Review

Multifunctional hydrogels for wound healing: Special focus on biomacromolecular based hydrogels

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Abstract

Hydrogels are widely used for wound healing applications due to their similarity to the native extracellular matrix (ECM) and ability to provide a moist environment. However, lack of multifunctionality and low mechanical properties of previously developed hydrogels may limit their ability to support skin tissue regeneration. Incorporating various biomaterials and nanostructures into the hydrogels is an emerging approach to develop multifunctional hydrogels with new functions that are beneficial for wound healing. These multifunctional hydrogels can be fabricated with a wide range of functions and properties, including antibacterial, antioxidant, bioadhesive, and appropriate mechanical properties. Two approaches can be used for development of multifunctional hydrogel-based dressings; taking the advantages of the chemical composition of biomaterials and addition of nanomaterials or nanostructures. A large number of synthetic and natural polymers, bioactive molecules, or nanomaterials have been used to obtain hydrogel-based dressings with multifunctionality for wound healing applications. In the present review paper, advances in the development of multifunctional hydrogel-based dressings for wound healing have been highlighted.

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1. Introduction

In recent years, extensive studies have focused on the design of appropriate wound dressings with various structures. Tissue engineering strategies by generation multifunctional artificial matrix can address some unmet requirements in wound healing. Engineering appropriate biomaterials that can mimic the surrounding ECM and support cellular function are critical for the formation of functional new tissues [1]. Recently, hydrogels, as one of the promising wound dressing materials, have received significant attention [2]. Hydrogels are defined as three-dimensional cross-linked polymeric network structures with the ability to hold a large amount of water in their networks and retain their structures after swelling. Thanks to their desirable properties such as biomechanical 3D structures, elasticity similarity to the native tissue, ability to provide a moist environment and absorb wound exudates porous structures, allowing for gaseous exchange for preventing the growth of anaerobic bacteria, acting as a barrier to prevent bacterial infections, and improving the epithelization and cell migration into the wound, hydrogels are one of the most promising materials for tissue regeneration specially wound healing applications [3–9]. Also, hydrogels can be prepared as an injectable form. Injectable hydrogels provide certain advantages such as filling the wound sites with irregular space, adherence to the wounds, and ability for in situ encapsulating of bio-active molecules and cells that are important for enhanced skin tissue regeneration [10]. However, conventional hydrogels have several drawbacks which may limit their applications for wound healing such as low mechanical properties and inability to mimic the native tissue micro-structure [11]. Also, the conventional hydrogels possess limited functionality and only able to provide a moist environment. To expand the potential applications of hydrogels in wound healing, several studies have developed multifunctional hydrogels with excellent performance to support wound healing by their multiple functions [12]. Multifunctional hydrogels, in addition to providing a suitable microenvironment mimicking the native tissue ECM, can have other functions that accelerate wound healing. In addition, these hydrogels can be designed in a bilayer manner to protect the infiltration of microbes and also prevent loss of hydrogel moisture. Various hydrogels have been also developed with inherent or additional antibacterial properties to inhibit infection and prevent the chronic phase of the wound. Some hydrogels have been fabricated with stimuli-responsive properties and can be triggered via various stimuli such as temperature, light, and pH to deliver therapeutic molecules, kill bacteria, and promote cellular proliferation. Also, smart hydrogel-based systems have been applied to recognize the pH changes in the wound environment and, at the same time, release bioactive molecules. The development of multifunctional hydrogels can be designed based on the chemical composition of the hydrogel’s constituent materials, modification of the materials, and incorporation of nanostructures in the hydrogels. Nanoreinforced hydrogels, as one of the rapidly emerging approaches, have been considered in developing multifunctional hydrogel with improved properties for wound healing applications. In the present review paper, we will focus on the advances in the wound healing field by using multifunctional hydrogels. In particular, we will highlight and summarize the nanoreinforced multifunctional hydrogels, as these structures have presented a promising paradigm shift in the field of wound healing.

2. The functional properties of multifunctional hydrogels for wound healing

Multifunctional hydrogel-based dressings have emerged as unique materials due to the combination of the required properties for effective wound healing [1,2,3,4]. Some contributing factors can influence the antibacterial, antioxidant, electrical, adhesiveness, mechanical, and tissue regeneration behavior of the fabricated hydrogels. These factors include the type of polymer used to prepare hydrogel, modification of the used polymers, incorporating nanomaterials and nanostructures, and also the addition of the active materials into the hydrogel network [5,6,7,8,9]. In the following subsections, we will highlight some recent studies and developments of multifunctional hydrogels that have been considered for wound healing. Fig. 1 represents various functions to develop multifunctional hydrogels for wound healing.

2.1. Antibacterial properties

Both acute and chronic wounds are susceptible to microbial infections that delay the healing process. So, it is important to incorporate antibacterial properties in the hydrogel dressings. In the previous studies, various antibacterial agents such as conventional antibiotics have been introduced in the hydrogels to prevent infections in the wound site, but there have some drawbacks such as antibiotic resistance. As a result, researchers have focused on developing new approaches to generate hydrogels with antibacterial properties. It is necessary to form hydrogel-based dressings with antibacterial properties to combat the multidrug-resistant bacterial infection that is one of the major problems in chronic wounds [13,14]. Antibacterial hydrogels have been generated by using inherent anti-infection properties of the applied polymers in the fabrication of hydrogels or incorporating antibacterial agents such as metallic nanoparticles (NPs), carbon-based nanomaterials, and nanofibers. Chitosan is a natural polymer with inherent antibacterial properties that is widely used in developing hydrogels for wound healing. The antibacterial properties of the chitosan-based dressings mainly rely on the interaction between the positive charge of chitosan and negative charge in bacteria cell membranes [15]. Other mechanisms such as inhibiting nucleic acid synthesis as a result of the binding of chitosan with RNA and DNA of bacteria and metal chelation ability of chitosan also have been described [16]. However, synthesizing chitosan hydrogels with satisfying mechanical properties and good water solubility is a challenge [17]. Chitosan can be modified with various functional groups to overcome these challenges. For example, the introduction of quaternary ammonium moiety into the backbone of chitosan leads to produce quaternized chitosan. Quaternization of chitosan is one of the most commonly used modifications approaches to obtain hydrogel with improved water solubility and antibacterial property that is used to fabricate chitosan-based dressings, especially injectable hydrogels [18]. The grafting electroactive polymers such as polyaniline on the backbone of
chitosan have also shown excellent antibacterial activity [19]. This can be due to the synergistic effects of positive-charged amino groups of chitosan and electron transfer between polyaniline and bacterial cells that lead to bacterial death [20].

Hydrogels based on nanomaterials have been extensively investigated for antibacterial properties. Nanocomposite hydrogels containing metal and metal oxide-based nanostructures such as zinc oxide nanoparticles (ZnO NPs) [21], silver NPs [22], gold NPs [23] have been considered for wound healing. The main antibacterial activities in most of the metallic NPs are governed by these mechanisms: attachment of NPs to bacterial membranes, disruption of the bacterial membrane, leakages of bacterial inner components, such as nucleic acids via the outer membrane and peptidoglycan layer, and finally the inhibition of protein synthesis. About the metallic oxide NPs, photocatalysis is the main antibacterial mechanism. The production of free radicals such as oxygen radicals and hydroxyl radicals, as a result of the effects of ultraviolet irradiation on metallic oxide NPs, leads to killing bacteria in a short time [14,24]. Various factors can influence the antibacterial properties of metal NPs. For example, in one study, the effect of shape and surface modification of gold NPs for wound healing application has been investigated. This study reported a thermosensitive hydrogel based on poloxamer 407 that included charged modified gold NPs (rods (AuNR) and spheres (AuNS)) for wound healing. Polyethylene glycol (PEG), polyallylamine hydrochloride (PAH), and polyacrylic acid (PAA) were used as neutral, positive, and negative charge modifiers, respectively. The results demonstrated that poloxamer 407 hydrogels loaded with PEG-modified AuNRs and PAH-modified AuNRs presented significant wound closure without scars 21 days post treatment. Additionally, PAH-AuNRs loaded hydrogel indicated high collagen deposition in comparison to other groups, confirming the healing of the wound. The antibacterial effects of the nanocomposite hydrogels, in vivo swapping of the wounds showed that PAH-AuNRs group had no bacterial growth, however, in PEG-AuNRs group, a low number of bacterial colonies was observed. In PAA-AuNRs, PEG-AuNSs, and poloxamer hydrogels, an observable growth of mixed bacteria was indicated [25]. Improved healing of wound treated by PAH-AuNRs-hydrogel can be attributed to its positive-charged groups that may adsorb and interact with the released proteins in the wound which lead to the development of gold–protein aggregates and organization of collagen. The wound healing effects in the case of PEG-AuNRs hydrogel can be explained by the hydrophilicity, high absorbent, and adhesiveness properties of PEG. Also, the strong antibacterial activity of AuNRs compared to AuNSs can be explained by the effect of nanoparticle morphology on wound healing [26].

Recently, multifunctional hydrogels with antibacterial properties have been developed [27] using various approaches. Localized photodynamic therapy (PDT) has been used for developing antibacterial hydrogels by incorporation photosensitizer agents such as metallic NPs and carbon-based nanomaterials. The activation of these nanostructures under the suitable wavelength irradiation and generating free radicals can kill the bacteria by oxidative damage [28]. Also, photothermal therapy (PTT), using the combination of near-infrared (NIR) and light-absorbing materials, have been applied to kill locally pathogenic bacterial via hyperthermia damage [29]. Induced PTT in the range of NIR (700–1100 nm) due to its advantages such as deep tissue penetration, negligible cytotoxicity, and very low tissue damage has been considered to kill microbes. Various materials such as transition metal chalcogenides, noble metal NPs, and carbon-based nanomaterials have been investigated as antibacterial agents for photothermal induced destruction of bacteria [27,28]. But, one of the main drawbacks of PTT is the high temperature required to kill bacteria, which can damage near healthy tissue [30]. Therefore, the combination of various antibacterial approaches such as PTT and PDT in a single system can be used to achieve a multifunctional dressing with appropriate outcomes for

Fig. 1. Schematic representation of various functions such as antioxidant effects, antibacterial activities, tissue adhesiveness, and mechanical properties to develop multifunctional hydrogels for wound healing.
The antibacterial materials used for the fabrication of multifunctional hydrogels.

<table>
<thead>
<tr>
<th>Material used</th>
<th>Antibacterial mechanisms</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan</td>
<td>Interaction between the positive charge of chitosan and negative charge in bacterial cell membranes; Inhibiting nucleic acid synthesis as a result of the binding of chitosan with RNA and DNA of bacteria, metal chelation</td>
<td>[15, 16]</td>
</tr>
<tr>
<td>Polyaniline grafted chitosan</td>
<td>Synergistic effects of chitosan and polyaniline</td>
<td>[19, 20]</td>
</tr>
<tr>
<td>Metallic NPs</td>
<td>Attaching to bacterial membranes, disrupting the integrity of the bacterial membrane, leakage of bacterial inner components such as nucleic acids, inhibition of protein synthesis, production of free radicals</td>
<td>[14, 24]</td>
</tr>
<tr>
<td>Photosensitizer agents such as</td>
<td>PDT, PTT</td>
<td>[28, 29]</td>
</tr>
<tr>
<td>metallic nanoparticles and</td>
<td></td>
<td></td>
</tr>
<tr>
<td>carbon based nanomaterials</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short amphiphilic peptides</td>
<td>Cationic residue</td>
<td>[32, 33]</td>
</tr>
</tbody>
</table>

of the interesting class of antioxidant hydrogels is polyphenol based hydrogels that can be used in wound healing due to their unique free radical scavenging through hydroxyl groups on their aromatic rings [41]. Gallic acid (GA) as a potent polyphenol has conjugated onto the gelatin chains to obtain antioxidant hydrogel with reduced burst leach of GA from the hydrogel network and the collagenase-inhibitory activities [43]. The collagenase inhibitory activity of the GA is due to its binding with collagenase via hydrophobic interaction, π-π stacking, and hydrogen bonding [44]. The addition of gelatin conjugated GA into the gelatin hydroxyl phenyl propionic acid hydrogel led to generating injectable and antioxidant hydrogel. The accelerating wound healing effect of GA modified hydrogel was observed by improved neovascularization, highly ordered align collagen fibers organization, and hair follicle formation [43].

Modified self-assembled peptide-based materials with antioxidant properties have used for wound healing. In one study, a hydrogel blend made from feruloyl-modified peptide and feruloyl-glycol chitosan resulted in an antioxidant supramolecular hydrogel due to the free radicals scavenging of the phenolic group of ferulic acid. The antioxidant property of this hydrogel was almost equivalent to curcumin, but in the case of feruloyl modified hydrogel, higher concentration was need [45]. Most of the antioxidant functionalized hydrogels have made by direct incorporating of the antioxidant agents into the hydrogels. Tannic acid (TA) is another polyphenol widely used in hydrogel systems to achieve functional hydrogels. Jing et al. used silk fibroin (SF) as a natural protein that was co-assembled with TA to obtain multifunctional self-assemble hybrid hydrogels for wound healing. A hydrogel containing TA with antibacterial, antioxidant, and anti-inflammatory properties was formed through the gelation of SF hydrogel via hydrophobic interactions, hydrogen bonding, and π-π stacking. This hybrid hydrogel showed homogeneous porous structures. Increasing the concentration of TA resulted in higher cross-linking and a more compact network structure with decreased pore size. They demonstrated that increasing the concentration of TA could enhance the adhesiveness of the hydrogels. The recovery of the external structure of the SF-TA hydrogel was observed after 30 min due to the self-healing properties of the scaffold. Furthermore, the addition of TA strongly enhanced the antibacterial activities of SF-TA hydrogel due to the sustained release of TA that resulted in a long-term antibacterial efficacy. Compared to the SF hydrogel without TA, the incorporation of TA did not induce any cytotoxic effects on fibroblast cell viability [46]. Curcumin, as one of the best natural antioxidant polyphenol, has been also encapsulated in hydrogels for wound healing. Curcumin has shown to reduce the healing time in puncture wound models by improving the restoration of the structural epidermis and enhancing deposition of collagen as well as the vascular density in the wound site [47–49]. However, the use of curcumin is associated with some limitations such as inducing mitochondrial dysfunctions at high dose, rapid metabolization, and low hydrophilicity and stability. To overcome these drawbacks, controlled release platforms by using nanotechnology and stimuli-responsive systems have been designed to prevent cytotoxicity and improve the therapeutic effects of curcumin. For example, in one study, matrix metalloproteinase 9 (MMP-9) responsive system based on thermoresponse poloxamer hydrogel and curcumin self-assemble NPs enclosed in the gelatin microspheres has developed. The aim of the inclusion of curcumin NPs into the gelatin microspheres was the controlled release of curcumin in the wound site. The improved ROS scavenging properties of this system was attributed to the protection of curcumin in the nanoparticle form [50]. In addition, mussel-inspired polydopamine (PDA) functionalized hydrogels have been used as antioxidant materials both in vitro and in vivo due to the presence of catechol hydroxyl groups that contribute in ROS scavenging by donating an electron to cause the free radical to a redox balance [51]. Zhang and co-workers incorporated arginine derivatives with antioxidant capability into the dopamine modified hyaluronic acid (HA) hydrogel for wound healing. The results showed that the introduction of arginine derivatives into the HA
hydrogel could induce antioxidant capability by decreasing the production of intracellular ROS, enhancing superoxide dismutase and glutathione peroxidase enzyme activities [40]. Using nanomaterials in the hydrogel network is a less common but a promising approach to obtain antioxidant hydrogels. Ceria nanocrystals have been used as antioxidant nanostructures in wound healing. Studies demonstrated their significant potential to reduce ROS in comparison to biological antioxidants. The antioxidant ability of ceria nanocrystals is originated from co-presence of Ce³⁺ (reduced) and Ce⁴⁺ (oxidized) at the surface of NPs that can reversibly bind to oxygen [52]. Incorporating or chemically conjugation of nanostructures in the matrix of the hydrogels can improve their efficacy and lead to their sustained release. For example, in a study, cerium oxide NPs were dispersed into a gelatin hydrogel to yield a nanocomposite hydrogel with sustained bioactivity [53]. Rutin is another bioactive molecule with antioxidant properties that can be used for the fabrication of multifunctional hydrogels for wound healing applications [54,55].

Fig. 2 represents various antioxidants to develop multifunctional hydrogels for wound healing.

2.3. Tissue adhesiveness

Tissue adhesiveness is one of the other criteria that can be considered when developing hydrogel-based wound dressings to provide the integration with the tissue. Hydrogels can display adhesive property by making chemical/physical linkages between the surrounding tissues and functional groups of the hydrogel [56–58]. However, due to the moist environment of the wound, it is important to design strong tissue adhesive hydrogels to remedy the weak interfacial adhesive forces derived from the moisture of the wound [59]. An ideal wound adhesive material should have strong tissue adhesiveness to seal the defect, support wound healing, have controlled degradation rate and high biocompatibility, prevent tissue inflammation, and provide appropriate mechanical characteristics [60]. There are several limitations such as toxicity and weak mechanical strength with the application of cyanoacrylate and fibrin adhesives, respectively [61]. The use of adhesive wound dressings based on mussel-inspired hydrogels has obtained significant attention. Mussel inspired materials are widely used to fabricate tissue adhesive hydrogels even in moist environments. The wet adhesive property of the mussels is due to the presence of a derivative of tyrosine, L-3,4-dihydroxyphenylalanine (L-DOPA) as a catechol-containing amino acid in their proteins [62]. The oxidation of catechol hydroxyl groups of DOPA to ortho-quinone under alkaline condition leads to the cross-linking of the adhesive proteins of mussels and, as a result, cohesion properties are supplied to the network of the proteins. Bio-adhesive interfacial adhesion mechanism of the DOPA functionalized materials is due to the covalent interaction of nucleophile groups of tissue surface such as –OH, –NH₂, –COOH, and –SH with the oxidized DOPA [63,64]. The macroscopic adhesion property of the DOPA based adhesive materials depend on the interfacial adhesion and bulk cohesion [59,65]. Therefore, improving the mechanical strength of the bulk part of DOPA modified polymers adhesive by nano fillers such as chitin nanocrystals can enhance the bulk cohesion due to the formation of strong physical and chemical interactions with the polymer [59]. DOPA functionalized materials are frequently used for developing wound healing adhesive hydrogels. Catechol functionalization of branched PEG by the non-degradable linker is one of the most approaches for fabricating mussel-inspired adhesive hydrogels. The use of degradable linkers such as enzymatic sensitive parts can be applied to obtain functional adhesive hydrogels with responsiveness to local inflammation in wound site [66]. Citric acid is another favorable candidate for fabricating DOPA based adhesive materials due to providing degradable ester bond formation, improving the hemocompatibility and hydrophilicity of the polymers, and making the possibility for bio-conjugation via reactive pendant sites [64]. However, the high cost and neurologi cal effects of dopamine [67,68] may limit its clinical applications.

Gelatin methacryloyl (GelMA)-based hydrogels have been used as one of the attractive adhesive materials for wound healing. Visible light cross-linkable GelMA hydrogel with rapid and strong adhesiveness to different shapes and sizes of wounds has been developed. The adhesive property of the GelMA hydrogels to different tissues is mainly stemmed from the induction of covalent bond formation as a result of produced radicals in the photo-crosslinking process, and mechanical interlocking between GelMA and the native tissue [69,70]. Chitosan is another used polymer for the preparation of adhesive hydrogels by modification with catechol groups or hydrocaffeic acid. In this context, hydrogel was prepared by integrating of hydrophobically modified chitosan lactate with hydrocaffeic acid-modified chitosan to obtain tissue adhesive hydrogels. The high adhesive properties of the prepared hydrogels were typically mediated by the covalent bonds between the reactive o-quinone groups of hydrocaffeic acid-modified chitosan and amine or thiol groups of tissue [71]. The incorporation of pyrogallol segments by the conjugation of gallic acid onto the chitosan backbone led to the formation of wet adhesive hydrogel with a bonding strength of 47 kPa that was higher than the commercially used fibrin glue. This could be attributed to the amine, deprotonated pyrogallol, and hydroxyl groups interactions with the tissue in the physiological condition [72]. TA-based materials are also very attractive for designing multifunctional hydrogels with the adhesive property. Major advantages of TA-based adhesive hydrogels are their biocompatibility, possessing a rich source of pyrogallol and catechol groups, availability, and low cost [73–75]. However, the low adhesive feature of the TA-based hydrogels could hinder their applications for wound healing. Incorporation of nanostructures within the TA-based hydrogels can enhance their tissue adhesive property. Recently, a study by Fan et al. was shown that the reinforcement of polyacrylamide-tannic acid hydrogels (PAAm-TA) with kaolin NPs could improve the adhesive strength of the prepared hydrogels (20 times higher than that of PAAm-TA) to porcine skin. This was due to the high toughness of the prepared nanocomposite hydrogel, and the hydrogen bonding and covalent interactions between chemical groups of the hydrogel and the skin surface. Also, the oxidation of some pyrogallol of TA into the reactive quinones in the presence of ammonium persulfate radicals, and O₂ led to further interaction between nanocomposite hydrogel and skin. The high toughness of the nanocomposite hydrogel as the result of the addition of kaolin NPs contributed to greater adhesive strength of PAAm-TA-kaolin nanoparticles [75]. Another strategy to achieve an adhesive and bioactive hydrogel was to incorporate an inorganic material such as bioglass (BG) into the oxidized sodium alginate. The fast ion exchange on the surface of BG can provide the required alkaline environment for the formation of an imine bond between the amino

![Fig. 2. Schematic representation of various antioxidant materials to develop multifunctional hydrogels for wound healing.](image-url)
groups of skin tissue and the aldehyde modified alginate hydrogel. Also, it was shown that the release of Si ions increased neo-vascularization and improved wound healing [56].

Besides designing adhesive materials for wound healing, syringe-injectability and rapid self-healing are other parameters that has been considered in developing hydrogels for wound healing. In this regard, Sun et al. described a multifunctional tissue adhesive hydrogels based on N-hydroxysuccinimide glutarate ester modified PEG (PEG-SG) and TA. It was demonstrated that TA, due to possessing hydroxyl groups, could efficiently contribute to the interaction with different surfaces. The strong adhesion property of these hydrogels originated from the formation of hydrogen bonds between hydroxyl groups of TA and \((\text{-CH}_2\text{-CH}_2\text{-O-})\) of PEG-SG and subsequent ester exchange between N-hydroxysuccinimide and amino \((\text{-NH}_2\text{-})\) of tissue proteins. Also, the effect of PEG with two, four, six, and eight arms was examined on adhesive strength, and the results showed that the eight-arm PEG/TA had higher adhesive property [76]. In another study, HA modified with epigallocatechin-gallate (EGCG) was used to generate tissue adhesive hydrogel via oxidation of 1,2,3-trihydroxyphenyl group of EGCG to form reactive quinone using an enzymatic reaction [77].

2.4. Mechanical properties

Generating mechanically strong dressings is another parameter that should be considered in developing functional hydrogels for wound healing. Various conventional strategies, such as increasing monomer concentration and cross-linking density, have been used to improve the mechanical properties of hydrogels, but forming a denser polymeric network may decrease the swellability. In this regard, using other approaches, such as double network hydrogels, macromolecular cross-linkers, nanostructure reinforced hydrogels, and metal coordination networks are promising strategies to improve the properties of hydrogels [78]. Doublenetwork hydrogels due to having two independently crosslinking networks, provide good mechanical performance. In a study, a double network hydrogel was made from 1) dynamic and reversible physically crosslinked (coordinate bond) between the carboxyl groups of acrylamide-modified HA and folate with Fe\(^{3+}\), and 2) chemical crosslinking network by free radical polymerization of acrylamide under ultraviolet light to form polycrylamide. The prepared hydrogel presented some favorable properties for wound healing applications, such as improved mechanical strength and toughness, self-healing properties, injectability, and pH-responsive release behavior [79]. The doublednetwork hydrogels with physical crosslinking could have properties such as good biocompatibility, injectability, rapid self-healing, and appropriate mechanical properties in comparison to single physical network hydrogels [80,81]. Cellulose has been used to form double-network hydrogels via aggregation of its chain and the concomitant hydrogen bonding between the hydroxyl groups of these chains as a result of pH reduction [82]. The hyperbranched polymers with terminal functionalized groups are applied for the fabrication of double network hydrogels. For example, Xu and co-workers used functionalized hyperbranched PEG to obtain double crosslinked hydrogel. Pre-crosslinking between the terminal aldehyde and diol groups of functionalized hyperbranched PEG formed cotton-shaped foam with high swellability. Also, UV mediated polymerization of terminal acrylate groups resulted in the formation of hydrogel with double crosslinked network and improved mechanical properties. The favorable water uptake and mechanical properties of the developed hydrogel on fibroblast cells plays a major role in wound healing.

The effect of stiffness of gelatin methacrylate (GelMA)-poly (ethylene glycol) diacrylate (PEGDA) composite hydrogels on the proliferation and angiogenic activity of fibroblasts has been studied. It was showed that there was a significant correlation between cell proliferation and the stiffness of fabricated hydrogel. The cell proliferation assay demonstrated an enhance in the cell growth and cell area with increasing the stiffness of the hydrogels (1.3 to 23 kPa). About the angiogenic activity of fibroblasts, there was no significant difference between the matrix stiffness and the number of the formed sprouts; however, a further increase in matrix hydrogel stiffness led to a decrease in sprout formation [86]. In another study, the authors investigated the stiffness of the interpenetrating networks (IPNs) of collagen-I and alginate hydrogels on dermal fibroblasts morphologies and regulation of inflammation mediators. They found that an increase in the stiffness of IPN hydrogels led to a change in cell morphology, which was a spherical shape in the fibroblasts. Later, the effect of the stiffness of the hydrogels on wound healing associated inflammation genes was investigated. The results demonstrated the capacity of the stiffer hydrogels (with modulus from 50 to 1200 Pa) to induce upregulation of interleukin 10 (IL-10) and cyclooxygenase-2 (COX-2) were involved in the inflammation cascades [87]. Also, hydrogel stiffness can influence various stages of wound healing such as growth factors secretion, collagen deposition, and scar formation. In this regard, Chen and co-workers studied the stiffness effect of hydrogel based on poly(N-isopropylacrylamide) (PNIPAM) and poly(amidamine) (PAA) on the wound healing. The prepared hydrogels were divided into three groups based on their storage modulus \(G^′\) (low stiffness < medium stiffness < high stiffness group). The medium-stiff group showed faster wound contraction compared to other groups that also was confirmed by the higher expression of \(\alpha\)-smooth muscle actin (\(\alpha\)-SMA) in the immunohistochemical analysis. The medium-stiff groups due to their moderate stiffness and generating suitable pulling effects, could potentially support wound contract in the early stage and even later one. The quantitative analysis of wound healing associated growth factors (TGF-1, bFGF) indicated their higher secretion in the medium stiff hydrogel treated groups [88]. The expression of TGF-\(\beta\)1 was typically associated with the myofibroblasts transition through regulating the expression of \(\alpha\)-SMA [89]. Also, the medium-stiff hydrogel displayed the highest collagen fibers deposition that could be due to the higher expression of TGF-\(\beta\)1 in this group. Furthermore, the medium-stiff hydrogel treated animals demonstrated lower scar formation. The could be attributed to the elevated secretion of bFGF in the medium-stiffness group that act as an inhibitor of endothelial/epithelial to mesenchymal transition [88,90]. Collagen arrangement also was affected by the mechanical properties of the developed hydrogels. It was indicated that softer hydrogels with low elastic modulus \(<12\) kPa could support the formation of densely packed and orderly collagen arrangement compared to stiffer hydrogels with elastic modulus \(>48\) kPa. The improved neo-vascularization of the softer hydrogels could be due to providing more oxygen and nutrients to the wound sites [91]. Although most of the studies indicated that softer hydrogels support higher cell viability and proliferation, more investigation on the effects of the mechanical properties of hydrogels on cell behavior and wound healing are required.

3. Multifunctional hydrogels for wound healing

Multifunctional hydrogels have been fabricated from either natural or synthetic polymers. There are several issues such as low mechanical properties and lack of bioactivity when using natural and synthetic based polymers, respectively. Therefore, the combination of both natural and synthetic polymers are frequently used to generate hydrogels with desirable characteristics [92,93]. Polysaccharides such as
alginate and chitosan have been widely applied as natural polymers to form hydrogels for wound healing applications. In this section, we will review multi-component homogenous and nanostructure based multifunctional hydrogels as promising candidates for wound healing.

3.1. Multi-component homogenous multifunctional hydrogels

Chitosan is one of the most important polymers for wound healing applications due to its promising properties such as biocompatibility, biodegradability, antimicrobial properties, hemostatic effect, and bio-adhesiveness [94–96]. Therefore, chitosan-based multifunctional hydrogels have been reported. Various physical and chemical crosslinking methods have been used to obtain chitosan hydrogels [97].

The chemical crosslinking methods including the use of 1) different chemical crosslinkers such as aldehyde groups to make covalent imine bonds with the amino groups of chitosan [98], and genipin [99], 2) photo-crosslinking by modifying chitosan with photoresponsive groups such as methacrylate [100,101], and 3) graft polymerization [102]. The physical crosslinking methods include non-covalent interactions such as the fabrication of temperature-sensitive chitosan hydrogels by using glycerophosphate [103]. The inherent antibacterial activity of chitosan makes it an ideal candidate for generating hydrogels for wound healing and tissue engineering. Various approaches are employed to improve the chitosan performance for the repair of skin tissue. In this regard, hydrogel based on quaternized chitosan (QCS)-graft-polyaniline was shown to improve the biocompatibility of QCS and enhance the cell proliferation due to the electroactivity of the polyaniline. Also, combination of QCS and polyaniline was resulted in improved antibacterial activity of the hydrogel [18]. Skin is an electrical sensitive tissue with own conductivity [104]. It is reported that the current at the wound location increases, so electrical stimulation may promote wound healing by influencing cell behavior [105]. Thus, developing functional wound dressings incorporated conductive materials can be a good candidate for wound healing. In a noteworthy example, Zhao and coworkers developed an injectable hydrogel from a mixture of QCS-graft-polyaniline and benzaldehyde group functionalized poly (ethylene glycol)-co-poly(glycerol sebacate) (QCS/PEGS-FA) for wound healing (Fig. 3). Accordingly, the prepared hydrogel presented good adhesiveness, fast self-healing property, effective blood clotting, great antibacterial effect, biocompatibility, and reactive species scavenging ability. Also, the QCS/PEGS-FA hydrogel displayed a highly dynamic chemical bond by aromatic Schiff base that can contribute to the self-healing and adhesive properties of the prepared hydrogel. The combination of the electroactivity of polyaniline with ionic conductivity

![Fig. 3. Schematic representation of QCS/PEGS-FA hydrogel synthesis. (a) Synthesis of QCS copolymer; (b) Synthesis of PEGS-FA copolymer; (c) Preparation of QCS/PEGS-FA hydrogel; (d) Photographs of PEGS-FA solution, QCS solution and hydrogel QCS3/PEGS-FA1.5. Scar bar: 5 mm. Reprinted from [10]. Copyright (2017) with permission from Elsevier.](image-url)
of QCS’ amino groups and quaternary ammonium groups resulted in the electroconductivity of the QCSP/PEGs-FA hydrogel that was in the range of human skin. Also, incorporation of polyaniline to hydrogels led to the formation of dressing with free radical scavenging ability. The in vivo wound healing assays showed that the use of QCSP/PEGs-FA hydrogel resulted in faster healing rate compared to control group and a commercial film dressing (Tegaderm™). The improved wound healing activity of QCSP/PEGs-FA hydrogel could be due to the incorporation of polyaniline into the hydrogel network that promoted antibacterial activity, cytocompatibility, and also induced electroactivity and antioxidant effect. In addition to the faster wound closure, the QCSP/PEGs-FA hydrogel suppressed inflammatory cell infiltration and promoted tissue regeneration [10].

Stimuli-responsive hydrogels [188,189] loaded with drugs are ideal biomaterials for diabetic wound healing via on-demand delivery of drugs and bioactive molecules in response to the elevated level of glucose and acidic environment of the wounds. Dual or multi-responsive hydrogels can respond to two or more environmental stimuli, therefore, they provide an alternative strategy to obtain multifunctional hydrogels for wound healing [106]. Phenylboronic acid (PBA) as one of the glucose-responsive structures has been widely investigated for various medical applications due to its unique properties such as easy preparation and good stability [107]. In this regard, in a study, injectable dual pH and glucose-responsive hydrogels were fabricated for diabetic wound healing [108]. This hydrogel was based on phenylboronic modified chitosan (CSPBA), poly(β-naphthyl alcohol) (PVA) and benzaldehydecapped poly(ethylene glycol) (OH-PEGCHO) by the crosslinking of pH-responsive benzoic-imine (Schiff’s base) and glucose-responsive phenylboronate ester. Also, insulin and fibroblast cells were incorporated in the hydrogel as bioactive agents. Insulin-releasing from hydrogel was accelerated by reducing the pH value or enhancing the glucose concentration in the environment. This phenomenon could be due to the hydrolysis of the hydrogel network (as a result of the instability of the Schiff’s base at low pH condition) and the high tendency of PBA groups to combine with glucose compared to the OH groups of PVA in the environment with the presence of glucose [109]. Three-dimensional encapsulation of L929 fibroblast cells within hydrogels presented good cell viability and proliferation; also, the cell growth was faster in the hydrogel group with lower polymer concentration and crosslinking density. This could be due to the looser network structure and the higher swelling property of the hydrogel that provided a suitable environment for the cell spreading. The addition of a low amount of glucose (3 mg/mL) to the developed hydrogel with lower polymer concentration and crosslinkage density led to accelerating the proliferation of the cells compared to other groups. The enhanced wound healing of the insulin/L929 incorporated hydrogel group can be described by the combination of the appropriate properties of the hydrogel dressing, drug, and cell effects [108]. In a recent study, Tian et al. developed “on-demand antimicrobial activity” and self-healing hydrogel containing HA, Fe, and EDTA based on coordination bond development between the Fe³⁺ and carboxyl groups of EDTA and HA. This degradable hydrogel was capable to locally release Fe³⁺ by the degradation of HA by hyaluronidase of bacteria. The released Fe³⁺ following by reducing them to Fe²⁺ and reaction with H₂O₂ to produce hydroxyl radical to destroy bacteria. Also, the downregulation of pro-inflammatory genes was observed in the HA-Fe-EDTA hydrogel treated group [110]. One of the important challenges in chronic wounds is increased levels of myeloperoxidase (MPO) and matrix metalloproteinases (MMPs) that are related to the infection and result in excessive degradation of the ECM and growth factors. Therefore, the development of functional hydrogels with antibacterial properties and controlling the infection-related proteolytic and oxidative enzymatic activities are essential for achieving satisfactory healing outcomes. It was reported that thiol exhibits inhibitory effects on MMPs [111], which makes them suitable for engineering hydrogel for wound healing. In one study, a multifunctional hydrogel was developed via enzymatic crosslinking of thiolated chitosan and gallic acid to obtain a hydrogel with antibacterial activity, antioxidant properties, MMP, and MPO controlling effects due to the presence of gallic acid and thiol content. Also, the chelation of Zn²⁺ via thiol groups and gallic acid could contribute to the inhibition capability of the hydrogel against MMPs from their active center [112].

Another stimuli-responsive multifunctional hydrogel with NIR/pH responsiveness was developed by Zhao et al. (Fig. 4). This multifunctional double-network hydrogel with photothermal, antibacterial, self-healing, injectability, tissue adhesiveness, and antioxidative activity was prepared by using poly(glycerol sebacate)-co-poly(ethylen glycol)-g-catechol prepolymer (PEGSD) and Ureido-pyrimidinone (UPy)-hexamethylene disiocyanate (HDI) synthon modified gelatin (GTU). The fabrication of multifunctional double-network hydrogel was based on the mixing of PEGSD, GTU, and FeC₃O to form coordination interaction between catechol of PEGSD and Fe³⁺ and quadrupole hydrogen bonding of GTU [81]. The modification of gelatin with UPy could yield a physically cross-linked hydrogel by quadrupole hydrogen bonding [113]. Development of physically double-network crosslinked PEGSD/GTU/Fe hydrogel showed shear-thinning property, which is important to achieve suitable injectable hydrogel. Developing injectable hydrogels with autonomously self-healing capability have considered in tissue engineering. The self-healing process of hydrogels is based on the reconnection of broken links in the hydrogel network that is mediated by noncovalent or covalent bonds [114]. The self-healing mechanism of the PEGSD/GTU/Fe hydrogel relied on the dynamic coordination between catechol groups of PEGSD and Fe³⁺ and quadrupole hydrogen bonding between UPy motifs in GTU [81,115]. The flexible polymer chains of PEGSD and also the physical double-network crosslinking of the hydrogel resulted in toleration compressing, cyclic bending, and stretching of PEGSD/GTU hydrogel. The adhesiveness of the prepared hydrogels was originated from two mechanisms: 1) the interfacial adhesion features of the prepared hydrogels due to the presence of many catechol groups and flexible polymer chains of PEGSD/Fe³⁺ network 2) introduction of UPy to gelatin which enhanced the cohesive strength of the PEGSD/GTU hydrogels. About the effect of UPy motif on adhesive properties, concentration-dependent behavior was observed. The coordination of catechol groups of PEGSD with Fe³⁺ provided multifunctionality for hydrogel via antioxidant activity, NIR/pH-sensitivity, and photothermal ability, which are beneficial for the skin repair. This hydrogel showed good antibacterial effects against *Escherichia coli* (83.9% killing ratio) and *Staphylococcus aureus* (85.0% killing ratio) after 1 min NIR application. The photothermal capability of the PEGSD/GTU hydrogel was mainly due to the very effective NIR absorption and thermal conversion of catechol–Fe³⁺ coordination [81].

One of the recent strategies in designing multifunctional hydrogels is the modification of polymers with dodecyl to improve the tissue adhesion, hemostasis, cell recruitment functions, and antibacterial properties. The dodecyl modification of chitosan provided excellent hemostasis function, owing to the anchoring of the dodecyl onto the lipid bilayer of the cell membrane. The antibacterial activity of this hydrogel was originated from the chitosan inherent bacterial destroying properties and coordinated effects of the bacterial anchoring capability of the dodecyl [116]. Using protein-based materials such as human serum albumin (HSA) due to its advantages such as biocompatibility, biodegradability, and bio-adhesiveness has been also applied to form hydrogels for wound healing [117]. It was demonstrated that released ions such as Ca and Si from bioglass (BG) could have a dual-functional role in the wound healing process by enhancing the angiogenesis and regulating the gelling process of the hydrogel-based dressings [118]. In a study, bioadhesive, bioactive, and controllable injectable composite hydrogel was developed for wound healing. This hydrogel was based on BG, HSA, and succinimidyl succinate modified poly(ethylene glycol) (SSPEG). The rapid ions exchange on the surface of BG causes the formation of an alkaline environment and thus the making of the amide bond between the free amino groups of the HAS and two arm succinimidyl active esters groups of SSPEG. Using the various amounts of BG, the gelling time of the composite
hydrogels was modified from 15 s for 1%BG/HAS/SSPEG and 6 min for 0.05% BG/HAS/SSPEG groups. It was presented that the variation in the amounts of the BG has not been effected in the adhesive capabilities of the composite hydrogels.

In vivo chronic wound model studies demonstrated the introduction of BG in the HAS/SSPEG hydrogel led to significantly more angiogenesis compared to HAS/SSPEG hydrogel [157]. To obtain hydrogel with appropriate tissue adhesiveness, excellent cell affinity, and self-healing ability, Han et al. developed a single network hydrogel using PDA–polyacrylamide. The preservation of sufficient free catechol groups by preventing the overoxidation of PDA during hydrogel preparation was a key factor in archiving multifunctional hydrogel. In vivo wound healing experiments demonstrated faster wound healing in PDA–polyacrylamide hydrogel compared to other groups. This was due to the excellent tissue adhesiveness, cell attachment, good affinity to ECM proteins, and protecting the bioactivity of the loaded epidermal growth factor (EGF) [57].
Sprayable hydrogels are another class of biomaterials that have been considered for wound healing due to their easy applicability. One important concern of these hydrogels can be their possible weak mechanical and adhesive properties and also lack of the antibacterial activity. In this regard, elastic, adhesive, and antimicrobial sprayable hydrogels have been developed using GelMA and methacryloyl-substituted re-combinant human tropoelastin (MeTro) as the backbones of the hydrogel and Tet213 (KRWWKWWRRC) antimicrobial peptide. Visible-light mediated photo-crosslinking of the hydrogel can overcome the cytotoxicity issues of UV light. Interestingly, the prepared composite hydrogels presented antimicrobial performance against both Gram-positive and Gram-negative bacteria. The adhesive properties of the composite hydrogels was better than commercially available tissue adhesives [69]. Using therapeutic molecules such as chemokines is another approach for wound healing. The incorporation and delivery of bioactive molecules such as chemokines within hydrogel systems, especially in situ forming hydrogels, have been widely used due to the protective role of these hydrogels. In this regard, a sprayable in situ forming hydrogels composed of gelatinhydroxyphenyl propionic acid (GH) and two types of chemokines (Interleukin 8(IL-8) and macrophage inflammatory protein-3α (MIP-3α)) were prepared via horseradish peroxidase (HRP)-catalyzed crosslinking. The release behaviors of GH/IL-8 and GH/MIP-3α showed 75.6% and 19.1% cumulative release (with maintaining their bioactivity) over 7 days, respectively. This observed delayed-release rate of MIP-3α may be attributed to the electrostatic interactions between residual carboxyl groups of GH polymer and MIP-3α. Implantation of these hydrogels in streptozotocin-induced type I diabetic mice showed increased wound closure in both GH/IL-8 and GH/MIP-3α groups compared to GH hydrogel. Most remarkably, the significant enhance in wound closure and re-epithelialization were observed in the GH/IL-8 group that may be due to its release behavior. It has been shown that IL-8 can recruit different cells, such as keratinocytes, mesenchymal stem cells (MSCs), and endothelial cells to the wound location [119]. Recently, enzymatic crosslinking hydrogels such as phenol-rich polymer mediated via the HRP approach has been used in biomedical applications. Indirect providing of H2O2 by galactose oxidase for HRP-mediated gelation was used to overcome the limitations of high localized concentration of H2O2 [120]. As an example, Wei and coworkers applied acrylamide monomer, low-molecular-weight gela-tors- (1,3,2,4-dibenzylidene-D-sorbitol dicarboxylic acid) (LMWG-DBS-COOH), and glucose oxidase (GOx)-glucose-Fe(III)-system to obtain functional double network hybrid hydrogel. The polymerization of acrylamide was initiated by the following reactions: oxidation of glucose by GOx, reduction of O2 to H2O2, and production of hydroxyl radicals (·OH) through fenton reaction. Also, a decrease in pH value as a result of the production of gluconic acid led to self-assembly of DBS-COOH to form the second network of the hybrid hydrogel with the appearance of ribbon shape. The in vivo studies of the prepared hydrogels on a full-thickness skin defect in a rat model were explored. It was shown that the use of diclofenac sodium loaded hybrid gel led to better wound closure, higher density of fibroblasts, thicker epithelium tissue, and smallest scar tissue compared to other groups [121].

Recently natural plant polyphenols have been widely investigated in preparation of functional hydrogels for wound healing. TA-based hydrogels due to their low-cost, biocompatibility, excellent antibacterial, anti-inflammatory, antioxidiant, and bioadhesive properties are frequently used in wound healing. Therefore, TA could potentially be used for the preparation of multifunctional hydrogels for wound healing [67]. Taking advantage of both TA and metal ions such as Zn2+, anti-bacterial hydrogels with controlled release of TA in response to acidic conditions can be formed for wound healing applications. At lower pH, the ionic interactions between Zn2+ and TA were disrupted which led to the release of TA [73]. Some disadvantages of inorganic nanomaterials such as non-degradability and non-specific biological toxicity limit the use of these materials as photothermal antibacterial agents. To overcome these limitations, recently, TA-Fe(III) composites in the agarose hydrogel (ATF) was used to investigate the photothermal treatment of wound infection. TA can efficiently chelate Fe (III) via digalloyl groups and form TA-Fe composites. Increasing pH values of ATF hydrogels led to improved mechanical properties due to the deprotonation of TA at higher pH, and as a result, availability of more phenolate groups for complexation with Fe(III), so more stable complex was formed. Also, ATF hydrogels showed the photothermal effect under NIR exposure at 808 nm with increased temperature to 58 °C. The antibacterial activity of ATF hydrogels with NIR light irradiation at the determined time displayed the killing of nearly 95% of bacteria [27]. Epigallocatechin-gallate (EGCG) is another polyphenolic compound that has been used for biomedical applications. Due to its radical scavenging [122] and mussel inspired adhesive properties, it has been highlighted as a promising material with the potential to yield multifunctional based hydrogels for wound healing. EGCG conjugated HA was used to prepared multifunctional hydrogel with adhesive, anti-inflammatory, and anti-oxidant properties that could support skin tissue regeneration [77].

Developing wound dressings based on smart hydrogels with the capability to detect pathogens and release antibacterial agents at the wound location could be an advanced strategy for wound management. In a study, a multifunctional hydrogel with bacterial infection detection (via colorimetric pH measurement) and delivery of antibacterial agents at the wound location was developed. This system was composed of 3D printed porous sensors based on gentamicin-loaded alginate fiber and mesoporous resin beads doped with a pH-responsive dye (Brilliant Yellow and naturally derived cabbage juice) for pH measurement. It was observed that pH values change in the range of 4.0–9.0 were visually detectable using a smartphone. Various factors such as the concentration of alginate, fiber diameter, and the thickness of the dressing were effective on the response time of the sensors [123]. Table 2 summarizes the used multi-component homogenous multifunctional hydrogels for wound healing.

3.2. Multifunctional hydrogels with nanostructural components

To date, multifunctional hydrogels based on various nanostructures have drawn noticeable attention as a promising approach to yield hydrogels with appropriate characteristics for wound healing [124,125]. Overall, the nanomaterials applied for the fabrication of multifunctional hydrogels for wound healing can be categorized into four groups: 1) nanofiber-based, 2) metal-based, 3) carbon-based, and 4) mineral-based. In this section, we introduce developments of the nanostructure-based multifunctional hydrogels for wound healing (Fig. 5).

3.2.1. Nanofiber based composites for wound healing applications

Recently, dressings based on both nanofibers and hydrogels have been considered for tissue regeneration and wound healing. In the present subsections, we focus on the current developments in the fabrication of nano reinforced composite hydrogels with appropriate properties for wound healing applications. We initially focus on different approaches to fabricate nanofiber-hydrogel based scaffolds, which mainly include bilayers, nanofiber reinforcement dressings, and self-assembled scaffolds as potential structures for wound healing applications considering the challenges of individual electrospin nanofibers and hydrogels. Nanofiber based multifunctional hydrogels can be divided into 3 approaches: integration of nanofibrous structures to hydrogels, nanofiber incorporated in hydrogels, and self-assemble peptide-based hydrogels. All of the listed approaches have been used to add functionality to the hydrogels. Various methods and approaches for the fabrication of nanofiber-hydrogel composites have been previously reviewed [126,127]. Among various nanostructures, electrospin nanofibers have attracted significant attention in wound healing [128].


2.3.1.1. Bilayer dressings based on nanofiber and hydrogels. Using hydrogels alone has some disadvantages such as low mechanical properties and susceptible to loss of moisture and bacterial penetration that limit their applications in wound healing. Various approaches such as chemical or physical modifications have been explored to overcome these challenges. Developments in the field of material design and nanotechnology have enabled researchers to fabricate composite and hybrid materials that can not only benefit individual structures of each material but also overcome their intrinsic defects [126]. In this regard, due to their biomimetic structures, asymmetric membranes have been investigated as wound dressings. The asymmetric membranes usually contain two parts: 1) a denser top layer to protect wounds from physical and chemical threats, prevent bacterial invasion, and control the gaseous exchanges [95,129], and 2) the bottom layer as a supporting part to improve cell infiltration and proliferation [130,131]. Structures such as nanofibers or films and hydrogels or sponges have been used in upper and lower layers, respectively. Hybridization of nanofibers with hydrogels has several advantages such as improving mechanical properties, biocompatibility, and functionality of scaffolds [132]. Various approaches such as concurrent electrospinning and electrospraying and direct polymerization of hydrogels on nanofibers have been used for the fabrication of fiber-hydrogel bilayer composites [127,133,134].

Among different methods such as phase separation, self-assembly, and electrospinning, the latter is one of the most common techniques to produce nanofibers from both synthetic and natural polymers. The advantages of electrospinning are its efficacy, simplicity, reproducibility, versatility, and preventing infiltration of microbes due to the small pore size between individual fibers. [128,135,136]. In one study, Jong Wook Kim et al. used electrospun keratin/chitosan nanofibers and GelMA hydrogel to obtain a bilayer scaffold (Fig. 6). Electrospun keratin/chitosan nanofibers and GelMA pre-polymer solution were seeded by keratinocyte and human dermal fibroblasts respectively. The cell-laden layers were used to make bilayer scaffold by photo crosslinking of GelMA under the electrospun keratin/chitosan nanofibers. Interestingly, the cells in each layer were able to proliferate, so provide the epidermis-like thin cell layer on keratin/chitosan nanofibers and dermis-like cell-laden GelMA hydrogel [137]. In another study, electrospun SF membranes were coated with alginate hydrogel containing amniotic fluid to obtain bioactive multifunctional dressing. The addition of alginate hydrogel to fibers led to a slight decrease in ultimate tensile strength and modulus and an increase in elongation-at-break of SF nanofibers. This was due to the coating of fibers with alginate hydrogel that could decrease fiber to fiber contacts and provided better slide between the fibers [138]. 3D-printing technologies have also been used to fabricate biomimetic multilayer scaffolds for the skin tissue regeneration, but these printed structures before the formation of microvascular networks cannot appropriately support living cells for in vivo applications. Another recent example of the bilayer membrane scaffold was reported by Jun Yang et al. They developed a bilayer membrane scaffold using poly (lactic-co-glycolic acid) (PLGA) nanofiber as the outer membrane and alginate hydrogel as the inner layer mimicking the skin epidermis and dermis. They demonstrated that the PLGA nanofiber and bilayer membrane scaffolds had lower vapor transmission rates in comparison to the alginate layer, so sufficient moisture could be provided for epithelial cell proliferation and migration. The maintenance of moist microenvironment in the alginate layer due to the protective role of PLGA nanofiber could accelerate neovascularization. The fabricated bilayer membrane degradation rate was also influenced by the designed structures; the degradation rate of PLGA nanofiber within 4 weeks was lower than the bilayer membrane and alginate hydrogel. The nanofiber membrane with lower degradation rate could cover the wound for a long time and act as a barrier to prevent wound infection, while the rapid degradation of the alginate layer was suitable for cell proliferation. The antibacterial assessment showed that S. aureus could penetrate the hydrogel, but not into the PLGA nanofiber or bilayer membrane due to their high- density nanofiber structure. Furthermore, the mechanical properties of the alginate hydrogel and bilayer membrane were compared with each other. Incorporation of the outer PLGA nanofiber enhanced tensile strength and thus could support the hydrogel layer [139]. To obtain nanofiber-hydrogel bilayer dressing without using any crosslinker, aldehyde, and amino groups containing components can be used. For instance, a bilayer dressing was fabricated by crosslinking oxidized dextran and gelatin via Schiff-base reaction between aldehyde and amino groups on a poly(-caprolactone-co-lactide)/poloxamer nanofibers [140].

2.3.1.2. Nanofiber reinforced hydrogel. To improve the mechanical properties of the hydrogels, various approaches such as double physical and chemical crosslinking have been used, but the designed hydrogels were unable to properly mimic the fibriber microstructure of the native ECM and tolerance the loading conditions [11]. In addition, it is required to design nanocomposite hydrogels with adequate cell adhesion and cell migration in their network to induce wound healing and tissue regeneration [141]. The native ECM of skin composed of an aqueous matrix reinforced with nanofibrous proteins, polysaccharides, and proteoglycans; as a result, the materials used to repair skin should simulate the architecture of ECM of skin [135,142]. Furthermore, the fibrous structure of ECM provides good strength, stiffness, and toughness for the tissues [11]. Recently, nanofiber-reinforced hydrogels have been used frequently in various tissue engineering applications due to their biomimetic structures, improved cell-matrix interactions, and enhanced mechanical properties [138]. Also, reinforcement of the hydrogel matrix by nanofibrous structure can improve the mechanical properties of scaffolds, provide high porosity, and high specific surface area [143]. Most of the tissues possess fiber-reinforced hydrogel structures, so mimicking this architecture by natural and synthetic nanofibers, can effectively improve the properties of scaffolds used for tissue repair [144]. Reinforcement of hydrogels with nanofibrous structures can also support cell attachment and mechanotransduction in tissue regeneration [11]. Cellulose nanofibers (CNF) as functional structures have been applied to modify the properties of hydrogels. CNFs have been used to fabricate nanocomposite

<table>
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<th>Table 2</th>
<th>Multi-component homogenous multifunctional hydrogels for wound healing.</th>
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<tr>
<td>Hydrogel composition</td>
<td>Functions</td>
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<tr>
<td>QESP/PEG5-FA</td>
<td>Good adherence, fast self-healing property, effective blood clotting, great antibacterial effect, biocompatibility, reactive species scavenging ability, electroactive pH-responsive, glucose-responsive, sensitive insulin releasing, accelerating the proliferation of the cells</td>
</tr>
<tr>
<td>Phenylboronic modified chitosan, PVA and benzaldehyde-capped PEG</td>
<td>Antibacterial activity, antioxidant properties, MMP and MPO controlling effects</td>
</tr>
<tr>
<td>Thiolated chitosan and gallic acid</td>
<td>N/P ratio, pH responsiveness, photothermal, antibacterial, self-healing, injectability, tissue adhesiveness, and antioxidant activity</td>
</tr>
<tr>
<td>PEGSD/GTU/Fe</td>
<td>Bioadhesive, bioactive, controllable injectability, angiogenesis</td>
</tr>
<tr>
<td>BC/HAS/SSPEG</td>
<td>Excellent tissue adhesiveness, the excellent cell attachment, good affinity to ECM proteins, and protecting the bioactivity of the loaded EGF</td>
</tr>
<tr>
<td>PDA-polyacrylamide</td>
<td>Adhesive, anti-inflammatory, and antioxidant properties</td>
</tr>
<tr>
<td>GelMA/MetRO</td>
<td>Sprayable hydrogels, elastic, adhesive, and antimicrobial</td>
</tr>
<tr>
<td>EGCG conjugated HA</td>
<td>Antibacterial activity, antioxidant properties</td>
</tr>
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hydrogels with greater reinforcement effects. Also, using CNFs for the preparation of bioink for 3D-printing have been considered due to their structural similarity to ECM and good mechanical properties. The important challenge for using CNFs as bioink is that to develop a printable formulation and finally obtain stable scaffolds. One approach to achieve a good CNF based 3D-printed hydrogels is to use a double crosslinking method with Ca\(^{2+}\) (in situ) and chemical crosslinking with 1,4-butanediol diglycidyl ether (post-printing) [145]. Another approach uses auxiliary material such as synthetic or natural polymers that are added to CNF-based bioinks to improve their printability. For instance, in a recent study, researchers used 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)-mediated oxidized CNF (1.0 w/v %) and low concentration of GelMA (≤1 w/v %) as a bioink to engineer constructs for wound healing. TEMPO modified CNFs provided immediate ionic crosslinking of printed scaffolds using CaCl\(_2\) solution (5%) to support the UV crosslinking of GelMA. Also, GelMA crosslinking by UV irradiation was carried out after completing the printing process. The improved alignments of CNF/GelMA scaffolds compared to CNF scaffolds could be explained by avoiding the generation of hydrogen bonds between CNFs as a result of the adsorption of GelMA onto CNFs. Following the encapsulation of mouse 3T3 fibroblasts, the CNF/GelMA scaffolds specially with CNF/GelMA ratios of 5:1 and 2:1 presented more cell adhesion than CNF hydrogel because of the RGD (arginine-glycine-aspartic acid) motifs of the GelMA [146]. Incorporation of antibacterial properties to the CNF/gelatin-based hydrogel was achieved through the introduction of aminated silver NPs into the hydrogel matrix. The organization of this hybrid hydrogel was based on the dynamic ionic bonds between carboxyl groups of functionalized CNF and gelatin with aminated silver NPs and also carboxylated CNF with amine groups of gelatin [147].

Bacterial cellulose (BC) has been also used to fabricate wound dressings due to its several advantages such as enhanced tensile strength, nanofibrous structure, and good biocompatibility [6]. For example, nanoﬁber-reinforced biomimetic hydrogel was developed based on alginate and TEMPO-oxidized modiﬁed bacterial cellulose (TOBC) and Zn\(^{2+}\) as antibacterial wound dressing. Briefly, the water suspension of BC was modiﬁed by TEMPO to obtain COOH groups on the nanofibers and then mixed with sodium alginate solution, D-glucono-δ-lactone (GDL) and calcium carbonate were added as crosslinkers to form the hydrogel.

![Fig. 5. Schematic representation of application of nanostructures for fabrication of multifunctional hydrogels that can be used in wound healing.](image)
The nano-fiber-reinforced alginate hydrogel presented porous structure. In addition, cell viability studies indicated that the incorporation of TOBC nano-fibers improved fibroblasts proliferation in comparison to alginate hydrogel. Also, the incorporation of Zn$^{2+}$ provided excellent antimicrobial and biological properties. The addition of TOBC nano-fibers until specific concentration to the alginate hydrogel network improved the tensile strength, while it decreased swellibility. The decrease in swelling properties could be due to the enhanced crosslinking between TOBC and Ca$^{2+}$ and the high crystallinity of nano-fibers [6].

3.2.1.3. Self-assembled nano-fibrous hydrogels. Peptide-based hydrogels, another type of nanostructured hydrogels, can be used for wound healing owing to their interesting properties such as molecular self-assembly and biofunctionality [33]. Peptide-based self-assembled nano-fibrous hydrogels can provide bio-effective peptide sequences such as laminin and collagen to mimic native ECM, in addition to creating a hydrated scaffold for improving cell proliferation. Amphiphilic peptides that can form nano-fibers contain four main segments: a hydrophobic part, a segment with ionizable side chain residues for solubility, a β-sheet-forming peptide that supports nano-fiber formation, and bio-active sequence for cellular responses [148]. Also, collagen as a major component of the ECM can be used to fabricate molecular self-assembled nano-fibrous hydrogels. At physiological environments via the molecular self-assembling, collagen can form hydrogels with aligned fibril constructions that mimick the ECM of tissues. However, weak mechanical properties and insufficient stability have limited the applications of nano-fibrous hydrogels. Biological modification of self-assembled collagen fibril hydrogels by the addition of non-toxic enzyme crosslinkers such as transglutaminase increased the thermostability of the scaffolds.

Antimicrobial peptides (AMP) have various advantages for wound healing applications. These include broad-spectrum antibacterial abilities and less drug resistance. ε-Poly-l-lysine (EPL) has been considered as one of the most important AMPs thanks to its unique properties such as broad-spectrum antimicrobial effects against both Gram-negative and Gram-positive bacteria, low-cost production by fermentation (produced by Streptomyces albulus), biocompatibility, and biodegradability [149]. Recently, antimicrobial self-assembled nano-fibrous hydrogels with broad-spectrum antibacterial properties and satisfying mechanical characteristics based on metallohydrogels have been investigated for wound healing. In this content, N-([fluorenyl-9-methoxycarbonyl]) (Fmoc)-modified amino acid metallohydrogels were developed by amino acid coordinated self-assembling with Ag$^+$. The anchoring of Ag$^+$ within the amino acid nano-fiber hydrogels could support the in situ production of Ag nanoparticles (AgNPs) to increase both mechanical and antibacterial properties of the fabricated hydrogels and prevent the self-aggregation of the AgNPs [150]. Self-assembled nano-fibrous hydrogels with pH and NIR responsivity have also utilized for controlled release of antibacterial agents. Jian-Hao Wang and coworkers reported pH-switchable antimicrobial hydrogel with nano-fibers containing self-assembled octapeptide (IKFQFHFD) for biofilm eradication and chronic wound healing (Fig. 7). At neutral pH, the IKFQFHFD formed a supramolecular hydrogel due to the hydrophobic...
\(\pi-\pi\) stacking and hydrogen bonding, but under acidic \(pH\) (\(pH -5.5\)) which attribute to infected chronic wounds, nanofiber networks destabilized because of the intermolecular electrostatic rejection. Also, cypate and prolinc were incorporated in supramolecular nanofiber networks as the photothermal agent and procollagen component, respectively, with acidic \(pH\) (\(pH -5.5\)) -sensitive release behaviors. The disassembly of IKFQFHFD based hydrogel at acidic \(pH\) led to the release of cypate in the infected site and destroyed the extracellular polymeric substances of the bacterial biofilm. Also, the antibacterial performance of the hydrogels enhanced by NIR laser [151].

3.2.2. Metal based nanocomposite hydrogels

Metal-based nanomaterials have attracted considerable interest in wound healing. Previous studies demonstrated that metallic NPs present lower bacterial resistance compared to conventional antibiotics due to their different antibacterial mechanisms [152]. Among metal-based nanomaterials, ZnO NPs have received significant attention due to their excellent properties such as: antimicrobial properties, low cytotoxicity, promoting the proliferation of fibroblasts, increasing angiogenesis, keratinocyte migration, and re-epithelialization. Therefore, there is a great interest in using ZnO-based nanocomposite hydrogels for wound healing applications [153–155]. For example, metal-organic frameworks (MOFs) nanomaterials prepared from different metals such as zinc have been investigated for biomedical applications. Also, the addition of photosensitizer agents such as chlorin e6 can make MOF as multifunctional nanostructure with advantages such as the ability for photodynamic therapy, and releasing of \(Zn^{2+}\) for antibacterial, and wound healing applications [156]. Surface modification of ZnO NPs with PDA can overcome the weak dispersion of these NPs in the hydrogel network. In this regard, multifunctional nanocomposite sprayable dressing based on PDA modified ZnO NPs, L-glutamic acid, and Kappa-carrageenan was developed by Tavakoli and coworkers. Methacylated Kappa-carrageenan (KaMA) provided a biomimetic hydrogel network by visible light-crosslinking. The addition of PDA modified ZnO NPs into the prepared hydrogel resulted in promoting tissue adhesive, blood clotting ability, and improved mechanical characteristics. It was shown that the addition of ZnO-PD NPs led to reducing in the viscosity of hydrogel as the shear rate enhanced from 0.01 to 100 \(cm^{-1}\) indicating the shear-thinning behavior of the nanocomposite hydrogel. Additionally, the microstructure of nanocomposite hydrogels showed that the introduction of ZnO-PD NPs decreased the pore size of the scaffold as a result of electrostatic interaction between PDA and KaMA chains. PDA modification reduced the antibacterial efficacy of ZnO NPs, but the incorporation of L-glutamic acid improved the antibacterial effect of nanocomposite hydrogel [157].

Other types of ZnO based NPs such as ZnO quantum dots (QD) can be used to improve the antibacterial activities of the hydrogels. For instance, ZnO QDs@graphene oxide (GO) nanocomposites were introduced to chitosan hydrogel to achieve multifunctional dressing with antibacterial, and photothermal effects for wound healing. The incorporation of these nanocomposites to the chitosan hydrogel network had several advantages such as: preventing the direct interaction of ZnO QDs@GO with wound site and inflammation, and controlling the release of \(Zn^{2+}\). The ZnO QDs@GO-CS hydrogel showed cytotoxicity to NIH-3T3 cells on the first day compared to the third and seventh days after treatment that may be due to the ROS production by ZnO QDs. Also, it was observed that NIR irradiation of ZnO QDs@GO-CS hydrogel (at 808 nm) led to more cell death because of the hyperthermia effects. However, the cytotoxicity of the chitosan hydrogel was more than the other treated groups. The wound healing effects of the ZnO QDs@GO-CS nanocomposite hydrogel could be explained by providing a moist environment and natural antibacterial activity of chitosan hydrogels, antibacterial performance of ZnO QDs attributed to the sustained release of \(Zn^{2+}\) in a \(pH\)-dependent manner, and PTT properties of GO under 808 nm light irradiation [158].

While a variety of conductive-adhesive hydrogels have shed light on the development of functional hydrogels for wound healing, the susceptibility of the conductive-adhesive hydrogels to bacterial infection and consequently, inflammation should be considered. Conductive hydrogels that are incorporated with

Figure 7. a) Conceptual illustration of \(pH\)-switchable antimicrobial IKFQFHFD-based nanofiber networks for biofilm eradication and chronic wound healing. b) Representative photographs and TEM images of hydrogel under different \(pH\) conditions. Molecular dynamic simulation of the aggregation of IKFQFHFD in water with different \(pH\) values was also given. c) Schematic representation of hydrogel-Cypate system for acidic \(pH\)-switchable photothermal damage of extracellular polymeric substances structure and biofilm eradication. Adapted with permission from [151].

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antibacterial agents such as silver NPs can potentially present good outcomes for wound healing. For instance, in a recent study, polydopamine modified silver nanoparticles (PDA@Ag NPs) incorporation was shown to contribute to the self-healing, self-adhesive, and excellent antibacterial properties of the hydrogel containing 3-aminophenylboronic acid, polyaniline, and polyvinyl alcohol. The antibacterial activity of the nanocomposite hydrogel was mainly due to the presence of Ag NPs. Benefiting from the multifunctional characteristics of the nanocomposite hydrogel such as electroactive and antibacterial properties, the significant neovascularization and collagen deposition in the PDA@Ag NPs incorporated hydrogel treated groups were observed on diabetic foot wounds [159]. Some issues of AgNPs such as cytotoxicity at their high concentrations and aggregation have limited their applications. In order to overcome these limitations, developing hybrid nanomaterials by various nanostructures have been proposed [152]. Yan et al. fabricated a sprayable hydrogel based on Ag NPs functionalized reduced GO nanosheets (AG) with antibacterial effect and poly(N-isopropylacrylamide-co-n-butylacrylate) (P(NIPAAm-co-NBA))-poly (ethylene glycol)-poly(N-isopropylacrylamide-co-n-butylacrylate) (PEP) hydrogel with sol–gel irreversibility [160]. In addition, in situ forming hydrogels with improved properties such as fast gelation with great stability and antibacterial have gained a lot of attention for wound healing compared to preformed hydrogels. Especially stable physically crosslinked hydrogels are promising candidates for tissue regeneration due to the absence of organic crosslinkers and their enhanced biocompatibility. In this context, functional thermo-responsive in situ forming hydrogels were prepared to generate an effective dressing for meticillin-resistant Staphylococcus aureus (MRSA) infected wound. Indeed, coordinated interactions between the collapsed PNIPAM chains and AG led to an increase in the stability of nanocomposite hydrogel at low temperature; however, this trend was AG-concentration dependent. Increasing the amount of AG until 0.75 wt% enhanced the tensile strength of the nanocomposite hydrogel, but at the concentration of 1 wt%, the tensile strength sharply reduced which was due to the inappropriate dispersion of extra amounts of AG in the hydrogel. This study indicated that AG/PEP nanocomposite hydrogel displayed higher antibacterial effects compared to both the PEP and PEP-rGO hydrogels groups. In vivo skin wound healing showed that the sprayed PEP-AG aqueous dispersion on the wound site in response to the local skin temperature could form irreversible hydrogel without using any crosslinker. AG/PEP nanocomposite hydrogel presented significantly faster MRSA infected wound closure in comparison to the untreated group after 12 days. Additionally, the in vitro studies showed that AG/PEP nanocomposite hydrogel supported cell viability over 98%, even at a high concentration of 200 μg/mL [160].

3.2.3. Carbon based nanomaterials

Carbon-based nanomaterials such as graphene, carbon nanotubes (CNT), and carbon dots display promising electrochemical, optical, and catalytic properties [161] that are widely used in the field of wound healing. Two-dimensional (2D) graphene sheet and GO have been considered as promising nanomaterials in tissue engineering. Due to its active functional groups, such as carboxyl, hydroxyl, and epoxy, GO is a good candidate for the loading of drugs and biological molecules [7,162]. The incorporation of GO nanosheets as 2D materials can make a dual network nanocomposite hydrogel without using any organic crosslinkers [160,163]. Various methods have been used for the fabrication of reduced GO (rGO) nanosheets that among them, green chemistry is a promising approach [164]. Due to the recent advances in the design of new nanomaterials to accelerate wound healing, carbon-based multifunctional nanostructures have been also received significant attention. For example, hybrid nanosheets of graphitic carbon nitride (g-C3N4)-Zn2+ @GO (SCN-Zn2+ @GO) were developed to take advantage of the synergistic effects of the photothermal and photodynamic antibacterial activity for wound healing. The induction of hyperthermia at 808 nm light irradiation due to the presence of GO can reduce the bacterial activity. On the other hand, the photocatalytic performance of the hybrid nanosheets at 660 nm can kill the bacteria via membrane damage, protein denaturation, and disturbance of bacterial metabolic pathways [165]. Considering the major drawbacks of conductive polymers (for instance polyaniline, polypyrrole, and polythiophene) such as poor solubility and non-degradability that limit their biomedical applications [104], wound dressings incorporating electroactive materials such as metal NPs and carbon nanomaterials can be a promising approach for accelerating wound healing. In particular, reducing GO by polydopamine (pGO) and the use of pGO for reinforcement have resulted in improved mechanical properties of the scaffold, uniformly distribution of GO in the scaffold to supply a channel for the transition of electrical signals, and scavenging ROS. The generated electrical conductivity in response to electrical stimulation has been utilized to improved the biological activity via increasing adhesion, proliferation, and aspect ratio of C2C12 cells [51]. Also, the chemical crosslinking and physical attachment between PDA or catechol groups and soft tissue enable satisfying wet bio-adhesiveness to soft tissues [7,166]. Thus, polydopamine based hydrogels have been incorporated in wound dressings to obtain adhesive scaffold. In a recent study, multifunctional injectable nanocomposite hydrogels with adhesive, hemostatic, antibacterial, and conductive properties were fabricated by HA-graft-dopamine (HA-DA) and rGO for wound healing. In this study, GO nanosheets were functionalized with dopamine (rGO@PDA) to obtain rGO with enhanced hydrophilicity. The formation of the nanocomposite hydrogel network was due to the oxidative coupling of catechol groups of rGO@PDA and HA-DA via H2O2/HRP (horseradish peroxidase) as an initiator. Due to the chemical crosslinking between rGO@PDA and HA-DA, the respective nanocomposite hydrogel had significantly higher stress at the same strain. The swelling ratio of hydrogels affected by the concentration of rGO@PDA, decreasing the amount of rGO@PDA enhanced the swelling ratio of the of the HA-DA/rGO hydrogels. Using rGO@PDA as a photothermal contrast agent in the presence of NIR irradiation for just 1 min, exhibited a significantly decrease in the bacteria survival ratio (64.1% for Escherichia coli and 65.4% for Staphylococcus aureus). In vitro studies demonstrated that these nanocomposite hydrogels provided higher cell proliferation than HA-DA hydrogel. For the in vivo wound healing study, the hydroxyproline quantity was assessed to investigate the collagen deposition in treated groups. It was observed that the collagen content in HA-DA/rGO was significantly higher than HA-DA hydrogel [7].

CNTs due to their electrical and photothermal antibacterial effects [167] can be used to generate nanocomposite hydrogels for wound healing. It has been demonstrated that the incorporation of CNTs to a glycol-chitosan hydrogel affected gelation time, porosity, and storage modulus of the resulting hydrogels [168]. In this content, Ravanbakhsh et al. explored the use of CNTs as nanoreinforcer for the production of glycol-chitosan based hydrogel to evaluate the effect of CNT concentration on cell migration. They demonstrated that CNT at a concentration of 250 μg/mL had the highest rate of cell migration, but this trend was not observed at high concentrations [141]. The effect of electrical conductivity on wound healing has been also demonstrated. CNTs due to their electrical properties can be applied as a conductive material in hydrogel-based dressings. The surface coating of CNTs by dopamine is an emerging approach to generate functional nanomaterial with photothermal induced antibacterial effects, adhesiveness, antioxidant properties, and good dispersivity in the hydrogel matrix. In one relevant study, Liang et al. fabricated gelatin-grafted dopamine/ PDA-coated CNTs/chitosan (GT-DA/CS) nanocomposite hydrogels for the healing of infected full-thickness wound. The incorporation of chitosan improved hydrogels’ mechanical properties. Grafting dopamine could provide excellent tissue adhesion, hemostatic and antioxidative properties. CNT-PDA incorporation within GT-DA/CS
hydrogels increased compression stress based on CNT-PDA concentration. It was also observed that shear adhesive strength and conductivity of GT-DA/CS hydrogel improved when CNT-PDA concentration was increased. Photothermal antibacterial performance of the prepared GT-DA/CS-CNT-PDA hydrogels showed that the addition of CNT-PDA to hydrogel led to significant enhanced photothermal-induced antibacterial effects without impacting the cellular ingrowth [169].

Carbon dots (CD) as zero-dimensional (0D) nanomaterial have been considered in various biomedical applications [170]. Recently, several studies demonstrated that CDs at low concentrations could display broad-spectrum antibacterial activity via entering the bacteria by diffusion, destroying the bacterial wall, binding to the DNA and RNA of bacteria, and finally killing the bacteria [171]. Antibacterial agent loaded hydrogels are effective, but they have drawbacks such as undesirable drug release and toxicity to normal cells [172]. So, developing smart hydrogels with antibacterial effects under external stimuli can be used as a promising [173]. Xiang et al. described the fabrication of smart injectable hydrogels made from DA and folic acid (FA) by zinc ions (DFT-hydrogel) that acted as a crosslinker for wound healing application. The formation of injectable hydrogels was based on the chelation of Zn$^{2+}$ by carboxyl and catechol groups of FA and PDA. Also, PDA functionalized carbon quantum dot (CDQ)-decorated ZnO NPs (CZnO) were incorporated in the prepared hydrogel to obtain a smart antibacterial nanocomposite hydrogel (Fig. 8). The use of carbon quantum dots was due to modifying the photocalytic performance of ZnO to respond to IR or visible light. The antibacterial performance of C/ZnO/DFT nanocomposite hydrogel was investigated by 660 nm plus 808 nm light. The results showed that the antibacterial rates of the C/ZnO/DFT nanocomposite hydrogel against S. aureus and E. coli were 99.9996% and 99.9998%, respectively. The antibacterial efficacy of C/ZnO/DFT nanocomposite hydrogel in darkness without light irradiation for S. aureus and E. coli were 78.9% and 70.7%, respectively. This could be due to the sustained release of Zn$^{2+}$, which was proven to have antibacterial effect. In vivo experiments using a wound model on infected rats demonstrated that the quantitative wound closure rates and collagen deposition of C/ZnO/DFT nanocomposite hydrogel were better than other groups [173]. In another study, Omidi et al. developed a pH-sensitive CD/chitosan nanocomposite hydrogel and investigated the physicochemical, mechanical, and antibacterial properties, pH sensitivity, and in vivo wound healing properties of the developed hydrogel. The CD/chitosan nanocomposite hydrogels were fabricated by the addition of different concentrations of CDs (from ammonium hydrogen citrate) to the chitosan solution. The microstructures of the fabricated CD/chitosan nanocomposite hydrogels showed that the incorporation of CDs led to an increase in the roughness and fracture of the hydrogel. The improved mechanical properties of the CD/chitosan nanocomposite hydrogel in comparison to the pure chitosan hydrogel could be explained by the inclusion of CDs to chitosan. It was also observed that increasing the concentration of CDs enhanced the inhibition zone. Also, the pH sensitivity of fluorescence intensities of CDs showed that in the range of 4–9 with increasing pH, the fluorescence intensity of the CDs was enhanced. This agrees with the range of pH oscillation in the wound healing process, so the prepared CD/chitosan nanocomposite hydrogel can have a great potential for monitoring pH in wound healing [174].

3.2.4. Mineral based nanomaterials

2D nanomaterials such as nanoclays with sheet or disc-like morphology and unique structural and surface properties have been used as promising biomaterials in the field of bioactive molecule release and regenerative medicine [175]. Also, these clays can uniformly disperse within the polymeric matrix, successfully interact with polymer interface, and act as crosslinker influence the hydrogel mechanical properties [176]. Between nanoclays, laponite (Na$^+$)[(Mg$_5$Li$_{0.3}$Si$_8$O$_{20}$(OH)$_4$)$_{10.7}$ due to its unique characteristics such as dual charged surface, absence of heavy metals, high cationic exchange, and low cytotoxicity in comparison to other 2D nanomaterials, are frequently used for biomedical applications [124,176]. Minerals, as the basic components of the laponite nanosheets present naturally in the human body. Also, these laponite nanosheets are biocompatible and biodegradable under physiological conditions [177]. It has been shown that incorporation of laponite in hydrogel can improve the physiological stability, injectability, and in vivo hemostatic performance [178]. The hemostatic performance in clays such as laponite and montmorillonite (MMT) is governed by their swellability, charged stimulation of activating blood coagulation, and disk-like structure [179,180]. Laponite is a good nanostructure as an auxiliary material for the direct 3D-printing of hydrogel composite scaffolds without using any support bath (self-supported approach). Laponite nanoclays, due to their favorable properties such as crystal structure, positive and negative charges, can mix with a variety of hydrogel inks. The ability of laponite in recovering its house-of-cards structure preserves the self-supporting capacity for direct 3D-printing of scaffolds in the air. Also, the improved mechanical properties by the addition of laponite can provide shape integrity of printed scaffolds after deposition [181]. Various nanoclays have been indicated for hemostasis applications. For example, zeolite can stimulate hemostasis via quick absorption of plasma and blood cells to speed up blood clotting through the release of Ca ions. The clotting mechanism of kaolinite (kaolin) [Al$_2$Si$_2$O$_5$(OH)$_4$] -iron oxide nanocomposite (α-Fe$_2$O$_3$-kaolinite) was ascribed to synergistic effects of kaolin and α-Fe$_2$O$_3$. These effects include adsorption of fluid to concentrated blood platelets, red blood cells (RBCs), and clotting factors, and induction of the intrinsic coagulation cascade by kaolin [182]. Laponite are used as a vehicle for sustained release of growth factors such as vascular endothelial growth factor (VEGF). The strong interaction between the growth factor and the laponite can result in no releasing VEGF. Injectable hydrogel contained VEGF incorporated laponite has investigated for angiogenesis. It was observed that the VEGF-laponite gels increased the tubulogenesis without releasing VEGF. The prolonged maintenance of VEGF at the implantation location led to enhanced efficacy [183].

In one study, Giriraj Lokhande and coworkers described the shear-thinning injectable hydrogels based on 2D laponite nanosilicates and R-carrageenan hydrogel for wound healing. These nanocomposite hydrogels were fabricated by the physically crosslinking of R-carrageenan using monovalent ions of exfoliated laponite. The increase in nanosilicates concentrations due to their charged nature and high surface area resulted in improved compressive modulus of the prepared hydrogels. The increased compressive modulus was because charged laponites provided more electrostatic interactions with R-carrageenan charged side groups. It was observed that the micro-architecture and pore size of the prepared hydrogel network were not influenced by the incorporation of 2D nanosilicates. In vitro studies showed that the nanocomposite hydrogels resulted in more cell adhesion in comparison to the R-carrageenan hydrogel due to having nanosilicates with electrostatically-driven adhesion sites for proteins. Additionally, the presence of laponites in the R-carrageenan hydrogel led to enhance platelets and red blood cell adhesion and decreased clotting time. Also, further reduction in the zeta potential of the R-carrageenan hydrogel as a result of the addition of nanosilicates was proposed as the reason for the activation of the coagulation pathway [124].

Changing the pH of chronic wounds as a result of infection has been considered to design smart dressings to release drugs into the wound site without any adverse effects. For instance, the fabrication of pH-responsive wound dressing for on-demand release of drugs using electronically controlling local pH was described by Klaee et al. The authors prepared pH-sensitive nanocomposite hydrogel based on PEDMA/laponite/chitosan NPs as drug vehicles, microfabricated electrodes serving as anode and cathode, and finally electronic circuitry. The addition of
laponite nanosilicate was for the induction of electrostatic interaction between the positive charge of chitosan NPs and negative charge of laponite to provide sustained drug release from chitosan NPs. Reduction of the size of the chitosan NPs due to their dehydration in pH 14 confirmed the release process of the drug. The use of an electrical voltage altered the local pH at the nanocomposite hydrogel coated the anode without affecting the wound pH. Moving negative ions to the anode produced basic pH around the anode. Incorporating chitosan NPs to PEGDA/laponite hydrogel increased the pore size and swelling ratio of the scaffold. Additionally, the burst swellability and rapid degradation of PEGDA/laponite NPs in basic pH could result in the faster drug release [184]. Although the use of laponite improved the mechanical properties of the hydrogels, it is important to prepare hydrogels with suitable adhesiveness for wound healing applications. To obtain a hydrogel with both appropriate mechanical properties and sufficient adhesiveness, Han and co-workers reported the development of a tough nanocomposite hydrogel with mussel-inspired adhesive property using PDA, nanoclay, and polyacrylamide (PAM). Intercalation of DA between the layers of nanoclays resulted in the formation of PDA-intercalated clay nanosheets with sufficient free catechol groups for adhesive properties. AM monomers were then incorporated and in situ polymerized to produce the nanocomposite hydrogel. In order to oxidize DA to form PDA, Fe(III) and sodium periodate also were applied as oxidative agents, but a freestanding hydrogel could not obtain and only a highly viscous fluid was formed. The dissolved ions from the clay served as an oxidation environment to make a stable PDA-clay-PAM scaffold. The fabricated PDA-clay-PAM hydrogel presented adherence to human skin with no irritation. Also, clay-PAM hydrogel without DA did not show adhesive properties and the adhesion capability of the PDA-clay-PAM hydrogel depended on the concentration of DA. But an increase in the concentration of nanoclays resulted in a decrease in adhesion strength of the prepared nanocomposite hydrogels. This could be due to the two reasons: 1. inducing overoxidation of the PDA and consequently lower available catechol groups, and 2. making highly crosslinked network by nanoclays which prevented the movement of the polymer chains at the adhesion interface. They indicated optimal weight ratios for DA/AM and clay/AM for achieving the maximum adhesion properties for hydrogel (0.8 and 10 wt%, respectively). The PDA-clay-PAM nanocomposite hydrogel demonstrated robust mechanical properties, including stretchable, resilient, and tough characteristics. The PDA-clay-PAM nanocomposite hydrogel containing EGF had prolonged release in comparison to the PAM hydrogel over 21 days that could be due to the catechol and quinone groups interactions of PDA with the proteins [185]. Recently, researchers have studied the use of multifunctional dressings for various applications such as treating skin cancer, improving wound healing, and preventing multi-drug resistant bacterial infection.
For example, Zhou et al. developed a multifunctional nano-composite hydrogel (Fig. 9) based on PDA modified bioactive glass NPs (BGN@PDA) incorporated in a hydrogel composed of F127-ε-Poly-ε-lysine (FEPL) and F127-Phe-CHO. This injectable nanocomposite hydrogel presented excellent self-healing property that could be due to the Schiff base between the amino group of FEPL and the aldehyde group of F127-Phe-CHO and BGN@PDA. The incorporation of BGN@PDA into the hydrogel resulted in an excellent photothermal property under NIR irradiation that could lead to significant A375 cancer cells killing. Also, C2C12 cell viability was greater than 80% indicating the good biocompatibility of the nanocomposite hydrogel. The resulting nanocomposite hydrogel showed considerable antibacterial performance with the killing efficiency up to 99% against E. coli and S. aureus which was mainly due to the presence of F127-Phe-CHO and F127-ε-Poly-ε-lysine. Furthermore, the in vivo full-thickness skin wound model studies demonstrated that the nanocomposite hydrogel could promote tissue regeneration via more collagen deposition, angiogenesis, and epidermis formation compared to other groups [187]. Table 3 summarizes the used multifunctional hydrogels with nanostructural components for wound healing.

4. Future perspectives

Despite the potential impact of multifunctional hydrogels, there are still challenges in applying them for wound healing applications. For future studies, developing advanced multifunctional hydrogels by adding the wound monitoring tools such as pathogenic infection detectors and at the same time smart drug releasing systems can be a promising approach. Taking the advantages of other biomacromolecules including gums, keratin, zein, and DNA will become an interesting approach for developing multifunctional hydrogels with good mechanical properties, self-healing, antioxidant, antibacterial, ECM mimicking, cell proliferation, tissue adhesive characteristics. DNA based approaches can be used for fabricating multifunctional hydrogels through providing biomacromolecule as the backbone of the dressings or DNA sequence encoding special proteins such as growth factors. In addition, more studies should be performed on the fabrication of multifunctional hydrogels which support scarless skin tissue regeneration. Advances in cell-laden hydrogels for tissue regeneration and using various stem cells not only provide accelerated wound healing but also support patient-specific treatments. New dressings with incorporated stem cell-derived exosomes are under investigation and designing multifunctional hydrogels using these vesicles will be the consideration of researchers in the future studies. Finally, the clinical translation capability and marketing of the prepared hydrogels should be considered to provide a better future for wound healing.

5. Conclusion

Conventional hydrogels offer superior advantages for the fabrication of wound dressings, but they suffer from some drawbacks that limit their applications for wound healing. The development of multifunctional hydrogels using various approaches has been provided a new vision in the field of wound healing which enabled them to provide effective skin regeneration. In this review paper, multifunctional hydrogels have been categorized by taking the advantages of their
Table 3
Multifunctional hydrogels with nanostructural components for wound healing.

<table>
<thead>
<tr>
<th>Hydrogel backbone</th>
<th>Type of nanostructure</th>
<th>Functions</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>GelMA</td>
<td>Keratin/chitosan nanofibers/bilayer scaffold</td>
<td>The cells in each layer were able to proliferate, provide the epidermis-like thin cell layer on keratin/chitosan nanofibers and dermis-like cell-laden GelMA hydrogel</td>
<td>[137]</td>
</tr>
<tr>
<td>Alginate</td>
<td>PLA nanofibers/bilayer membrane</td>
<td>Provide sufficient moisture for epithelial cells proliferation and migration, accelerate neovascularization, cover the wound for a long time and act as a barrier to prevent wound infection</td>
<td>[139]</td>
</tr>
<tr>
<td>Alginate</td>
<td>TEMPO-oxidized modified bacterial cellulose, Zn^{2+}/nanofiber reinforced hydrogel</td>
<td>Provide sufficient moisture for epithelial cells proliferation and migration, accelerate neovascularization, cover the wound for a long time and act as a barrier to prevent wound infection</td>
<td>[6]</td>
</tr>
<tr>
<td>Peptide (IKQPHFD)/cytate/proline</td>
<td>Self-assembled nanofibrous hydrogel</td>
<td>pH-switchable antimicrobial property, pH-sensitive release of bioactive molecules, destroy the extracellular polymeric substances of the bacterial biofilm, NIR responsive, contain procollagen component</td>
<td>[151]</td>
</tr>
<tr>
<td>Methacrylated kappa-carrageenan/l-glutamic acid</td>
<td>PDA modified ZnO NPs</td>
<td>Good dispersity of nanoparticles in the hydrogel network, promoting tissue adhesion, blood clotting ability, suitable mechanical characteristics, antibacterial efficacy, promote cell viability, collagen deposition</td>
<td>[157]</td>
</tr>
<tr>
<td>Chitosan</td>
<td>ZnO QDs/graphene oxide (GO) nanocomposites</td>
<td>Antibacterial, photothermal effects, sustained release of Zn^{2+} in a pH-dependent manner</td>
<td>[158]</td>
</tr>
<tr>
<td>3-aminophenylboronic acid/Polyaniline/PVA</td>
<td>PDA modified silver nanoparticles</td>
<td>Self-healing property, self-adhesive, excellent antibacterial properties, electroactive, promote neovascularization and collagen deposition</td>
<td>[159]</td>
</tr>
<tr>
<td>HA-graft-dopamine (HA-DA)</td>
<td>Reduced GO (rGO)</td>
<td>Injectable, adhesive, hemostatic, antibacterial, conductive properties, promote collagen deposition</td>
<td>[7]</td>
</tr>
<tr>
<td>Gelatin-grafted dopamine/Chitosan</td>
<td>PDA-coated CNTs</td>
<td>Excellent tissue adhesion, hemostatic, antioxidative properties, photothermal antibacterial performance without impacting the cellular ingrowth</td>
<td>[169]</td>
</tr>
<tr>
<td>DA/folic acid/zinc ions</td>
<td>PDA functionalized carbon quantum dot (CQD)-decorated ZnO NPs</td>
<td>Injectable, great photothermal and photodynamic responsive antibacterial performance,</td>
<td>[173]</td>
</tr>
<tr>
<td>8-carrageenan PEGDA</td>
<td>Laponite</td>
<td>More cell adhesion, decrease clotting time</td>
<td>[124]</td>
</tr>
<tr>
<td>F127-e-Poly-l-lysine/F127-Phe-CHO</td>
<td>Laponite/Chitosan NPs</td>
<td>On-demand release of drugs using electronically controlling local pH</td>
<td>[184]</td>
</tr>
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Table 3: Multifunctional hydrogels with nanostructural components for wound healing.

**Declaration of competing interest**

The authors have no conflict of interest to declare.

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**References**


H. Wu, F. Li, S. Wang, L. Xu, J. Li, Y. Li, et al., Ceria nanocrystals decorated mesoporous silica nanosphere based ROS-scavenging tissue adhesive for highly efficient regenerative wound healing, Biomaterials 151 (2018) 66–77.


M. Kim, Y. Ahn, K. Lee, W. Jung, C. Cha, In situ facile-forming chitosan hydrogels


