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Drug therapies and delivery mechanisms to treat perturbed skin wound healing

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ABSTRACT

Acute wound healing is an orderly process of four overlapping events: haemostasis, inflammation, proliferation and remodelling. A drug delivery system with a temporal control of release could promote each of these events sequentially. However, acute wound healing normally proceeds very well in healthy individuals and there is little need to promote it. In the elderly and diabetics however, healing is often slow and wounds can become chronic and we need to promote their healing. Targeting the events of acute wound healing would not be appropriate for a chronic wound, which have stalled in the proinflammatory phase. They also have many additional problems such as poor circulation, low oxygen, high levels of leukocytes, high reactive oxygen species, high levels of proteolytic enzymes, high levels of proinflammatory cytokines, bacterial infection and high pH. The future challenge will be to tackle each of these negative factors to create a wound environment conducive to healing.

Contents

Abbreviations: ROS, Reactive oxygen species; MMPs, Matrix metalloproteinases; ECM, Extra-cellular matrix; DFU, Diabetic foot ulcer; PU, Pressure ulcer; VLU, Venous leg ulcer; IL-1β, Interleukin 1 beta; TNF-α, Tumour necrosis factor alpha; PDGF, Platelet-derived growth factor; FGF, Fibroblast growth factor; FGFR, Fibroblast growth factor receptor; VEGF, Vascular endothelial growth factor; EGF, Epidermal growth factor; HB-EGF, Heparin-binding EGF-like growth factor; TGF-β, Transforming growth factor beta; BMP, Bone morphogenetic protein; uPA, Urokinase-type plasminogen activator; TIMP, Tissue inhibitors of matrix metalloproteinases; RNS, Reactive nitrogen species; Cx43, Connexin 43; Cx43 AsODN, Cx43-specific antisense oligodeoxynucleotide; CCL2, Chemokine ligand 2; ATP, Adenosine triphosphate; ZO-1, Zonular Occludin-1; DOX, Doxycycline; a-1 PI, a-1 antitrypsin; MiR, microRNA; HIF, Hypoxia-induced factor; DFO, Deferoxamine; PHMB, Polyhexamethylene biguanide; DNA, Deoxyribonucleic acid; AMPs, Antimicrobial peptides; HA, Hyaluronic acid; PLA, Poly-lactic acid; PCL, Polycaprolactone; PGA, Poly-glycolic acid; PEG, Polyethylene glycol; 3D, Three-dimensional; PLGA, Poly (lactic-*co*-glycolic acid); LbL, Layer-by-layer.

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1. Introduction: physiology of wound healing

The skin is an intricate structure composed of two main layers – epidermis and dermis with the subcutaneous fats beneath the dermis (Fig. 1) [1]. In a healthy individual, wound healing occurs through four temporarily and spatially overlapping phases namely haemostasis, inflammation, proliferation, and remodelling [1–4]. Haemostasis occurs immediately following an injury. The constriction of blood vessels and formation of a blood clot prevent further blood loss and provide a temporary barrier to the outside world [5]. Growth factors and cytokines secreted by platelets attract fibroblasts, immune cells and endothelial cells to activate the healing cascade [4]. During the subsequent inflammation phase, blood vessels increase in permeability, allowing enzymes and immune cells to reach the injury site with neutrophils predominantly present in the first three days and monocytes arriving subsequently. Neutrophils release reactive oxygen species (ROS) and matrix metalloproteinases (MMPs) that remove wound debris while concurrently removing any bacterial contamination [6,7]. Upon arrival at the injury site, monocytes differentiate into macrophages that phagocytose spent neutrophils and non-viable tissue components [6]. Additionally, the release of various growth factors and cytokines initiates the recruitment of fibroblasts, keratinocytes and endothelial cells to repair the damaged blood vessels [1,7]. This inflammatory phase subsides and the proliferative phase takes over. Fibroblasts migrate into the wound bed to lay down extracellular matrix that serves as a scaffold for the keratinocytes to crawl over [3,4]. Angiogenesis occurs with the formation of new blood vessels in the granulation tissue of the wound bed [4,8,9]. *Re*-epithelialization occurs concurrently with keratinocytes at the wound edge proliferating and migrating over the wound bed [3,4]. Finally, only after the wound closes, some of the fibroblasts differentiate into myofibroblasts and contract down the granulation tissue [10–12]. The extracellular matrix (ECM) undergoes remodelling. Collagen gets extensively remodelled and replaced but never manages to achieve a basket weave-like appearance of normal skin but instead tends to form laminar arrays of a scar tissue, lacking the tensile strength of normal skin [4,10,13].

2. Chronic wounds and elements of perturbed wound healing

Chronic wounds typically fail to heal in an orderly and timely manner, usually within 3 months of tissue injury [14]. These wounds commonly fail to proceed beyond the inflammation phase and experience chronic inflammation, which perpetuates the degree of tissue damage [15–17]. Generally, chronic wounds can be divided into three most common categories - diabetic foot ulcers (DFU), pressure ulcers (PU) and venous leg ulcers (VLU). Despite the differences in their molecular etiology, these wounds share common elements, as summarised in Fig. 2, that perturb the wound healing cascade [14].

2.1. Dysregulation of pro-inflammatory cytokines

Pro-inflammatory cytokines interleukin 1 beta (IL-1β) and tumour necrosis factor alpha (TNF-α) are essential in the early phases of wound healing because they initiate neutrophil recruitment, their maturation as well as increase permeability of blood vessels [18]. Although IL-1β and TNF-α are key for wound healing, the overproduction and prolonged expression to these cytokines increases tissue destruction due to the overactivation of immune cells. Further, these cytokines trigger the production of proteases, which breakdowns the ECM [19,20].

An analysis of chronic wound fluids found significantly elevated levels of pro-inflammatory cytokines IL-1, IL-6 and TNF-α [21]. When compared to fluids from mastectomy wounds, the natural inhibitors of these cytokines, P55 and IL-1 receptor antagonist, are less prevalent in chronic wound fluids [22]. Interestingly, the level of these cytokines decreased in fluids upon healing of these chronic wounds [21].

2.2. Altered availability and activity of growth factors

Growth factors initiate many complex biological and molecular events underlying wound healing. Platelet-derived growth factor (PDGF) is one of the initial factors produced upon wound injury, which triggers a range of cellular responses throughout all phases of the wound healing process [23]. The binding of PDGF to their complementary family of receptor tyrosine kinases activates several signalling pathways that enhances cellular migration and proliferation [24]. Concurrently, the expression and phosphorylation of PDGF receptors α and β is significantly upregulated to bring about timely wound healing [23,24]. In contrast, the underproduction of PDGF and excessive protease-mediated degradation of both PDGF and PDGF receptors result in depressed levels of PDGF and PDGF receptors within the chronic wound beds [25].

Fibroblast growth factors (FGF) 1, 2, 7, 10 and 22 are expressed upon dermal injury [26]. Once liberated from the ECM, FGF ligands bind and activate FGF receptors (FGFR) in a heparan sulfate proteoglycan dependent manner. These receptor-ligand interactions activate downstream signalling of multiple pathways that enhance cellular migration,

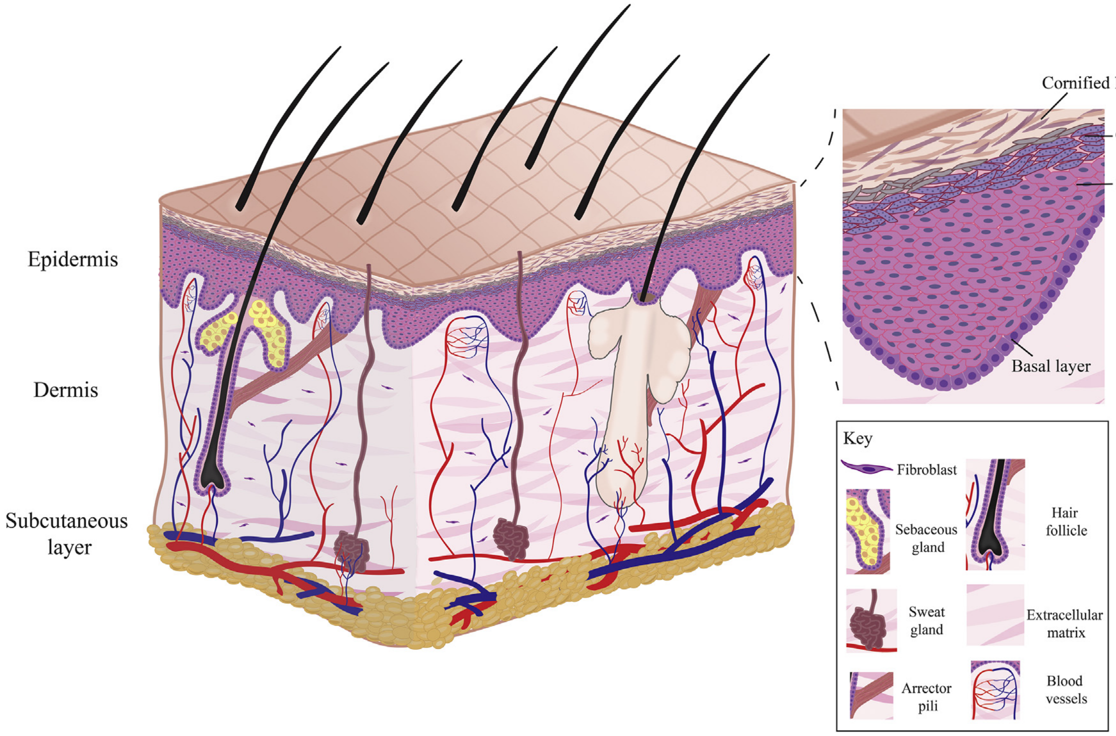


Fig. 1. An illustration of the skin anatomy in human.

proliferation, production of growth factors, epithelization as well as stimulating the production of proteases involved in remodelling the wound bed [4]. Additionally, FGF 7, 10 and 22 upregulate ROS protective enzymes and reduce the level of inflammatory mediators triggered during the injury [4,27]. However, in chronic wounds, this FGF-FGFR mediated signalling is impaired [28]. Typically, in chronic wound fluids, levels of FGF remain low in the presence of elevated inhibitory heparan sulfate levels [29,30].

Vascular endothelial growth factors (VEGF) are heparin-binding glycoproteins that exert their functions by binding to multiple cell-surface tyrosine kinases receptors to mediate angiogenesis [31–33]. Upon activation of their receptors, VEGF triggers multiple events required for successful angiogenesis, increasing permeability of vessels, degrading basement membrane by proteases, promoting endothelial cell migration as well as enhancing proliferation of vascular cells within the wound bed [28,34]. Additionally, VEGF initiates migration and activation of monocytes, production of proteases, proliferation of fibroblast, formation of scars and mobility of keratinocytes for wound re-epithelialization [31–33,35]. Although there is an elevated level of VEGF mRNAs in chronic wounds, the level of VEGF remains low due to the high level of proteolytic activities within the wound bed [36].

Epidermal growth factors (EGF) are membrane-bound and their activation involve proteases such as MMPs. EGF, heparin-binding EGF-like growth factor (HB-EGF) and TGF-α are the three main members of the EGF family that are essential in mediating the wound healing cascade [23,28,37–39]. These factors are important inflammatory response modulators, stimulate re-epithelization, attribute to angiogenesis as well as the formation of granulation tissue [38,40–44].Since chronic wounds have inadequate levels of EGF and EGFR, the normal injury and repair process in these wounds fail to initiate [39,44].

The transforming growth factor beta (TGF-β) family plays multiple regulator roles in modulating wound healing responses and scarring [45,46].In particular, TGF- β1–3, bone morphogenetic proteins (BMPs) and activins are the main members that implement major wound healing processes [28,47]. These factors are activated mainly by proteases such as MMP-2 and MMP-9 to trigger signalling pathways leading to cytoskeleton rearrangements, cell motility induction and transcriptional machinery activation [48,49]. TGF- β1–3 are key for recruitment of the inflammatory cells and fibroblasts to the wound bed [28]. They facilitate the migration of keratinocytes for re-epithelization as well as the formation and remodelling of ECM [48]. The maintenance of the stem cells within the hair follicles and regulation of matrix assembly are mediated by BMPs [48,50] while activins are mainly involved in inducing keratinocyte differentiation and the subsequent increase in matrix deposition by fibroblasts [47,51]. In chronic wounds, levels of both TGF- β ligands and receptor density are significantly lower than healing wounds [28,52].

2.3. Modified expression of proteases

Proteases and their corresponding inhibitors play critical roles in maintaining the balance between ECM degradation and deposition [53,54]. This equilibrium is necessary for coordinated healing of wounds [54]. However, this balance is disrupted in chronic wounds [54,55]. The degradation and remodelling of the ECM by MMPs is a key process during wound healing [53,54,56]. This process triggers the influx of leukocytes, angiogenesis and re-epithelialization [54,57]. In particular MMPs are capable of degrading ECM components and facilitate several pathways that lead to the regeneration of injured tissue. The clearance of damaged protein and destruction of the provisional matrix allows cellular migration into the wound bed and the subsequent formation of granulation tissue [55,58,59]. Beyond this, MMPs also degrade growth factors and their receptors along with angiogenic factors to influence cellular behaviour throughout the wound healing process [53]. Other proteases that are involved in wound healing include the serine proteases that are expressed by neutrophils. These serine proteases include elastase, cathepsin G as well as urokinase-type plasminogen activator (uPA) which degrade growth factors and hence, bring about a reduction in the bioactivity of these growth factors [60]. A controlled expression of proteases is crucial to the wound healing cascade. Elevated and prolonged expression of MMPs disrupts the balance between tissue breakdown and repair and thus, lead to excessive ECM degradation that is closely associated with delayed healing [55,56,60]. Similarly, high

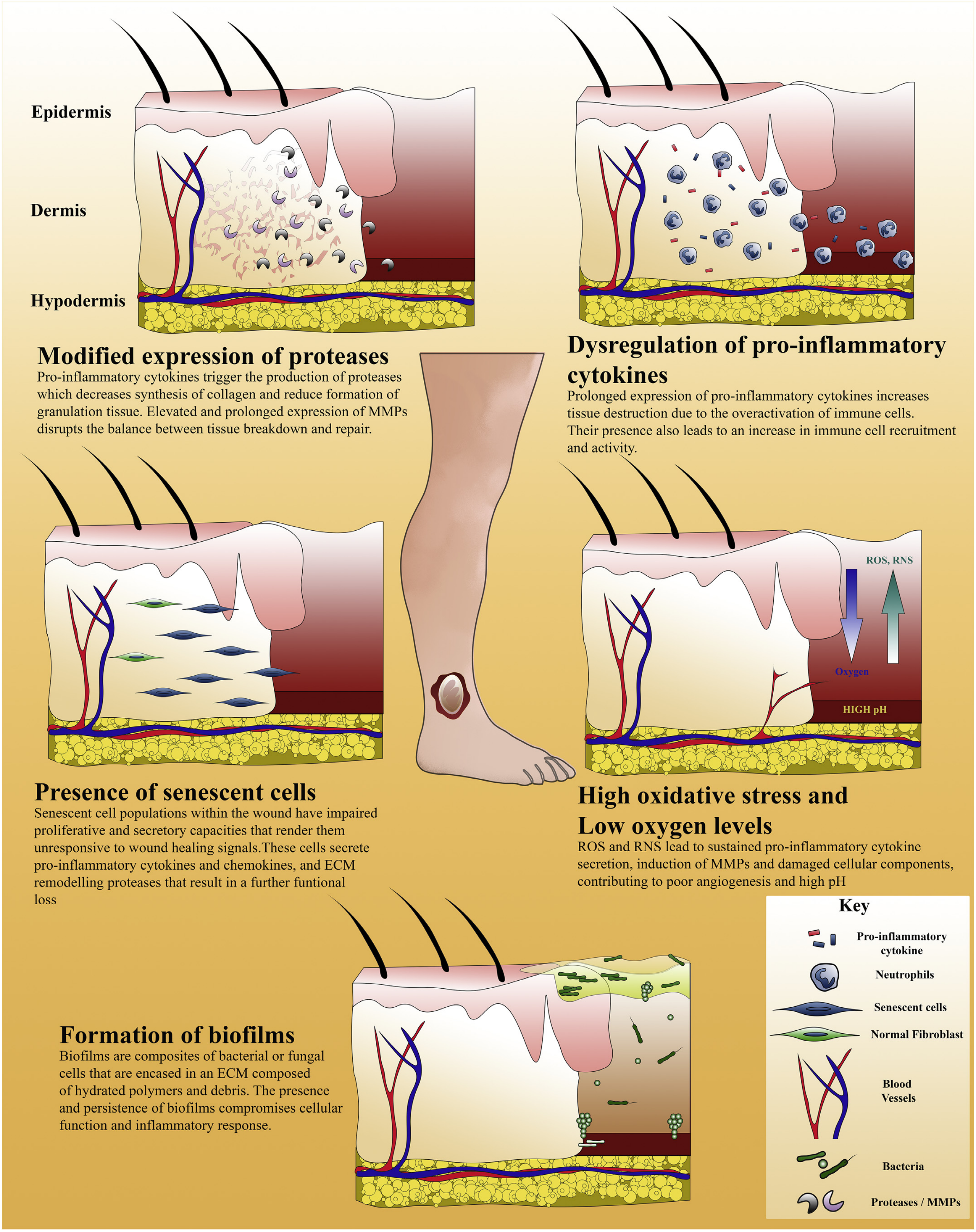


Fig. 2. An illustration that summarises the elements of perturbed healing found in chronic wounds.

expression of serine proteases decreases the bioavailability and bioactivity of growth factors that are crucial to wound healing [61].

In chronic wounds, the prolonged exposure of these wounds to pro-inflammatory cytokines, IL-1 and TNF-α, act to stimulate the production of MMPs while inhibiting the synthesis of the tissue inhibitors of matrix metalloproteinases (TIMPs) [19–21]. Compared to acute wounds, the protease activity in chronic wounds is significantly elevated – 116 fold higher in chronic wound fluids than in mastectomy fluids [62]. Higher levels of MMP-1, MMP-2 and MMP-9 were found in fluid of PU as compared to fluids from acute surgical wounds [63]. In addition, elevated levels of MMPs, neutrophil elastase and cathepsin G were found within the granulation tissue of PUs [63]. Similarly, in both DFUs and VLUs, the levels MMP-2, MMP-9 and neutrophil elastase remained elevated compared to healing wounds [64]. Conversely, the concentration of TIMP-1 decreased in these chronic wounds [65]. Elevated protease activity in chronic wounds degrade proteins necessary for wound healing and hence, contributing to the failure of chronic wounds to heal [64].

2.4. High oxidative stress

ROS and reactive nitrogen species (RNS) are pivotal in the orchestration of the normal wound healing response [66–70]. These molecules are secondary messengers to many inflammatory cell types and non-lymphoid cells, which are involved in the repair process [67,71]. ROS also regulates angiogenesis at the wound site and ensures optimal blood perfusion into the wound bed [72]. In addition, ROS acts as early host defense against infection through induction of ROS bursts via phagocytes [73].

Oxidative stress is mediated by excessive production of ROS and RNS [74]. Although these molecules are involved in physiological regulatory circuits, the enhanced ROS and RNS concentrations can cause severe damage to cellular components, driving wounds into non-healing state [74,75]. In chronic wounds, the elevated and sustained levels of ROS and RNS have been associated with impaired wound healing [76,77]. Excessively high level of ROS and RNS lead to sustained pro-inflammatory cytokine secretion, induction of MMPs, undesired modification and degradation of ECM proteins and impairment of fibroblasts and keratinocytes [78–80]. Furthermore, such high levels of oxidative stress activate pro-apoptotic proteins that subsequently induce cell death [81]. While low levels of ROS and RNS are essential in stimulating effective wound healing, an excessively high level of these species causes cellular damage and impaired wound healing.

2.5. Presence of senescent cells

Chronic wounds are characterized by senescent cell populations with impaired proliferative and secretory capacities that render them unresponsive to wound healing signals [82–86]. The perpetual chronic inflammatory environment within chronic wounds result in high oxidative stress that lead to DNA damage-related cell cycle arrest, driving cell populations into a senescent phenotype [87]. This diminished proliferative capacity of senescent fibroblasts, keratinocytes, endothelial cells and macrophages is directly correlated to perturbed ECM production and remodelling, poor neovascularization and angiogenesis, insufficient growth factor production and inability to re-epithelialize over the wound bed [88–92]. In addition, these cells secrete pro-inflammatory cytokines and chemokines as well as ECM remodelling proteases that further traps the cells in the wound in a vicious cycle of progressive functional loss [93].

2.6. Low oxygen levels

Oxygen is a key moderator of wound healing [94,95]. It is a requirement for various processes in the wound healing cascade including collagen deposition, re-epithelization, resistance to infection and angiogenesis [94,96,97]. Hence, a lack of oxygen supply impedes wound healing. Most chronic recurrent wounds, especially PUs, are a result of local tissue ischemia or ischemia-reperfusion injury. The skin and local vasculature become scarred and atrophic, forming permanent obstacles to the transport of oxygen and the eventual chronic hypoxic environment [98]. Furthermore, in the chronic inflammatory environment of these wounds, there exists an imbalance between the supply of oxygen and the high energy demand of the healing tissue. The increased utilization of oxygen by hypermetabolic regenerating tissue coupled with the sustained and elevated production of ROS by phagocytes further reduces the oxygen availability in the injured tissue [99–103].

2.7. Impaired angiogenesis

Angiogenesis is impaired in chronic wounds and thus, causing further tissue damage that results from chronic hypoxia and insufficient micronutrient delivery [104,105]. In particular, patients with diabetes show abnormal angiogenesis in various organs [104]. Under high glucose conditions, the normal angiogenic process is inhibited resulting in poor formation of new blood vessels and a subsequent decrease in entry of growth factors to activate the wound healing cascade [106].

2.8. High pH in wound beds

The pH of a wound can affect many factors including oxygen release, protease activity, angiogenesis and bacterial toxicity [107–109]. Upon injury, the pH of wounds increases [110]. However, as the wound progresses towards healing, the pH moves to neutral and then becomes acidic [111]. Healing of wounds typically occurs in an acidic environment. On the contrary, chronic non-healing wounds have an elevated alkaline environment within the range of 7.2 to 8.9 [112–114]. Excessive breakdown of the ECM and growth factors occurs more readily under alkaline conditions as MMPs show peak enzymatic activity at alkaline pH. Typically, MMP-2 and neutrophil elastase show peak activity at pH 8.0 and pH 8.3 respectively [108]. The pH environment also influences oxygen delivery to damaged tissues. Oxygen delivery to damaged tissue is dependent on both perfusion and diffusion. The reduction of pH increases the release of oxygen to these tissues [114]. Therefore, in chronic wounds, not only is oxygen perfusion perturbed, the alkaline environment also results in the reduction of oxygen diffusion to the wound bed. Additionally, high pH in the wound bed also increases toxicity of bacterial end products, prevents the destruction of abnormal collagen in the wound bed, hinders functional angiogenesis and reduces fibroblast activity [114,115].

2.9. Formation of biofilms

Biofilms are composites of bacterial or fungal cells that are encased in an ECM composed of hydrated polymers and debris [116]. *Pseudomonas aeruginosa and Staphylococcus aureus* are two bacteria that can commonly be identified in chronic wounds [117]. Biofilms differ from planktonic bacteria phenotypically and are also far more resistant to antibiotics [118].

Chronic wounds possess an ideal environment for biofilm formation. The necrotic tissue and debris provide surfaces for bacterial attachment [116]. Typically, open wounds are more susceptible to infection due to impaired host immune response [116,119,120]. The presence and persistence of biofilms in chronic wounds severely compromises cellular function and worsens inflammatory response [121,122]. Notably, the levels of pro-inflammatory cytokines and growth factors significantly increase in the presence of bacteria and their endotoxins, driving wounds into a chronic inflammatory phase [121,122]. Additionally, in the presence of biofilms, MMP expression increases, causing excessive degradation of ECM and proteins necessary for timely wound healing [123].

3. Current therapeutics to promote healing in chronic wounds

Various therapeutic have been developed over the years in an attempt to restore the dysregulated metabolic and signalling processes in a chronic wound environment. These strategies, as summarised in Table 1, revolve around dampening inflammation, encouraging re-epithelialization, minimizing oxidative stress, boosting oxygen levels, promoting angiogenesis, enhancing fibroblast migration, increasing bioactivity and bioavailability of growth factors as well as eliminating microbial infestation in the wound bed.

3.1. Gap junction targeted therapy

Gap junctions are intercellular channels that mediate both electrical and biochemical coupling through the exchange of ions, secondary messengers and small metabolites [124]. These junctions are formed by two connexons where one is provided by each cell. Gap junction intercellular communication is key in regulating cellular differentiation, apoptosis, cell migration and intracellular signalling [125]. Notably, connexin 43 (Cx43) remains elevated in chronic wounds [126]. This sustained elevated expression hinders re-epithelization, prolongs inflammation and can facilitate the spread of apoptotic signals to surrounding healthy tissues [126].

The application of Cx43-specific antisense oligodeoxynucleotides (Cx43 AsODN) on both acute and chronic wounds showed significant improvements to both the rate and quality of healing [127]. In these wounds, the antisense treatment dampens inflammation by reducing the number of neutrophils and level of pro-inflammatory cytokines such as TNF-α and chemokine ligand 2 (CCL2) at the injury site. Keratinocyte proliferation and migration were encouraged with the downregulation of Cx43. Concurrently, the rate of angiogenesis and migration of fibroblast into the wound were enhanced [126–128].

Table 1
A summary of therapeutic molecules to promote wound healing.

Therapeutic molecules	Major effects on wound healing	References
Cx43 AsODN	• Reduces the number of neutrophils	[126–128]
Mimetic peptides (GAP26 and GAP27)	• Reduces levels of pro-inflammatory cytokines • Increases keratinocyte proliferation and migration	[135,136]
ACT1 peptide	• Increases rate of re-epithelisation • Reduces scar formation	[141]
DOX	• Targets microbial infections • Inhibits matrix destruction by proteases • Reduces oxidative stress	[142–147]
FGF-1, 2 and 7	• Promote wound re-epithelisation • Improve angiogenesis	[28,149–151]
MiR-424	• Promotes angiogenesis	[153]
MiR-31	• Increases cellular proliferation • Enhances rate of re-epithelisation	[154]
Anti-miRs (against miR-221 and miR-222)	• Combat bacterial infections via increase expression of NO	[155,156]
Oxygen	• Accelerates rate of wound closure	[159,160]
Purified porcine haemoglobin (Granulox by SastoMed)	• Reduces pain score • Encourages angiogenesis	[162]
DFO	• Recruits endothelial cells for the formation of blood vessels • Reduces oxidative stress	[163]
Topical antibiotics (E.g. Mupirocin)	• Combats bacterial infections through destruction of vital bacteria components such as the bacterial cell wall	[164]
PHMB		[165]
Ionized silver		[166,167]
Povidone iodine		[168]
AMPs	• Destroy pathogens • Enhance re-epithelisation • Promote granulation tissue formation • Encourage angiogenesis	[170–172,175]

Individual, undocked connexons have significant roles in signalling as well [129]. The transient opening of a hemichannel allows the extracellular release of signalling molecules such as adenosine triphosphate (ATP) when cells or tissues are under stress. If these channels are opened for prolonged amount of time, cells undergo programmed cell death and may cause further tissue injury [130]. To prevent or reduce the opening of connexons during tissue injury, short peptides that mimic the extracellular loops of connexins were applied to temporary block connexon opening and prevent the eventual formation of gap junctions [131,132]. In particular, GAP26 and GAP27 are two mimetic peptides that have been reported to block the communication properties of docked gap junctions without affecting the structures of connexins [133,134]. The application of GAP26 and GAP27 to cultures of human fibroblasts and keratinocytes found a reduction in gap junction communication and accelerated cellular migration [135,136].

ACT1, an internalization protein antennapedia that is linked to the last nine amino acids, RPRPDDLEI, of the Cx43 tail, has been used as an alternative peptide approach to target the interaction between the C-terminal of Cx43 with Zonular Occludin-1 (ZO-1) [137]. Rather than blocking the connexons, the ACT1 peptide competes out Cx43 C-tail interactions with the PDZ2 domain on ZO-1, the matricellular protein CCN3, 14–3-3 proteins as well as protein kinases – all of which are closely associated with tissue response to an injury [137–140]. Similar to the Cx43 antisense treatment, the ACT1 treatment resulted in an accelerated rate of healing of acute and chronic wounds while reducing scar formation [141].

3.2. Anti-inflammatory small molecular drug

Doxycycline (DOX) is a chemically modified tetracycline compound that is widely used to treat infections caused by micro-organisms in chronic wounds [142]. Besides its antimicrobial activity, MMP activities are also inhibited by DOX through the chelation of calcium and zinc ions, which inhibits matrix destruction mediated by MMPs [143–145]. In addition, DOX prevents the degradation of the host defense protein a-1 antitrypsin (a-1 PI) which inhibits neutrophil elastase activity [146]. Thus, the degradation and inhibition of TIMPs is minimized. Beyond that, DOX reduces oxidative stress in chronic wounds by scavenging ROS and reducing cytokine-induced NO production [147].

3.3. Growth factor delivery

The delivery of exogenous growth factors to chronic wound bed aids in the restoration of crucial growth factor required for timely wound repair [148]. FGF-1 and 2 have been used for treatment of chronic wounds, with a slight acceleration in healing rates being observed [28,149]. On the other hand, FGF-7 was shown to enhance the repair of VLUs by promoting wound epithelialization [149]. To overcome the instability of VEGF in the protease-rich chronic wound environment, biologically active and protease-resistant VEGF isoforms were delivered to improve angiogenesis in chronic wounds [150,151].

3.4. MicroRNAs(miRs)

Gene expression in the wound healing process is extensively regulated by non-coding microRNAs (miRs). Many miRs expressed during the normal wound healing process are dysregulated during chronic wound healing [152]. The introduction of miR-424 in chronic wound beds promotes angiogenesis through the stabilization of hypoxia-induced factor (HIF) which subsequently triggers the transcription of VEGF [153]. On the other hand, the delivery of miR-31 induces the up-regulation of both TGF-β1 and TGF-β2 in chronic wounds. This elevation of miR-31 increases cellular proliferation and rate of re-epithelialization

[154]. On the other hand, the silencing of both miR-221 and miR-222 via anti-miR delivery aids in increasing the expression of nitric oxide – a potent antibacterial agent crucial to combating infection in chronic wounds [155,156].

3.5. Oxygen delivery

Administration of oxygen has been shown to accelerate the healing process of chronic wounds [157,158]. In particular, the use of oxygenated dressings has encouraged wound healing, lowered pain score and improves histological profiles [159,160]. Notably, the delivery of dissolved oxygen at pH 5.5 via Oxy Dressing has increased intracellular ATP levels without affecting ROS production, cell proliferation and viability. The acidic environment provided by this dressing also aids in suppressing wound infection, increases antimicrobial activity, enhances re-epithelialization and angiogenesis [161].

An alternative method to boost oxygen availability at chronic wounds is through the application of purified haemoglobin to these injured tissues. The haemoglobin spray by Granulox (SastoMed) comprises of purified porcine haemoglobin. Upon application, the purified haemoglobin binds to oxygen from the atmosphere and diffuses it into the wound bed to accelerate wound healing [162].

3.6. Pro-angiogenic small molecular drug

The topical application of deferoxamine (DFO) is a potential therapeutic approach to improving healing in chronic wounds. In particular, DFO act as an iron chelator and effective HIF-1α inducer and stabilizer. The DFO-induced HIF-1a stabilization circumvents the chronic hypoxic milieu of PU, VLU and DFU, thereby stimulating new vessels, enhancing cell motility and the subsequent recruitment of endothelial precursors, which are essential to the healing phase of cutaneous wounds. Furthermore, DFO prevents iron release in the tissue of PU, VLU and DFU patients, which indirectly inhibit the generation of iron-induced ROS that overload the wound's antioxidant capacities [163]. This reduction in oxidative stress aids in the modulation of inflammation in chronic wounds.

3.7. Antimicrobial agents

Minimizing microbial infections in the wound bed is essential towards proper wound healing. Topical antibiotics such as mupirocin, retapamulin, bacitracin, neomycin, gentamycin and polymyxin B have significantly reduced the incidence of infections and are commonly used as part of standard wound care management. These antibiotics are bactericidal at high concentrations, inhibit bacterial protein biosynthesis, interfere with bacterial cell wall development and destroy bacterial cell membrane. However, the overuse of antibiotics presents several potential complications for chronic wound patients including bacterial resistance, contact allergy, and further moisture reduction in wound bed [164].

Moving away from antibiotics, several new agents have been reported. Polyhexamethylene biguanide (PHMB), a synthetic positively charged polymer with a hydrophobic backbone and cationic groups separated by hexamethylene chains, binds to negatively charged bacterial cell wall. Upon PHMB's attachment to the acidic membrane elements of the bacteria, the bacteria membrane becomes ridged, causing the separation of the individual membrane lipids and the eventual dissolution of the bacterial cell [165]. Ionized silver is another antibacterial agent that attacks at least three bacterial cellular components namely, cell membrane, deoxyribonucleic acid (DNA) and cytoplasmic organelles [166,167]. However, for chronic wounds, the lack of moisture might render silver to be ineffective as the antibacterial properties are prominent only in its ionized state. Iodine on the other hand, is a natural and non-metallic element that has several antimicrobial actions. It blocks bacterial cell efflux pumps, interferes with cellular respiratory processes, changes DNA structure, and denatures proteins. Although high concentration of iodine is cytotoxic and can delay healing, the use of low concentration, slow-releasing iodophors such as povidone iodine improve healing rates and are effective as highly potent antimicrobials with a broad spectrum of activity, including antibiotic-resistant strains [168].

Apart from synthetic and small molecular weight molecules, antimicrobial peptides (AMPs) are increasingly coming into focus as new treatment strategies for bacterial infections in skin wounds. AMPs are small, cationic, amphiphilic peptides formed with 10 to 50 amino acids that function as effector agents of innate mammalian immune system [169]. These host defense peptides directly interact with and interrupt anionic phospholipid membranes to destroy pathogens – enabling AMPs to target multi-resistant pathogens and display a wide spectrum microbicidal activity against both bacteria and fungi [170,171]. Many AMPs also act as wound healing peptides and therefore, demonstrating antimicrobial and tissue regeneration properties which are highly desired therapeutic properties for encouraging healing in chronic wounds [172]. Currently, >20 AMPs have been identified in the skin [173]. In DFUs, endogenous AMPs, β-defensins hBD-1, hBD-2, hBD-3 and hBD-4, and cathelicidin LL-37, were of insufficient levels to contain infection and promote proper wound healing [174]. Although these AMPs are expressed in DFUs, they are either expressed at low levels or cleaved and degraded by the proteases within the hostile microenvironment of DFU [174]. Hence, when compared to healthy skin, DFU biopsies presented significantly lower levels of these AMPs, contributing to the DFU pathogenesis [174]. To compensate for the low levels of cathelicidin LL-37, LL-37 AMPs were transfected, with adenoviral vectors, into excisional wounds in diabetic mice. Upon treatment, the wounds revealed a significant enhancement in re-epithelialization, granulation tissue formation and angiogenesis [175]. Unfortunately, similar wound responses were not observed with topical application of LL-37 AMPs as these topically-administered exogenous peptides undergo degradation in the harsh microenvironment of chronic wounds [174,176].

4. Therapeutic delivery systems used in chronic wound treatment

Therapeutic delivery systems are particularly important in the aspect of wound regeneration as the ineffective vasculature in chronic wounds prevent effective delivery of drugs to the healing tissue when the therapeutic is administered systemically. A higher dosage of therapeutics is often required for systemic delivery to account for the breakdown and non-specific diffusion of molecules to the intended site of delivery. Such high dosage can induce undesirable off target side effects. Furthermore, biological components have short half-lives. Coupled with the complexity and dynamics of the wound healing environment, a sophisticated drug delivery system is required to better deliver these active factors in proper dosage and timely fashion to the wound bed.

Currently, materials used in chronic wound care aim to fulfil two different purposes. 1: a temporary dressing over the wound area to form a barrier against microbes while maintaining a suitable environment within the wound area to support the healing process, and 2: a scaffold that serves a dermal substitute to host endogenous cell migration and proliferation, facilitating wound closure. Beyond that, these materials can double up to serve as localised delivery systems to replenish cells with regenerative capacity and deliver therapeutics to the chronic wound bed. Delivering cells to the hostile non-healing wound environment remains challenging. Enhancement to the therapeutic potential of cell-based therapies can be achieved with the use of localised delivery systems which promote cell viability, adhesion, proliferation, and migration [177,178]. Various works that adopted such combinatory approach of delivering cells via localised delivery systems have reported increased rate of wound closure in diabetic rat models [179–184] as well in patients with VLU and DFU [185,186]. However, in this review, we have restricted our discussion scope to acellular delivery systems as we acknowledge the limitations of cellular-based therapies for chronic wound healing – high cost, limited shelf life as well as increased likelihood of inducing inflammatory and immunogenic responses [178,187].

4.1. Temporary wound dressing

An ideal wound dressing forms a temporary barrier to cover the wound to prevent the growth of microbes, maintain the moisture in the wound while allowing oxygen access into the regenerating tissue without interfering with the wound healing process [188]. To avoid complications during dressing removal, the choice of material to fabricate dressing should be immunocompatible, non-degradable, unfavourable for cellular attachment and growth. Wound dressings are key towards exudate management. Exudates of wounds contain a large quantity of inflammatory cytokines and chemokines that are favourable for bacterial growth. The optimal wound dressing material should efficiently remove wound exudates without removing excessive amount of moisture from the wound area [189]. Additionally, the choice of material should facilitate delivery of drugs and biological factors by preserving the bioactivity of these therapeutics while releasing them at a desired rate.

Several types of wound dressings made from various materials are used for standard chronic wound care. In particular, medical gauze is the most commonly used wound dressing product. Fabricated from woven or nonwoven natural or synthetic fibers, the gauze aids in the absorption of wound exudates while keeping the wound environment moist [190]. However, the gauze serves as a poor barrier towards microbes and might cause the removal of nascent tissues in the wound bed upon when it is changed. Furthermore, the lack of elasticity of the gauze confines the movement of the body part, causing discomfort to patients. In contrast, thin, transparent film dressings, made of polymeric materials such as polyurethane, provide elasticity that allows for comfortable movement of affected body parts [191]. These thin transparent dressings are semi-permeable to gaseous exchange and light penetration while blocking the entry of microbes. The transparency of these dressings facilitate the inspection and assessment of wounds. However, the thin film lacks efficient absorption of wound exudates. To overcome this, foams, made of polymeric material, have the advantages of thin transparent dressings yet have the ability to absorb more wound exudates [192]. Besides maintaining moisture and permitting exchange of gases at the wound bed, these foams provide cushioning to the wounded tissue and thus, protecting the wound bed from secondary mechanical injury. Alginate dressings and hydrocolloid dressings are some alternatives to foams that have high absorbency of exudates while facilitating gaseous exchange [193,194].

Therapeutics can either be coated on or incorporated into wound dressings. Upon an injury, the breach of the skin barrier allows bacteria and other microbes to enter the wound. Hence, wound dressings with antimicrobial agents are key to preventing subsequent entry of microbes while eliminating residual microbes residing within the wound bed. PHMB has been incorporated into gauze and foam dressings to remove wound exudate while facilitating the destruction of bacteria [165]. These dressings do not release PHMB. Rather, bacteria are killed in the compartment above the wound in the dressing. As PHMB attaches to the membrane of the bacteria, the facilitated dissolution of the bacterial cell remains localised to the dressing and no residual organisms are left alive to facilitate resistance towards PHMB. In a clinical trial with PHMB foam dressings, a significant reduction in superficial bacterial burden was observed – one-sixth the amount of polymicrobial organisms as compared to control foam dressings [165]. However, no significance was found in the rate of wound closure between the wounds treated with PHMB foam dressings and the control foam dressings. Other antimicrobial agents such as silver have also been incorporated into foam and alginate dressings so that the ionized silver can be released slowly in response to wound exudate [166].

4.2. Scaffold as a dermal substitute

Traditional wound dressings protect the wound bed and provide a favourable environment to encourage wound healing. However, these dressings are unable to replace lost tissue, which is a situation prevalent in chronic wounds where the dermis is severely damaged. As such, scaffolds that serve as artificial dermal substitutes become necessary to help repair chronic wounds.

Scaffolds can mimic the ECM in native tissue and hence, play important roles in tissue regeneration. They can serve to provide structural support for cells to reside, contribute to the mechanical properties of tissues, provide bioactive cues for cells to respond to their microenvironment while acting as bioactive factors and therapeutics reservoirs. Importantly, for wound healing, scaffolds provide a flexible physical environment for remodelling in response to tissue dynamic processes [195].

Fabrication of scaffolds for the treatment of chronic wounds should involve materials that facilitate tissue regeneration, restore tissue function and promote a rapid healing process. Ideally, these materials should possess a degradation rate that matches with the rate of tissue growth. Additionally, both the material and its by-products should not induce toxicity nor adverse immune response. Many scaffold materials can induce a foreign body reaction and impair the healing process. Importantly, these materials should integrate and adhere well to the surrounding native tissue while ensuring that no additional mechanical stress is imposed on the wound or the surrounding intact tissue.

The materials utilized in scaffold fabrication can be generally placed in two categories – naturally derived biopolymers and synthetic polymers. Naturally derived biopolymers that have been used as scaffolds for wound regeneration include collagen, silk, hyaluronic acid (HA), dextran, fibrin, gelatin and chitosan [196]. On the other hand, polyesters such as poly-lactic acid (PLA), polycaprolactone (PCL) and poly-glycolic acid (PGA) are some of the more commonly used synthetic polymers for wound regeneration [197]. Depending on the choice of material coupled with the fabrication technique, scaffolds can exist as gels, sponges or fibrous membranes.

4.2.1. Hydrogels

Hydrogels are chemically cross-linked three-dimensional network structures that are highly porous and hydrophilic [198]. Usually, ultraviolet light or chemical crosslinkers are used to induce the formation of this three-dimensional network. Their structure facilitates gas exchange while maintaining fluid balance at the wound area. More importantly, the porous structure appropriately mimic the ECM structure and functionality, promoting cell migration, growth and maturation [199]. Additionally, the high porosity of the hydrogels and the moist environment it creates helps to deliver the encapsulated therapeutics into the wound in a sustained manner. Moreover, thermoresponsive and injectable hydrogels, which allow site-targeted yet minimally invasive scaffold implantation, are especially desirable to fill the wound bed [200].

Pluronic F127 is a commonly used thermoresponsive hydrogel that is nontoxic. It forms a free-flowing solution below ambient room temperature and sets into a gel at body temperature. This gel can serve as a drug depot at the site of administration that slowly and continuously releases drug into the surrounding tissue [201]. Pluronic F127 has recently been used for delivery of dissolved oxygen in an attempt to restore the poor oxygen levels to chronic wound beds. Marketed as Oxy Dressing, oxygenated water was combined with Pluronic F127 and applied on wounds [161]. Oxy Dressing has demonstrated that the levels of oxygen, through this Pluronic F127 delivery system, remain stably above 30 mg/ml for at least 30 h [161]. Furthermore, the standalone use of Pluronic F127 has shown a significant rate of wound closure along with high vascular density and collagen formation within the wound bed [202]. Concurrently, in vitro studies with dissolved oxygen revealed elevated levels of intracellular ATP without affecting ROS production, cell proliferation and cell viability [161]. Hence, the delivery of dissolved oxygen using Pluronic F127 may yield better wound healing response compared to using the gel alone. However, in vivo studies in wound models should be done to further verify this. Pluronic F127 has also been used to deliver Cx43 AsODN to downregulate Cx43 protein levels in the wound edge keratinocytes and fibroblasts whilst reducing the inflammatory upregulation of Cx43 in the blood vessels. The delivery of Cx43 AsODN with Pluronic F127 extended the relatively short half-life of about 1.5 h to >6 h. Wounds treated with Cx43 AsODN Pluronic gels were observed to be less inflamed, produced less exudates, as well as enhanced re-epithelisation and granulation tissue formation [127,203].

Polyethylene glycol (PEG) is one of the most utilized hydrophilic polymers for therapeutic delivery and it is known for its biocompatibility. As an antimicrobial and anti-inflammatory drug, doxycycline was delivered via a disulfide cross-linked PEG [204]. This hydrogel delivery system demonstrated a sustained release of doxycycline for up to 10 days in vitro and further enhanced skin permeation of doxycycline, promoting the wound healing process [204].

In recent years, three-dimensional (3D) bioprinting has emerged as a promising fabrication tool in tissue engineering and regenerative medicine. This technology provides a flexible, automated, on-demand, reproducible yet economical method for fabricating spatially organized, mechanically stable and biologically compatible hydrogel constructs that closely mimic the native organization of healthy tissues [205,206]. Synthetic and/or naturally derived polymers are used as bioinks to print skin substitutes, precisely tailored to each region and wound depth, with embedded ECM components, therapeutics, and growth factors [206]. Notably, 3D bioprinting overcomes the limitations of conventional hydrogel fabrication techniques – pore sizes can be more accurately controlled and increased to hundreds of microns [207], while enabling spatial patterns of therapeutics to be precisely engineered so as to guide cell migration, alignment and organization [208–210].

Owing to its similarity to the native ECM, gelatin has been favoured as a biomaterial for wound healing. Unfortunately, the poor mechanical stability and rapid degradation rate of gelatin limited its use as a dermal substitute and a controlled drug delivery system [211]. To overcome these limitations, silk was introduced and mixed with gelatin to form a bioink. Silk exhibits high biocompatibility and mechanical stability, as well as controlled drug release kinetics [212,213]. To further encourage skin repair, FGF-2 was chemically incorporated into the bioink. Using a pneumatic bio-printing system, this bioink was extruded to form a porous grid scaffold that has demonstrated sustained release of FGF-2 over 12 days – with a burst release of 32% over the first 24 h and a subsequent 5% day-by-day release over the next 11 days. Upon implantation into full-thickness wounds at the back of rats, FGF-2 incorporated gelatin-silk 3D-printed hydrogel exhibited an accelerated rate of re-epithelization, increased rate of collagen deposition and angiogenesis at post-implantation day 14 when compared with wounds that were implanted with gelatin-silk 3D-printed hydrogel without the incorporation of FGF-2 [207].

4.2.2. Lyophilised sponges

Lyophilised sponges also possess three-dimensional porous networks. Solutions of polymers to fabricate these scaffolds are first frozen before undergoing the freeze-drying process [214]. The lower the freezing temperature, the smaller the ice crystal formation. However, ice crystals can grow larger with longer freezing periods. During the freeze-drying process, the extreme low temperature within the vacuum chamber causes these ice crystals to vaporize, creating pores within the scaffold. These pore sizes range from 50 μm to several millimetre and can support cell migration and growth [214]. Additionally, their high porosity and well interconnected pores can absorb large amounts of wound exudate while permitting gaseous exchange. Similar to hydrogels, naturally derived biopolymers have mainly been used to fabricate these sponge-like scaffolds for wound healing applications. In comparison with hydrogels, these lyophilized sponged offer larger pore sizes which further facilitate cellular ingrowth. Furthermore, these sponges can contain both soluble and insoluble therapeutics with high therapeutics-loading capacity [215]. Stability of therapeutics was also reported to be better in lyophilized form when compared to semisolid-based formations [215].

Growth factor delivery has achieved limited success in clinical trials. This failure can be attributed to insufficient retention of growth factors within the wound bed and the exposure of growth factors to MMP-mediated degradation [28,39]. To address these problems, growth factors were incorporated and encapsulated within lyophilized sponges. Enhanced angiogenesis and granulation tissue formation was observed when EGF releasing sponges, made of HA and collagen, were implanted into full thickness wounds of diabetic mice [216].

4.2.3. Fibrous scaffolds

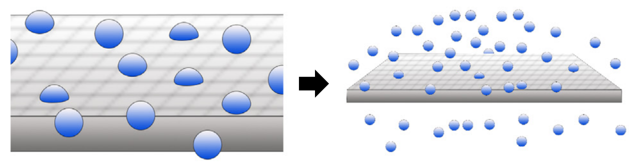
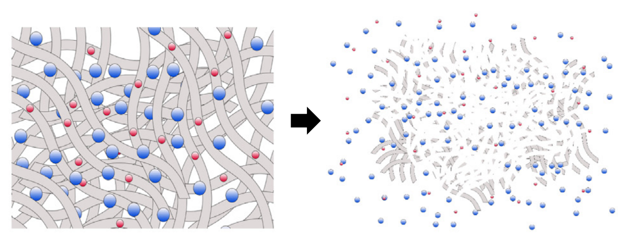
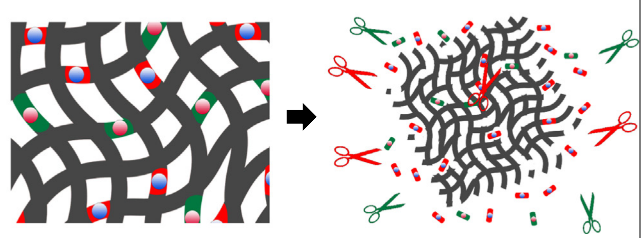
Fibrous scaffolds have nano to micro-architectures that mimic the natural collagen fibers in ECM [217,218]. Natural collagen fibers play essential roles in maintaining the structural integrity and strength of the skin. Hence, these biomimicking fibrous scaffolds provide topographical cues to influence cell migration, alignment, morphology and function towards wound regeneration [219]. These scaffolds can be fabricated using various techniques namely, electrospinning, self-assembly and phase separation [220,221]. Among them, the electrospinning method offers great versatility and reproducibility to fabricate fibrous scaffolds. This technique is simple yet efficient and forms fibers based on an electrohydrodynamic phenomenon whereby the charged polymer solution is extruded, from the spinneret tip, by electrostatic forces to form fibers which are subsequently attracted to an oppositely charged metallic collector [222,223]. Both naturally derived biopolymers and synthetic polymers can be electrospun [222–224]. Typically, organic solvents are used to dissolve these polymers to obtain polymer solutions that are then used for electrospinning. These solvents are mostly evaporated during the electrospinning process [225]. A mixture of both naturally derived biopolymers with synthetic polymers improves the hydrophilicity and alters degradation rate of these electrospun scaffolds [219]. All electrospun scaffolds display high surface area to volume ratio and high porosity for fluid and gaseous exchange. Additionally, as drug depots, these scaffolds have shown great promising in preserving and releasing therapeutics over a sustained period of time [226].

As a natural protein fibre obtained from the cocoons of the *Bombyx mori* silkworms, silk has attracted much interest due to its high mechanical strength and biocompatibility. EGF encapsulated electrospun silk nanofibers were fabricated and implanted into the wounds of mice. These functionalized silk scaffolds demonstrated a sustained release of EGF over a period of 7 days, with at least 25% of theoretical loaded EGF being released. Upon the application of these EGF-functionalised silk electrospun scaffolds, an increased in the rate of wound healing, collagen synthesis, re-epithelization, dermis proliferation, and reduced scar formation was observed [227,228]. Hence, wound closure was significantly enhanced by 90% with the treatment of these EGF loaded fibrous scaffolds [227].

4.3. Composite delivery systems

Microparticles and nanoparticles are frequently used in controlling the release of therapeutics especially in tissue regeneration [229]. Therapeutics are adsorbed onto the surface or encapsulated within the core of these particles. The release of these therapeutics usually features a burst that can be associated with the therapeutics adsorbed on the surface of the particles, followed by a sustained release which can be associated with the degradation of the polymer and the release of the therapeutics from the core of the particle. Similarly, these particles can either be fabricated from naturally derived biopolymers or synthetic polymers. In particular, for tunable drug delivery, chitosan and poly(lactic-*co*-glycolic acid) (PLGA) are favoured [229]. During in vivo delivery,

Table 2
A summary of therapeutic delivery mechanisms.

	Release Method	Schematic Representation
Passive	Diffusion	 time = 0 → time = t
Passive	Diffusion and polymer breakdown	 time = 0 → time = t
Active	Stimuli-triggered release	 Absence of stimuli → Presence of stimuli

these particles are susceptible to clearance and hence, to localize the delivery of these particles, they are frequently combined with scaffolds and used as composite delivery systems.

The release of therapeutics from hydrogels, lyophilized sponges and fibrous scaffolds occurs primarily through diffusion and degradation of polymers. Other mechanisms of therapeutic release from matrices and scaffolds are listed in Table 2. Altering the physical characteristics of these scaffolds to reduce the rate of diffusion and degradation without compromising cell ingrowth can be challenging. Hence, therapeutics can be first adsorbed or encapsulated in particles prior to incorporating them into the scaffold matrix. These particles serve to control the release of the therapeutics while the scaffolds serve primarily as a structure to support tissue regeneration. Multiple therapeutics can also be incorporated into such composite delivery systems to promote the various phases of wound healing.

Curcumin could be used as a topical wound healing reagent for the treatment of inflammatory conditions. A dual drug-loaded in situ gel-formation nanoparticle/hydrogel system was developed for the dual delivery of EGF and curcumin into wounds [230]. EGF and curcumin were co-encapsulated in PLA-Pluronic 10R5 copolymer (Mn $= 19.8 \times 10^3$) before being dispersed into thermosensitive and biocompatible PLA-Pluronic 10R5 copolymer (Mn $= 4.6 \times 10^3$) to form an in situ gel-forming composite. This composite delivery system displayed a slow cumulative release both EGF and curcumin over a period of 8 days, when compared to free and unbounded EGF and curcumin [230]. In an established excisional full-thickness wound model, this composite delivery system significantly enhanced rate of wound closure by 30% with an increase in rate of granulation tissue formation, collagen deposition, and angiogenesis, when compared to any single therapeutic delivery [230].

5. Therapeutic delivery strategies to promote chronic wound regeneration

5.1. Targeted delivery

Wound healing is a complex process that involves haemostasis, angiogenesis and the eventual restoration of the skin barrier function. In order for the proper occurrence of these healing phases, the presence of growth factors and cytokines is required. In the absence or lack of these biochemical cues, the healing process is derailed, leading to the formation of a chronic, non-healing wound. As such, therapeutic intervention becomes necessary to improve the physiological processes leading to wound healing. Different therapeutics are required at each phase. Hence, the ability to administer drugs specific to each phase is crucial to enhancing proper wound healing in chronic wounds.

Standard chronic wound care and management in hospitals often first involves the debridement of nonviable tissue and slough along with bacterial biofilms that prolong the inflammatory response in the chronic wound [231,232]. As a dermal substitute, scaffolds containing specific therapeutics required for that particular phase of healing can be implanted in the wound bed. Depending on the type of chronic wound, compression dressings, or foam-based dressings can be used to form a temporary barrier against microbes while maintaining moisture within the wound area. The former induces external compression to mitigate the hydrostatic pressure into the skin and subcutaneous tissues underlying VLUs while the latter aids in off-loading excessive pressure at extremities [233,234]. These dressings should contain antimicrobial agents to combat infection of microbes. As part of chronic wound care, these dressings are typically changed every seven days. During this change of dressings, clinicians can re-access the healing status of the wound and introduce scaffolds with therapeutics that are more suitable to induce the next phase of wound healing. Existing scaffolds within the wound bed can either be removed or designed to be degraded within seven days (i.e. before every change of dressing).

Although targeted delivery of therapeutics can deliver specific therapeutics to target or induce a particular phase of wound healing, there are several limitations involved. Firstly, the wound assessment by clinicians is subjective. Given the complexity of wound healing process, some phases tend to be overlapping. Hence, accurate assessment and determination of the wound healing phase can be challenging. As a result, clinicians may face difficulties to implant scaffolds with the most appropriate therapeutics into the wound bed. Secondly, the constant change of dressing and scaffolds might cause disturbance and destroy newly formed tissues in the wound bed and edges. Lastly, the effectiveness of such targeted delivery of therapeutics heavily rely on patient compliance. A lack of follow-ups would mean that the next phase of therapeutics cannot be timely introduced into the wound bed.

5.2. Spatial-temporal delivery within the wound bed

Human skin consists of two major distinct layers – a stratified, cellular epidermis and an underlying dermis of connective tissue [235]. The epidermis consists of stratified layers of keratinocytes that forms an impermeable barrier to the external environment. In contrast to the thin epidermis, the dermis forms the bulk of the skin tissue and contains numerous cells including dermal dendrocytes, macrophages and fibroblasts. Beneath the dermis lies the hypodermis where hair follicles, blood vessels and nerves extend from and into the dermis.

While controlled therapeutic delivery systems can deliver therapeutics locally at the wound bed over a sustained period of time, most systems fail to consider the multi-layered anatomy within the skin tissue. Scaffolds used as controlled therapeutic delivery for wound regeneration should be divided into three layers. The upper layer should include therapeutics that encourage re-epithelization such as the antisense Cx43asODN. The bottom layer should contain therapeutics that promote vascularization such as dissolved oxygen or porcine haemoglobin while the middle layer should have therapeutics that dampen inflammation (e.g. doxycycline) and promote angiogenesis (e.g. DFO or VEGF). Furthermore, not all components of the skin regenerate at the same rate. Hence, therapeutics that target the later phases of wound healing (e.g. angiogenesis and remodelling) should have delayed release compared to therapeutics that are meant to dampen inflammation and promote re-epithelization.

The layer-by-layer (LbL) technique can help to create distinct layers within a single scaffold. Recently, a single epidermal-dermal substitute, comprising of a bioactive LbL membrane on top of a porous dermal scaffold using HA and poly-L-lysine (PLL), was fabricated using spray-assisted LbL assembly [236]. The porous HA scaffold was first formed by lyophilization of a HA solution, which acts as a negatively charged substrate in which aerosolized solutions of the positively charged PLL and the negatively charged HA were sprayed sequentially. These oppositely charged species absorbed and grew on the substrate, forming a distinct LbL film above the HA porous scaffold. Beyond creating distinct layers, the LbL technique also allows different therapeutics and polymers of varying degradation profile to be incorporated at different layers during the fabrication process. Using such technique, the spatiotemporal delivery of therapeutics within the wound bed can be more precisely engineered for promoting chronic wound regeneration.

5.3. Stimuli-responsive delivery

Wound healing involves a dynamic series of events. An optimal therapeutic delivery system for wound healing should sequentially and selectively release therapeutics in response to biochemical cues relating to the condition and stage of healing, such as antimicrobial agents, cytokines, growth factors and other small molecule drugs in a controlled way in order to guide the wounds into the necessary conditions for healing. The sequential release of therapeutics is particularly crucial to acute wounds and not chronic wounds. Unlike acute wound healing, the healing phases in chronic wounds do not occur sequentially (i.e. progression from haemostasis, inflammation, proliferation then remodelling). Perturbed healing in chronic wounds is multifactorial and the various elements of delayed healing may prevail at any point of the healing process. Hence, the lack of sequence in how each element prevails would render sequential delivery ineffective. In contrast, selective and responsive delivery is of more paramount importance to regeneration of chronic wounds. The various elements of perturbed healing serve as triggers to stimulate the sequential delivery of therapeutics in response to the local environment of the wound bed as and when they are needed.

Chronic wounds have high levels of matrix degrading enzymes (e.g. MMP-1, MMP-2 and MMP-9) and pro-inflammatory enzymes (e.g. neutrophil elastase and cathepsin G). The use of protease cleavable peptides that link suitable therapeutics to the polymeric backbones can result in the formation of polymers with a therapeutic release rate that is matched proportionally to the concentration of proteases in the wound. In particular, a recent study has demonstrated the feasibility of an MMP-trigger delivery system that delivers TIMPs after myocardial infarction. A full-length TIMP-3 recombinant protein was encapsulated in a HA-based hydrogel that contained MMP-cleavable peptide crosslinks [237]. The presence and concentration of MMP after myocardial infarction influenced the rate of TIMP-3 release from the HA hydrogel. A similar methodology can be adopted to promote chronic wound regeneration. MMP-cleavable peptides recognized by MMP-1, MMP-2 and MMP-9 can be conjugated to the HA structure. Within the hydrogel, therapeutics that target the inflammatory phase (for instance Cx43asODN and doxycycline) could be released upon the cleavage of the peptide crosslinks by these MMPs.

Chronic wounds are also plagued with low oxygenation levels and presence of bacteria, both elements cause a significant increase in pH. PH responsive materials are often ionizable polymers that are weak acids or bases. These materials function through a change in their ionization state that results in changes in the polymer conformational state [238]. In particular, such change in conformation state for hydrogels mean a change in swelling behaviour which can be utilized in controlling the diffusion rate of therapeutics out of the hydrogel and into the wound bed. To demonstrate the feasibility of delivering drugs in response to an alkaline pH, super porous hydrogels were formulated via free radical polymerization of acrylamide and methacrylic acid [239]. These hydrogels only swell in alkaline pH environment and displayed rapid swelling kinetics. Hence, similar methodology could

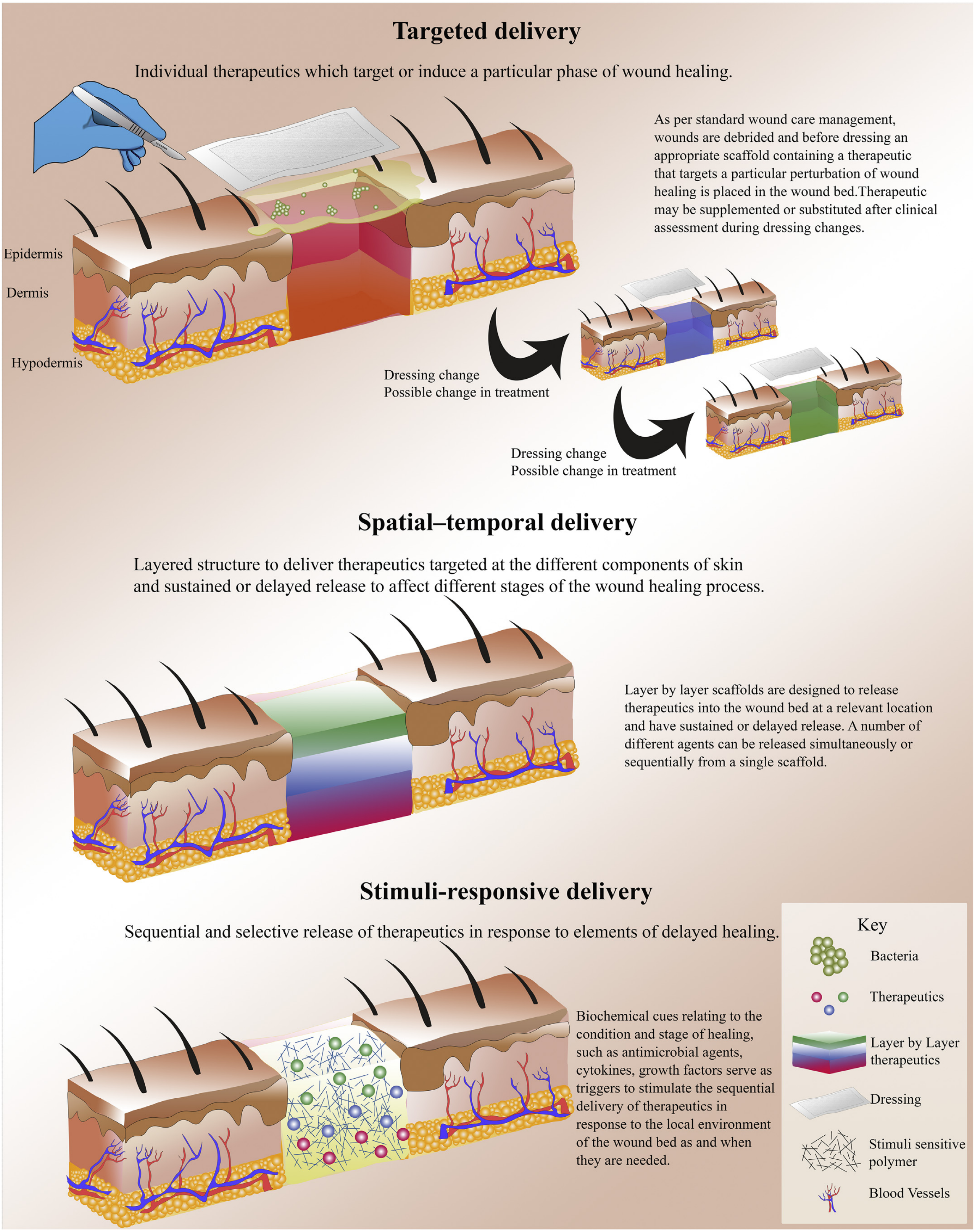


Fig. 3. An illustration that summarises the various delivery strategies for chronic wound care and regeneration.

be adopted to deliver antimicrobial therapeutics and dissolved oxygen in response to the high pH.

A summary illustrating all three delivery strategies can be found in Fig. 3.

6. Conclusion

Temporal control of drug delivery could promote the sequential events of acute wound healing. However, acute wound healing is not the major area of need. The largest unmet need is to find a way to promote chronic wound healing. This is a much more complicated task as not only has the wound stalled in the proinflammatory phase but there are many additional problems that are not found in acute wounds, each of which need to be targeted. Development of wound healing material that can react to an anomaly and release drugs to correct (but retain them if they are not required) will be the future challenge for treating chronic wounds. Such materials could target a single or multiple anomalies by utilizing different release triggers generated by the anomaly.

Declaration of interest

None to declare.

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