*-infected mouse wound model [161,162]. Unfortunately, high cytotoxicity of cationic polymeric carriers caused by strong interaction between cellular membrane and the carriers often contributes to collateral tissue damage of injured sites upon administration. Therefore, cationic polymers have been modified with an extender unit to have low cytotoxicity with high transfection efficiency [83,84]. Cho et al. reported that polyethyleneimine (PEI) modified with sorbitol reduced the cytotoxicity compared with free PEI [83]. In poly(sorbitol-*co*-PEI) (PSPEI) chain, the sorbitol units spatially segregated the cationic units and thus reduced local the cationic charge density of the polymer. Moreover, PSPEI become structurally flexible compared to PEI, which leads to improved interaction with nucleic acids and higher in vitro transfection efficiency. For scar reduction, connective tissue growth factor (CTGF) siRNA was used to inhibit CTGF-mediated TGFβ signaling which is associated with collagen overexpression in dermal scarring. Thus, siRNA-specific reduction in CTGF expression through PSPEI dramatically diminished wound contraction during skin regeneration of wounds in mice. Similarly, introduction of a β-cyclodextrin (CD) core to a cationic *star*-shaped polymer (β-CD-$(D_3)_7$) significantly reduces cytotoxicity but improves interaction with nucleic acids due to their dense molecular architecture with moderate flexibility [84,163,164]. Of note is that the in vitro gene silencing efficiency of β-CD-$(D_3)_7$ was comparable to

Table 1
Nano- or micro-sized drug delivery carriers for wound healing.

Nanosized drug carrier				
Types	Materials	Drugs	Advantages	Drawbacks
Polymeric	Degradable polymers	Antibacterial peptides; LL37 [155] Growth factors; rhEGF [156]	- Versatile degradation kinetics - Controlled drug release properties - Inherent wound healing property by supplying lactate byproducts	- Collateral tissue damage caused by acidic byproducts
	Cationic polymers	Anionic antibiotics; PT [160–162] Small interfering RNA; CTGF siRNA [83], MMP-9 siRNA [84]	- Intrinsic bio-adhesiveness to the mucosal surface of wound	- Dose limit due to their inherent cytotoxicity
Lipoid	Lipids	Antibacterial agents; LL37, Serpin A1 [165]	- Increased residence time due to similar lipoid composition to the skin - Superior drug penetration into skin - Strong hydrophobic interaction with drugs	- Usage of organic solvent or oil for particle formation - Drug leakage - Coalescence issue
	Liposomes	Lipophilic drug; Curcumin [170] Growth factors; FGF-2 [169,171]	- Easy surface modification of liposomal membrane	- Rapid drug release - Stability and coalescence issue
Inorganic	Gold, silicon, quantum dots	Small interfering RNA; GM3S siRNA [85], Flii siRNA [86] Antibodies; FnAb [191] Antimicrobial agents; SFT[179], APA[183], NO[197], AmB [198], curcumin [199]	- Tunable size with low size dispersity - Straightforward functionalization - Multifunctional capabilities	- Aggregation due to low surface colloidal stability - Inherent cytotoxicity - Toxic byproduct
	Silver	Silver ion [270,300]	- Inherent high antimicrobial activity	
	Ceria	Ceria ion [208,209]	- Scavenging ROS - Improving angiogenesis by modulating intracellular oxygen environment	
	Vanadium pentoxide, iron oxide, graphene oxide	Hydrogen peroxide [214–216]	- Peroxidase-like activity - Catalysts to enhance antibacterial performance of H2O2 at low dose	
Microsized carrier				
Drug enapsulated	Crosslinkable polymers	Highly water-labile drugs; Gentamycin sulfate [217], sodium hydrosulfide [219]	- Physical protection of drugs against hostile environment of wound beds - Low burst release - Prolonged drug release due to slow water penetration	- Unable to protect drugs after release - Low intracellular uptake efficiency of drugs
Coacervate-based	Heparin or heparan sulfate with polycations	Growth factors; VEGF, HGF, FGF-2, PDGF [226–228]	- Able to encapsulate macromolecules with high efficiency - Simple assembly in a purely aqueous environment - Extended half-life of growth factors in wound beds	- Difficulty in controlling uniform particle size - Issues of coalescence, phase separation, and precipitation during assembly

Table 2
Various strategies of substrate-mediated drug delivery for wound healing.

Approach	Purpose	Strategies	Drawbacks
Physical drug encapsulation in a scaffold for slow drug release	- Drug delivery for extended time periods - Long-term wound care	- Direct embedding of drugs in scaffolds [264–266] - Drug incorporation in nano−/micro-particles and then embedded in scaffolds [263,268] - Coating drug-incorporated scaffolds with degradable materials [269–271]	- Drug release largely depended on simple diffusion - Non-precise control of drug release profile
Tuning drug release from scaffolds for stage-wise drug delivery	- Programmable drug release to simulate physiologically relevant time courses of wound healing process	- Binary release of growth factors [177] - Sequential release of multiple growth factors [272] - Layer-by-layer deposition of multiple drugs with independent control over the drug release rates in each layer [276–279] - Degradation and drug release of hydrolytically degradable scaffolds manipulated by crosslinking density and porosity of the scaffolds [280] - Cell-mediated degradation and drug release of degradable scaffolds made of ROS-sensitive materials [281–283] - Biodegradable scaffolds made of polysaccharides/-peptides with a retention-and-release function [285,286]	- Complicated process of drug incorporation - Drug loss or deformation while post-modification step of scaffolds - Potential tissue damages caused by byproducts upon degradation of the scaffolds
Stimuli-responsive drug release for on-demand drug delivery	- Programmable drug delivery on-demand based on wound characteristics	Scaffolds with the capability of sensing the physiological condition of the wound - pH- and glucose-sensitive injectable hydrogels [290] - MMP-responsive nanofibers or hydrogels [11,291–295] On-demand drug release triggered by exogenous stimuli such as - Enzymes [296,297] - Irradiation of NIR [298,299] or visible light [300] - Electric current stimulation [301]	- Variations of physiological condition of the wound among patients

that of Lipofectamine 2000. Furthermore, β-CD-$(D_3)_7$/MMP-9-siRNA complexes significantly accelerated wound closure of chronic ulcers in diabetic rats in 7 days post-wounding by inhibiting abnormal overexpression of MMP-9 in chronic diabetic wounds.

Polymer-based drug carriers are versatile and can be precisely tailored depending on drug properties or target cell types. Currently, most of polymeric nanoparticles have been applied to deliver molecules such as peptides, nucleic acids, and antibiotics in the context of wound healing. Encapsulating large sized molecules such as growth factors or enzymes into nano-sized particles is more challenging and relatively limited. The latter would be a fertile research direction as more potent biologics are being identified for treatment of chronic wounds.

4.3. Lipid-based nanoparticles

Lipid-based nanoparticles are also attractive for the topical treatment of skin diseases because the small particle size and lipoidic composition of the nanoparticles ensure close contact between the nanoparticles and the wound sites, which leads to an increased residence time of the nanoparticles in the wound bed. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) have been used in this context. SLNs exhibit a slower drug release because their ordered molecular conformation and condensed structure tends to retain a part of the incorporated drug inside the lipid core due to the strong hydrophobic interactions between the lipid and the drug. Fumakia et al. developed a SLN formulation that can simultaneously deliver LL37 and Serpin A1 to the chronic wound sites [165]. The SLN formulation was able to achieve >80% protein encapsulation efficiency and the solid lipid core prevented drug leakage and coalescence issue, resulting in a slow release of both LL37 and A1 controlled by drug diffusion through the rigid lipid matrix structure. LL37 is an endogenous host defense peptide possessing antimicrobial activity and Serpin A1 is an elastase inhibitor that helps the chronic wound healing process by reducing overexpression of inflammatory mediators. Thus, simultaneous delivery of LL37 and Serpin A1 promoted wound closure as well as synergistically enhanced antibacterial activity against *S. aureus* and *E. coli* in

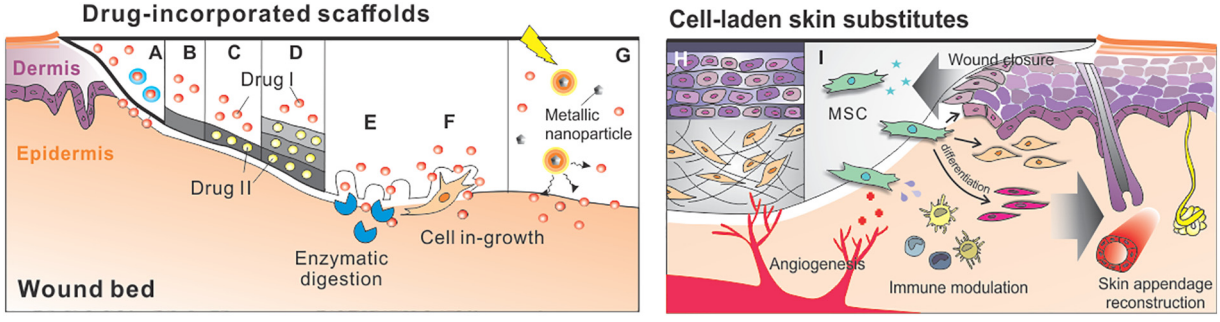

Fig. 3. Schematic illustrating drug delivery strategies for cutaneous wound healing. Controlled drug release can be achieved by (A) direct embedding of drugs or nanoparticulated drugs in scaffolds, or immobilization of drugs on the surface of scaffolds, (B) covering drug-embedded scaffolds with degradable polymer, (C, D) multiple drug incorporation and release in accordance with different degradation rates of each coating layer. Drug release upon degradation of scaffolds can be induced by (E) enzymatic digestion and (F) host cell in-growth during tissue regeneration. In addition, (G) external stimuli (e.g., UV, NIR, and enzymes) can trigger drug release on-demand. Cell-laden substitutes rapidly recover massive tissue loss by filling up with (H) somatic cells (e.g., fibroblasts and keratinocytes) or (I) stem/progenitor cells.

comparison to LL37 or Serpin A1 alone. Both SLN and NLC are generally prepared through the emulsification-ultrasonication method; however, the difference is that organic solvent is required for SNL preparation while a liquid lipid (oil) is used to formulate NLC. Therefore, although wound healing efficiencies of both SNL and NLC formulations incorporating growth factors are similar [166], NLC formulation is often chosen over SLN because the use of organic solvent is not required for NLC preparation and growth factor incorporation [167].

Liposomes have been chosen for transdermal drug delivery due to their superior drug penetration into the skin. However, topical use of the liposome is often hampered by their rapid drug release and coalescence of the particles. Thus, advanced liposome-based formations such as semisolid phospholipid vesicles or gel core liposomes have been developed to have high drug loading level, long-term stability, and controlled drug release in wound sites [168,169]. Xu et al. developed liposomes with hydrogel core of silk fibroin (SF-LIP) by gelation of liquid SF inside vesicle after forming the common liposomes [169]. SF-LIP was capable of encapsulating bFGF with high efficiency and improved the stability of bFGF in wound fluid by 3-folds higher than free bFGF. Therefore, the in vitro cell proliferation activity and in vivo re-epithelization rate of bFGF-incorporated SF-LIP were superior to those of the conventional liposome formulation incorporating bFGF. Furthermore, bFGF-incorporated SF-LIP not only accelerated the wound closure of mice with deep second degree scald, but also induced angiogenesis by improving the stability of bFGF.

The other way to improve liposome delivery to wound sites is multifunctionalization of liposomal membranes with various bioactive molecules, such as ligands, receptors, polysaccharides or lipophilic drugs. Hyaluronan-functionalized liposomes have shown to be superior to liposomes for lipophilic drug delivery [170]. For example, hyaluronan stabilized dispersion of a lipophilic drug, curcumin, improved encapsulation as well as vesicle stability due to the surface exposure of polyanionic hyaluronan in comparison with liposomes. Furthermore, hyalurosomes without curcumin could improve skin re-epithelization due to its inherent tissue restoring properties from hyaluronan and synergistically accelerated in vivo wound regeneration with curcumin. In another example, Das et al. functionalized liposomes with a co-receptor protein for FGF-2, syndecan-4, to improve FGF-2 delivery efficacy for diabetic wound healing [171]. FGF-2 uptake in diabetic ulcers is limited because the level of syndecan-4 is largely reduced in the skin of patient with type 2 diabetes. To overcome the inherent resistance to growth factor signaling, FGF-2 was delivered by syndecan-4-functionalized liposomes and FGF-2 uptake was increased by enhancing the binding of FGF-2 to its receptor. This approach can be considered for further development of therapeutics that can effectivly treat chronic wounds in different disease states.

4.4. Inorganic nanoparticles

Various inorganic nanoparticles such as gold, silica, iron oxide, and quantum dots, have emerged as attractive drug carriers due to their high surface area, tunable size with low size dispersity, straightforward functionalization, and multifunctional capabilities. Especially, metallic and metal oxide nanoparticles such as silver, copper oxide, zinc oxide, and titanium dioxide nanoparticles have been used as potential alternatives for the treatment of drug-resistant bacterial infections due to their inherent high antimicrobial activity [172,173].

Among them, gold nanoparticles are particular interest as drug carriers due to the ease of synthesis, tunable size and shape, flexible surface modification and bioconjugation and tunable optical and electronic properties [174–176]. For drug delivery, gold nanoparticles act as a core where drugs can be immobilized at high concentration per surface area. Gold nanoparticles functionalized with densely packed and highly oriented siRNA have shown to penetrate the epidermal barrier of both intact mouse and human skin, enter keratinocytes, and efficiently down-regulate gene targets [85]. Although they are highly anionic, additional chemical modifications or transfection agents are not required to facilitate transportation through the stratum corneum or entry into cells. The topical application of ganglioside monosialic acid 3 synthase (GM3S) siRNA-immobilized gold nanoparticles (siGM3S-AuNPs) decreased local GM3S expression by >80% at the wound edge, which leads to increased keratinocyte migration and proliferation and the full healing of the splinted full-thickness wounds in diabetic mice within 12 days. As the complete wound closure in diabetic mice takes over 2 weeks [177], gene delivery via gold nanoparticles significantly accelerated the wound healing process.

Also, gold nanoparticles have been used for delivery of antimicrobial agents to improve the healing of infected wounds because the small size and high surface area provides large contact area with bacteria, which leads to the destruction of the permeability and respiration functions of bacteria membranes [178,179]. Gold nanodots (Au NDs, ~2.5 nm) immobilized with an antimicrobial peptide, surfactin (SFT) had much lower values of the minimal inhibitory concentration (>80-fold) than free SFT [179]. Due to the synergistic effect of SFT and Au NDs on the disruption of the bacterial membrane, SFT-Au NDs exhibited greater antimicrobial activity even to the multidrug-resistant (MDR) bacteria. Moreover, in a rat model of methicillin-resistant *S. aureus*-infected wounds, SFT-Au NDs showed fast wound healing, epithelialization, and efficient collagen production. Jiang's group has developed a series of antibacterial gold nanoparticles coated with small antibacterial intermediates to fight against a MDR bacteria-caused wound infection [180–182]. One of the antibacterial intermediates, 6-aminopenicillanic acid (APA)-coated gold nanoparticles (APA-Au NPs) exhibited remarkable antibacterial activity and even more effective than the commercial silver nanoparticles [183]. Furthermore, APA-AuNP-embedded wound dressings (electrospun polycaprolactone/gelatin nanofibers) significantly enhanced the healing of the MDR bacteria-infected wounds in rats compared with the dressing containing APA alone. These studies demonstrated that drug-conjugated gold nanoparticles not only improve extracellular drug penetration into wound sites, but also remarkably accelerate the healing of infected wounds by efficient destruction of bacterial membrane functions.

Silicon-based nanoparticles (Si NPs) are also promising drug carriers due to their high surface area, biocompatibility and degradability. The highly porous structure of Si NPs allows entrapment of a variety of therapeutic cargoes including proteins. Furthermore, the pore size can be tuned from a few nanometers to a few microns achieving surface areas of up to 800 m^2/g [184]. Degradation of Si NPs is tunable depending on the pore size and chemistry, upon which nontoxic silicic acid is produced[185,186]. Various small molecular weight payloads, including proteins and oligonucleotides, can be loaded with high efficiency and these payloads can be released as the nanoparticles degrade [187–189]. Porous Si microparticles loaded with a therapeutic antibody, Infliximab, released the antibody with zero-order release kinetics over the course of 8 days [190]. Critically, the released antibody remained functional and was able to sequester TNF-α in human chronic wound bed over a weeklong timeframe. Porous Si NPs incorporating Flightless I (Flii) siRNA or Flii neutralizing antibodies (FnAbs) significantly decreased Flii activity in human chronic wounds [86,191]. Flightless (Flii), a cytoskeletal actin remodeling protein, is a negative regulator of wound healing and its level is elevated in human chronic wounds. Thus, the treatment of Flii siRNA- or FnAb-loaded porous Si NPs significantly improved the healing of chronic wounds in diabetic mice compared to controls treated with drugs alone. Importantly, Si NPs were able to store drugs within pores and efficiently protected them from enzymatic degradation in wound site.

Silane hydrogel nanoparticle is one type of silicon-based nanoparticles and widely used for drug delivery due to the simple sol-gel process of nanoparticle preparation [192]. In particular, surface modification and drug loading/release profile are easy to be manipulated by using organic additives [193–196]. For example, incorporation of PEG and chitosan to silane gels forms a strong glass-like hydrogen bonding network

and consequently inhibits rapid drug release typically observed in these nanoparticles because of their large pores [193]. For burn wound infections, various agents such as nitric oxide, curcumin, and amphotericin B (AmB) can be delivered by silane hydrogel nanoparticles [192,193,197,198]. Upon administration to wound bed which comprises an aqueous milieu, the hydrogen bonding network of the silane nanoparticle would be disrupted, leading to extended drug release [193]. In contrast to free AmB, AmB-encapsulated silane nanoparticles enhanced Candida spp. killing efficacy and reduced fungal biofilm metabolic activity due to their enhanced permeation capacity and controlled release of AmB in wound sites [198]. Thus, in a mouse model of the burn wounds infected with *C. albicans*, the treatment of AmB-encapsulated silane nanoparticles exhibited fast efficiency in fungal clearance and consequently have more advanced re-epithelization as compared to the AmB solution and untreated infected control. Similarly, curcumin-incorporated silane nanoparticles significantly reduced bacterial burden in a mouse model of burn wounds infected with methicillin-resistant *Staphylococcus aureus* (MRSA), which led to enhanced wound healing [199].

Reactive oxygen species (ROS)-modulating therapies are emerging for augmentation of wound repair and regeneration. ROS such as singlet oxygen, hydroxyl radicals, and superoxide radicals have excellent antibacterial properties [200,201]. When the skin is injured, high amount of ROS is produced at the wound site as part of a defense mechanism against invading pathogens [202,203]. However, elevated ROS production in chronic wounds triggers deleterious effects such as cellular senescence, fibrotic scarring, and inflammation. Therefore, optimal balance between the constructive and destructive capacity of ROS is essential for successful healing and regeneration of injured tissue. Inorganic materials with the ability to manage ROS level and control oxidative damage in the microenvironment of wounds would be appealing. Cerium oxide nanoparticles can scavenge ROS and protect cells against oxidative stress because the Ce3+ (reduced) and Ce4 + (oxidized) states can co-exist to bind oxygen reversibly [204–207]. Therefore, in wound healing, cerium oxide nanoparticles facilitate the proliferation and migration of keratinocytes and fibroblasts. They also enhance angiogenesis by modulating intracellular oxygen environment [208]. Wu et al. integrated ceria with mesoporous silica nanoparticle (MSN) to achieve rapid closure of deep wound due to nanobridging effect of the MSN between the particle and the tissue matrix [209]. The ROS-scavenging tissue adhesive MSN-Ceria composites accelerated the wound repair of a full-thickness dorsal wound in rats owing to the synergistic effect of nanobridging for barrier function restoration and ROS-scavenging for oxidative stress microenvironment modulation.

Hydrogen peroxide (H_2O_2) treatment is one of the ROS-modulating therapies for wound disinfection. It has been proven to be an alternative of antibiotics against antibiotic-resistant bacteria. However, the use of high concentration of H_2O_2 (0.5–3%, ca. 166 mM-1 M) not only often causes inflammation, but is also harmful to healthy tissues and even delays wound healing [210,211]. Artificial enzyme mimetics such as vanadium pentoxide, iron oxide, and graphene have been developed as catalysts to enhance antibacterial performance of H_2O_2, in which H_2O_2 could be catalyzed into the more toxic ROS species such as hydroxyl radical (•OH) [212–214]. Since the OH radicals have a higher antibacterial activity, the conversion of H_2O_2 into OH improves the antibacterial performance of H_2O_2 at low dose in the presence of catalysts. Wang et al. fabricated graphitic carbon nitride-immobilized gold nanoparticles (g-C3N4@AuNPs) as an artificial enzyme mimic [215]. The nanocomposites exhibited enhanced peroxidase catalytic activity due to the positive synergistic coupling effect of gold nanoparticle to stabilize the free radicals and intrinsic peroxidase-like activity of graphitic carbon nitride [216]. The nanocomposites activated biologically relevant amounts of H_2O_2 (100 μM) for defending bacterial infection in vivo, which not only exhibited exiting antibacterial activity, but also avoided side effects from high concentration of H_2O_2. Similarly, graphene quantum dots (GQD) possess higher peroxidase-like activity than graphene oxide due to their excellent electron transportation property [214]. GQDs are tiny pieces of graphene defined as a 0-D material with characteristics derived from both graphene and carbon dots. With the assistance of GQDs (100 μg/ml), H_2O_2 (1 mM) significantly increased the antibacterial activity against both *E. coli* and *S. aureus*, while a relatively higher H_2O_2 concentration (100 mM-1 M) could decrease the survival rate of the bacterial only to 10% in the absence of GQD addition. Furthermore, the GQD-based antibacterial system was able to break down the existing biofilm and prevent formation of new biofilm as well because OH radicals generated from H_2O_2 have a higher activity to oxidize nucleic acids, proteins, and polysaccharides in the matrix of biofilm [212]. GQD-absorbed cotton fabric was prepared as a dressing and it showed excellent antibacterial property in vivo with the assistance of low concentration of H_2O_2 (100 μM) which was 10^4–10^6-fold lower than the H_2O_2 concentration commonly used in wound disinfection. These studies demonstrate that inorganic nanoparticles can act as drug carriers as well as therapeutics at the same time due to their unique inherent therapeutic properties for wound repair.

5. Microcarriers

When intracellular delivery is not required, microspheres are advantageous because of better control of the drug release profile, particularly with low burst release. As gentamycin sulfate (GS) was incorporated to gelatin microspheres (GMs), and then embedded in a silk fibroin (SF) scaffolds, the drug release rate of GS-incorporated GM/SF (GS/GM/SF) scaffolds was significantly slower than that of GS-incorporated SF (GS/SF) scaffolds without microencapsulation [217]. Furthermore, the GS/GM/SF scaffolds not only significantly reduced burn infection by *P. aeruginosa*, but also accelerated re-epithelialization rates of infected wounds than the GS/SF or SF scaffolds. This study demonstrated that microsphere encapsulation can achieve longer-term release of effective concentration of antibiotics, thereby reducing the risk of bacterial infection.

Microencapsulation of highly water-labile drugs is particularly useful for improving their bioavailability at the wound site. The administration of exogenous H_2S donors is known to improve angiogenesis of diabetic wound healing by extending the activation of cellular ERK1/2 and p38. Sodium hydrosulfide (NaHS) is frequently used as a H_2S donor; however, NaHS is a highly water-labile compound and begin to generate H_2S during the encapsulation process when exposed to water. Furthermore, a burst release of H_2S in wound site would cause cytotoxicity [218]. Lin et al. reported an emulsion method for microparticle preparation which comprises hydrophobic phase-change materials (PCMs) of 1-tetradecanol and paraffin wax to encapsulate NaHS, an exogenous H_2S depot [219]. PCMs can serve as depots of NaHS and barriers to water during the emulsification process. In addition, H_2S release can be controlled by tuning the water penetration into the PCM depot by the use of binary mixture of PCMs with different hydrophobicity. In the wound bed, the hydrophobicity of NaHS-encapsulated microparticles impedes water penetration, which leads to a prolonged H_2S production. The sustained release of H_2S accelerated the healing of full-thickness wounds in diabetic mice by promoting epidermal/endothelial cell proliferation and migration as well as angiogenesis. Microencapsulation not only simply delays the drug release, but also precisely controls drug release by modulating degradability. Poly(ethylene glycol) (PEG) microspheres containing VEGF-binding peptides (VBPs) can control VEGF-dependent angiogenesis by varying degradation rate of the microspheres [220]. Non-degradable VBP microspheres enhanced VEGF sequestration and in vivo neovascularization while the microspheres with fast-degrading crosslinks reduced VEGF signaling of endothelial cells. Therefore, the dynamic regulation of growth factor sequestration and activity thorough the microspheres can be advantageous to enhance angiogenesis of the wound healing process and to reduce scar formation as well.

Growth factor delivery through a heparin-based coacervate system have been shown to accelerate wound healing by sustained release and extended half-life of growth factors in wound bed [221–223]. Many therapeutic growth factors such as VEGF, heparin-binding EGF-like growth factor (HB-EGF), and hepatocyte growth factor (HGF), FGF-2, and PDGF have a natural affinity for heparan sulfate which is a glycosaminoglycan in the extracellular matrix [224]. Heparin has similar structure and functionality as heparan sulfate and protect growth factors from proteolytic degradation. Thus, many growth factor delivery systems employ heparin or heparan sulfate moiety to preferentially load, stabilize heparin-binding growth factors, and extend their half-lives in harsh condition, like wound beds. In addition, the specific heparin binding of growth factors also helps to prevent large burst release while providing tunable release. Coacervates are formed by charge neutralization and phase separation of a polymer-rich phase from the bulk water when solutions of a cationic polymer and an anionic polymer are mixed. A synthetic polycation, poly(ethylene argininylaspartate diglyceride) (PEAD), is often chosen due to its low cytotoxicity and good in vivo biocompatibility [225]. Upon addition to the anionic heparin in aqueous solution, PEAD formed a complex coacervate via polyvalent charge attraction and loaded heparin-binding growth factors with high efficiency. Co-delivery of VEGF and HGF through the heparin-based coacervate system exhibited linear release profile of two factors over 3 weeks and induced strong angiogenic effects on endothelial cell proliferation and tube formation in vitro [226]. FGF2-loaded coacervates exhibited an initial burst release during the first 24 h by simple diffusion and subsequent a slow sustained release for 16 days due to the degradation, dissolution, and/or erosion of coacervates over time [227]. Controlled release of FGF-2 from the coacervate significantly accelerated cutaneous wound healing in mice with improved granulation tissue formation and angiogenesis. Platelet-rich plasma (PRP) is a fraction of blood plasma containing various therapeutic growth factors, which has high potential for wound healing. Heparin-binding proteins such as PDGF and VEGF in PRP became incorporated into the coacervate with 60% of loading efficiency [228]. Furthermore, platelet-derived proteins incorporated into the coacervate were slowly released over 3 weeks in vitro. In a porcine wound model, PRP-incorporated coacervate significantly increased the rate of wound re-epithelialization by 35% compared to free PRP. In addition, wounds treated with PRP coacervate exhibited increased collagen alignment and an advanced state of vascularity compared with free PRP. Unlike covalent attachment of heparin to a surface or inside of a scaffold, the coacervate platform employs heparin in free-form which maintains its native functionality and selectivity for heparin-binding proteins. Furthermore, the heparin-binding complexes are mobile and able to more freely interact with cell surface receptors in comparison to the immobilized heparin/growth factor complexes.

6. Drug-incorporated scaffolds

6.1. Wound dressing materials

Ideal wound dressing exhibits good biological compatibility, biodegradability, water adsorption and retention properties, low cytotoxicity, nonstick ability, and antibacterial effects. It prevents the wound from being infected, allows gas exchange, could be removed easily, adsorbs excrescent wound exudate, and remains a part of the exudate to maintain local moisture of the wound, which accelerates wound healing. Moreover, the materials can serve as a depot for delivering a diversity of therapeutics including growth factors, small molecule drugs, nucleic acids, or cells. Therefore, drug-incorporated wound dressings synergistically enhance the wound healing process.

6.2. Traditional dressings

Traditional dressings, such as gauze or gauze-woven cotton composite dressings, are used as primary or secondary dressings for protecting the wound from contaminations [153,229]. These materials are characterized with their low cost, easy use and convenient fabrication. However, the common disadvantages of traditional dressings including ischemia/necrosis, need for frequent changes and adherence to the wound bed have restricted their usage in wound management. Thus, effort has been made to address these disadvantages by grafting the gauze-cotton composite with non-adhesive inner surface, which are fabricated to relieve the pain or minimize the damage to the renewed skin when removing the dressings [230].

6.3. Artificial dressings

Artificial dressings are semi-occlusive or occlusive, available in the form of film, foam, hydrogel, or hydrocolloid [153,229]. After revolution of the production of wound dressings from a passive material to active and functionalized ones, these non-biological, polymeric wound dressings have a large share for wound dressing applications due to their appealing biological advantages. For instance, electrospun nanofibrous scaffold has received significant attention as wound dressing materials due to its unique micro- and nano-scaled fibrous structure resembling native ECM. Its highly porous structure can enhance the fluid adsorption and facilitate oxygen, water and nutrient exchange [231,232]. Moreover, a high surface area of the nanofibrous scaffold not only facilitates cell attachment, but also offers large payload of therapeutic agents. However, cellular infiltration into the scaffold is often restricted due to the inter-fiber network with nanoscale sized pores. Thus, low density nanofibrous scaffolds [233,234] or short nanofiber fragments [235] have been developed for free cellular infiltration and proliferation in the scaffold during tissue regeneration. Hydrogels with high water content have been proposed as a promising biomaterial suitable for dressing membranes because wound dehydration would disturb ideal moist healing environment and delay wound healing. Hydrogel can maintain a moist environment at the wound interface, allow gaseous exchange, act a barrier to microorganisms, remove excess exudates, and be easily removed without trauma. However, the low mechanical strength of hydrogels restricts their use alone as a wound dressing. Biomaterial scientists have modified synthetic hydrogels composed of biological polymers from natural sources, such as collagen and chitosan, to generate novel dressing materials for wound healing [230,236]. These natural polymers specifically chitosan, its derivatives, and glucan drastically enhanced the biological activities of poly(vinyl alcohol) (PVA) blended hydrogels compared with PVA alone, showing enhanced therapeutic impact on wound dressing applications [230,236].

In situ forming hydrogels, particularly, offer several advantages for wound healing applications including closer contact with surrounding tissues, good tissue conformity by accommodating large and irregular wound defects, and enhanced patient compliance. Therapeutics or cells can be easily encapsulated prior to gelation and the mixtures can be applied on the top of wound sites via injection or a spray and crosslinked by exogenous stimuli such as temperature changes, light irradiation, and addition of enzymes. Many photocrosslinkable hydrogels have used ultraviolet (UV) light for robust crosslinking. However, UV light induces DNA and tissue damage [237–239], affects cell metabolic activity [240], and suppresses the immune system in vivo [241] at the irradiation site. Recently, a visible light-activated photo-initiator system has been developed to eliminate the biosafety concerns associated with UV light [242]. Enzyme-catalyzed crosslinking of the hydrogel can also minimize damages of the wound sites by conducting gelation under mild physiological conditions and enables easy manipulation, adjustable gelation time, and mechanical properties [9,10,243]. Horseradish peroxidase (HRP)-triggered in situ crosslinkable gelatin-hydroxyphenyl propionic acid (GH) was prepared by crosslinking of

phenol moieties of the gelatin derivative in the presence of HRP and H_2O_2 via enzyme-catalyzed oxidative reaction [243]. Two chemokines (interleukin-8 and macrophage inflammatory protein-3α) were loaded into GH hydrogels by in situ crosslinking on dorsal wounds in diabetic mice, resulted in facilitated cell infiltration and promoted wound healing with enhanced re-epithelialization/neovascularization and increased collagen deposition, compared with no treatment of the GH hydrogel alone. A dopamine-modified ε-poly-L-lysine-polyethylene glycol-based hydrogel (PPD hydrogel) could also form in situ by HRP-mediated crosslinking of catechol moieties of the dopamine [9]. The PPD hydrogels had superior wet tissue properties because the cationic amine residues of lysine groups can display hydrated cations from the tissue surface, allowing catechol to cohere via binding affinity with various nucleophiles (e.g. amid bond, thiol, and amines) of biological molecule on tissue surface [244–246]. Due to the catechol-lysine cooperation effect, the inherent interfacial interactions of hydrogels such as hydrogen bonding, π–π and/or cation–π interaction are also partially contributed to this strong adhesion strength. In addition, the PPD hydrogels exhibited outstanding anti-infection property due to the inherent antibacterial ability of ε-poly-L-lysine.

Recently, conductive scaffolds have been proposed for active wound dressing materials. When skin is injured, endogenous electrical field (EF) is generated via transepithelial potential (TEP) differences at the damaged epithelial layer [247,248]. Differential ionic gradient at the wound bed induces TEP differences and TEP disruption induces endogenous EF[249]. The EF participates in skin wound healing by stimulating fibroblast proliferation and differentiation into myofibroblast[59], keratinocyte migration [250], and angiogenesis [251]. Bhang et al. reported a piezoelectric dermal patch which can generate electrical field (EF) under small mechanical deformations, thus avoiding the use of subsidiary, external electrical devices to induce EF [252]. The dermal patch composed of multilayers of zinc oxide nanorods and PDMS generated 900 mV of electrical potential by animal motion when it was applied to the wound bed at the back of mice, resulting in facilitated wound closure. Additionally, electroactive wound dressings can regulate the overproduction of ROS at the wound sites by scavenging free radicals. Zhao et al. developed injectable conductive hydrogels based on quaternized chitosan-g-polyaniline (QCSP) and demonstrated that the introduction of polyaniline to the hydrogel can endow the material with good free radical scavenging capacity [253]. In comparison to chitosan hydrogel, polyaniline-grafted chitosan hydrogels showed faster wound closure of full thickness wounds of mice in all the healing stages. This study demonstrated that electroactivity of a conductive scaffold can contribute to promote the wound healing process due to its antioxidant property.

6.4. Biological dressings

Several more recent developments in wound care dressings have emphasized on integrating growth factors, enzymes, or antimicrobial compounds into the wound dressing itself. These biological dressings, as discussed previously, are known for their biocompatibility, biodegradability and similarity to macromolecules recognized by the human body [254]. The biological dressings as called "xenograft dressings" are regarded as the most suitable materials for complete healing of deep, chronic wounds and burns. An important consideration in the design of biological dressings is their ability to control infection. Commercially available antimicrobial dressings include Cutisorb™, Iodosorb, Actisorb Silver 220. Another new generation product, Acticoat, uses novel silver-coating technologies in a dressing designed to prevent wound adhesion, control bacterial growth and facilitate burn wound care. Although the antiseptic-impregnated biological dressings may not be appropriate for broad-spectrum application to healing wounds, these wound care materials may be intended for the use over partial or full thickness wounds such as pressure ulcers, venous ulcers, diabetic ulcers and chronic lower leg ulcers where infection can be a problem, especially with formation of biofilms. Meanwhile, hundreds of studies have demonstrated that growth factors can augment all aspects of tissue repair in normal and impaired healing models [255,256]. A proper balance of cytokines and growth factors promotes recruitment of functional cells (epithelial cells, fibroblasts, and endothelial cells) to the wound bed, which later stimulates their proliferation, and have profound influence on the extracellular matrix deposition. Presently, recombinant human PDGF, FGF and EGF are studied extensively for their application in growth factor-mediated wound repair [63,153]. Among which, PDGF and EGF are FDA approved and commercially available in human applications.

Another bioactive property of the biological dressings is believed to interact with cells or matrix proteins in the wound bed to promote healing. The ECM is a complex mixture of structural and functional proteins. These molecular components are secreted by host cells and arranged in tissue-specific patterns, which are responsive to and optimized for the physiologic and biomechanical requirements of each tissue and organ. The three-dimensional (3D) ultrastructure of ECM also provides a scaffold for guiding cell organization, growth and differentiation during the process of wound healing. Currently, available acellular ECM scaffolds with clinical data to support their efficacy include porcine-derived small intestinal submucosa (e.g. Oasis), porcine urinary bladder matrix (e.g. Matristem), bovine dermis (e.g. Primatrix and Matriderm), equine pericardium, and amniotic membrane (e.g. Epifix) [257,258]. These decellularized biological products serve as temporary substrates into which cells can migrate and proliferate in a well-organized and controlled fashion, thereby promoting granulation tissue development and tissue regeneration. Although there are no guidelines that clearly recommend the use of ECM scaffolds for wound healing, their benefit in acute wounds and burns has been demonstrated in several clinical studies [257,258]. However, the pathophysiology of chronic difficult-to-heal wounds is extremely complex, arising from varied etiologies and combined co-morbidities of diabetes, neuropathy, immunosuppression, vascular deficiencies, and increased bacterial load that disrupt healing. These wounds also suffer from severe molecular and cellular deficiencies and are, unfortunately, heterogeneous across the patient population. Additionally, during decellularization, ECM often loses their structure and composition, resulting in largely reduced growth factor/cytokine level in the ECM scaffolds [259].In this regard, the application of ECM-based scaffolds alone appears to be limited for chronic wound care due to a lack of interaction with recipient cells and tissues. To address this challenge, advances in cell biology and tissue engineering have led to a pronounced increase in the quantity and efficacy of biological wound grafts by revitalization of the biodegradable matrix with autologous cellular elements (differentiated or stem cells) [260]. These living skin equivalents not only address the deficient ECM of damaged tissue by adding a collagen matrix but also introduce immune-privileged living cells that proliferate and actively synthesize growth factors, cytokines, and ECM components, as well as providing an optimal wound healing environment [63,258,260]. Biological bioengineered dressings are suitable for repair of chronic ulcers such as diabetic foot ulcer (DFU) and pressure ulcer (PU), but generally require a vascularized wound bed. Apligraf is an FDA approved skin equivalent substitute containing an epidermal keratinocyte layer and a dermal layer of fibroblast-seeded collagen. In a clinical trial, Apligraf is indicated for treatment of partial & full-thickness skin ulcers due to venous insufficiency and DFU that have not appropriately responded to conventional therapy [261,262]. More bioengineered skin substitutes commercially available include: Dermagraft composed of a cryopreserved, absorbable 3D polyglactin mesh substrate seeded with human dermal fibroblasts, and Alloderm™ comprised of normal human fibroblasts with all cellular materials removed. Given the complexity of cutaneous wound healing process, the cell-based biological dressings offer a huge potential in the field of chronic wound management and are thought to act in a number of ways to improve wound healing. Specifically, the combined mode of using live cells can overcome the delivery

limitations of direct cytokine and growth factor treatment, e.g. low stability, short in vivo half-life and high risk of carcinogenesis, leading to better therapeutic outcomes.

6.5. Substrate-mediated drug delivery for wound healing

6.5.1. Physical drug encapsulation in a scaffold for slow drug release

The scaffolds serve as depots for bioactive molecules, preserving their stability and function and enabling sequential release for extended time periods. Physical drug encapsulation in a scaffold can shield the drug from degradation in the harsh environment of chronic wounds while also providing more sustained, localized drug administration as compared to a bolus drug delivery. Xiao et al. achieved slow release of copper ions from copper benzene tricarboxylate ($Cu_3(BTC)_2$, known as HKUST-1) nanoparticles by entrapping within an antioxidant citrate-based hydrogel [263]. Poly(polyethylene glycol citrate-co-N-isopropylacrylamide) (PPCN), a thermos-responsive biodegradable polymer was used as a vehicle to slowly release drugs at the wound site. PPCN has been shown to efficiently entrap and slowly release bioactive stromal cell-derived factor-1 (SDF-1) for at least 3 weeks in vitro without a burst release due to the physical entrapment as well as the electrostatic interactions between positively charged SDF-1 and the negatively charged polymer network of PPCN [264–266]. It also has intrinsic antioxidant properties due to the diol-citrate ester in its backbone that may reduce oxidative stress at the wound. Likewise, PPCN could entrap HKUST-1 nanoparticles by gelation at approximately 27 °C. The entrapment of the nanoparticles in PPCN hydrogels can slow down their degradation by stabilizing in protein-rich condition and thus prevent premature release of the cargo, leading to slow release of copper ions. Cytotoxicity and apoptosis caused by copper ions were significantly reduced while dermal cell migration in vitro and wound closure rates in vivo were enhanced.

Nitric oxide (NO)-releasing materials are often embedded in a scaffold to avoid a fast NO release in wound beds. Viscous ointment has been used as a storage of NO-releasing materials and sustains the release of NO by physically preventing rapid contact between NO donors and water. An ointment containing NO-loaded zeolite powder released NO over at least 3 h upon contact with moisture. Considering that NO-loaded zeolite powder released nearly all NO within 1 h of contact with moisture, embedding into a hydrophobic ointment base significantly slows the transport of water to the zeolite molecules [267]. Likewise, PEG-based ointment embedding NO-releasing polymer (FBN) composed of Pluronic F127, PEI and N-diazeniumdiolates (NONOates) not only facilitated NO release in a slow manner, but also served as a moisturizer to enhance the wound healing [268]. In comparison to sole FBN, FBN-embedded ointment enhanced the amount of NO release by 10-fold and increased overall duration around 8-fold. The FBN-embedded ointment also improved the healing activity in the early phase of wound healing in mice compared to PEG ointment alone.

Drug release from scaffolds can be manipulated by coating the drug-embedded scaffolds with degradable materials. Drug release profiles of surface-coated scaffolds are largely influenced by various parameters including the degradability and hydrophobicity of coating materials, thickness and the number of coated layers, and porosity of the coated layer. When a collagen scaffolds with antisense oligonucleotide (asODN) was coated with a single layer of PLGA, asODN was released over 95% within the first 2 days [269]. Scaffolds coated with polycaprolactone (PCL) eluted incorporated asODN at a slower rate than those coated with PLGA, and four layers of PCL coating exhibited a more sustained release profile in which asODN elution began to plateau around 6–7 days. In another example, polymeric ultra-thin film incorporating silver sulfadiazine (AgSD) was coated with polylactide (PLA) nanosheets to control silver ion release [270]. The thickness of the PLA nanosheet layer was varied by changing the concentration of PLA solution and the release rate of silver ion decreased as the thickness of PLGA nanosheet increased; the AgSD-loaded nanosheet with a thickness of 77 nm released 74% of silver ion for 3 days, whereas that with a thickness of 146 nm released 33% of silver ion for 3 days and continued to release Ag ion for over a week. Without coating, all silver ion released within 1 day. Thus, the AgSD-loaded films coated with PLA nanosheets improved wound healing of a partial-thickness burn wound in mice by reducing the probability of infections in wound during the first 3 days after injury, the high risk-period of infection. Drug elution rates of the scaffolds coated with hydrolytically degradable materials are also influenced by the hydrophilicity of the scaffold. As two types of films made of polyurethane (PU) with different water uptake (Tecoflex SG-80A (6.2%) and Tecophilic SP-60D-20 (22.5%)) was dip-coated with PLGA solution containing NO donors, the PLGA-coated films made of higher water uptake PU had higher NO release and a longer duration than those made of lower water uptake PU [271]. High water uptake of the scaffolds accelerated hydrolysis of the PLGA coating, thereby not only accelerated NO release but also providing an acidic environment to accelerate decomposition of NO donor.

6.5.2. Tuning drug release from scaffolds for stage-wise drug delivery

Multiple growth factors encapsulation in a single nanofibrous scaffold with a different releasing pattern has been developed for stage-wise delivery of growth factors to synchronize with the wound healing process. Choi et al. achieved binary release profile of bFGF and EGF by encapsulating bFGF in the core and immobilizing EGF on the surface of the nanofiber post-coaxial electrospinning [177]. The core-encapsulated bFGF showed an initial burst release profile and approximately 30% of the encapsulated bFGF was released in the first 12 h, whereas the surface-immobilized EGF was rarely released; <2% of the immobilized EGF was released in 7 days. Therefore, the initial burst of bFGF of the nanofibers enhanced the proliferation of epidermal cells in the early wound repair of a full thickness burn wound in diabetic mice. Furthermore, covalently immobilized EGF on the nanofibers continually stimulated the EGF receptors of keratinocytes and thus accelerated keratinocyte migration and proliferation during the entire wound healing process. Thus, the programmable release of multiple growth factors through the electrospun nanofibrous scaffolds not only allows for growth factor delivery in a stage-wise manner but also induces a synergistic effect with nanofibrous structure of the scaffolds on the improved efficiency of chronic wound healing. Similarly, Lai et al. created a slower release pattern of growth factors by embedding growth factor-preloaded nanoparticles in nanofibers [272]. EGF and bFGF were directly embedded in the nanofibers while VEGF and PDGF-BB was encapsulated in the gelatin nanoparticles and then electrospun with collagen and hyaluronic acid to form nanofibers. Therefore, VEGF and PDGF-BB were slowly and gradually released up to one month as the gelatin particle degraded whereas the release profile of EGF and bFGF showed relatively faster initial release. In vivo, VEGF and bFGF stimulate the recruitment of endothelial cells, while PDGF is responsible for the stabilization of new blood vessels [273]. In addition, EGF and PDGF act synergistically to stimulate the proliferation of epithelial cells and thus modulating the subsequent re-epithelialization process [274]. Thus, the sequential release of these growth factors simulate the physiologically relevant time course of wound healing [275].

Hammond's group reported a method for coating dressings using the layer-by-layer (LbL) process to enable both sustained release and independent control over the release kinetics of multiple drugs. Self-assembled nanometer-scale coatings of a nylon dressing can incorporate and release therapeutically relevant quantities of drugs in a controllable fashion to yield rapid chronic wound closure. They constructed a hydrolytically degradable siRNA depot on top of a commercially available nylon bandage by LbL assembly [276]. The first film assembled directly on the woven nylon substrate with a two-component film of poly(β-amino ester)2 and dextran sulfate. On top of this degradable undercoating, an siRNA-containing LbL film composed of chitosan layer and siRNA layer was assembled. By varying the number of layers of each component architecture independently, release profiles were tunable

ranging from hours to weeks. The diabetic wounds treated with MMP-9 siRNA-incorporated LbL film exhibited significant inhibition of MMP-9 expression and the overall protease activity was diminished by 60%, which allowed more granulation tissue to form in the wound bed and collagen accumulation in the re-epithelialized tissue was 5-fold more than untreated wounds. LbL-assembled film on scaffolds can coordinate distinct release via surface-based erosion but interlayer diffusion often occurs during the construction of the LbL film [277,278], resulting in a polymer blends that lacks distinct release kinetics of each drug. Thus, for more independent control over the release rates of incorporated drugs in each layer, a diffusion barrier was introduced to the LbL structures. Therapeutic dressings were fabricated using a repeating tetralayer architecture that consists of hydrolytically degradable poly (β-amino ester), poly(acrylic acid) (PAA), VEGF and/or PDGF-BB, and heparin sulfate (HS) [279]. PDGF-BB film was first assembled on a nylon dressing and a PAAC layer replaced PAA layer in every third tetralayer of the PDGF-BB section of the film as a dithiol-crosslinked diffusion barrier. Five more PAAC layers were created prior to deposition of VEGF-containing film on top of the PDGF-BB film to reduce interfilm diffusion and enable independent control of release kinetics of each growth factor. Therefore, VEGF released first could stimulate the initial formation of new vasculature in wounds of diabetic mice and PDGF-BB subsequently maturated the vessels via PDGF-BB-mediated mural cell recruitment. This combinational treatment of proangiogenic growth factors promoted significant increases in the formation of granulation tissue and neovasculature in chronic wounds of diabetic mice when compared to single growth factor delivery.

Synthetic degradable scaffolds can tune drug release based on the desired dose and time by optimally matching rates of scaffold degradation with tissue in-growth. Degradation and drug release of hydrolytically degradable scaffolds can be manipulated by crosslinking density and porosity of the scaffolds. Porous polyester urethane (PEUR) scaffolds were fabricated for substrate-mediated transfection of PHD2 siRNA to wound sites [280]. Polyester triol prepolymers were mixed with trehalose, porogen, and siRNA-encapsulated nanoparticles and fabricated into scaffolds through a reactive foaming process with two different isocyanate-containing crosslinkers, lysine triisocyanate (LTI) or hexamethylene diisocyanate trimer (HDIt). The siRNA release was controlled through tuning the quantity of porogen added during PEUR scaffold fabrication and by alteration of the isocyanate-containing crosslinkers. HDIt induced the scaffolds to be more hydrophobic and thus degrading more slowly. PEUR scaffolds with higher amount of porogen had larger pores and exhibited a fast siRNA release and a more transient in vivo gene silencing efficiency while those with lower amounts of porogen produced twice higher in vivo gene silencing at 35 days due to slower siRNA release. In the other study, ROS-degradable scaffolds made of poly(thioketal urethane) (PTK-UR) promoted even more robust tissue regeneration of diabetic wounds in rats than PEUR scaffolds [281]. PTK-UR is degraded by reactive oxygen species (ROS) generating by cells because the thioketal moiety of the polymer is sensitive to hydroxyl radicals [282,283]. Relative to ester-based PEUR materials, the PTK-UR enabled better matched rates of degradation and cell infiltration during the wound healing process. Furthermore, PTK-UR scaffolds incorporating PHD2 siRNA significantly promoted blood vessel growth and encouraged a more robust healing response in diabetic rats.

Biodegradable scaffolds made of polysaccharides/peptides containing bioactive sequences can effectively replicate the functions of many ECM proteins and help to promote wound repair. As discussed above, heparin-based materials have been used due to their affinity for numerous growth factors that are key biological mediators of the wound healing process. Affinitive bindings of heparin to growth factors not only protect the growth factors from degradation, but also enhance the interaction with cellular receptors [224]. In addition, the release of heparin-binding growth factors can be modulated in dependence on the sulfation pattern of heparin-based materials [284]. Specific removal of single sulfate groups mediating protein-GAG affinity (6-O– or N-sulfate) resulted in higher VEGF release rates while removal of both moieties reduced the growth factor binding efficiency. Heparin-mimetic peptide nanofibers have shown to promote in vivo angiogenesis through the retention of endogenously produced growth factors such as VEGF, HGF, and FGF-2 and thus enhancing their presentation and stimulating autocrine signaling from the cells at the wound site [285]. In contrast, a non-bioactive control gel (bearing a similar secondary structure for self-assembly but no heparin-mimetic motifs) exhibited no improvement over sucrose-treated controls, suggesting that the bioactive sequences of heparin-mimetic motifs are effective in promoting wound healing by sequestrating endogenous growth factors and releasing them in response to the progression of wound healing. Similarly, Wang's group recently developed a galacturonic acid-containing polysaccharide, named EUP3, which preferentially binds PDGF-BB [286]. They prepared an electrospun hydrogel sponge comprising EUP3 and gelatin to achieve a 'retention-and-release' function. Upon administration of the EUP3/gelatin scaffolds, EUP3 started to sequester endogenous PDGF-BB into the scaffold ('retention'). As healing continued, gelatin was gradually degraded and liberated the PDGF-BB/EUP3 complexes to the wound site ('release'). The EUP3/gelatin scaffolds facilitated PDGF-BB-mediated cellular functions and accelerated the repair of a full-thickness skin wound in mice with enhanced neovascularization, controlled immune activation and improved neotissue formation without the addition of exogenous growth factors.

6.5.3. Stimuli-responsive drug release for on-demand drug delivery

The effectiveness of the therapy depends on maintaining sufficient drug concentration in the wound site throughout the treatment. Therefore, a drug delivery system that is programmed to deliver drugs on-demand based on the wound characteristics is advantageous particularly for chronic wounds. Diabetic ulcers have distinctive physiological microenvironment such as acidic pH, high levels of glucose and protease, in particular, MMPs [287–289]. Thus, scaffolds with the capability of sensing the physiological condition of the wound such as pH, glucose, and MMP can achieve on-demand drug delivery for wound healing. Zhu's group developed pH- and glucose-sensitive injectable hydrogels through the covalent crosslinking of imine bond and phenylboronate ester using phenylboronic-modified chitosan (CSPBA), poly(vinyl alcohol) (PVA) and benzaldehyde-capped PEG (OHC-PEG-CHO) [290]. Imine bond is stable at physiological pH while labile at mildly acidic. Boronic acid derivatives binds to diol moieties of glucose with high affinity through reversible boronate ester formation. Thus, the introduction of phenylboronic acid moieties and imine bonds (Benzoic-imine bonds) into the scaffolds enabled dual responsive drug release at diabetic ulcers. Insulin and fibroblasts were incorporated into the hydrogels during the in situ crosslinking. Decreasing the media pH or increasing the glucose level led to the accelerated release of insulin from hydrogels. Therefore, the insulin/fibroblast-incorporated hydrogels exhibited enhanced wound healing of a streptozotocin-induced diabetic animal model due to sustained and triggered drug release from the hydrogels.

Kim et al. developed MMP-responsive non-viral gene delivery systems for wound healing of diabetic ulcers. PCL electrospun nanofibrous mesh was surface-modified with linear PEI (LPEI) via MMP-cleavable peptide as a linker [291–293]. Therapeutic nucleic acids such as plasmids encoding human EGF (phEGF) and MMP-2 siRNAs were electrostatically incorporated to LPEI-modified nanofibrous mesh for enhanced wound healing by EGF production or MMP-2 inhibition at wound sites. The release of gene complexes was significantly increased in diabetic mice with a full-thickness wounds where MMP-2 expression level is highly elevated due to the enzymatic digestion of the MMP-cleavable linkage between the mesh and LPEI. Thus, the nanofibrous meshes accelerated the wound recovery of the diabetic ulcers by balancing expression or suppression of target genes associated with abnormality of diabetic ulcers [292,293]. They also prepared MMP-responsive hydrogel-like clusters of gene complexes comprising four-

arm PEG-MMP-2 siRNA conjugates (4PEG-siRNA) and LPEI [294]. Because the four-arm PEG was conjugated to siRNA via MMP-cleavable peptides, the 4PEG-siRNA/LPEI clusters were efficiently dissociated in MMP-rich environments of dorsal wounds in diabetic animals. The diabetic wounds treated with 4PEG-MMP-2 siRNA/LPEI clusters exhibited faster wound closure rate and the re-epithelialized tissue highly expressed cytokeratin with a lower level of MMP-2 expression than those treated with MMP-2 siRNA/LPEI complexes. Furthermore, the introduction of MMP-cleavable linkers into scaffolds enables the cell-based remodeling by dermal fibroblasts of the wound site [11]. StarPEG-heparin hydrogels crosslinked with MMP-cleavable peptides accelerated dermal fibroblast infiltration into the hydrogels and the cells actively degraded the hydrogels by secreting MMPs, which enhanced the release of heparin and heparin-binding growth factors at wound sites [295]. Similarly, hyaluronic acid hydrogels crosslinked via MMP-degradable peptide could be fully degraded by both hyaluronidases and MMPs in splinted full-thickness wounds in diabetic mice, resulted in accelerated release of proangiogenic plasmids encoding VEGF and subsequent promoted angiogenesis during wound healing of diabetic ulcers [11].

Exogenous stimuli such as addition of enzymes, irradiation of near-infrared irradiation (NIR) or visible light, and electric current stimulation can trigger on-demand drug release of the scaffolds at wound sites. Specific enzyme-responsive scaffolds could deliver drugs sustainably under the physiological conditions and the amount of delivered drugs could be readily controlled by the enzyme concentration. For local delivery of nitric oxide (NO) to wound sites, a self-assembling short peptide hydrogel was crosslinked with β-galactosidase-responsive caged NO donor [296]. Upon the addition of β-galactosidase, NO release from the hydrogel was triggered because the enzyme removes the sugar capping group on the caged NO donor, leading to the release of NO in a controllable way. The release rate of NO was precisely tuned by adjusting the concentration of β-galactosidase treated to the hydrogel. Similarly, electrospun PCL mesh coated with a NO-releasing material, NONOate grafting chitosan (CS-NO) was prepared to control NO release by the catalyst of β-galactosidase [297]. The controlled release of NO enhanced macrophage recruitment, re-epithelialization, angiogenesis, and collagen synthesis, resulted in promoted wound healing of a full thickness wound in mice. These NO delivery systems are a promising approach especially for chronic wound caused by ischemia.

For controllable drug release of the scaffolds mediated by photothermal performance under near-infrared irradiation (NIR), semiconductor cuprous sulfide nanoparticles have been used as a photothermal agent which converts the NIR energy into heat and increases local temperature. Wang et al. developed a NIR-responsive electrospun nanofibrous mesh by incorporating Cu_2S nanoparticles [298]. Under NIR irradiation, the mesh exhibited controllable photothermal performance, which resulted in high level of skin tumor cell death at cutaneous defects in a diabetic mouse model after surgical excision of skin cancer. Moreover, skin tissue regeneration of tumor-induced wounds was improved by angiogenesis effect of Cu ions released from the scaffolds. Li et al. fabricated a NIR-driven coordinated CuS nanoparticle for on-demand release of CO_2 [299]. Dopamine-containing hollow CuS nanoparticles incorporated bicarbonate (BC) through the coordination of the ferric ion (Fe3+) bridged between dopamine and BC. Due to the photothermal transduction of the CuS nanoparticles upon NIR irradiation, local temperature of the wound was elevated to 42 °C and BC was subsequently decomposed into CO_2 at the wound site. The controlled release of CO_2 induced a weak acidic microenvironment in blood, promoting microcirculation and giving the blood perfusion phenomenon to accelerate the incisional wound healing in mice. Furthermore, only single time irradiation of NIR light was needed for this CO_2 delivery system to achieve enhanced wound recovery. Semiconductor nanoparticles have also been employed for a visible light-triggered drug delivery system due to their excellent photocatalytic activity. Mao et al. reported a carboxymethyl cellulose (CMC) hydrogel incorporated with Ag/Ag@AgCl/ZnO hybrid composites which broad antibacterial efficiency under visible light irradiation [300]. Introducing semiconductor nanomaterials, ZnO, to Ag nanocomposites, generated more ROS during photoexitation of ZnO, which could enhance antimicrobial activity under visible light irradiation. Furthermore, silver and zinc ion were sustainably released over a period of 21 days because the reversible swelling-shrinking transition of the CMC hydrogel triggered by pH changes in the biological environment, thereby producing the synergistic antibacterial effects and accelerated wound healing of *S. aureus*-infected wounds in rats.

Recently, Mostafalu et al. proposed a wound dressing integrated with electronic system which could remotely control multiple drug release (Fig. 4) [301]. The dressing was comprised of individually addressed functional fibers, assembled into fabrics using a textile fabrication process. The functional thread is composed of a conductive core thread as a microheater which was coated with a hydrogel layer of alginate/PEG diacrylate (Alg/PEGDA) carrying thermosensitive particles. The particles were formed with poly(N-isopropylacrylamide) (PNIPAM) and PEGDA for a significant release at 42 °C and low release rate at 25 °C and 37 °C. In addition, two different antibiotics (cefazolin and vancomycin) and VEGF were respectively loaded into the particles. The dressing was connected to a microcontroller that can wirelessly transfer commands from an external source such as a smartphone. Therefore, each thread was able to be individually triggered to allow the release of multiple drugs in different stages of the wound healing without disturbing the release profile of other fibers. On-demand release of antibiotics and VEGF from the dressing not only eliminated

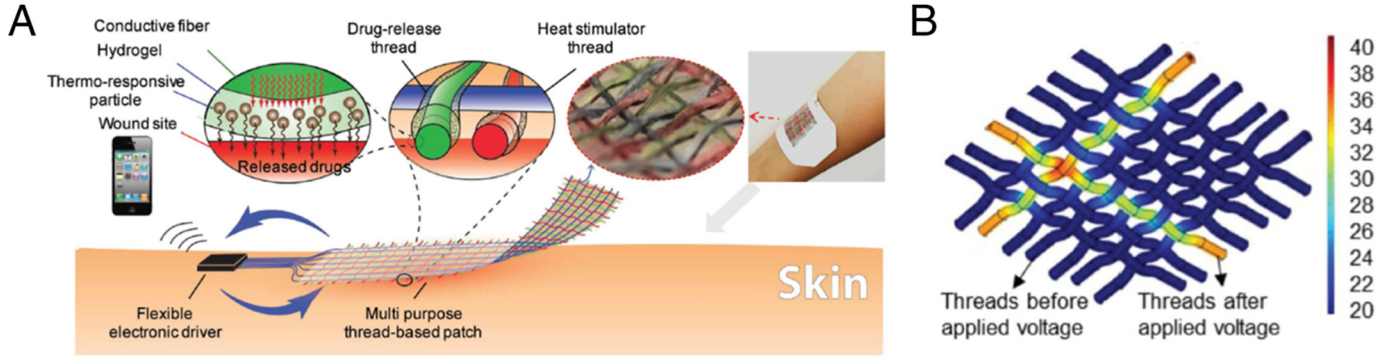

Fig. 4. On-demand drug delivery using a textile dressing. (A) Schematics of a multipurpose thread-based patch for the transdermal drug delivery in which a hydrogel layer carrying thermoresponsive particles are coated on a flexible thread-based heater. (B) Numerical simulation showing the interference of the individual active threads. The colorful bar shows the temperature distribution. Adapted from ref. [301] with permission from WILEY-VCH publishing group.

bacterial infection but also enhanced angiogenesis in vitro and improved healing rate of a murine model of diabetic wounds. Taken together, the studies demonstrate that multiple drug release of stimuli-responsive scaffolds in an on-demand fashion based on the wound characteristics not only increases the efficacy of the treatment, but also decreases the side effects such as occurrence of drug resistant organisms.

7. Cellular skin substitutes

Cell-based therapy approaches for wound healing are advantageous over delivering single soluble factors in that administered cells can respond to the local environment and release multiple factors.

7.1. Cell source

7.1.1. Somatic cells

Human skin cells, fibroblasts and keratinocytes, are the first source used to fabricate cell-laden substitutes. Fibroblasts are the most abundant cells in the dermis and synthesize most of the extracellular matrix component (collagen, elastin, laminin, fibronectin and glycosaminoglycans), while keratinocytes provide a barrier-protective function of the skin. During wound healing process, fibroblasts synthesize collagen and differentiation into myofibroblastic phenotype to facilitate wound closure in response to paracrine signaling from keratinocytes and inflammatory cells. Fibroblasts and keratinocytes communicate with each other via double paracrine signaling loops, known as cross talk, which leads to the recruitment of cells necessary for complete wound closure. Thus, bilayered cellular construct containing both fibroblasts and keratinocytes have been developed for repair and regeneration of skin with large wounds such as burns, pressure sores, and diabetic ulcers. When the construct applied to the wound site, the cells supply signaling molecules, growth factors, and extracellular matrix proteins aiding in skin tissue regeneration process. There are commercially available products incorporating keratinocytes (e.g. EpiCel), fibroblast (e.g. Dermagraft), or both keratinocytes and fibroblasts (e.g. Apligraft).

7.1.2. Stem cells and progenitors

Although skin cells are good autologous cell sources, the use of somatic cells is impractical for extensive wound treatment due to limited donation sites and high risk for a secondary morbidity where cells are harvested. Thus, mesenchymal stem cells (MSC) are a promising alternative for chronic wound healing because they are capable of robust ex vivo expansion while maintaining the ability to differentiate into cells of epidermal and dermal lineages [302]. Even skin appendages such as hair follicles, sweat glands, and microvessels can be rapidly regenerated in the MSC-treated wounds. Most stem cells studied in wound healing are MSC, adipose-derived stem cells (ASC), endothelial progenitor cells (EPC), and umbilical cord perivascular cells (UCPC). Epidermal stem cells and progenitor cells from patient epidermis are also considered as autologous cell sources for chronic wound healing. Clinical evidence suggests that hair follicle progenitor cells contribute to the re-epithelization of wounds [303]. In a clinical pilot study, autologous scalp follicular grafts transplanted into the wound be of chronic leg ulcers demonstrated 4-fold higher wound closure rate in comparison to the no treatment ulcers [304]. A full-thickness scalp burn treated with a collagen-glycosaminoglycan neodermis containing hair follicle micrografts was regenerated to a normal multilayered epidermis derived from hair follicle progenitor cells [305], suggesting that epidermal stem and progenitors are largely replaced by epidermal progeny following wound repair [19].

The administration of stem cells not only can accelerate wound healing, but also enhance wound healing quality and physiological function of the regenerated skin through direct differentiation and paracrine signaling [302,306,307]. Especially, MSC exhibit a number of trophic functions to enhance tissue regeneration, such as promoting wound closure, enhancing angiogenesis, modulating inflammatory response, and reducing scar formation [26,27,308]. Variable expression of migratory and attractant cytokines produced by MSC accelerates recruitment of macrophages and endothelial cell as well as dermal fibroblasts and keratinocytes migration toward wound sites, resulting in promoted wound closure. In addition, MSC enhance angiogenesis during the wound healing process by secreting significantly high level of the pro-angiogenic cytokines, such as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and basic fibroblast growth factor (bFGF), and adrenomedullin to promote vascular stability and vasoprotection. MSC also participate in reducing scar formation by secreting a variety of cytokines and growth factors that have anti-fibrotic properties, including HGF, IL-10, and adrenomedullin [308–311]. Nitric oxide produced by MSC can scavenge reactive oxygen species (ROS) in wound sites, which prevent fibrogenesis and accumulation of fibrotic tissues caused by prolonged ROS exposure during wound healing. Interestingly, in response to inflammatory environment of the wound, MSC are activated to initiate immunomodulatory functions by producing ample amounts of immunoregulatory factors, and thereby regulate inflammation and reduce inflammation-related scar formation. Wounds treated with MSC demonstrated a significantly lower the number of inflammatory cells and a decrease level of pro-inflammatory cytokines such as IL-1 and TNF-α, resulting in a faster wound healing rate of burn wounds in rats [20].

7.2. Reprogrammed cells (engineered cells)

7.2.1. Genetically engineered MSC

MSC are attractive candidates to promote wound healing due to their ability to self-renew migrate toward injury sites and secrete paracrine signals to attract other cell types to the wound bed. However the poor viability of MSC at the transplanted site often decreases their therapeutic potential. In addition the amounts of paracrine signals secreted from MSC are often insufficient for a therapeutic effect on wound healing especially in chronic wounds and it is difficult to control their levels of expression/secretion to achieve physiologically adequate concentration. The level of growth factor/cytokine secretion varies depending on the state of the cells and their passage number [312]. To amplify paracrine signal production and improve the survival of MSC in a transplantation site stem cells are genetically engineered to overexpress desired therapeutic factors in wound site. The survival rate of MSC genetically engineered with stromal cell-derived factor-1 (SDF-MSC) in wounds was significantly high compared with that of normal MSC [33]. In addition SDF-MSC secreted a high level of VEGF HGF and IL-6 and formed larger number of blood vessels in wound beds which leads to faster wound healing than normal MSC treatment. VEGF-overexpressing adipose-derived stromal cells (ASC) significantly accelerated wound closure rate increased normal-appearing blood vessel formation and induced more mature collagen fiber formation in the wound site in comparison to non-engineered ASC [21]. Dox-inducible HGF-secreting human umbilical cord blood-derived MSCs express HGF for long-term in a controllable manner which promoted in vivo angiogenesis in a mouse hindlimb ischemia model [34]. The inducible growth factor expression of engineered MSCs would be a useful therapeutic modality for the treatment of wounds requiring stage-wise treatment of growth factors

7.2.2. Induced pluripotent stem cells (generated from somatic cells)

Adult somatic cells can be reprogrammed into induced pluripotent stem cells (iPSC) with similar characteristics to embryonic stem cells (ESC) in terms of morphology, self-renewal, and differentiation capacity [313]. Unlike ESC, iPSC are not only free from ethical issues, but also can be propagated as autologous cells, which can avoid the complication of immune rejection. Thus, iPSC-derived skin cells could be a promising cell source for customized cellular therapies to treat non-healing wounds. IPSC can be generated by transduction of somatic cells with a combination of reprogramming factors (such as Oct3/4, Sox2, Klf4,

and c-Myc, or alternatively Oct3/4, Sox2, Nanog, and Lin28). IPSC generate a wide range of differentiated cell types including keratinocytes [22] and melanocytes [23,314]. Bilousova et al. developed the methods for the differentiation of iPSC into a multipotent keratinocyte stem cells, and keratinocyte stem cells derived from iPSC are able to regenerate the epidermis, hair follicles, and sebaceous glands in an in vivo graft assay in mice [22]. Ohta et al. developed methods to generate large numbers of autologous melanocytes from human iPSC [314]. Major challenges in the utility of iPSC for regenerative medicine are i) the extremely low yield of reprogramming from somatic cells to iPSC, and ii) low differentiation efficiency of iPSC into the cell type of interest. The majority of the studies use skin fibroblasts as the parental cells due to the ease of skin biopsy; however, the reprogramming efficiencies of adult human fibroblasts using Yamanaka four factors (Oct 4, Sox2, Klf4, and c-Myc) is very low at under 0.01% [313–316]. Although a recent study reported ~1% reprogramming efficiency using neonatal/juvenile human keratinocytes, the reprogramming efficiency of adult human keratinocytes is still unclear [317]. Furthermore, epigenetic differences occurred during the reprogramming process in iPSC cause variations in the genome stability of iPSC lines and in their differentiation potential [318–321]. Thus, these problems cause a highly heterogeneous cell population along with the remaining undifferentiated iPSC, which could lead to teratoma formation in vivo. Thus, the importance of developing efficient methods for reprogramming and differentiating iPSC should be further emphasized for iPSC-based therapy.

Several attempts have been made to derive functional stem cells from iPSC for skin tissue regeneration. MSC derived from iPSC (iPSC-MSC) obtain most mesenchymal characteristics of the naïve BM-MSC [28,322,323]. In addition, they show much greater proliferation and self-renewal capacity than BM-MSC, diminish histocompatibility-mediated immune response, and no longer tumorigenic [28]. Thus, studies have indicated that iPSC-MSC exert stronger therapeutic effects in tissue repair than BM-MSC [28,324]. Zhang et al. reported that exosomes derived from human iPSC-MSC facilitated cutaneous wound healing in a rat model through a paracrine signaling, resulting in accelerated re-epithelialization, reduced scar widths, and the promotion of collagen maturity [325]. Yang et al. demonstrated that human iPSC-derived epithelial stem cells (iPSC-EpSC) are capable of reconstituting the epithelial components of the hair follicle and interfollicular epidermis in the skin of immune-deficient mice [29]. These iPSC-based approaches can generate large number of human autologous cells for wound healing through cell transplantation, and suggest the potential use of skin stem cells in iPSC-based therapy for advanced wound treatments for degenerative skin disorders.

7.2.3. Transdifferentiated cells (Directly conversed cells)

Recently, it has been shown that transient induction of pluripotency-related genes generates a developmental plastic state which is amenable to transdifferentiate mouse embryonic fibroblasts (MEF) into other cell types in a directed manner [326–328]. Importantly, in contrast to iPSC, transient induction of pluripotency-associated transcription factors in MEF prevents teratoma formation in vivo [329,330]. Lacovides et al. reported the conversion of MEF into functional keratinocytes via the transient expression of pluripotency factors, Sox2, Oct4 and Klf4, coupled with directed differentiation [331]. Induced keratinocytes morphologically resemble primary keratinocytes, express keratinocyte-specific markers and regenerate a fully stratified epidermis in vivo. In order to completely bypass a pluripotent intermediate state, direct lineage conversion between somatic cells of different types has been reported and demonstrated that direct conversion approach can minimize unnecessary ex vivo manipulation of cells, thereby reducing the risk of damage accumulation during reprogramming [327]. Chen et al. reported that the combination of p63, a master regulator of epidermal development and differentiation, and Klf4, a regulator of epidermal differentiation is sufficient to convert dermal fibroblasts to a keratinocyte phenotype [332]. In addition, induced keratinocytes are similar to keratinocytes in global gene expression, morphology, chromatin state, as well as protein expression. Yang et al. generated functional melanocytes by direct reprogramming of mouse and human fibroblasts in a combination of three factors, MITF, Sox10, PAX3. Induced melanocytes acquired phenotypical and functional characteristics of primary melanocytes, and they generated pigmented epidermis and hair follicles in skin reconstitution assays in nude mice [333]. Although skin cell generation via direct reprogramming has not been actively demonstrated so far, the direct reprogramming approach for wound healing is an exciting direction due to the potential to provide autologous skin cells and facilitate large-scale skin cell regeneration.

7.2.4. Genetically corrected cells

IPSC-derived skin cells could be a promising cell source for customized cellular therapies to treat non-healing wounds. In particular, genetically corrected autologous iPSC of patients with skin diseases can be used for a permanent corrective therapy for chronic wounds resulting from genetic predisposition. Genetic mutations can be readily repaired in iPSC using various genome-editing tools, such as zinc finger nucleases (ZFN), transcription activator-like effector nuclease (TALEN), or clustered, regularly interspaced, short palindromic repeats (CRISPR).

Epidermolysis bullosa (EB) is a family of rare genetic disorders characterized by structural and mechanical fragility of the integuments, leading to recurrent skin and mucosal blistering and erosions that severely impair EB patients' quality of life. Junctional EB is caused by mutation in LAMA3, LAMB3, or LAMC2, which encode laminin-332, a heterotrimeric protein consisting of α3, β3, and γ2 chains. Because laminin-332 is highly reduced, blister formation within the lamina lucida of the basement membrane occurs upon minor trauma, resulting in chronic wounds and subsequent recurrent infections and scarring. So far, there is no cure for junctional EB, but transplantation of autologous epidermal stem cells which are genetically modified are able to restore a normal epidermis on a junctional EB patient in a phase I/II trial, implying the possibility of permanent local treatment for chronic wounds of junctional EB [35]. Bauer et al. reported that transplantation of epidermal sheets made of gene-corrected epidermal stem cells improves wound closure of a large non-healing epidermal ulceration in a clinical trial [35]. Epidermal stem cells, obtained from an adult patient's palm, were transduced with a retroviral vector expressing LAMB3 cDNA (encoding LAM5- β3), and employed to fabricate epidermal sheets [35,36]. In skin wounds of the EB patient, genetically modified cells expressed a higher amount of laminin-332- β3 than normal keratinocytes and regenerated a normal epidermis and a normal dermal-epidermal junction. In addition, self-renewing transduced epidermal stem cells induced long-term maintenance of the regenerated epidermis.

Recessive dystrophic EB (RDEB) is caused by mutations in the gene COL7A1 encoding type VII collagen, resulting in severe blistering and chronic wounds in response to mechanical stress. Sebastiano et al. generated patient-derived COL7A1-corrected epithelial keratinocyte and prepared cell sheets for autologous grafting [37]. IPSC derived from RDEB patients were first generated and corrected mutations in the COL7A1 locus of RDEB-derived iPSC by adenovirus-associated viral variant-mediated targeting. The repaired iPSC were differentiated into stratifying and graftable keratinocytes which secreted wild-type type VII collagen, resulting in the formation of stratified epidermis in vivo in mice. Wenzel et al. generated iPSC from fibroblasts of COL7A1 mutant mice and repaired the COL7A1 genomic locus by Flpe recombinase (Flpo)-mediated excision of a PEGneo cassette carried by the hypomorphic mice [38]. Genetically repaired iPSC could be differentiated into functional fibroblasts that re-expressed and secreted type VII collagen. Intradermal injection of fibroblasts derived from genetically repaired iPSC resulted in long-term restoration of type VII collagen deposition and resistance to mechanical resistance to skin blistering in mice with RDEB. Webber et al. corrected COL7A1 mutations of fibroblasts from an RDEB patient using the CRISPR/Cas9 system and the corrected fibroblasts were used for generating iPSC [39]. The resulting iPSC-derived keratinocyte

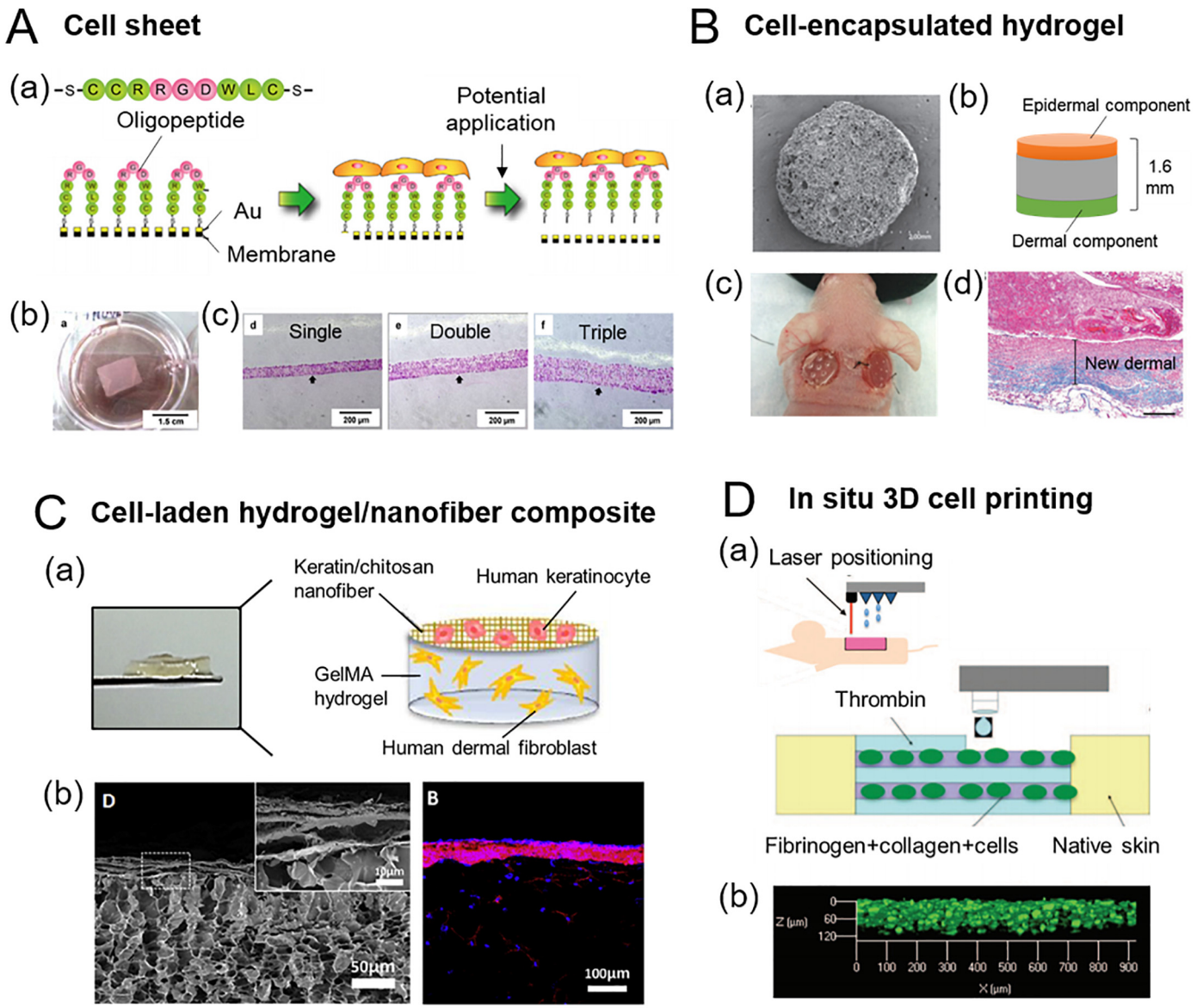

Fig. 5. Cell-based strategies for skin tissue regeneration. (A) Cell-sheets. (a) Schematic of electrochemical detachment of cells from a gold-coated membrane substrate. (b) Human neonatal skin fibroblasts sheet after detachment for transplantation. (c) H&E staining of cross sections of stacked fibroblast sheets: single, double and triple layered sheets. Figure adapted from Enomoto et al. [353]. (B) Cell-encapsulated hydrogel. Human fibroblasts and keratinocytes are encapsulated in a pullulan-gelatin hydrogel. (a) SEM image and (b) schematic of the cell-encapsulated hydrogel. (c) Mouse dorsal skin wounds treated with the cell-encapsulated hydrogel (6 mm in diameter). (d) Trichrome-stained images of the regenerated skin tissue treated with the cell-encapsulated hydrogel, showing a thick dermal layer regeneration on day 14 post-administration. Figure adapted from Nicholas et al. [362]. (C) Cell-laden hydrogel/nanofiber composite. (a) Photo and schematic image of bi-layer scaffold composed of human hair keratin/chitosan nanofiber and gelatin methacrylate hydrogel. Immortalized keratinocytes (HaCaT cells) and primary human dermal fibroblasts are separately encapsulated into the composite to mimic the epidermis and dermis layers of the skin tissue structure. (b) SEM and CLSM images (red: F-actin, blue: nucleus) of the composite, showing its bi-layered structure. Figure adapted from Kim et al. [40]. (D) In situ 3D cell printing. (a) Schematic of the in situ 3D cell printing process with amniotic fluid-derived stem cells and ECM consisting of composite hydrogels and thrombin. (b) Side view of a CLSM image of the wound containing the gels with green fluorescently labeled cells post-printing. Figure adapted from Skardal et al. [41].

exhibited characteristic epithelial morphology and expressed keratinocyte-specific genes and transcription factors in vitro.

8. Skin equivalents

The limitation of current biomaterial-based skin substitutes is poor vascularization, which incurs long healing period and unsatisfactory cosmetic outcome [334]. Stem cells hold great potential for tissue regeneration as they have excellent immunocompatiblity, secrete numerous growth factors, and produce matrices. Therefore, skin equivalents can be developed by using stem cells and their complexes with biomaterials. In this part, we summarize the skin equivalents generated with cells or through their combination with biomaterials, such as cell sheets, cell-hydrogels, cell-scaffolds, and cell-laden 3D-printed constructs (Fig. 5). Moreover, the pre-vascularization techniques used for the success of skin equivalents are detailed.

8.1. Scaffold-free cell sheets for skin

Scaffold-free skin equivalents make full use of cellular functions and their secreted ECMs that mimic native skin tissue [335]. Among the scaffold-free methods, cell sheet technology is one of the most advanced approaches due to its simplicity of process compared with other skin substitutes [336], excellent fusion with native skin tissue [337,338], and possibility of fabricating customized neotissue ECM from patient's own cells without foreign body rejection [339].

Cell sheets classically form two or three (few layers) dimensional geometry under culture in appropriate conditions without scaffolds. Various cell types, including skin keratinocytes, fibroblasts, and stem cells (i.e. ASCs), are grown on a plastic culture plate to undergo self-organization (tissue patterning) to mimic their naive structure [340,341]. In fact, those cells form a sheet-like structure in a monolayer at first [342,343], which becomes a multilayer at high confluency after long term culture and produces ECM, finally ready for implantation in target skin wound [344]. Using the cell sheet technology, growth-arrested allogenic neonatal keratinocytes and fibroblasts were prepared as cell-based skin regenerative additives (i.e. HP802–247) which is currently in phase III clinical trials [345,346]. Not only the cell sheets from a single cell type, but bilayered cell sheets made of both fibroblasts and keratinocytes, were also introduced, which showed enhanced skin regeneration in chronic wounds compared with fibroblast or keratinocyte only cell sheet due to biomimetic cross-talks between the cells [347]. To further enhance the regenerative capacity of the multi-layered cell sheets, various moulds for layering, rolling, or draping were used [335]. However, it is still very challenging to produce over tens of layers, which is considered necessary for complete replacement of damaged skin wound [334]. Moreover, the enzymatic detachment using trypsin or dispase damages cell sheet integrity and biological functions. On the other hand, the use of temperature-responsive polymers such as poly (N-isoproplyacrylamide) (PNIPAM) has accelerated the cell sheet technology [348,349]. PNIPAM allows detachment of cell sheets by a slight decrease in temperature (from 37 to 32 °C) [350], which helps preservation of structure, lineage phenotype and regenerative capability [351,352]. One interesting study reported electrochemical detachment using gold-coated membrane substrate modified with an oligopeptide layer, which can be noninvasively detached by applying a negative electrical potential to cleave the gold–thiolate bond from oligopeptide [353].

However, scaffold-free cell sheets face technical challenges such as prolonged in vitro culture period (~a few weeks) and restricted volume for implantation as well as inherent physical weakness and poor vascularization [340]. Therefore, biomaterials and tissue engineering technologies have been introduced to overcome such limitations of scaffold-free cell sheets [354,355]. For example, thin layers of biomaterials in the form of hydrogels, nanofibers, or their mixtures were used as a substrate for cell sheets, which could reinforce the physical integrity, mechanical property and vascularization [356,357]. Moreover, layering of cells by micro-patterning or printing technology and direct incubation of cells in 3D hydrogels were combined with cell sheet engineering, which could increase the cell sheet layers and physical integrity [358].

8.2. Cell-laden hydrogels and hydrogel/nanofiber hybrids

Hydrogels can safely load cells and biological factors, and can conform to defect sites and possibly degradable to accommodate skin regeneration [359,360]. Many hydrogel compositions have been used to incorporate and deliver cells for skin repair, including natural (alginate, collagen type 1, HA, chitosan) and synthetic (poly (vinyl alcohol) (PVA), poly (ethylene glycol) (PEG), polycaprolactone (PCL), polylactic acid (PLA), and poly-L-lactic acid (PLLA)) polymers and their composites [361,362].

Typical cell-hydrogel based skin substitutes comprise fibroblasts/keratinocytes layer-by-layer cultured on collagen hydrogel using transwell and air lifting. Although the method is time-consuming (~14 days) and laborsome (~6 steps), many studies have used different types of cells (i.e. MSCs) over skin cells to generate stem cell-enabled biomimetic ECM structure and skin regeneratives [363–365]. MSCs in the hydrogel can regulate wound healing through a series of paracrine growth factors (e.g., TGF-β, FGF), and differentiate into certain cell types involved in wound healing, such as keratinocytes, fibroblasts, and endothelial cells, thereby accelerating wound closure via enhanced vascularization, less granulation tissue formation and improved re-epithelialization [366,367].

Among various hydrogels, in situ forming hydrogels such as PNIPAM, polyesters, chitosan, polyphosphazenes, polycarbonates, polycyanoacrylates, polyorthoesters, poly(ethylene glycol)-b-poly(l-alanine), polypeptides and their composites are attractive for skin regeneratives [342,368]. They can encapsulate cells in a solution state by simply mixing and become gel upon injection in body temperature or pH conditions. One interesting study reported in situ forming hydrogels via an enzymatic cross-link with horseradish peroxidase (HRP) [369]. In particular, the hydrogels made of gelatin-hydroxyphenyl propionic acid were engineered with two different stiffness levels (1.1 kPa and 6.2 kPa). The soft hydrogel facilitated more proliferation of dermal fibroblasts and their synthesis of ECM components, as compared with the stiffer hydrogel. In vivo transplantation of the fibroblast-encapsulated soft hydrogels to a punched skin in mice accelerated collagen deposition and neovascularization, and consequently improved wound closing. Further, decellularized-tissues combined with synthetic hydrogel composition were developed to provide enriched biomimetic-cell-adhesive matrix. When decellularized adipose tissue was combined with photo-cross-linkable methacrylated glycol chitosan or chondroitin sulfate the regeneration of skin wound was significantly accelerated in diabetic mice wound model [370].

A bilayered nanofiber/hydrogel hybrid skin graft can mimic the skin tissue anatomy, consisting of epidermis and dermis, and the biological and mechanical characteristics. The upper layer of skin, epidermis, made of the basement membrane and keratinocytes, prevents infection, and protects from external stimuli by covering the wound, which can be mimicked with densely structured film or electrospun nanofiber. On the other hand, the lower layer, dermis, absorbs and retains the wound exudates at large volume, and promotes fibroblast proliferation and keratinocyte migration in 3D culture condition, which can be designed with porous hydrogel or sponge structure.

Among the combinations, the nanofiber/hydrogel hybrids were shown to integrate well to result in skin mimic substitutes [371]. Although electrospun nanofibers or hydrogels have been widely investigated for wound dressings, the individual system has been suboptimal in terms of cell encapsulating ability, 3D layering and physical stability [230,372]. Kim et al. fabricated nanofiber-hydrogel hybrid made of human hair keratin/chitosan nanofiber and gelatin methacrylate (GelMA) hydrogel [40]. After grafting cells to the nanofiber, the GelMA hydrogel with micropores of 10–20 μm was combined to promote the skin-biomaterial interfacial property in vitro. Similarly, Pan et al. developed a hybrid made of electrospun PLCL/Poloxamer nanofiber and dextran/gelatin hydrogel for skin regeneration [373]. The hybrid scaffolds exhibited enhanced mechanical properties (~80% increase) and in vitro proliferation and differentiation of adipose-derived stem cells, proving the potential for temporary skin replacement.

8.3. 3D printed skin substitutes

3D cell printing, known as 3D bioprinting, has gained great interest in engineering various tissues including skin [335]. The technology offers rapid, versatile, and customizable manufacturing of scaffolds that can in situ incorporate cells and biofactors, and potentially mimic the native tissue anatomy and function [374]. 3D bioprinting generally uses layer-by-layer technique to print cells with bioinks to make native tissue-like structures [375], therefore the method is considered optimal to create two different parts of skin substitutes, epidermis and dermis, at a time.

Skin fibroblasts and keratinocytes embedded in collagen were laser-assisted-bioprinted in 3D, which showed uniform cell distribution, high cell proliferation and gap junctions among incorporated cells [376]. Inkjet-based 3D printing by thermal inkjet was adapted due to great

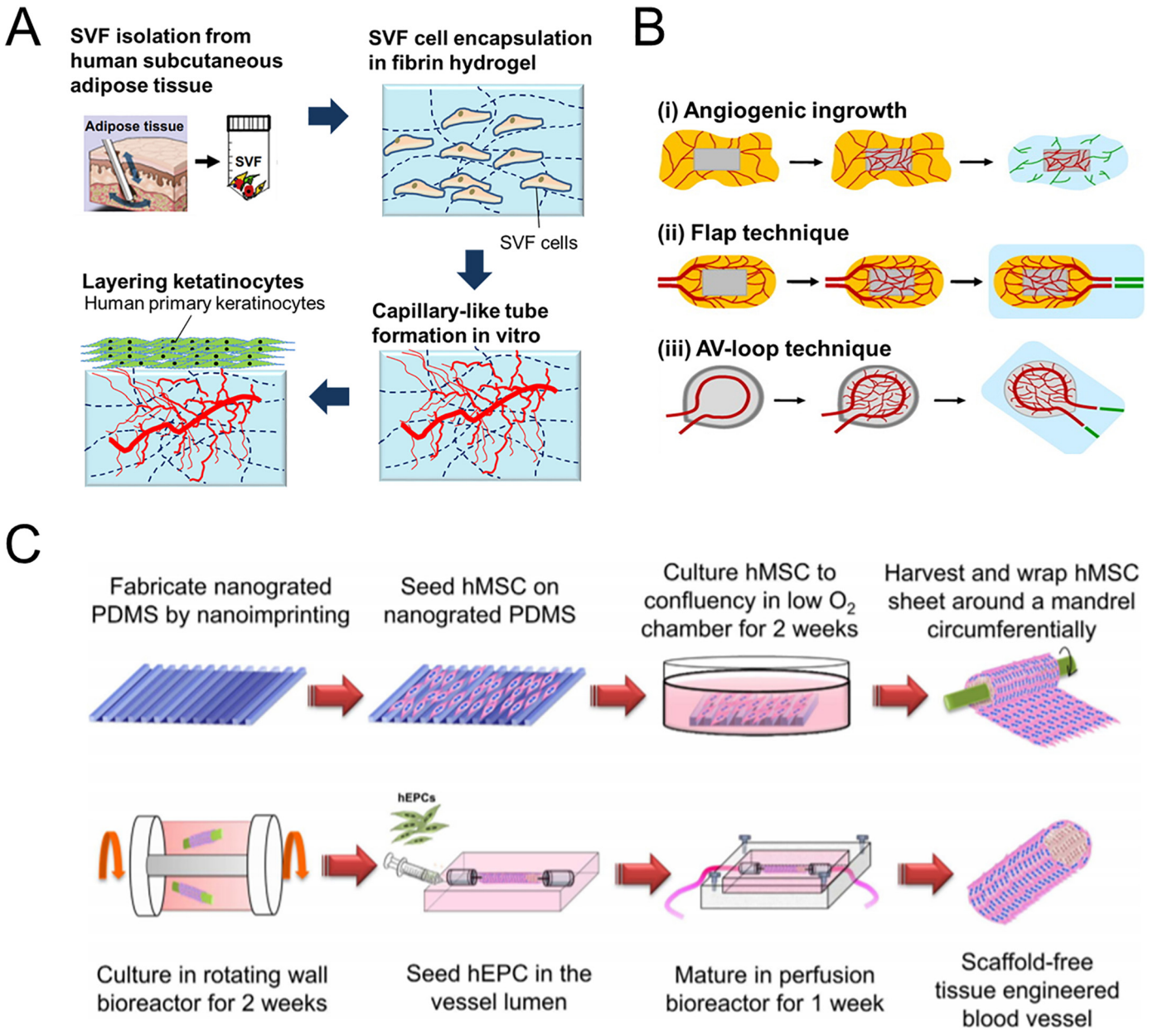

Fig. 6. Pre-vascularization strategies. (A) Delivery of tube forming cells. After hydrogels prevascularized by tube forming components (SVF: stromal vascular fraction), they were covered with human primary keratinocyte as skin substitute complex. Figure adapted from Klar et al. [392] (B) In-vivo skin bioreactor: (i) Ingrowth of newly developing microvessels in the scaffold, (ii) Use of scaffold-muscle flap complex including vein and vessels, and (iii) scaffold-spontaneous sprouting of vessels by AV-loop technique. After complete vascularization, the implanted biomaterial complex is transferred to the recipient site (blue), where the preformed microvasculatures inside the implant finally are interconnected to the microvessels of the recipient tissue (green) by inosculation. Figure adapted from Laschke et al. [386] (C) Formation of tissue engineered blood vessel (TEBV). Overall experimental scheme of fabricating an hMSC-based scaffold-free TEBV was described. Adapted from Jung et al. [403].

cyto-compatibility and convenience compared to other piezoelectric, and electromagnetic approaches [377]. Extrusion-based 3D printed skin substitutes were also developed and generally used for fabricating skin substitute with their great versatility, using skin cells with various bioinks such as collagen [378], fibrin-collagen [41], photo-crosslinkable gelatin methacrylamide (GelMA)/collagen [379], and self-healing hydrogel of chitosan, poly(acrylic acid) and ferric ions [380]. More recently, a single-step hybrid printing method using bioink and biopolymer mesh was developed, which uses extrusion-based and inkjet-based dispensing modules at the same time to print dermal fibroblasts and epidermal keratinocytes, respectively [381]. Gelatin or collagen type 1 hydrogel was used as a bioink and PCL mesh was used as a framework to support mechanically preventing the contraction of collagen during tissue maturation. The system could overcome time-consuming fabrication of 3D printing or conventional cell-hydrogel multilayering method [382]. The single-step hybrid printing system showed a skin-mimicking morphology and functionality. Apart from 3D bioprinting for skin substitute, bio-functional additives that can (selectively) modulate biological activities such as tyrosinase, silica based nanoparticles, peptides, single chemicals and other biofactors were also investigated in the 3D printed skin substitute [378,383].

Through 3D bioprinting, a skin mimic with appropriate anatomical structure along with vascularization is possible. Huang et al. reported 3D printed epidermal sweat glands, by using gelatin and alginate hybrid gels to encapsulate cells and construct cell-laden 3D structures [384]. The developed 3D constructs with 300-μm pore size supported the cellular self-organization and formed sweat gland tissues that were stable long-term and elicited bio-inductive cues upon degradation.

Interestingly, the glandular morphogenesis was only seen in the 3D conditions. Furthermore, the controlled delivery of growth factors (i.e. BMP-4) was able to provide a therapeutic effect on sweat gland and skin regeneration in burned paws of mice. In another example, melanin-producing skin cells (or melanocytes) and keratinocytes were precisely distributed by a 3D bioprinting in a hierarchical porous collagen-based dermis structure containing fibroblasts [378]. The approach shows promise in fabricating a tissue-engineered skin with pigmentation in a skin defect animal model that is comparable to native human skin.

8.4. Pre-vascularized skin equivalents

Successful treatment of full thickness skin wounds is still challenging due largely to lack of an adequate vascularization of skin substitute. Poor vascularization of cell-laden skin substitutes leads to cell death and eventually a failure which is primarily caused by hypoxia and lack of nutrients around the implanted cells [385]. Therefore, prevascularization has emerged as a promising approach for the tissue-engineered skin substitutes to regenerate particularly large skin defects (Fig. 6). Prevascularization is a concept, where therapeutic angiogenesis is induced in engineered scaffolds either by growth factor delivery or by angiogenic potent cells, to achieve higher tissue interactions with preformed microvasculatures and adequate blood supply after implantation [386]. With the prevascularization of skin substitutes it is possible to accelerate skin tissue regeneration and decrease scar formation, leading to rapid adaptation to clinical settings [387].

Thus far, three major strategies have been reported; (i) in vitro culture of cells and their delivery in the form of spheroids or sheets with layered matrix produced from tube-forming cells, (ii) in situ neovascularization using in vivo bioreactor, and (iii) ex vivo tube formation of tissue engineered blood vessel (TEBV) using ex vivo bioreactor and their hybrid with conventional skin substitutes.

The first approach of prevascularization introduces conventional in vitro cultures of tube-forming cells in the form of spheroids or sheets together with skin substitutes. Seeding of vessel-forming cells such as endothelial cells (ECs), endothelial progenitor cells (EPCs), stem cells, or their combination with mural cells (vascular smooth muscle cells, pericytes, and their precursor from the mesenchyme) onto hydrogels of natural/synthetic origin is commonly applied [388–392]. Spheroids of vessel-forming cells have also shown promising results due to intensive cell-cell and cell-matrix interactions in 3D arrangement [393]. Thanks to their in vivo mimic 3D environments, spheroids have higher angiogenic potential than 2D cultured cells, including accelerated formation of prevascularized matrix and higher in vitro production of angiogenic growth factors [394,395]. Implantation of the spheroids made from skin cells and tube forming cells (at various ratios) into the dorsal skinfold chamber resulted in rapid vascularization and skin tissue formation due to enhanced prevascularization [396]. During the preparation of cell sheets or cell-hydrogel complexes, the introduction of tube forming cells was shown to be highly effective for prevascularization. The bioreactor systems could synergize the generation of perfusable blood vessels in skin substitutes under in vitro cultures [397].

The second approach of in situ prevascularization uses the body as a bioreactor for the generation of functional microvasculatures within tissue constructs. The simplest approach is the implantation of skin substitutes into an easily accessible and well-vascularized tissue of live species, such as a subcutaneous site or a muscle pouch [398,399], which induces random ingrowth of newly developing microvasculatures from the surrounding host tissue. After complete vascularization, the implanted skin substitute is transferred to the recipient site, where the blood perfusion of the preformed microvasculatures inside the skin substitutes is finally restored by inoculation. However, this approach is time-consuming (needs at least three surgical interventions), and faces ingrowth of unspecific granulation tissues and possibly delayed blood perfusion of a construct after implantation (from the incidental interconnection of sprouting microvessels) [400].

For this reason, a direct surgical anastomosis of tissue constructs at defect sites using flap technique and arteriovenous (AV)-loop technique was developed for immediate reperfusion after implantation [401]. The flap and AV-loop techniques need different anatomical implantation sites, muscle and arteriovenous fistula, respectively. While the flap technique involves significant tissue loss and deformity at the donor site, the AV-loop technique doesn't require surrounding muscle tissue and thus has been intensively studied for skin tissue engineering [402]. However, both techniques need at least two surgical steps, requiring further optimization for clinical applications [386].

Tissue engineered blood vessel (TEBV) is a promising option for prevascularization of skin substitutes. Some of the TEBVs have been developed to mimic native human vessels in terms of mechanical properties and vasoactivities of biochemicals, finding possible applications in vascular defects and drug screening [403,404]. TEBVs can be produced from tube-forming cells such as ECs, EPCs, and stem cells in conjunction with smooth muscle cells (SMCs) and fibroblasts with or without the help of scaffolds [405], when cultured on 2D dish, pseudo 3D circumferential mandrel, or rotating/perfusion bioreactors for maturation. In principle, when the TEBVs are engineered into skin equivalents or combined with other skin substitutes, a prevascularization is possible, enabling direct implantation to full thickness skin wounds and successful regeneration. However, formidable hurdles remain for clinical application, including long-time culture of cells and cell-constructs to generate functional TEBV-skin substitutes, and the regulatory approval for such complex designs.

9. Summary and perspectives

Among various innovative strategies of drug delivery for the cutaneous wound healing, scaffold-mediated drug delivery approaches are particularly suited for improving wound healing because the scaffold can act as a barrier to protect against infections and to maintain moisture environment in addition to serving as a drug reservoir to provide wound-stage specific biochemical cues and drugs. Cell-laden scaffold can further bring the power of cell therapy and tissue engineering to bear, and it is particularly advantageous for regeneration of large area skin defects. Furthermore, genetically engineered cell sources could address the limitations of wound healing specific to the type of wound and injury state, and allow for potential patient-specific wound therapy. This future generation of therapeutics will require an effective intracellular delivery system for genome editing tools such as CRISPR/Cas9 or TALEN. It will further benefit from scaffold-mediated genetic engineering of the wound microenvironment to achieve fetal wound healing characteristics so that adult wound healing with complete regeneration of skin appendages without impairment or scarring may be possible.

Advances in 3D bioprinting can offer precise cell patterning in predefined spatial locations, which enables the recapitulation of architectural organization of native skin. However, current bioinks for skin cell printing are mostly alginate, collagen, fibrin or their mixture, which remain suboptimal. The field awaits the development of more bioactive bioinks to advance 3D-bioprinted constructs for wound healing. For functional recapitulation of native skin, various cell types including fibroblasts, keratinocytes, epidermal stem/progenitors, and endothelial cells need to be used in combination with optimal bioinks in order to faithfully mimic skin-specific microenvironment containing microvessels, hair follicles, and sweat glands. Nevertheless, 3D printing with or without cells holds intriguing potential for wound healing. One can imagine in the not-too-distant future that doctors would be able to use a hand-held 3D bioprinter to apply a cell-laden dressing on the wounds of patients in the operation room or in the field.

Many innovative strategies have been applied to tackle the cutaneous wound healing due to easy accessibility to the target tissue. However, because of the typical non life-threatening scenario, the

benefit-to-risk consideration demands that these treatment strategies in the vast majority of cases be highly cost-effective. This presents a different but equally formidable challenge for biomaterials researchers and biomedical engineers to translate the technologies. However, given the scale and increasing prevalence of various types of cutaneous wounds, it will be a challenge worthy of close collaboration among researchers of many disciplines.

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References

[1] J.V. Dovi, L.K. He, L.A. DiPietro, Accelerated wound closure in neutrophil-depleted mice, J. Leukoc. Biol. 73 (2003) 448–455.
[2] P. Martin, D. D'Souza, J. Martin, R. Grose, L. Cooper, R. Maki, S.R. McKercher, Wound healing in the PU. 1 null mouse—tissue repair is not dependent on inflammatory cells, Curr. Biol. 13 (2003) 1122–1128.
[3] T. Swanson, D. Keast, R. Cooper, J. Black, D. Angel, G. Schultz, K. Carville, J. Fletcher, Ten top tips: identification of wound infection in a chronic wound, Wounds (2) (2015).
[4] C.K. Sen, G.M. Gordillo, S. Roy, R. Kirsner, L. Lambert, T.K. Hunt, F. Gottrup, G.C. Gurtner, M.T. Longaker, Human skin wounds: a major and snowballing threat to public health and the economy, Wound Repair Regen. 17 (2009) 763–771.
[5] K. Jarbrink, G. Ni, H. Sonnergren, A. Schmidtchen, C. Pang, R. Bajpai, J. Car, The humanistic and economic burden of chronic wounds: a protocol for a systematic review, Syst. Rev. 6 (2017) 15.
[6] W. Chen, A. Palazzo, W.E. Hennink, R.J. Kok, Effect of Particle size on Drug Loading and Release Kinetics of Gefitinib-Loaded PLGA Microspheres, Mol. Pharm. 14 (2017) 459–467.
[7] A. Gonzalez-Pujana, E. Santos, G. Orive, J.L. Pedraz, R.M. Hernandez, Cell microencapsulation technology: current vision of its therapeutic potential through the administration routes, J. Drug Deliv. Sci. Technol. 42 (2017) 49–62.
[8] R.M. Olabisi, Cell microencapsulation with synthetic polymers, J. Biomed. Mater. Res. A 103 (2015) 846–859.
[9] R. Wang, J. Li, W. Chen, T. Xu, S. Yun, Z. Xu, Z. Xu, T. Sato, B. Chi, H. Xu, A biomimetic mussel-inspired ε-Poly-l-lysine hydrogel with robust tissue-anchor and anti-infection capacity, Adv. Funct. Mater. (2017) 27.
[10] C. Dhand, M. Venkatesh, V.A. Barathi, S. Harini, S. Bairagi, E. Goh Tze, N. Leng, K.Z.W. Muruganandham, M. Low, X.J. Fazil, D.K. Loh, S.P. Srinivasan, R.W. Liu, N.K. Beuerman, S. Verma, R. Lakshminarayanan Ramakrishna, Bio-inspired crosslinking and matrix-drug interactions for advanced wound dressings with long-term antimicrobial activity, Biomaterials 138 (2017) 153–168.
[11] T. Tokatlian, C. Cam, T. Segura, Porous hyaluronic acid hydrogels for localized non-viral DNA delivery in a diabetic wound healing model, Adv. Healthcare Mater. 4 (2015) 1084–1091.
[12] D.L. Steed, Clinical evaluation of recombinant human platelet-derived growth factor for the treatment of lower extremity ulcers, Plast. Reconstr. Surg. 117 (2006) 143S–149S (discussion 150S-151S).
[13] D.L. Steed, J.B. Goslen, G.A. Holloway, J.M. Malone, T. Bunt, M.W. Webster, Randomized prospective double-blind trial in healing chronic diabetic foot ulcers: CT-102 activated platelet supernatant, topical versus placebo, Diabetes Care 15 (1992) 1598–1604.
[14] H. Uchi, A. Igarashi, K. Urabe, T. Koga, J. Nakayama, R. Kawamori, K. Tamaki, H. Hirakata, T. Ohura, M. Furue, Clinical efficacy of basic fibroblast growth factor (bFGF) for diabetic ulcer, Eur. J. Dermatol. 19 (2009) 461–468.
[15] A.J. Marti-Carvajal, C. Gluud, S. Nicola, D. Simancas-Racines, L. Reveiz, P. Oliva, J. Cedeno-Taborda, Growth factors for treating diabetic foot ulcers, Cochrane Database Syst. Rev. 10 (2015), CD008548.
[16] J.-L. Richard, C. Parer-Richard, J.-P. Daures, S. Clouet, D. Vannereau, J. Bringer, M. Rodier, C. Jacob, M. Comte-Bardonnet, Effect of topical basic fibroblast growth factor on the healing of chronic diabetic neuropathic ulcer of the foot: a pilot, randomized, double-blind, placebo-controlled study, Diabetes Care 18 (1995) 64–69.
[17] T.J. Wieman, J.M. Smiell, Y. Su, Efficacy and safely of a topical gel formulation of recombinant human platelet-derived growth factor-BB (becaplermin) in patients with chronic neuropathic diabetic ulcers: a phase III randomized placebo-controlled double-blind study, Diabetes Care 21 (1998) 822–827.
[18] A.J. Martí-Carvajal, M.X. Rojas-Reyes, L. Reveiz, N. Rodriguez-Malagon, J. Cedeño-Taborda, Growth factors for treating diabetic foot ulcers, Cochrane Database Syst. Rev. 6 (2010).
[19] G. Mascre, S. Dekoninck, B. Drogat, K.K. Youssef, S. Brohee, P.A. Sotiropoulou, B.D. Simons, C. Blanpain, Distinct contribution of stem and progenitor cells to epidermal maintenance, Nature 489 (2012) 257–262.
[20] L. Liu, Y. Yu, Y. Hou, J. Chai, H. Duan, W. Chu, H. Zhang, Q. Hu, J. Du, Human umbilical cord mesenchymal stem cells transplantation promotes cutaneous wound healing of severe burned rats, PLoS One 9 (2014) e88348.
[21] A. Nauta, C. Seidel, L. Deveza, D. Montoro, M. Grova, S.H. Ko, J. Hyun, G.C. Gurtner, M.T. Longaker, F. Yang, Adipose-derived Stromal Cells Overexpressing Vascular Endothelial Growth factor Accelerate Mouse Excisional Wound Healing, Mol. Ther. 21 (2013) 445–455.
[22] G. Bilousova, J.A. Chen, D.R. Roop, Differentiation of mouse induced pluripotent stem cells into a multipotent keratinocyte lineage, J. Investig. Dermatol. 131 (2011) 857–864.
[23] S. Ohta, Y. Imaizumi, W. Akamatsu, H. Okano, Y. Kawakami, Generation of human melanocytes from induced pluripotent stem cells, Methods Mol. Biol. 989 (2013) 193–215.
[24] S. Balaji, S.G. Keswani, T.M. Crombleholme, The role of mesenchymal stem cells in the regenerative wound healing phenotype, Adv. Wound Care (New Rochelle) 1 (2012) 159–165.
[25] M.N. Walter, K.T. Wright, H.R. Fuller, S. MacNeil, W.E. Johnson, Mesenchymal stem cell-conditioned medium accelerates skin wound healing: an in vitro study of fibroblast and keratinocyte scratch assays, Exp. Cell Res. 316 (2010) 1271–1281.
[26] Y.K. Jeon, Y.H. Jang, D.R. Yoo, S.N. Kim, S.K. Lee, M.J. Nam, Mesenchymal stem cells' interaction with skin: Wound-healing effect on fibroblast cells and skin tissue, Wound Repair Regen. 18 (2010) 655–661.
[27] C. Nie, D. Yang, J. Xu, Z. Si, X. Jin, J. Zhang, Locally administered adipose-derived stem cells accelerate wound healing through differentiation and vasculogenesis, Cell Transplant. 20 (2011) 205–216.
[28] L.G. Villa-Diaz, S.E. Brown, Y. Liu, A.M. Ross, J. Lahann, J.M. Parent, P.H. Krebsbach, Derivation of mesenchymal stem cells from human induced pluripotent stem cells cultured on synthetic substrates, Stem Cells 30 (2012) 1174–1181.
[29] R. Yang, Y. Zheng, M. Burrows, S. Liu, Z. Wei, A. Nace, W. Guo, S. Kumar, G. Cotsarelis, X. Xu, Generation of folliculogenic human epithelial stem cells from induced pluripotent stem cells, Nat. Commun. 5 (2014) 3071.
[30] T. Freyman, G. Polin, H. Osman, J. Crary, M. Lu, L. Cheng, M. Palasis, R.L. Wilensky, A quantitative, randomized study evaluating three methods of mesenchymal stem cell delivery following myocardial infarction, Eur. Heart J. 27 (2006) 1114–1122.
[31] C. Krisp, F. Jacobsen, M.J. McKay, M.P. Molloy, L. Steinstraesser, D.A. Wolters, Proteome analysis reveals antiangiogenic environments in chronic wounds of diabetes mellitus type 2 patients, Proteomics 13 (2013) 2670–2681.
[32] S. Khanna, S. Biswas, Y. Shang, E. Collard, A. Azad, C. Kauh, V. Bhasker, G.M. Gordillo, C.K. Sen, S. Roy, Macrophage dysfunction impairs resolution of inflammation in the wounds of diabetic mice, PLoS One 5 (2010) e9539.
[33] Y. Nakamura, H. Ishikawa, K. Kawai, Y. Tabata, S. Suzuki, Enhanced wound healing by topical administration of mesenchymal stem cells transfected with stromal cell-derived factor-1, Biomaterials 34 (2013) 9393–9400.
[34] H.K. Chang, P.H. Kim, H.M. Cho, S.Y. Yum, Y.J. Choi, Y.S. Son, D.B. Lee, I.S. Kang, K.S. Kang, G. Jang, J.Y. Cho, Inducible HGF-secreting Human Umbilical Cord Blood-derived MSCs Produced via TALEN-mediated Genome Editing Promoted Angiogenesis, Mol. Ther. 24 (2016) 1644–1654.
[35] L. De Rosa, S. Carulli, F. Cocchiarella, D. Quaglino, E. Enzo, E. Franchini, A. Giannetti, G. De Santis, A. Recchia, G. Pellegrini, M. De Luca, Long-term stability and safety of transgenic cultured epidermal stem cells in gene therapy of junctional epidermolysis bullosa, Stem Cell Rep. 2 (2014) 1–8.
[36] L. LAMA, Closure of a large chronic wound through transplantation of gene-corrected epidermal stem cells, J. Investig. Dermatol. 2 (2016).
[37] V. Sebastiano, H.H. Zhen, B. Haddad, E. Bashkirova, S.P. Melo, P. Wang, T.L. Leung, Z. Siprashvili, A. Tichy, J. Li, M. Ameen, J. Hawkins, S. Lee, L. Li, A. Schwertschkow, G. Bauer, L. Lisowski, M.A. Kay, S.K. Kim, A.T. Lane, M. Wernig, A.E. Oro, Human COL7A1-corrected induced pluripotent stem cells for the treatment of recessive dystrophic epidermolysis bullosa, Sci. Transl. Med. 6 (2014).
[38] D. Wenzel, J. Bayerl, A. Nyström, L. Bruckner-Tuderman, A. Meixner, J.M. Penninger, Genetically corrected iPSCs as cell therapy for recessive dystrophic epidermolysis bullosa, Sci. Transl. Med. 6 (2014) 264ra165.
[39] B.R. Webber, M.J. Osborn, A.N. McElroy, K. Twaroski, C.-I. Lonetree, A.P. DeFeo, L. Xia, C. Eide, C.J. Lees, R.T. McElmurry, CRISPR/Cas9-based genetic correction for recessive dystrophic epidermolysis bullosa, NPJ Regenerative Med. 1 (2016) 16014.
[40] J.W. Kim, M.J. Kim, C.S. Ki, H.J. Kim, Y.H. Park, Fabrication of bi-layer scaffold of keratin nanofiber and gelatin-methacrylate hydrogel: Implications for skin graft, Int. J. Biol. Macromol. 105 (2017) 541–548.
[41] A. Skardal, D. Mack, E. Kapetanovic, A. Atala, J.D. Jackson, J. Yoo, S. Soker, Bioprinted amniotic fluid-derived stem cells accelerate healing of large skin wounds, Stem Cells Transl. Med. 1 (2012) 792–802.
[42] M.P. Curran, G.L. Plosker, Bilayered bioengineered skin substitute (Apligraf): a review of its use in the treatment of venous leg ulcers and diabetic foot ulcers, BioDrugs 16 (2002) 439–455.
[43] S.B. Mahjour, X. Fu, X. Yang, J. Fong, F. Sefat, H. Wang, Rapid creation of skin substitutes from human skin cells and biomimetic nanofibers for acute full-thickness wound repair, Burns 41 (2015) 1764–1774.
[44] S.A. Eming, P. Martin, M. Tomic-Canic, Wound repair and regeneration: mechanisms, signaling, and translation, Sci. Transl. Med. 6 (2014) 265sr266.
[45] M.H. Kim, W. Liu, D.L. Borjesson, F.R. Curry, L.S. Miller, A.L. Cheung, F.T. Liu, R.R. Isseroff, S.I. Simon, Dynamics of neutrophil infiltration during cutaneous wound

healing and infection using fluorescence imaging, J. Invest. Dermatol. 128 (2008) 1812–1820.
[46] M. Artuc, B. Hermes, U.M. Steckelings, A. Grutzkau, B.M. Henz, Mast cells and their mediators in cutaneous wound healing–active participants or innocent bystanders? Exp. Dermatol. 8 (1999) 1–16.
[47] T.J. Koh, L.A. DiPietro, Inflammation and wound healing: the role of the macrophage, Expert Rev. Mol. Med. 13 (2011) e23.
[48] C.M. Minutti, J.A. Knipper, J.E. Allen, D.M. Zaiss, Tissue-specific contribution of macrophages to wound healing, Semin. Cell Dev. Biol. 61 (2017) 3–11.
[49] B.C. Wulff, T.A. Wilgus, Mast cell activity in the healing wound: more than meets the eye? Exp. Dermatol. 22 (2013) 507–510.
[50] M.F. Ng, The role of mast cells in wound healing, Int. Wound J. 7 (2010) 55–61.
[51] P. Martin, S.J. Leibovich, Inflammatory cells during wound repair: the good, the bad and the ugly, Trends Cell Biol. 15 (2005) 599–607.
[52] T.N. Demidova-Rice, M.R. Hamblin, I.M. Herman, Acute and impaired wound healing: pathophysiology and current methods for drug delivery, part 1: normal and chronic wounds: biology, causes, and approaches to care, Adv. Skin Wound Care 25 (2012) 304.
[53] S.A. Eming, T. Krieg, J.M. Davidson, Inflammation in wound repair: molecular and cellular mechanisms, J. Invest. Dermatol. 127 (2007) 514–525.
[54] A.J. Singer, R.A. Clark, Cutaneous wound healing, N. Engl. J. Med. 341 (1999) 738–746.
[55] J. Folkman, M. Klagsbrun, Angiogenic factors, Science 235 (1987) 442–448.
[56] M.G. Tonnesen, X. Feng, R.A. Clark, Angiogenesis in wound healing, J. Investig. Dermatol. Symp. Proc. (2000) 40–46.
[57] V.S. Rajkumar, X. Shiwen, M. Bostrom, P. Leoni, J. Muddle, M. Ivarsson, B. Gerdin, C.P. Denton, G. Bou-Gharios, C.M. Black, Platelet-derived growth factor-β receptor activation is essential for fibroblast and pericyte recruitment during cutaneous wound healing, Am. J. Pathol. 169 (2006) 2254–2265.
[58] J.H. Distler, A. Jungel, L.C. Huber, U. Schulze-Horsel, J. Zwerina, R.E. Gay, B.A. Michel, T. Hauser, G. Schett, S. Gay, O. Distler, Imatinib mesylate reduces production of extracellular matrix and prevents development of experimental dermal fibrosis, Arthritis Rheum. 56 (2007) 311–322.
[59] J.J. Tomasek, G. Gabbiani, B. Hinz, C. Chaponnier, R.A. Brown, Myofibroblasts and mechano-regulation of connective tissue remodelling, Nat. Rev. Mol. Cell Biol. 3 (2002) 349–363.
[60] M. Schäfer, S. Werner, Cancer as an overhealing wound: an old hypothesis revisited, Nat. Rev. Mol. Cell Biol. 9 (2008) 628–638.
[61] V. Falanga, Wound healing and its impairment in the diabetic foot, Lancet 366 (2005) 1736–1743.
[62] D.A. Dubay, M.G. Franz, Acute wound healing: the biology of acute wound failure, Surg. Clin. North Am. 83 (2003) 463–481.
[63] R.G. Frykberg, J. Banks, Challenges in the treatment of chronic wounds, Adv. Wound Care (New Rochelle) 4 (2015) 560–582.
[64] S.M. McCarty, S.L. Percival, Proteases and delayed Wound Healing, Adv. Wound Care (New Rochelle) 2 (2013) 438–447.
[65] I. Ben-Porath, R.A. Weinberg, The signals and pathways activating cellular senescence, Int. J. Biochem. Cell Biol. 37 (2005) 961–976.
[66] L.Y. Bourguignon, Matrix hyaluronan-activated CD44 signaling promotes keratinocyte activities and improves abnormal epidermal functions, Am. J. Pathol. 184 (2014) 1912–1919.
[67] H. Cook, P. Stephens, K.J. Davies, D.W. Thomas, K.G. Harding, Defective extracellular matrix reorganization by chronic wound fibroblasts is associated with alterations in TIMP-1, TIMP-2, and MMP-2 activity, J. Investig. Dermatol. 115 (2000) 225–233.
[68] D. Telgenhoff, B. Shroot, Cellular senescence mechanisms in chronic wound healing, Cell Death Differ. 12 (2005) 695–698.
[69] I.B. Wall, R. Moseley, D.M. Baird, D. Kipling, P. Giles, I. Laffafian, P.E. Price, D.W. Thomas, P. Stephens, Fibroblast dysfunction is a key factor in the non-healing of chronic venous leg ulcers, J. Investig. Dermatol. 128 (2008) 2526–2540.
[70] A. Kümin, M. Schäfer, N. Epp, P. Bugnon, C. Born-Berclaz, A. Oxenius, A. Klippel, W. Bloch, S. Werner, Peroxiredoxin 6 is required for blood vessel integrity in wounded skin, J. Cell Biol. 179 (2007) 747–760.
[71] S. Braun, C. Hanselmann, M.G. Gassmann, U. auf dem Keller, C. Born-Berclaz, K. Chan, Y.W. Kan, S. Werner, Nrf2 transcription factor, a novel target of keratinocyte growth factor action which regulates gene expression and inflammation in the healing skin wound, Mol. Cell. Biol. 22 (2002) 5492–5505.
[72] G.P. Ladwig, M.C. Robson, R. Liu, M.A. Kuhn, D.F. Muir, G.S. Schultz, Ratios of activated matrix metalloproteinase-9 to tissue inhibitor of matrix metalloproteinase-1 in wound fluids are inversely correlated with healing of pressure ulcers, Wound Repair Regen. 10 (2002) 26–37.
[73] G.S. Schultz, R.G. Sibbald, V. Falanga, E.A. Ayello, C. Dowsett, K. Harding, M. Romanelli, M.C. Stacey, L. Teot, W. Vanscheidt, Wound bed preparation: a systematic approach to wound management, Wound Repair Regen. 11 (Suppl. 1) (2003) S1–28.
[74] M.A. Loot, S.B. Kenter, F.L. Au, W.J. van Galen, E. Middelkoop, J.D. Bos, J.R. Mekkes, Fibroblasts derived from chronic diabetic ulcers differ in their response to stimulation with EGF, IGF-I, bFGF and PDGF-AB compared to controls, Eur. J. Cell Biol. 81 (2002) 153–160.
[75] R. Vasquez, B.J. Marien, C. Gram, D.G. Goodwin, J.O. Menzoian, J.D. Raffetto, Proliferative capacity of venous ulcer wound fibroblasts in the presence of platelet-derived growth factor, Vasc. Endovasc. Surg. 38 (2004) 355–360.
[76] I.B. Wall, R. Moseley, D.M. Baird, D. Kipling, P. Giles, I. Laffafian, P.E. Price, D.W. Thomas, P. Stephens, Fibroblast dysfunction is a key factor in the non-healing of chronic venous leg ulcers, J. Invest Dermatol. 128 (2008) 2526–2540.
[77] H. Cook, K.J. Davies, K.G. Harding, D.W. Thomas, Defective extracellular matrix reorganization by chronic wound fibroblasts is associated with alterations in TIMP-1, TIMP-2, and MMP-2 activity, J. Invest Dermatol. 115 (2000) 225–233.
[78] W.J. Ennis, A. Sui, A. Bartholomew, Stem cells and healing: impact on inflammation, Adv. Wound Care (New Rochelle) 2 (2013) 369–378.
[79] F. Cianfarani, G. Toietta, G. Di Rocco, E. Cesareo, G. Zambruno, T. Odorisio, Diabetes impairs adipose tissue-derived stem cell function and efficiency in promoting wound healing, Wound Repair Regen. 21 (2013) 545–553.
[80] L. Rodriguez-Menocal, M. Salgado, D. Ford, E. Van Badiavas, Stimulation of skin and wound fibroblast migration by mesenchymal stem cells derived from normal donors and chronic wound patients, Stem Cells Transl. Med. 1 (2012) 221–229.
[81] O. Stojadinovic, H. Brem, C. Vouthounis, B. Lee, J. Fallon, M. Stallcup, A. Merchant, R.D. Galiano, M. Tomic-Canic, Molecular pathogenesis of chronic wounds: the role of beta-catenin and c-myc in the inhibition of epithelialization and wound healing, Am. J. Pathol. 167 (2005) 59–69.
[82] M.L. Usui, J.N. Mansbridge, W.G. Carter, M. Fujita, J.E. Olerud, Keratinocyte migration, proliferation, and differentiation in chronic ulcers from patients with diabetes and normal wounds, J. Histochem. Cytochem. 56 (2008) 687–696.
[83] K.-H. Cho, B. Singh, S. Maharjan, Y. Jang, Y.-J. Choi, C.-S. Cho, Local delivery of CTGF siRNA with Poly (sorbitol-co-PEI) reduces scar contraction in cutaneous wound healing, Tissue Eng. Regener. Med. 14 (2017) 211–220.
[84] N. Li, H.C. Luo, C. Yang, J.J. Deng, M. Ren, X.Y. Xie, D.Z. Lin, L. Yan, L.M. Zhang, Cationic star-shaped polymer as an siRNA carrier for reducing MMP-9 expression in skin fibroblast cells and promoting wound healing in diabetic rats, Int. J. Nanomedicine 9 (2014) 3377–3387.
[85] P.S. Randeria, M.A. Seeger, X.Q. Wang, H. Wilson, D. Shipp, C.A. Mirkin, A.S. Paller, siRNA-based spherical nucleic acids reverse impaired wound healing in diabetic mice by ganglioside GM3 synthase knockdown, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 5573–5578.
[86] C.T. Turner, M. Hasanzadeh Kafshgari, E. Melville, B. Delalat, F. Harding, E. Mäkilä, J.J. Salonen, A.J. Cowin, N.H. Voelcker, Delivery of Flightless I siRNA from porous silicon nanoparticles improves wound healing in mice, ACS Biomater. Sci. Eng. 2 (2016) 2339–2346.
[87] M.A. Loots, E.N. Lamme, J. Zeegelaar, J.R. Mekkes, J.D. Bos, E. Middelkoop, Differences in cellular infiltrate and extracellular matrix of chronic diabetic and venous ulcers versus acute wounds, J. Invest Dermatol. 111 (1998) 850–857.
[88] M.S. Ågren, H.H. Steenfos, S. Dabelsteen, J.B. Hansen, E. Dabelsteen, Proliferation and mitogenic response to PDGF-BB of fibroblasts isolated from chronic venous leg ulcers is ulcer-age dependent, J. Investig. Dermatol. 112 (1999) 463–469.
[89] P.C. Kuo, C.H. Kao, J.K. Chen, Glycated type 1 collagen induces endothelial dysfunction in culture, In Vitro Cell Dev. Biol. Anim. 43 (2007) 338–343.
[90] K.L. Reigle, G. Di Lullo, K.R. Turner, J.A. Last, I. Chervoneva, D.E. Birk, J.L. Funderburgh, E. Elrod, M.W. Germann, C. Surber, R.D. Sanderson, J.D. San Antonio, Non-enzymatic glycation of type I collagen diminishes collagen-proteoglycan binding and weakens cell adhesion, J. Cell. Biochem. 104 (2008) 1684–1698.
[91] H. Liao, J. Zakhaleva, W. Chen, Cells and tissue interactions with glycated collagen and their relevance to delayed diabetic wound healing, Biomaterials 30 (2009) 1689–1696.
[92] C. Holmes, J.S. Wrobel, M.P. Maceachern, B.R. Boles, Collagen-based wound dressings for the treatment of diabetes-related foot ulcers: a systematic review, Diabetes Metab. Syndr. Obes. 6 (2013) 17–29.
[93] J. Fletcher, Wound assessment and the TIME framework, Br. J. Nurs. 16 (2007) 462–464, 446.
[94] S. Greatrex-White, H. Moxey, Wound assessment tools and nurses' needs: an evaluation study, Int. Wound J. 12 (2015) 293–301.
[95] C. Dowsett, N. Groemann, K. Harding, Taking wound assessment beyond the edge, Wounds Int. 6 (2015) 19–23.
[96] A.S. Halim, T.L. Khoo, A.Z. Saad, Wound bed preparation from a clinical perspective, Indian J. Plast Surg. 45 (2012) 193–202.
[97] M. Granick, J. Boykin, R. Gamelli, G. Schultz, M. Tenenhaus, Toward a common language: surgical wound bed preparation and debridement, Wound Repair Regen. 14 (Suppl. 1) (2006) S1–10.
[98] B.M. Madhok, K. Vowden, P. Vowden, New techniques for wound debridement, Int. Wound J. 10 (2013) 247–251.
[99] V. Langer, P.S. Bhandari, S. Rajagopalan, M.K. Mukherjee, Enzymatic debridement of large burn wounds with papain-urea: is it safe? Med. J. Armed Forces India 69 (2013) 144–150.
[100] D. Blueman, C. Bousfield, The use of larval therapy to reduce the bacterial load in chronic wounds, J. Wound Care 21 (2012) 244–253.
[101] G. Cazander, K.E. van Veen, A.T. Bernards, G.N. Jukema, Do maggots have an influence on bacterial growth? A study on the susceptibility of strains of six different bacterial species to maggots of Lucilia sericata and their excretions/secretions, J. Tissue Viability 18 (2009) 80–87.
[102] R.A. Sherman, Maggot versus conservative debridement therapy for the treatment of pressure ulcers, Wound Repair Regen. 10 (2002) 208–214.
[103] P.E. Prete, Growth effects of Phaenicia sericata larval extracts on fibroblasts: mechanism for wound healing by maggot therapy, Life Sci. 60 (1997) 505–510.
[104] K. Honda, K. Okamoto, Y. Mochida, K. Ishioka, M. Oka, K. Maesato, R. Ikee, H. Moriya, S. Hidaka, T. Ohtake, K. Doi, T. Fujita, S. Kobayashi, E. Noiri, A novel mechanism in maggot debridement therapy: protease in excretion/secretion promotes hepatocyte growth factor production, Am. J. Physiol. Cell Physiol. 301 (2011) C1423–C1430.
[105] K.Y. Mumcuoglu, J. Miller, M. Mumcuoglu, M. Friger, M. Tarshis, Destruction of bacteria in the digestive tract of the maggot of Lucilia sericata (Diptera: Calliphoridae), J. Med. Entomol. 38 (2001) 161–166.
[106] M.J. van der Plas, J.T. van Dissel, P.H. Nibbering, Maggot secretions skew monocyte-macrophage differentiation away from a pro-inflammatory to a pro-angiogenic type, PLoS One 4 (2009) e8071.

[107] R.J. Linger, E.J. Belikoff, Y. Yan, F. Li, H.A. Wantuch, H.L. Fitzsimons, M.J. Scott, Towards next generation maggot debridement therapy: transgenic Lucilia sericata larvae that produce and secrete a human growth factor, BMC Biotechnol. 16 (2016) 30.
[108] T. Mustoe, Understanding chronic wounds: a unifying hypothesis on their pathogenesis and implications for therapy, Am. J. Surg. 187 (2004) 65S–70S.
[109] S. Guo, L.A. Dipietro, Factors affecting wound healing, J. Dent. Res. 89 (2010) 219–229.
[110] O. Chow, A. Barbul, Immunonutrition: role in wound healing and tissue regeneration, Adv. Wound Care (New Rochelle) 3 (2014) 46–53.
[111] N.J. Trengove, M.C. Stacey, D.F. McGechie, S. Mata, Qualitative bacteriology and leg ulcer healing, J. Wound Care 5 (1996) 277–280.
[112] R. Zhao, H. Liang, E. Clarke, C. Jackson, M. Xue, Inflammation in chronic wounds, Int. J. Mol. Sci. (2016) 17.
[113] P.G. Bowler, B.I. Duerden, D.G. Armstrong, Wound microbiology and associated approaches to wound management, Clin. Microbiol. Rev. 14 (2001) 244–269.
[114] P.G. Bowler, B.J. Davies, The microbiology of infected and noninfected leg ulcers, Int. J. Dermatol. 38 (1999) 573–578.
[115] S.L. Percival, S.M. McCarty, B. Lipsky, Biofilms and wounds: an overview of the evidence, Adv. Wound Care (New Rochelle) 4 (2015) 373–381.
[116] D.G. Greenhalgh, Models of wound healing, J. Burn Care Rehabil. 26 (2005) 293–305.
[117] C.D. Roberts, D.J. Leaper, O. Assadian, The role of topical antiseptic agents within antimicrobial stewardship strategies for prevention and treatment of surgical site and chronic open wound infection, Adv. Wound Care (New Rochelle) 6 (2017) 63–71.
[118] S.L. Percival, S. Finnegan, G. Donelli, C. Vuotto, S. Rimmer, B.A. Lipsky, Antiseptics for treating infected wounds: Efficacy on biofilms and effect of pH, Crit. Rev. Microbiol. 42 (2016) 293–309.
[119] X. Chen, H.J. Schluesener, Nanosilver: a nanoproduct in medical application, Toxicol. Lett. 176 (2008) 1–12.
[120] P.L. Phillips, Q. Yang, S. Davis, E.M. Sampson, J.I. Azeke, A. Hamad, G.S. Schultz, Antimicrobial dressing efficacy against mature Pseudomonas aeruginosa biofilm on porcine skin explants, Int. Wound J. 12 (2015) 469–483.
[121] R. Goldman, Growth factors and chronic wound healing: past, present, and future, Adv. Skin Wound Care 17 (2004) 24–35.
[122] N.J. Trengove, H. Bielefeldt-Ohmann, M.C. Stacey, Mitogenic activity and cytokine levels in non-healing and healing chronic leg ulcers, Wound Repair Regen. 8 (2000) 13–25.
[123] L.F. Brown, B. Berse, R.W. Jackman, K. Tognazzi, E.J. Manseau, D.R. Senger, H.F. Dvorak, Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in adenocarcinomas of the gastrointestinal tract, Cancer Res. 53 (1993) 4727–4735.
[124] C.J. Powers, S.W. McLeskey, A. Wellstein, Fibroblast growth factors, their receptors and signaling, Endocr. Relat. Cancer 7 (2000) 165–197.
[125] P. Lindahl, B.R. Johansson, P. Leveen, C. Betsholtz, Pericyte loss and microaneurysm formation in PDGF-B-deficient mice, Science 277 (1997) 242–245.
[126] C. Sundberg, M. Branting, B. Gerdin, K. Rubin, Tumor cell and connective tissue cell interactions in human colorectal adenocarcinoma. Transfer of platelet-derived growth factor-AB/BB to stromal cells, Am. J. Pathol. 151 (1997) 479–492.
[127] M. Sadagurski, S. Yakar, G. Weingarten, M. Holzenberger, C.J. Rhodes, D. Breitkreutz, D. Leroith, E. Wertheimer, Insulin-like growth factor 1 receptor signaling regulates skin development and inhibits skin keratinocyte differentiation, Mol. Cell. Biol. 26 (2006) 2675–2687.
[128] S. Rabhi-Sabile, D. Pidard, J. Lawler, P. Renesto, M. Chignard, C. Legrand, Proteolysis of thrombospondin during cathepsin-G-induced platelet aggregation: functional role of the 165-kDa carboxy-terminal fragment, FEBS Lett. 386 (1996) 82–86.
[129] H. Lin, B. Chen, W. Sun, W. Zhao, Y. Zhao, J. Dai, The effect of collagen-targeting platelet-derived growth factor on cellularization and vascularization of collagen scaffolds, Biomaterials 27 (2006) 5708–5714.
[130] S. Rhee, F. Grinnell, P21-activated kinase 1: convergence point in PDGF- and LPA-stimulated collagen matrix contraction by human fibroblasts, J. Cell Biol. 172 (2006) 423–432.
[131] J.S. Vande Berg, M.A. Rose, P.L. Haywood-Reid, R. Rudolph, W.G. Payne, M.C. Robson, Cultured pressure ulcer fibroblasts show replicative senescence with elevated production of plasmin, plasminogen activator inhibitor-1, and transforming growth factor-beta1, Wound Repair Regen. 13 (2005) 76–83.
[132] D.L. Steed, Clinical evaluation of recombinant human platelet-derived growth factor for the treatment of lower extremity diabetic ulcers. Diabetic Ulcer Study Group, J. Vasc. Surg. 21 (1995) 71–78 (discussion 79-81).
[133] D.L. Steed, D. Donohoe, M.W. Webster, L. Lindsley, Effect of extensive debridement and treatment on the healing of diabetic foot ulcers. Diabetic Ulcer Study Group, J. Am. Coll. Surg. 183 (1996) 61–64.
[134] L.L. Zhao, J.D. Davidson, S.C. Wee, S.I. Roth, T.A. Mustoe, Effect of hyperbaric oxygen and growth factors on rabbit ear ischemic ulcers, Arch. Surg. 129 (1994) 1043–1049.
[135] M.C. Robson, L.G. Phillips, A. Thomason, B.W. Altrock, P.C. Pence, J.P. Heggers, A.F. Johnston, T.P. McHugh, M.S. Anthony, L.E. Robson, et al., Recombinant human platelet-derived growth factor-BB for the treatment of chronic pressure ulcers, Ann. Plast. Surg. 29 (1992) 193–201.
[136] R.S. Rees, M.C. Robson, J.M. Smiell, B.H. Perry, Becaplermin gel in the treatment of pressure ulcers: a phase II randomized, double-blind, placebo-controlled study, Wound Repair Regen. 7 (1999) 141–147.
[137] S. Barrientos, H. Brem, O. Stojadinovic, M. Tomic-Canic, Clinical application of growth factors and cytokines in wound healing, Wound Repair Regen. 22 (2014) 569–578.
[138] T. Ohura, T. Nakajo, T. Moriguchi, H. Oka, M. Tachi, N. Ohura Jr., R. Nogami, S. Murayama, Clinical efficacy of basic fibroblast growth factor on pressure ulcers: case-control pairing study using a new evaluation method, Wound Repair Regen. 19 (2011) 542–551.
[139] W.G. Payne, D.E. Ochs, D.D. Meltzer, D.P. Hill, R.J. Mannari, L.E. Robson, M.C. Robson, Long-term outcome study of growth factor-treated pressure ulcers, Am. J. Surg. 181 (2001) 81–86.
[140] X. Fu, Z. Shen, Y. Chen, J. Xie, Z. Guo, M. Zhang, Z. Sheng, Randomised placebo-controlled trial of use of topical recombinant bovine basic fibroblast growth factor for second-degree burns, Lancet 352 (1998) 1661–1664.
[141] J.L. Richard, C. Parer-Richard, J.P. Daures, S. Clouet, D. Vannereau, J. Bringer, M. Rodier, C. Jacob, M. Comte-Bardonnet, Effect of topical basic fibroblast growth factor on the healing of chronic diabetic neuropathic ulcer of the foot. A pilot, randomized, double-blind, placebo-controlled study, Diabetes Care 18 (1995) 64–69.
[142] H. Uchi, A. Igarashi, K. Urabe, T. Koga, J. Nakayama, R. Kawamori, K. Tamaki, H. Hirakata, T. Ohura, M. Furue, Clinical efficacy of basic fibroblast growth factor (bFGF) for diabetic ulcer, Eur. J. Dermatol. 19 (2009) 461–468.
[143] C. Yao, P. Yao, H. Wu, Z. Zha, Acceleration of wound healing in traumatic ulcers by absorbable collagen sponge containing recombinant basic fibroblast growth factor, Biomed. Mater. (1) (2006) 33–37.
[144] R. Gomez-Villa, F. Aguilar-Rebolledo, A. Lozano-Platonoff, J.M. Teran-Soto, M.R. Fabian-Victoriano, N.S. Kresch-Tronik, X. Garrido-Espindola, A. Garcia-Solis, A. Bondani-Guasti, G. Bierzwinsky-Sneider, J. Contreras-Ruiz, Efficacy of intralesional recombinant human epidermal growth factor in diabetic foot ulcers in Mexican patients: a randomized double-blinded controlled trial, Wound Repair Regen. 22 (2014) 497–503.
[145] K.H. Park, S.H. Han, J.P. Hong, S.K. Han, D.H. Lee, B.S. Kim, J.H. Ahn, J.W. Lee, Topical epidermal growth factor spray for the treatment of chronic diabetic foot ulcers: a phase III multicenter, double-blind, randomized, placebo-controlled trial, Diabetes Res. Clin. Pract. 142 (2018) 335–344.
[146] J.R. Hanft, R.A. Pollak, A. Barbul, C. van Gils, P.S. Kwon, S.M. Gray, C.J. Lynch, C.P. Semba, T.J. Breen, I. Phase, Trial on the safety of topical rhVEGF on chronic neuropathic diabetic foot ulcers, J. Wound Care 30-32 (2008) 34–37.
[147] K.M. Lacci, A. Dardik, Platelet-rich plasma: support for its use in wound healing, Yale J. Biol. Med. 83 (2010) 1–9.
[148] A. Martinez-Martinez, F. Ruiz-Santiago, J. Garcia-Espinosa, Platelet-rich plasma: myth or reality? Australas. Radiol. 60 (2018) 465–475.
[149] J.P. McAleer, S. Sharma, E.M. Kaplan, G. Persich, Use of autologous platelet concentrate in a nonhealing lower extremity wound, Adv. Skin Wound Care 19 (2006) 354–363.
[150] S. Salemi, C. Rinaldi, F. Manna, G.F. Guarneri, P.C. Parodi, Reconstruction of lower leg skin ulcer with autologous adipose tissue and platelet-rich plasma, J. Plast. Reconstr. Aesthet. Surg. 61 (2008) 1565–1567.
[151] S.M. O'Connell, T. Impeduglia, K. Hessler, X.J. Wang, R.J. Carroll, H. Dardik, Autologous platelet-rich fibrin matrix as cell therapy in the healing of chronic lower-extremity ulcers, Wound Repair Regen. 16 (2008) 749–756.
[152] D.J. Leaper, G. Schultz, K. Carville, J. Fletcher, T. Swanson, R. Drake, Extending the TIME concept: what have we learned in the past 10 years? Int. Wound J. 9 (2012) 1–19.
[153] S. Dhivya, V.V. Padma, E. Santhini, Wound dressings - a review, Biomedicine (Taipei) 5 (2015) 22.
[154] T.S. Stashak, E. Farstvedt, A. Othic, Update on wound dressings: Indications and best use, Clin. Tech. Equine Practice 3 (2004) 148–163.
[155] K.K. Chereddy, C.H. Her, M. Comune, C. Moia, A. Lopes, P.E. Porporato, J. Vanacker, M.C. Lam, L. Steinstraesser, P. Sonveaux, H. Zhu, L.S. Ferreira, G. Vandermeulen, V. Preat, PLGA nanoparticles loaded with host defense peptide LL37 promote wound healing, J. Control. Release 194 (2014) 138–147.
[156] Y. Chu, D. Yu, P. Wang, J. Xu, D. Li, M. Ding, Nanotechnology promotes the full-thickness diabetic wound healing effect of recombinant human epidermal growth factor in diabetic rats, Wound Repair Regen. 18 (2010) 499–505.
[157] P.E. Porporato, V.L. Payen, C.J. De Saedeleer, V. Préat, J.-P. Thissen, O. Feron, P. Sonveaux, Lactate stimulates angiogenesis and accelerates the healing of superficial and ischemic wounds in mice, Angiogenesis 15 (2012) 581–592.
[158] C.-M. Lehr, J.A. Bouwstra, E.H. Schacht, H.E. Junginger, In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers, Int. J. Pharm. 78 (1992) 43–48.
[159] V. Grabovac, D. Guggi, A. Bernkop-Schnürch, Comparison of the mucoadhesive properties of various polymers, Adv. Drug Deliv. Rev. 57 (2005) 1713–1723.
[160] S. Liu, K. Fukushima, S. Venkataraman, J.L. Hedrick, Y.Y. Yang, Supramolecular nanofibers self-assembled from cationic small molecules derived from repurposed poly(ethylene teraphthalate) for antibiotic delivery, Nanomedicine 14 (2018) 165–172.
[161] K. Fukushima, S. Liu, H. Wu, A.C. Engler, D.J. Coady, H. Maune, J. Pitera, A. Nelson, N. Wiradharma, S. Venkataraman, Y. Huang, W. Fan, J.Y. Ying, Y.Y. Yang, J.L. Hedrick, Supramolecular high-aspect ratio assemblies with strong antifungal activity, Nat. Commun. 4 (2013) 2861.
[162] S.Q. Liu, S. Venkataraman, Z.Y. Ong, J.M. Chan, C. Yang, J.L. Hedrick, Y.Y. Yang, Overcoming multidrug resistance in microbials using nanostructures self-assembled from cationic bent-core oligomers, Small 10 (2014) 4130–4135.
[163] T.K. Georgiou, M. Vamvakaki, C.S. Patrickios, E.N. Yamasaki, L.A. Phylactou, Nanoscopic cationic methacrylate star homopolymers: synthesis by group transfer polymerization, characterization and evaluation as transfection reagents, Biomacromolecules 5 (2004) 2221–2229.

[164] S.-A. Cryan, A. Holohan, R. Donohue, R. Darcy, C.M. O'Driscoll, Cell transfection with polycationic cyclodextrin vectors, Eur. J. Pharm. Sci. 21 (2004) 625–633.
[165] M. Fumakia, E.A. Ho, Nanoparticles encapsulated with LL37 and serpin A1 promotes wound healing and synergistically enhances antibacterial activity, Mol. Pharm. 13 (2016) 2318–2331.
[166] G. Gainza, M. Pastor, J.J. Aguirre, S. Villullas, J.L. Pedraz, R.M. Hernandez, M. Igartua, A novel strategy for the treatment of chronic wounds based on the topical administration of rhEGF-loaded lipid nanoparticles: in vitro bioactivity and in vivo effectiveness in healing-impaired db/db mice, J. Control. Release 185 (2014) 51–61.
[167] G. Gainza, D.C. Bonafonte, B. Moreno, J.J. Aguirre, F.B. Gutierrez, S. Villullas, J.L. Pedraz, M. Igartua, R.M. Hernandez, The topical administration of rhEGF-loaded nanostructured lipid carriers (rhEGF-NLC) improves healing in a porcine full-thickness excisional wound model, J. Control. Release 197 (2015) 41–47.
[168] M.L. Manca, M. Manconi, A.M. Falchi, I. Castangia, D. Valenti, S. Lampis, A.M. Fadda, Close-packed vesicles for diclofenac skin delivery and fibroblast targeting, Colloids Surf. B Biointerfaces 111 (2013) 609–617.
[169] H.L. Xu, P.P. Chen, D.L. ZhuGe, Q.Y. Zhu, B.H. Jin, B.X. Shen, J. Xiao, Y.Z. Zhao, Liposomes with silk fibroin hydrogel core to stabilize bFGF and promote the wound healing of mice with deep second-degree scald, Adv. Healthc Mater. (2017) 6.
[170] M.L. Manca, I. Castangia, M. Zaru, A. Nacher, D. Valenti, X. Fernandez-Busquets, A.M. Fadda, M. Manconi, Development of curcumin loaded sodium hyaluronate immobilized vesicles (hyalurosomes) and their potential on skin inflammation and wound restoring, Biomaterials 71 (2015) 100–109.
[171] S. Das, G. Singh, M. Majid, M.B. Sherman, S. Mukhopadhyay, C.S. Wright, P.E. Martin, A.K. Dunn, A.B. Baker, Syndesome therapeutics for enhancing diabetic wound healing, Adv. Healthc Mater. 5 (2016) 2248–2260.
[172] S.M. Dizaj, F. Lotfipour, M. Barzegar-Jalali, M.H. Zarrintan, K. Adibkia, Antimicrobial activity of the metals and metal oxide nanoparticles, Mater. Sci. Eng. C Mater. Biol. Appl. 44 (2014) 278–284.
[173] M. Rai, A. Yadav, A. Gade, Silver nanoparticles as a new generation of antimicrobials, Biotechnol. Adv. 27 (2009) 76–83.
[174] S.S. Agasti, A. Chompoosor, C.C. You, P. Ghosh, C.K. Kim, V.M. Rotello, Photoregulated release of caged anticancer drugs from gold nanoparticles, J. Am. Chem. Soc. 131 (2009) 5728–5729.
[175] P. Ghosh, G. Han, M. De, C.K. Kim, V.M. Rotello, Gold nanoparticles in delivery applications, Adv. Drug Deliv. Rev. 60 (2008) 1307–1315.
[176] E. Katz, I. Willner, Integrated nanoparticle-biomolecule hybrid systems: synthesis, properties, and applications, Angew. Chem. Int. Ed. Engl. 43 (2004) 6042–6108.
[177] J.S. Choi, S.H. Choi, H.S. Yoo, Coaxial electrospun nanofibers for treatment of diabetic ulcers with binary release of multiple growth factors, J. Mater. Chem. 21 (2011) 5258–5267.
[178] M. Comune, A. Rai, K.K. Chereddy, S. Pinto, S. Aday, A.F. Ferreira, A. Zonari, J. Blersch, R. Cunha, R. Rodrigues, J. Lerma, P.N. Simoes, V. Preat, L. Ferreira, Antimicrobial peptide-gold nanoscale therapeutic formulation with high skin regenerative potential, J. Control. Release 262 (2017) 58–71.
[179] W.Y. Chen, H.Y. Chang, J.K. Lu, Y.C. Huang, S.G. Harroun, Y.T. Tseng, Y.J. Li, C.C. Huang, H.T. Chang, Self-assembly of antimicrobial peptides on gold nanodots: against multidrug-resistant bacteria and wound-healing application, Adv. Funct. Mater. 25 (2015) 7189–7199.
[180] Y. Zhao, Y. Tian, Y. Cui, W. Liu, W. Ma, X. Jiang, Small molecule-capped gold nanoparticles as potent antibacterial agents that target gram-negative bacteria, J. Am. Chem. Soc. 132 (2010) 12349–12356.
[181] Y. Zhao, Z. Chen, Y. Chen, J. Xu, J. Li, X. Jiang, Synergy of non-antibiotic drugs and pyrimidinethiol on gold nanoparticles against superbugs, J. Am. Chem. Soc. 135 (2013) 12940–12943.
[182] Y. Feng, W. Chen, Y. Jia, Y. Tian, Y. Zhao, F. Long, Y. Rui, X. Jiang, N-Heterocyclic molecule-capped gold nanoparticles as effective antibiotics against multi-drug resistant bacteria, Nanoscale 8 (2016) 13223–13227.
[183] X. Yang, J. Yang, L. Wang, B. Ran, Y. Jia, L. Zhang, G. Yang, H. Shao, X. Jiang, Pharmaceutical intermediate-modified gold nanoparticles: against multidrug-resistant bacteria and wound-healing application via an electrospun scaffold, ACS Nano 11 (2017) 5737–5745.
[184] L.T. Canham, Properties of Porous Silicon, Institution of Electrical Engineers, 1997.
[185] S.D. Alvarez, A.M. Derfus, M.P. Schwartz, S.N. Bhatia, M.J. Sailor, The compatibility of hepatocytes with chemically modified porous silicon with reference to in vitro biosensors, Biomaterials 30 (2009) 26–34.
[186] L.T. Canham, Bioactive silicon structure fabrication through nanoetching techniques, Adv. Mater. 7 (1995) 1033–1037.
[187] J. Chhablani, A. Nieto, H. Hou, E.C. Wu, W.R. Freeman, M.J. Sailor, L. Cheng, Oxidized porous silicon particles covalently grafted with daunorubicin as a sustained intraocular drug delivery system, Invest. Ophthalmol. Vis. Sci. 54 (2013) 1268–1279.
[188] J.R. Link, M.J. Sailor, Smart dust: self-assembling, self-orienting photonic crystals of porous Si, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 10607–10610.
[189] E.C. Wu, J.H. Park, J. Park, E. Segal, F. Cunin, M.J. Sailor, Oxidation-triggered release of fluorescent molecules or drugs from mesoporous Si microparticles, ACS Nano 2 (2008) 2401–2409.
[190] S.J. McInnes, C.T. Turner, S.A. Al-Bataineh, M.J.A. Leccardi, Y. Irani, K.A. Williams, A.J. Cowin, N.H. Voelcker, Surface engineering of porous silicon to optimise therapeutic antibody loading and release, J. Mater. Chem. B 3 (2015) 4123–4133.
[191] C.T. Turner, S.J. McInnes, E. Melville, A.J. Cowin, N.H. Voelcker, Delivery of flightless I neutralizing antibody from porous silicon nanoparticles improves wound healing in diabetic mice, Adv Healthc Mater (2017) 6.
[192] R. Gupta, A. Kumar, Bioactive materials for biomedical applications using sol–gel technology, Biomed. Mater. 3 (2008) 034005.
[193] A.J. Friedman, G. Han, M.S. Navati, M. Chacko, L. Gunther, A. Alfieri, J.M. Friedman, Sustained release nitric oxide releasing nanoparticles: characterization of a novel delivery platform based on nitrite containing hydrogel/glass composites, Nitric Oxide 19 (2008) 12–20.
[194] T. Keeling-Tucker, M. Rakic, C. Spong, J.D. Brennan, Controlling the material properties and biological activity of lipase within sol-gel derived bioglasses via organosilane and polymer doping, Chem. Mater. 12 (2000) 3695–3704.
[195] S. Shtelzer, S. Rappoport, D. Avnir, M. Ottolenghi, S. Braun, Properties of trypsin and of acid-phosphatase immobilized in sol-gel glass matrices, Biotechnol. Appl. Biol. 15 (1992) 227–235.
[196] M.A. Brook, Y. Chen, K. Guo, Z. Zhang, J.D. Brennan, Sugar-modified silanes: precursors for silica monoliths, J. Mater. Chem. 14 (2004) 1469–1479.
[197] L.R. Martinez, G. Han, M. Chacko, M.R. Mihu, M. Jacobson, P. Gialanella, A.J. Friedman, J.D. Nosanchuk, J.M. Friedman, Antimicrobial and healing efficacy of sustained release nitric oxide nanoparticles against *Staphylococcus aureus* skin infection, J. Invest Dermatol. 129 (2009) 2463–2469.
[198] D.A. Sanchez, D. Schairer, C. Tuckman-Vernon, J. Chouake, A. Kutner, J. Makdisi, J.M. Friedman, J.D. Nosanchuk, A.J. Friedman, Amphotericin B releasing nanoparticle topical treatment of Candida spp. in the setting of a burn wound, Nanomedicine 10 (2014) 269–277.
[199] A.E. Krausz, B.L. Adler, V. Cabral, M. Navati, J. Doerner, R.A. Charafeddine, D. Chandra, H. Liang, L. Gunther, A. Clendaniel, Curcumin-encapsulated nanoparticles as innovative antimicrobial and wound healing agent, Nanomedicine 11 (2015) 195–206.
[200] F.C. Fang, Antimicrobial actions of reactive oxygen species, MBio (2) (2011).
[201] F. Vatansever, W.C. de Melo, P. Avci, D. Vecchio, M. Sadasivam, A. Gupta, R. Chandran, M. Karimi, N.A. Parizotto, R. Yin, Antimicrobial strategies centered around reactive oxygen species–bactericidal antibiotics, photodynamic therapy, and beyond, FEMS Microbiol. Rev. 37 (2013) 955–989.
[202] P. Niethammer, C. Grabher, A.T. Look, T.J. Mitchison, A tissue-scale gradient of hydrogen peroxide mediates rapid wound detection in zebrafish, Nature 459 (2009) 996–999.
[203] M. Schafer, S. Werner, Oxidative stress in normal and impaired wound repair, Pharmacol. Res. 58 (2008) 165–171.
[204] S.M. Hirst, A. Karakoti, S. Singh, W. Self, R. Tyler, S. Seal, C.M. Reilly, Bio-distribution and in vivo antioxidant effects of cerium oxide nanoparticles in mice, Environ. Toxicol. 28 (2013) 107–118.
[205] C. Korsvik, S. Patil, S. Seal, W.T. Self, Superoxide dismutase mimetic properties exhibited by vacancy engineered ceria nanoparticles, Chem. Commun. (Camb) (2007) 1056–1058.
[206] G. Pulido-Reyes, I. Rodea-Palomares, S. Das, T.S. Sakthivel, F. Leganes, R. Rosal, S. Seal, F. Fernandez-Pinas, Untangling the biological effects of cerium oxide nanoparticles: the role of surface valence states, Sci. Rep. 5 (2015) 15613.
[207] E.G. Heckert, A.S. Karakoti, S. Seal, W.T. Self, The role of cerium redox state in the SOD mimetic activity of nanoceria, Biomaterials 29 (2008) 2705–2709.
[208] S. Chigurupati, M.R. Mughal, E. Okun, S. Das, A. Kumar, M. McCaffery, S. Seal, M.P. Mattson, Effects of cerium oxide nanoparticles on the growth of keratinocytes, fibroblasts and vascular endothelial cells in cutaneous wound healing, Biomaterials 34 (2013) 2194–2201.
[209] H. Wu, F. Li, S. Wang, J. Lu, J. Li, Y. Du, X. Sun, X. Chen, J. Gao, D. Ling, Ceria nanocrystals decorated mesoporous silica nanoparticle based ROS-scavenging tissue adhesive for highly efficient regenerative wound healing, Biomaterials 151 (2018) 66–77.
[210] B.C. Dickinson, C.J. Chang, Chemistry and biology of reactive oxygen species in signaling or stress responses, Nat. Chem. Biol. 7 (2011) 504–511.
[211] A.E. Loo, Y.T. Wong, R. Ho, M. Wasser, T. Du, W.T. Ng, B. Halliwell, Effects of hydrogen peroxide on wound healing in mice in relation to oxidative damage, PLoS One 7 (2012) e49215.
[212] L. Gao, K.M. Giglio, J.L. Nelson, H. Sondermann, A.J. Travis, Ferromagnetic nanoparticles with peroxidase-like activity enhance the cleavage of biological macromolecules for biofilm elimination, Nanoscale 6 (2014) 2588–2593.
[213] F. Natalio, R. Andre, A.F. Hartog, B. Stoll, K.P. Jochum, R. Wever, W. Tremel, Vanadium pentoxide nanoparticles mimic vanadium haloperoxidases and thwart biofilm formation, Nat. Nanotechnol. 7 (2012) 530–535.
[214] H. Sun, N. Gao, K. Dong, J. Ren, X. Qu, Graphene quantum dots-band-aids used for wound disinfection, ACS Nano 8 (2014) 6202–6210.
[215] Z. Wang, K. Dong, Z. Liu, Y. Zhang, Z. Chen, H. Sun, J. Ren, X. Qu, Activation of biologically relevant levels of reactive oxygen species by Au/g-C3N4 hybrid nanozyme for bacteria killing and wound disinfection, Biomaterials 113 (2017) 145–157.
[216] J. Tian, Q. Liu, A.M. Asiri, A.H. Qusti, A.O. Al-Youbi, X. Sun, Ultrathin graphitic carbon nitride nanosheets: a novel peroxidase mimetic, Fe doping-mediated catalytic performance enhancement and application to rapid, highly sensitive optical detection of glucose, Nanoscale 5 (2013) 11604–11609.
[217] Y. Lan, W. Li, Y. Jiao, R. Guo, Y. Zhang, W. Xue, Y. Zhang, Therapeutic efficacy of antibiotic-loaded gelatin microsphere/silk fibroin scaffolds in infected full-thickness burns, Acta Biomater. 10 (2014) 3167–3176.
[218] B. Olas, Hydrogen sulfide in signaling pathways, Clin. Chim. Acta 439 (2015) 212–218.
[219] W.-C. Lin, C.-C. Huang, S.-J. Lin, M.-J. Li, Y. Chang, Y.-J. Lin, W.-L. Wan, P.-C. Shih, H.-W. Sung, In situ depot comprising phase-change materials that can sustainably release a gasotransmitter H2S to treat diabetic wounds, Biomaterials 145 (2017) 1–8.
[220] D.G. Belair, M.J. Miller, S. Wang, S.R. Darjatmoko, B.Y. Binder, N. Sheibani, W.L. Murphy, Differential regulation of angiogenesis using degradable VEGF-binding microspheres, Biomaterials 93 (2016) 27–37.
[221] H. Chu, N.R. Johnson, N.S. Mason, Y. Wang, A [polycation:heparin] complex releases growth factors with enhanced bioactivity, J. Control. Release 150 (2011) 157–163.

[222] N.R. Johnson, T. Ambe, Y. Wang, Lysine-based polycation:heparin coacervate for controlled protein delivery, Acta Biomater. 10 (2014) 40–46.
[223] N.R. Johnson, Y. Wang, Coacervate delivery of HB-EGF accelerates healing of type 2 diabetic wounds, Wound Repair Regen. 23 (2015) 591–600.
[224] I. Capila, R.J. Linhardt, Heparin-protein interactions, Angew. Chem. Int. Ed. Engl. 41 (2002) 391–412.
[225] H. Chu, J. Gao, Y. Wang, Design, synthesis, and biocompatibility of an arginine-based polyester, Biotechnol. Prog. 28 (2012) 257–264.
[226] H.K. Awada, N.R. Johnson, Y. Wang, Dual delivery of vascular endothelial growth factor and hepatocyte growth factor coacervate displays strong angiogenic effects, Macromol. Biosci. 14 (2014) 679–686.
[227] J. Wu, J. Ye, J. Zhu, Z. Xiao, C. He, H. Shi, Y. Wang, C. Lin, H. Zhang, Y. Zhao, Heparin-based coacervate of FGF2 improves dermal regeneration by asserting a synergistic role with cell proliferation and endogenous facilitated VEGF for cutaneous wound healing, Biomacromolecules 17 (2016) 2168–2177.
[228] D.W. Long, N.R. Johnson, E.M. Jeffries, H. Hara, Y. Wang, Controlled delivery of platelet-derived proteins enhances porcine wound healing, J. Control. Release 253 (2017) 73–81.
[229] M.K. Strecker-McGraw, T.R. Jones, D.G. Baer, Soft tissue wounds and principles of healing, Emerg. Med. Clin. North Am. 25 (2007) 1–22.
[230] E.A. Kamoun, E.-R.S. Kenawy, X. Chen, A review on polymeric hydrogel membranes for wound dressing applications: PVA-based hydrogel dressings, J. Adv. Res. 8 (2017) 217–233.
[231] H.N. Kim, Y. Hong, M.S. Kim, S.M. Kim, K.Y. Suh, Effect of orientation and density of nanotopography in dermal wound healing, Biomaterials 33 (2012) 8782–8792.
[232] H. Xu, F. Lv, Y. Zhang, Z. Yi, Q. Ke, C. Wu, M. Liu, J. Chang, Hierarchically micro-patterned nanofibrous scaffolds with a nanosized bio-glass surface for accelerating wound healing, Nanoscale 7 (2015) 18446–18452.
[233] B.A. Blakeney, A. Tambralli, J.M. Anderson, A. Andukuri, D.J. Lim, D.R. Dean, H.W. Jun, Cell infiltration and growth in a low density, uncompressed three-dimensional electrospun nanofibrous scaffold, Biomaterials 32 (2011) 1583–1590.
[234] J.M. Coburn, M. Gibson, S. Monagle, Z. Patterson, J.H. Elisseeff, Bioinspired nanofibers support chondrogenesis for articular cartilage repair, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 10012–10017.
[235] H.S. Kim, H.S. Yoo, Surface-polymerized biomimetic nanofibrils for the cell-directed association of 3-D scaffolds, Chem. Commun. (Camb) 51 (2015) 306–309.
[236] E.A. Kamoun, X. Chen, M.S.M. Eldin, E.-R.S. Kenawy, Crosslinked poly (vinyl alcohol) hydrogels for wound dressing applications: a review of remarkably blended polymers, Arab. J. Chem. 8 (2015) 1–14.
[237] C. Kielbassa, L. Roza, B. Epe, Wavelength dependence of oxidative DNA damage induced by UV and visible light, Carcinogenesis 18 (1997) 811–816.
[238] X. Kong, S.K. Mohanty, J. Stephens, J.T. Heale, V. Gomez-Godinez, L.Z. Shi, J.S. Kim, K. Yokomori, M.W. Berns, Comparative analysis of different laser systems to study cellular responses to DNA damage in mammalian cells, Nucleic Acids Res. 37 (2009) e68.
[239] C.G. Williams, A.N. Malik, T.K. Kim, P.N. Manson, J.H. Elisseeff, Variable cytocompatibility of six cell lines with photoinitiators used for polymerizing hydrogels and cell encapsulation, Biomaterials 26 (2005) 1211–1218.
[240] S.J. Bryant, C.R. Nuttelman, K.S. Anseth, Cytocompatibility of UV and visible light photoinitiating systems on cultured NIH/3T3 fibroblasts in vitro, J. Biomater. Sci. Polym. Ed. 11 (2000) 439–457.
[241] R. Prasad, S.K. Katiyar, Crosstalk among UV-induced inflammatory mediators, DNA damage and epigenetic regulators facilitates suppression of the immune system, Photochem. Photobiol. 93 (2017) 930–936.
[242] N. Annabi, D. Rana, E.S. Sani, R. Portillo-Lara, J.L. Gifford, M.M. Fares, S.M. Mithieux, A.S. Weiss, Engineering a sprayable and elastic hydrogel adhesive with antimicrobial properties for wound healing, Biomaterials 139 (2017) 229–243.
[243] D.S. Yoon, Y. Lee, H.A. Ryu, Y. Jang, K.M. Lee, Y. Choi, W.J. Choi, M. Lee, K.M. Park, K.D. Park, J.W. Lee, Cell recruiting chemokine-loaded sprayable gelatin hydrogel dressings for diabetic wound healing, Acta Biomater. 38 (2016) 59–68.
[244] B.K. Ahn, S. Das, R. Linstadt, Y. Kaufman, N.R. Martinez-Rodriguez, R. Mirshafian, E. Kesselman, Y. Talmon, B.H. Lipshutz, J.N. Israelachvili, J.H. Waite, High-performance mussel-inspired adhesives of reduced complexity, Nat. Commun. 6 (2015) 8663.
[245] G.P. Maier, M.V. Rapp, J.H. Waite, J.N. Israelachvili, A. Butler, B.I.O.L.O.G.I.C.A.L. ADHESIVES, Adaptive synergy between catechol and lysine promotes wet adhesion by surface salt displacement, Science 349 (2015) 628–632.
[246] S. Seo, S. Das, P.J. Zalicki, R. Mirshafian, C.D. Eisenbach, J.N. Israelachvili, J.H. Waite, B.K. Ahn, Microphase behavior and enhanced wet-cohesion of synthetic copolyampholytes inspired by a mussel foot protein, J. Am. Chem. Soc. 137 (2015) 9214–9217.
[247] R. Nuccitelli, A role for endogenous electric fields in wound healing, Curr. Top. Dev. Biol. 58 (2003) 1–26.
[248] B. Reid, M. Zhao, The electrical response to injury: molecular mechanisms and wound healing, Adv. Wound Care (New Rochelle) 3 (2014) 184–201.
[249] J. Dubé, O. Rochette-Drouin, P. Lévesque, R. Gauvin, C.J. Roberge, F.A. Auger, D. Goulet, M. Bourdages, M. Plante, L. Germain, Restoration of the transepithelial potential within tissue-engineered human skin in vitro and during the wound healing process in vivo, Tissue Eng. A 16 (2010) 3055–3063.
[250] D.R. Trollinger, R.R. Isseroff, R. Nuccitelli, Calcium channel blockers inhibit galvanotaxis in human keratinocytes, J. Cell. Physiol. 193 (2002) 1–9.
[251] X. Li, J. Kolega, Effects of direct current electric fields on cell migration and actin filament distribution in bovine vascular endothelial cells, J. Vasc. Res. 39 (2002) 391–404.
[252] S.H. Bhang, W.S. Jang, J. Han, J.K. Yoon, W.G. La, E. Lee, Y.S. Kim, J.Y. Shin, T.J. Lee, H.K. Baik, Zinc oxide nanorod-based piezoelectric dermal patch for wound healing, Adv. Funct. Mater. (2017) 27.
[253] X. Zhao, H. Wu, B. Guo, R. Dong, Y. Qiu, P.X. Ma, Antibacterial anti-oxidant electroactive injectable hydrogel as self-healing wound dressing with hemostasis and adhesiveness for cutaneous wound healing, Biomaterials 122 (2017) 34–47.
[254] G.D. Mogoşanu, A.M. Grumezescu, Natural and synthetic polymers for wounds and burns dressing, Int. J. Pharm. 463 (2014) 127–136.
[255] M. Tamari, Y. Nishino, N. Yamamoto, M. Ueda, Acceleration of wound healing with stem cell-derived growth factors, Int. J. Oral Maxillofac. Implants 28 (2013) e369–e375.
[256] L. Jiang, Y. Dai, F. Cui, Y. Pan, H. Zhang, J. Xiao, F.U. Xiaobing, Expression of cytokines, growth factors and apoptosis-related signal molecules in chronic pressure ulcer wounds healing, Spinal Cord 52 (2014) 145–151.
[257] A.M. Eweida, M.K. Marei, Naturally occurring extracellular matrix scaffolds for dermal regeneration: do they really need cells? Biomed. Res. Int. (2015) (2015) 839694.
[258] L.E. Dickinson, S. Gerecht, Engineered biopolymeric scaffolds for chronic wound healing, Front. Physiol. 7 (2016) 341.
[259] P.M. Crapo, T.W. Gilbert, S.F. Badylak, An overview of tissue and whole organ decellularization processes, Biomaterials 32 (2011) 3233–3243.
[260] A. Pourmoussa, D.J. Gardner, M.B. Johnson, A.K. Wong, An update and review of cell-based wound dressings and their integration into clinical practice, Ann. Transl. Med. (2016) 4.
[261] M. Edmonds, G. European, Australian Apligraf diabetic foot ulcer study, Apligraf in the treatment of neuropathic diabetic foot ulcers, Int J Low Extrem Wounds 8 (2009) 11–18.
[262] A. Veves, V. Falanga, D.G. Armstrong, M.L. Sabolinski, Graftskin, a human skin equivalent, is effective in the management of noninfected neuropathic diabetic foot ulcers, Diabetes Care 24 (2001) 290–295.
[263] J. Xiao, S. Chen, J. Yi, H.F. Zhang, G.A. Ameer, A cooperative copper metal–organic framework-hydrogel system improves wound healing in diabetes, Adv. Funct. Mater. (2017) 27.
[264] Y. Zhu, R. Hoshi, S. Chen, J. Yi, C. Duan, R.D. Galiano, H.F. Zhang, G.A. Ameer, Sustained release of stromal cell derived factor-1 from an antioxidant thermoresponsive hydrogel enhances dermal wound healing in diabetes, J. Control. Release 238 (2016) 114–122.
[265] C. Dealwis, E.J. Fernandez, D.A. Thompson, R.J. Simon, M.A. Siani, E. Lolis, Crystal structure of chemically synthesized [N33A] stromal cell-derived factor 1alpha, a potent ligand for the HIV-1 "fusin" coreceptor, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 6941–6946.
[266] R. Sadir, F. Baleux, A. Grosdidier, A. Imberty, H. Lortat-Jacob, Characterization of the stromal cell-derived factor-1alpha-heparin complex, J. Biol. Chem. 276 (2001) 8288–8296.
[267] M. Neidrauer, U.K. Ercan, A. Bhattacharyya, J. Samuels, J. Sedlak, R. Trikha, K.A. Barbee, M.S. Weingarten, S.G. Joshi, Antimicrobial efficacy and wound-healing property of a topical ointment containing nitric-oxide-loaded zeolites, J. Med. Microbiol. 63 (2014) 203–209.
[268] Y. Kang, J. Kim, Y.M. Lee, S. Im, H. Park, W.J. Kim, Nitric oxide-releasing polymer incorporated ointment for cutaneous wound healing, J. Control. Release 220 (2015) 624–630.
[269] D.J. Gilmartin, A. Soon, C. Thrasivoulou, A.R. Phillips, S.N. Jayasinghe, D.L. Becker, Sustained release of Cx43 antisense oligodeoxynucleotides from coated collagen scaffolds promotes wound healing, Adv. Healthc Mater 5 (2016) 1786–1799.
[270] K. Ito, A. Saito, T. Fujie, K. Nishiwaki, H. Miyazaki, M. Kinoshita, D. Saitoh, S. Ohtsubo, S. Takeoka, Sustainable antimicrobial effect of silver sulfadiazine-loaded nanosheets on infection in a mouse model of partial-thickness burn injury, Acta Biomater. 24 (2015) 87–95.
[271] E.J. Brisbois, J. Bayliss, J. Wu, T.C. Major, C. Xi, S.C. Wang, R.H. Bartlett, H. Handa, M.E. Meyerhoff, Optimized polymeric film-based nitric oxide delivery inhibits bacterial growth in a mouse burn wound model, Acta Biomater. 10 (2014) 4136–4142.
[272] H.J. Lai, C.H. Kuan, H.C. Wu, J.C. Tsai, T.M. Chen, D.J. Hsieh, T.W. Wang, Tailored design of electrospun composite nanofibers with staged release of multiple angiogenic growth factors for chronic wound healing, Acta Biomater. 10 (2014) 4156–4166.
[273] D. Bouïs, Y. Kusumanto, C. Meijer, N.H. Mulder, G.A. Hospers, A review on pro-and anti-angiogenic factors as targets of clinical intervention, Pharmacol. Res. 53 (2006) 89–103.
[274] S. Bennett, G. Griffiths, A. Schor, G. Leese, S. Schor, Growth factors in the treatment of diabetic foot ulcers, Br. J. Surg. 90 (2003) 133–146.
[275] C. Fischbach, D.J. Mooney, Polymeric systems for bioinspired delivery of angiogenic molecules, Polymers Regener. Med. (2006) 191–221.
[276] S.A. Castleberry, B.D. Almquist, W. Li, T. Reis, J. Chow, S. Mayner, P.T. Hammond, Self-assembled wound dressings silence MMP-9 and improve diabetic wound healing in vivo, Adv. Mater. 28 (2016) 1809–1817.
[277] K. Uhlig, N. Madaboosi, S. Schmidt, M.S. Jäger, J. Rose, C. Duschl, D.V. Volodkin, 3d localization and diffusion of proteins in polyelectrolyte multilayers, Soft Matter 8 (2012) 11786–11789.
[278] C. Vogt, V. Ball, J. Mutterer, P. Schaaf, J.C. Voegel, B. Senger, P. Lavalle, Mobility of proteins in highly hydrated polyelectrolyte multilayer films, J. Phys. Chem. B 116 (2012) 5269–5278.
[279] B.D. Almquist, S.A. Castleberry, J.B. Sun, A.Y. Lu, P.T. Hammond, Combination growth factor therapy via electrostatically assembled wound dressings improves diabetic ulcer healing in vivo, Adv. Healthc Mater 4 (2015) 2090–2099.

[280] C.E. Nelson, A.J. Kim, E.J. Adolph, M.K. Gupta, F. Yu, K.M. Hocking, J.M. Davidson, S.A. Guelcher, C.L. Duvall, Tunable delivery of siRNA from a biodegradable scaffold to promote angiogenesis in vivo, Adv. Mater. 26 (2014) 607–614, 506.
[281] J.R. Martin, C.E. Nelson, M.K. Gupta, F. Yu, S.M. Sarett, K.M. Hocking, A.C. Pollins, L.B. Nanney, J.M. Davidson, S.A. Guelcher, Local delivery of PHD2 siRNA from ROS-degradable scaffolds to promote diabetic wound healing, Adv. Healthcare Mater. 5 (2016) 2751–2757.
[282] J.R. Martin, M.K. Gupta, J.M. Page, F. Yu, J.M. Davidson, S.A. Guelcher, C.L. Duvall, A porous tissue engineering scaffold selectively degraded by cell-generated reactive oxygen species, Biomaterials 35 (2014) 3766–3776.
[283] M.S. Shim, Y. Xia, A reactive oxygen species (ROS)-responsive polymer for safe, efficient, and targeted gene delivery in cancer cells, Angew. Chem. Int. Ed. Engl. 52 (2013) 6926–6929.
[284] U. Freudenberg, A. Zieris, K. Chwalek, M.V. Tsurkan, M.F. Maitz, P. Atallah, K.R. Levental, S.A. Eming, C. Werner, Heparin desulfation modulates VEGF release and angiogenesis in diabetic wounds, J. Control. Release 220 (2015) 79–88.
[285] F. Yergoz, N. Hastar, C.E. Cimenci, A.D. Ozkan, T. Tekinay, M.O. Guler, A.B. Tekinay, Heparin mimetic peptide nanofiber gel promotes regeneration of full thickness burn injury, Biomaterials 134 (2017) 117–127.
[286] Q. Li, Y. Niu, H. Diao, L. Wang, X. Chen, Y. Wang, L. Dong, C. Wang, In situ sequestration of endogenous PDGF-BB with an ECM-mimetic sponge for accelerated wound healing, Biomaterials 148 (2017) 54–68.
[287] V. Izzo, M. Meloni, E. Vainieri, L. Giurato, V. Ruotolo, L. Uccioli, High matrix metalloproteinase levels are associated with dermal graft failure in diabetic foot ulcers, Int. J. Low Extr. Wound 13 (2014) 191–196.
[288] C. McArdle, K.M. Lagan, D.A. McDowell, The pH of wound fluid in diabetic foot ulcers-the way forward in detecting clinical infection? Curr. Diabetes Rev. 10 (2014) 177–181.
[289] L. Yazdanpanah, M. Nasiri, S. Adarvishi, Literature review on the management of diabetic foot ulcer, World J. Diabetes 6 (2015) 37–53.
[290] L. Zhao, L. Niu, H. Liang, H. Tan, C. Liu, F. Zhu, pH and glucose dual-responsive injectable hydrogels with insulin and fibroblasts as bioactive dressings for diabetic wound healing, ACS Appl. Mater. Interfaces 9 (2017) 37563–37574.
[291] H.S. Kim, H.S. Yoo, MMPs-responsive release of DNA from electrospun nanofibrous matrix for local gene therapy: in vitro and in vivo evaluation, J. Control. Release 145 (2010) 264–271.
[292] H.S. Kim, H.S. Yoo, In vitro and in vivo epidermal growth factor gene therapy for diabetic ulcers with electrospun fibrous meshes, Acta Biomater. 9 (2013) 7371–7380.
[293] H.S. Kim, H.S. Yoo, Matrix metalloproteinase-inspired suicidal treatments of diabetic ulcers with siRNA-decorated nanofibrous meshes, Gene Ther. 20 (2013) 378–385.
[294] H.S. Kim, Y.J. Son, H.S. Yoo, Clustering siRNA conjugates for MMP-responsive therapeutics in chronic wounds of diabetic animals, Nanoscale 8 (2016) 13236–13244.
[295] A. Watarai, L. Schirmer, S. Thönes, U. Freudenberg, C. Werner, J.C. Simon, U. Anderegg, TGFβ functionalized starPEG-heparin hydrogels modulate human dermal fibroblast growth and differentiation, Acta Biomater. 25 (2015) 65–75.
[296] J. Gao, W. Zheng, J. Zhang, D. Guan, Z. Yang, D. Kong, Q. Zhao, Enzyme-controllable delivery of nitric oxide from a molecular hydrogel, Chem. Commun. (Camb) 49 (2013) 9173–9175.
[297] X. Zhou, H. Wang, J. Zhang, X. Li, Y. Wu, Y. Wei, S. Ji, D. Kong, Q. Zhao, Functional poly (ε-caprolactone)/chitosan dressings with nitric oxide-releasing property improve wound healing, Acta Biomater. 54 (2017) 128–137.
[298] X. Wang, F. Lv, T. Li, Y. Han, Z. Yi, M. Liu, J. Chang, C. Wu, Electrospun micropatterned nanocomposites incorporated with Cu2S nanoflowers for skin tumor therapy and wound healing, ACS Nano 11 (2017) 11337–11349.
[299] W.P. Li, C.H. Su, S.J. Wang, F.J. Tsai, C.T. Chang, M.C. Liao, C.C. Yu, T.T. Vi Tran, C.N. Lee, W.T. Chiu, T.W. Wong, C.S. Yeh, CO2 delivery to accelerate incisional wound healing following single irradiation of near-infrared lamp on the coordinated colloids, ACS Nano 11 (2017) 5826–5835.
[300] C. Mao, Y. Xiang, X. Liu, Z. Cui, X. Yang, K.W.K. Yeung, H. Pan, X. Wang, P.K. Chu, S. Wu, Photo-inspired antibacterial activity and wound healing acceleration by hydrogel embedded with Ag/Ag@AgCl/ZnO Nanostructures, ACS Nano 11 (2017) 9010–9021.
[301] P. Mostafalu, G. Kiaee, G. Giatsidis, A. Khalilpour, M. Nabavinia, M.R. Dokmeci, S. Sonkusale, D.P. Orgill, A. Tamayol, A. Khademhosseini, A textile dressing for temporal and dosage controlled drug delivery, Adv. Funct. Mater. (2017) 27.
[302] M. Sasaki, R. Abe, Y. Fujita, S. Ando, D. Inokuma, H. Shimizu, Mesenchymal stem cells are recruited into wounded skin and contribute to wound repair by transdifferentiation into multiple skin cell type, J. Immunol. 180 (2008) 2581–2587.
[303] L.H. Peng, Z.Y. Mao, X.T. Qi, X. Chen, N. Li, Y. Tabata, J.Q. Gao, Transplantation of bone-marrow-derived mesenchymal and epidermal stem cells contribute to wound healing with different regenerative features, Cell Tissue Res. 352 (2013) 573–583.
[304] F. Jimenez, C. Garde, E. Poblet, B. Jimeno, J. Ortiz, M.L. Martinez, A. Gutierrez-Rivera, V. Perez-Lopez, U. Etxaniz, C. Naveda, J.L. Higuera, N. Egues, E. Escario, A. Izeta, A pilot clinical study of hair grafting in chronic leg ulcers, Wound Repair Regen. 20 (2012) 806–814.
[305] H.A. Navsaria, N.O. Ojeh, N. Moiemen, M.A. Griffiths, J.D. Frame, Reepithelialization of a full-thickness burn from stem cells of hair follicles micrografted into a tissue-engineered dermal template (Integra), Plast. Reconstr. Surg. 113 (2004) 978–981.
[306] S.H. Lee, S.Y. Jin, J.S. Song, K.K. Seo, K.H. Cho, Paracrine effects of adipose-derived stem cells on keratinocytes and dermal fibroblasts, Ann. Dermatol. 24 (2012) 136–143.
[307] S. Schlosser, C. Dennler, R. Schweizer, D. Eberli, J.V. Stein, V. Enzmann, P. Giovanoli, D. Erni, J.A. Plock, Paracrine effects of mesenchymal stem cells enhance vascular regeneration in ischemic murine skin, Microvasc. Res. 83 (2012) 267–275.
[308] W.M. Jackson, L.J. Nesti, R.S. Tuan, Mesenchymal stem cell therapy for attenuation of scar formation during wound healing, Stem Cell Res Ther 3 (2012) 20.
[309] L. Li, Y. Zhang, Y. Li, B. Yu, Y. Xu, S. Zhao, Z. Guan, Mesenchymal stem cell transplantation attenuates cardiac fibrosis associated with isoproterenol-induced global heart failure, Transpl. Int. 21 (2008) 1181–1189.
[310] L. Chen, E.E. Tredget, P.Y. Wu, Y. Wu, Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing, PLoS One 3 (2008) e1886.
[311] L. Li, S. Zhang, Y. Zhang, B. Yu, Y. Xu, Z. Guan, Paracrine action mediate the antifibrotic effect of transplanted mesenchymal stem cells in a rat model of global heart failure, Mol. Biol. Rep. 36 (2009) 725–731.
[312] S. Smith, W. Neaves, S. Teitelbaum, Adult versus embryonic stem cells: treatments, Science 316 (2007) 1422–1423.
[313] K. Takahashi, K. Tanabe, M. Ohnuki, M. Narita, T. Ichisaka, K. Tomoda, S. Yamanaka, Induction of pluripotent stem cells from adult human fibroblasts by defined factors, Cell 131 (2007) 861–872.
[314] S. Ohta, Y. Imaizumi, Y. Okada, W. Akamatsu, R. Kuwahara, M. Ohyama, M. Amagai, Y. Matsuzaki, S. Yamanaka, H. Okano, Y. Kawakami, Generation of human melanocytes from induced pluripotent stem cells, PLoS One 6 (2011) e16182.
[315] Y. Zhao, X. Yin, H. Qin, F. Zhu, H. Liu, W. Yang, Q. Zhang, C. Xiang, P. Hou, Z. Song, Y. Liu, J. Yong, P. Zhang, J. Cai, M. Liu, H. Li, Y. Li, X. Qu, K. Cui, W. Zhang, T. Xiang, Y. Wu, Y. Zhao, C. Liu, C. Yu, K. Yuan, J. Lou, M. Ding, H. Deng, Two supporting factors greatly improve the efficiency of human iPSC generation, Cell Stem Cell 3 (2008) 475–479.
[316] M. Nakagawa, M. Koyanagi, K. Tanabe, K. Takahashi, T. Ichisaka, T. Aoi, K. Okita, Y. Mochiduki, N. Takizawa, S. Yamanaka, Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts, Nat. Biotechnol. 26 (2008) 101.
[317] T. Aasen, A. Raya, M.J. Barrero, E. Garreta, A. Consiglio, F. Gonzalez, R. Vassena, J. Bilic, V. Pekarik, G. Tiscornia, M. Edel, S. Boue, J.C. Izpisua Belmonte, Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes, Nat. Biotechnol. 26 (2008) 1276–1284.
[318] X. Kang, Q. Yu, Y. Huang, B. Song, Y. Chen, X. Gao, W. He, X. Sun, Y. Fan, Effects of integrating and non-integrating reprogramming methods on copy number variation and genomic stability of human induced pluripotent stem cells, PLoS One 10 (2015), e0131128.
[319] I. Garitaonandia, H. Amir, F.S. Boscolo, G.K. Wambua, H.L. Schultheisz, K. Sabatini, R. Morey, S. Waltz, Y.C. Wang, H. Tran, T.R. Leonardo, K. Nazor, I. Slavin, C. Lynch, Y. Li, R. Coleman, I. Gallego Romero, G. Altun, D. Reynolds, S. Dalton, M. Parast, J.F. Loring, L.C. Laurent, Increased risk of genetic and epigenetic instability in human embryonic stem cells associated with specific culture conditions, PLoS One 10 (2015), e0118307.
[320] J. Na, D. Baker, J. Zhang, P.W. Andrews, I. Barbaric, Aneuploidy in pluripotent stem cells and implications for cancerous transformation, Protein Cell 5 (2014) 569–579.
[321] B.Y. Hu, J.P. Weick, J. Yu, L.X. Ma, X.Q. Zhang, J.A. Thomson, S.C. Zhang, Neural differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 4335–4340.
[322] Q. Lian, Y. Zhang, J. Zhang, H.K. Zhang, X. Wu, Y. Zhang, F.F. Lam, S. Kang, J.C. Xia, W.H. Lai, K.W. Au, Y.Y. Chow, C.W. Siu, C.N. Lee, H.F. Tse, Functional mesenchymal stem cells derived from human induced pluripotent stem cells attenuate limb ischemia in mice, Circulation 121 (2010) 1113–1123.
[323] G. Bilousova, D.H. Jun, K.B. King, S. De Langhe, W.S. Chick, E.C. Torchia, K.S. Chow, D.J. Klemm, D.R. Roop, S.M. Majka, Osteoblasts derived from induced pluripotent stem cells form calcified structures in scaffolds both in vitro and in vivo, Stem Cells 29 (2011) 206–216.
[324] S. Buccini, K.H. Haider, R.P. Ahmed, S. Jiang, M. Ashraf, Cardiac progenitors derived from reprogrammed mesenchymal stem cells contribute to angiomyogenic repair of the infarcted heart, Basic Res. Cardiol. 107 (2012) 301.
[325] J. Zhang, J. Guan, X. Niu, G. Hu, S. Guo, Q. Li, Z. Xie, C. Zhang, Y. Wang, Exosomes released from human induced pluripotent stem cells-derived MSCs facilitate cutaneous wound healing by promoting collagen synthesis and angiogenesis, J. Transl. Med. 13 (2015) 49.
[326] S.H. Orkin, K. Hochedlinger, Chromatin connections to pluripotency and cellular reprogramming, Cell 145 (2011) 835–850.
[327] O. Bar-Nur, C. Verheul, A.G. Sommer, J. Brumbaugh, B.A. Schwarz, I. Lipchina, A.J. Huebner, G. Mostoslavsky, K. Hochedlinger, Lineage conversion induced by pluripotency factors involves transient passage through an iPSC stage, Nat. Biotechnol. 33 (2015) 761–768.
[328] S. Kelaini, A. Cochrane, A. Margariti, Direct reprogramming of adult cells: avoiding the pluripotent state, Stem Cells Cloning 7 (2014) 19.
[329] A. Margariti, B. Winkler, E. Karamariti, A. Zampetaki, T.-n. Tsai, D. Baban, J. Ragoussis, Y. Huang, J.-D.J. Han, L. Zeng, Direct reprogramming of fibroblasts into endothelial cells capable of angiogenesis and reendothelialization in tissue-engineered vessels, Proc. Natl. Acad. Sci. 109 (2012) 13793–13798.
[330] M. Abad, L. Mosteiro, C. Pantoja, M. Canamero, T. Rayon, I. Ors, O. Grana, D. Megias, O. Dominguez, D. Martinez, M. Manzanares, S. Ortega, M. Serrano, Reprogramming in vivo produces teratomas and iPS cells with totipotency features, Nature 502 (2013) 340–345.
[331] D. Iacovides, G. Rizki, G. Lapathitis, K. Strati, Direct conversion of mouse embryonic fibroblasts into functional keratinocytes through transient expression of pluripotency-related genes, Stem Cell Res Ther 7 (2016).

[332] Y. Chen, D.S. Mistry, G.L. Sen, Highly rapid and efficient conversion of human fibroblasts to keratinocyte-like cells, J. Invest Dermatol. 134 (2014) 335–344.
[333] R.F. Yang, Y. Zheng, L. Li, S.J. Liu, M. Burrows, Z. Wei, A. Nace, M. Herlyn, R.T. Cui, W. Guo, G. Cotsarelis, X.W. Xu, Direct conversion of mouse and human fibroblasts to functional melanocytes by defined factors, Nat. Commun. (2014) 5.
[334] J.R. Dias, P.L. Granja, P.J. Bártolo, Advances in electrospun skin substitutes, Prog. Mater. Sci. 84 (2016) 314–334.
[335] G.D. DuRaine, W.E. Brown, J.C. Hu, K.A. Athanasiou, Emergence of scaffold-free approaches for tissue engineering musculoskeletal cartilages, Ann. Biomed. Eng. 43 (2015) 543–554.
[336] K.W. Ng, D.W. Hutmacher, Reduced contraction of skin equivalent engineered using cell sheets cultured in 3D matrices, Biomaterials 27 (2006) 4591–4598.
[337] K.A. Athanasiou, R. Eswaramoorthy, P. Hadidi, J.C. Hu, Self-organization and the self-assembling process in tissue engineering, Annu. Rev. Biomed. Eng. 15 (2013) 115–136.
[338] L.E. Fitzpatrick, T.C. McDevitt, Cell-derived matrices for tissue engineering and regenerative medicine applications, Biomater. Sci. 3 (2015) 12–24.
[339] A.F. LAPLANTE, L. GERMAIN, F.A. AUGER, V. MOULIN, Mechanisms of wound reepithelialization: hints from a tissue-engineered reconstructed skin to long-standing questions, FASEB J. 15 (2001) 2377–2389.
[340] I. Elloumi-Hannachi, M. Yamato, T. Okano, Cell sheet engineering: a unique nanotechnology for scaffold-free tissue reconstruction with clinical applications in regenerative medicine, J. Intern. Med. 267 (2010) 54–70.
[341] Y.-C. Lin, T. Grahovac, S.J. Oh, M. Ieraci, J.P. Rubin, K.G. Marra, Evaluation of a multilayer adipose-derived stem cell sheet in a full-thickness wound healing model, Acta Biomater. 9 (2013) 5243–5250.
[342] E.J. Yun, B. Yon, M.K. Joo, B. Jeong, Cell therapy for skin wound using fibroblast encapsulated poly(ethylene glycol)-poly(l-alanine) thermogel, Biomacromolecules 13 (2012) 1106–1111.
[343] T. Leangarun, P. Chumroenrattanakorn, D. Koolpirak, K. Pasuwat, Automation for fabrication of functional tissues by stacking cell sheet, International Conference on Electronics, Information, and Communications (ICEIC) 2016, pp. 1–4.
[344] S.W. Lane, D.A. Williams, F.M. Watt, Modulating the stem cell niche for tissue regeneration, Nat. Biotechnol. 32 (2014) 795.
[345] R.S. Kirsner, W. Vanscheidt, D.H. Keast, J.C. Lantis 2nd, C.R. Dove, S.M. Cazzell, M. Vartivarian, M. Augustin, W.A. Marston, N.D. McCoy Bs, D.D. Cargill Ph, T.D. Lee Mshs, J.E. Dickerson Jr., H.B. Slade Md, Phase 3 evaluation of HP802-247 in the treatment of chronic venous leg ulcers, Wound Repair Regen. 24 (2016) 894–903.
[346] R.S. Kirsner, W.A. Marston, R.J. Snyder, T.D. Lee, D.I. Cargill, H.B. Slade, Spray-applied cell therapy with human allogeneic fibroblasts and keratinocytes for the treatment of chronic venous leg ulcers: a phase 2, multicentre, double-blind, randomised, placebo-controlled trial, Lancet 380 (2012) 977–985.
[347] A.M. Wojtowicz, S. Oliveira, M.W. Carlson, A. Zawadzka, C.F. Rousseau, D. Baksh, The importance of both fibroblasts and keratinocytes in a bilayered living cellular construct used in wound healing, Wound Repair Regen. 22 (2014) 246–255.
[348] M. Silva, L. Daheron, H. Hurley, K. Bure, R. Barker, A.J. Carr, D. Williams, H.-W. Kim, A. French, P.J. Coffey, Generating iPSCs: translating cell reprogramming science into scalable and robust biomanufacturing strategies, Cell Stem Cell 16 (2015) 13–17.
[349] J. Yang, M. Yamato, T. Shimizu, H. Sekine, K. Ohashi, M. Kanzaki, T. Ohki, K. Nishida, T. Okano, Reconstruction of functional tissues with cell sheet engineering, Biomaterials 28 (2007) 5033–5043.
[350] H. Liu, S. Wang, Poly(N-isopropylacrylamide)-based thermo-responsive surfaces with controllable cell adhesion, SCIENCE CHINA Chem. 57 (2014) 552–557.
[351] T. Okano, N. Yamada, H. Sakai, Y. Sakurai, A novel recovery system for cultured cells using plasma-treated polystyrene dishes grafted with poly (N-isopropylacrylamide), J. Biomed. Mater. Res. A 27 (1993) 1243–1251.
[352] J.Y. Sim, J. Moeller, K.C. Hart, D. Ramallo, V. Vogel, A.R. Dunn, W.J. Nelson, B.L. Pruitt, Spatial distribution of cell–cell and cell–ECM adhesions regulates force balance while maintaining E-cadherin molecular tension in cell pairs, Mol. Biol. Cell 26 (2015) 2456–2465.
[353] J. Enomoto, N. Mochizuki, K. Ebisawa, T. Osaki, T. Kageyama, D. Myasnikova, T. Nittami, J. Fukuda, Engineering thick cell sheets by electrochemical desorption of oligopeptides on membrane substrates, Regenerative Therapy 3 (2016) 24–31.
[354] J. Fukuda, A. Khademhosseini, J. Yeh, G. Eng, J. Cheng, O.C. Farokhzad, R. Langer, Micropatterned cell co-cultures using layer-by-layer deposition of extracellular matrix components, Biomaterials 27 (2006) 1479–1486.
[355] S. Kainuma, S. Miyagawa, S. Fukushima, J. Pearson, Y.C. Chen, A. Saito, A. Harada, M. Shiozaki, H. Iseoka, T. Watabe, H. Watabe, G. Horitsugi, M. Ishibashi, H. Ikeda, H. Tsuchimochi, T. Sonobe, Y. Fujii, H. Naito, K. Umetani, T. Shimizu, T. Okano, E. Kobayashi, T. Daimon, T. Ueno, T. Kuratani, K. Toda, N. Takakura, J. Hatazawa, M. Shirai, Y. Sawa, Cell-sheet therapy with omentopexy promotes arteriogenesis and improves coronary circulation physiology in failing heart, Mol. Ther., 23 374–386.
[356] D. Jiang, Y. Qi, N.G. Walker, A. Sindrilaru, A. Hainzl, M. Wlaschek, S. MacNeil, K. Scharffetter-Kochanek, The effect of adipose tissue derived MSCs delivered by a chemically defined carrier on full-thickness cutaneous wound healing, Biomaterials 34 (2013) 2501–2515.
[357] K.W. Ng, W. Tham, T.C. Lim, D. Werner Hutmacher, Assimilating cell sheets and hybrid scaffolds for dermal tissue engineering, J. Biomed. Mater. Res. A 75A (2005) 425–438.
[358] F. Anjum, N.A. Agabalyan, H.D. Sparks, N.L. Rosin, M.S. Kallos, J. Biernaskie, Biocomposite nanofiber matrices to support ECM remodeling by human dermal progenitors and enhanced wound closure, Sci. Rep. 7 (2017) 10291.
[359] S. Huang, X. Fu, Naturally derived materials-based cell and drug delivery systems in skin regeneration, J. Control. Release 142 (2010) 149–159.
[360] Q. Liu, Y. Huang, Y. Lan, Q. Zuo, C. Li, Y. Zhang, R. Guo, W. Xue, Acceleration of skin regeneration in full-thickness burns by incorporation of bFGF-loaded alginate microspheres into a CMCS–PVA hydrogel, J. Tissue Eng. Regen. Med. 11 (2017) 1562–1573.
[361] K.-H. Jeong, D. Park, Y.-C. Lee, Polymer-based hydrogel scaffolds for skin tissue engineering applications: a mini-review, J. Polym. Res. 24 (2017) 112.
[362] M.N. Nicholas, M.G. Jeschke, S. Amini-Nik, Cellularized bilayer pullulan-gelatin hydrogel for skin regeneration, Tissue Eng. A 22 (2016) 754–764.
[363] S. Chen, J. Shi, M. Zhang, Y. Chen, X. Wang, L. Zhang, Z. Tian, Y. Yan, Q. Li, W. Zhong, Mesenchymal stem cell-laden anti-inflammatory hydrogel enhances diabetic wound healing, Sci. Rep. 5 (2015) 18104.
[364] K.C. Rustad, V.W. Wong, M. Sorkin, J.P. Glotzbach, M.R. Major, J. Rajadas, M.T. Longaker, G.C. Gurtner, Enhancement of mesenchymal stem cell angiogenic capacity and stemness by a biomimetic hydrogel scaffold, Biomaterials 33 (2012) 80–90.
[365] K. Xu, D.A. Cantu, Y. Fu, J. Kim, X. Zheng, P. Hematti, W.J. Kao, Thiol-ene Michael-type formation of gelatin/poly (ethylene glycol) biomatrices for three-dimensional mesenchymal stromal/stem cell administration to cutaneous wounds, Acta Biomater. 9 (2013) 8802–8814.
[366] J.A. Burdick, R.L. Mauck, S. Gerecht, To serve and protect: hydrogels to improve stem cell-based therapies, Cell Stem Cell 18 (2016) 13–15.
[367] M.P. Lutolf, P.M. Gilbert, H.M. Blau, Designing materials to direct stem-cell fate, Nature 462 (2009) 433–441.
[368] S.S. Liow, Q. Dou, D. Kai, A.A. Karim, K. Zhang, F. Xu, X.J. Loh, Thermogels: in Situ Gelling Biomaterial, ACS Biomater. Sci. Eng. 2 (2016) 295–316.
[369] Y. Lee, J.W. Bae, J.W. Lee, W. Suh, K.D. Park, Enzyme-catalyzed in situ forming gelatin hydrogels as bioactive wound dressings: effects of fibroblast delivery on wound healing efficacy, J. Mater. Chem. B 2 (2014) 7712–7718.
[370] S. Chen, J. Shi, M. Zhang, Y. Chen, X. Wang, L. Zhang, Z. Tian, Y. Yan, Q. Li, W. Zhong, M. Xing, L. Zhang, L. Zhang, Mesenchymal stem cell-laden anti-inflammatory hydrogel enhances diabetic wound healing, Sci. Rep. 5 (2015) 18104.
[371] C.A. Brohem, L.B. Cardeal, M. Tiago, M.S. Soengas, S.B. Barros, S.S. Maria-Engler, Artificial skin in perspective: concepts and applications, Pigment Cell Melanoma Res. 24 (2011) 35–50.
[372] J. Dias, P. Granja, P. Bártolo, Advances in electrospun skin substitutes, Prog. Mater. Sci. 84 (2016) 314–334.
[373] J.-f. Pan, N.-h. Liu, H. Sun, F. Xu, Preparation and characterization of electrospun PLCL/Poloxamer nanofibers and dextran/gelatin hydrogels for skin tissue engineering, PLoS One 9 (2014) e112885.
[374] S.V. Murphy, A. Atala, 3D bioprinting of tissues and organs, Nat. Biotechnol. 32 (2014) 773.
[375] J. Li, M. Chen, X. Fan, H. Zhou, Recent advances in bioprinting techniques: approaches, applications and future prospects, J. Transl. Med. 14 (2016) 271.
[376] L. Koch, A. Deiwick, S. Schlie, S. Michael, M. Gruene, V. Coger, D. Zychlinski, A. Schambach, K. Reimers, P.M. Vogt, B. Chichkov, Skin tissue generation by laser cell printing, Biotechnol. Bioeng. 109 (2012) 1855–1863.
[377] X. Cui, T. Boland, D.D. D'Lima, M.K. Lotz, Thermal Inkjet Printing in Tissue Engineering and Regenerative Medicine, Recent Patents on Drug Delivery & Formulation, Vol. 6, 2012 149–155.
[378] N. Wei Long, Q. Jovina Tan Zhi, Y. Wai Yee, N. May Win, Proof-of-concept: 3D bioprinting of pigmented human skin constructs, Biofabrication 10 (2018), 025005.
[379] H.B. Zhang, S. Yong, X. Tianlong, Y. Ruixue, Y. Shimo, J. Wei, W. Zhang, Tyrosinase doped bioink for 3D bioprinting of living skin constructs, Biomed. Mater. 13 (2018) 035008.
[380] M.A. Darabi, A. Khosrozadeh, R. Mbeleck, Y. Liu, Q. Chang, J. Jiang, J. Cai, Q. Wang, G. Luo, M. Xing, Skin-inspired multifunctional autonomic-intrinsic conductive self-healing hydrogels with pressure sensitivity, stretchability, and 3D printability, Adv. Mater. 29 (2017) 1700533.
[381] J.-S.L. Byoung Soo Kim, Ge Gao, Dong-Woo Cho, Direct 3D cell-printing of human skin with functional transwell system, Biofabrication (2017) 9.
[382] F. Meier, M. Nesbit, M.-Y. Hsu, B. Martin, P. Van Belle, D.E. Elder, G. Schaumburg-Lever, C. Garbe, T.M. Walz, P. Donatien, T.M. Crombleholme, M. Herlyn, Human melanoma progression in skin reconstructs, Am.J. Pathol. 156 (2000) 193–200.
[383] Y.-J. Choi, H.-G. Yi, S.-W. Kim, D.-W. Cho, 3D cell printed tissue analogues: a new platform for theranostics, Theranostics 7 (2017) 3118–3137.
[384] S. Huang, B. Yao, J. Xie, X. Fu, 3D bioprinted extracellular matrix mimics facilitate directed differentiation of epithelial progenitors for sweat gland regeneration, Acta Biomater. 32 (2016) 170–177.
[385] Y. Ikada, Challenges in tissue engineering, J. R. Soc. Interface (3) (2006) 589–601.
[386] M.W. Laschke, M.D. Menger, Prevascularization in tissue engineering: current concepts and future directions, Biotechnol. Adv. 34 (2016) 112–121.
[387] M. Costa, R.P. Pirraco, M.T. Cerqueira, R.L. Reis, A.P. Marques, Growth factor-free pre-vascularization of cell sheets for tissue engineering, in: K. Turksen (Ed.), Stem Cell Heterogeneity: Methods and Protocols, Springer New York, New York, NY 2016, pp. 219–226.
[388] J. Liu, C. Liu, B. Sun, C. Shi, C. Qiao, X. Ke, S. Liu, X. Liu, H. Sun, Differentiation of rabbit bone mesenchymal stem cells into endothelial cells in vitro and promotion of defective bone regeneration in vivo, Cell Biochem. Biophys. 68 (2014) 479–487.
[389] S.M. Watt, F. Gullo, M. van der Garde, D. Markeson, R. Camicia, C.P. Khoo, J.J. Zwaginga, The angiogenic properties of mesenchymal stem/stromal cells and their therapeutic potential, Br. Med. Bull. 108 (2013) 25–53.
[390] M. Cherubino, L. Valdatta, R. Balzaretti, I. Pellegatta, F. Rossi, M. Protasoni, A. Tedeschi, R.S. Accolla, G. Bernardini, R. Gornati, Human adipose-derived stem cells promote vascularization of collagen-based scaffolds transplanted into nude mice, Regen. Med. 11 (2016) 261–271.

[391] Y. Wu, L. Chen, P.G. Scott, E.E. Tredget, Mesenchymal Stem Cells Enhance Wound Healing through Differentiation and Angiogenesis, Stem Cells 25 (2007) 2648–2659.
[392] A.S. Klar, S. Güven, T. Biedermann, J. Luginbühl, S. Böttcher-Haberzeth, C. Meuli-Simmen, M. Meuli, I. Martin, A. Scherberich, E. Reichmann, Tissue-engineered dermo-epidermal skin grafts prevascularized with adipose-derived cells, Biomaterials 35 (2014) 5065–5078.
[393] V. Femke, J.P.-V.S. Sandra, F. Eric, W.V.N. Johan, E.R.H. Steven, O.P.H. Stefan, J.V.M.V.O. Gerjo, Prevascular structures promote vascularization in engineered human adipose tissue constructs upon implantation, Cell Transplant. 19 (2010) 1007–1020.
[394] M.W. Laschke, M.D. Menger, Life is 3D: boosting spheroid function for tissue engineering, Trends Biotechnol. 35 (2017) 133–144.
[395] A.L. Torres, S.J. Bidarra, M.T. Pinto, P.C. Aguiar, E.A. Silva, C.C. Barrias, Guiding morphogenesis in cell-instructive microgels for therapeutic angiogenesis, Biomaterials 154 (2018) 34–47.
[396] M.W. Laschke, T.E. Schank, C. Scheuer, S. Kleer, S. Schuler, W. Metzger, D. Eglin, M. Alini, M.D. Menger, Three-dimensional spheroids of adipose-derived mesenchymal stem cells are potent initiators of blood vessel formation in porous polyurethane scaffolds, Acta Biomater. 9 (2013) 6876–6884.
[397] K. Sakaguchi, T. Shimizu, S. Horaguchi, H. Sekine, M. Yamato, M. Umezu, T. Okano, In vitro engineering of vascularized tissue surrogates, Sci. Rep. 3 (2013) 1316.
[398] M.W. Laschke, M. Rucker, G. Jensen, C. Carvalho, R. Mulhaupt, N.C. Gellrich, M.D. Menger, Improvement of vascularization of PLGA scaffolds by inosculation of in situ-preformed functional blood vessels with the host microvasculature, Ann. Surg. 248 (2008) 939–948.
[399] H. Kokemueller, S. Spalthoff, M. Nolff, F. Tavassol, H. Essig, C. Stuehmer, K.-H. Bormann, M. Rücker, N.-C. Gellrich, Prefabrication of Vascularized Bioartificial Bone Grafts In Vivo for Segmental Mandibular Reconstruction: Experimental Pilot Study in Sheep and First Clinical Application, International Journal of Oral and Maxillofacial Surgery, Vol. 39, 2010 379–387.
[400] J. Rouwkema, A. Khademhosseini, Vascularization and angiogenesis in tissue engineering: beyond creating static networks, Trends Biotechnol. 34 (2016) 733–745.
[401] X. Sun, W. Altalhi, S.S. Nunes, Vascularization strategies of engineered tissues and their application in cardiac regeneration, Adv. Drug Deliv. Rev. 96 (2016) 183–194.
[402] M. Lovett, K. Lee, A. Edwards, D.L. Kaplan, Vascularization strategies for Tissue Engineering, Tissue Engineering. Part B, Reviews 15 (2009) 353–370.
[403] Y. Jung, H. Ji, Z. Chen, H.F. Chan, L. Atchison, B. Klitzman, G. Truskey, K.W. Leong, Scaffold-free, human mesenchymal stem cell-based tissue engineered blood vessels, Sci. Rep. 5 (2015) 15116.
[404] C. Fernandez, R. Yen, S. Perez, H. Bedell, T. Povsic, W. Reichert, G. Truskey, Human vascular microphysiological system for in vitro drug screening, Sci. Rep. 6 (2016) 21579.
[405] H. Ji, L. Atchison, Z. Chen, S. Chakraborty, Y. Jung, G.A. Truskey, N. Christoforou, K.W. Leong, Transdifferentiation of human endothelial progenitors into smooth muscle cells, Biomaterials 85 (2016) 180–194.