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Advances in biomedical applications of self-healing hydrogels

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Hydrogels are important biomaterials that have several applications in drug and cell delivery, tissue engineering, three-dimensional (3D) printing and more recently, in sensing and actuating applications. With the advent of self-healing hydrogels, it is becoming possible to have smarter materials with sustainable mechanical properties under stress and also added functionalities. The mechanisms responsible for the self-healing behavior of these materials are related to their internal structure and processes triggered by damage they may sustain. These mechanisms rely on either chemical bonding or physical interactions of the structural components of hydrogels, or on both. Many self-healing hydrogels have been developed and tested *in vitro* and in animals. However, there are still challenges, especially with healing characteristics that need to be addressed and investigated in animal experiments before their clinical applications can be initiated, for which a multidisciplinary approach is required. In the current paper, various biomedical applications of self-healing hydrogels are discussed in detail, highlighting current challenges and future prospects.

1. Introduction

Tissue damage can be sustained due to trauma or disease, and the human search for appropriate biomaterials to treat such tissue defects has continued for thousands of years.[1] Advances in Biomaterials Science enabled the development of tissue reconstructive strategies[2] and tissue engineering procedures[3] that can provide living constructs and also allow the delivery of different agents such as cells,[4] drugs,[5,6] cytokines[7] and polynucleotides[8] for therapeutic, diagnostic[9] or theranostic[10] purposes. There is a class of biomaterials, hydrogels, which is becoming increasingly important for its role as a filler,[11] and for the delivery of various drugs, biomolecules and cells.[12–14] Hydrogels are characterized by their close structural resemblance of native extracellular matrix in terms of swellability, porosity, and biocompatibility,[15,16] and various types of hydrogels have been successfully used in tissue regeneration[17] and three-dimensional (3D) bioprinting.[18,19] Although attractive in lending their flexibility and availability in an injectable format, and their easy delivery using minimally invasive procedures with subsequent solidification using one of the crosslinking techniques,[20] hydrogels suffer from limited mechanical properties, especially when they are subjected to mechanical stress.[21,22] As such, damage may lead to the loss of their function, their lifespan and successful biomedical applications.[23]

Over the past few years, significant progress in the production of advanced functional hydrogels with adjustable chemical,

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physical and biological characteristics has been achieved.[15,16] With the advent of self-healing hydrogels,[24,25] it is becoming possible to develop biomaterials and living constructs that can maintain their function and structure mimicking the self-healing properties of native tissues. Thus, developing mechanically robust hydrogels that possess self-healing capacity will expand their lifetime and reliability for use in various biomedical applications (Fig. 1). Besides the extended lifetime of self-healing hydrogels, their safety profile can also be better than conventional hydrogels.[26] Different methods can be used to achieve self-healing behavior in the hydrogels, which involve either chemical or physical mechanisms or both.[27]

They can be synthesized using dynamic covalent bonds, non-covalent interactions, or both.[28,29] There are very good review papers that describe in detail the mechanisms and synthesis of self-healing hydrogels.[24,25] However, a review dedicated to the discussion of biomedical application is lacking. Therefore, with the increasing interest in this group of smart biomaterials, there is a need to review these works to further help advance developments in this field and aid their translation to clinical applications in the future. This paper goes through these reports and analyzes the potential use of self-healing hydrogels for: (i) the delivery of cells,[4] drugs,[12–14] biomolecules[7] and polynucleotides,[8] (ii) engineering tissues for the development of regenerative therapeutics[9] or tissue models,[30,31] and (iii) the fabrication of sensors[32] and actuators.[33] There are many challenges that

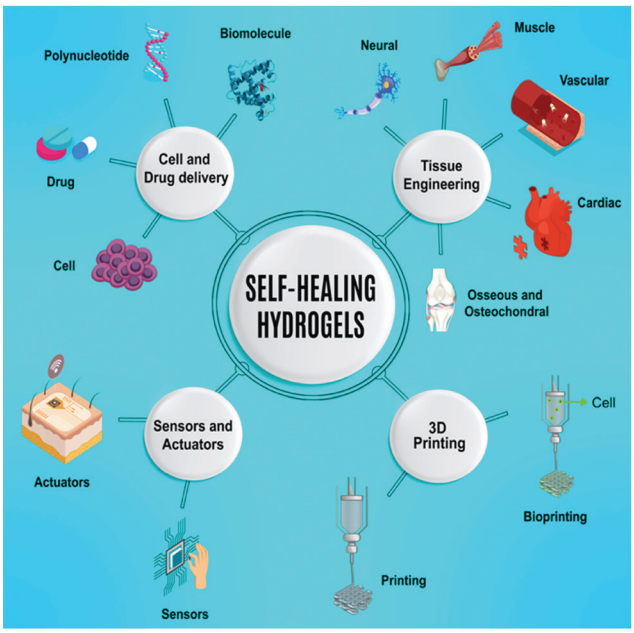

Fig. 1 Various biomedical applications of self-healing hydrogels.

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currently face these material applications, which are discussed along with future prospects. This review will likely serve as a good reference for scientists and clinicians who work or have an interest in this interesting group of biomaterials.

2. Mechanisms

To obtain a self-healing material, two main strategies can be used, namely, having an external mechanism or an internal mechanism, which is a property of the material itself. In the former, a composite material is used, *e.g.* a filler that can help in the healing of the material following the development of a break.[34] Since this requires the use of another material, this paper will focus on discussing the mechanisms of the latter. In brief, these can be broadly classified into chemical and physical crosslinking mechanisms, and they include dynamic covalent, physical bonds, or a combination of both (Fig. 2, Table 1). For more details, readers are referred to excellent reviews on the mechanisms of fabricating self-healing hydrogels.[24,35,36] Dynamic covalent bonds, such as imine bonds,[28,29] metal-coordination interactions,[37] disulfide bonds,[38] and Diels–Alder reactions,[39] are stable with slow dynamic equilibriums, whereas non-covalent interactions, such as hydrophobic,[40] host–guest,[41] ionic[42] or electrostatic interactions,[43] or hydrogen (H) bonds,[44–46] are fragile or less stable with rapid dynamic equilibriums. Self-healing mechanisms can also be either autonomous or non-autonomous.[47]

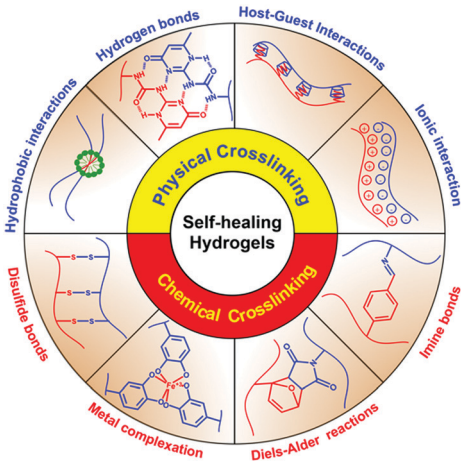

Fig. 2 Different chemical and physical mechanisms for synthesizing self-healing hydrogels.

2.1. Chemical bonds

Chemical bonds, such as in Schiff bases, imine formation, metal–ligand interactions, disulfide bonds, and Diels–Alder reactions are commonly used in synthesizing self-healing hydrogels. To prepare self-healing hydrogels using Schiff bases and their derivatives, the main synthetic strategy utilizes primary amine functional groups, or polymers containing amino groups, such as polyacrylamide, chitosan and with other polymers containing aldehyde groups, such as dextran,[48] oxidized alginate,[49] and hyaluronic acid[50] with sodium periodate. The Schiff base or imine formation is achieved through nucleophilic attack by primary amines on the aldehyde or ketone electrophilic carbon atoms. The reaction takes place either under acidic or neutral pH, producing water as a by-product. In the presence of water, hydrolysis can occur, and therefore, dynamic equilibrium can be achieved by adjusting the conditions of the reaction.

There have been many studies exploring various biomedical applications of imine formation-based self-healing hydrogels such as those containing hydrazones and oximes. Hydrazones and oximes are formed by reacting aldehydes or ketones with hydrazides, and aminooxy compounds, respectively. It should be noted that hydrazones and oximes are more stable than imines.

Metal-complexation is another dynamic covalent mechanism, where the transition metal binds with its ligands *via* donor–acceptor interactions to form a metal–ligand complex. Ligands are donors and give two or more electrons to the acceptor, the transition metal (also known as chelation). The resulting metal–ligand complexes are reversible, elastic and adhesive. Chelation is widely utilized to produce self-healing hydrogels, adhesives and elastic materials. The perfect example in nature is mussel's feet that can adhere to any dry or aqueous surface through the chelation of Fe^{3+} ions and catechol ligands.[59] Like catechols, other ligands such as pyrogallol or terpyridine groups can also be utilized for the production of self-healing hydrogels.

Table 1 Summary of mechanisms of self-healing hydrogels showing the type of the material, and the corresponding mechanism of self-healing

No.	Crosslinking method	Self-healing mechanism	Material	Ref.
1	Chemical	Imine bond	DF-PEG and glycol chitosan	51
2		Imine bond	Benzaldehyde-polyethylene glycol (PEG-BA) with carboxymethyl chitosan (CMC)	52
3		Imine bond	Poly(ethylene oxide-*co*-glycidol)-CHO [poly(EO-*co*-Gly)-CHO] with glycol chitosan (GC)	53
4		Imine derivative bond	Oxidized hyaluronic acid-gelatin-adipic acid dihydrazide (oxi-HAG-ADH)	50
5		Metal-complexation	Modified-chitosan pyrrole-Fe^{3+}	54
6		Disulfide bonds	8-Arm-PEG-S-TP (thiopyridyl-PEG)-doxycycline-H_2O_2	55
7		Diels–Alder	Hyaluronan methylfuran (HA-mF)	39
8	Physical	Hydrogen bonds	Modified hyaluronic acid (HA) with 1,6-hexamethylenediamine (HMDA) and cytosine (C) or guanosine (G) (HA-HMDA-C/G)	56
9		Hydrogen bonds	2-(Dimethylamino)-ethyl methacrylate (DMAEMA) with 2-(3-(6-methyl-4-oxo-1,4-dihydropyrimidin-2-yl)ureido)ethyl methacrylate (SCMHBMA)	57
10		Host–Guest interactions	Modified poly(acrylic acid) with cyclodextrins (pAA-CDs) and pAA with ferrocene (pAA-Fc) (pAA-6βCD-pAA-Fc.)	58
11		Host–Guest interactions	HA with adamantanes (Ad-HA) and HA with β-cyclodextrins (CD-HA) ((CD-HA-Ad-HA))	41

Another type of dynamic covalent bond is the disulfide bond. The reversible mechanism of this system is simply based on the exchange reaction between thiol and disulfide groups. The low activation energy needed by thiol groups to break the disulfide bonds serves as the driving force of the reaction. The thiol–disulfide exchange reactions are the nucleophilic substitution reactions in which the attacking nucleophiles are the thiolates. It is also worth mentioning that these reactions are spontaneous and sensitive to changes in pH, temperature, or redox potential.[60] The thermally reversible Diels–Alder reaction is another dynamic covalent chemistry-based reaction that can be used for the synthesis of self-healing hydrogels.[61,62] It is a one-step process that takes place between a conjugated diene and a dienophile that can be either an alkene or an alkyne. In Diels–Alder reactions, newly formed σ-bonds can break at temperatures higher than 100 °C and can reform at lower temperatures;[63,64] thus, it is possible to fabricate self-healing hydrogels that can break and heal by adjusting the temperature.[63,65] The Diels–Alder reaction is the best and maybe the only efficient method that can be employed to synthesize self-healing hydrogels for use in electronic packaging and structural materials.[66,67] Using this method, the production of highly pure hydrogels can be achieved without having side reactions.

2.2. Physical interactions

Physical crosslinking such as hydrophobic interactions, ionic interactions, host–guest interactions and hydrogen bonds can be used to produce self-healing hydrogels. Hydrogen bonds are universal and are present in the structures of many natural and biological compounds such as proteins or enzymes.[68] Hydrogen bonds are usually formed between carboxyl and amide groups (NH···O═C), hydrogen and fluorine (H···F) or hydroxyl groups (OH···OH). The typical bond energy of a hydrogen bond is between 4 kJ mol^{-1} and 60 kJ mol^{-1}. For comparison, the bond energy of a carbon–carbon bond is about 350 kJ mol^{-1}. Although the interaction energy of individual hydrogen bonds is relatively low, the use of multiple hydrogen bonds makes the structure relatively stronger to hold the backbone of the polymeric hydrogels and enable them to heal *via* reversible dynamic equilibrium. Hydrogen bonds can break and reform rapidly in picosecond time scale. Hence, this mechanism has been used in the synthesis of self-healing hydrogels,[57,69] and other smart biomaterials.[70]

Hydrophobic interactions are another strategy for synthesizing self-healing hydrogels. Hydrophobic interactions are generated by the incorporation of hydrophobic monomers within the hydrophilic polymer network in aqueous media with the help of surfactants such as sodium dodecyl sulfate (SDS). This takes place through cyclic dissociation and re-association of the micelles.

When self-healing hydrogels are formed by using host–guest interaction mechanism, specific non-covalent interactions are formed between macrocyclic hosts and smaller guest molecules. Examples of macrocyclic hosts include cucurbit[*n*]urils (CB), cyclodextrins (CD), and crown ethers; examples of guest molecules include alcohols, acids, amines, amino acids, or less polar molecules such as alkyls, cycloalkanes, and aromatic molecules.[71,72] Host–guest interactions cover a range of hydrogels used for biomedical applications such as bioimaging and 3D printing,[41] as well as drug and gene delivery.[73,74] Electrostatic interactions between oppositely charged polymer chains or ionic interaction between ions and charged polymer chains can also be used to produce self-healing hydrogels. In the ionic interaction, the mobility of the un-crosslinked polymer chains and the migration of the free ions are responsible for the dynamic reversibility of the ionic bonding of the polymer.[75]

3. Applications

3.1. Cell delivery

Intravenous and intra-arterial injections are commonly used routes for cell delivery.[76] Nevertheless, substantial loss of cells following transplantation and/or their accumulation in undesired sites still present a major issue[76,77] leading to problems such as aberrant growth.[78] Injectable self-healing hydrogels can be used as vehicles for cell delivery and tissue regeneration[48,79–86] (Table 2). Such materials gained attention as cell carriers due to their ease of fabrication, suitable mechanical properties, cell protection and retention, biocompatibility, injectability and bioactivity.[87] However, none of the self-healing hydrogels has reached the clinical trial stage. In this section, we summarize the important biomedical applications of self-healing hydrogels that are used as cell carriers.

3.1.1. Cardiovascular regeneration. Generating vasculature is essential to ensure nutrient and oxygen (O_2) supply to engineered and transplanted tissues. Self-healing hydrogels can protect and retain delivered cells from shear stress while maintaining their integrity independent of gelation kinetics. For example, cell viability was found to be 82% when endothelial progenitor cells (ECFCs) were injected subcutaneously in immune-deficient mice using a gelatin-based self-healing hydrogel (Fig. 3).[84] Most of the cells were homogenously distributed throughout the hydrogel. After 4–6 h of ECFC encapsulation, formed cell vacuoles started to coalesce, resulting in the formation of a complex and extensive vascular meshwork within the hydrogel. Lumenized vessels with distributed CD31+ cells were observed after three days in culture. It was also found that the *in vitro* incubation of these cell-loaded hydrogels, prior to injection, resulted in rapid vasculogenesis *in vivo* with the formation of larger and more mature vessels ($>50\ \mu m^2$). In another study, self-healing hydrogels were investigated for use in myocardial repair. An HA-based hydrogel encapsulating murine ECFCs was used for the treatment of experimental myocardial infarction in rats, by injection into the border zone of the ischemic myocardium. It was found that there was a significant cell migration from the ECFCs-loaded hydrogel after 48 h with enhanced vasculogenesis in the ischemic myocardium occurring after 28 days. A significant reduction in myocardial scar formation, improved ejection fraction of the left ventricle and improved contractility were observed 28 days post-injection.[88] In another study, chitosan-*graft*-aniline tetramer (CS-AT) poly(ethylene glycol) dialdehyde (PEGDA)-based self-healing hydrogels were used as vehicles for the injection of skeletal and cardiac myoblasts.[89] Good cell

Table 2 Summary of self-healing materials that have been used for cancer combination therapy. They were used to deliver one, two or three types of cells. Delivered cells included human mesenchymal stem cells (hMSCs), bone marrow-derived mesenchymal stem cells (BMSCs), murine neural stem cells (mNSCs), human induced pluripotent stem cells (hiPSCs), human endothelial progenitor cells (hEPCs), mouse myoblasts (C2C12), rat cardiac myoblasts (H9c2), human umbilical vein endothelial cells (HUVECs), dermal fibroblasts (hDFBs), murine myoblasts (mMBs). Delivered biomolecules include one, or more than one type of cytokines. Delivered biomolecules include b fibroblast growth factor (bFGF), FGF-2, platelet-derived growth factor BB (PDGF-BB), vascular endothelial growth factor (VEGF), human epidermal growth factor (hEGF), interleukin 10 (IL-10) and anti-transforming growth factor beta 1 (TGF-β1). Delivered drugs and pharmaceutical agents include doxorubicin (DOX), docetaxel (DTX), Taxol, Decorin, mitoxantrone, 5-aminosalicylic acid (5-ASA), chlorhexidine acetate (CHA) or amoxycillin. Delivered polynucleotides include DNA such as bacteriorhodopsin (HEBR) plasmid or RNA such as small interfering RNA (siRNA). Element delivery includes silver (Ag^{+}) and ferric (Fe^{3+}) ions. Agents from these various categories were also combined and delivered using self-healing materials. These include combining cells with biomolecules, drugs or cytokines, or with both cells with polynucleotides, cytokines with pharmaceutical agents, or cytokines with elements

No.	Cargo	Cargo details	Material	Application	Ref.
1	Cells	hEPCs	Gelatin (Gel) + oxidized dextran (oxDex)	Cardiovascular regeneration	84
2		mEPCs	Adamantane + β-cyclodextrin modified hyaluronic acid	Cardiovascular regeneration	88
3		C2C12 myoblasts + H9c2 cardiac myoblasts	Chitosan-*graft*-aniline tetramer (CS-AT) + poly(ethylene glycol) dialdehyde (PEGDA)	Cardiovascular regeneration	89
4		mCCs	4 arm star PEG + vinyl sulfone + short dithiol	Osteochondral regeneration	82
5		Stem cells		Cartilage	85
6		mNSCs	*N*-Carboxyethyl chitosan (CEC) + oxidized sodium alginate (OSA)	Neural regeneration	83
7		mNSCs	Glycol–chitosan (GCS) + DF-PEG	Neural regeneration	79
8		mNSCs	Glycol–chitosan (GCS) + DF-PEG + cellulose nanofiber (CNF)	Neural regeneration	86
9		hMSCs	Adamantane + β-cyclodextrin modified hyaluronic acid	Stem cell therapy (cartilage regeneration)	79
10		mMBs and HUVECs	*N*-Carboxyethyl chitosan (CS) + dextran-*graft*-tetraaniline (Dex)	Skeletal muscle regeneration	48
11		hMSCs, hDFBs and hCCs	Carboxymethyl cellulose dialdehyde (CMC-D) + carboxymethyl chitosan (CMCh)	Stem cell therapy (Wound healing)	80
12	Biomolecules	FGF-2	*N*-Succinyl-chitosan (SCS) + Benzaldehyde-terminated polyethylene glycol (BAPEG)	Wound healing	90
13		IL-10 and anti-TGF-β1	Hyaluronic acid	Immune-based therapy (kidney disease)	91
14		HGF and IGF-1	Ureido-pyrimidinone (UPy) + PEG	Cardiac regeneration	92
15		AMSCs-exo	Pluronic®F127 + oxidative hyaluronic acid (OHA) + epsilon-poly-L-lysine (EPL)	Wound healing and skin regeneration	93
16		BMP-4	Chondroitin sulfate + *N*-maleoyl alanine terminated PEG + Pluronic®F127 + adipic dihydrazide	Bone regeneration	94
17	Drugs	DOX	Carboxyethyl-modified chitosan (CMC) + aldehyde modified hyaluronic acid (A-HA)	Anticancer therapy	95
18		DOX	Poly(γ-*o*-nitrobenzyl-L-glutamate) + PEG-*b*-poly(γ-*o*-nitrobenzyl-L-glutamate)	Anticancer therapy	96
19		DOX	*N*-Carboxyethyl chitosan (CEC) + dibenzaldehyde-terminated poly(ethylene glycol) (PEGDA)	Anticancer therapy	97
20		DOX	Hydrazide functionalized hydroxylethyl grafted poly(aspartic acid) (PAEH) + poly(ethylene glycol) dialdehyde (PEGDA)	Anticancer therapy	98
21		Taxol	Glycol–chitosan (GCS) + DF-PEG	Anticancer therapy	99
22		Decorin	Gellan gum + NaCl	Wound healing	100
23		Mitoxantrone	Alginate + calcium	Anticancer therapy	101
24		Curcumin	Quaternized chitosan (QCS) + benzaldehyde-terminated Pluronic®F127 (PF127-CHO)	Anticancer therapy + wound healing	102
25		DOX and Amoxicillin	Chitosan-grafted-dihydrocaffeic acid (CS-DA) + oxidized pullulan (OP)	Anticancer and antibacterial therapy	103
26		DOX and DTX	Chitosan (CS) + difunctional PEG (DF–PEG–DF)	Anticancer therapy	104
27		DOX and DTX	Chitosan–catechol + Fe^{3+}	Anticancer therapy	99
28		5-ASA and Epsilon-poly-L-lysine (EPL)	Poly(glycerol sebacate) + ureido-pyrimidinone (UPy)	Drug delivery and antibacterial surface functionalization	105
29	Polynucleotides	siRNA	β-Cyclodextrin (CD) and adamantane (Ad) + polyethylenimine (PEI)	Optogenetic tool delivery	106
30	Combination	hMSCs + dexamethasone	Gelatin and acrylate β-cyclodextrin	Drug delivery and cell carrier	107
31	Combination	hMSCs + dexamethasone	Gelatin and acrylate β-cyclodextrin	Drug delivery and cell carrier	107
32		mFBs, rhodamine B and cisplatin and L929	Glycol chitosan (GCS) + dibenzaldehyde terminated poly(*N*-isopropyl acrylamide)-*co*-poly(acrylic acid) (DF poly(NIPAM-*co*-AA))	Drug delivery and cell carrier	108
33		FBs + hEGF	Gelatin (GE) + oxidized alginate (OSA) + adipic acid dihydrazide (ADH)	Growth factor delivery and cell carrier	109
34		bFGF-PLGA micro-spheres + CHA	Aminated gelatin (NGel) + adipic acid dihydrazide (ADH) + oxidized dextran (ODex)	Wound healing	110
35		PDGF-BB and Fe^{3+}	(EDTA)-Fe^{3+} + hyaluronic acid (HA)	Anti-infection and wound healing (skin regeneration)	111
36		BMSCs (rat and human) + TGF-β1 + kartogenin	Gelatin + acrylate β-cyclodextrin	Osteochondral regeneration	85

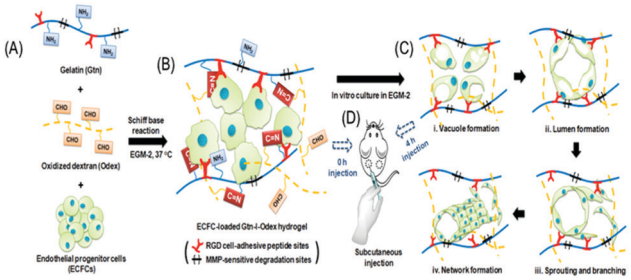

Fig. 3 Schematic illustration showing the preparation method and properties of the ECFC-loaded Gtn-l-Odex hydrogel. First, cells were mixed with a polymer solution (A). Second, the hydrogel was crosslinked (B) *via* the dynamic imine chemical bonding that formed between the amino groups (in Gtn) and aldehyde groups (in Odex). (C) Vascular morphogenesis of endothelial progenitor cells (ECFCs) that were cultured in the hydrogel. (i) Vacuole formation in the encapsulated ECFCs. (ii) Merging of vacuolated ECFCs leading to lumen formation. (iii, iv) Tubulogenesis occurring through the sprouting and branching of the vascular network. (D) Schematic showing the injectability of the introduced self-healing hydrogel in mice at 0 h and 4 h *in vitro* culture time. Reproduced from ref. 84, with permission from Elsevier, 2018.

Fig. 4 An injectable hydrogel was formed by mixing *N*-carboxyethyl chitosan and oxidized sodium alginate. Reproduced from ref. 83, with permission from Nature Publishing Group, 2016.

viability and proliferation of the co-cultured cells were observed after three days of culture. These hydrogels also exhibited antibacterial activity, adhesiveness and biodegradability. The hydrogels were also found to protect cells during injection into the rats.

3.1.2. Skeletal muscle regeneration. Tissues like muscles are subjected to tensile forces (stretching) that can cause, with time, the rupture of hydrogel polymer chains. Unlike traditional hydrogels, self-healing hydrogels are developed as flexible, stronger and longer-lasting materials with more breaking down and building up, thus mimicking skeletal muscle physiological conditions during training. Cytocompatible dextran-*graft*-aniline tetramer-*graft*-4-formylbenzoic acid and *N*-carboxyethyl chitosan-based hydrogels were developed for use in skeletal muscle repair. They were used to encapsulate human umbilical vein endothelial cells (HUVECs) and C2C12 myoblasts and inject them into rats having volumetric muscle loss. The hydrogel was demonstrated to maintain cell viability and proliferation capacity. Four weeks after implantation, enhanced myofibers/capillary density and new tissue formation were observed in the muscle defects of the rats.[48]

3.1.3. Neural tissue regeneration. Delivering neural stem cells (NSCs) into lesion sites where they can proliferate, differentiate and integrate with the host tissue constitutes a potential approach for the treatment of neural disorders. Biodegradable and soft hydrogels (0.1–1 kPa) mimicking native brain tissues are sought to favor neural cell differentiation and function following injection. Unlike classical hydrogels, self-healing ones can be injected to deliver neural cells *via* loaded-fragments that can self-heal, while protecting cells, into an integrated structure at the lesion site. In their work, Tseng and colleagues reported a chitosan-based hydrogel presenting self-healing properties even after high strain-induced structural damage.[112] Once encapsulated within the hydrogel, NSCs started to form spheroids that grew with time and expressed neural differentiation markers that include β-tubulin and Map2 (mature neuronal marker). Compared to acellular hydrogels, NSC spheroid-loaded hydrogels revealed a better healing effect (*i.e.* coiling contraction, hatching and recovery rates) in the damaged central nervous system of zebrafish. In another work, oxidized sodium alginate and *N*-carboxyethyl chitosan were mixed to form an injectable self-healing hydrogel for the delivery of NSCs (Fig. 4).[83] The hydrogel supported homing, proliferation (up to 5 days) and differentiation of encapsulated NSCs with a marked expression of neuronal markers nestin and β-III tubulin but not glial marker GFAP, indicating the promotion of NSC differentiation into neurons rather than to glial cells. NSC-loaded hydrogels that were injected into mice with the focal cerebral ischemia model led to complete filling up of the lesion site one week after injection, with cells uniformly distributed throughout the hydrogel.[83] To improve neural regeneration, cellulose nanofibers (CNF) were added to chitosan-based self-healing hydrogels. Depending on the CNF concentrations, chitosan-based hydrogels were found to enhance NSCs proliferation (up to 28 days) as well as mitochondrial and non-mitochondrial functions along with an increase in NSCs GFAP and CNPase glial marker- and β-tubulin (early) and Map2 (mature) neural marker expression at protein and mRNA levels. Six days after injection into experimental brain injury in adult zebrafish, NSCs-loaded hydrogels led to better results as compared to control (untreated) group, with improved survival rate (70% *vs.* 20% respectively) and recovery of swimming activity (65% *vs.* 20% respectively).[86] It was concluded that hydrogels with 0.09 wt% exhibited the best conditions, leading to optimized hydrogel self-healing, *in vitro* NSC differentiation and *in vivo* neural regenerative effects. In another study, glycol-chitosan (GCS) was mixed with a DF-PEG-based self-healing hydrogel for neural regeneration.[81] NSCs embedded in this hydrogel exhibited significant neural differentiation. *In vitro* results demonstrated the formation of spheroids that grew with time and the expression of β-tubulin and Map2 (mature neuronal marker) by NSCs embedded in hydrogels. The hydrogels also showed a good healing effect with high hatching and recovery rates (90% and 81% respectively) in zebrafish-damaged central nervous system (after 48 h).

3.1.4. Chondral and osteochondral regeneration. Various natural and synthetic hydrogel-based materials have been

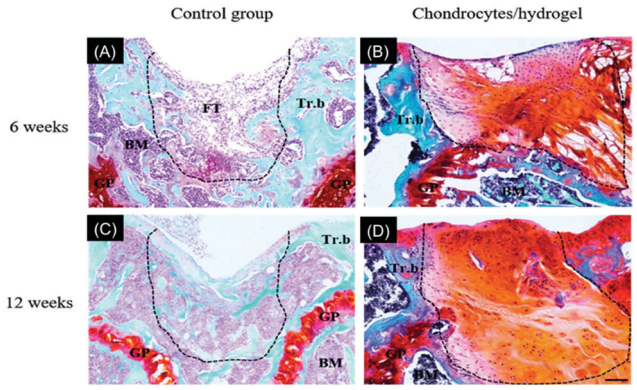

Fig. 5 The use of chondrocyte-containing self-healing 4-arm star poly-(ethylene glycol) (PEG) modified with vinyl sulfone in the treatment of experimental articular cartilage in mice. Images of Safranin O-stained sections of the defects at six weeks (A and B) and at 12 weeks (C and D) postoperative, using both treated and control sides. Newly formed tissue is shown in the area defined by the dashed line. Bone marrow (BM), trabecular bone (Tr.b), growth plate (GP), and fibrous tissue (FT) are shown. The scale bar is 100 μm. Reproduced from ref. 82, with permission from Elsevier, 2017.

applied as cell carriers for cartilage repair.[113] Nevertheless, conventional hydrogels have a high risk of failure once applied in load-bearing sites, because they lack the physical properties required to protect encapsulated cells. Therefore, the use of a self-healing hydrogels represents a good option with the potential for application in osteochondral regeneration[79,82,85] For example, Wang *et al.* developed a cartilage-like construct using a hydrogel based on 4-arm star PEG functionalized with vinyl sulfone and short dithiol crosslinkers.[82] The hydrogel was used to deliver chondrocytes into osteochondral defects in immunodeficient mice. After 12 weeks, increased proliferation, chondrogenic differentiation and ECM deposition were observed. Cartilage repair was achieved with hyaline cartilage formation that was integrated with the surrounding cartilage tissue (Fig. 5).[82] In another study, a self-healing and injectable supramolecular gelatin hydrogel, free of chemical crosslinking, was prepared to deliver stem cells and chondrogenic agents such as transforming growth factor-1 (TGF-β1) or kartogenin (KGN).[85] Such a hydrogel can encapsulate bone marrow derived MSCs and be handily injected to completely fill up the cartilage defect and adhere to the surrounding cartilage without affecting the viability and function of the encapsulated cells (up to 14 days). After 28 days of implantation in the subcutis of nude mice, MSC-laden hydrogels were stained positive for type II collagen and chondroitin sulfate. When used for the treatment of experimental osteochondral defects in the knees of rats, there was fully regenerated cartilage that was well integrated with the surrounding tissue after six weeks postoperatively.

3.1.5. Wound healing. The use of stem cells for the treatment of wounds represents an attractive option that utilizes the potential of stem cells in participating in the healing process through differentiation into appropriate cell types and through the secretion of paracrine factors. Self-healing hydrogels enable cell encapsulation through the formation of crosslinks between polymer chains prior to cell delivery into the wound. Alternatively, *in situ* gelation of the cell carrier can also be used. For example, carboxymethyl cellulose dialdehyde and carboxymethyl chitosan were mixed and used for *in situ* gelation, which readily occurred under *in vitro* physiological conditions without the need for the use of external stimuli.[80] This material was found to support encapsulated MSC viability and proliferation for up to five days. The *in vitro* chemotactic and proliferative response of fibroblasts to paracrine factors secreted by the transplanted MSCs confirmed the ability of the hydrogel to support and maintain the paracrine function of encapsulated MSCs.

3.2. Drug delivery

Conventional systemic drug delivery systems still suffer from different limitations such as the lack of target specificity, bioavailability, and premature elimination. The use of smart biomaterials may help to improve drug bioavailability, uptake and release, which are likely going to reduce their undesirable effects and optimize their target specificity.[114] Self-healing hydrogels have emerged as important potential drug carriers that combine biocompatibility, injectability, biodegradability, long-term operability, tissue adhesion, along with tunable cargo-release and stimuli-responsiveness.[95–97,99–105,107,115,116] Thus far, several hydrogels have been investigated, such as those containing polyethylene glycol (PEG), hydroxyethyl methacrylate (HEMA), and gellan gum (GG) and they are approved by the US Food and Drug Administration (FDA) for use as injectable drug delivery systems.[117] Although many of these PEG-based,[96,99,104,106,115,118] or GG-based[100] drug delivery self-healing hydrogels have been investigated, none of them has reached clinical studies or been approved by the FDA as yet. Drug delivery self-healing hydrogels *in vitro* and *in vivo* studies are presented in Table 2.

3.2.1. Cancer therapy. Among the various modalities available for the treatment of cancer, chemotherapy is considered an important one.[119] Unfortunately, chemotherapy is limited by its systemic toxicity. Therefore, the use of hydrogel-based drug delivery systems may help to reduce the occurrence of the undesirable effects of chemotherapy and improve its efficacy, tissue distribution and pharmacokinetics.[12]

When injected directly into the target location, drug-laden hydrogels with prolonged lifetimes can help to avoid the undesirable loss of the administered drug and thus improve the therapeutic efficacy of the drug and reduce the systemic toxicity. In one study, it was found that the use of encapsulated docetaxel (DTX) and doxorubicin (DOX) had a significantly better anti-cancer effect (reduction in cell number by 94.6%) as compared to the use of free drugs (reduction of 56.4% and 54.9% for DOX and DTX respectively) on human breast cancer cells.[104] However, the lack of self-healing properties in conventional hydrogels is likely to affect the release of the encapsulated drugs and thus their therapeutic efficacy. Self-healing hydrogels were proposed as ideal drug carriers because they are characterized by resistance to stress-induced cracks, shear-thinning injectability and homogeneous distribution within tissue defects.[98]

Having stimuli-responsiveness in the drug-releasing self-healing hydrogels can be utilized to trigger the release of the

loaded drug by either local stimuli such as changes in pH or temperature, or by external triggers such as light, electric field or acoustic waves.[95–97] Due to the acidic environment of the tumor tissue, pH-sensitive selective drug delivery *via* self-healing hydrogels has received considerable attention.[120] For example, this was demonstrated by Qu *et al.* who showed the higher cumulative release of DOX at the pH of 4.4 as compared to release at physiological pH (96% *vs.* 40%, respectively) after 96 h.[102] Interestingly, compared to free DOX, the self-healing hydrogel-encapsulated DOX showed a better anticancer effect on human hepatocellular liver carcinoma cells. Localized, on-demand therapeutic release can also be achieved through selective exposure of drug-loaded self-healing hydrogels to light. For example, UV irradiation-assisted DOX 24 h release from a self-healing glutamate-based hydrogel was much more (80%) as compared to hydrogels that were not exposed to UV irradiation (10%). Such an increase in DOX release resulted in an enhanced rate of apoptosis of cervical epithelioid carcinoma cells (61%) as compared to cells where no UV irradiation was used.[96] Ultrasound-mediated drug delivery from self-healing hydrogels is also another safe approach for localized drug administration. In one study, the effectiveness of the release of Mitoxantrine from an alginate-based self-healing hydrogel was demonstrated by using pulsatile ultrasound as a trigger. It was found that more (50%) cumulative release was achieved when US was used as compared to no use of US (30%) after 24 h *in vivo*.[101] It resulted in a significantly reduced viability of human breast cancer cells after 12 h in culture. There was also significant apoptosis in tumor xenografts 8 days after their implantation in mice, and reduced tumor growth was seen six months post-treatment. Thus far, self-healing hydrogels have been explored for the delivery of single anticancer drugs,[95–97,101,116] and also for the delivery of a combination of drugs.[99,104] Combining two or more drugs has been considered to have a profound impact on the treatment of cancer because of their synergistic effect. For example, Xie *et al.* showed that the release of DOX and DTX from a chitosan-PEG based self-healing hydrogel was synergistic, with the release of DTX being accelerated by the release of DOX.[104] Compared to single drugs, two-drug co-delivery resulted in the increased delivery of both DOX (48.9% to 67.6%) and DTX (78.2% to 83.7%) in 30 days (Fig. 6). In another study, Yavari *et al.* utilized catechol-Fe(III) complexation to produce a catechol containing chitosan-based self-healing hydrogel (CAT-Gel), which was employed for the combined delivery of DOX and DTX.[99] The resulting hydrogel was retained in the subcutis of mice for more than 40 days. As a carrier of DOX and DTX, it led to sequential and sustained drug release, which consequently led to achieving a synergistic therapeutic effect on murine lung and breast cancer cells *in vitro*. They also showed that the combined delivery of DTX and DOX by self-healing hydrogels showed a significantly enhanced antitumor activity and tumor size reduction as compared to the delivery of DOX or DTX alone, when it was used for the treatment of breast cancer xenografts implanted in nude mice. Furthermore, the combination of different chemotherapeutic agents, and also of chemotherapeutic agents with another cancer therapy modality can have a synergistic

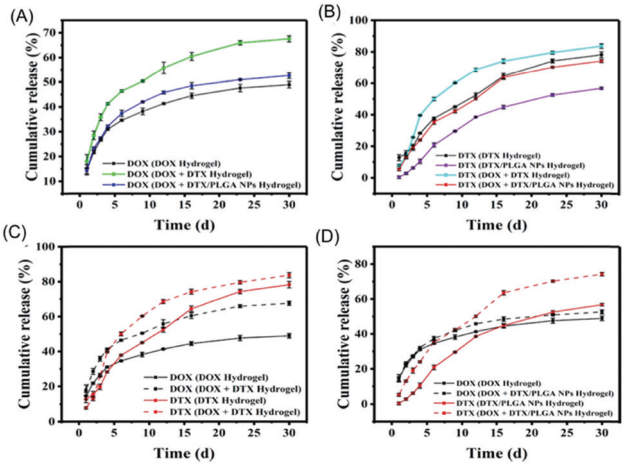

Fig. 6 Profiles of doxorubicin (DOX) (A) and docetaxel (DTX) (B) cumulative release from different hydrogel formulations at 37 °C. Synergistic release of DOX and DTX from native (C) and composite (D) hydrogel formulations was also observed. Reproduced from ref. 104, with permission from the American Chemical Society, 2017.

effect in cancer treatment. For example, superparamagnetic particles and two chemotherapeutic drugs (DOX and paclitaxel) were loaded into a PEG-cyclodextrin self-healing hydrogel.[121] It was possible to induce hyperthermia using an alternating magnetic field and the release of loaded drugs.[98] *In vitro* results showed that the anticancer treatment could be improved when hypothermia was combined with chemotherapy. More examples of combinatorial and multimodal cancer therapy using self-healing hydrogels for the treatment of cancer are provided in Table 2.

Although there have been several studies thus far, most of these were carried out *in vitro*. More *in vivo* studies are required to be able to draw a sound conclusion and help subsequent applications in the clinic. Moreover, these *in vivo* studies relied on the use of subcutaneous xenograft ectopic tumor models,[122] which do not necessarily have the same specific circumstances that orthotopic tumor models may have. *In vitro* studies that have been conducted so far have focused mainly on the evaluation of drug release profiles, biomaterial behavior and cytocompatibility.[120] *In vivo* studies were limited to the use of small animals.[104] Among these, self-healing hydrogels produced by physical interactions seemed to have fast self-healing behavior,[123] and self-healing hydrogels produced by chemical bonds were the strongest.[26] Regarding biocompatibility, chitosan-based hydrogels have been the best among hydrogels studied so far.[99] The most promising materials relied generally on Schiff base chemistry mechanisms.[103] However, all of these need to be investigated in animals and for longer follow-up times to prove both biocompatibility and efficiency.

3.2.2. Wound healing. Adhesive and biocompatible hydrogels with high water content, porous structure and appropriate swelling ratio offer great potential for application in the treatment of wounds. Although hydrogel-based dressings are currently in clinical use,[124] their lack of enough strength to withstand the repetitive motion of the skin represents an important limitation, which may lead to reduced therapeutic efficacy and discomfort.

Therefore, the use of durable and self-healing hydrogels can be useful to circumvent this problem. When loaded with agents that may enhance wound healing, the effect of self-healing hydrogels can be further enhanced.[125] In one study, Chouhan *et al.* used gellan gum supplemented with sodium chloride to develop Decorin-releasing self-healing hydrogel eye drops for the prevention of corneal scarring.[100] The developed hydrogel was transparent and well retained after it was applied to the ocular surface of freshly enucleated pig eye. It was also found to be cytocompatible when tested using human corneal fibroblasts *in vitro*. The cumulative release of Decorin was 45% after 3 h and led to enhanced re-epithelialization in an *ex vivo* rat corneal injury model (85% after 4 days). To regenerate skin flaps, a PEG-based self-healing hydrogel crosslinked with silver ions (Ag^{+}) was developed and utilized to carry Mangiferin liposomes (MF-Lip), in an attempt to combine the skin flap cytoprotective properties of MF-Lip with the anti-bacterial effects of Ag^{+}.[115] With almost 67% entrapment efficiency, the PEG-based hydrogel exhibited an MF-Lip cumulative and sustained release of about 95% after 7 days. *In vitro*, the release of MF-Lip enhanced the viability of HUVECs, which were cultured under either hypoxic or normoxic conditions. An increased expression of the angiogenic factors VEGF and bFGF was also observed. When injected into a random-pattern skin flap rat model, MF-Lip loaded hydrogels were able to increase skin flap microvessel density and skin flap survival rate, to decrease skin flap inflammation and to reduce skin flap infection.

3.2.3. Antibacterial delivery. It should be noted that with the use of localized delivery of antibacterial therapy directly to the site of infection, the therapeutic window of intensity can be increased by administering ultra-high doses of antibiotics, which would otherwise not be possible by using systemic approaches, where the occurrence of side effects limits the dosage that can be administered.[126] The use of self-healing hydrogels for antibiotic delivery offers several advantages including injectability, tissue adhesion and durability. Such properties are likely to endow such hydrogels with long-term function.[124,127]

Designed mucoadhesive and self-healing hydrogels based on oxidized pullulan (OP) and chitosan were found to have good injectability, adhesiveness, morphology, and rheological characteristics. With a cumulative release of 75% after 36 h, the use of Amoxicillin-loaded hydrogels led to a significant antibacterial effect (after two days) on cultured *S. aureus* and *E. coli* as compared to Amoxicillin-free hydrogels.[103] In addition to carrying drugs, self-healing hydrogels can undergo surface modification to confer on them antimicrobial function. For example, Wu *et al.* coated epsilon-poly-L-lysine (EPL) antimicrobial polymers on glycerol-based hydrogels by taking advantage of the physical interactions that exist between the units. The developed material showed good *in vitro* cytocompatibility and *in vivo* biocompatibility, and exhibited rapid self-healing and good mechanical properties. EPL-grafted hydrogels led to 100% killing of cultured *E. coli* (Fig. 7).[105]

3.2.4. Bone regeneration. Owing to their ease of fabrication, biocompatibility and bioactivity, crosslinked gelatin-based hydrogels have gained major attention.[128] Unfortunately, the resulting

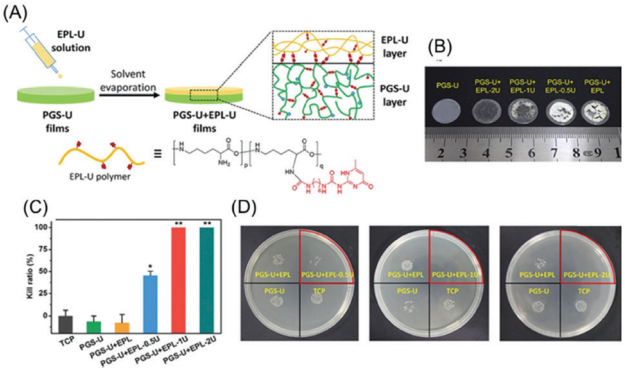

Fig. 7 (A) Schematic showing the coating of poly(glycerol sebacate) (PGS-U) films with epsilon-poly-L-lysine (EPL-U) due to the physical interactions that exist between the ureido-pyrimidinone (UPy) units. (B) Images of films made from the polymers that were studied in this research. (C) After the incubation of *E. coli* for 1 h, the PGS-U film and PGS-U + EPL film did not show any antibacterial effect; however, PGS-U + EPL-0.5U films, showed 45% bacterial killing efficacy and both PGS-U + EPL-1U and PGS-U + EPL-2U groups demonstrated 100% antimicrobial activity. (D) No colony-forming units were observed in cultures of the materials with 100% antibacterial activity. Reproduced from ref. 105, with permission from Elsevier, 2016.

hydrogels are fragile for post-gelation processing and they often fail to support even low compressive or tensile strain, making them unsuitable for use in demanding applications, *e.g.* in the treatment of load-bearing tissues such as bone.[129] Therefore, self-healing gelatin and acrylate β-cyclodextrin (β-CD) hydrogels with mechanical properties better than native gelatin-based hydrogels were developed by using guest–host macromere approach for potential use in bone regeneration.[107] These hydrogels can maintain their structure and thus, their cargo, to support compressive and tensile strain, and to self-heal when they sustain a fracture. The weak guest–host crosslinks between the gelatinous aromatic residues and β-CD were found to improve human mesenchymal stem cell (hMSC) infiltration, migration and spreading into the hydrogel without suffering structural damage. The use of β-CD conferred to the hydrogel the capability to carry, absorb (up to 80%) and release (up to 14 days) hydrophobic small molecules such as the anti-inflammatory drug, Dexamethasone (Dex). However, one has to be careful not to suppress regenerative macrophages (MP2) to the extent that may affect the regeneration capacity.[130,131] In this study, it was found that Dex-loaded hydrogels in the dose of 0.8 mM boosted encapsulated hMSCs osteogenic differentiation with enhanced expression of osteogenic gene markers *in vitro*. *In vivo*, it was also found that acellular Dex-loaded hydrogels can support tissue mineralization and regeneration which may indicate safe and possible polarization of MPs in regenerative and osteogenic direction (Fig. 8).

3.3. Biomolecule delivery

Delivering therapeutic biomolecules such as growth factors and other peptides plays an important role in tissue and regenerative engineering.[132] Biomolecules are known to have low stability, a short half-life that is highly dependent on the environmental

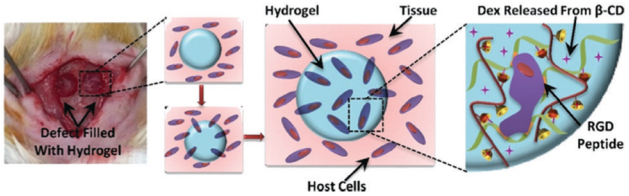

Fig. 8 Bone defect filled with dexamethasone (Dex)-laden gelatin-based hydrogel (HGM) that can enhance the host cell migration and deliver Dex in a controllable manner for *in situ* bone tissue regeneration. Reproduced from ref. 86, with permission from Elsevier, 2016.

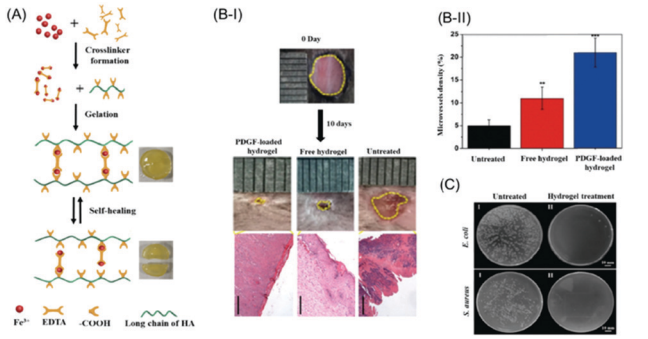

Fig. 9 Hyaluronic acid (HA)-based self-healing hydrogel for antibacterial and skin regeneration applications. (A) Formation and self-healing mechanism of the modified HA based hydrogel crosslinked *via* Fe + 3 (HA-Fe + 3) hydrogel. (B-I) Platelet-derived growth factor (PDGF)-loaded HA-Fe + 3 hydrogel wound healing and (B-II) angiogenesis abilities upon implantation on infected peeled mice backs (after 10 days) and (C) HA-Fe + 3 hydrogel antibacterial activity as shown by footages of E. coli cfu on agar plates from diluted bacterial suspension with (I) and without (II) hydrogel treatment. Reproduced from ref. 111, with permission from the American Chemical Society, 2018.

situation (*e.g.* pH, and temperature and enzymes) and efficient therapeutic effects only when delivered at sufficiently high doses, which may also lead to unwanted side effects.[133,134] This is largely related to the type of carrier used for controlled biomolecule release.[135] In light of these limitations, self-healing hydrogels can potentially be used to exert appropriate control over the delivery of growth factors,[85,90–92,109–111] cytokines[91] and exosomes.[93] However, none of the biomolecule releasing self-healing hydrogels have reached clinical application (Table 2). Important experimental applications of biomolecule delivery using self-healing hydrogels are discussed in the following sections.

3.3.1. Wound healing. The process of wound healing involves inflammation, proliferation and remodeling stages, where various factors, *i.e.* cells, growth factors and cytokines participate in a well-orchestrated manner to repair injured tissues.[136] Delivering therapeutic biomolecules, *i.e.* growth factors, and cytokines at the wound site is crucial for boosting the healing process and avoiding the development of complications such as chronic inflammation or infection.[124,127] The use of self-healing hydrogels represents an attractive option to safely deliver sensitive biomolecules by protecting them from degradation and to maintain their appropriate and sustained release required for achieving normal wound healing.[137] Among the growth factors important for wound healing, FGF-2 and bFGF are known to boost cell proliferation and migration and to regulate angiogenesis and wound healing.[138] For the delivery of FGF-2, a self-healing chitosan-based hydrogel was developed in one study, and it had two distinct FGF release profiles with 25% at 30 min and 70% cumulatively at 72 h.[90] *In vitro*, the use of FGF-loaded hydrogels led to increased viability of fibroblasts and HUVECs cells, and enhanced expression of tissue repair factors such as TGF- and COL-I and angiogenic factors such as VEGF and urokinase plasminogen activator (μPA). When injected into large experimental abdominal wall defects in rats, FGF-loaded hydrogel led to granulation tissue formation, with vascularized ECM and the deposition of highly oriented collagen fibers, owing to the stimulated recruitment of cells responsible for angiogenesis and repair.

To achieve slower and prolonged release, PLGA encapsulation of bFGF and bFGF in PLGA microsphere, with subsequent loading into a self-healing gelatin hydrogel was also explored.[110] Sustained release of bFGF for up to 12 days *in vitro* was achieved. When injected into full-thickness skin wounds in rats, bFGF-loaded hydrogels led to faster wound healing, as compared to unloaded hydrogels. Increased fibroblast proliferation and collagen deposition were observed. To deliver PDGF-BB, Tian *et al.* produced a hyaluronic acid (HA)-based self-healing hydrogel.[111] They demonstrated that it is possible to have a sustained and pH-dependent release of PDGF-BB (95% after 10 days at pH 6.8), and to enhance healing and angiogenesis after 10 days of its application in the treatment of the peeled backs of mice (Fig. 9).

Recently, self-healing hydrogels made of Pluronic®F127 (F127), oxidative HA and EPL were utilized to deliver MSC-derived exosomes to assist wound healing.[93] It was found that released exosomes enhanced *in vitro* cell proliferation, migration and tube formation capability of HUVECs. *In vivo*, exosome-loaded hydrogels enhanced the healing of experimental diabetic wounds in mice, leading to an 88.6% closure rate after 14 days of treatment. The formation of abundant granulation tissue, deposition of COL-I and III, formation of blood vessels and skin re-epithelization upon application were observed.

3.3.2. Bone regeneration. The use of biomolecule-releasing self-healing hydrogels offers a good option for the treatment of complex bone defects. Because self-healing hydrogels can resist mechanical stress and maintain their structure, they can be useful in protecting their cell- or biomolecule-cargo from damage.[139,140] They can also maintain appropriate and sustained release of agents required for bone healing.[87,141] To this end, a hydrogel made from chondroitin sulfate (ChS), which is known for its regenerative properties, was developed.[94] The hydrogel exhibited a good rat-derived MSC cytocompatibility. Excellent self-healing, injectability and *in vivo* tissue adhesion properties were also demonstrated in experiments in mice. The use of the hydrogel to deliver BMP-4 into murine bone defects led to bone tissue formation and significant decrease in defect size, at 12 weeks following treatment.

3.3.3. Cardiac regeneration. Heart regenerative therapeutics are required to treat conditions such as those occurring after the heart sustains an ischemic insult as may occur after myocardial infarction.[142] For this purpose, biomolecules are delivered using

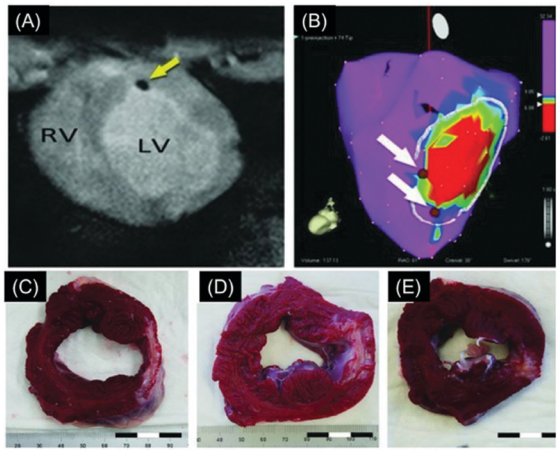

Fig. 10 (A) Cardiac magnetic resonance imaging (MRI), where the yellow arrow indicates the ureido-pyrimidinone-based hydrogel. (B) Electromechanical mapping of the left ventricle; the white arrows indicate the UPy-hydrogel + hepatocyte growth factor/insulin-like growth factor injections. (C–E) The appearance of the scar in all three treatment groups; (C) UPy-hydrogel, (D) hepatocyte growth factor/insulin-like growth factor, and (E) UPy-hydrogel + hepatocyte growth factor/insulin-like growth factor injections. Adapted from ref. 92, with permission from Wiley, 2014.

different hydrogel-based systems.[143] In a new study, a self-healing injectable hydrogel was developed by bonding UPy units with PEG chains.[92] It was found that the gel heals within minutes at 37 °C with a storage modulus of more than 10 kPa, which closely matches the stiffness of cardiac tissue. The hydrogel was used as a carrier for insulin-like growth factor (IGF-1) and hepatocyte growth factor (HGF), and it showed an *in vitro* release of these molecules for seven days, with an initial burst (41% and 27%, respectively) followed by a sustained release. When injected into a porcine chronic cardiac ischemia model, HGF and IGF-1 loaded hydrogel led to favorable remodeling and reduction in the cardiac scar and recruitment of active and recovering cardiomyocytes seen at 28 days after injection (Fig. 10).

3.3.4. Immune therapy. Tissue injury following obstructive nephropathy triggers a series of deleterious events including macrophage infiltration, apoptosis and fibrosis, leading to organ dysfunction;[144] therefore, delivering anti-inflammatory and anti-fibrosis biomolecules was suggested. For example, a minimally invasive and sustained delivery of anti-TGFβ, IL-10, or both molecules is possible by using an HA-based self-healing hydrogel.[91] It was found that on using this system, an efficient release of IL-10, anti-TGF-b or combined IL-10 and anti-TGF-b (90% at two weeks) can be achieved without any competition between the two molecules.

When injected into murine model of chronic renal disease, it was found that drug release occurred through erosion of the hydrogel and it took up to 18 days for total clearance. Quantitative histological analysis showed a significantly reduced macrophage infiltration, at 21 and 35 days after injection with the combination of IL-10 and anti-TGF-β, or with anti-TGF-β-laden hydrogels. Interestingly, it was found that the delivery of IL-10 or anti-TGF-β also led to decreased fibrosis on days 21 and 35, whereas the co-delivery of IL-10 and anti-TGF-showed an increased rate of fibrosis as compared to single drug delivery in this study.

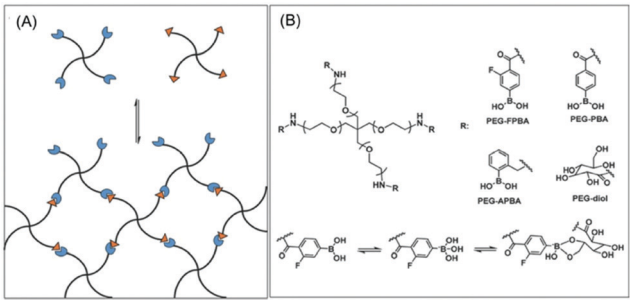

Fig. 11 (A) The gelation mechanism between the functionalized PEG bearing PBAs and diols. (B) Chemical structures and possible interactions between PEG–APBA and PEG–diols. Reproduced from ref. 153, with permission from Wiley, 2015.

3.3.5. Treatment of diabetes. Diabetes is projected to rise to 642 million of the world population by 2040,[145] and so the development of more efficient blood glucose level controlling systems is highly required.[146–148] A considerable number of diabetes patients depend on insulin treatment to control their glycemic levels.[149] Insulin is a protein with hydrophobic and hydrophilic functional groups that can form stable complexes with polymeric hydrogels. Hence, for on-demand insulin delivery in response to high blood glucose levels, the use of materials such as glucose-responsive hydrogels was explored.[150–152] Recently, the interest in the use of injectable self-healing hydrogels for insulin delivery emerged to achieve normoglycemic levels. Generally, glucose-responsive self-healing hydrogels can mainly be synthesized *via* dynamic covalent bonding of boronic acid-based polymers or by host–guest interactions. In one study, a glucose-responsive self-healing hydrogel was produced by using dynamic covalent bond formation between *cis*-diol-modified PEG and phenylboronic acid (Fig. 11).[153] The viscosity of the polymer solutions was low enough to allow the injection of the gels using standard needles. Synthesized hydrogels were capable of releasing insulin in response to high glucose concentrations due to the dynamic chemistry between glucose and boronic acid functional groups of the hydrogel. Authors suggested that this system can potentially be used to reduce high blood glucose levels in diabetic patients.

3.4. Polynucleotide delivery

The delivery of polynucleotides such as DNA or siRNA offers a versatile approach to guide *in situ* cell function.

Following systemic delivery by injection, these molecules are quickly cleared, degraded, or complexed with other proteins, preventing them from reaching their target sites to have the intended effect.[154] Similar to protein and peptide biomolecules, polynucleotide delivery can benefit from the use of self-healing hydrogels, which can help polynucleotides to be retained at the target site, protect them from degradation and prolong their therapeutic lifespan. Interesting examples of potential applications of polynucleotide delivering self-healing hydrogels include the treatment of cardiac[106] and neural[118] conditions (Table 2).

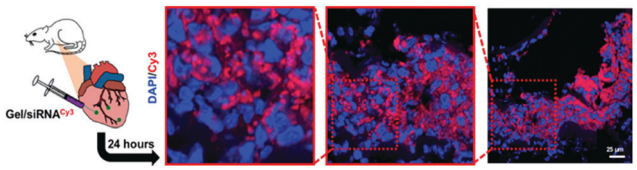

Fig. 12 Injection of the self-healing hydrogel loaded with cyanine 3 (Cy3)-labeled siRNA enhances the uptake of siRNA in rat myocardium. Immunohistochemistry of the heart 24 h post injections. Reproduced from ref. 156, with permission from the American Chemical Society, 2017.

As far as clinical translation is concerned, none of these self-healing preparations has reached the clinical application stage.

3.4.1. Cardiac therapy. The use of small interfering RNA (siRNA) gained a lot of attention for use in therapeutic approaches aiming to silence specific genes that are involved in disease development. It was noted that siRNA is rapidly cleared from circulation following systemic delivery; otherwise, it tends to readily aggregate with serum proteins or accumulate in vital organs such as the lungs, kidney, or liver.[155] To get siRNA to a target organ, Wang *et al.* developed a self-healing hydrogel by conjugating β-CD and adamantane (Ad) to polyethyleneimine (PEI) and 8-arm PEG.[156] Release assays revealed that a sustained release of active siRNA with ability to transfect cells was achieved, with less than 10% of the loaded molecule released after two weeks. Compared to CD-PEI or Ad-PEG alone, the complex hydrogel increased siRNA uptake by HT1080 human fibrosarcoma cells *in vitro* without leading to cytotoxicity. When injected into the left ventricular myocardium of rats, Cyanine 3 (Cy3)-labeled-siRNA-loaded hydrogels promoted Cy3-siRNA uptake by rat cardiomyocytes. More interestingly, Cy5.5-siGFP-loaded hydrogels promoted GFP knockdown in rat cardiomyocytes after 24 h and for up to seven days with the Cy5.5 signal seen with decreased expression of GFP in cells uptaking the siRNA (Fig. 12).

3.4.2. Neuromodulation. Optogenetics such as bacteriorhodopsin (HEBR) plasmid delivery, offers a unique method for neuromodulation, which allows targeted and fast control (excitation or inhibition) of neural events.[157] Delivering such small molecules into neural cells is still a challenge due to various limitations such as cell toxicity or damage as well as challenges facing *in vivo* delivery of these genetically modified cells.

To overcome these limitations, a self-healing chitosan hydrogel was developed to encapsulate HEBR and neural stem cells (NSCs) and deliver the resulting neuromodulated cells.[118] It was found that cell transfection was significantly improved following injection of the loaded hydrogels as compared to free plasmid transfection on culture plastic (80% *vs.* 50% of transfected cells), which is possibly due to the effect of shear stress during the injection process. Upon transfection, the transfected NSC exhibited an increase in their survival and differentiation within the hydrogel, with increased protein and gene expression of nestin, β-tubulin, MAP2, and GFAP after 10 days. When injected into zebrafish that have neural disorders, green light stimulated HEBR/CNS-loaded chitosan-based hydrogels colocalized the hind-, mid- and forebrain areas, and promoted the repair of the central nervous system with a hatching rate of about 58% after 10 days.

3.5. Tissue engineering, regenerative therapy and tissue models

Because self-healing hydrogels behave like native tissues in the sense that they can heal and recover their integrity following damage or stress, they have attracted increasing attention for use in tissue engineering and regenerative therapy.[87,141,158] Despite the progress made in this direction, only *in vitro* and *in vivo* studies, and no clinical trials, have been performed so far.

3.5.1. Skeletal muscle tissue engineering. Skeletal muscle represents about half of the body mass and it is subjected to stress and injury. Despite its ability to adapt, skeletal muscle fails to sufficiently regenerate in the case of severe injury or ischemia, such as occurs in cases of volumetric muscle loss or myopathies.[159] Therefore, various therapeutic approaches have been explored,[160,161] including the use of biomaterials. Among these materials, natural hydrogels have emerged as promising candidates, especially when they have adequate elasticity and injectability. Such biomaterials can be utilized for the delivery of cells and other agents to the site of injury, for stimulating muscle endogenous regenerative capacity.[162]

Unfortunately, these natural hydrogels still suffer from slow gelation and low mechanical properties, especially when one thinks of applications in the muscle, where continued exposure to stress is expected.[164] Therefore, self-healing hydrogels with controllable gelation kinetics and sufficient mechanical strength are required for achieving satisfactory skeletal tissue engineering.[87] Self-healing and conductive hydrogels made of *N*-carboxyethyl chitosan and dextran-*graft*-tetraaniline showed great potential for use in skeletal muscle regeneration.[48] Their biocompatibility and regenerative boosting effect were demonstrated when they were injected into experimental rat volumetric muscle defects and followed for 28 days (Fig. 13). In another work, McKinnon *et al.* synthesized a tunable multi-arm PEG-based self-healing hydrogel with viscoelastic properties that mimic those of the native muscle.[165] The hydrogel was found to enhance myoblast

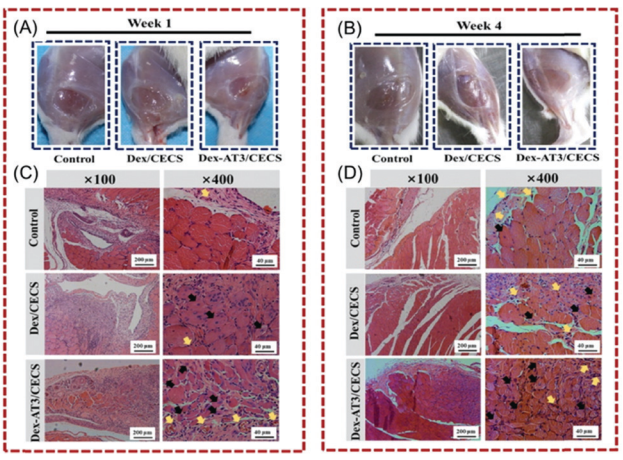

Fig. 13 Muscle regeneration after volumetric muscle loss and treatment for one week (A) and four weeks (B). Representative histological images of buffer solution (PBS) and self-healing hydrogel-injected rats after one week (C) and four weeks postoperative (D) are also shown. Reproduced from ref. 48, with permission from Elsevier, 2019.

proliferation and the formation of multinucleated myotubes after 10 days in culture.

3.5.2. Vascular tissue engineering. The survival and success of engineered tissues are highly dependent on the formation of a functional vascular network that would anastomose with the vasculature of the host in order to ensure blood supply to the implanted construct.[166–170] Engineering vascularized tissues for *in vivo* implantation[171] or for *in vitro* tissue modeling[172] requires the use of suitable materials that have appropriate biological and mechanical properties. Hydrogels have often been utilized for the development of vascular tissue because of their native ECM-mimicking features such as water content, bioactivity and elasticity.[173] Because of their modest mechanical performance when exposed to shear stress and their uncontrollable gelation time, further development is needed before their use as injectable matrices for vascular tissue engineering can be successfully undertaken.[174] Self-healing hydrogels can overcome these limitations and maintain their integrity and mechanical properties independent of the gelation kinetics. Hsieh *et al.* found that a self-healing composite hydrogel made of chitosan and fibrin can be used to engineer vascular networks.[163] After 24 h in culture, endothelial cells (ECs) in the hydrogel started to form spheroids and then a capillary-like network. The highest mean branching point density and branch length occurred after 72 h in culture, and cells embedded in the hydrogel exhibited greater expression levels of vascular gene markers, basal glycolysis, and a decrease in basal mitochondrial respiration compared to cells in the control hydrogels. It was also interesting to note that even a cell-free composite hydrogel led to an increased number of newly-formed capillaries and branching points 24 h following injection into zebrafish, as compared to chitosan hydrogels or PBS injections. More interestingly, the use of a composite hydrogel led to recovery of the function of ischemic hind limbs of mice 15 days after injection (Fig. 14). Although this is an encouraging result, more preclinical studies are needed to lay a basis for clinical translation.

3.5.3. Neural tissue engineering. Neurological disorders affect millions of people worldwide and can lead to permanent cognitive, motor and psychotic dysfunction. With limited regenerative capacity, tissue complexity, donor-site morbidity and limited autologous availability, repairing neurological disorders still present a challenge for scientists.[175] To overcome these limitations, efforts have been made to engineer and deliver appropriate biomaterials that can boost the regenerative capacity of the native nervous tissues.[176,177] The use of hydrogels is attractive because they have physicochemical properties that can support cell growth and host tissue regeneration[178] and they can be delivered by using a minimally invasive approach.[179] The gelation time of conventional hydrogels is, however, hard to control and it requires the use of non-physiological triggers, such as changes in the pH or temperature.[20] Thus, self-healing hydrogels can be used in a fragmented state with subsequent integration to form a gel at the application site without the need for any nonphysiological triggers to induce *in situ* gelation.[180] It was reported in several studies that neural proliferation and differentiation are boosted by the use of electrically conductive materials[181] such as graphene-based materials.[182] It was found that graphene-based self-healing hydrogels can lead to good cellular attachment and PC-12 neural cell growth on the seen after 48 h in culture.[182] In addition to conductivity, mechanical properties of hydrogels that are close to those of native neural tissues are sought. Hydrogel degree of stiffness[183] and mechanical performance have been found to have an important role in guiding neural stem cell proliferation[184] and differentiation,[185] and these properties should be carefully considered when designing or selecting a self-healing hydrogel for use in neural tissue engineering. In this regard, hydrogels composed of amyloid nanofibrils were suggested because they have mechanical properties that may mimic those of the native neural tissues.[186] Amyloids are self-assembling peptide aggregates and, therefore, they have the potential to be used as smart materials for biomedical applications.[187,188] When studied *in vitro*, these amyloid-based hydrogels were found to support neuronal cells and fibroblast attachment and spreading in both 2D and 3D cultures.[189] The stiffness of hydrogels was tuned to suit stem cell differentiation. After seven days of culturing on the softest (peptide concentrations = 6 mg ml^{-1}) amyloid-based hydrogels, hMSCs exhibited the highest cell elongation and spreading, as well as enhanced expression of neuronal protein and gene markers and decreased expression of GFAP astrocyte markers.

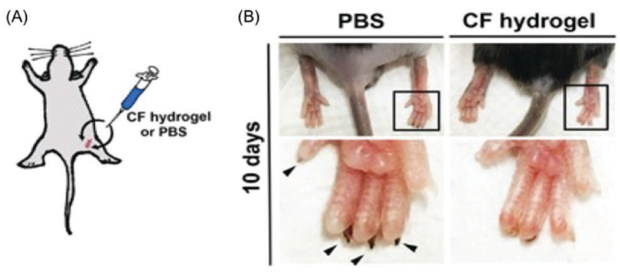

Fig. 14 (A) Injection of the self-healing hydrogel for the treatment of ischemic hindlimbs of mice, 48 hours after surgery. (B) Representative images of mouse ischemic hindlimbs after injection with buffer solution (control group) or self-healing hydrogel (experimental group) are shown. The experimental group showed limb salvage without nail loss in comparison with the control group. Reproduced from ref. 163, with permission from Nature Publishing Group, 2017.

3.5.4. Cardiac tissue engineering. The heart may be damaged by conditions such as myocardial infarction, which constitutes a serious health challenge and represents a leading cause of death worldwide.[190] Myocardial infarction results in the death of cardiac myocytes, matrix degradation and fibrous tissue scar formation, which subsequently lead to cardiac dysfunction and failure.[191] Current clinical therapies focus on the restoration of the blood flow to ischemic tissue, which does not necessarily lead to cardiac tissue regeneration or restoration of function.[192] Therapeutic approaches based on drugs, growth factors or cell delivery are insufficient to restore cardiac function.[193]

Owing to their amenability for use in minimally invasive procedures, regenerative properties and their ability to carry therapeutic agents, hydrogels have been investigated for their use in cardiac tissue repair and regeneration.[192–194] However, conventional hydrogels are likely to encounter repeated mechanical loads due to continuous and repetitive cardiac contraction, leading to their

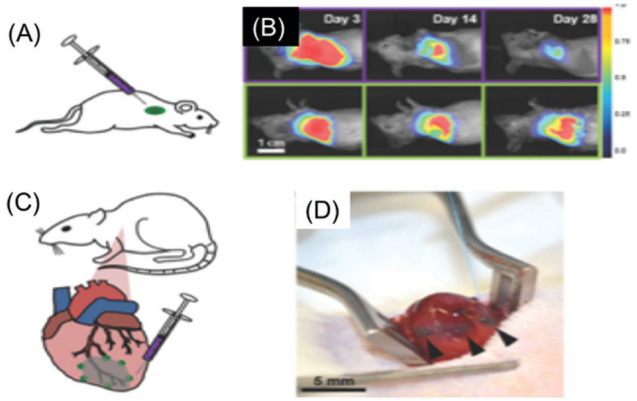

Fig. 15 (A) Injection of self-healing hydrogel into the right flank of mice. (B) Fluorescence imaging of the injected mice. (C) Injection of self-healing hydrogel into a rat infarct model and (D) visualization of the heart after injection. Reproduced from ref. 196, with permission from Wiley, 2014.

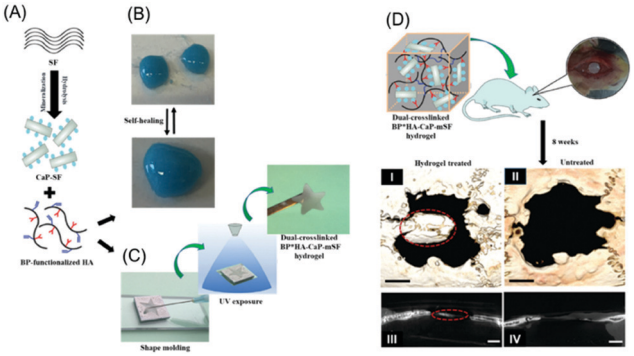

Fig. 16 (A) Fabrication of bisphosphonate functionalized hyaluronic acid calcium phosphate-coated silk fibroin (BP*HA-CaP-mSF) hydrogel microfibers. (B) BP*HA-CaP-mSF hydrogel self-healing ability and (C) moldability properties and the formation of a robust dually crosslinked BP*HA-CaP-mSF hydrogel after exposure to ultra-violet (UV) light. (D) Accelerated bone repair observed following BP*HA-CaP-mSF hydrogel 8 weeks after implantation into rat cranial critical bone defect as highlighted by micro-CT analysis: (DI-DII) 3D reconstruction views and (DIII-DIV) sagittal cross-section. Reproduced from ref. 208, with permission from Wiley, 2017.

breakage and loss of function.[87,195] Therefore, the use of self-healing hydrogels can withstand mechanical loads and function well under such challenging conditions for much longer periods of time. It was also demonstrated that self-healing hydrogels such as HA-based ones can have a high target site retention (>98%) and it can be useful for cardiac tissue engineering.[196] Compared to untreated hydrogels that were injected into an experimental myocardial infarction lesions in mice, dual-crosslinked hydrogels led to ventricular remodeling with decreased scar formation and infarct size and increased vascular density in the border zone, contractility, fractional shortening and increased ejection fraction 28 days following injection (Fig. 15).

Because cardiac tissue has also an efficient conductive system, electroconductive biomaterials have been investigated and they were shown to enhance the commitment of stem cells to the cardiac phenotype.[197,198] To this end, a self-healing electroconductive chitosan/graphene oxide composite hydrogel was developed.[195] This hydrogel enhanced embryonic stem cell-derived fibroblast and cardiomyocyte adhesion, viability and proliferation. Interestingly, cultured cardiomyocytes were uniformly distributed in the hydrogel and well connected. They were spontaneously beating with rates close to those of the normal human heart, which made this material interesting for further testing *in vivo*. One also has to consider that electroconductive materials may have adverse effects in the possible generation of arrhythmias in the treated heart. This a critical issue that requires animal experiments and careful evaluation.

3.5.5. Osseous and osteochondral tissue engineering. Bone tissue engineering aims to develop reliable bone tissue that can restore bone discontinuity and function; yet, many fundamental problems remain to be solved before wider clinical applications can be achieved.[166,199] Having biomaterials that can mimic native tissues in having appropriate porosity,[127,200] mechanical strength,[201,202] and self-healing capability[87,113] will enable successful bone tissue engineering. Therefore, self-healing hydrogels have recently been investigated for use in bone tissue regeneration.[203–209] In one study PEG-based self-healing hydrogel was investigated for use in bone tissue engineering.[206,207] Upon crosslinking with polydopamine-coated layered double hydroxides, the resulting hydrogel exhibited self-healing abilities and adhesion to bone surface under wet conditions. When cultured with hMSCs, the hydrogel supported cell adhesion, spreading and the formation of an extensive actin cytoskeleton along with an increased Runx2 protein expression after 14 days of incubation in osteoinductive culture media.[206] Self-healing hydrogels can also be combined with drugs such as bisphosphonates (BP) to inhibit osteoclasts and osteolysis.[210,211]

Recently, BPs were grafted to hyaluronic acid (HA) and the resulting biomaterial was mixed with calcium phosphate and magnesium nanoparticles to form a self-healing hydrogel[205] suitable for use in bone engineering.[203–205,208] When the mixture was used for the treatment of experimental femoral bone defects in rats, trabecular-like bone formation was seen throughout the hydrogel. Calcified bone was seen throughout the bone defects at 28 days following injection with the material.[205] The mixture was injected into the osteonecrosed femoral head of rabbits and induced the formation of a bony-tissue at the defect site at 60 days post-treatment.[203] It was also found that when the HA-based hydrogel was mixed with BP-Mg^{2+}, it could support the spreading and viability of MSCs and their osteogenic commitment.[204] In their work, Shi *et al.* synthesized a self-healing hydrogel made of BP and acrylamide-functionalized HA supplemented with CaP-coated silk fibroin microfibers (mSF).[208] Cell proliferation and attachment along with osteogenic differentiation and cell cluster formation and mineralized mSF were seen after 14 days of culture. When implanted in critical-sized cranial bone defects in rats, the HA/mSF-based hydrogel led to the formation of new vascularized bone tissue, four to eight weeks after implantation (Fig. 16).

3.6. Use of self-healing hydrogels for 3D printing and 3D bioprinting

Self-healing hydrogels represent an attractive group of smart materials that can be used in 3D printing.[214,215] The use of

self-healing materials in 3D printing of soft and dynamic scaffolds will enable us to improve the properties and durability of the printed constructs.[216] The use of self-healing hydrogels in 3D printing is still in its early stages, with only a few studies published so far.[20]

3.6.1. Three-dimensional printing. To develop a self-healing substrate for 3D printing, a strong thermoplastic self-healing hydrogel based on the formation of multiple hydrogen bonds between glycinamide functional groups of *N*-acryloyl glycinamide (NAGA) was developed, and it was found to heal and recover its initial mechanical strength owing to the reconstitution of hydrogen bonds.[217] The hydrogel was used for extrusion-based 3D printing of multiform shapes and patterns. To modify the properties of the hydrogel, 2-acrylamide-2-methylpropanesulfonic acid (AMPS) was used to control the swelling, and poly-(3,4-ethylenedioxythiophene)–poly(styrenesulfonate) (PEDOT/PSS) was used to tune the conductivity of the hydrogels. The resulting electroconductive self-healing PNAGA-poly(AMPS)/PEDOT/PSS hydrogel was blended with activated charcoal and used for extrusion-based 3D printing of electrodes. To tune water absorption ability and conductivity of the hydrogel, 2-acrylamide-2-methylpropanesulfonic acid (AMPS) was used, and to tune aqueous dispersion in the hydrogel poly(3,4-ethylenedioxythiophene)–poly(styrenesulfonate) (PEDOT/PSS) was used. Graphene oxide (GO) integrated into chemically crosslinked polyacrylic acid (PAA) was also used for the 3D printing of electronic skin and electronic nose.[218] Smaldone and coworkers developed a new self-healing biomaterial by using temperature-responsive covalent bonding to enhance both the strength and the toughness of the material.[219] To accomplish this, they used furan-maleimide Diels–Alder (fmDA) adducts as a crosslinking method. When a blend of fmDA and polylactide (PLA) was used for 3D printing, increased inter-filament adhesion strength along the z-axis and ultimate tensile strength of the material were achieved (Fig. 17).[212] In another study, Smaldone and coworkers changed the crosslinking degree of PLA and mending agent to develop a new ink suitable for processing by a conventional FFF 3D printer and produced constructs having almost uniform mechanical strength along all directions.[219] In another study, ethylenediamine (EDA) was used to crosslink benzaldehyde-functionalized poly(2-hydroxyethyl methacrylate) (PHEMA) and produce a 3D printable self-healing organogel.[220] Self-healing behavior was found to occur at room temperature without the need for external additives, leading to 98% recovery of the modules of the material after induced damage. In another study, authors reported a self-healing and 3D-printable gel, which was prepared by the cross-linking of poly(*n*-hydroxyethyl acrylamide-*co*-methyl vinyl ketone) (PHEAA-*co*-PMVK) with bifunctional hydroxylamine.[213] Subjecting the 3D printed constructs to thermally induced phase separation (TIPS) helped in the formation of oxime crosslinks and hydrogen bonds (Fig. 18). The 3D-printed objects exhibited rapid and full self-healing characteristics and significant swelling capacity. The morphological changeability of the 3D printed constructs in response to environmental stimuli such as changes in temperature, moisture, pH, or light,[221,222] is known as four-dimensional (4D) printing, which has been recently explored.[223–225] Invernizzi *et al.* synthesized a 3D printable temperature-responsive shape memory and self-healing hydrogel by using polycaprolactone (PCL) and 2-ureido-4[1*H*]-pyrimidinone (UPy). The self-healing characteristics of the 3D printed constructs were achieved by having methacrylate bearing UPy (UPyMA) functional groups in the material.[226] Highley *et al.* synthesized a self-healing hydrogel by using β-cyclodextrin (β-CD) or adamantane (Ad)-modified HA (CD–HA and Ad–HA) and used intermolecular host–guest interactions (between β-CD and Ad moieties).[227] As shown in Fig. 19, their formulation exhibited shear-yielding, self-healing and deformation resistance during the printing process. Wang *et al.* also developed a self-healing hydrogel that has anti-inflammatory and antibacterial properties.[228] This hydrogel was produced by the copolymerization of *N*-acryloyl glycinamide (NAGA) and 1-vinyl-1,2,4-triazole (VTZ). They have used this material for 3D printing and because of its multiple functions, it can be utilized for different biomedical applications.

3.6.2. Three-dimensional bioprinting. To achieve successful and good quality 3D bioprinting, bioink continuous and steady flow, rapid gelation, and the fidelity of the resulting constructs are required.[229–231] To this end, bioinks with self-healing properties are highly desirable because they can be

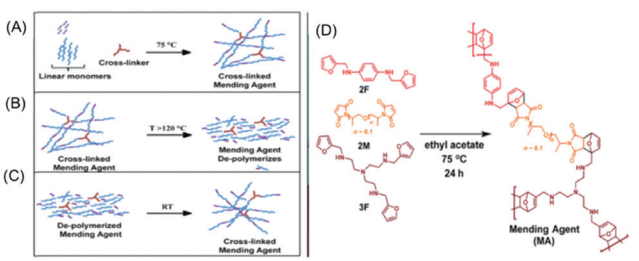

Fig. 17 (A) A schematic of the crosslinking mechanisms and formed final products at (A) 75 °C, (B) >120 °C and (C) room temperature. (D) Chemical structures showing the crosslinking process *via* the furan-maleimide Diels–Alder reaction. Reproduced from ref. 212, with permission from the American Chemical Society, 2016.

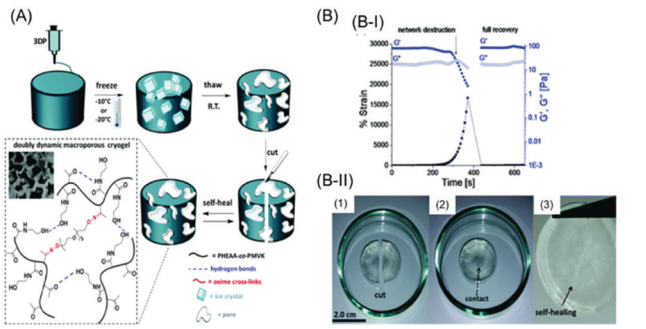

Fig. 18 (A) The process of preparing self-healing biomaterial crosslinked *via* the freeze–thaw method. The inset shows the chemical structure and morphology of the produced material. (B-I) Rheological behavior of the self-healing hydrogels shows a rapid recovery after high strain destruction. (B-II) Qualitative assessment of the self-healing properties of the 3D printed hydrogel. Reproduced from ref. 213, with permission from the Royal Society of Chemistry, 2018.

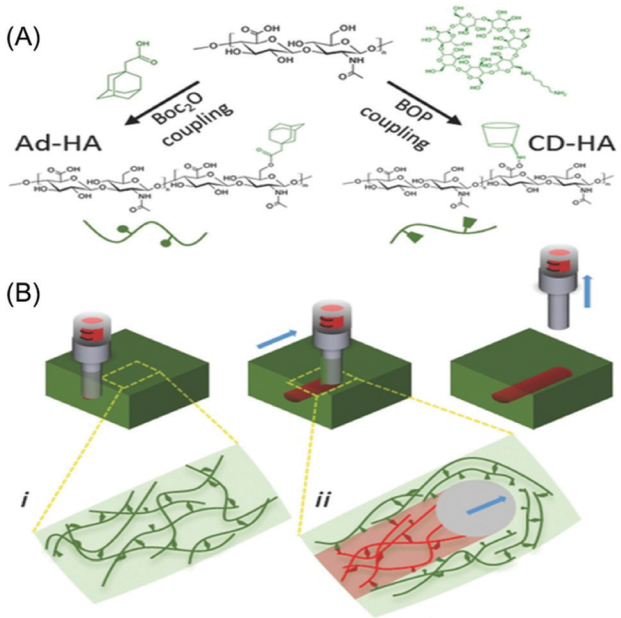

Fig. 19 (A) The chemical structure of hyaluronic acid (HA) conjugated with β-cyclodextrin and adamantine that can be crosslinked upon mixing *via* host–guest interactions. (B) Schematic illustration of the 3D printing process of the hydrogel into a support gel (green), Scalebar: 200 μm. Reproduced from ref. 227, with permission from Wiley, 2015.

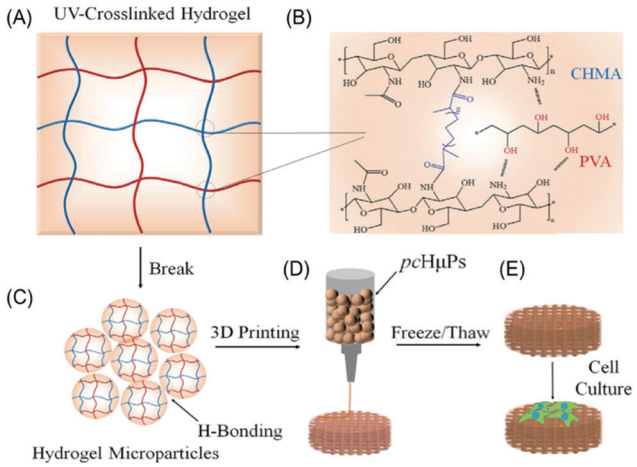

Fig. 20 Schematic illustration of 3D printable self-healing hydrogel. The network (A) and crosslinking method (B) of self-healing hydrogels. (C) The hydrogel can be broken into microgels and assembled into bulk gels *via* H-bonding. (D) Synthesized hydrogel can be used for the 3D printing of scaffolds. (E) Freeze/thawing is used to enhance cell growth and improve the mechanical properties. Reproduced from ref. 232, with permission from Wiley, 2020.

used to produce constructs that mimic the native healing of tissues.

So far, only a few studies have been published on the use of self-healing materials in 3D bioprinting,[20] owing to the challenges associated with the designs and procedures.[233] Hence, new materials and crosslinking methods need to be developed to make progress in this field. Among the few studies, Zhang *et al.* 3D bioprinted self-healing structures that contained pre-crosslinked microparticles (pcHμPs) made of polyvinyl alcohol (PVA) and chitosan methacrylate (CHMA) (Fig. 20). Constructs with excellent shape accuracy could be quickly produced. The 3D bioprinted scaffolds supported bone-marrow-derived MSC growth and spheroid formation. It is worth noting that recent work has also suggested the possibility of producing a durable self-healing PVA-based hydrogel to be used as bioink in the future.[234] Kim *et al.*[235] mixed adipic acid dihydrazide (ADH)-functionalized chitosan with oxidized hyaluronan (OHA) to form a hydrogel. When used in 3D bioprinting, these hydrogels exhibited a rapid self-healing behavior owing to the used crosslinking method. Neither the material nor the crosslinking method had a major impact on mouse chondrogenic cell viability (80% viability) or their chondrogenic differentiation.

3.7. Sensors and soft actuators

Hydrogels with attractive properties such as flexibility, fatigue-resistance, conductivity and self-adhesiveness have great potential in the field of health-monitoring systems.[202,236] While conventional hydrogels are likely to present weak mechanical properties and suffer from serious damage following physical stimulation, self-healing hydrogels have the advantage of compensating incidental scratches or mechanical cuts (Table 3). However, most of the investigations of self-healing hydrogels for the production of sensors and actuators are limited to *in vitro* experiments.[237] In the following sections, we will highlight the potential use of self-healing hydrogels as sensors and soft actuators.

3.7.1. Sensors

3.7.1.1. Motion sensing. Several self-healing sensors capable of detecting body motion and converting it into a measurable signal have been developed.[239–241] For human motion sensing, conductive self-healing hydrogels can be good candidates due to their flexibility, and adhesiveness.[242] For instance, Zhang *et al.* developed a sensor by using dopamine-modified hyaluronic acid (DHA) and poly(acrylic acid) (PAA) self-healing hydrogel. This material produced reproducible and distinct signal patterns during physiological movements such as swallowing, blood pressure variation and breathing, when they were attached to the throat, wrist or chest, respectively. In another study, an adhesive, conductive and self-healing hydrogel was synthesized by combining polydopamine (PDA) with a mixture of PVA and functionalized single-wall carbon nanotubes.[238] The resulting hydrogel exhibited excellent cytocompatibility, good mechanical and electrical self-healing (99% recovery in 2 s) properties, and a robust adhesiveness to organic and inorganic surfaces along with the possibility to support up to 250 g in weight and multiple adhesion/peeling cycles. Such properties provided the hydrogel with the potential to be utilized in the development of wearable soft strain sensors that can be used to monitor different human physiological motions including chewing, breathing, as well as finger and elbow bending (Fig. 21).

3.7.1.2. Temperature sensing. Self-healing hydrogels can also be useful for temperature sensing.[243,244] For instance, Kweon *et al.* developed a temperature sensor by using sodium tetraborate,

Table 3 Summary of self-healing hydrogels that were used as sensors

No.	Material	Purpose	Main outcome	Ref.
1	Poly(acrylic acid) + dopamine-functionalized hyaluronic acid + Fe^{3+}	Strain sensors (body motion)	– Capacitance changes following bending angles when attached to the finger or knee – Reproducible and distinct signal patterns during physiological movements like swallowing, blood pressure variation and breathing when attached to the throat, wrist and chest, respectively	240
2.	Lauryl methacrylate + polyacrylamide + chitosan + carboxyl-functionalized multi-walled carbon nanotubes	Strain sensors (body motion)	– Strong adhesion and conductivity properties, with an increase in resistance when fixed to a finger with different bending angles – Resistance signal patterns during physiological motions such as talking, breathing and blood pressure variation when attached to the throat, rib cage and wrist, respectively	241
3	Gelatin + Fe^{3+} + lithium chloride + poly(acrylamide-*co*-dopamine).	Strain sensors (body motion)	– Good conductivity and adhesion on different matrixes (*i.e.* plastic, wood, ceramics) – Demonstrated ability to monitor human body movements *via* adhering the hydrogel to human joints (wrist, throat, finger and neck) with various curving angles or to the throat for strain sensitivity	265
4.	Alginate + gelatin + polypyrrole	Strain sensors (body motion)	– Ability to serve as a conductive repairable circuit with changes in resistance and electrical properties once attached on fingers, depending on the type of movement and bending angles	266
5	Polydopamine-coated talc nanoflakes + polyacrylamide	Strain sensors (body motion)	– Great stretchability, adhesiveness to various substrates and improved cell affinity to human HEF1 fibroblasts – High conductivity and response (increase in hydrogel resistance) to mechanical compression – High sensitivity and strain-dependent resistance and ability to detect human motions such as deep breath and finger, knee or elbow bending	238
6	Polypyrrole + double-bond decorated chitosan + poly(acrylic acid) + Fe^{3+}	Strain sensors (body motion)	– Mechanical and electrical self-healing properties (100% in 2 min) and ultrastretchability (1500%) – Confirmed conductivity in circuit with increased electrical performance following compression – Suitability as 3D printing sensors with high sensitivity and ability to detect human motions such as respiration (inhalation/exhalation), wrist pulse, finger and bicep bending	267
7	Single wall carbon nanotube + polyvinyl alcohol + tetrafunctional borate ions	Strain sensors (body motion)	– Mechanical (100% in 60 s) and electrical (98% in 3.2 s) self-healing and stretchability (1000%) – Confirmed conductivity in a complete circuit composed of a light-emitting diode bulb – Ability to detect human motion including finger, knee, elbow and neck bending/release	268
8	Cellulose nanofibers + graphene + poly(vinyl alcohol)-borax	Strain sensors (body motion)	– High stretchability (1000%), excellent viscoelasticity (3.7 kPa) and self-healing ability (97.7% in 20 s) – Good conductivity with increased performance following applied tensile stress – Ability to detect human motion as hand gestures and finger/arm bending with different angles	269
9	MXene (Ti3C2Tx) nanosheets + pristine crystal clay hydrogel	Strain sensors (body motion)	– Stretchability (up to 3400%), instantaneous self-healing and great adhesiveness to various surfaces – Ability to act as a sensing material for motion speed/direction, to monitor body motions (*i.e.* hand gestures, finger bending, facial expressions) or vital signals such as human pulse and for anti-counterfeiting and phonatory recognition	270
10	κ-Carrageenan + polyacrylamide	Strain sensors (body motion)	– Good stretchability, conductivity and mechanical/conductivity recovering – Suitable for 3D printing with a maintain of the mechanical/conductivity features – Ideal for the detection of body motion including finger and wrist bending/relaxing	271
11	Guar gum + glycerol + borax	Strain sensors (body motion)	– Good stretchability, adhesiveness, anti-freezing, conductivity and self-healing (<15 s) capacities – Ability to detect body motion, with good repeatability, such as finger bending/relaxing	272
12	Polyvinyl alcohol + borax + ethylene glycol + reduced graphene oxide	Strain sensors (body motion)	– Flexibility and resistance to various deformations (*i.e.* stretching, twisting, compression and slicing at low temperature) – Ability to monitor, with a temperature range of -10 to 60 °C, various human motions including palm posture (forefinger joint) and breathing (chest)	273
13	Functionalized single-wall carbon nanotube + polyvinyl alcohol + polydopamine	Strain sensors (body motion)	– Good mechanical/electrical self-healing (99% in 2 s), resistance (up to 250 g in weight) and cytocompatibility (>100% after 72 h) – Robust adhesiveness to organic and inorganic surfaces along with the possibility for multiple adhesion/peeling cycles – Ability to monitor (in wire and wireless mode) various human physiological motions including chewing, breathing, finger and elbow bending	274

Table 3 (*continued*)

No.	Material	Purpose	Main outcome	Ref.
14.	Poly(vinyl alcohol) + poly(acrylic acid) + Fe^{3+}	Strain, pressure and temperature sensor	– Good conductivity properties with changes in resistance upon tensile stain, compressive pressure or variation in temperature – Resistive strain and pressure sensing even under mechanical fatigue and thermal stress (90 °C)	244
15	Polyaniline nanofibers + polyacrylic acid + Fe^{3+}	Strain and temperature sensor	– High adhesiveness to different substrates even under wet conditions – High sensitivity and low limit of strain detection with potential usage for personalized signature identification, anti-counterfeiting and phonatory recognition – Good thermo-sensation with reproducible temperature-discrimination capacity during repetitive cold and heat stimulations	275
16	Amorphous calcium carbonate nanoparticles + polyacrylic acid + alginate	Strain sensor (body motion and pressure)	– Good viscoelasticity, cytocompatibility, stretchability, compliance and self-healability – High-pressure sensitivity allowing to detect gentle finger touch, human motion (speaking, finger bending and blood pressure) and water droplets (rain mimicking)	246
17	Poly(*N*-isopropylacrylamide) + poly(vinyl alcohol) + sodium tetraborate decahydrate + poly(sodium acrylate)	Touch and temperature sensor	– High optical transparency (91%), stretchability (up to 600% strain), electrical conductivity, bendability and self-healing capabilities (<7 s) – Low critical solution temperature behavior at a temperature of 30.1 °C with fully reversible and reproducible transparent/opaque transitions – Ability to act as a touchpad even in bend state or isotropic deformation and to monitor the location of touch point and temperature stimuli variation with a response time of 0.3 s	243
18	Polydopamine + graphene oxide + polyacrymide	Sensor for invasive systems	– Good electeroconductivity with quick recovery after hydrogel stretching/compressing – Mechanical/electroconductivity self-healing and adhesiveness capacities (up to 30 kPa) – Ability to detect the electromyographic signal during relaxation tension of arm without losing sensitivity after adhesion/strip-off cycles – *In vivo* biocompatibility along with excellent signals from the dorsal muscle after implantation (higher than commercial surface electrodes)	247
19.	Tannic acid-coated cellulose nanocrystals + poly(acrylic acid) + aluminum ions	Sensor for invasive systems	– Good elasticity, stretching abilities (up to 2900%) and fatigue resistance with a recovery ratio of 95.2% – Mechanical (88% in 30 min) and conductivity (97.1% in 3 s) self-healing and adhesiveness capacities (up to 30 kPa) – Adhesion to various surfaces (*i.e.* glass, PTFE, rubber, human skin) with 350 g weight load support – High strain sensitivity and ability to detect large but also subtle motions.	276

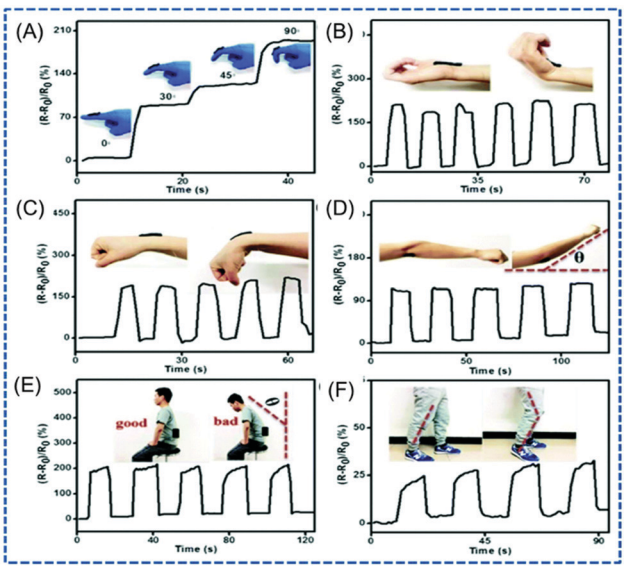

Fig. 21 Application of self-healing hydrogel in the detection of resistance changes in the finger (A), wrist (B), opisthenar (C), elbow (D), back (E) and knee (F) joint motions. Reproduced from ref. 238, with permission from Wiley, 2017.

sodium polyacrylate (SA), decahydrate (borax) and poly(vinyl alcohol) (PVA) hydrogel. The stretchability (up to 600% strain), high optical transparency (91%), electrical conductivity, bendability and self-healing properties in <7 s of the sensor were demonstrated. Authors showed that the synthesized materials can act as a touchpad even in the bent state or isotropic deformation, and can monitor the touch point position and variation in temperature stimuli with a reaction time of 0.3 s. Using polyaniline nanofibers, PAA and ferric ions, Ge *et al.* proposed a fiber-reinforced and thermosensitive self-healing hydrogel capable of realizing various functionalities.[245] The resulting hydrogel exhibited the ability to detect the electromyographic signal during the relaxation tension of the arm without losing sensitivity after adhesion/strip-off cycles. The capability to develop sensors that can reproducibly discriminate changes in temperature following stimulation by heat or cold in a highly sensitive manner demonstrates the potential of this hydrogel for use in personalized signature identification, anti-counterfeiting and phonatory recognition.

3.7.1.3. Pressure and strain sensing. Self-healing hydrogels can be designed in a way that they respond to pressure or strain stimuli, and transfer them into electrical signals.[244,246] Wu *et al.*

developed a viscoelastic, cytocompatible, and stretchable, self-healing calcium carbonate (ACC)/PAA/alginate-based hydrogel.[246] This hydrogel successfully responded to changes in human blood pressure with stable and repeatable capacitance changes. In another study, Shin *et al.* used a physically crosslinked PVA and chemically crosslinked poly(acrylic acid) containing Fe^{3+} ions to develop a self-healing hydrogel.[244] This hydrogel could detect the strain and pressure even under mechanical fatigue and thermal stress (90 °C). The hydrogel tolerated tensile loading–unloading cycles (strain of 100 to 500%).

3.7.1.4. Cellular signal sensing. Han *et al.* synthesized a hydrogel using a mixture of PDA, GO and polyacrylamide, which displayed high toughness, self-healing and good adhesiveness to various surfaces including soft tissues.[247] The latter was able to regulate the fate of cultured bone marrow stem cells by favoring their adhesion and growth rate following hydrogel-induced electrical signals. Interestingly, the resulting hydrogel was shown to be biocompatible when implanted in critical-sized osteochondral defects in rabbits, and it was also able to detect electrical signals, in a more accurate way than industrial surface electrodes, upon implantation in rabbit dorsal muscles. In another report, a self-healing hydrogel was made by combining GO with chemically crosslinked PAA to produce the GO–PAA hydrogel, and it was used for developing wearable electronic biosensors; *e.g.*, for the detection of pulse rate, finger movements and toxic gas emission (Fig. 22).[218] More details of self-healing hydrogel-based sensors are included in Table 3.

3.7.2. Soft actuators. There are some excellent reviews on using self-healing hydrogels for the production of actuators.[248,249] However, most of these papers focused on the material properties rather than the applications. Because of their properties of swelling and shrinking depending on the amount of water they may have, hydrogels have recently attracted more attention for producing soft actuators.[250] Employing electricity, magnetism, strain, heat, pH, molecular interactions and ionic strength can be used to influence the amount of water in the hydrogel.[251,252] Elastic deformation of the hydrogels enables shape-switching of hydrogel networks, *i.e.*, shape-memory behavior (*via* reversible covalent or physical cross-links). By changing the balance between hydrophobic and hydrophilic forces in the structure, the healing rate of the hydrogel can be increased. There are several potential applications of self-healing hydrogels as actuators, which most importantly include soft robotics,[253] wearable devices[254] and tissue repair and regeneration,[255] details of which are discussed below. Other applications are expected to emerge in the future.

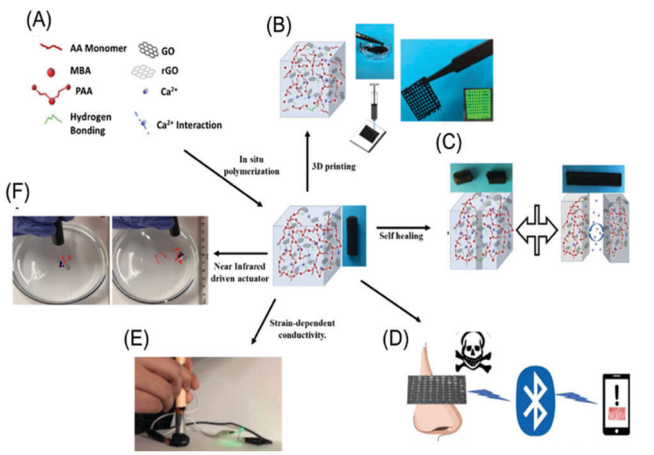

Fig. 22 Graphene oxide (GO)/polyacrylic acid (PAA) self-healing hydrogel-based sensor and actuator. (A) Composition and formation of the GO/PAA hydrogel. (B and C) GO/PAA 3D printing and self-healing abilities. (D) The use of GO/PAA as an electronic nose to wirelessly detect toxic ammonia gas. (E) GO/PAA strain-dependent conductivity demonstration. (F) Rocket-like GO/PAA hydrogel-based actuator driven by neat infrared laser irradiation. Adapted from ref. 218, with permission from the Royal Society of Chemistry, 2019.

3.7.2.1. Soft Robotics. Much progress has been made in the field of soft-robotics and the generation of robot-machine interfaces.[256] Owing to their high swellability, flexibility and self-healing characteristics, these hydrogels have been explored for use in the development of soft robotics and artificial muscle.[257] Because self-healing hydrogels are more robust than conventional hydrogels, with the ability to recover their properties following injury, and repeated stimulations or solicitations,[258–262] their potential to be used as soft robotics was suggested by different investigations.[263] For example, Qin *et al.* developed an anisotropic silver nanoparticle polyacrylamide (SNPP)-based hydrogel with self-healing and controllable solvent-responsive mechanical actuating properties.[264] Because of their uniform silver lamellar nanostructure, the hydrogels exhibited distinct anisotropic behavior. In addition to self-healing, these properties conferred on the hydrogel attractive and complicated bending actuation capabilities such as robotic palm-bending-fingers-clamping and arm lifting-like behaviors when exposed to poor solvent stimuli. Authors have suggested that such hydrogels can be potentially used as smart soft robots. To develop a hydrogel that can maintain its properties and perform well in a wider range of environmental conditions, a highly stretchable (up to 2700%), self-healing and ultra-tough glycerol-based hydrogel was developed.[277] It was made by adding boron nitride nanosheets into poly(acrylamide-*co*-maleic anhydride) and post-treatment with glycerol/water. The resulting hydrogel exhibited resistance to dehydration at temperatures as high as 60 °C, and to freezing at temperatures as low as −45 °C. Stimulated by dryness or heat, the resulting hydrogel-based actuator was able to form complex shapes and perform well in gripping, releasing and even driving a ball. This can be applied to the development of soft robots that can actuate according to the degree of humidity and temperature, and be used in situations such as where cooling is needed for cellular and tissue preservation.

3.7.2.2. Wearable devices. There is an increasing interest in developing wearable devices for various biomedical applications such as monitoring,[278] and combined monitoring and actuation.[279] However, several challenges currently face the development of this field. Among the most important challenges is the difficulty of having a soft and elastic material that can resume its original shape after deformation. The other challenge is related to the

capability of the material to withstand the rigors of the daily activities of the patient. Therefore, various flexible systems were investigated to develop materials that can function even when they are twisted, bent, stretched, or broken. For instance, self-healing electro-conductive hydrogels were developed, and they are expected to have an important role in developing a new generation of smart wearable devices.[242] Using an electro-responsive hydrogel, actuators that can convert electrical energy into mechanical energy, by undergoing matrix deformations, can be developed. These actuators are compatible with electronics and power supply batteries, which is a major advantage. Dielectric elastomer actuators (DEAs) have gained much attention as actuators using their electrical potential. In their study, Gao *et al.* developed a stretchable and self-healing (*via* hydrogen bonding) hydrogel for use as a compliant electrode in DEAs.[277] Made of carbon nanotubes (CNTs) and PVA, the resulting hydrogel exhibited a stretchability of about 200% and a small relative resistance change ($\sim$1.20). Interestingly, when the hydrogel electrodes were connected to the dielectric polymer, a strain of more than 40% was achieved, which is much higher than what can be achieved by using non-CNT-containing PVA electrodes. Other researchers went further and developed smart hydrogels in order to cover a multitude of applications. The resulting hydrogel, made by combining GO with a chemically crosslinked PAA hydrogel, exhibited excellent self-healing and conductivity properties (*i.e.* $\sim$88.02%, 87.47% and 86.93% maintenance of its tensile strain, tensile strength and toughness, respectively). In addition, such a dually-crosslinked hydrogel demonstrated high stretchability (2500%), toughness ($\sim$9.73 MJ m^{-3}) and Young's modulus of about 753.667 KPa. Owing to its capability to absorb near infrared radiation, GO–PAA hydrogel-based actuators could be tailored to different configurations and be robot driven when irradiated by the laser source, *i.e.* laser-driven actuators.[218] This new hydrogel can potentially be utilized for the development of wearable actuators, with reduced requirement for continuous repair and thus leading to reduced costs and longer service life. In another study, Turng *et al.* utilized PAAc that was crosslinked with reduced graphene oxide (rGO) to produce a tough hydrogel.[195] Moreover, the use of rGO nanosheets rendered the hydrogel electroconductive. However, because of the sensitivity of electro-conductive properties of the hydrogels to strain, even small strain may significantly affect the conductivity or resistance of the network.

3.7.2.3. Soft tissue repair and regeneration. The interest in employing self-healing hydrogels in the repair and regeneration of various tissues is growing.[233] Currently available self-healable hydrogels unfortunately have fracture energies that are much lower ($<$10 J m^{-2}) than those of that of the skin, muscle, tendon and cartilage (kJ m^{-2} regime), and thus research is required to enhance the mechanical properties of self-healing hydrogels.[24] One approach may employ nanostructures for improving the hydrogel mechanical properties. It is possible to develop nanocomposite hydrogels that have lower (LCST) and upper critical solution temperatures (UCST) by utilizing materials such as *N*,*N*-dimethylacrylamide (DMAA) or *N*-isopropylacrylamide (NIPAM). In addition to reinforcing the hydrogels, the incorporation of nanomaterials such as carbon nanotubes, graphene and GO can result in strong self-healing hydrogels that are also photo-thermo-responsive.[249] For example, Wang *et al.* developed temperature-sensitive hydrogel trunks that have three parts; one thermo-responsive part is comprised of GO and PNIPAM/clay, the second thermo-responsive part is comprised of PDMAA/clay, and the third part (at the end) is composited with Fe_2O_3 nanoparticles.[280] Higher temperature leads to the shrinkage of the PNIPAM/clay network, and the expansion of the PDMAA/clay network. Consequently, GO in the hydrogels leads to the conversion of near-infrared (NIR) radiation into heat, which results in the shrinkage or expansion of the nanocomposite parts of the hydrogel. Thus, the successive use of NIR radiation can be employed for the actuation of the trunks. Magnetic manipulation of the trunks can be achieved through Fe_2O_3 nanoparticles to enable the dislocation of cargos. Utilizing NIR as the driving energy and control signal for the soft actuator makes this self-healing hydrogel potentially useful in tissue engineering because of NIR safety[281] and its capability to penetrate tissues and organs.[282] Lin *et al.* found that polyacrylic acid (PAAc) chains are capable of interacting with Fe^{3+} to gain additional crosslink density and ultrahigh strength properties.[283] The PAAc/clay and Fe^{3+} crosslinked PAAc/clay (F-AAc) hydrogels showed different responsiveness to changes in pH and/or ionic strength. Thus, as the sticky PAAc and F-AAc composite hydrogels are assembled into bilayers, reversible shape morphing and actuation are achieved upon changes in pH and ion strength. Bilayer actuators with various shapes have been fabricated and reversibly actuated by changing the environmental conditions. Developing self-healing hydrogels that have ultrahigh strength properties is attractive as they can be potentially applied in the repair of muscle, tendon and cartilage. However, the use of bilayer actuators is limited to sticky materials, and the sticky interface may be sensitive to pH, ionic strength, or temperature, which could limit their practical applications.

3.8. Other applications

Achieving adhesion between surfaces and soft materials using gels is still a challenging task.[284] Hydrogel-based adhesives are sensitive to stress and damage and hence, the use of self-healing hydrogels can be a good option. Hydrogels that can sense environmental stress and self-heal after damage emerged as potential candidates for the development of surface adhesives.[285–288] Without surface pre-functionalization, Liu and Scherman demonstrated that a cucurbit [8]uril (CB[8])-based supramolecular hydrogel network can act as a tough and self-healing adhesive for diverse nonporous (*i.e.* glass, stainless steel and aluminum) and porous (wood and bone) substrates.[285] Using dynamic Schiff crosslinking and hydrogen bonds between dopamine-grafted oxidized sodium alginate and polyacrylamide chains, it is also possible to develop a self-healing hydrogel that exhibits successful adhesion to various organic (*e.g.* skin) and inorganic substrates, including glass, water-filled plastic bottles and metals.[286] This is due to the high tendency of the catechol groups of dopamine to interact with the surface of these materials.

Self-healing hydrogels were also successfully used as hemostatic materials.[287,288] In their work, Huang *et al.* used carboxymethyl chitosan and benzaldehyde-terminated four-arm PEG to develop a self-healing (94 ± 9.8% after 12 h under physiological conditions) injectable hydrogel with good mechanical strength (3162.06 ± 21.06 Pa).[287] Such properties are sought for materials intended for use in the management of bleeding. Besides its ability to encapsulate cells, the resulting chitosan-based hydrogel exhibited good cytocompatibility and its use led to significantly decreased blood loss (0.29 ± 0.11 g) and shortened hemostasis time (120 ± 10 s), as compared to the use of conventional surgical gauze (0.65 ± 0.10 g and 167 ± 21 s, respectively), when applied to bleeding experimental liver injury in rabbits.[287] A self-healing hydrogel was produced by mixing dialdehyde-functionalized PEG solutions with an agarose-ethylenediamine conjugate. Its excellent adhesion ability was demonstrated in porcine skin, and its hemostatic effect was demonstrated in a rabbit liver hemorrhage model by decline in the amount of bleeding (0.19 ± 0.03 g) and significant decrease in hemostasis time (<10 s), as compared to the use of sterile gauze (0.71 ± 0.09 g and 72 ± 11 s, respectively).[288] This hydrogel can potentially be utilized as a hemostatic sealant for application in emergency situations such as for controlling bleeding from the liver or from other friable tissues after major trauma.

In recent work, Multanen and Bhushan added silica nanoparticles and a fluorosilane overcoat to a polyacrylamide hydrogel to develop a hydrogel-based coating system with self-healing, icephobic, liquid repellent and self-cleaning abilities.[289] The addition of fluorosilane provided hydrophobicity with a low tilt angle and oleophobicity. The anti-icing properties, confirmed at temperatures down to −60 °C, showed that supercooled water droplets were able to roll over the frozen liquiphobic hydrogel and bounce off the surfaces. Interestingly, this type of self-healing hydrogel was also shown to maintain liquiphobicity (toward water or oil) even at high temperatures (up to 95 °C). More interestingly, this hydrogel exhibited self-cleaning properties and demonstrated the rolling behavior of water droplets on the surface, resulting in an effective removal of pre-added contaminating silicon carbide particle debris from the surface of the hydrogel based-coating.[289] Such self-healing hydrogels can potentially be used for cellular and tissue cryopreservation.

4. Challenges and future outlook

Over the past few years, self-healing hydrogels have attracted major attention as important soft materials for use in significant biomedical applications; however, they are still associated with limitations and face many challenges that need to be addressed. Various chemical and physical crosslinking methods or their combinations have been applied in the production of self-healing hydrogels. Most of the dynamic covalent binding and physical binding are reversible, which confers desirable characteristics for synthesizing self-healing hydrogels.[22] In physical binding, metal–ligand binding has been used more often than the other methods for fabricating self-healing systems. However, one of the difficulties with metal–ligand-induced crosslinking is the controllability of the long-term stability of the final product. For example, in all previous works on catechol-based hydrogels, self-healing ability maybe lost over time. This is due to the relative ease with which catechol groups can be oxidized to quinone.[290] To avoid the oxidation of catechol, one can use catechol derivatives or analogues such as chlorocatechol, nitrocatechol, and 3-hydroxy-4-pyridinone.[291] Using dynamic chemical crosslinking for the synthesis of self-healing materials was found to enhance their mechanical properties. However, further development is still required. For designing self-healing hydrogels that are made *via* dynamic covalent binding, there is a need to study the balance between having an appropriate recovery rate and sufficient mechanical properties in the resulting hydrogels. The self-healing ability and the mechanical robustness of hydrogels are two characteristics that are in opposition. This implies that strong and stable crosslinking interactions can provide mechanical strength but may limit the polymer chain movements, which are needed for the recovery of the hydrogel networks.[292] The healing speed of the hydrogels is another important property that needs to be taken into consideration. This is especially important for implantable self-healing hydrogels because they can be exposed to constant mechanical stress such as may occur in blood vessels, skin, muscle and cartilage.

To date, only a few studies on self-healing hydrogels have been performed *in vivo*; therefore, more animal experiments are needed to assess the biocompatibility and the feasibility of the application of self-healing hydrogels for various therapeutic purposes. It should be noted that many types of self-healing hydrogels cannot be applied *in vivo* because some of the crosslinks are not stable in the body environment.[293] For possible clinical translation, the ability of self-healing hydrogels to maintain their mechanical properties and biocompatibility in clinical applications remains to be proved.[294] The crosslinking of hydrogels with stronger bonds to develop tough self-healing hydrogels is needed.[24] There are some reports on overcoming this challenge *via* the use of double network strategy,[295] or the addition of fillers[21,296] to the polymeric networks, *e.g.*, by using nanoparticles; however, there is still a major problem in having self-healing hydrogels that are strong enough to endure high shear stress with high fracture energy of over 1 kJ. To better understand the effect of crosslinking reactions and interactions that exist at the damage interfaces of hydrogels, the use of simulation and modeling programs such as molecular dynamics modeling should be further explored. This will help to identify the right polymeric systems needed for the target application. Performing simulations for a better understanding of self-healing mechanisms is crucial not only for the development of new self-healing hydrogels but also to optimize the system for boosting their performance.[297] The use of appropriate software can help to elucidate various important parameters on the binding sites to design more advanced self-healing hydrogels.

Methods that are used for assessing the behavior of self-healing hydrogels vary from one center to the other and from one study to another.[298] This may make it difficult to compare reported results on the healing abilities of different hydrogels. Therefore, standardized testing and characterization methods

of self-healing hydrogels require further development. Rheological property tests are important and they must be used to quantitatively evaluate the healing properties and recovery rate of the self-healing hydrogels. Qualitative assessment of hydrogel properties, although necessary, it is not sufficient to properly demonstrate the healing characteristics of the hydrogel. It should be noted that cyclic strain time sweep tests are commonly used for studying the behavior of self-healing hydrogels.[299] In the future, uniform and standardized protocols and parameters should be employed for conducting such tests in order to have comparable results of testing the healing properties of hydrogels. Consistency in rheological tests will not only allow us to quantitatively compare the self-healing characteristics of hydrogels of differing material formulations, but also enhance the applications of self-healing hydrogels in different biomedical areas, particularly including their use in as smart bioinks in the advancing field of 3D and 4D printing.[300] Thus far, reports on the use of self-healing hydrogels in 3D bioprinting are limited,[233,235] and there is great potential in advancing this further and developing smart constructs[301] or influencing cell fates[302] by employing self-healing bioinks.

To enhance the biomedical applications of the self-healing hydrogels in the future, we need to also improve their biodegradability, biocompatibility and multifunctionality. Natural polymers such as polypeptides and polysaccharides can be employed in the synthesis of self-healing hydrogel networks to enhance hydrogel properties. For biodegradable self-healing hydrogels, it is also important to optimize the biodegradability to ensure that the biodegradable hydrogel remains stable in physiological conditions for the time required to provide the required function. The synthesis of autonomous self-healing hydrogels that do not require external triggers in a facile way is a challenging task,[303] and it needs development.

In order to use self-healing hydrogels in drug delivery systems, burst drug release from the hydrogel is one limitation that needs to be addressed to produce an efficient controlled releasing system. Synthesizing smart hydrogels that have both shape memory and self-healing properties, without these affecting each other, should be further investigated.[33,234,304,305] The next generation of self-healing materials should have a greater intrinsic healing capacity even under harsher environments. This is even more important for the self-healing materials that are intended to be implanted *in vivo*. Most of the reported metal ion-containing self-healing hydrogels are not stable *in vivo* because of possible exchange reactions with the cations present in the body, such as sodium ions. Furthermore, there are difficulties in the large-scale production of self-healing hydrogels, which need further research and development to enable industrial production and the wider use of this attractive group of biomaterials.[216] In the future, more attention should be paid to the development of multifunctional smart hydrogels that have other functions such as magnetism, conductivity, and luminescence besides the self-healing behavior. This will be particularly useful for the utilization of self-healing materials in the development of biosensors, artificial muscle, actuators and other devices with optoelectronic capabilities.[306] Researchers from different backgrounds such as physics, chemistry, engineering and biology should work together to overcome these challenges to advance this field. Such interdisciplinary collaborations will help faster translation of the technology to clinical settings. The involvement of translational researchers and clinicians will help to develop novel applications of these hydrogels such as soft robotics, human-machine interaction systems and electrical skin.

From a clinical perspective, the integration of biomaterials has been a major problem. This has been attributed to the type of cellular and tissue reactions a biomaterial may elicit in the body, which varies with ''bioinert'' materials, bioactive and biodegradable ones.[307–311] Various methods have been explored to control or enhance the development of favorable tissue reactions that can lead to implant bonding to tissues and prevent the formation of interfacial fibrous tissue with subsequent implant loosening and failure.[312–314] Other strategies involved the utilization of tissue reaction modulating agents such as anti-inflammatory[315–317] or antiosteolytic agents.[210,318,319] The use of self-healing hydrogels has a good potential to address this problem, which is of major clinical significance. Such hydrogels can be applied as coatings[320–322] on various implants as either monofunctional or multifunctional coating with drug-releasing properties. Therefore, the use of multifunctional self-healing hydrogels can have a role to play in enhancing the biocompatibility and the integration of implants in the body. Certainly, the involvement of translational researchers and clinicians in a multidisciplinary approach is required to expedite the application of these exciting materials and their transfer to industrial production and translation to the clinic.

5. Conclusions

Recent developments in biomaterials science have led to the development of self-healing hydrogels that were exploited for use in drug release, cellular release, wound healing, regenerative therapeutics, sensors, actuators and other biomedical applications. Most studies evaluated the behavior and characteristics of these materials *in vitro* and *in vivo* but none has made it to the clinic so far. By avoiding irreversible damage in a biomaterial, self-healing hydrogels can have an extended lifetime in the body. Strong biomaterials can be produced, especially with the use of hydrogen bonding in the self-healing materials; however, there are still challenges that need to be addressed, including the strength and durability of these hydrogels. There is also an active area of research that deals with adding more functionalities to the self-healing hydrogels such as adhesiveness, electroconductivity, magnetism, and luminescence, which will make these materials more efficient candidates for developing new applications. In the future, the behavior and integration of implants in the body may be improved with the aid of multifunctional self-healing biomaterials.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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