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Development of novel microenvironments for promoting enhanced wound healing

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Abstract

Purpose of Review—Nonhealing wounds are a significant issue facing the healthcare industry. Materials that modulate the wound microenvironment have the potential to improve healing outcomes.

Recent Findings—A variety of acellular and cellular scaffolds have been developed for regulating the wound microenvironment, including materials for controlled release of antimicrobials and growth factors, materials with inherent immunomodulative properties, and novel colloidal-based scaffolds. Scaffold construction methods include electrospinning, 3D printing, decellularization of extracellular matrix, or a combination of techniques. Material application methods include layering or injecting at the wound site.

Summary—Though these techniques show promise for repairing wounds, all material strategies thus far struggle to induce regeneration of features such as sweat glands and hair follicles. Nonetheless, innovative technologies currently in the research phase may facilitate future attainment of these features. Novel methods and materials are constantly arising for the development of microenvironments for enhanced wound healing.

Keywords

Wound microenvironment; Wound modulation; Chronic wounds; Biomaterials; Biomaterials for wound healing; Novel wound healing materials

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Introduction

Degenerative nonhealing wounds are a significant issue in the medical industry. A retrospective study of chronic wounds using data collected in 2014 determined that 14.5% of Medicare beneficiaries in the United States, or 8.2 million patients, were diagnosed with at least one wound or wound-related infection. This resulted in an average annual wound care cost of $2.4 billion and a midrange healthcare cost estimate of $31.7 billion that year[1,2]. In an effort to repair damaged dermal tissue, healthcare providers desire wound dressings, artificial skin substitutes, or alternative means of protecting the wound from infection while enhancing the body's natural wound healing mechanisms. For this reason, several strategies for wound repair have been developed, including controlled-release materials for antimicrobial and growth factor delivery, various hydrogels and scaffolds produced via electrospinning and 3D printing, materials derived from cell extracellular membranes, and nanomaterial and colloidal approaches. In this review, we discuss the wound healing cascade and methods by which biomaterials can be used to modulate the wound microenvironment. We additionally describe traditional and emergent materials used to enhance the natural wound healing process.

Introduction to Skin Physiology and the Wound Healing Process

The skin is composed of three sections arranged in order from exterior to interior: the epidermis, dermis, and hypodermis (Figure 1). The epidermis is the protective outer barrier primarily formed from keratinocytes, which are cells that range from cuboidal to flattened in shape and are tightly-packed. This layer is stratified into four sections from exterior to interior: the stratum corneum, stratum granulosum, stratum spinosum, and stratum basale. Once keratinocyte progenitor cells differentiate in the stratum basale, keratinocytes continue to progress outwards through the layers as they mature until cell death occurs in the stratum corneum, where the cells become the body's protective exterior layer[3–7]. The dermal layer determines the mechanical properties of the skin and is comprised primarily of extracellular matrix (ECM), the fibroblast cells which secrete the ECM and growth factors, and immune cells. It is divided into the papillary, subpapillary, and reticular layers, and is separated from the epidermis by a basement membrane[4,5,7–10]. The hypodermis is composed of adipocyte cells in a fatty layer, and is primarily used for cushioning and insulation of the body[4,8,11]. Upon injury, the structural integrity of the skin is compromised and must be repaired via the wound healing process.

Any breach in the skin barrier necessitates wound healing. While damaged tissue from acute punctures to the epidermis such as cuts and minor burns can be repaired efficiently, damage to the dermal layer can result in the formation of disorganized scar tissue. Additionally, large or infected dermal abrasions, large burns, ulcers, or certain pathologies that inhibit cell migration such as diabetes, can result in the development of chronic non-healing wounds from acute wounds[7,12,13]. Typically, wound healing is a process characterized by four stages: hemostasis, inflammation, cell proliferation and reepithelialization, and tissue maturation and remodeling[7,14]. When there is damage to the skin (Figure 2), bleeding occurs and the coagulation cascade is activated, promoting primary and secondary hemostasis.

Primary hemostasis is the process by which blood flow to the wound area is blocked by a temporary platelet “plug” formed by platelets that are activated by von Willebrand factor (VWF) at injured vessel walls. Platelets are anuclear blood cells derived from bone marrow megakaryocytes which circulate in an inactive state until activated by VWF[15–17]. Once platelets deform and spread along the vascular wall and aggregate to occlude the wound site, secondary hemostasis occurs to create a fibrin-rich clot. During this process, culmination of the coagulation cascade leads to thrombin enzyme generation/activation: thrombin cleaves fibrinogen into fibrin strands, and fibrin is crosslinked to the platelet “plug” to form a complete clot for hemostasis[16,17]. Platelet-fibrin interactions play a critical role in determining clot properties and, in turn, the hemostatic and pro-healing properties of the wound microenvironment. Via actin/myosin-generated contractile mechanisms, platelets pull on the fibrin strands in the clot through a process known as clot retraction, which decreases clot surface area, increases clot stability, and promotes blood flow to carry nutrients to the healing tissue[16,18,19]. Inflammation also occurs shortly after injury, overlapping temporally with hemostasis, where immune cells such as neutrophils and macrophages are recruited to clean the wound site of bacteria and debris and secrete growth factors to stimulate the subsequent cell proliferation phase of wound repair[7,20]. During the cell proliferation phase, fibroblasts from the surrounding tissue are drawn into the clot by macrophage growth factors and secrete ECM, forming replacement connective tissue[4,21]. Re-epithelialization then occurs as fibroblasts, keratinocytes, and other surrounding epithelial cells migrate into the ECM and form rudimentary granulation tissue, while neovascularization simultaneously occurs to provide nutrient and waste transport to the wound site[4,7,22–24]. The maturation phase then ensues, where fibroblasts in the granulation tissue differentiate into myofibroblasts, which contract the wound tissue and generate copious quantities of ECM, thereby replacing the predominantly collagen type III matrix with mechanically stronger type I collagen[4,20].

The myriad processes involved in wound healing provide numerous opportunities to engineer the microenvironment and promote greater healing efficiency. Materials can be used to introduce growth factors, stem cells, antimicrobial agents, structural ECM, or active ECM modifiers to the wound site[7]. Some materials are also designed to enhance aspects of the wound healing cascade by delivering necessary proteins for thrombus development or replacing components with artificial substitutes. Moderation of wound pH, temperature, oxygen and carbon dioxide composition, microbe and cell content, and hydration are also critical considerations for enhancement of the natural wound healing process[4]. In subsequent sections, we provide an overview of material-based strategies for engineering the wound microenvironment to promote enhanced healing.

Overview of Biomaterial Strategies for Promoting Wound Repair

Just as there are a vast array of steps in the wound healing process, there are myriad strategies in development to enhance these steps to enable efficient wound microenvironment modulation and improve wound closure rates. This includes materials employed for controlled release to prevent infection and promote wound healing, such as hydrogel scaffolds and nanomaterials, and development of means for producing these

materials, such as electrospinning, 3D bioprinting, and cell and tissue-derived production. These methods will be detailed in the following sections.

Controlled Release Materials

The human body employs a plethora of bioactive molecules to initiate and regulate the wound healing cascade. Controlled release materials enable the delivery of concentrated doses of these molecules to the wound site at a tunable rate, rather than in a single bolus injection. Proteins such as growth factors facilitate various steps along the wound healing cascade and have been extensively incorporated into controlled release materials for wound healing. Immunomodulatory and antimicrobial agents have also been incorporated into scaffolds to prevent bacterial proliferation and the formation of biofilms at the wound site, as well as to reduce the immunogenic response elicited by implantation of the therapeutic material[25]. These various controlled release molecules are detailed here.

There are several families of growth factors (Table 1) that have significant effects upon wound healing time and success rates, including Epidermal Growth Factor (EGF), Fibroblast Growth Factor (FGF), Transforming Growth Factor-β (TGF-β), Platelet Derived Growth Factor (PDGF), and Vascular Endothelial Growth Factor (VEGF). For the EGF family, primary wound healing proteins include EGF, Transforming Growth Factor-α (TGF-α), and Heparin Binding EGF (EGF-HB), all of which bind EGF receptors (EGFRs) to increase keratinocyte migration and proliferation in the wound site[26–30]. The FGF family for wound healing primarily consists of FGF-2, which promotes migration of keratinocytes and fibroblasts and stimulates the production of ECM components, as well as FGF-7 and FGF-10, which increase migration and proliferation of keratinocytes and participate in the reduction of harmful reactive oxygen species (ROS) in the wound site[30–34]. Within the TGF-β family, TGF-β1 is the primary wound healing molecule and has a significant role in inflammation, angiogenesis, and reepithelialization[30,35,36]. Activins and Bone Morphogenic Proteins (BMPs) are other members of the TGF-β family, which influence expression of growth factors in dermal fibroblasts and keratinocyte differentiation[30,37–39]. PDGF has a role in each phase of the wound healing cycle by stimulating inflammatory cell and fibroblast migration to the wound site upon injury, promoting angiogenesis and vessel maturation, upregulating critical reepithelialization factors, and upregulating collagen remodeling factors[29,30,40–44]. As for the VEGF family, VEGF-A stimulates angiogenesis via endothelial cell migration and proliferation[30,45–47], VEGF-C recruits hematopoietic and inflammatory cells to the wound site and stimulates angiogenesis[30,48,49], and Placental Growth Factor (PLGF) promotes granulation tissue formation, maturation, and vascularization[50,51]. In chronic wounds, the concentrations of these factors are decreased with respect to a healthy individual, and their delivery to the wound site is therefore believed to be of potential benefit to a patient[30]. Several methods of incorporating these growth factors into scaffolds have been proposed to combat half-life and clearance issues, including direct addition of factors during scaffold production, loading of factors in solution into scaffolds via absorption, encapsulating factors into nanoparticles, and conjugating factors to scaffold crosslinkers[52].

Regarding controlled release of immunoregulatory molecules, popular materials include honey, chitosan, silver nanoparticles and ions, copper, zinc, antibiotics, and antiseptics[7,53]. Honey has been found to dehydrate bacteria and extract wound exudate, while chitosan interrupts the phospholipid bilayer of the bacterial cell wall and silver is thought to interrupt the DNA replication process and cause destruction of the bacterial plasma membrane[7,54–56]. Zinc has demonstrated antibacterial capabilities and promotes keratinocyte migration, while copper promotes fibroblast proliferation[53,57,58]. Common antibiotics include minocyclin, vancomycin, ciprofloxacin, and streptomycin, while antiseptics such as chlorhexidine, hydrogen peroxide, and poly(hexamethylene) biguanide hydrochloride (PHMB) are used[53,59–65]. Often, these molecules are core components of the scaffold itself or loaded into scaffolds via similar means as the growth factors above for direct administration and controlled release into the wound site, where they enact immunomodulatory, microbicidal, or pro-angiogenic and pro-cell viability effects. At times, combinations of molecules are delivered as a multifaceted approach to combating infection and chronic wounds simultaneously.

Hydrogels

Hydrogels are a commonly used scaffolding material in wound healing that can be directly applied to the wound or in some cases injected into tissue for self-assembly[4]. Hydrogels are hydrophilic three dimensional networks of crosslinked polymers that swell extensively in water, are suitable for use in all stages of the wound healing process, are composed of a large array of bioinert substances, and are capable of being loaded with controlled release materials[7,66]. Hydrogels are formed by crosslinking polymers into a 3-dimensional gel matrix through physical or chemical means, which improves their strength, resistance to degradation, and tunability of mechanical properties to the specific environment in which they will be used. Physical crosslinking entails utilizing the polymer's reversible solid-gel transition phase, entangling polymer chains, hydrogen bonding, hydrophobic interaction, and crystallite formation[66,67]. Chemical crosslinking is non-reversible and involves the covalent binding of the polymers using a crosslinking agent, as well as various other polymerization approaches[7,67]. Some popular natural polymers for the development of pro-healing hydrogels include alginate, chitosan, gelatin, collagen, hyaluronic acid (HA), and cellulose, while synthetics include polyethylene glycol (PEG), polyvinyl alcohol (PVA), polynipam (pNIPAm), and polystyrene (PS)[16,66].

Nanomaterials

Nanomaterials, such as nanofibers and nanoparticles, are increasingly popular materials for use in drug delivery and scaffolding for wound repair. Utilizing materials with nanoscale features enables direct material-to-cell interaction mechanisms which may not be possible with larger-scale materials. Scaffolds and particles can be loaded with therapeutic molecules for controlled release approaches[68].

Nanofibers can be constructed from many of the same materials as hydrogels to obtain natural biodegradable, synthetic, and hybrid characteristics in an effort to mimic ECM in the native tissue environment and exhibit adjustable porosity. The nanofiber physical

characteristics and fiber alignment have a distinct impact upon immune response, angiogenesis in the wound site, and cell viability, proliferation, and migration[68]. The most common production method for these fibers is the process of electrospinning, where in short, a voltage is generated over a polymeric solution as it is being ejected from a syringe and forces droplets into a Taylor cone shape and down into a collector, where the polymers are then elongated, dried, cooled, and spun into fibers[7,69]. Electrospinning enables tight control over fiber alignment and spatial arrangement, and the morphology of the resultant fiber can be edited by adjusting the concentration of the polymer, polymer type(s) used, collector electric field, temperature, and humidity[7,68–72]. Unlike hydrogels however, many electrospun nanofiber scaffolds are unable to absorb sufficient quantities of wound exudate and maintain a moist environment conducive to wound healing[7]. Beyond electrospinning, plant and bacterial cellulose fibers and self-assembling peptides can be used as nanofiber materials for wound repair[7]. Despite their organic origin, these forms of cellulose have both been found to not exhibit immunologic effects when utilized as scaffolding material[73,74].

There are a number of different nanoparticle strategies in development for the delivery of therapeutic molecules into the wound site, with benefits such as controlled and targeted drug delivery, protection of molecules from degradation, decreased toxicity, and easy integration with other wound healing techniques[68,75]. Nanoparticle types include: micelles, self-assembled core-shell structures; polymeric nanoparticles composed of biodegradable polymers; nanoemulsions, nanometric droplets dispersed into another immiscible liquid; liposomes, spherical lipid bilayer structures; cyclodextrins, hollow particles of cyclic oligosaccharides from glucopyranose; and inorganic nanoparticles, composed of metals[68]. Application of these nanoparticle types in wound healing have been recently reviewed by Alberti *et al*[68]. Beyond use for drug delivery, nanoparticles can be used to recapitulate cellular properties involved in the wound healing cascade, as evidenced by Brown *et al.*'s Platelet-like-Particles[16].

Some strategies involve hybrid scaffolds that consist of hydrogels and microparticles in combination[76]. For instance, scaffolds have been developed that are composed of microgels crosslinked together to form highly degradable, injectable scaffolds. Zhou *et al.* investigated this idea by mixing degradable PEG-lactic acid microgels with non-degradable PEG microgels in multiple ratios for injection, self-assembly into a scaffold, and tunable degradation. Microgels were fabricated at 100%, 75%, 50%, 25%, and 0% biodegradability and characterized to find that a higher biodegradable polymer composition degrades faster, fibroblasts encapsulated in the gels during the production process experienced roughly the same viability in each scaffold at 14 days, and that a slower degradation rate improved cell proliferation *in vitro*[77]. Riley *et al.* discusses injectable hydrogel microparticles (HMPs) which jam above a specific packing fraction into a bulk product, forming a granular scaffold. HMPs having a diameter greater than 10μm and a packing fraction of 0.58 self-assemble into these granular hydrogels, which switch from a liquid to a solid once injected and are not required to gel or crosslink *in vivo*[78]. Darling *et al.* demonstrates this process using injectable, HA-based spatially patterned hydrogel microparticles. The granular gel had adjustable layering and orientation capabilities *in vitro* and *in vivo* post-injection, and researchers discovered that layering gels in a specific arrangement in the syringe prior to injection can allow for a single injection to achieve complex hydrogel fabrication

arrangements in an *in vivo* mouse model[79]. A more detailed review of colloidal-based scaffolds for wound healing is discussed in later sections. Overall, components with nanoscale features have shown much promise towards the creation of pro-healing materials, as they allow for excellent control over structures on similar length scales as cell-ECM interactions.

3D Bioprinting

While nanofibers and nanoparticles can be used to control nanoscale features presented to cells, traditional scaffold fabrication from these building blocks, such as electrospinning or simple particle mixing of nanoparticles with bulk hydrogels, have major limitations in patterning. 3D bioprinting, which is an additive manufacturing technology that facilitates controlled deposition of living cells, biomaterials and factors into three dimensional scaffolds, can be used to gain spatial control over patterning of these building blocks[80,81]. Bioprinting is a premier scaffold construction technique which can be used to fabricate the interconnected architecture of the epidermis, dermis, and hypodermis, while simultaneously ejecting the pertinent cells of each layer into the necessary spatial arrangement[80,82]. Kim *et al.* utilized minced, decellularized porcine skin tissue as a basis for their bioink and incorporated human neonatal dermal fibroblasts to the ink prior to printing a 3D construct. Once gelled, human keratinocytes were ejected on the top layer of the construct to form a monolayer. After a period of seven days, the cell viability of the construct was found to be 90%, and quantities of collagen, GAGs, and hyaluronic acid were similar to that of native skin, though elastin and DNA concentrations were substantially lower. Compared to a collagen skin construct, the bioink construct demonstrated significantly lower contraction after 10 days in culture, greater epidermal thickness, and increased ECM secretion. For *in-vivo* evaluation, the group loaded their construct with either adipose-derived stem cells (ASCs), endothelial progenitor cells (EPCs), or both simultaneously, and applied their constructs to a mouse wound model. The combined bioink-ASC-EPC construct demonstrated vastly improved wound closure effectiveness at 93% closure after 21 days compared to a buffer control at 70% closure. Altogether, the group demonstrated the viability of a 3D printing bioink to support the development of a full-thickness skin substitute[83].

There are four primary printing methods that have been used for creating pro-healing materials, including inkjet, extrusion, laser-assisted, and Digital Light Processing (DLP)[80,84]. Synthetic polymers such as polycaprolactone (PCL) and poly(lactic-co-glycolic) acid (PLGA), and natural polymers such as chitosan, collagen, and fibrin, have previously been used as bioinks for 3D bioprinting[85,86], however much room for improvement in biologically active bioinks still remains. Some other drawbacks of 3D bioprinting are that the cell types capable of being printed in appropriate quantities are still largely in development, creating vasculature in a short time for patient-specific needs is a challenge, and current techniques lack the addition of hair follicles, sweat glands, and other features from natural skin, though this is being investigated by the addition of stem cells to scaffolds[80,87,88].

Cell and Tissue-Derived Scaffolds

Beyond the bottom-up type approaches described thus far, top down approaches to create biologic-derived scaffolds either from cell culture or whole tissue-derived scaffolds have also shown great promise for directing wound healing responses. Such materials typically take the form of ECM that is decellularized to remove immunogenic material, while leaving the physiological scaffolding structure intact. Such scaffolds can be obtained by decellularizing cell-derived ECMs or entire tissues/organs. Different tissues have different structural compositions and morphologies, and therefore decellularized tissues from one organ may not be conducive to the growth of cells from another organ[89]. A decellularized ECM from an organ with a basement membrane and mechanical properties similar to those of the different layers of skin, or decellularized skin itself, can therefore be seeded with skin progenitor cells and used as a scaffold for wound regeneration with off-the-shelf capabilities[14,90].

Tissue sources for decellularization can be xenogeneic, allogeneic, or even autologous, and all cellular components that can incite an immune response must be removed from the ECM scaffold prior to application[89,91]. This is typically done through a combination of physical and chemical means, whereby a chemical such as a surfactant, hypotonic or hypertonic solution, or biological agent (enzyme) is used to disrupt membranes, lyse cells, and remove a majority of genetic material, and then a physical treatment such as freeze-drying is applied to remove the remaining material[92,93]. Upon completion of decellularization protocols, it is important to analyze the decellularized matrix to ensure that it has not been damaged by processing, as some chemical and physical treatments have been shown to harm certain types of ECM[89]. If the matrix is found to be structurally intact, a patient's progenitor cells can be reseeded into the ECM in a bioreactor and directed to differentiate into target cells that repopulate the matrix, manipulate the structure into the necessary skin conformation, and endow it with the patient's immune signature[94]. Some commercial decellularized pro-healing products include AlloDerm, FlexHD, and Matriderm[95].

Commercial Wound Dressings

There are numerous commercially available wound dressings and scaffolds on the market utilizing several technologies reviewed here for the treatment of wounds, including living skin substitutes, acellular naturally-derived scaffolds, and biopolymeric scaffolds. Some popular living skin substitutes include Apligraf (Novartis), a bovine collagen matrix cultured with neonatal fibroblasts and keratinocytes for partial and full thickness wounds; Dermagraft (Organogenesis), neonatal fibroblasts cryopreserved on a polyglactin scaffold; and Theraskin (Solsys Medical), cryopreserved skin harvested from cadavers[96–98]. Some examples of acellular naturally-derived scaffolds include Promogran (Acelity), a freeze-dried collagen and cellulose composite material for enhancing cell migration; Integra (Integra), a collagen, glycosaminoglycan, and silicone scaffold that promotes regeneration while being biodegradable; and DermACELL (LifeNet Health), a decellularized dermal allograft to support cell ingrowth[97–99]. Finally, commonly used biopolymeric scaffolds include Talymed (Marine Polymer Technologies), shortened N-acetyl glucosamine fibers from algae for cell migration stimulation, and Hyalomatrix (Medline), hyaluronic and silicone membrane that is

biodegradable[98]. Commercially available options are sure to increase in coming years as the novel material approaches described in this review become mature technologies.

Novel Directions for Modulating the Wound Microenvironment

The technologies described thus far provide an overview of many of the methods by which the wound microenvironment may be engineered to promote enhanced healing. Within these more established fields, there are numerous novel materials and methods in development that have the potential to shape the regenerative medicine field, including the use of bioactive scaffolds, hydrogels for cell delivery, and colloidal-based materials. Recent significant advancements include Microporous Annealed Particle hydrogels for injection and *in vivo* crosslinking[76], artificial microgels which recapitulate platelet function *in vivo*[19,100,101], and injectable pH-switchable scaffolds[102]. The following sections will detail specific examples in these areas which have been demonstrated to be of potential benefit to wound reepithelialization and tissue remodeling.

Bacterial Implications on Scaffold Design

Consideration of bacteria in wounds as well as the potential to harness bacteria themselves for production of pro-healing scaffold material highlight the key role of bacteria in scaffold design for modulating wound microenvironments. Several bioactive scaffolds of various compositions are currently in development, with significant categories including those with antibacterial qualities, major structural components designed from chitosan and bacterial cellulose, and other hydrogels fabricated from a vast array of components. Some scaffolds are injectable and others have been developed to be layered directly onto the wound, some utilize controlled therapy release and others rely on native tissue stimulation from the scaffold, but all are designed to elicit beneficial tissue repair responses once placed into the wound when compared to traditional dressing methods.

Antibacterial Scaffolds

An array of antimicrobial scaffolds has been recently devised which demonstrate wound healing capabilities while preventing the development of biofilms and infection at the wound site[102–105]. A number of materials have been employed for use as scaffolding components, which exhibit high levels of biocompatibility and incorporate metals and proteins to prevent the proliferation of various species of bacteria. Hydrogel scaffolds composed of collagen, alginate, or a combination of hyaluronic acid (HA) and corn silk extract (CSE) were loaded with gallium ions or silver particles, and cell viability and antimicrobial capability were assessed. For the collagen-gallium scaffold, a concentration of 0.025% gallium had an antibacterial potency of >90% against *S. aureus* and *P aeruginosa* and a slight adverse impact on cell proliferation *in vitro* compared to the control group, while *in vivo* tests in rats demonstrated a marked decrease in bacterial CFUs over a seven day duration compared to the control[105]. For the injectable collagen and alginate gels loaded with either silver salt formulation lactate or saccharinate, *in vitro* results demonstrated that both collagen-silver scaffolds and alginate-silver scaffolds were effective against *S. epidermitis* and *P. aeruginosa* bacteria compared to the control, though alginate-silver scaffolds were far more dose-dependent. Cell viability was heightened at lower silver doses, with alginate hydrogels

generally exhibiting superior performance to collagen[103]. The thermosensitive (CSE) and HA gels loaded with silver nanoparticles exhibited a strong antimicrobial effect at silver concentrations ≥1.7 μg/mL against *E. coli*, *S. Aureus*, *B. subtilis*, and *P Aeruginosa*, and *in vitro* cell viability was 100% for silver-containing hydrogels at 24 hours (same as control) but dropped to 80% viability at 72 hours (lower than control)[104]. These results indicate that metallic antimicrobials may have minor adverse effects upon cell viability but overall exhibit strong protection from wound infection.

An interesting technology developed by Wang *et al.* involves the development of a self-assembled antimicrobial octapeptide, IKFQFHFD (Ac-Leu-Lys-Phe-Gln-Phe-His-Phe-Asp-NH_2), which can be used to produce a pH-switchable hydrogel. The hydrogel itself has been engineered to be biocompatible at neutral pH and have antimicrobial properties in acidic pH, while also having the capability of being loaded with biomaterials for wound healing applications. This hydrogel was tested against *S. aureus*, *E. coli*, *P. aeruginosa*, and *B. subtilis*, and shown to have strong antimicrobial features at low pH. Biocompatibility was also analyzed using a live/dead assay, and cell viability was determined to be 84% at pH 5.5 and 95% at pH 7.4. Used alone, the IKFQFHFD hydrogel functioned poorly to eradicate a fully formed MRSA biofilm, but when loaded with a photothermal dye and irradiated to induce heat damage, the hydrogel-dye-radiation combination was found to be sufficient for destruction of the biofilm, with the hydrogel acting as a controlled-release material with change in pH. The group confirmed the feasibility of the hydrogel on an *in vivo* diabetic mouse wound model by testing the combined hydrogel loaded with the cypate dye and proline, an amino acid associated with wound healing and radiation treatment, and found that the technique significantly reduced wound healing time compared to control groups, reaching 100% closure around day 20 compared to around 45% closure for the PBS control on the same day[102].

Naturally-derived chitosan scaffolds have also been used extensively as antimicrobial scaffolds to promote healing. Chitosan is a natural scaffolding material derived from the exoskeletons of arthropods. Recent advancements have seen the utilization of chitosan as a major co-component of hydrogels for conferring antibacterial and cell proliferative qualities, and gels have been developed for loading, injection, and dressing purposes. Researchers have conjugated chitosan with pluronic 123 polymer and loaded it with curcumin or combined it with HA and a catechol-loaded terpolymer for controlled release, and have crosslinked it with gelatin and combined it with lactose-crosslinked gelatin to produce a two-layered structure. *In vitro* experiments for each type of scaffold revealed that cell viability at 3 days was at least 90% for those including chitosan. *In vivo* experimentation was carried out in mice, rats, and *ex vivo* human skin explants, and resulted in some scaffolds performing better than the control groups, such as the curcumin-loaded gel[106] and catechol-loaded terpolymer[107], while the gelatincrosslinked[108] scaffold performed more poorly than the controls. These results indicate that while chitosan-mixed scaffolds may function well in an *in vitro* environment, further experimentation must be conducted on their use in *in vivo* situations.

Bacterial Cellulose Scaffolds

While the quest for antibacterial molecules and scaffolding materials continues, novel applications for leveraging bacterial products for scaffolding have also arisen, the most predominant of which is bacterial cellulose. Bacterial cellulose is a naturally-derived material used as a combination material in scaffolding due to its biodegradability, biocompatibility, and high mechanical strength. It has recently been combined with copolymer P(3HB/4HB), a polyhydroxyalkanoate (PHA) derived from microbes and having similar qualities to bacterial cellulose itself, to develop scaffolds for managing burns[109]. It was also recently conjugated with resveratrol, a non-cytotoxic naturally-derived chemical that was thought to reduce inflammation and stimulate angiogenesis[110]. Both studies demonstrated favorable results with the use of bacterial cellulose as a scaffolding material, but according to *in vivo* experimental results in mouse models, the resveratrol-loaded bacterial cellulose scaffold may have inhibited wound reepithelialization compared to the scaffold alone by day 14, with 10% of the wound remaining without resveratrol and 20% remaining with resveratrol. P(3HB/4HB) combined with bacterial cellulose exhibited vastly improved wound healing by day 14 and when combined with fibroblasts, the wound was nearly completely sealed by this time. These results demonstrate that bacterial cellulose is a viable natural scaffolding material for wound healing that can exhibit loading potential for advanced therapeutics[109,110].

Cell Delivery Scaffolds

Many of the scaffolds discussed thus far can be used alone or in combination with cell or cell-derived factor delivery to enhance healing outcomes. Common strategies include delivering fibroblasts, keratinocytes, stem cells, and platelet rich plasma (PRP) via scaffolds directly to the wound site. As previously discussed, fibroblasts and keratinocytes are primary cell types of the skin layers that produce ECM and form the protective physical outer layer of the skin, and are therefore important for delivery. Stem cells are also critical to wound healing applications and can be found in all three layers of the skin, though they are primarily located in the epidermis[4]. Platelet-rich plasma is another cell-rich product that can be delivered in scaffolds to exhibit wound healing potential. PRP is obtained from centrifugation of autologous patient blood and contains a high concentration of platelets and growth factors beneficial to wound reepithelialization[111]. PRP regulates inflammation, stimulates angiogenesis, aids in remodeling of tissue, is cost-effective, and non-immunogenic for autologous use[111–113]. PRP can be delivered topically or in scaffolds and dressings[111].

Many types of stem cells have been investigated for use in pro-healing scaffolds. Adult stem cells, which can be derived from hair follicle, epidermis, and mesenchymal stem cells (MSCs), are popular selections and have been shown to secrete various growth factors and cytokines that aid in the wound repair process[114,115]. These stem cell sources can differentiate into keratinocytes[116]. When delivered into the proper environment that has correct mechanical characteristics, temperature, pH, and oxygen concentration that recapitulate the skin microenvironment, stem cells can be induced to differentiate into target skin cells[4]. However, delivery of stem cells without a carrier material has been shown to

result in low retention of cells at the wound site and a high loss of viability. Therefore, much effort has recently been dedicated to optimizing scaffold design for stem cell delivery/retention at the wound site via methods such as encapsulation and topical application. For example, a scaffold for encapsulation and delivery of MSCs in a thermo-sensitive chitosan/glycerol phosphate sodium/cellulose nanocrystal hydrogel was recently devised by Xu *et al.* This material combination enabled the formation of a viable, injectable, self-healing and self-assembling scaffold *in vivo*, while encapsulating MSCs for direct delivery into the wound site. The scaffold demonstrated similar cell viability and wound closure rates to the control group, reduction of inflammation, and promotion of angiogenesis[117].

Another injectable scaffold, developed by García *et al.*, activated the immunomodulatory functions of encapsulated MSCs by tethering interferon gamma (IFN-γ), a pro-inflammatory stimulus, to a PEG hydrogel. The IFN-γ has been found to cause MSCs to secrete immunoactive factors including indoleamine, programmed death ligand-1 (PD-L1), and prostaglandin E2 (PGE2) in a process called licensing. Encapsulated MSCs were found to secrete significantly increased concentrations of immunoactive factors in scaffolds with IFN-γ compared to scaffolds without after four days in culture, and *in vivo* studies in immunodeficient and immunocompetent mouse intestinal mucosal wounds five days after treatment with the hydrogel demonstrated significant wound closure rate increases over controls such as hydrogels with MSCs but without IFN-γ, hydrogels alone, MSCs alone, and no treatment. Also, wounds examined after four weeks showed the continued presence of MSCs at the wound site[118]. While these studies were performed using intestinal mucosal wounds, similar approaches could be useful for modulating the healing environment in skin wounds.

Scaffolds for stem cell delivery which are topically applied have also been described. Huang *et al.* produced a 3D bioprinted PCl and bacterial cellulose scaffold seeded with MSCs, thereby creating a skin substitute. The mechanical properties were found to be similar to those of native human skin, cells seeded onto the scaffold showed a 99% viability after three days, cells demonstrated a paracrine effect *in vitro* for factor release, and *in vivo* results indicated a much greater wound closure rate in 10 days using the artificial skin (95%) compared to PBC scaffold (75%) and no wound coverage (58%)[119]. Together, these scaffolds represent multiple means of delivering MSCs into a wound and demonstrate the influence these cells have on wound healing kinetics compared to acellular scaffolds of the same materials.

Colloidal-Based Wound Healing Materials

A number of colloidal-based materials have been produced which modify the wound microenvironment, either via delivery of bactericidal and growth factors using nanoparticle approaches, or by using the nanoparticles themselves as a direct method of bolstering the natural wound healing process. These treatments can be applied directly to the skin or injected into the wound site with minimal toxicity to the patient.

As previously discussed, growth factors and bactericidal chemicals are valuable components for drug delivery to wound sites, which may have a significant impact on wound closure

rates, cell viability, and cell proliferation. Some labs have developed nanoparticles for delivery of these components, including bactericidal terbium oxide (Tb_4O_7) nanoparticles and antimicrobial and growth factor-delivering PLGA nanoparticles[120,121]. The Tb_4O_7 NPs were found to have intrinsic oxidase-like activity at acidic pH levels and behave as enzymes (nanozymes), while the PLGA NPs encapsulated VEGF, basic fibroblast growth factor (bFGF), and antimicrobial peptide (AMP) K4, and were designed to protect their cargo from enzymatic degradation. The Tb_4O_7 NPs reduced the survival of *S. aureus* and *E. coli* to 10% at a concentration of 100 μg/mL and 50 μg/mL respectively, while PLGA-K4 NPs reduced survival of *S. aureus* and gram-negative *P. Aeruginosa* to 40% and 30% respectively. *In vitro* viability of HUVECs was shown to be >80% for all concentrations of Tb_4O_7 NPs used, with <20% of wound area and <5% of bacterial concentration remaining after day five compared to PBS treatment in mice, while PLGA-K4 NPs showed improved *in vivo* cell viability and 24 hour scratch-wound migration rates over PLGA-NP control, though the antibacterial K4 appeared to counteract the effects of the growth factor slightly.

Another popular colloidal material for wound healing is bioactive glass, which exhibits antibacterial and angiogenic properties. Some labs have complexed silver and gold with bioactive glass to produce micro and nano-particles for both injection and topical application for therapeutic purposes. In one case, silver was incorporated directly into glass nanoparticles, and these particles were shown to cause a nearly five-orders-of-magnitude decrease in CFU concentration of *S. aureus* compared to no treatment, while no effect was shown against *P. aeruginosa* in a 3D tissue engineered infected skin model. Cell metabolic rate was shown to be the same between the experimental group and the control group, indicating that there was no *in vitro* cytotoxicity[122]. In another case, Mârza *et al.* produced an ointment by encapsulating gold nanoparticles in glass and milling into a powder, which was then added to Vaseline. The resultant ointment was then tested for cell viability of keratinocytes *in vitro,* and demonstrated over 100% viability after 24 hours, indicating that cell proliferation occurred. For *in vivo* studies, ointment was topically applied on days 0, 3, and 7, and BG-Vaseline and BG-AUNP-Vaseline groups performed equally as well in mouse wound closure testing and exhibited more rapid wound closure rates early in the treatment process than the control of no treatment[123]. These uses of bioactive glass as colloids for enhanced wound regeneration demonstrate its promise as an effective synthetic wound microenvironment modulator.

Other colloidal-based wound therapies focus on injection of colloids directly at the wound site to form a scaffold, which incites the body's cells to modify the scaffold structure. One approach takes advantage of several of the technologies previously described, including *in vivo* microgel self-assembly and use of MSCs, which were co-injected with the scaffold. Koh *et al.* developed mechanically tunable PEG-based Microporous Annealed Particle (MAP) microgels which could be injected and enzymatically crosslinked *in vivo*, creating pores between particles capable of entrapping and protecting MSCs that were co-injected with the microgels, as well as oxygen and nutrients necessary for cell proliferation (Figure 3). After performing the injection in mice and comparing with a nonporous scaffold and PBS-only treatments, MAP/MSC scaffolds demonstrated enhanced cell retention, migration, and proliferation at the scaffold site, proving this co-injection system to be a useful cell delivery and scaffolding method[124]. Leveraging MAP technology, Isaac *et al.* produced a

PEG MAP hydrogel via electrospinning which can be annealed *in situ* using bio-orthogonal tetrazine click chemistry (TzMAP hydrogel). A tetrazine-norbornene reaction can be activated by injecting PEG-norbornene microgels and PEG-di-tetrazene crosslinker into the wound site and allowing a 3D scaffold to form. The group found that this material could act as a controlled-release material for PDGF-BB, and cell viability data collected 24 hours after encapsulating periodontal ligament stem cells into the hydrogels during the microporous annealing step *in vitro* was determined to be 87%[125]. These experiments demonstrate the potential of the MAP technology for the advancement of colloidal-based wound healing approaches. Another synthetic colloidal approach involves the construction of ultralow crosslinked (ULC) poly(N-isopropyl-acrylamide) (pNIPAm) microgels to create synthetic platelet-like particles (PLPs) for simulating platelet interactions in the wound environment. As previously described, platelets are a critical component of the wound healing cascade and aid in hemostasis and clot retraction. Nandi *et al.* detailed the process of developing these PLPs and found that the ULC base particle is capable of deforming like an activated platelet, ULCs conjugated to fibrin-targeting antibodies to form PLPs are capable of generating contractile forces and enhancing fibroblast migration *in vitro*, and PLPs demonstrate enhanced wound closure rates *in vivo* over saline controls[101]. Additional studies have corroborated these results, as well as exhibited the antimicrobial activity of these PLPs when complexed with colloidal gold[19,100]. Another similar colloidal technology produced by Muhamed *et al.* is injectable fibrin-based nanoparticles (FBNs) which have similar porosity and composition to the native clot structure and are designed to be integrated into clot assembly. *In vitro* analysis showed that FBNs support fibrin scaffold formation and increase scaffold formation rates, while *in vivo* analysis in mice indicated that growth factors could be covalently bound to FBNs to increase wound closure rates over controls[126]. Colloidal-based wound healing therapies are an innovative and evolving component of microenvironment moderation, offering an alternative approach to traditional scaffold development therapies. Colloids allow for flexibility in material choices, high tunability of the underlying material mechanical properties, and delivery of therapeutic molecules to the wound site. They have the ability to combine many of the properties overviewed in previous sections of this review, increasing therapeutic potential.

Conclusions and Future Outlook

Biomaterials are critical tools to overcoming chronic nonhealing wounds, which account for a significant portion of annual medical costs and patient morbidity. This review has highlighted several strategies currently under investigation for combating wounds and moderating the wound microenvironment, including wound dressings, hydrogels, nanomaterials, and scaffolds produced via electrospinning, 3D printing, and acellular materials. Emerging strategies for modulating the wound environment, including novel methods for enhancing delivery of MSCs into the wound, various antimicrobial and growth factor biomolecules for addition to dressings and scaffolds, and novel colloidal scaffold-based approaches were discussed.

Despite the vast array of materials developed for this issue, there are still areas requiring further investigation. Materials have managed to replicate the basic characteristics of skin, but incorporation of advanced features such as hair, hair follicles and their stem cells, and

sweat glands into artificial dermal scaffolds has yet to be successfully achieved. Scaffolding materials such as chitosan also require further investigation, as their *in vivo* viability may require further testing and analysis. Novel technologies such as injectable self-assembling scaffolds, granular jamming microparticle hydrogels, and MAP hydrogels offer a promising outlook for the advancement of the field. Additionally, combinations of existing and emerging technologies, such as bioprinting of microparticle hydrogels and the inclusion of drug-eluting nanoparticles into bulk hydrogels and/or electrospun fibers to create composite materials, offer a myriad of options for expanding the possibilities of wound microenvironment modification. Overall, biomaterials strategies for modulating the wound microenvironment have the potential to extensively improve treatment outcomes for patients with chronic non-healing wounds.

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References

1. Nussbaum SR et al. An Economic Evaluation of the Impact, Cost, and Medicare Policy Implications of Chronic Nonhealing Wounds. Value Heal 21, 27–32 (2018).
2. Sen CK Human Wounds and Its Burden: An Updated Compendium of Estimates. Adv. Wound Care 8, 39–48 (2019).
3. Zhong SP, Zhang YZ & Lim CT Tissue scaffolds for skin wound healing and dermal reconstruction. Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology (2010). doi:10.1002/wnan.100
4. Castaño O, Pérez-Amodio S, Navarro-Requena C, Mateos-Timoneda MÁ & Engel E Instructive microenvironments in skin wound healing: Biomaterials as signal releasing platforms. Adv. Drug Deliv. Rev. 129, 95–117 (2018). [PubMed: 29627369] Offers a comprehensive overview of the wound healing process, important growth factors for promoting tissue repair and regeneration, and currently used commercial tissue products.
5. Mathes SH, Ruffner H & Graf-Hausner U The use of skin models in drug development. Advanced Drug Delivery Reviews (2014). doi:10.1016/j.addr.2013.12.006
6. Bouwstra JA & Honeywell-Nguyen PL Skin structure and mode of action of vesicles. in Advanced Drug Delivery Reviews (2002). doi:10.1016/S0169-409X(02)00114-X
7. Son YJ et al. Biomaterials and controlled release strategy for epithelial wound healing. Biomater. Sci. (2019). doi:10.1039/c9bm00456d
8. Gilaberte Y, Prieto-Torres L, Pastushenko I & Juarranz Á Anatomy and Function of the Skin. in Nanoscience in Dermatology (2016). doi:10.1016/B978-0-12-802926-8.00001-X
9. Jones I, Currie L & Martin R A guide to biological skin substitutes. British Journal of Plastic Surgery (2002). doi:10.1054/bjps.2002.3800
10. Madison KC Barrier function of the skin: 'La Raison d'Être' of the epidermis. J. Invest. Dermatol. (2003). doi:10.1046/j.1523-1747.2003.12359.x
11. Fenner J & Clark RAF Anatomy, Physiology, Histology, and Immunohistochemistry of Human Skin. in Skin Tissue Engineering and Regenerative Medicine (2016). doi:10.1016/B978-0-12-801654-1.00001-2
12. Nuschke A Activity of mesenchymal stem cells in therapies for chronic skin wound healing. Organogenesis (2014). doi:10.4161/org.27405
13. Martin P & Nunan R Cellular and molecular mechanisms of repair in acute and chronic wound healing. Br. J. Dermatol. 173, 370–378 (2015). [PubMed: 26175283]

14. Xue M & Jackson CJ Extracellular Matrix Reorganization During Wound Healing and Its Impact on Abnormal Scarring. Adv. Wound Care (2015). doi:10.1089/wound.2013.0485
15. Haji-Valizadeh H, Modery-Pawlowski CL & Sen Gupta A A factor VIII-derived peptide enables von Willebrand factor (VWF)-binding of artificial platelet nanoconstructs without interfering with VWF-adhesion of natural platelets. Nanoscale 6, 4765–4773 (2014). [PubMed: 24658160]
16. Nandi S & Brown AC Platelet-mimetic strategies for modulating the wound environment and inflammatory responses. Exp. Biol. Med. 241, 1138–1148 (2016).
17. Yau JW, Teoh H & Verma S Endothelial cell control of thrombosis. BMC Cardiovascular Disorders (2015). doi:10.1186/s12872-015-0124-z
18. Cines DB et al. Clot contraction: Compression of erythrocytes into tightly packed polyhedra and redistribution of platelets and fibrin. Blood 123, 1596–1603 (2014). [PubMed: 24335500]
19. Sproul EP, Nandi S, Roosa C, Schreck L & Brown AC Biomimetic Microgels with Controllable Deformability Improve Healing Outcomes. Advanced Biosystems 2, (2018).
20. Sanon S, Hart DA & Tredget EE Molecular and Cellular Biology of Wound Healing and Skin Regeneration. in Skin Tissue Engineering and Regenerative Medicine (2016). doi:10.1016/B978-0-12-801654-1.00002-4
21. P. B. Wound healing and the role of fibroblasts. J. Wound Care (2013). doi:10.12968/jowc.2013.22.8.407
22. Goh ET, Kirby G, Rajadas J, Liang XJ & Tan A Accelerated Wound Healing Using Nanoparticles. in Nanoscience in Dermatology (2016). doi:10.1016/B978-0-12-802926-8.00023-9
23. Kumar P et al. Role of angiogenesis and angiogenic factors in acute and chronic wound healing. Plast. Aesthetic Res. (2015). doi:10.4103/2347-9264.165438
24. Reinke JM & Sorg H Wound repair and regeneration. Eur. Surg. Res. 49, 35–43 (2012). [PubMed: 22797712]
25. Demidova-Rice TN, Hamblin MR & Herman I Acute and impaired wound healing: pathophysiology and current methods for drug delivery, part 2: role of growth factors in normal and pathological wound healing: therapeutic potential and methods of delivery. Adv. Ski. wound care 25, 304–314 (2012).
26. Martin P Wound healing - Aiming for perfect skin regeneration. Science (80-.). (1997). doi:10.1126/science.276.5309.75
27. McCawley LJ, O'Brien P & Hudson LG Epidermal growth factor (EGF)- and scatter factor/hepatocyte growth factor (SF/HGF)mediated keratinocyte migration is coincident with induction of matrix metalloproteinase (MMP)-9. J. Cell. Physiol. 176, 255–265 (1998). [PubMed: 9648913]
28. Hudson LG & McCawley LJ Contributions of the epidermal growth factor receptor to keratinocyte motility. Microscopy Research and Technique (1998). doi:10.1002/(SICI)1097-0029(19981201)43:5<444::AID-JEMT10>3.0.CO;2-C
29. Rheinwald JG & Green H Epidermal growth factor and the multiplication of cultured human epidermal keratinocytes. Nature (1977). doi:10.1038/265421a0
30. Barrientos S, Stojadinovic O, Golinko MS, Brem H & Tomic-Canic M Growth factors and cytokines in wound healing. Wound Repair and Regeneration (2008). doi:10.1111/j.1524-475X.2008.00410.x
31. Grellner W, Georg T & Wilske J Quantitative analysis of proinflammatory cytokines (IL-1β, IL-6, TNF-α) in human skin wounds. in Forensic Science International (2000). doi:10.1016/S0379-0738(00)00218-8
32. Sogabe Y, Abe M, Yokoyama Y & Ishikawa O Basic fibroblast growth factor stimulates human keratinocyte motility by Rac activation. Wound Repair Regen. (2006). doi:10.1111/j.1743-6109.2006.00143.x
33. Di Vita G et al. Cytokines and growth factors in wound drainage fluid from patients undergoing incisional hernia repair. Wound Repair Regen. (2006). doi:10.1111/j.1743-6109.2006.00120.x
34. Sivamani RK, Garcia MS & Rivkah Isseroff R Wound re-epithelialization: Modulating keratinocyte migration in wound healing. Frontiers in Bioscience (2007). doi:10.2741/2277
35. Kane CJM, Hebda PA, Mansbridge JN & Hanawalt PC Direct evidence for spatial and temporal regulation of transforming growth factor β1 expression during cutaneous wound healing. J. Cell. Physiol. (1991). doi:10.1002/jcp.1041480119

36. Kopecki Z et al. Collagen loss and impaired wound healing is associated with c-Myb deficiency. J. Pathol. (2007). doi:10.1002/path.2113
37. McDonnell MA, Law BK, Serra R & Moses HL Antagonistic effects of TGF/β1 and BMP-6 on skin keratinocyte differentiation. Exp. Cell Res. 263, 265–273 (2001). [PubMed: 11161725]
38. D'Souza SJA, Pajak A, Balazsi K & Dagnino L Ca2+ and BMP-6 Signaling Regulate E2F during Epidermal Keratinocyte Differentiation. J. Biol. Chem. (2001). doi:10.1074/jbc.M100780200
39. Seishima M et al. Activin A induces terminal differentiation of cultured human keratinocytes. J. Invest. Dermatol. (1999). doi:10.1046/j.1523-1747.1999.00558.x
40. Jinnin M et al. Regulation of fibrogenic/fibrolytic genes by platelet-derived growth factor C, a novel growth factor, in human dermal fibroblasts. J. Cell. Physiol. 202, 510–517 (2005). [PubMed: 15389578]
41. Heldin CH & Westermark B Mechanism of action and in vivo role of platelet-derived growth factor. Physiological Reviews (1999). doi:10.1152/physrev.1999.79.4.1283
42. Lindahl P, Johansson BR, Levéen P & Betsholtz C Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. Science (80-.). (1997). doi:10.1126/science.277.5323.242
43. Sundberg C, Branting M, Gerdin B & Rubin K 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. (1997).
44. Sanjay K, Ly QP, Rothman VL & Tuszynski GP Thrombospondin-1 promotes proliferative healing through stabilization of PDGF. J. Surg. Res. (2002).
45. Suzuma K, Takagi H, Otani A & Honda Y Hypoxia and vascular endothelial growth factor stimulate angiogenic integrin expression in bovine retinal microvascular endothelial cells. Investig. Ophthalmol. Vis. Sci. (1998).
46. Senger DR et al. Stimulation of endothelial cell migration by vascular permeability factor/vascular endothelial growth factor through cooperative mechanisms involving the α(v)β3 integrin, osteopontin, and thrombin. Am. J. Pathol. (1996).
47. Pepper MS, Ferrara N, Orci L & Montesano R Potent synergism between vascular endothelial growth factor and basic fibroblast growth factor in the induction of angiogenesis in vitro. Biochem. Biophys. Res. Commun. (1992). doi:10.1016/0006-291X(92)92277-5
48. Witzenbichler B et al. Vascular Endothelial Growth Factor-C (VEGF-C/VEGF-2) Promotes Angiogenesis in the Setting of Tissue Ischemia. Am. J. Pathol. 153, 381–394 (1998). [PubMed: 9708799]
49. Cao Y et al. Vascular endothelial growth factor C induces angiogenesis in vivo. Proc. Natl. Acad. Sci. U. S. A. (1998). doi:10.1073/pnas.95.24.14389
50. Cianfarani F et al. Placenta growth factor in diabetic wound healing: Altered expression and therapeutic potential. Am. J. Pathol. (2006). doi:10.2353/ajpath.2006.051314
51. Carmeliet P et al. Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. Nat. Med. (2001). doi:10.1038/87904
52. Wang Z et al. Novel biomaterial strategies for controlled growth factor delivery for biomedical applications. NPG Asia Mater. 9, e435–17 (2017).
53. Anjana J, Rajan VK, Biswas R & Jayakumar R Controlled Delivery of Bioactive Molecules for the Treatment of Chronic Wounds. Curr. Pharm. Des. 23, 3529–3537 (2017). [PubMed: 28472912]
54. Majtan J Honey: An immunomodulator in wound healing. Wound Repair and Regeneration (2014). doi:10.1111/wrr.12117
55. Feng QL et al. A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. J. Biomed. Mater. Res. (2000). doi:10.1002/1097-4636(20001215)52:4<662::AID-JBM10>3.0.CO;2-3
56. Muñoz-Bonilla A & Fernández-García M Polymeric materials with antimicrobial activity. Progress in Polymer Science (Oxford) (2012). doi:10.1016/j.progpolymsci.2011.08.005
57. Sudheesh Kumar PT et al. Flexible and microporous chitosan hydrogel/nano ZnO composite bandages for wound dressing: In vitro and in vivo evaluation. ACS Appl. Mater. Interfaces (2012). doi:10.1021/am300292v

58. Gopal A, Kant V, Gopalakrishnan A, Tandan SK & Kumar D Chitosan-based copper nanocomposite accelerates healing in excision wound model in rats. Eur. J. Pharmacol. (2014). doi:10.1016/j.ejphar.2014.02.033
59. Payne DN, Babb JR & Bradley CR An evaluation of the suitability of the European suspension test to reflect in vitro activity of antiseptics against clinically significant organisms. Lett. Appl. Microbiol. (1999). doi:10.1046/j.1365-2672.1999.00482.x
60. Llorens E, Calderón S, Del Valle LJ & Puiggalí J Polybiguanide (PHMB) loaded in PLA scaffolds displaying high hydrophobic, biocompatibility and antibacterial properties. Mater. Sci. Eng. C (2015). doi:10.1016/j.msec.2015.01.100
61. Obermeier A et al. Novel high efficient coatings for anti-microbial surgical sutures using chlorhexidine in fatty acid slow-release carrier systems. PLoS One (2014). doi:10.1371/journal.pone.0101426
62. Pawar HV, Tetteh J & Boateng JS Preparation, optimisation and characterisation of novel wound healing film dressings loaded with streptomycin and diclofenac. Colloids Surfaces B Biointerfaces (2013). doi:10.1016/j.colsurfb.2012.08.014
63. Unnithan AR et al. Wound-dressing materials with antibacterial activity from electrospun polyurethane-dextran nanofiber mats containing ciprofloxacin HCl. Carbohydr. Polym. (2012). doi:10.1016/j.carbpol.2012.07.071
64. Japoni A, Farshad S, Ziyacyan M & Ziaian S Detection of Van-positive and negative Vancomycin Resistant Entrococci and their antibacterial susceptibility patterns to the newly introduced antibiotics. Pakistan J. Biol. Sci. (2009). doi:10.3923/pjbs.2009.844.851
65. Aoyagi S, Onishi H & Machida Y Novel chitosan wound dressing loaded with minocycline for the treatment of severe burn wounds. Int. J. Pharm. (2007). doi:10.1016/j.ijpharm.2006.09.016
66. Francesko A, Petkova P & Tzanov T Hydrogel Dressings for Advanced Wound Management. Curr. Med. Chem. 25, 5782–5797 (2019).
67. Maitra J & Shukla VK Cross-linking in Hydrogels - A Review. Am. J. Polym. Sci. 4, 25–31 (2014).
68. Alberti T et al. Nanotechnology: A Promising Tool Towards Wound Healing. Curr. Pharm. Des. 23, 3515–3528 (2017). [PubMed: 28472915] Offers an overview of nanoscale approaches to wound healing, including nanofibers and various nanoparticle advancements.
69. Reneker DH & Yarin AL Electrospinning jets and polymer nanofibers. Polymer (2008). doi:10.1016/j.polymer.2008.02.002
70. Lu W, Sun J & Jiang X Recent advances in electrospinning technology and biomedical applications of electrospun fibers. J. Mater. Chem. B (2014). doi:10.1039/c3tb21478h
71. Ribeiro C, Sencadas V, Ribelles JLG & Lanceros-Méndez S Influence of processing conditions on polymorphism and nanofiber morphology of electroactive poly(vinylidene fluoride) electrospun membranes. Soft Mater (2010). doi:10.1080/1539445X.2010.495630
72. Thompson CJ, Chase GG, Yarin AL & Reneker DH Effects of parameters on nanofiber diameter determined from electrospinning model. Polymer (Guildf). (2007). doi:10.1016/j.polymer.2007.09.017
73. Jang WD, Hwang JH, Kim HU, Ryu JY & Lee SY Bacterial cellulose as an example product for sustainable production and consumption. Microb. Biotechnol. 10, 1181–1185 (2017). [PubMed: 28695653]
74. Barud HS et al. Antimicrobial bacterial cellulose-silver nanoparticles composite membranes. J. Nanomater 2011, (2011).
75. Garcia-Orue I et al. Nanotechnology approaches for skin wound regeneration using drug-delivery systems. in Nanobiomaterials in Soft Tissue Engineering: Applications of Nanobiomaterials (2016). doi:10.1016/B978-0-323-42865-1.00002-7
76. Daly AC, Riley L, Segura T. & Burdick JA Hydrogel microparticles for biomedical applications. Nat. Rev. Mater (2019). doi:10.1038/s41578-019-0148-6Describes the development of Hydrogel MicroParticle technologies (HMPs). HMPs can be formulated in a variety of sizes, can be used to encapsulate biologics, and can self-assemble in vivo into granular hydrogels. This new class of particles is at the forefront of novel therapeutic technologies.

77. Zhou W, Stukel J, Alniemi A & Willits RK Novel microgel-based scaffolds to study the effect of degradability on human dermal fibroblasts. Biomed. Mater 13, (2018).
78. Riley L, Schirmer L & Segura T Granular hydrogels: emergent properties of jammed hydrogel microparticles and their applications in tissue repair and regeneration. Curr. Opin. Biotechnol. 60, 1–8 (2019). [PubMed: 30481603]
79. Darling NJ, Sideris E, Hamada N, Carmichael ST & Segura T Injectable and Spatially Patterned Microporous Annealed Particle (MAP) Hydrogels for Tissue Repair Applications. Adv. Sci 5, 1–8 (2018).Describes the development of patternable granular scaffolds which can be injected and maintain their pattern in vivo. These hydrogels can be used to create physical and chemical gradients and mimic native skin architecture.
80. He P et al. Bioprinting of skin constructs for wound healing. Burn. Trauma 6, 1–10 (2018).
81. Murphy SV & Atala A 3D bioprinting of tissues and organs. Nature Biotechnology (2014). doi:10.1038/nbt.2958
82. Binder K & Skardal A Human Skin Bioprinting: Trajectory and Advances. in Skin Tissue Engineering and Regenerative Medicine (2016). doi:10.1016/B978-0-12-801654-1.00020-6
83. Kim BS et al. 3D cell printing of in vitro stabilized skin model and in vivo pre-vascularized skin patch using tissue-specific extracellular matrix bioink: A step towards advanced skin tissue engineering. Biomaterials 168, 38–53 (2018). [PubMed: 29614431]
84. Zhu W et al. 3D printing of functional biomaterials for tissue engineering. Current Opinion in Biotechnology (2016). doi:10.1016/j.copbio.2016.03.014
85. Tarassoli SP et al. Skin tissue engineering using 3D bioprinting: An evolving research field. J. Plast. Reconstr. Aesthetic Surg 71, 615–623 (2018).
86. Lee V et al. Design and fabrication of human skin by three-dimensional bioprinting. Tissue Eng. - Part C Methods (2014). doi:10.1089/ten.tec.2013.0335
87. Yu H et al. Isolation of a novel population of multipotent adult stem cells from human hair follicles. Am. J. Pathol. (2006). doi:10.2353/ajpath.2006.051170
88. Hassan W, Dong Y & Wang W Encapsulation and 3D culture of human adipose-derived stem cells in an in-situ crosslinked hybrid hydrogel composed of PEG-based hyperbranched copolymer and hyaluronic acid. Stem Cell Res. Ther. (2013). doi:10.1186/scrt182
89. Badylak SF, Freytes DO & Gilbert TW Extracellular matrix as a biological scaffold material: Structure and function. Acta Biomater. 5, 1–13 (2009). [PubMed: 18938117]
90. Brown B, Lindberg K, Reing J, Stolz DB & Badylak SF The basement membrane component of biologic scaffolds derived from extracellular matrix. Tissue Eng. (2006). doi:10.2193/0091-7648(2006)34[519:TNEAEA]2.0.CO;2
91. Erdag G & Morgan JR Allogeneic versus xenogeneic immune reaction to bioengineered skin grafts. Cell Transplant. (2004). doi:10.3727/000000004783983594
92. Cui H, Chai Y & Yu Y Progress in developing decellularized bioscaffolds for enhancing skin construction. J. Biomed. Mater. Res. - Part A 107, 1849–1859 (2019).
93. Gilpin A & Yang Y Decellularization Strategies for Regenerative Medicine: From Processing Techniques to Applications. BioMed Research International (2017). doi:10.1155/2017/9831534
94. Ozguldez HO, Cha J, Hong Y, Koh I & Kim P Nanoengineered, cell-derived extracellular matrix influences ECM-related gene expression of mesenchymal stem cells. Biomater. Res. 22, 1–9 (2018). [PubMed: 29308274]
95. Parmaksiz M, Dogan A, Odabas S, Elçin AE & Elçin YM Clinical applications of decellularized extracellular matrices for tissue engineering and regenerative medicine. Biomed. Mater. 11, (2016).
96. Zaulyanov L & Kirsner RS A review of a bi-layered living cell treatment (Apligraf) in the treatment of venous leg ulcers and diabetic foot ulcers. Clinical interventions in aging (2007). doi:10.2147/ciia.2007.2.1.93
97. Gaspar-Pintiliescu A, Stanciuc A-M & Craciunescu O Natural composite dressings based on collagen, gelatin and plant bioactive compounds for wound healing: A review. Int. J. Biol. Macromol. 138, 854–865 (2019). [PubMed: 31351963]
98. Dickinson LE & Gerecht S Engineered biopolymeric scaffolds for chronic wound healing. Front. Physiol 7, (2016).

99. Gottleib ME, Furman J, Gottlieb ME & Furman J Successful Management and Surgical Closure of Chronic and Pathological Wounds using Integra®. J. Burn. Surg. Wound Care (2004).
100. Sproul EP et al. Development of Biomimetic Antimicrobial Platelet-Like Particles Comprised of Microgel Nanogold Composites. Regen. Eng. Transl. Med. (2019). doi:10.1007/s40883-019-00121-6
101. Nandi S et al. Platelet-like particles dynamically stiffen fibrin matrices and improve wound healing outcomes. Biomater. Sci. 7, 669–682 (2019). [PubMed: 30608063]
102. Wang J et al. pH-Switchable Antimicrobial Nanofiber Networks of Hydrogel Eradicate Biofilm and Rescue Stalled Healing in Chronic Wounds. ACS Nano (2019). doi:10.1021/acsnano.9b05608Describes the production and testing of a self-assembling custom hydrogel wound dressing with antimicrobial properties activated only in acidic environments. The dressing is capable of drug loading and delivery, and of overcoming biofilms in vivo.
103. Ceresa C, Fracchia L, Marchetti A, Rinaldi M & Bosetti M Injectable scaffolds enriched with silver to inhibit bacterial invasion in tissue regeneration. Materials (Basel). 12, (2019).
104. Makvandi P, Ali GW, Della Sala F, Abdel-Fattah WI & Borzacchiello A Biosynthesis and characterization of antibacterial thermosensitive hydrogels based on corn silk extract, hyaluronic acid and nanosilver for potential wound healing. Carbohydr. Polym. 223, 115023 (2019). [PubMed: 31427021]
105. Xu Z et al. Preparation and characterization of a gallium-loaded antimicrobial artificial dermal scaffold. Mater. Sci. Eng. C 105, 110063 (2019).
106. Pham L et al. A dual synergistic of curcumin and gelatin on thermal-responsive hydrogel based on Chitosan-P123 in wound healing application. Biomed. Pharmacother. 117, 0–11 (2019).
107. Puertas-Bartolomé M et al. Bioadhesive functional hydrogels: Controlled release of catechol species with antioxidant and antiinflammatory behavior. Mater. Sci. Eng. C 105, 110040 (2019).
108. Garcia-Orue I et al. Development of Bioinspired Gelatin and Gelatin/Chitosan Bilayer Hydrofilms for Wound Healing. Pharmaceutics 11, 314 (2019).
109. Volova TG, Shumilova AA, Nikolaeva ED, Kirichenko AK & Shishatskaya EI Biotechnological wound dressings based on bacterial cellulose and degradable copolymer P(3HB/4HB). Int. J. Biol. Macromol. 131, 230–240 (2019). [PubMed: 30872059]
110. Meng E et al. Bioapplications of bacterial cellulose polymers conjugated with resveratrol for epithelial defect regeneration. Polymers (Basel). 11, (2019).
111. Chicharro-Alcántara D et al. Platelet rich plasma: New insights for cutaneous wound healing management. J. Funct. Biomater 9, (2018).
112. Lacci KM & Dardik A Platelet-rich plasma: Support for its use in wound healing. Yale Journal of Biology and Medicine (2010).
113. Andia I & Abate M Platelet-rich plasma: Underlying biology and clinical correlates. Regenerative Medicine (2013). doi:10.2217/rme.13.59
114. Dash BC et al. Stem cells and engineered scaffolds for regenerative wound healing. Bioengineering 5, 1–19 (2018).
115. Duscher D et al. Stem Cells in Wound Healing: The Future of Regenerative Medicine? A Mini-Review. Gerontology (2016). doi:10.1159/000381877
116. dos Santos JF, Borçari NR, da Silva Araújo M & Nunes VA Mesenchymal stem cells differentiate into keratinocytes and express epidermal kallikreins: Towards an in vitro model of human epidermis. J. Cell. Biochem. 120, 13141–13155 (2019). [PubMed: 30891818]
117. Xu H et al. Enhanced cutaneous wound healing by functional injectable thermo-sensitive chitosan-based hydrogel encapsulated human umbilical cord-mesenchymal stem cells. Int. J. Biol. Macromol. 137, 433–441 (2019). [PubMed: 31271797]
118. García JR et al. IFN-γ-tethered hydrogels enhance mesenchymal stem cell-based immunomodulation and promote tissue repair. Biomaterials 220, 119403 (2019). [PubMed: 31401468] Discusses the development of an injectable hydrogel suitable for the encapsulation and delivery of mesenchymal stem cells (MSCs) to the wound area. MSCs play important roles in tissue repair and regeneration, and it is therefore crucial that reliable methods of transporting MSCs into a wound with high viability are examined.

119. Huang R et al. The topography of fibrous scaffolds modulates the paracrine function of Ad-MSCs in the regeneration of skin tissues. Biomater. Sci. 7, 4248–4259 (2019). [PubMed: 31393466]
120. Vijayan A, James PP, Nanditha CK & Vinod Kumar GS Multiple cargo deliveries of growth factors and antimicrobial peptide using biodegradable nanopolymer as a potential wound healing system. Int. J. Nanomedicine 14, 2253–2263 (2019). [PubMed: 30992665]
121. Li C et al. Bactericidal effects and accelerated wound healing using Tb 4 O 7 nanoparticles with intrinsic oxidase-like activity. J. Nanobiotechnology 17, 1–10 (2019). [PubMed: 30612562]
122. Zheng K et al. Ag modified mesoporous bioactive glass nanoparticles for enhanced antibacterial activity in 3D infected skin model. Mater. Sci. Eng. C 103, 109764 (2019).
123. Mârza SM et al. Skin wound regeneration with bioactive glass-gold nanoparticles ointment. Biomed. Mater 14, (2019).
124. Koh J et al. Enhanced In Vivo Delivery of Stem Cells using Microporous Annealed Particle Scaffolds. Small 15, 1970208 (2019).
125. Isaac A et al. Microporous Bio-orthogonally Annealed Particle Hydrogels for Tissue Engineering and Regenerative Medicine. (2019). doi:10.1021/acsbiomaterials.9b01205Describes a novel application of the MAP hydrogels described in reference 79. Spontaneous thiol-norbornene click chemistry technology is leveraged to create self-assembling microporous scaffolds. This technology allows for the production of scaffolds with a variety of properties by using functionalized components and loading of cells and therapeutics.
126. Muhamed I, Sproul EP, Ligler FS & Brown AC Fibrin Nanoparticles Coupled with Keratinocyte Growth Factor Enhance the Dermal Wound-Healing Rate. ACS Appl. Mater. Interfaces 11, 3771–3780 (2019). [PubMed: 30604611]

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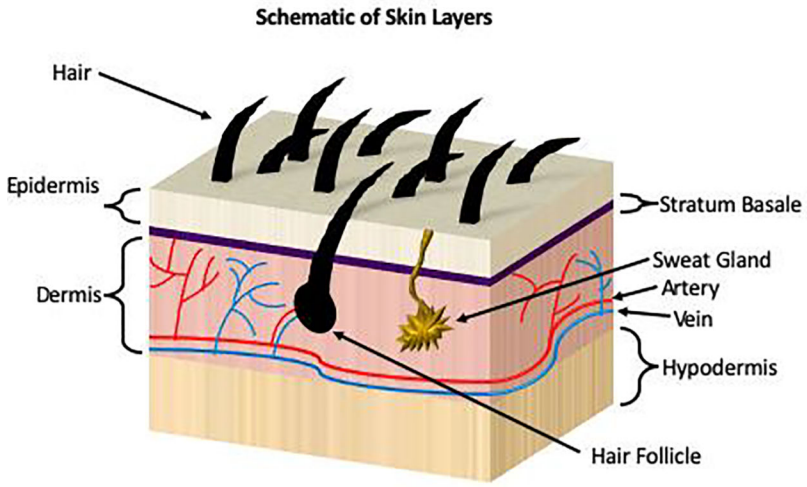

Figure 1:
Schematic of the major skin layers, including the epidermis, basement membrane, dermis, and hypodermis.

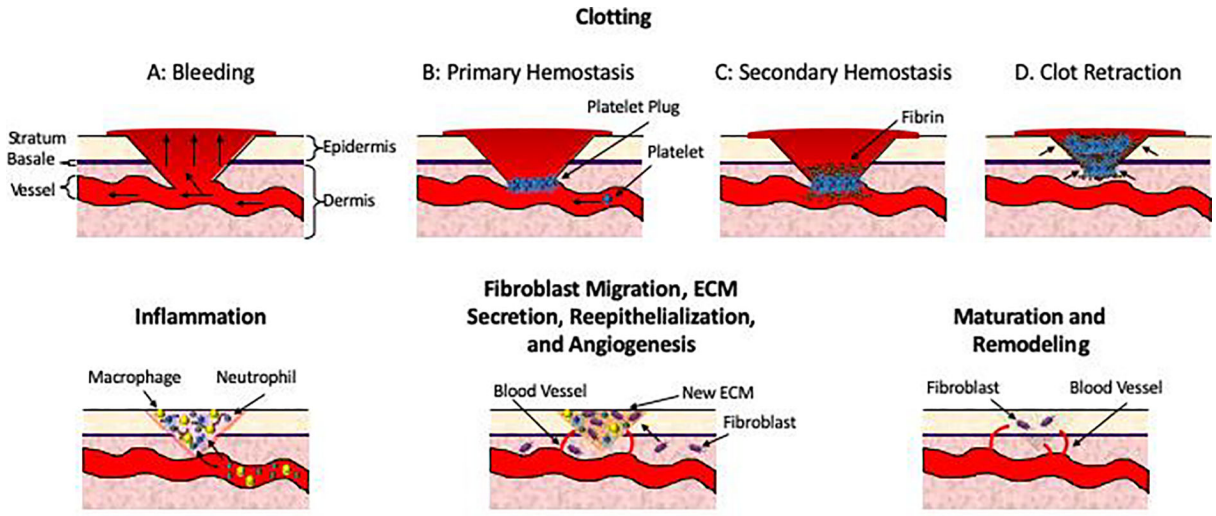

Figure 2:

Schematic of the wound healing process, depicting the stages of clotting, inflammation, fibroblast migration, reepithelialization, and angiogenesis and maturation. These phases overlap temporally to promote repair, replacement, and remodeling of damaged skin tissue. Upon injury, bleeding occurs, and hemostasis follows to stop the bleeding. Primary hemostasis occurs and consists of the formation of a platelet plug to occlude the wound site. Fibrin formed from the cleaving of fibrinogen by thrombin is crosslinked with the platelet plug to form a more robust clot in Secondary Hemostasis, and this clot is later retracted by platelets. Subsequently, inflammation occurs to remove pathogens and damaged material from the wound site, and fibroblasts migrate into the clot to secrete ECM while angiogenesis occurs to bring nutrients into the new tissue. Over longer time scales (weeks/months) this new tissue matures and is remodeled by local cells.

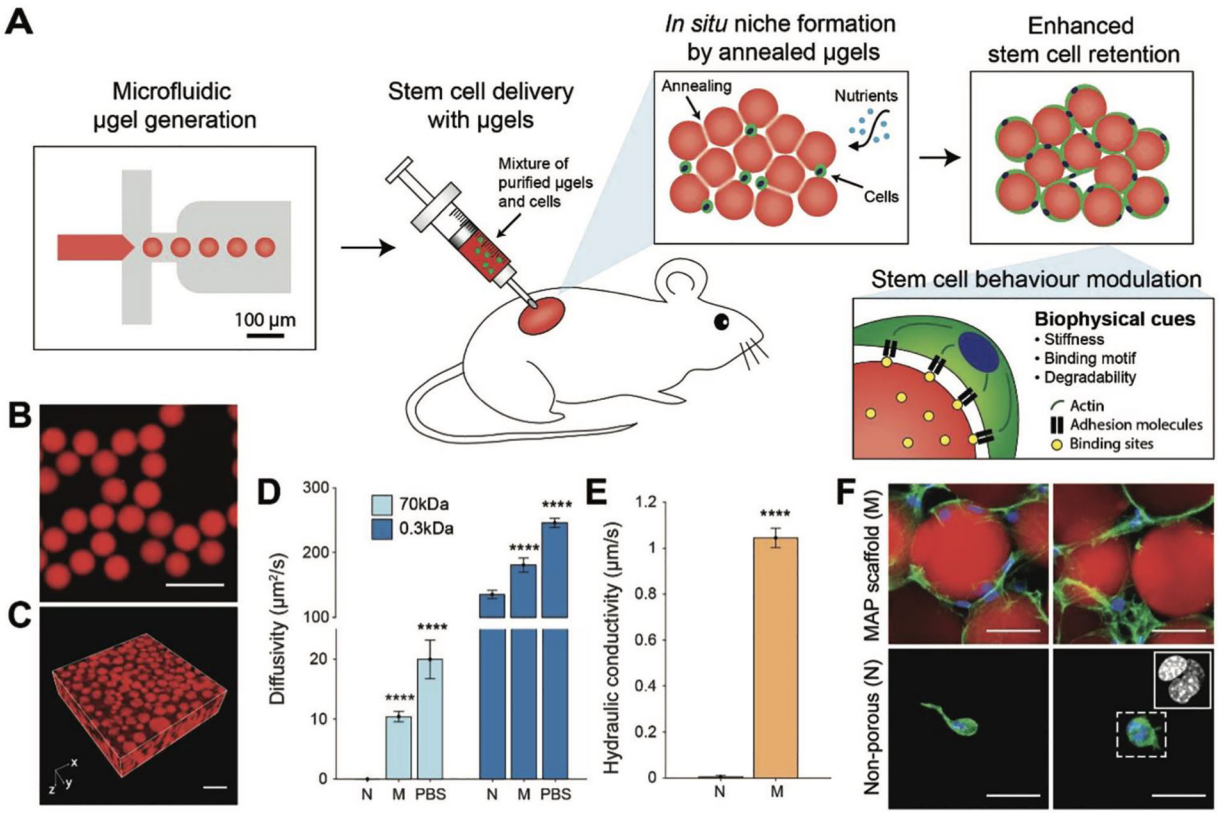

Figure 3:

Mesenchymal stem cell (MSC) delivery and in situ niche creation strategy using annealed monodisperse hydrogel particles. A) An artificial stem cell niche is created in situ by annealing a suspension of injectable monodisperse hydrogel particles. Highly monodisperse particle building blocks enable the generation of a pore network in a highly controllable manner, which promotes the transport of oxygen and nutrients as well as cell migration. The biophysical properties of building blocks are modulated to enhance the functions of the transplanted MSCs. B) Monodisperse hydrogel particles or μgels produced by the microfluidic device. Scale bar: 200 μm. C) Tissue scaffold assembled from monodisperse μgels. Scale bar: 200 μm. D) Diffusivity of 70 kDa dextran and 0.3 kDa FITC in nonporous scaffolds (N), MAP scaffolds (M) and PBS (n =4–7). Data are presented as average ±s.d. Statistical significance based on one-way ANOVA followed by Tukey's HSD post hoc test (significance compared to N, ****p <0.0001). E) Hydraulic conductivity of PBS through the nonporous scaffold (N) and MAP scaffold (M) at atmospheric pressure (n =3). Data are presented as average ±s.d. Statistical significance based on standard two-tailed Student t-test (****p <0.0001). F) Fluorescent images of MSCs in vitro cultured in microporous scaffolds and nonporous scaffolds at week 2. Blue, nucleus; Green, actin; Red, gel. Scale bar: 50 μm. Reproduced from reference 124 Koh, J. *et al.* Enhanced In Vivo Delivery of Stem Cells using Microporous Annealed Particle Scaffolds, *Small* **15**, 1970208, ©Wiley, 2019 with permission from Wiley Online Library, copyright (2019).

Table 1.

Growth Factors and their Effects on Wound Healing

Growth Factor Family	Growth Factor	Effects	Source
Epidermal Growth Factor (EGF)	Epidermal Growth Factor (EGF)	Bind EGF receptors (EGFRs) to increase keratinocyte migration and proliferation in the wound site.	[26, 27, 28, 29, 30]
	Transforming Growth Factor-α (TGF-α)		
	Heparin Binding Epidermal Growth Factor (EGF-HB)		
Fibroblast Growth Factor (FGF)	Fibroblast Growth Factor-2 (FGF-2)	Promotes migration of keratinocytes and fibroblasts, and stimulates the production of ECM components	[30, 31, 32, 33, 34]
	Fibroblast Growth Factor-7 (FGF-7)	Increase migration and proliferation of keratinocytes and participate in the reduction of harmful reactive oxygen species (ROS) in the wound site.	
	Fibroblast Growth Factor-10 (FGF-10)		
Transforming Growth Factor-β (TGF-β)	Transforming Growth Factor-β1 (TGF-β1)	Primary wound healing molecule, has a significant role in inflammation, angiogenesis, and reepithelialization.	[30, 35, 36]
	Activins	Influence expression of growth factors in dermal fibroblasts and keratinocyte differentiation.	[30, 37, 38, 39]
	Bone Morphogenic Proteins (BMPs)		
Platelet Derived Growth Factor (PDGF)	Platelet Derived Growth Factor (PDGF)	Stimulates inflammatory cell and fibroblast migration to the wound site upon injury, promotes angiogenesis and vessel maturation, upregulates critical reepithelialization factors, and upregulates collagen remodeling factors.	[29, 30, 40, 41, 42, 43, 44]
Vascular Endothelial Growth Factor (VEGF)	Vascular Endothelial Growth Factor-A (VEGF-A)	Stimulates angiogenesis via endothelial cell migration and proliferation.	[30, 45, 46, 47]
	Vascular Endothelial Growth Factor-C (VEGF-C)	Recruits hematopoietic and inflammatory cells to the wound site and stimulates angiogenesis.	[30, 48, 49]
	Placental Growth Factor (PLGF)	Promotes granulation tissue formation, maturation, and vascularization.	[50, 51]