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Immunomodulatory Hydrogels: Advanced Regenerative Tools for Diabetic Foot Ulcer

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Diabetic foot ulcer (DFU) is one of the most common complications of diabetes, bringing physical and mental challenges for patients due to the lack of efficient curative therapy. Despite considerable advances in pharmacological and surgical approaches, clinical trials for DFU patients remain disappointing due to the local overactive and excessive inflammation. Immunomodulatory hydrogels has significant advantages to overcome the clinical challenge of DFUs therapy. Here, recent fabrication and regenerative advances in the utilization of functional hydrogels for altering the immune microenvironment of DFUs are comprehensively reviewed. The pathological features and the healing processes of DFUs, followed by summarizing the physicochemical properties essential for the design of regenerative hydrogels for immunomodulation in DFUs, are briefly introduced. Then, the potential immuno-therapeutic modalities of hydrogels and emerging trends used to treat DFUs via multitherapeutic approaches and enhanced efficacy and safety are discussed. Taken together, by linking the structural properties of hydrogels to their functions in DFU therapy with a particular focus on immunomodulatory stimuli, this review can promote further advances in designing advanced hydrogels for DFUs, resulting in improved diabetic wound repair through translation into clinical setting in the near future.

1. Introduction

With the advent of the global aging society, the therapeutic pressure of diabetic foot ulcers (DFUs) is increasingly severe. Around 25% of diabetic patients develop DFUs in their lifetime, and DFUs had become one of the most common and serious complications of diabetes.[1] Excessive inflammatory responses and abnormal immune microenvironments are the main pathological features of diabetic wounds.[2] Wound healing is a complex multicellular and metabolic process, and mainly comprises four overlapping and continuous phases: hemostasis, inflammation, proliferation, and remodeling.[3] In diabetic wounds, the high glucose toxicity, the toxic effect of advanced glycation end-products (AGEs), and the continuously excessive inflammatory immune microenvironment are the three main mechanisms of refractory diabetic

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wounds, and the disturbed immune microenvironment is the most leading factor among the pathological changes of DFUs.[4] The continuous release of inflammatory factors leads to an inflammatory cascade reaction, and the high proinflammatory state reduces collagen deposition, impairs granulation tissue formation and vascular maturation, and thereby impeding the process of diabetic wound healing.

The crosstalk between immune cells and various stem cells provides the "soil environment" for wound healing, among which macrophages are the main factor involved in the regulation of local immune response and the process of wound healing.[5] A huge number of recruited and tissue-colonized macrophages respond to microenvironmental signals and secrete various chemokines, proinflammatory and anti-inflammatory mediators, proteases, cytokines, and extracellular matrix (ECM) components through dynamic and continuous phenotypic differentiation to participate in and regulate various stages of wound healing.[6] Recruited monocyte-derived macrophages and tissue-resident macrophages are the essential key players in response to tissue damage. Refractory diabetic wounds can be exacerbated due to the improper activation of macrophages. Due to the microenvironment stimuli, the M1 macrophages can accumulate in diabetic wounds and impair the transition of immune microenvironment from a proinflammatory to an anti-inflammatory state.[7] Consequently, a highly inflammatory microenvironment will be formed and maintained in the wound site, identified by the increased levels of Interleukin 1 (IL-1) and tumor necrosis factor-α (TNF-α), proinflammatory cytokines produced by M1 macrophages, in diabetic wounds.[8]

Immunomodulatory strategies control the immune responses after tissue damage by providing an anti-inflammatory microenvironment.[9] Recently, biomaterials-based immunomodulation has attracted extensive research interest[10] using hydrogels, which are composed of hydrophilic polymer chains and can replenish water in the wound. The stability of the hydrogel, achieved through their cross-linked network, facilitates its local use in wound management.[11] Compared with other systems, hydrogels can be used in combination with bioactive molecules or cells and have unique advantages in tissue repair and regeneration.[12] The bioactive molecules loaded within hydrogels can be delivered in a sustained manner, thus enhancing the effectiveness of the therapy.[13] Hydrogels can also be used to encapsulate stem cells to maximize their therapeutic potential in mediation of the immune microenvironment and promote epithelialization, ECM production, and maturation, with the ultimate goal of accelerating diabetic wound healing. Currently, the emerging focus is on the significant roles of hydrogel-based immunomodulation strategies for DFUs treatment.[14] For instance, Mei et al.[14] recently reported an injectable photocrosslinking silk hydrogel system for treating the diabetic wound. Mechanistically, the hydrogel system enhanced diabetic-wound healing via spatiotemporal immunomodulation in a diabetic mouse model. In another study, Hauk et al.[15] developed a collagen/hyaluronan-based hydrogel system, which could accelerate diabetic wound healing by suppression of M1 macrophage activation and promotion of M2 macrophage polarization. Despite extensive studies on hydrogel-based immunomodulatory approaches, according to our knowledge, the use of these immunomodulatory functional hydrogels for the treatment of DFUs has not been adequately explored.

Herein, we summarize the advanced strategies developed for accelerating diabetic wound healing based on the utilization of different immunomodulatory hydrogels. First, the pathological features of DFUs and the related immunomodulation strategies are discussed. Second, the cutting-edge knowledge on the required properties for the design of functional hydrogels for immunomodulation in DFUs are reviewed, followed by discussing recently developed therapeutic modalities of functional-hydrogels-based immunomodulation in DFUs. Finally, advanced wound healing technologies with their potential for clinical translation and emerging hydrogel-based immunomodulatory directions for DFU therapy are discussed.

2. The Pathological Features and the Healing Processes of DFUs

2.1. Overview of Wound Repair and Regeneration

Generally, the process of wound repair includes four overlapping and continuous stages: hemostasis, inflammation, proliferation, and remodeling.[16] The complicated process is tightly mediated by multiple cells, and various bioactive factors, cellular molecules, and cytokines that are secreted to enhance wound repair and tissue regeneration.[17] When tissue damage occurs, the hemostasis stage can stop the bleeding and minimize hemorrhage, which is a short period maintained through the contraction of blood vessels, platelet aggregation, and blood clot formation.[18] Furthermore, the inflammation stage occurs immediately after tissue damage, which is crucial in deciding whether the wound could achieve rapid healing or becoming a chronic wound.[19] Multiple inflammatory cells, immune cells, molecules, and chemokines are recruited and activated during the homeostasis and inflammation stages.[20] These bioactive factors also enhance the proliferation, migration, and function of fibroblasts, vascular endothelial cells, and keratinocytes, leading to the initiation of the next stage, which is the proliferation phase.[21] Finally, the neo-vascularization and ECM deposition promote the formation of granulation tissue and provide a new substrate for keratinocyte migration in the remodeling phase for collagen deposition.[22]

2.2. Skin Immune Niche and DFUs

Skin immune niche includes innate and adaptive immune cells that reside across the epidermis and dermis layers, which provide functional interaction with tissue cells, such as fibroblasts, keratinocytes, and vascular endothelial cells to maintain immune homeostasis and promote tissue regeneration (**Figure 1**).[23] Emerging evidence indicates the eminent roles of the immune cells and the immune microenvironment in wound repair and regeneration.[24] Each of the classical wound healing phases of hemostasis, inflammation, proliferation, and remodeling involves immunomodulation.[25] DFUs usually involves immune dysfunctions exemplified by the failed

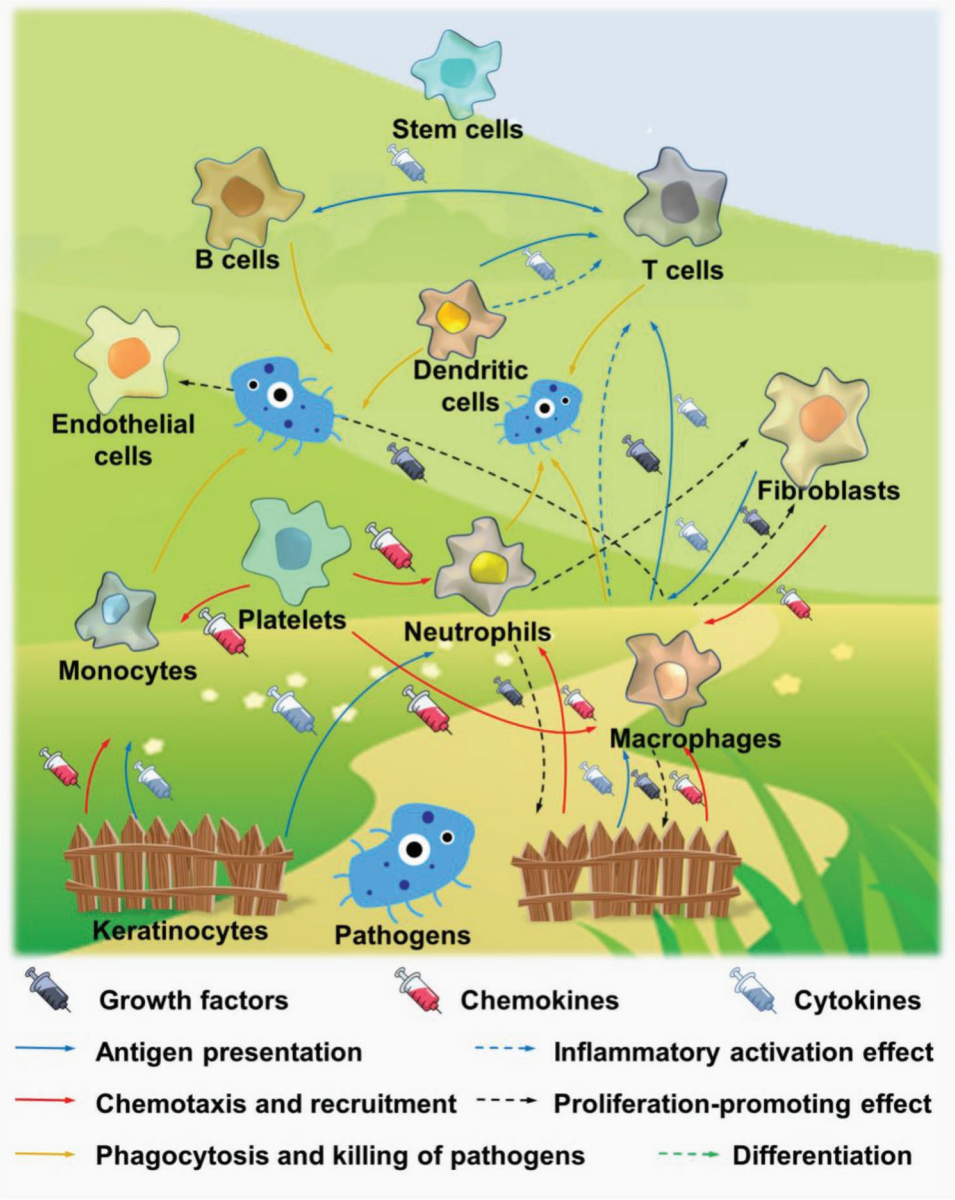

Figure 1. The dynamic immunocytes crosstalk during tissue repair and regeneration. Innate and adaptive immune cells and the tissue functional cells coordinately construct the immune microenvironment to maintain immune homeostasis and promote tissue repair and regeneration through the secretion of bioactive factors and cellular interactions.

transition of macrophages' phenotypes under pathological conditions.[26] The pathophysiological phenomena associated with DFUs include persistent and excessive inflammation, abnormal immune microenvironment, multiple infections, impaired angiogenesis, and delayed re-epithelialization.[27] DFUs are not capable to enter the proliferative stage due to the multiple cellular factors. Promoting the transition of diabetic wounds from the inflammatory stage to the proliferative stage represents a potential strategy for accelerating wound healing.[28] Therefore, altering signaling pathways that accelerate this transition may become therapeutic targets in enhancing tissue regeneration through both immune and nonimmune approaches.

2.3. Hydrogels-Based Immunomodulatory Therapies for DFUs

Tissue regeneration is a complicated and tightly-mediated process and cellular immunomodulation plays a prominent role in the entire process.[29] The concept of the immune microenvironment is initially proposed in the tumor, which is referred to the surrounding microenvironment of cells and the secreted bioactive factors.[29] It was well-documented that tumors can influence their microenvironment by mediating cell signaling transduction to promote tumor angiogenesis and induce immune tolerance, while immune cells in the microenvironment can further influence the growth and function of cancer cells.[30] Currently, the accumulative interest is toward

understanding the role of the immune microenvironment in the mediation of inflammatory diseases, such as oxidative stress damage, Crohn's disease, and DFUs.[31] For example, Song et al. have highlighted the prominent role of the immune microenvironment in the regulation of diabetic wound healing and summarized the advanced therapies targeting immune cells to promote diabetic wound healing.[32] Therefore, the precise therapeutic strategy based on the regulation of the immune microenvironment will bring new ideas for basic research and clinical translation and treatment of DFUs.

During the past decades, bioactive materials are emerging as a promising strategy to eliminate the translational gap by resolving the huge challenges associated with DFUs treatment.[33] Especially, hydrogels are widely utilized to enhance tissue regeneration and play as functional vehicles for drug delivery in preclinical DFUs studies.[34] Our previous studies indicated that the multifunctional hydrogels can load and sustainably release active small molecules or extracellular vesicles to regulate the local immune microenvironment of diabetic wounds, especially mediation of the phenotypic polarization and reprogramming of macrophages, and further regulate the function of vascular endothelial cells or fibroblasts, and ultimately significantly promote the healing of diabetic wounds.[35] As shown in **Figure 2**, the hydrogel systems currently applied for tissue regeneration include hydrogel, hydrogel–cell, hydrogel–molecule, hydrogel–exosome, and combined systems.[36] In comparison with other biomaterials, hydrogels have eminent advantages in terms of their in vivo and environmental toxicity, making functional hydrogels popular and valuable for tissue repair and regeneration.[37]

3. Required Properties of Functional Hydrogels for Immunomodulation in DFUs

3.1. Hydrogels for Immunomodulation Based on the Chemical Properties

It has been revealed that the chemistry and crosslinking methods of hydrogels are involved in regulating the immune response in DFUs.[9] To date, a variety of biomaterials, including natural or synthetic materials, have been used to develop hydrogels for the treatment of DFUs. It is well-recognized that natural materials, such as collagen, hyaluronic acid (HA), chitosan, and alginate, provide significant biocompatibility and cell affinity. On the other hand, synthetic materials, such as poly(vinyl alcohol) (PVA), poly(ethylene glycol) (PEG), poly(vinyl pyrrolidone) (PVP), and polyurethane (PU), are characterized by high homogeneity and controllable degradability.[38] Researchers are constantly attempting to design functional hydrogels with bioactive materials for immunomodulation in DFUs.

Among various natural materials, the decellularized ECM, a mixture of crosslinked bioactive proteins that are able to guide cell migration, proliferation, and differentiation, has exhibited immunomodulatory properties.[39] Recently, a study conducted by Vriend et al. demonstrated that porcine skin-derived ECM hydrogels failed to result in better dermal wound healing, as observed through the immune response and matrix remodeling.[40] The results indicated that the components of the ECM seem too complicated and that the application of ECM hydrogels might induce both proinflammatory and prohealing phenotypes of immune cells. HAs, a major glycosaminoglycan of the ECM, have been widely used to develop hydrogels for DFUs

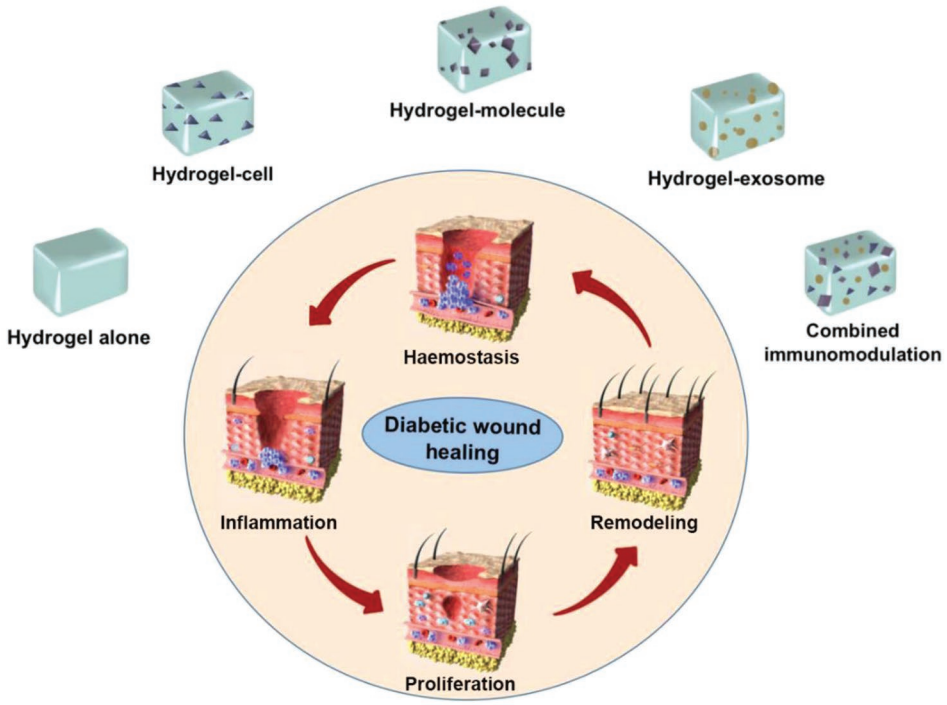

Figure 2. Hydrogel-based therapies are applied in four classical stages of tissue repair and regeneration.

and are reported to play a role in regulating macrophage activation and reprogramming. It is proposed that low molecular weight HA (5 kDa) promoted M1 polarization, as evidenced by enhanced expression of proinflammatory genes, such as *NOS2, TNF*, *IL12b*, and *CD80*, and upregulated secretion of proinflammatory cytokines, such as nitric oxide (NO) and TNF-α. On the other hand, high molecular weight HA (3000 kDa) induced M2 polarization, as confirmed by the upregulation of anti-inflammatory genes, including *ARG1, IL10*, and *MRC1*.[41] In view of the role of high molecular weight HA in regulating immune cell behaviors, it has been widely used to fabricate functional hydrogels for immunomodulation in chronic wounds. For instance, Liu et al. developed a Cu^{2+}/high molecular weight HA/PVA hydrogel to regulate M2 phenotype macrophages for improvement of the immune-microenvironment in the wound bed. The hydrogel was able to polarize M1 macrophages into M2 macrophages and maintain the consistent phenotype of M2 macrophages in the wound site, representing an effective treatment strategy for a DFUs.[42] In a recent study, Xu et al. modified HA methacrylate (HAMA) with phenylboronic acid (PBA) and fabricated a glucose-responsive HAMA-PBA/catechin (HMPC) hydrogel to treat diabetic wounds. The HMPC hydrogel exhibited superior glucose-responsive catechin release behavior, which could effectively scavenge reactive oxygen species (ROS). The developed HMPC hydrogel was also able to promote angiogenesis, and reduce inflammatory responses (as shown by decreased IL-6 levels and increased IL-10 levels), resulting in rapid diabetic wound healing in vivo (**Figure 3**A).[43] In addition, Qin et al. fabricated an HA-gelatin (HA-GEL) hydrogel to mimic the ECM and demonstrated that the hydrogel improved wound healing in diabetic mice by promoting cell survival, adhesion, and proliferation and reducing the level of inflammatory chemokines in the wound bed.[44] Silk fibrin (SF), a natural amino acid polymer, is another promising biomaterial for the development of functional hydrogels for DFUs. The nuclear factor kappa-B (NF-κB) signaling pathway is involved in SF-induced wound healing.[45] The immunomodulatory function of SF has been revealed recently.[46] Chouhan et al.

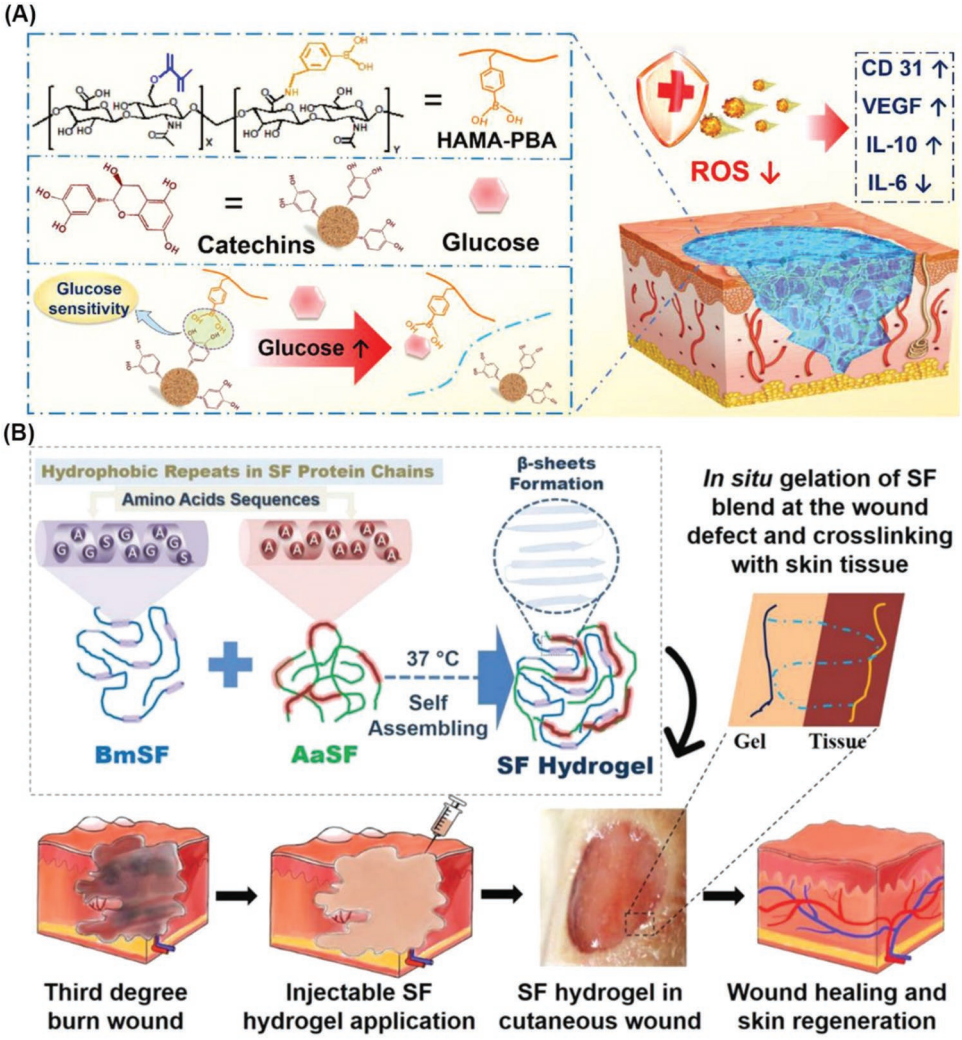

Figure 3. Hydrogel chemistry-based immunomodulation. A) Schematic illustration of the HAMA/PBA/catechin hydrogel for the treatment of diabetic wounds. Reproduced with permission.[43] Copyright 2022, Elsevier. B) Preparation of the SF hydrogels to accelerate wound healing through promotion of the transitions from the inflammation to proliferation stage. Reproduced with permission.[47] Copyright 2018, Wiley-VCH GmbH.

developed in situ forming SF hydrogel by using blends of SF solutions isolated from Bombyx mori and Antheraea assama, which showed inherent self-assembly and crosslinking due to the formation of β-sheet structures. Compared to collagen hydrogels, the SF hydrogel was more favorable to fibroblast proliferation and keratinocyte migration. Moreover, the SF hydrogel was shown to accelerate wound healing through promotion of the transitions from the inflammation to proliferation stage, as confirmed by the downregulation of TNF-α and CD163 expression (Figure 3B).[47]

Recently, several synthetic materials have been shown to have the ability to regulate the immune response. For instance, MXene nanomaterials have drawn the attention of researchers due to their metallic conductivity, hydrophilicity, and high plasticity.[48] Mxene quantum dots (MQDs) provide a more specific surface area and material-cell interactions and have been reported to have immunomodulation ability. Rafieerad et al. demonstrated that the titanium carbide (Ti_3C_2) MXene MQDs selectively inhibited the activation of $CD4^+IFN\text{-}\gamma^+$ T-lymphocytes while promoting the proliferation of immunosuppressive $CD4^+CD25^+FoxP3^+$ regulatory T-cells. Moreover, they incorporated the Ti_3C_2 MQDs into a chitosan-based hydrogel and found that the composite hydrogel showed increased conductivity while maintaining thermosensitivity and injectability.[49] Rafieerad et al. developed tantalum-carbide ($Ta_4C_3T_x$) MQDs and found that these MQDs could be ingested spontaneously into endothelial cells and alter cell surface receptor expression to inhibit the activation of proinflammatory T-lymphocytes.[50] In view of these findings, MXene hydrogels represent a promising strategy for the treatment of DFUs. The application of synthetic polymers for immunomodulation in DFUss has sparked a significant interest. However, the underlying mechanism by which synthetic polymers modulate immune responses remains unclear and needs to be studied in the future. Besides, the synthetic polymers used must be biocompatible and biodegradable to avoid a drastic host defensive response.[51] Therefore, in the near future, increasing research will be carried out to develop more efficient and less expensive synthetic polymer-based hydrogels that can regulate the immune response in the wound site for DFUs healing.

3.2. Hydrogels for Immunomodulation Based on the Physical Properties

Immune responses in the wound area can also be regulated by the physical properties of hydrogels. To investigate the impact of hydrogel stiffness on macrophage polarization, Chen et al. incubated bone marrow-derived macrophages on polyacrylamide hydrogels with different substrate stiffnesses (2.55 ± 0.32 kPa, 34.88 ± 4.22 kPa, and 63.53 ± 5.65 kPa). It was found that the expression of CD206 and secretion of IL-4 and TGF-β were increased in macrophages cultured on hydrogels with medium stiffness compared to those cultured on hydrogels with low stiffness. The underlying mechanism was elucidated, as moderate stiffness could alternatively induce M2 macrophage polarization through the ROS-initiated NF-κB signaling pathway.[52] Coburn et al. also found similar results that the anti-inflammatory activity of macrophages increased with increasing hydrogel stiffness (from 1.11 ± 0.344 kPa to 7.86 ± 0.999 kPa), as observed through the secretion of TNF-α and IL-10.[53] In another study, Choi et al. demonstrated that dendritic cells (DCs) stressed by stiffer hydrogels exhibited decreased migration ability compared to those stressed by soft hydrogels.[54] These results highlighted the significance of hydrogel stiffness and can be used to guide the design of functional hydrogels for immunomodulation in DFUs.

In addition, the porosity and pore size of hydrogels have been shown to modulate the immune response. Hydrogels with a high porosity or larger pore size favor immune cell infiltration.[55] It has been demonstrated that macrophage polarization is also associated with the porosity and pore size of scaffolds. For instance, Li et al.[56] prepared 3D-printed polycaprolactone/PEG/hydroxyapatite (PCL/PEG/HA) scaffolds with different pore sizes (209.9 ± 77.1 μm, 385.5 ± 28.6 μm, and 582.1 ± 27.2 μm) and reported that scaffolds with larger pore sizes significantly reduced the foreign body response and promoted M2 macrophage infiltration (**Figure 4**). In another study, Chen et al. investigated the impact of pore size of poly (2-hydroxyethyl methacrylate) (pHEMA) and poly(dimethylsiloxane) (PDMS) scaffolds on DC maturation and recruitment in vitro and in vivo. They confirmed that scaffolds with smaller pore sizes (20 μm) favored DC activation and enrichment compared with scaffolds with larger pore sizes (90 μm), regardless of the polymer used.[57] However, how the porosity and pore size of hydrogels affect inflammatory reactions remains unclear. Detailed investigation of the underlying mechanisms by which the porosity and pore size of hydrogels regulate immune cell activity is necessary. Meanwhile, the topography of the hydrogels was observed to regulate the macrophage response. Singh et al. conducted a study in which they investigated the impact of micropatterned surfaces of the hydrogel on macrophage activation. They demonstrated that macrophages cultured on microgrooves/ridges and micropillars expressed distinct gene profiles that were related to primary metabolic processes. However, no clear polarization toward M1 or M2 phenotypes was observed.[58] In addition, the impact of other hydrogel surface properties, such as roughness and hydrophilicity, on immune cell behavior also needs further investigation.

It is suggested that the physical properties of immune modulation need to be considered in the development of functional hydrogels for DFUs. However, how physical cues are transferred into intracellular molecular signals which impact immune cell behaviors has not been fully revealed and needs further investigation. Furthermore, whether the effects of the physical properties of hydrogels on immunomodulation in DFUs can be achieved in vivo also remains to be elucidated.

4. Potential Therapeutic Modalities of Functional-Hydrogel-Based Immunomodulation

4.1. Hydrogels Alone

There are lines in the literature showing that hydrogels constituted by various bioactive substances possess pleiotropic effects including immunoregulation against the development of multiple diseases.[59] On account of the dissimilar actions

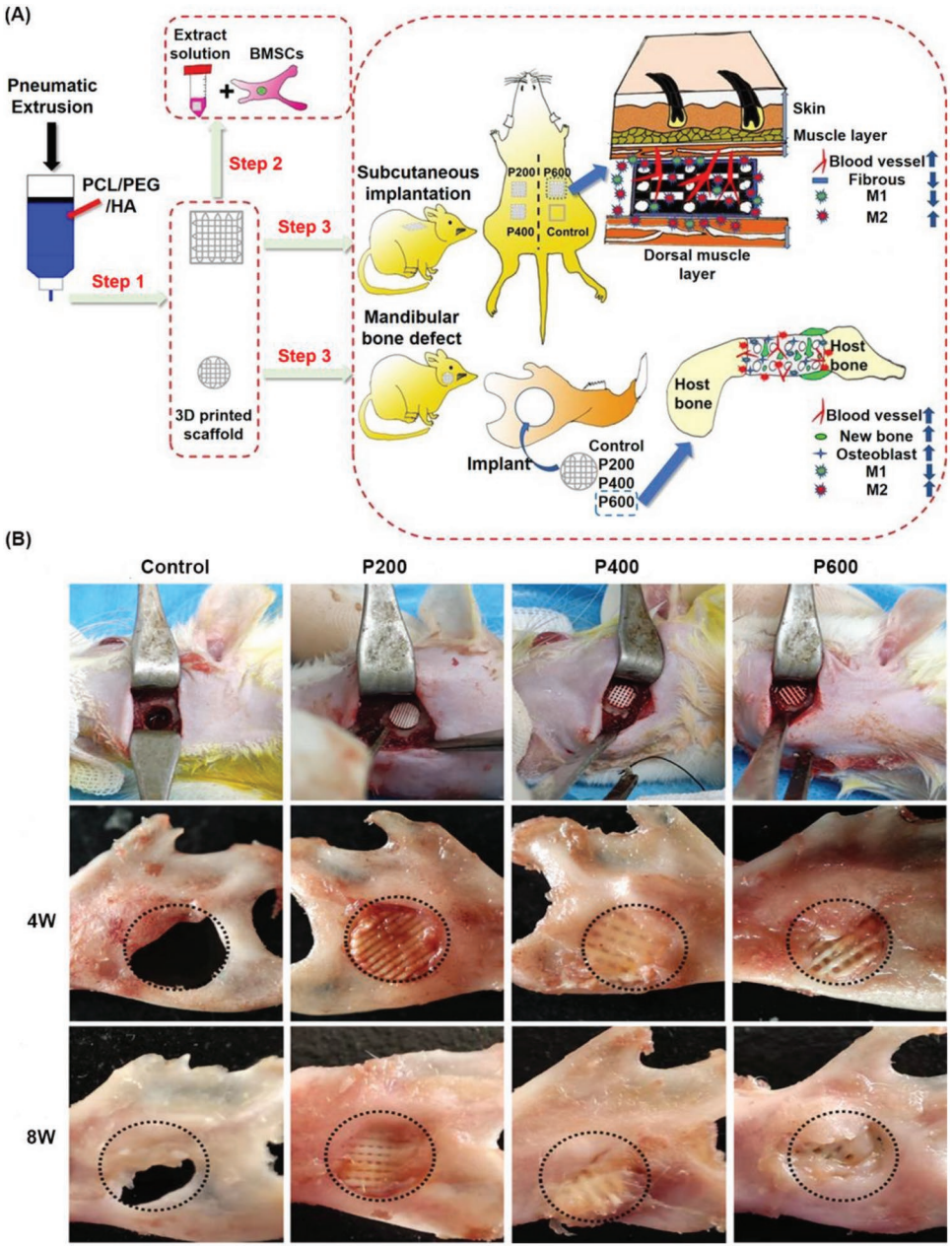

Figure 4. Hydrogels with a high porosity or larger pore size favor immune cell infiltration. A) Schematic representation of preparation of the P600 PCL/PEG/HA scaffold with larger pore size and their beneficial roles in bone regeneration. B) Representative images of scaffolds with different pore sizes (P200, P400, and P600) implanted into the rat mandibular defect area, and the general images of mandibular bone defect samples at 4 and 8 weeks after injury. A,B) Reproduced with permission.[56] Copyright 2018, Wiley-VCH GmbH.

produced by immune responses on the progression of different disorders, the therapeutic benefits of hydrogels could be ascribed to promotive roles or inhibitory functions on the immune system.[60] As it is known that persistent infection and failure of neovascularization, provoked by dysregulated immunological reactions, are deeply implicated in the pathogenesis of DFUs, one pivotal mechanism by which hydrogels exert pro-healing activities is associated with improving the imbalance of immune homeostasis.[61] Hybrid hydrogels consisting of cationic chitosan/anionic dextran and elastin-based hydrogels recruit macrophages and neutrophils in the wound site, which is profitable for eliminating invading pathogens and necrotic tissues (**Figure 5**A,B).[62] Dendritic hydrogel and sodium alginate hydrogel weaken the generation of IL-1β and NO, and facilitate M2 phenotype polarization of macrophages separately to ameliorate local inflammatory responses for enhancing the survival of keratinocytes, fibroblasts, and endothelial cells (ECs).[63] Additionally, certain types of hydrogels have been reported to trigger angiogenic initiation and prohibit scar formation during the process of skin regeneration via increasing M2 macrophage accumulation.[64] Thus, by means of regulating the immune microenvironment of different phases,

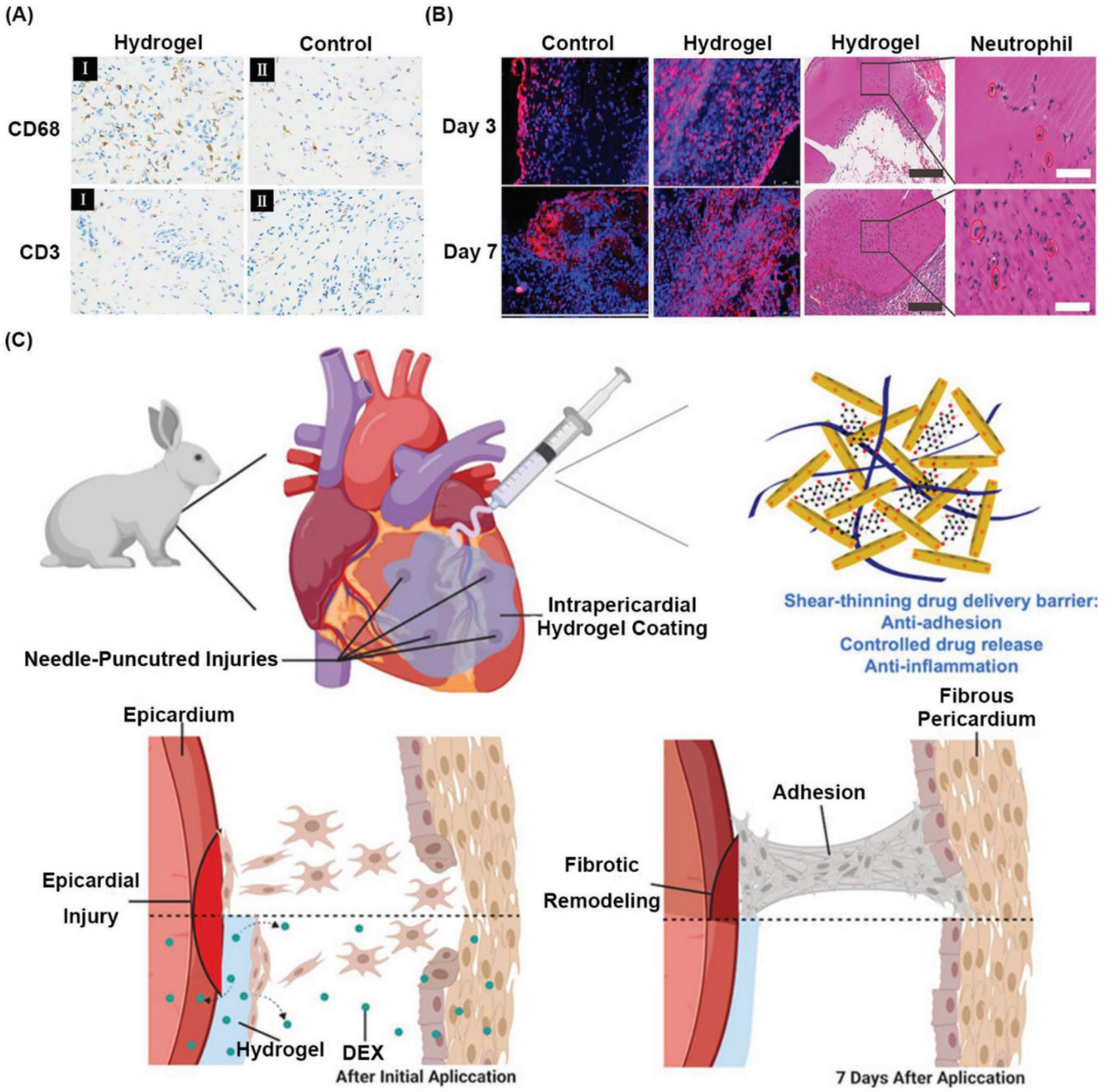

Figure 5. The immunoregulatory roles produced by hydrogels in inflammation alleviation and tissue regeneration. A) CD68+ and CD3+ were chosen as the most reliably specific markers of macrophages and T lymphocytes, respectively, which played a vital function in the healing process. Cationic chitosan/anionic dextran hydrogel treatment induces the accumulation of CD68+ and CD3+ cells in the wound, which promoted pro-inflammatory activities and elimination of invading pathogeons. Reproduced with permission. [62a] Copyright 2018, American Chemical Society. B) The recruitment and infiltration of neutrophils and macrophages (CD68+) to the wound sites and elastin-based hydrogels at different timepoints, which is crucial for eliminating invading pathogens and accelerated wound healing. Red immunofluorescence: CD68 positive; red-circles: neutrophils. Reproduced with permission. [62b] Copyright 2022, Elsevier. C) The schematic illustration of hydrogel formulation and its injection to the injured intrapericardial space that subsequently form a protective hydrogel-coating in the epicardium that prevents adhesion formation through suppression of local inflammation. Reproduced with permission. [65b] Copyright 2021, American Chemical Society.

these hydrogels display positive roles in contributing to DFUs healing. Other disorders like bone and cartilage defects, spinal cord and nerve injury, colon ulcer, intrapericardial adhesion, dental pulp, and skeletal muscle damage also present mitigatory tendencies when confronted with the immune adjustment actions of hydrogels.[65] 3D-printed chitosan/silk fibroin, phenylboronic acid-crosslinked polyvinyl alcohol@gelatin colloids, photocrosslinked methacrylated gelatin, sulfated chitosan, or periosteal extracellular matrix-based hydrogels are witnessed to accelerate osteogenesis through boosting IL-10 and M2

biomarkers CD206 and Arg1 production while decreasing IL-1β and M1 indicators CD86 and iNOS expression.[66] After being injected into the affected area, hydrogel barrier comprising silicate nanodisk and polyethylene glycol restrains tissue adhesion through the suppression of local inflammatory response (Figure 5C).[65b] The repair rate of dental-pulp is increased on the presence of immunosuppressive effects derived from the fibrin hydrogel, as seen by the elevation of M2/M1 ratio and proportion reduction of APCs, myeloid DCs, T cells, and B cells.[67]

In addition, the nanofibrous hydrogels, resembling the dressings which absorb wound exudates and keep the moist environment of the wound area, display regulatory roles in the immune system to accelerate the healing process.[68] For instance, a nanofibrous hydrogel constituted by electrospun thioether grafted hyaluronic acid nanofibers covers the damaged region and facilitates the diabetic wound repair via shortening the inflammation phase during and promoting stage transition to the regeneration phase through ameliorating ROS-induced inflammatory reactions and accelerating M1 macrophages reprogramming into the M2 phenotype.[69] The sulfonated nanocellulose-based nanofibrous hydrogels are found to induce macrophages polarization to M2 state participating in tissue remodeling, as seen by CD31+ area and blood vessel number increase, iNOS expression reduction and CD163 content elevation. [70]

Considering the favorable biocompatibility, biodegradability, low immunogenicity, and satisfying safety, hydrogels have been utilized to act as delivery vectors via encapsulating various cell types and vaccines for abrogating foreign body response and then improving curative efficacies.[71] Pancreatic islets, mesencgymal stem cells (MSCs), adipose stem cells, and neural stem/progenitor cells are regarded as the common implant agents loading in hydrogels to fulfill the purpose of tissue regeneration.[72] Vaccines against chronic infections and tumors are demonstrated to be more prone to achieve the desired results of successful immunization through forming complexes with hydrogels.[73] On the one hand, the controlled release endows continuously immunostimulatory properties of vaccines. On the other hand, several kinds of hydrogels are able to serve as auxiliaries to amplify innate and adaptive immune reactions. For instance, by virtue of recruiting and maturing DCs, promoting the expansion of cytotoxic T cells, depleting myeloid suppressor cells, regulatory T cells (Tregs), and M2-like tumor-associated macrophages, regulating Th1, Th2, and Th17 activities, inducing generation and secretion of antibodies and cytokines, a few hydrogels composed of thermally responsive alginate-collagen, hyaluronic acid, glycol chitosan/calcium alginate, quaternized chitosan, melittin-$RADA_{32}$ hybrid peptide and, etc., are capable of effectively activating the local and systemic immune system.[74] Inspired by the multifarious immunoregulatory actions produced by hydrogels, plentiful studies have been performed to investigate the implication of their physicochemical features in immune modulation, which uncover that factors including stereochemical structure, stiffness, peptide chirality, pH value, size, elasticity, shape, charge trait, surface, and crosslinking chemistry are critically involved.[75] With respect to the molecular mechanisms underlying the modulatory roles of hydrogels in immunocytes activities, although NF-κB, YAP, mitogen-activated protein kinases (MAPKs), PI3K/Akt, mammalian target of rapamycin (mTOR), Rho-associated kinase (ROCK), and NLRs signal molecules have been discovered to be associated with activation or inhibition of immune reactions, the specific pathways involved in are still needed to be further explored.[76]

In addition to inhibiting inflammatory responses and facilitating regenerative processes, the immunoregulatory hydrogels are reported to display promotive roles in the initial hemostasis stage of wound healing. Lignin and cellulose-based natural polymer hydrogels have shown high adhesion to the bleeding site and decrease the bleeding time and blood loss, which are favorable for wound repair.[77] A self-healing hydrogel constructed by Schiff-base reaction between oxidized hyaluronic acid and N-carboxyethyl chitosan is capable of serving as a physical barrier of wound spaces to prevent the bleeding by forming into suitable sizes and shapes and absorbing the exudate to swell and compress the wound and encapsulate the blood cells without any leakage.[78] Apart from rapidly swelling and compress of the hemorrhage site, the hybrid hyaluronic acid-polyurethane hydrogel is able to activate the endogenous coagulation system to induce the occurrence of quick hemostasis, which perform a synergetic effect with the anti-inflammatory function of the hydrogel for promoting the wound healing.[79]

4.2. Hydrogels as Cellular Delivery Vehicles

Accumulating preclinical evidence highlights the hydrogel carrier as a crucial delivery strategy for cell therapy. Encapsulated in porous microparticles, transplanted cells overcome the adversities of poor survival and low retention in the host niche.[80] Due to the self-renewal capacity and versatile biological functions, especially potent immune mediation via controlling immunocytes growth, migration, differentiation, and maturation, stem cells from bone marrow, umbilical cord, adipose and neural tissues are the main populations embraced in hydrogels applied for regeneration.[81] With the subcutaneous injection of thiol-functionalized hyaluronic acid hydrogels carrying adipose MSCs, repressed expression of IL-13, chemokine ligand 11 (CCL11), and CCL24, the weakening of epidermal thickness and mast cell permeation are seen in the target region, accompanied by the amelioration of atopic dermatitis (**Figure 6**A,B).[82] Induced pluripotent stem cells released from the biocompatible hydrogel reduced the infiltration of neutrophils and CD4+ and CD8+ T cells in the myocardium to trigger cardiovascular repair postmyocardial infarction (Figure 6C).[83] Through transfecting with lentiviral vector loading IL-4 gene, the genetically modified MSCs display strengthened immunosuppressive abilities, characterized by a markedly increase of IL-4 expression and macrophage polarizing to M2 phenotype, thereby favoring inflammation ablation and bone defect healing.[84] There is evidence showing that hydrogels coloading endothelial progenitor cells and MSCs possess superior effects against lipopolysaccharide (LPS)-evoked endotoxemia, and the inner mechanism might be attributed to macrophages status reprogramming from M1 to M2 and production of IL-4 and IL-10 stimulated by MSCs protecting endothelial progenitor cells from apoptosis and angiogenesis promotion, which then

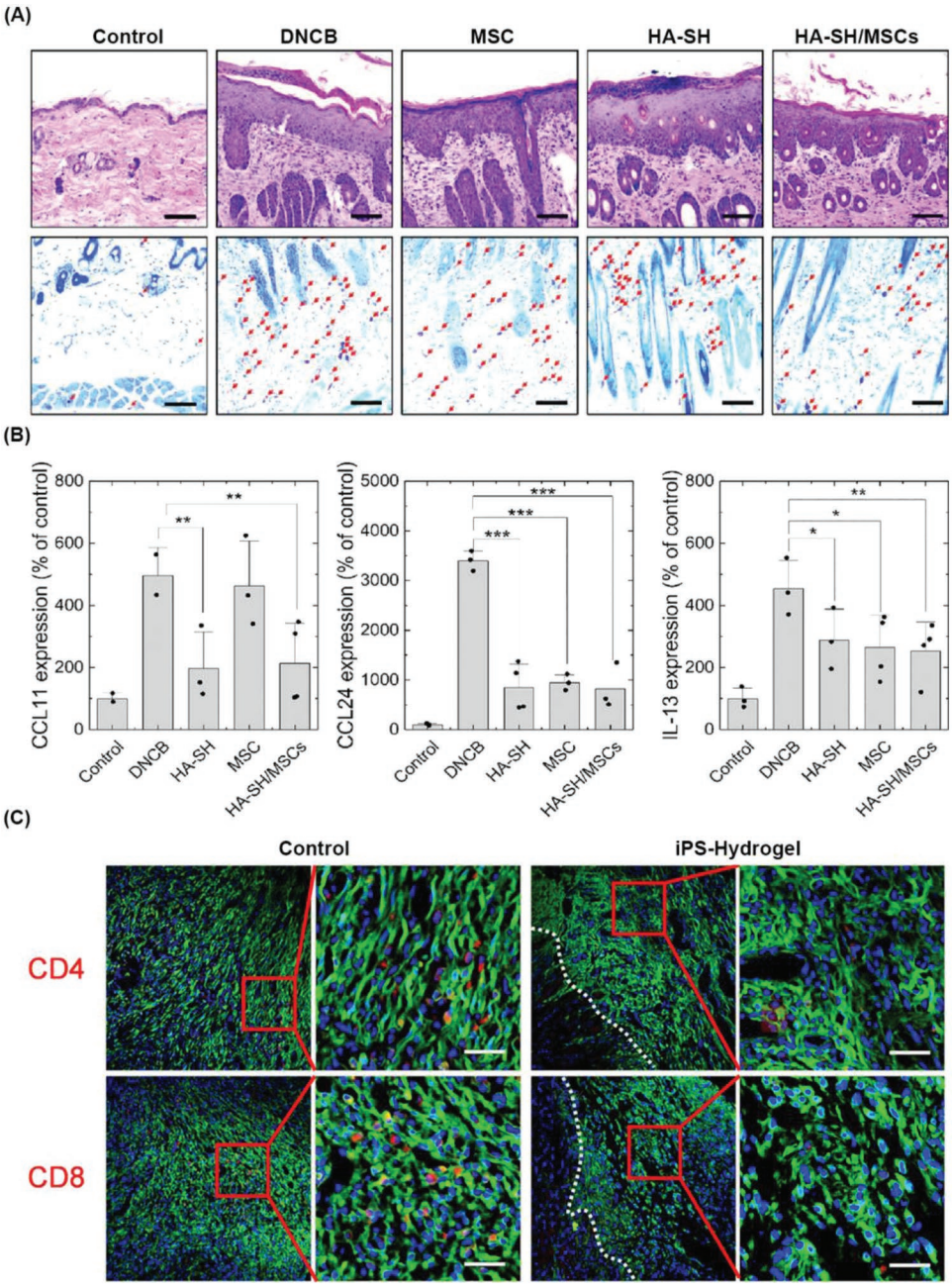

Figure 6. The regulatory effects of stem cells loaded in hydrogels on the immune responses. A,B) Administration of 1-Chloro-2,4-dinitrobenzene (DNCB) induces dermal injury and inflammation response. The intervention of MSCs alone fails to markedly ameliorate the development of dermatitis. Treatment of hydrogels carrying MSCs decreases epidermis thickness, mast cell accumulation, and generation of pro-inflammatory cytokines in the wound area. Reproduced with permission.[82] Copyright 2022, American Chemical Society. C) Injection of induced pluripotent stem cells encapsulated in hydrogels reduces the infiltration of CD4+ T cells and CD8+ T cells in the injured site of myocardial infarction. Reproduced with permission.[83] Copyright 2021, Springer Nature.

facilitates these anti-inflammatory substances to enter the circulation and amplify the antiendotoxic roles of MSCs.[85] Moreover, in view of their direct immunosuppressive actions, Tregs are identified as another cell cohort embedded in hydrogels to deal with immune hyperactivation-related illnesses and preserve the survival of local autologous graft without affecting the systemic immune system.[86] It has been illustrated that immunotherapy holds great promise for cancer obliteration by activating immune cells and proteins to accurately and specifically destroy tumor cells, unlike conventional radiation and chemotherapy.[87] As previously depicted, several kinds of immunocytes including Chimeric Antigen Receptor T (CAR T) cells, antigen-loaded DCs, natural killer cells (NKs), and cytotoxic T cells have been entrapped in hydrogels for transferring to the target circumstance, which potentiates immune killing effects toward malignancies in the host in an exogenous supplement

manner.[88] Despite that, none of above cell groups enclosed in hydrogels are present for DFU management, and instead, M2 macrophages-laden hydrogels have been reported to enhance the healing process of chronic diabetic wounds. Specifically, after being seeded on the high-molecular-weight hyaluronic acid hydrogel, the M2 macrophages inhibit the accumulation of pro-inflammatory cytokines IL-6, IL-1β, and TNF-α and enhance the release of pro-proliferative factors, such as IL-4 and VEGF to facilitate the angiogenesis and skin regeneration.[42] Additionally, MSCs-encapsulated hydrogels might be another feasible option, given that this kind of hybrid mitigates aberrant immune microenvironment to abolish skin inflammation responsible for the pathophysiological progression of DFU and promote angiogenesis and tissue regeneration.[82]

4.3. Hydrogels as Vehicles for the Delivery of Bioactive Molecules

Many studies have revealed that an increasing number of bioactive factors that can be used to treat ailments and improve health rely on their efficient immunoregulatory functions, such as interleukins, chemokines, phytochemicals, microRNAs, metal ions, gas molecules, monoclonal antibodies, bacterial components, cellular lysates, colony-stimulating factors, adhesion molecules, statins, hypoglycemic medications, and immunosuppressants.[89] Because of the own drawbacks of certain molecules like poor dissolution in water, burst release, unguided dispersion, and rapid degradation, hydrogels-related delivery approach substantially counteract these disadvantages, as confirmed by an increase in solubility, stability, and bioavailability.[90] Preclinical data unravel that bioactive substances liberated from hydrogels prohibit the development of DFUs primarily through antagonizing inflammation responses by mediating macrophage phenotype switching and cytokines secretion.[61] With an elevation of CD206, Arg-1, IL-10, and transforming growth factor Beta (TGF-β) expression and a reduction of CD86, iNOS, IL-1β, and TNF-α in the macrophage cohort, Paeoniflorin loaded in hydrogels produces positive roles in accelerating M1 reprogramming to M2 condition via influencing activities of STAT1/6, which, in turn, induces angiogenesis and re-epithelialization and leads to cutaneous healing in the diabetic wound area (**Figure 7**A).[91] High-sulfated hyaluronan discharged from the hydrogel located in the damaged site of diabetic mice was observed to favor would closure via a mechanism that might be dependent on enhancing anti-inflammatory M2 macrophage differentiation and subsequently level elevation of RELMa and IL-10 and content decrement of S100A9 and NLRP3 (Figure 7B).[15] Macrophages migration and infiltration, intracellular NF-κB signal axis activation, and pro-inflammatory cytokines expansion evoked immune disorder was dramatically alleviated by functional factors including epigallocatechin gallate, miR-223, and thrombin-derived peptide, accounting for their contributing roles in infectious inflammation restraint and skin repair (Figure 7C).[89a,92] Owing that a stage featured by weak immune response exists during the pathophysiological processes of DFUs, which aggravates microbial invasion and delays wound healing. In addition, intercellular adhesion molecule 1 (ICAM-1), Ag^+ or Zn^{2+}-laden hydrogels have been developed to recruit neutrophils, monocytes, and lymphocytes in the infected area for eliminating pathogens and improving tissue injury (Figure 7D).[93] Other groups of illnesses widely subjected to molecular immunotherapy mainly include bone defect, cartilage damage, and malignant tumors, indicating that immunoregulation dysfunction is a common predisposing event involved in the pathogenesis of tissue regeneration retardation or dysplasia enlargement, figuring out the inner interactions among them is worthy to provide solid foundations for developing reliable molecules targeting specific immune cells and signal pathways associated with DFUs initiation and progression.[94]

Except for single bioactive factors carried in hydrogels, two or more molecules have been found to be coentrapped in hydrogels for encumbering cellular pathological processes. According to the findings from recent studies, there are differences in the modulation of immune reactions among certain hydrogels loading multiple agents, i.e., one substance participates in mediating the contents and activities of immunocytes and cytokines and the other materials directly or indirectly affect immune functions, or produce therapeutic actions toward DFUs independent of immunoregulation.[95] It is deciphered that the combination of IL-10 with AT-RvD1 greatly enhances the accumulation of CD206+ macrophages, IL-10 expressing DCs, and Th cells with anti-inflammatory profiles in the wound site to launch pro-regenerative activities.[96] Supplement of Ag particles exerts bacterial-killing effects which compensate for the disability of inflammation-related infection suppression resulting from metformin hydrogel-induced M2 macrophage polarization, neutrophil extracellular traps (NETs) formation suppression, and neutrophils activation and degranulation in the diabetic trauma region.[95] Not only through releasing H_2S to stimulate macrophage phenotype shifting and inflammation abolishment in damaged areas, but dopamine-hyaluronic acid hydrogel also emits VEGF to promote cellular proliferation and skin repair (Figure 7E).[97] Of note, multilayer hydrogels with disparate physicochemical properties, alien degradation durations, and irrelevant drug release onset of each sheet have been observed currently.[98] On account of the diverse immunoreaction features in different phases of DFUs development, it is imperative to seek suitable bioactive molecules-encapsulated hydrogels performing immune-mediating capacities in a spatiotemporal way. In addition, there is evidence that hydrogels-carrying substances exert immunomodulatory roles varying depending on local microenvironment profiles, ascertaining whether there are differences in protective mechanisms against immune dysfunction between molecules usage alone and their combination with hydrogels is necessary to in-depth understand the pathogenic processes of DFUs and obtain superior treatment modalities.[99]

Given that the adverse effects caused by oxidative stress and deficient angiogenesis on tissue regeneration, immunomodulatory hydrogels possessing antioxidative and proangiogenic abilities per se or loading ROS scavengers and pro-angiogenic agents have been developed to enhance the beneficial effects on wound healing (**Table 1**). Not only releasing IL-33 to induce ILC2s and Tregs accumulation and M1-to-M2 transition in the wound microenvironment, the DNA hydrogels but also directly eliminate endogenous ROS from HaCaT cells to suppress oxidative

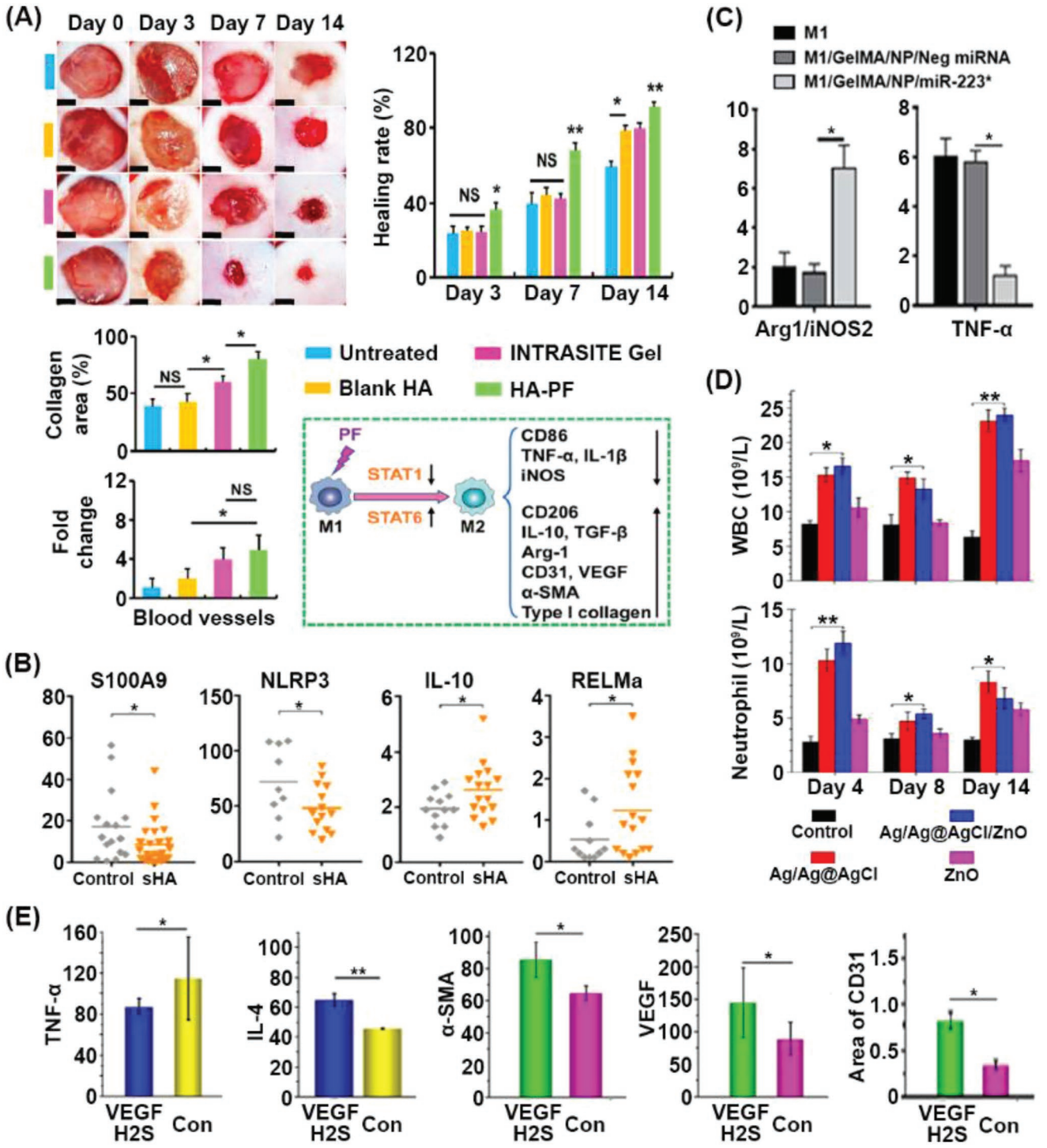

Figure 7. Bioactive molecules loaded in hydrogel improve immune disorder to promote wound healing. A) Paeoniflorin encapsulated in hydrogel facilitates the process of wound repair and increases collagen deposition and angiogenesis. Reproduced with permission.[91a] Copyright 2021, Elsevier. Administration of paeoniflorin changes the value of the M2/M1 ration and the content of pro- and anti-inflammatory factors and affects the activities of STAT1 and STAT6 in macrophages upon stimulation with inflammatory factors. Reproduced with permission.[91b] Copyright 2021, Elsevier. B) The expression profiles of genes involved in inflammation development after intervention with the hydrogel containing high-sulfated hyaluronan. Reproduced with permission.[15] Copyright 2021, Elsevier. C) The phenotype reprogramming and production of proinflammatory TNF-α in macrophages are mediated by miR-223* hydrogel. Reproduced with permission.[89a] Copyright 2019, Wiley-VCH GmbH. Thrombin-derived peptide (TCP-25) hydrogel decreases the NF-κB/AP-1 activity in the wound area. Reproduced with permission.[92b] Copyright 2020, American Association for the Advancement of Science. D) Ag^+ or Zn^{2+} ions discharged from the hydrogel exert contributing roles in the promotion of immune cell recruitment in the damaged region for antagonizing infection and accelerating wound healing. Reproduced with permission.[93b] Copyright 2017, American Chemical Society. E) The hydrogel carrying H_2S and VEGF induces the initiation of anti-inflammatory activities and simultaneously directly facilitates the process of angiogenesis. Reproduced with permission.[97] Copyright 2022, Elsevier.

Table 1. The antioxidative and proangiogenic effects from immunoregulatory hydrogels to facilitate wound healing.

Hydrogel composition	Encapsulated agents	Immune regulation	ROS scavenging	Angiogenesis	Refs.
DNA polymeric chains	IL-33	IL-33 induces recruitment of ILC2s and Tregs and M2 macrophages polarization	DNA hydrogel itself promotes the clearance of intracellular ROS	—	[100]
Poly(ethylene glycol), 2,2-bis(hydroxymethyl) propionic acid	–	The hydrogel reduces the expression of IL-1β	The hydrogel decreases the level of reactive nitrogen species and mitochondrial ROS	—	[63]
Heparin, hyaluronic acid	Curcumin	Heparin inhibits the spread of MCP-1 and TNF-α, curcumin suppresses macrophages accumulation and triggers M1 reprogramming into M2 phenotype	Curcumin prohibits the generation of ROS in macrophages	—	[101]
Zwitterionic polysaccharide	ADSC	The cell-hydrogel complex increases the level of CD206 and decreases the level of CD68 and TNF-α in the wound area	—	The cell-hydrogel complex promotes the expression of bFGF, VEGF, α-SMA, and CD31 in the skin tissue	[105a]
Polymers carbopol 940	CuSQ	CuSQ inhibits the expression of TNF-α and MMP-9	—	CuSQ increases the expression of VEGF and TGF-β1 in the wound site	[104a]
Poloxamer 407, hyaluronic acid	Ginsenoside Rg3	Ginsenoside Rg3 suppresses the activities of MAPK and NF-κB pathway in the skin defect region	—	Ginsenoside Rg3 increases the expression of HIF-1α, VEGF, collagen I, and collagen III	[103]
Elastin, gelatin	—	The hydrogel enhances the expression of IL-4, IL-10, and TGF-β and reduces the expression of iNOS, TNF-α, and IL-6 in macrophages	—	The hydrogel enhances the tube formation functions of ECs and increases the level of CD31 in the wound area	[62b]
Silk fibroin	Metformin	The drug-hydrogel complex promotes M1 phenotype conversion to M2 state and decreases the formation of NETs and release of neutrophil elastase and MPO	—	The drug-hydrogel facilitates the migration of ECs and induces the expression of VEGF and α-SMA in the diabetic skin tissue	[14]
Chitosan, carboxymethyl cellulose	MSC-derived exosomes	The exosomes increases the level of CD206 and reduces the level of TNF-α, IL-1β, and iNOS in macrophages	—	The exosomes enhances the activity of PI3K/AKT pathway and increases the expression of VEGF and TGF-β1 in ECs	[105b]
Hyaluronic acid	Paeoniflorin	Paeoniflorin facilitates macrophages polarization to M2 phenotype	—	Paeoniflorin promotes the expression of VEGF, CD31, α-SMA, and collagen I in the skin tissue	[91a]
Chitosan, collagen	Serp-1	Serp-1 promotes M2 macrophages formation	—	Serp-1 induces CD31 and VEGF expression in the wound region	[102a]
Poly(ethylene glycol), diacrylate	SDF-1α	SDF-1α induces the accumulation of anti-inflammatory monocytes		SDF-1α increases the density of CD31+ vessels	[107]
4-arm-thiolated poly(ethylene glycol), silver nitrate	4-octyl itaconate	4-octyl itaconate decreases the level of IL-1β and IL-6	4-octyl itaconate activated the Keap1/Nrf2 pathway to induce the expression of antioxidative HO-1, NQO1, SOD, and CAT	4-octyl itaconate ameliorates ECs apoptosis and facilitates the expression of cellular VEGF and bFGF	[106a]
Alginate, hyaluronic acid, poly-lysine	Curcumin, EGCG	The phytochemicals increase the level of TNF-α and decrease the level of IL-10 in the wound site	The phytochemicals promote the clearance of ROS	The phytochemicals facilitate the expression of CD31 in the damaged area	[106b]

stress-provoked local inflammation responses and cellular injury.[100] Another kind of cationic hydrogels formed by amino-functional hyperbranched dendritic-linear-dendritic copolymers are capable of prohibiting reactive nitrogen species and mitochondrial ROS generation and proinflammatory cytokines secretion in human keratinocytes and then increasing cellular viability.[63] It is reported that the heparin/hyaluronic acid hydrogel system disrupts the influx of immune cells and captures TNF-α and MCP-1 at the injured area, and the curcumin, possessing potent antioxidative capacities, is released from the hydrogel complex to reduce ROS production in macrophages and enhance fibroblasts survival, all of which are beneficial for re-epithelialization, ECM remodeling and wound closure.[101] In terms of the hydrogel complexes implicated in angiogenesis, both of the hydrogel compositions and loaded bioactive substances are uncovered to exhibit regulatory roles.[14,62b,102] Findings from Peng et al. show that poloxamer 407/hyaluronic acid hydrogel complex releases ginsenoside Rg3 to trigger collagen deposition and angiogenesis, and the inner mechanism might be attributed to the drug-induced HIF-1α/VEGF

axis activation.[103] Other bioactive molecules like growth factors, energy metabolic products, and metallic compounds have been indicated to be loaded in hydrogels to evoke immune suppression and enhance ECs proliferation and migration, then resulting in wound repair acceleration.[91a,104] Moreover, there is evidence suggesting that hydrogel systems carrying adipose-derived stem cells, MSC-derived exosomes or engineered macrophages display pro-regenerative effects on wound sites, which to some extent is associated with angiogenesis enhancement caused by encapsulated agents-derived pro-proliferative cytokines.[105] Recently, the drug-encapsulated hydrogel systems possessing immunosuppression, ROS scavenging and pro-angiogenic abilities have also been reported to exert desirable effects on facilitating diabetic wound healing, by virtue of the suppression of inflammation augmentation and faster advent of regeneration phase resulted from the multifunctional synergistic actions of hydrogel complexes.[106]

4.4. Hydrogels as Exosomes Delivery Vehicles

Considerable documentations reveal that exosomes, a series of subcellular structures detected in most body fluids with a size distribution ranging from 50 to 120 nm, package biomacromolecules like lipids, nucleic acids, and proteins of source cells in phospholipid bilayer membranes and transfer them to adjacent cells for regulating intercellular physiological and pathological activities.[108] Given that internal cargos and compositions of exosomes are derived from secretory cells, stem/progenitor cells released exosomes are reported to similarly facilitate tissue regeneration by implementing regulatory roles in immune responses, with preferable efficacy and less systemic rejection in a way of encapsulating in hydrogels.[109] Apart from inducing angiogenesis, MSCs exosomes expelled from hydrogels produce anti-inflammatory actions in the local injured site via enhancing M2 state formation and repressing M1 type switching of macrophages, followed by contents reduction IL-1, TNF-α, IL-1β, and toll-like receptor 4 (TLR4) and level elevation of IL-10, Arg1, and Fizz, thereby leading to the skin would repair and fibrotic scar inhibition.[110] For investigating immunoregulatory mechanisms of exosomes shed from hydrogels involved in tissue healing, extensive experiments have been performed and discovered that miRNAs including miR-451a, miR-181b, and miR-1246 enclosed in exosomes are of vital importance, which enter target macrophages and affect expressions and functions of proteins responsible for phenotype polarization, such as MIF, PRKCD, and AKT.[111] Furthermore, there are interventions which increase the level of immunosuppressive signal molecules like PD-L1 in source cells on purpose, resulting in the presence of abundant target proteins loaded in exosomes. Then, after discharged from hydrogels, exosomes are captured by surrounding T cells, which favors PD-L1 binds to PD-1 on the membranes of T cells, accompanied by transduction of activation inhibition signals and initiation of expression abrogation of proinflammatory factors, thus triggering skin wound and bone defect healing.[112] These findings suggest that genetically engineering exosomes carried in hydrogels might represent a therapeutic tendency toward DFUs, due to the novelty, stability, convenience, effectiveness, and precision guidance of this approach.

4.5. Combined Immunomodulatory Therapies

However, hydrogels based on immunomodulatory strategies assume one of the most effective pathways to treat diabetic foot ulcers. Still, they suffer from limited therapeutic effects to regulate chronic inflammation and impaired neovascularization for diabetic wounds. On the other hand, bacterial infection is more suspected in the hyperglycemia microenvironment leading to excessive ROS generation which synergistically could impede the process of wound healing by hindering angiogenesis and aggravating inflammation. Therefore, it is an urgent need to design multifunctional hydrogels that could regulate inflammatory reactions efficiently while simultaneously destroying bacteria and ROS in the wound area to induce neovascularization and promote wound healing. In the following section, we discuss recent studies about the combination of hydrogel-based immunotherapy and diverse emerging methods for ROS scavenging and bacteria elimination. In 2021, Tu et al. reported an M2 macrophage-polarized anti-inflammatory, antioxidant, and antibacterial hydrogel (GDFE) for inducing angiogenesis and facilitating diabetic wound healing.[113] The GDFE hydrogel was prepared through the Schiff-base reaction between F127-ε-polylysine (FE) and polydopamine-modified graphene oxide (GO) (**Figure 8**A). The antioxidant ability of the hydrogel was investigated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Results revealed that GDFE hydrogel could significantly reduce the absorption peak at 516 nm and confirmed the excellent antioxidant efficiency of hydrogel up to 92% in comparison to 96% for ascorbic acid (VC) as a commercial product (Figure 8B,C). The scavenging free radical property of the hydrogel is attributed to the 1,2-dihydroxybenzene in polydopamine which oxidized to quinones, a powerful ROS capturer. Since bacteria interfere with the healing process and aggravate inflammation in the hyperglycemic environment, the in vitro antibacterial assessment of the hydrogel was carried out against *Escherichia coli* (*E. coli*, representative of gram-negative bacteria), *Staphylococcus aureus* (*S. aureus*, representative of gram-positive bacteria), and methicillin-resistant *Staphylococcus aureus* (MRSA, drug-resistant bacteria). The results showed over 99% rate of bacterial killing, confirming the strong antibacterial efficiency of GDFE. This property originated from EPL in FE, a polycationic polypeptide that could damage bacterial cell membranes due to its opposite charge. The macrophage modulation effect was evaluated by incubating RAW264.7 macrophages with GDFE, GFE, FE, and Control group (M0 macrophages) for 48 h. As shown in Figure 8D, GO-modified FE (GFE) and GDFE hydrogels revealed higher elongation compared to the Control group. Further assessment of macrophage polarization based on immunofluorescence staining of CD206 as a surface marker of M2 macrophages exhibited higher fluorescent intensity of CD206 for macrophages treated with GFE and GDFE hydrogels (Figure 8E). The anti-inflammatory activity of the hydrogel is due to the presence of GO which could up-regulate M2 macrophage marker genes. The in vivo diabetic wound healing results also supported in vitro results and revealed that wounds treated with GDFE had a faster wound closure with re-epithelization and neovascularization in contrast to other groups. Moreover, the inflammatory evaluation of the wound showed a lower level of TNF-α in the costaining with F4/80 in

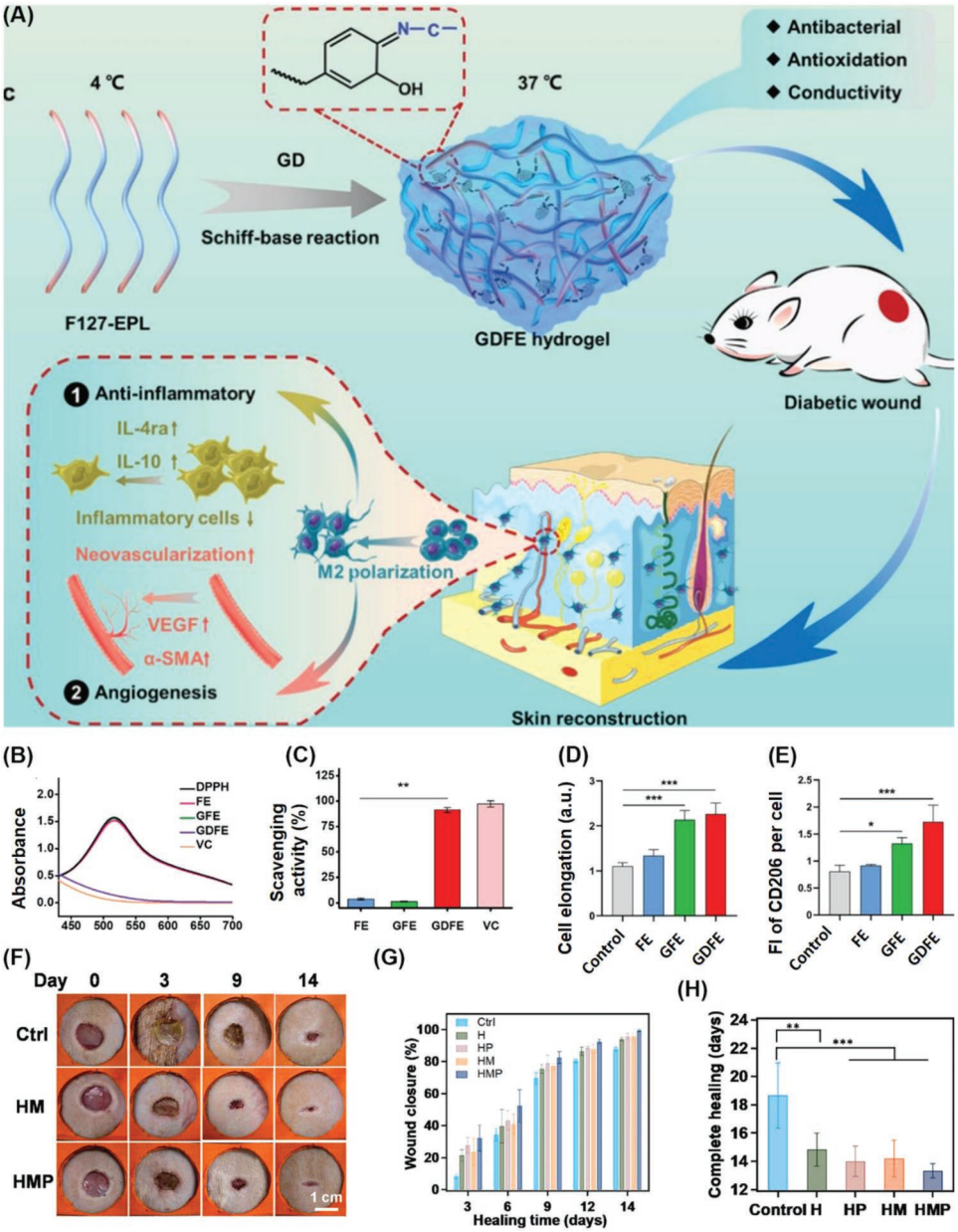

Figure 8. A) Schematic image of GDFE hydrogel synthesis for promoting diabetic wound healing. a) Schematic illustration of GD synthesis. b) Schematic illustration of FE hydrogel synthesis. c) Schematic illustration of GDFE hydrogel synthesis. B) UV–visible spectra of DPPH, FE, GFE, GDFE, and VC. C) Free radical scavenging performance of FE, GFE, GDFE, and VC. D) Quantitative data of morphological change of RAW264.7 macrophages stimulated by different hydrogels. E) Quantitative data of immunofluorescence intensity of CD206. A,E) Reproduced with permission.[113] Copyright 2021, Wiley-VCH GmbH. F) Macroscopic image of MRSA-infected wound after various treatments at different time points. G) The rate of wound closure for different treatments on days 3, 6, 9, 12, and 14. H) Time of complete healing wounds treated with different hydrogels and no treatment (Control group). F–H) Reproduced with permission.[114] Copyright 2022, Elsevier.

the GDFE hydrogel. Also, the immunofluorescence staining and quantitative results of M1 marker F4/80/INOS2 and M2 marker F4/80/ARG exhibited lower expression of INOS2 and higher ARG in GDFE hydrogel. Taken together the results proposed GDFE as a multifunctional hydrogel with antibacterial, antioxidant, and macrophage polarization properties that could remarkably promote diabetic wound healing without loading any bioactive drug.[114] Also, in another attempt, EPL in combination with Ibuprofen (IBU) as an anti-inflammatory drug was encapsulated into an ECM-mimicking hydrogel. The hydrogel revealed a 95.8% wound closure rate in comparison to commercial 3 M wound dressing (88.4%) on day 14 by possessing a combination of antibacterial and reprogramming macrophage properties.[115]

Since the excessive ROS in the wound microenvironment disturbs endothelial function and also promotes inflammation result in impeding wound healing. It has been reported that antioxidant hydrogels could down-regulate the proinflammatory

factors. Based on these concepts, Liu et al. fabricated PVA based hydrogel cross-linked by a ROS-responsive linker loaded with mupirocin (MP) and granulocyte-macrophage colony-stimulating factor (MP+G@Hydrogel) as the antibiotic and growth factor, respectively. The hydrogel exhibited great ROS scavenging ability both in vitro and in vivo due to the presence of the ROS-responsive linker. The in vivo infected diabetic wound healing results showed about 37% healing at day 8 for MP+G@Hydrogel compared to about 7% for PBS. The hydrogel in response to the ROS present in the wound environment could up-regulate the macrophage polarization toward M2-type, enhance the generation of blood vessels, and eliminate *S. aureus* infection through the therapeutic effect of MP, resulting in an excellent strategy to treat DFUs.[116] In another study conducted by Gao et al. manganese dioxide (MnO_2) nanosheets as an inorganic nanoenzyme were utilized to scavenge ROS and also generate O_2. The HM hydrogel was fabricated through cross-linking of poly(PEGMA-*co*-GMA-*co*-AAm) (PPGA) polymers by hyperbranched poly-*L*-lysine (HBPL) modified MnO_2. The antioxidant results revealed complete scavenging H_2O_2 and $^{\bullet}O_2^-$ clearance for HM in comparison to H hydrogel (crosslinking of PGCA and pure HBPL). This is due to the catalase and peroxidase mimicking the effect of MnO_2 and could produce oxygen simultaneously. Also, by taking advantage of amine groups of HBPL both H and HM hydrogels exhibited remarkable DPPH and $^{\bullet}OH$ clearance efficiency leading to the elimination of various kinds of ROS. Furthermore, due to the importance of the antibacterial activity of the hydrogel in diabetic wounds, the bacterial transcriptome was evaluated. Transcriptome sequencing demonstrated that HBPL could down-regulate the most encoding pathogenesis of MRSA by inhibiting the quorum sensing system and stimulating a proinflammatory response. The wound healing capacity of the HM hydrogel was carried out in an MRSA-infected cutaneous wound model. In order to facilitate the healing process, pravastatin sodium was loaded into the hydrogel (HMP hydrogel) to benefit from inducing NO synthesis by endothelial nitric oxide synthase. The in vivo macroscopic and quantitative results of wound healing of HMP hydrogels showed faster wound healing due to the synergistic antibacterial and antioxidant activity followed by down-regulating inflammatory factors and macrophage polarization toward M2-phenotype as well as supplying more oxygen and NO (Figure 8F–H).[114]

5. Emerging Immunomodulatory Trends in DFUs Treatments

In recent years, photothermal therapy (PTT) has attracted much attention as an alternative antibiotic therapy that could overcome drug-resistant complications.[117] PTT is a noninvasive strategy that could eliminate a broad spectrum of bacteria by converting near-infrared (NIR) light to heat.[118] On the other hand, it has been reported that mild heat stimulation at about 40–41 °C could induce angiogenesis and also decrease inflammation which is vital for the wound healing process.[119] In this regard Yuan et al.[120] introduced an M2 macrophage-polarized anti-inflammatory antibacterial antioxidant hydrogel in combination with mild photothermal therapy and angiogenic drug [deferoxamine (DFO)] to treat DFUs. For hydrogel preparation, first, the mesoporous PDA NPs (MPDA NPs) formed imine bonds with DFO (M@D). Then, the HTHE-M@D hydrogel was prepared via enzymatic cross-linking of epigallocatechin gallate dimer-grafted hyaluronic acid (HA-EGCG) and tyramine-grafted human-like collagen (HLC-TA) and integrating M@D (**Figure 9**A). The HTHE-M@D hydrogel exhibited robust photothermal properties with different amounts of M@D under 808 nm NIR laser irradiation with 0.190, 0.277, and 0.369 W power densities (Figure 9B,C). The required temperature for the mild photothermal therapy was provided at the concentration of 2.0 mg mL^{-1} M@D with a 0.190 W power density. The cytocompatibility assay also revealed remarkable cell proliferation with a concentration of 2.0 mg mL^{-1} M@D. Further, the antibacterial and antioxidant capacity of the hydrogel was assessed because as mentioned above the hyperglycemia microenvironment increases the potential infection risk and also induces ROS production. As shown in Figure 9D, the in vitro and in vivo antibacterial results showed great antibacterial activity for HTHE-M@D hydrogel in comparison to HTHE and other groups. However, no significant difference was observed in HTHE-M@D and HTHE-M@D + L (exposed to NIR laser) groups, indicating that mild photothermal therapy did not significantly affect bacteria killing due to mild hyperthermia. The in vivo results were also in good agreement with the in vitro studies (100% antibacterial activity for HTHE-M@D and 88.3 for the HTHE group). The strong antibacterial efficacy of the HTHE-M@D hydrogel is due to the presence of ECGC which could inhibit the biosynthesis of cellular components and also affect the expression of proteins contributing in the formation of the bacterial cell membrane. Moreover, it would be amplified by the presence of PDA benefited from the bacterial cell adhesion effect. In the following, the DPPH assay was evaluated to determine the antioxidant efficacy. The HTHE-M@D hydrogel showed significant antioxidant activity of about 71–90% for 0.5–4 mg mL^{-1} concentration of M@D. The hydroquinone moieties, imine, and catechol groups of PDA and galloyl moiety in the D ring and the hydroxyl groups in the B ring of EGCG could eliminate free radicals endowing the HTHE-M@D hydrogel with remarkable antioxidant activity. Furthermore, the macrophage transformation activity of the HTHE-M@D hydrogel by CD206 immunofluorescence staining of macrophages demonstrated that HTHE and HTHE-M@D hydrogels could maintain M2 phenotype successfully after 72 h in comparison to the IL-4 control group. LPS and IL-4 promote M1 and M2 phenotypes, respectively. The expression level of CD206 for HTHE and HTHE-M@D hydrogels was significantly increased compared to the LPS control group (72 h). This is due to the High-molecular-weight HA that could transform M1 to M2 macrophages by inducing M2 phenotype-specific gene expression. Also, the hematoxylin-eosin (H&E), Masson's trichrome, and MOMA-2 immunofluorescence staining, a marker of macrophages and monocytes, exhibited less recruited macrophages around the subcutaneously injected site of HTHE-M@D hydrogel. This is attributed to the anti-inflammation properties of HLC, EGCG, and DFO. To determine the combined mild photothermal therapy and HTHE-M@D hydrogel effect on angiogenesis, in vitro tube formation experiments with endothelial cells and in vivo chicken embryo

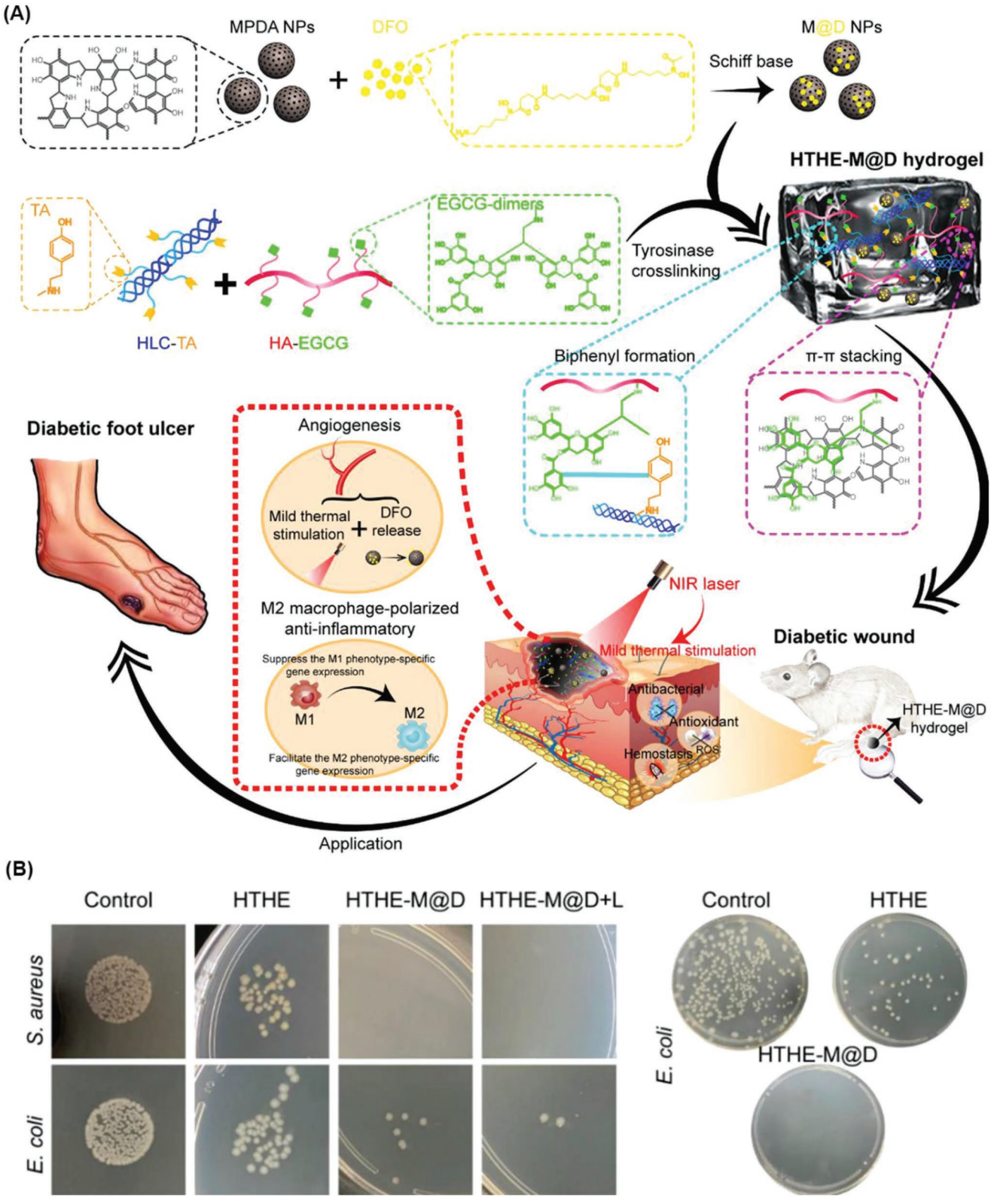

Figure 9. A) Schematic representation of the HTHE-M@D hydrogel synthesis and mechanism of diabetic foot wound healing through the combined effect of the hydrogel and mild heat stimulation. B) Photographs of the in vitro bacterial viability of *S. aureus* and *E. coli*, and the in vivo bacterial viability of *E. coli*. Reproduced with permission.[120] Copyright 2022, Elsevier.

allantoic membrane (CAM) tests were carried out. The results indicated that the HTHE-M@D + L group possessed the most vasculogenic index through the effect of DFO and mild photothermal therapy on the upregulation of angiogenic factors and expression of eNOS. Overall, the combination of HTHE-M@D hydrogel and mild thermal stimulation could treat diabetic wounds by promoting impaired angiogenesis and macrophage transformation into M2-type. [120]

The foot wound models of diabetic rats also showed the fastest wound closure for the HTHE-M@D + L group and almost healed completely on day 13 in comparison to other groups. In another study, Guo et al. utilized methacrylated gelatin (GelMA) and oxidized dextran (oDex) and loaded it with black phosphorus (BP) nanosheets and zinc oxide nanoparticles (ZnO NPs). The Gel/BP/ZnO + NIR hydrogel revealed excellent antibacterial property both in vitro and in vivo due to the combination of photothermal activity of BP under the 808 nm NIR laser irradiation and ZnO release which increase the phagocytosis of bacteria by macrophages. The in vitro bacterial survival rate of Gel/BP/ZnO + NIR hydrogel was less than 5% compared to 20% without NIR irradiation. Taken together, the Gel/BP/ZnO + NIR hydrogel could promote infected wound healing with the fastest closure rate (64%) due to the immunoregulation effect of ZnO in combination with mild heat stimulation which could ablate inflammation and induce angiogenesis resulting in shortening bacteria infected wound healing time.[121] Further to increase the antibacterial killing efficacy of broad spectrum-bacteria by photothermal therapy, Liu et al. provided a poly(N-acryloyl glycinamide) (PNAGA) polymer mixing with PDA-coated gold nanorods (Au@PDA NRs) and coated with *E.coli* or *S. aureus*-activated macrophage membrane (called E/SMM-PNAGAAu@PDA hydrogel) which could target specific bacteria due to the expression of a specific bacterial receptor on macrophage membrane (**Figure 10**A). Then the accumulated bacteria in the hydrogel microenvironment would be destroyed under NIR irradiation therapy. The killing rate of hydrogel under NIR irradiation was 98.4% and 97.6% for *E. coli* and *S. aureus*, respectively (Figure 10B). This feature is in contribution of the angiogenic-inducing effect of photothermal therapy to facilitate diabetic wound healing.[122]

6. Perspectives and Clinical Translation

Wound healing is a complex cascade relying on multiple steps, including coagulation, inflammation, matrix synthesis, angiogenesis, and tissue remodeling to optimally restore tissue integrity and function. In diabetic wounds, the above highly coordinated process is affected by persistent inflammation and excessive production of reactive oxygen species, which leads to long-term microenvironment disturbance and impedes wound healing.[123] To promote diabetic wound healing, accumulative research on immunomodulatory functional hydrogels, loaded with a variety of bioactive cytokines and molecules has driven

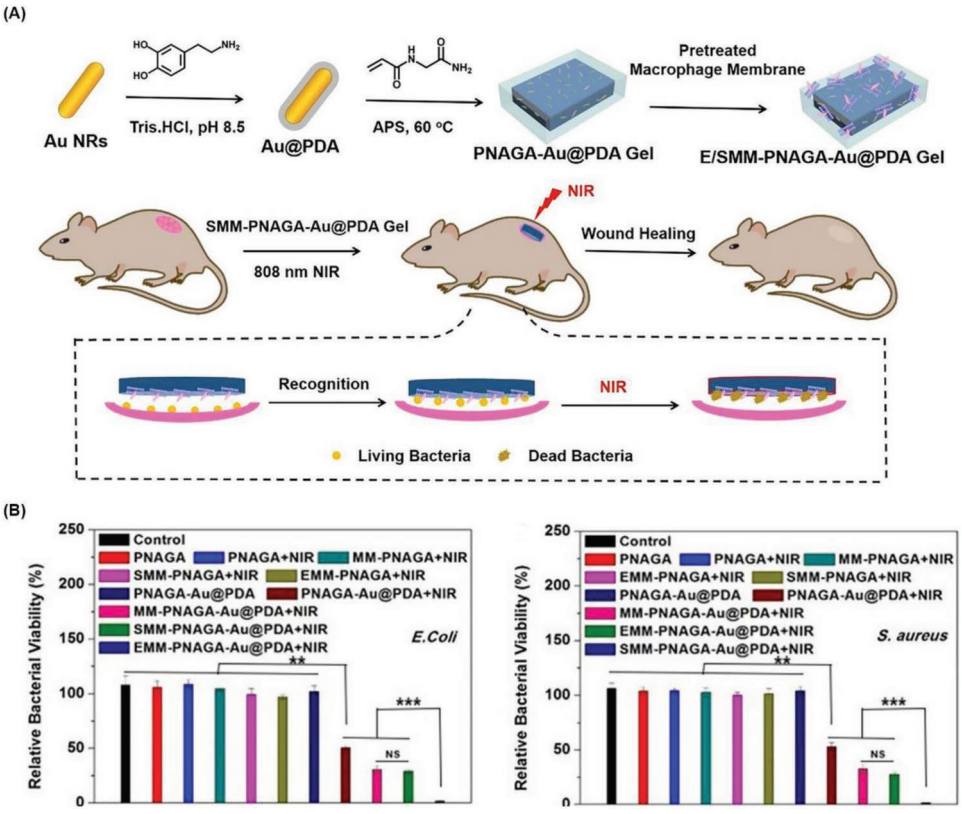

Figure 10. A) Schematic illustration of the design of the MM-PNAGA-Au@PDA hydrogel with specific recognition ability of bacteria. B) Relative bacterial viability of *E. coli* and *S. aureus* after 30 min incubation with different samples and exposure to NIR irradiation with a power density of 2 W cm^{-2} for 5 min for +NIR groups. Reproduced with permission.[122] Copyright 2021, Elsevier.

the design and development of immune-responsive wound dressings. Therefore, extensive stride in the understanding of cutting-edge hydrogel-based immunomodulatory strategies and the underlying molecular mechanisms will promote prominent development in the management of diabetic wound healing.

The activation and functional maintenance of the immune system is closely associated with tissue regeneration. Emerging evidence indicates the huge potential of immunomodulation in the promotion of wound healing, and multiple biomedical strategies are reported for the amelioration of excessive and persistent immune responses in chronic refractory wounds. In this review, we comprehensively summarized the recent developments in immunomodulatory strategies based on functional hydrogels for diabetic wound healing. With the ongoing understanding of the relationship between hydrogel-mediated immunomodulation and diabetic wound healing, functional hydrogel-based immunomodulatory strategies could be safely translated into clinical applications with more possibility.

Recently, to enhance the immunomodulatory function of hydrogels, advanced manufacturing techniques, such as 3D bioprinting, are utilized in the preparation of hydrogels for tissue regeneration.[124] For instance, Wang et al. reported a new bioink for the preparation of functional hydrogels with 3D bioprinting. The new bioink is based on two immiscible aqueous phases of GelMA and dextran, further endowed with antibacterial and anti-inflammatory properties.[124] In addition, smart materials and chemical systems, which can detect, record, analyze, and respond to information from the surrounding environment have attracted accumulative interest. Preparing smart hydrogel dressings with flexible electronics becomes a critical direction for diabetic wound treatments.[125] Hydrogel-based smart wound dressings can dynamically record wound status and provide functional responses, which represents a promising direction for developing smart drug systems. Electrical stimulation can facilitate wound healing with high efficiency and limited side effects. A recent study from professor Ali Khademhosseini's group reported a flexible electrical hydrogel patch for accelerating wound healing.[126] The conductive hydrogel was synthesized using silver nanowire and methacrylated alginate, which was optimized to enable printing on medical-grade patches for personalized wound treatment. In vitro enhanced secretion of growth factors with promoted cell proliferation and function was found in response to electrical stimulation. Furthermore, the in vivo results indicated an accelerated wound closure rate in rodents.[126]

Delivery of bioactive factors is a crucial function of hydrogels in the promotion of diabetic wound healing. Multiple functional hydrogels have been designed as a delivery platform by preventing bioactive factors from protease degradation. However, the microenvironment-responsive release of bioactive factors to maintain immunotherapy is a great challenge, which is one of the main obstacles to the clinical translation of hydrogel-based immunotherapy. To overcome this challenge, various stimuli-responsive hydrogels were developed and more bioengineered techniques were applied in the design of wound dressings.[18,127] For instance, a recent study introduced multiple stimuli-responsive MXene-based hydrogels for promoting chronic wound healing, which has highly efficient photo- and magnetic-responsive drug delivery function.[127] Consequently, this MXene-based hydrogel system exhibits multiple response capabilities and controllable drug delivery ability, which can effectively deliver drugs to the wound site and accelerate wound healing. It provides powerful tools to endow hydrogels with stimuli-responsive and controllable drug delivery characteristics, thereby achieving enhanced immunoregulation potential.

In the near future, more in-depth knowledge of molecular mechanisms on biomaterials-mediated immunotherapy and the advances in bioengineering techniques will motivate researchers to devise more effective immune engineering approaches, eliminate the translational gap between basic studies and clinical studies, and eventually improve the therapeutic efficacy of hydrogels. However a series of hitherto unresolved issues and considerations should be addressed before the successful translation of hydrogels for DFUs into clinical practice:

i) DFUs are widely characterized by multiple-drugs-resistant bacterial infections and abnormal immune microenvironment. Therefore, immunomodulatory hydrogels with broad-spectrum antibacterial activity are highly desired to be designed and developed.
ii) The safety of immunomodulatory hydrogels may raise concerns and over-suppression of immune response may also revoke certain immune diseases and cancers. Therefore, immunomodulatory hydrogels with precise and targeted properties are needed to preemptively avoid unintended health issues.
iii) The widely used murine or rodent models of diabetic wound poorly mimic human wound due to the considerable involvement of contraction instead of re-epithelialization. Therefore, more appropriate animal models are highly desirable for recapitulating the complex pathological microenvironment of human DFUs and their response to treatment. Meanwhile, the combination of emerging experimental techniques including organs-on-chips may represent a promising direction for verification of in vivo assays.
iv) Emerging evidence indicates that superficial fascia mobilization after skin damages plays a dominant role in tissue regeneration and scar formation. Future studies are desirous to uncover the effects of immunomodulatory hydrogels supplied as ECM scaffold by replacing wound ECM from the superficial fascia. It may provide new opportunities for facilitating the healing of DFUs with reduced scar formation.
v) Ultimately, as DFUs are caused by multiple pathological factors, immunomodulatory hydrogels with multifunctional properties (antibacterial, pro-angiogenesis, pro-repair, and ROS scavenging, etc.) is highly desired. In addition, reducing synthesis costs will facilitate the successful application of immunomodulatory hydrogels in the clinical practice of DFUs.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

angiogenesis, antibacterial, diabetes, diabetic wound, hydrogels, immunomodulation

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