Self-healing biomaterials

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Self-Healing Biomaterials: From Molecular Concepts to Clinical Applications

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Biomaterials are being applied in increasingly complex areas such as tissue engineering, bioprinting, and regenerative medicine. For these applications, challenging—or even contradictory—combinations of biomaterial properties are often required which cannot be met by conventional biomaterials. During the past decade, several new concepts have been developed to render biomaterials self-healing, thereby offering new opportunities to improve the functionality of traditional biomaterials in terms of their mechanical, handling, and biological properties. Consequently, various types of self-healing polymeric, ceramic, or composite biomaterials have been developed. Nevertheless, despite the rapid emergence of the field of self-healing biomaterials, this field of research has not been reviewed during the recent years. Therefore, this article provides a critical overview of recent progress in the field of self-healing biomaterials research by discussing both extrinsic and intrinsic self-healing systems. While the extrinsic self-healing section focuses on self-healing dental materials and orthopedic bone cements that rely on release of healing liquids from embedded microcapsules, the section on intrinsic self-healing materials mainly discusses concepts for self-healing of polymeric biomaterials that are either hydrated (hydrogels) or nonhydrated (e.g., films and coatings). Finally, benefits of the self-healing feature for biomaterials are discussed, and directions for future research and developments are outlined.

were selected “off-the-shelf” based on the ingenuity of the surgeons.^[1] During the last century, significant medical and technological progress has paved the way for the field of biomaterials research and the emergence of a biomaterials industry. Various synthetic or natural biomaterials have been developed which have enabled treatment of a wide range of medical conditions. Nevertheless, biomaterials are being considered for increasingly complex indications, which has increased the requirements for biomaterials considerably during the past decades.^[2] Consequently, challenging—or even contradictory—combinations of biomaterial properties are often required which cannot be met by conventional biomaterials. For instance, biomaterials are desired which combine injectability, mechanical strength, and degradability with toughness to avoid mechanical damage following crack propagation. To meet these strict requirements, biomaterials are needed which can adapt to the implantation site and are able to heal themselves upon mechanical damage.

1. Introduction

Throughout history, the strive for longer and healthier life has urged mankind to develop materials that can restore the function of lost or damaged body parts. Initially, these materials

While the majority of synthetic biomaterials does not recover from mechanical damage, natural tissues display a remarkable capacity for self-healing such as the spontaneous self-repair of bone fractures or ruptured skin. This self-healing ability is the ultimate solution of nature for continued survival based

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on adaptation and remodeling of damaged tissues.^[3] Recently, a new generation of man-made materials has evolved with the capacity to self-heal. These self-healing materials are characterized by their ability to recover their original properties after damage, particularly in terms of mechanical properties.^[4] Generally, these materials heal themselves via either “extrinsic” or “intrinsic” mechanisms. While materials with an extrinsic self-healing capacity require external aids such as capsules containing healing agents to facilitate the process of self-healing,^[5] materials with an intrinsic self-healing capacity can self-heal without any external aid.^[6] Several strategies have been explored so far to develop self-healing materials for various applications.^[4] To the best of our knowledge, the first scientific article describing self-healing man-made materials designed specifically for biomedical applications was published in 1998, when Koole et al.^[7] reported the intrinsic self-healing of punctured holes in a polymeric drug reservoir capsule. In 2001, White et al.^[5] reported a polymer-matrix composite capable of extrinsic self-healing owing to the incorporation of a microencapsulated healing agent, which was not, however, specifically designed for biomedical applications. In a first review article dedicated to self-healing biomaterials published in 2010, Brochu et al.^[8] explained how the development of synthetic biomaterials with an autonomous capability for self-healing would benefit implants used for tissue replacement. A main motive for the interest in such self-healing implants was extension of the lifetime of biomaterials, since in situ detection of mechanical damage in medical implants and subsequent replacement of damaged prostheses are highly challenging. Since 2012, the field of self-healing biomaterials has grown more rapidly, as evidenced by an analysis of recent publication data (Figure 1). New concepts for self-healing have been developed and new self-healing biomaterials have been synthesized for several biomedical application areas. Most prominently, supramolecular polymeric biomaterials are increasingly considered for biomedical conditions, which offers the possibility to synthesize biomaterials with intrinsic self-healing capacity that can adjust

Figure 1. Annual number of publications on self-healing biomaterials since 2005 (obtained from Web of Science in December 2017 using self-healing and biomaterials as keywords).

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themselves to the implantation site and can recover from potential mechanical damage.^[9] Until recently, improved durability was considered the main promise of self-healing biomaterials.

Although this field of research is still in its infancy, recent literature indicates that self-healing biomaterials can offer strong benefits compared to non-self-healing biomaterials in terms of their processing, handling, biological and clinical performance.

Despite the rapid emergence of the field of self-healing biomaterials, this field of research has not been reviewed during the past years. Therefore, this progress report provides an overview of recent progress in the field of self-healing biomaterials research. Moreover, potential benefits of self-healing biomaterials are discussed and directions for future research and developments are outlined. This progress report does not focus on studies related to self-healing materials not intended for use in biomedical applications since these studies have been reviewed previously.^[4a,10]

2. Self-Healing and Its Characterization

Self-healing materials are able to recover their functionality after damage.^[4a] In view of the importance of mechanical properties of biomaterials, the self-healing capacity of biomaterials is commonly determined by evaluating the recovery of their mechanical properties after mechanical damage. Nevertheless, to test the self-healing of other functional properties, different characterization strategies should be developed. For example, biomaterials used for electroconductive cardiac patches should provide electrical conductivity in addition to mechanical support.^[11] Therefore, recovery of their mechanical as well as electrical properties should be quantified to obtain a full overview of their self-healing performance. In addition, the type of damage that a biomaterial experiences depends on the specific application. For example, while hydrogels used for minimally invasive treatment are prone to shear-induced failure during injection, a biomedical coating on a metallic implant is prone to delamination. Therefore, these differences should be taken into account for the design and characterization of self-healing biomaterials.

Both qualitative and quantitative tests are used to evaluate the self-healing capacity of biomaterials. Qualitative methods include inspection of the integrity of a biomaterial after damage with the naked eye or using microscopy. This type of evaluation analyzes, e.g., crack closure after scratching a biomaterial surface or shape stability of a biomaterial after cutting and subsequent fusing. Quantitative methods often measure the percentage of recovery of a specific physical quantity after self-healing (e.g., strength or elastic modulus). Such measurements can be performed for instance using compressive, tensile, or three-point bending tests after cutting-induced mechanical damage or crack propagation. Furthermore, self-healing of hydrogels upon shear-induced failure is commonly measured using rheological tests by applying cycles of low and high oscillatory strains. In addition to the self-healing efficiency, the kinetics of self-healing of biomaterials determines their applicability as well. The self-healing kinetics is particularly important since excessively slow self-healing can result in premature disintegration of a biomaterial under dynamic in vivo conditions. Another important factor determining the self-healing capacity of biomaterials relates to the time period during which a material is damaged. As an example, this factor corresponds

to the time during which two pieces of a biomaterial are kept separated from each other after cutting-induced failure but prior to self-healing.^[12]

Generally, it should be emphasized that standard protocols have not yet been established for the quantification of the self-healing capacity of biomaterials, which can be explained by the fact that research on self-healing biomaterials has emerged only during the past decade. Consequently, various studies have tested different experimental variables for the mechanical characterization of self-healing, which complicates direct comparison between reported results considerably. For example, different degrees of oscillatory strain (ranging from 50%^[13] to more than 1000%^[14]) have been used to destruct gel networks during rheological characterization of self-healing. Nevertheless, the effect of the degree of destructive straining on the self-healing efficiency of hydrogels is usually not studied. Accordingly, a systematic study on quantification of the self-healing behavior of biomaterials is essential to establish standard protocols for the characterization of the self-healing behavior of biomaterials. These protocols should also take account of the environmental conditions to which biomaterials are exposed in vivo. For instance, the self-healing capacity of dental and orthopedic biomaterials is commonly quantified by measuring static mechanical properties such as flexural strength, flexural modulus, and fracture toughness of notched specimens.^[15] However, cyclic or dynamic testing conditions should be employed since these tests are more similar to clinical loading scenarios by mimicking, e.g., mastication in dentistry or locomotion in orthopedics.^[16] Accordingly, these standard protocols would improve our understanding of self-healing properties of current and future self-healing biomaterials and allow direct comparison between reported results in the literature. In view of the above-mentioned lack of standard protocols, the following sections will not compare self-healing behaviors of different material systems. Instead, an overview of the current state-of-the-art in this emerging research field will be presented by describing how molecular concepts on self-healing biomaterials are being translated toward clinical applications in regenerative medicine. Specifically, extrinsic and intrinsic self-healing biomaterials will be reviewed in Sections 3 and 4, respectively.

3. Extrinsic Self-Healing Biomaterials

Extrinsic self-healing biomaterials do not exhibit an intrinsic capability to self-heal after damage. Consequently, these systems need additional healing agents to enable self-healing. The most common strategy toward design of extrinsic self-healing biomaterials was pioneered by White and co-workers^[5,17] and entails the incorporation of capsules containing healing liquids into a non-self-healing matrix (**Figure 2**). In these biomaterials, mechanical damage induces rupture of the capsules and release of their cargo into microcracks to enable self-healing. To this end, a catalyst is typically incorporated in the matrix to accelerate the process of self-healing by catalyzing a polymerization reaction in microcracks following release of the healing agent from the capsules.^[5]

In this section, this capsule-based approach for the development of extrinsic self-healing biomaterials is reviewed with a

Figure 2. Schematic illustration of the capsule-based concept for self-healing. A) Mechanical damage causes crack formation. B) Crack propagation ruptures the microcapsules, releasing their cargo into the crack. C) The released healing agent reacts with the embedded catalyst, filling the crack through a polymerization reaction. Reproduced with permission.^[5] Copyright 2001, Springer Nature: Nature.

specific focus on applications in dentistry and orthopedics. These biomaterials typically consist of (i) a matrix phase containing specific catalysts, (ii) healing liquids encapsulated in (iii) the embedded microcapsules. These components are discussed in the following subsections. The composition of these extrinsic self-healing biomaterials for dentistry and orthopedic applications are listed in **Table 1**.

3.1. Matrix Phase

The most commonly applied monomers for polymerization of resins in dental composites are bisphenol A glycidyl methacrylate (bis-GMA) and urethane dimethacrylate (UDMA). Both of these dimethacrylate monomers have a high molecular weight resulting in high viscosity and reduced polymerization shrinkage compared to methyl methacrylate (MMA).^[24] To improve the clinical handling properties of these resins, monomers of lower molecular weight are embedded as viscosity modifiers such as MMA, ethylene glycol dimethacrylate (EDMA) and tri(ethylene glycol) dimethacrylate (TEGDMA).^[24]

Inorganic glass fillers are essential additives to reduce the polymerization shrinkage and the coefficient of thermal expansion, while improving the mechanical properties and aesthetic features of the composite material.^[24] In addition, the inclusion of metals such as barium and strontium renders dental resin-based composites radiopaque. As mentioned above, the surface of the glass particles is coated with a silane coupling agent such as 3-(triethoxysilyl)propyl methacrylate to improve the mechanical properties of resin-based dental composites by forming covalent bonds between the glass surface and the resin matrix.^[15a,17,18]

Table 1. Composition of extrinsically self-healing dental materials and orthopedic bone cements.

	Matrix composition ^{a)}	Healing liquid composition ^{a)}	Microcapsule composition	Microcapsule size [μm]	Type of catalyst/initiator	Filler particles	Reference
Dental materials	Bis-GMA:TEGDMA (1:1)	TEGDMA	PUF	70 ± 24/≈25	BPO/DHEPT	BBSG	[15a–d]
	Bis-GMA:TEGDMA (7:3)	DCPD	PUF	50–300	–	–	[18]
	Bis-GMA:UDMA:TEGDMA (1:1:1)	DCPD	PUF	–	Grubbs' catalyst	Silanized BBSG	[19]
	Bis-GMA:HEMA (1:1)	Polyacrylic acid	Silica	180 (unsilanized) ^{a)} 20 (silanized) ^{a)}	–	SFASG	[15f]
	PENTA, di- and trimethacrylate resin	TEGDMA	PU	≈0.6 ^{a)}	–	Silicon dioxide	[20]
Orthopedic bone cements	PMMA	Bis-EMA+TMPET/TMPTA	PUF	106 ± 24	BPO/MBDMA	–	[21]
	MMA-co-Sty, PMMA	Anisole/dissolved PMMA	PU/UF	≈300 ^{a)}	BPO/amine	–	[17]
	PMMA	OCA	PU	121 ± 24	–	–	[22]
	PMMA	DCPD	PUF	226 ± 51	Grubbs' catalyst	–	[23]

BBSG: barium borosilicate glass; Bis-EMA: bisphenol A ethoxylate dimethacrylate; Bis-GMA: bisphenol A glycidyl dimethacrylate; BPO: benzoyl peroxide; DCPD: dicyclopentadiene; DHEPT: *N,N*-dihydroxyethyl-*p*-toluidine; HEMA: hydroxyethyl methacrylate; MBDMA: 4,4'-methylenebis(*N,N*-dimethylaniline); MMA-co-Sty: methyl methacrylate styrene copolymer; OCA: 2-octyl cyanoacrylate; PENTA: dipentaerythritol pentaacrylate monophosphate; PMMA: poly(methyl methacrylate); PUF: poly(urea formaldehyde); PU: polyurethane; PU/UF: polyurethane/poly(urea-formaldehyde); SFASG: strontium fluoroaluminosilicate glass; TEGDMA: tri(ethylene glycol) dimethacrylate; TMPET: trimethylolpropane ethoxylate triacrylate; TMPTA: trimethylolpropane triacrylate; UDMA: urethane dimethacrylate; ^{a)}Estimated from scanning electron micrographs.

Catalysts are embedded in the matrix of self-healing dental resin-based composites.^[5] The chemical nature of these catalysts depends on the specific healing agent released from microcapsules. White et al.^[5] exploited ring-opening metathesis polymerization (ROMP) of dicyclopentadiene (DCPD) to fill microcracks. For dental restorative material, Grubbs' catalyst, i.e., a transition metal carbene complex, was used as the catalyst to trigger this polymerization reaction, achieving a recovery of 57% of the original fracture toughness.^[19] However, the use of Grubbs' catalyst in dental materials remains debated considering the toxicity and high cost of this catalyst. More recently, Xu and co-workers introduced benzoyl peroxide (BPO) in the resin matrix to trigger self-healing based on free-radical polymerization of TEGDMA released from microcapsules.^[15a–d] BPO is preferred as a catalyst since it has been used extensively in dental materials.^[15b]

As aforementioned, bone cements as employed in orthopedic surgery to anchor prostheses to bone. The matrix phase of bone cements is prepared by mixing a powder with a liquid phase. The powder phase consists of prepolymerized poly(methyl methacrylate) (PMMA) particles,^[17,22a,c,23] barium sulfate as radiopacifier, and BPO as initiator.^[10a] The liquid phase usually contains MMA as main component, *N*, *N*-dimethyl-*p*-toluidine as accelerator and hydroquinone as stabilizer to prevent premature polymerization. In order to improve the resistance of bone cements against abrasive wear, bone cements have been developed which contain poly(methyl methacrylate-*co*-styrene) (P(MMA-ST)) copolymeric beads instead of PMMA.^[25]

3.2. Healing Liquids

3.2.1. Chemical Composition of Healing Liquids

Research on self-healing dental and orthopedic biomaterials was inspired by the study of White et al.^[5] on self-healing systems comprising poly(urea formaldehyde) (PUF) capsules containing DCPD as a healing liquid. Depending on the chemical nature of the specific acrylic resin, the healing liquid to be released into microcracks varied among the reported studies. The most commonly applied chemical reaction to induce self-healing is ROMP,^[26] which relies on a transition metal catalyst (e.g., ruthenium-based Grubbs' catalyst). ROMP proceeds rapidly at ambient conditions resulting in low shrinkage levels.^[5] However, due to the high cost of Grubbs' catalyst and toxicity of both DCPD and Grubbs catalyst, alternative microcapsules filled with TEGDMA monomers have been developed.^[15a,c–e,20,27] In the studies of Wu et al.^[15] the polymerization of these monomers was initiated by BPO, thereby avoiding the use of Grubbs' catalyst to trigger the polymerization. Huyang et al.^[15f] designed and developed self-healing dental composites based on the release of aqueous solutions of polyacrylic acid (as healing liquid) from silica microcapsules. This healing liquid triggered a self-healing mechanism based on the hardening of glass ionomer cements (GICs). Finally, good solvents for the matrix^[17] as well as an established acrylate-based adhesive^[22c] have also been proposed as healing liquids, including anisole, a nontoxic solvent for PMMA,^[17] and 2-octyl cyanoacrylate (OCA, commercial name Dermabond), a widely used tissue adhesive.^[22a,c]

3.2.2. Physicochemical Properties of Healing Liquids

To guarantee efficient self-healing, shells of microcapsules should break upon propagation of microcracks, thereby releasing the healing liquid which subsequently penetrates through capillary action into the empty space formed by the propagating microcrack.^[15b] Therefore, the healing liquids should exhibit a low viscosity and allow for wetting of the exposed crack surfaces to enable sufficient penetration of the cracks. Finally, sufficient molecular interactions between the healing liquid polymerizing in the microcracks and the surrounding resin matrix should be created. Acrylic healing liquids which are generally explored for application in dentistry^[15a–d,18–20] are chemically similar to the surrounding matrix. This allows the formation of strong bonds with the surrounding organic matrix, for instance through reaction with unreacted monomers residing in the matrix. Moreover, it should be emphasized that dental resin-based composites contain large amounts of glass filler particles (ranging between 50 and 80 vol%). Consequently, healing liquids should ideally also adhere to glass filler particles, although this aspect has not yet been recognized in research on self-healing dental composites. For the self-healing concept based on filling of microcracks with GIC as reported by Huyang et al.,^[15f] the affinity between the hydrophobic resin matrix and the hydrophilic GIC-based filling agent is lower, which might compromise the mechanical integrity of the resulting composite. Gladman et al.^[17] reported a solvent-bonding approach to create a strong interface between the matrix of acrylic bone cements and the crack-filling agents by releasing anisole as solvent for PMMA from double-shell wall pol(urethane)/urea-formaldehyde (PU/UF) microcapsules, which resulted in self-healing of up to 80% of the original fracture toughness.

Due to the potential leaking of monomers to the surrounding tissues, healing liquids should also be sufficiently biocompatible. To avoid the use of toxic DCPD and Grubbs' catalyst, TEGDMA can be used as healing liquid with BPO as initiator in combination with DHEPT as a catalyst.^[15a–d] Alternatively, a healing liquid which can polymerize with water or ambient moisture in bone cement matrices without any catalyst can be used. Anisole, as a good solvent for PMMA, is also approved by the Food and Drug Administration as a food additive, which may also justify its use as healing liquid for bone cements.^[17]

3.3. Microcapsules

3.3.1. Chemical Composition of Microcapsules

Most self-healing biomaterials in dentistry and orthopedics rely on the application of microcapsules based on PUF or PU. PU forms flexible, optically transparent and hermetic microcapsule shells by interface polymerization. PU-based microcapsules are, however, mechanically weak and tend to agglomerate.^[28] As an alternative, an inorganic approach by using silica microcapsules for application in dental self-healing composites was adopted.^[15f]

3.3.2. Physicochemical Properties of Microcapsules

Morphological features of microcapsules, which control both the self-healing efficacy and mechanical properties of the resulting biomaterials, include their size, shape, and shell thickness. The microcapsule size determines the volume of encapsulated healing liquid as well as the mechanical properties of the microcapsule-containing biomaterial.^[10a] Small microcapsule sizes are generally preferred to improve the dispersion of microcapsules and infiltration of the healing liquid into small microcracks.^[29] Since most self-healing biomaterials contain microcapsules formed by means of emulsification, the shape of these microcapsules is controlled by parameters of the healing liquid such as viscosity and surface tension.^[30] In most studies on self-healing microcapsule system, the shape of microcapsules prepared by emulsification is spherical. Furthermore, the thickness of the microcapsule shell has a strong influence on the process of self-healing since this parameter determines the stability of the microcapsules. When microcapsule shells are too thin, healing liquids are released prematurely upon mixing with the matrix phase. Excessively thick microcapsule shells, on the other hand, compromise the self-healing efficacy by hampering the release of healing liquid upon microcracking. For each capsule-based self-healing system, an optimal balance should be found between microcapsule strength versus fragility.^[17,29] The kinetics of the release of healing liquid from microcapsules is not only controlled by the chemistry and thickness of the microcapsule shells, but also by the bond strength between the microcapsule shell and surrounding organic matrix. Sufficient capsule-matrix bonding is crucial to enable release of the healing liquid upon microcracking. Melamine has been used to modify urea-formaldehyde microcapsules and increase the bond strength between the microcapsule and the resin matrix composed of Bis-GMA and TEGDMA.^[18] Other groups used γ -glycidoxypolytrimethoxy silane to provide additional epoxy functional groups to enhance the bond strength between PUF microcapsules and epoxy matrices.^[31] Using a similar method, modified PUF microcapsules were modified with a silane-coupling agent to improve the interfacial adhesion between the capsules and the surrounding organic matrix.^[32] However, in some studies no catalyst or initiator was incorporated into the matrix,^[18,20] hence the effect of the improved bond strength on the self-healing efficiency could not be evaluated.

3.3.3. Encapsulation Techniques

Several methods have been developed to prepare microcapsules.^[30] The most commonly applied methods are in situ and interfacial polymerization. In situ polymerization has been applied to prepare UF, melamine-formaldehyde (MF), melamine-urea-formaldehyde (MUF), and phenol-formaldehyde (PF) microcapsules.^[10a] Interfacial polymerization has been used to form, e.g., PU microcapsules.^[10a] To form more robust shell walls, Caruso et al.^[33] combined these two polymerization methods to produce PU/UF microcapsules. The double-walled microcapsules retained the excellent interfacial bonding of urea-formaldehyde,^[33] while its surface roughness enhanced the mechanical adhesion.^[34]

4. Intrinsic Self-Healing Biomaterials

Intrinsic self-healing biomaterials exhibit an intrinsic capability to self-heal after damage without the need of additional healing agents. The most common strategy for the development of such biomaterials is based on the use of noncovalent interactions which enable reformation of broken bonds in a biomaterial due to their reversibility. The following sections provide an overview of hydrated (hydrogels, Section 4.1) and nonhydrated biomaterials (Section 4.2) that exhibit an intrinsic capability to self-heal.

4.1. Hydrogels

Hydrogels are 3D interconnected networks dispersed in an aqueous phase. Their viscoelastic nature, high water content and possibility for functionalization using bioactive moieties render hydrogels highly favorable for biomedical applications. These features of hydrogels mimic the natural environments of cells in various tissue types. Consequently, hydrogels exhibit a tremendous potential for use as synthetic extracellular matrices.^[35] Furthermore, with the emergence of minimally invasive treatments and (bio)printing technologies as well as the in vitro culture of (stem) cells, there is a growing demand for injectable biomedical hydrogels.^[36] To this end, several studies^[37] have used liquid gel precursors that can be injected and subsequently crosslinked at the target site. Nevertheless, postinjection crosslinking of polymer solutions is often problematic due to limited access to the injection site (e.g., light penetration depth), slow crosslinking kinetics, and potential cytotoxicity of cross-linking reagents. Therefore, shear-thinning and self-healing hydrogels that can be injected and immediately recover their mechanical properties upon extrusion are highly advantageous for these applications.^[10d] The self-healing capability is particularly important, since shear-thinning behavior renders a hydrogel injectable, but self-healing behavior is necessary to allow for recovery of mechanical integrity after injection.^[38] Furthermore, besides the recovery of their mechanical properties, self-healing and adaptive biomedical hydrogels can have additional biological benefits (e.g., cell ingrowth) that may benefit regenerative medicine.^[39]

4.1.1. Noncovalently Crosslinked Hydrogels

The use of noncovalent interactions is a common and effective strategy for the synthesis of materials with self-healing capability. Accordingly, several investigations have successfully exploited various types of reversible bonds for the development of self-healing biomedical hydrogels. **Figure 3** illustrates common strategies used for the production of noncovalently crosslinked hydrogels with self-healing capability.

Electrostatic Interactions: Electrostatic interactions between oppositely charged groups are widely employed by nature in biological processes such as protein folding.^[40] These reversible attractive forces have also been used to form hydrogels with self-healing capability. A promising example of such self-healing biomaterials was reported by Sun et al.,^[41] who used

Figure 3. Common strategies for the synthesis of noncovalently crosslinked hydrogels with self-healing capability.

polyampholytes made by a random copolymerization of cationic and anionic monomers such as 3-(methacryloylamino) propyl-trimethylammonium chloride and *p*-styrene sulfate, respectively. In these hydrogels, the random presence of cationic and anionic groups alongside the polymer chains enabled the formation of hydrogels based on a combination of strong and weak bonds between different ionic groups. While the strong bonds rendered the hydrogels highly elastic (elastic modulus up to 8 MPa), the reversible weak bonds were responsible for the high toughness (up to 4000 J m⁻²) and self-healing capability (self-healing efficiency up to ≈99%) of these biomaterials. Nevertheless, electrostatic forces are screened in solutions with high ionic concentrations^[40] and therefore they might not be sufficient to maintain and/or self-heal the integrity of a hydrogel network under physiological conditions. Indeed, polyampholyte gels^[40] displayed inferior mechanical properties, reduced self-healing efficiency and enhanced swelling upon immersion in salt solutions (NaCl concentration ≥ 0.1 M). Moreover, the authors found that strong bonds hinder the self-healing of hydrogels; the use of more hydrophobic monomers resulted in stiffer hydrogels, but these more hydrophobic systems displayed poor self-healing efficiencies (≈30%). In another study, an electrostatically crosslinked and injectable hydrogel was developed based on negatively charged dextran sulfate and 4-arm poly(ethylene glycol) (PEG) linkers modified with peptide sequences containing repeating units of lysine or arginine and alanine.^[42] After disruption of the hydrogel using large shear strains, the solid-like gel network immediately reformed, but recovered its original viscoelastic properties completely after 20 min.

Metal Coordination Interactions: Coordination between metallic cations and organic ligands is a promising crosslinking strategy for the formation of self-healing hydrogels. A well-known example of these interactions in nature is the coordination between Fe³⁺ ions and catechol groups in the cuticle of

mussel byssal threads which, as reported by Harrington et al.,^[43] can act as reversible load-bearing bond imparting hardness, extensibility and self-healing capability to these natural materials. These Fe³⁺-catechol crosslinks have been successfully exploited by Holten-Anderson et al.^[14] for the preparation of self-healing hydrogels by adding Fe³⁺ cations to 4-arm PEG polymers that were end-functionalized with catechol groups. By tuning the pH from acidic to basic, different crosslinking species of mono-, bis-, and tris catechol-Fe³⁺ were formed. Interestingly, hydrogels with near-covalent crosslinks were obtained through tris-catechol-Fe³⁺ coordination showing similar stiffness as covalently crosslinked PEG-catechol hydrogels, while maintaining the ability to recover their initial stiffness within minutes after network failure induced by destructive shearing up to ≈2000%. In another study, Shafiq et al.^[44] used a nitrocatechol derivative to end-functionalize 4-arm PEG. This functionalization strategy not only enabled the formation of self-healing hydrogels based on reversible catechol-Fe³⁺ coordination, but also allowed phototriggered degradation of the hydrogels owing to the photocleavable *o*-nitrophenyl ethyl groups. Consequently, this strategy can be used for the on-demand degradation of this type of hydrogels for in vitro or in vivo applications. Moreover, other studies^[45] have adopted another mussel-inspired approach based on histidine-divalent cation coordination for the development of self-healing hydrogels. Interestingly, one study^[45c] reported that the mechanics of histidine-based hydrogels can be effectively controlled by tuning the relative concentration of different divalent cations used for crosslinking of the gel network.

Bisphosphonates are commonly used drugs for the treatment of osteoporosis which exhibit a strong binding affinity toward the calcium phosphate phase of bone.^[46] The strong but reversible coordination bonds between bisphosphonate groups and Ca²⁺ ions can act as highly effective crosslinks for the development of self-healing hydrogels. Noncovalently

crosslinked hydrogels have been synthesized by mixing bisphosphonate-functionalized hyaluronic acid and Ca^{2+} ions which showed shear-thinning and self-healing behavior.^[47] The bisphosphonate- Ca^{2+} coordination strategy has been also successful for the production of a synthetic self-healing hydrogel through bisphosphonate functionalization of star-shaped PEG.^[48]

Alginate is a natural polymer which can also form a gel through noncovalent crosslinking with Ca^{2+} ions. The gel formation of alginate gels is based on coordination of hydroxyl and carboxylates groups with Ca^{2+} ions.^[49] Huebsch et al.^[50] have shown that ultrasound can be used for disruption of alginate gel networks for on-demand drug delivery, but the gel network can self-heal owing to the reversibility of alginate- Ca^{2+} crosslinks. Consequently, this mechanism could be successfully used in vivo for treatment of xenograft tumors by enabling improved spatiotemporally controlled delivery of anticancer drug mitoxantrone.

Hydrophobic Interactions: Hydrogels formed based on hydrophobic forces are another class of self-healing gels which are relevant for the biomedical field. These hydrogels can be synthesized by modification of various polymers with nonpolar groups such as alkyl chains. For instance, self-healing hydrogels have been made by incorporation of hydrophobic *n*-alkyl (meth)acrylate sequences in polyacrylamide chains through a micellar polymerization method.^[51] Interestingly, the self-healing ability of these hydrogels was improved with increasing the length of alkyl chains, with the highest level of self-healing observed for C18 alkyl chain.

Host-guest complexation based on hydrophobic forces has been also used for the preparation of biomedical hydrogels with self-healing capacity. A commonly used host molecule that can be employed for this purpose is cyclodextrin (CD). CDs are cyclic structures composed of repeating units of D-glucopyranoside linked by a 1,4-glycosidic linker. Kai et al.^[52] developed a noncovalently crosslinked hydrogel by first grafting PEG methyl ether methacrylate onto a lignin biopolymer using atom transfer radical polymerization, followed by addition of α -CD. Only 1% (w/v) of this copolymer was sufficient to form self-healing hydrogels through the inclusion complexation of PEG branches in the cavity of α -CD. An advantage of developing supramolecular hydrogels using CD is the possibility to tune the cavity size of CD to match different guest molecules. Accordingly, Peng et al.^[53] introduced larger β -CD host functionalities in a random polymer based on poly(*N,N*-dimethylacrylamide-*r*-glycidol methacrylate- β -CD) (P(DMA-*r*-GMA-CD)). Consequently, these β -CD moieties complexed with ferrocene groups of another random polymer, i.e., poly(*N,N*-dimethylacrylamide-*r*-2-hydroxyethylmethacrylate-Fc) (P(DMA-*r*-HEMA-Fc)), enabling formation of hydrogels. The resulting hydrogels could rapidly self-heal after shear-induced destruction and also could respond to electrochemical stimuli.

Hydrogen Bonding Interactions: A hydrogen bond can be regarded as a dipole-dipole interaction in which a hydrogen atom attached to an electronegative atom ("donor") interacts with another electronegative atom in its vicinity ("acceptor"). In general, hydrogen bonds are very directional and exhibit strengths between 4 and 120 kJ mol⁻¹,^[54] which render these noncovalent interactions ideal for the design of hydrogel networks. Consequently, supramolecular hydrogel networks have

been developed using multiple hydrogen bonding ureido-pyrimidinone (UPy) units coupled to a PEG chain via alkyl-urea spacers.^[55] In this system, the hydrogen bonds between UPy or urea moieties are shielded from water molecules due to a hydrophobic environment formed by the alkyl spacers. Consequently, UPy dimerization and lateral hydrogen bonds rising from the urea moieties lead to formation of 1D stacks that assemble into fibers. Subsequently, the entanglement of these dynamic fibers in water leads to formation of hydrogels.^[56] These dynamic hydrogel networks have been shown to self-heal after both cutting^[57] or shear-induced^[55] mechanical damage, and have already been translated toward practical applications in vivo^[55] aimed at delivering growth factors and decreasing infarct scar in a pig myocardial infarction model.

Other Noncovalent Interactions: Self-assembling peptides or proteins that form supramolecular structures are able to form self-healing hydrogels which are suitable for biomedical applications.^[58] These supramolecular peptide assemblies are mostly formed by a combination of hydrogen bonding, hydrophobic interactions and electrostatic interactions. Clarke et al.^[59] developed a self-healing hybrid hydrogel based on multiple β -sheet forming peptides coupled to the backbone of a poly(glycolic acid) (PGA) polymer. Reversible cross-links were formed in water via the intermolecular hydrogen bonds and additional hydrophobic interactions between β -sheets, allowing this hydrogel to recover its mechanical properties after network disruption (200% strain, 30 min recovery). Another example of such system is a hydrogel assembled from a short synthetic peptide sequence based on N-Pro-Ser-Phe-Cys-Phe-Lys-Phe-Glu-Pro-C.^[58a] These amphiphilic peptides self-assemble into β -sheet and β -turn structures which assemble into nan-fibrous structures at a high concentration via electrostatic and hydrophobic interactions. Consequently, the 3D percolation of these nanofibers led to the formation of a self-healing hydrogel network which could afford controlled release of a hydrophobic drug, and assisted animal hemostasis. Self-healing peptide-based hydrogels have also been made of β -sheet forming amyloid peptides in which hydrophobic, π - π interactions and hydrogen bonding are the driving forces behind the network formation.^[58b] These hydrogels have been shown to support attachment and spreading of various cell types and could be used as a synthetic extracellular matrix for stem cell differentiation. Molecular recognition by proteins is another mechanism that has been used for the formation of self-healing hydrogels. Accordingly, injectable and self-healing hydrogels have been developed by using two polymeric components containing Trp-Trp or Pro-rich peptide domains which have a high and specific binding affinity toward each other.^[60] In these systems, the gel is formed by simple mixing of the two components without the need of environmental triggers, which is highly beneficial for cell encapsulation. Lu et al.^[61] employed a similar principle by using RII α subunit of cAMP-dependent kinase A ("docking") and anchoring domain of A-kinase anchoring protein ("locking") to prepare two-component systems based on a Dock-and-Lock mechanism. These hydrogels revealed a higher elasticity (G' from 10 to 1000 Pa) as compared to similar systems and could quickly (<6 s) recover their viscoelastic properties after shear-induced mechanical failure.

Figure 4. Common strategies for the synthesis of covalently crosslinked hydrogels with self-healing capability.

Several studies have used combinations of noncovalent interactions to develop self-healing biomedical hydrogels. Although a higher density of hydrophobic interactions can enhance the mechanical properties of hydrogels, hydrophobic modifications of polymers beyond a certain threshold render them insoluble in water and unsuitable for the preparation of hydrogel. Therefore, Zhang et al.^[62] used hydrophobic UPy groups as well as the coordination of carboxylic acid groups of gelatin with Fe^{3+} ions to develop a self-healing and mechanically robust gelatin-based hydrogel. In another study, Li et al.^[63] developed an injectable, self-healing and thermosensitive hydrogel through the self-assembly of a copolymer consisting of a hydrophilic and catechol-modified PEG block and a thermosensitive poly[2-(methacryloyloxy)-ethyl] trimethylammonium iodide block. The gel formation and self-healing in this system was achieved based on hydrogen bonding and aromatic interactions including π - π stacking between the catechol groups. A similar hydrogel system was developed by Li et al.^[64] using a copolymer made of catechol-functionalized poly(*N*-isopropylacrylamide) (PNIPAM) and PEG.

4.1.2. Covalently Crosslinked Hydrogels

Covalent crosslinking of hydrogels typically results in the formation of static gel networks with nonreversible bonds that do not facilitate self-healing. Recently, however, covalent crosslinking using dynamic covalent bonds has emerged which can enable formation of mechanically robust and self-healing hydrogels. This synthesis strategy for the development of biomedical hydrogels is typically carried out by exploiting Dies-Alder (DA), Schiff-base, or thiol-disulfide exchange

reactions. This paragraph highlights recent progress using this strategy for development of self-healing biomedical hydrogels. **Figure 4** illustrates common strategies used for the production of self-healing biomedical hydrogels based on covalent crosslinks.

Dies-Alder Reactions: DA reactions between a diene and a dienophile have been used to prepare hydrogels, but high temperatures ($>100\text{ }^{\circ}\text{C}$) were needed to introduce reversibility.^[65] To address this problem, Wei et al.^[66] developed biomedical hydrogels using fulvene-modified dextran and dichloromaleic-acid-modified PEG. These hydrogels showed dynamic crosslink formation and self-healing behavior upon shear-induced or cutting-induced failure at room temperature without the need of external stimuli. Interestingly, in this study, scanning electrochemical microscopy was used to demonstrate the self-healing process of scratched hydrogels in situ by correlating spatial and temporal electrochemical signals with the topography of the samples.

Schiff-Base Reactions: Schiff-base reactions involve the selective reaction of an electrophilic carbon, such as ketone or aldehyde, with a nucleophilic species, such as amine or hydrazine, thereby forming reversible quasicovalent imine or hydrazone linkages. Therefore, covalently yet reversibly crosslinked hydrogel networks can be synthesized through simple mixing of components containing these reactive groups. Accordingly, Yang et al.^[67] mixed solutions of benzaldehyde-bifunctionalized PEG and glycol chitosan, which resulted in a fast formation (150 s) of self-healing gels based on reversible imide bonds. A similar self-healing hydrogel system has been developed by reacting benzaldehyde-bifunctionalized PEG with glycol chitosan and fibrinogen, showing angiogenic effects in vivo.^[68] Finally, Lu et al.^[69] used acylhydrazone-based crosslinking

with or without additional *in vivo* DA crosslinking to produce dynamic and self-healing hydrogels. These hydrogels were loaded with bone morphogenetic protein-4 and injected into bone defects to promote bone regeneration *in vivo*.

Thiol–Disulfide Exchange Reactions: Dynamic covalent crosslinks based on thiol–disulfide exchange reactions have also been used to produce self-healing hydrogels for biomedical applications. In the presence of nucleophilic thiols at neutral or alkaline pH, disulfide bonds can be broken and reformed producing dynamic and reversible hydrogel networks. Nevertheless, in these hydrogels, thiolate groups are likely to experience aerial oxidation leading to the loss of the dynamic and the self-healing features. A promising strategy to overcome this issue entails capping of reactive thiolate groups with Au(I) or Ag(I) ions, which can protect the thiolates from aerial oxidation without hindering the thiolate/disulfide exchange.^[70] Dynamic hydrogels have been synthesized using this strategy, which exhibit a fast recovery after network disruption by a large strain (800%) or by cutting.^[70a] Frequency-dependent stiffness was observed for this hydrogel; at low frequencies the exchange of Au-S/SS occurred fast enough to re-arrange while the network was less dynamic at high frequencies due to the slow exchange reactions and chain entanglement. Consequently, *ex vivo* investigations^[70b] revealed a high potential for these hydrogels to be used as shock-absorbing biomaterials for the treatment of intervertebral disc degeneration due to their self-healing and frequency-dependent stiffness behavior.

4.1.3. Combination of Noncovalent and Covalent Interactions

An emerging strategy for the development of self-healing biomedical hydrogels is based on combining covalent with noncovalent interactions. One strategy is to use (photo-initiated) chemical reactions to induce covalent bonding between polymers and crosslinking species, and these species noncovalently interact with each other, leading to the formation of noncovalently crosslinked hydrogels. Another strategy is to combine covalent and noncovalent crosslinks to produce single or double network self-healing gels with enhanced mechanical properties (**Figure 5**). Generally, “weaker” supramolecular interactions introduce reversibility and thixotropic behavior in these systems, whereas “stronger” covalent linkages can strengthen the network of the final construct. Furthermore, the entanglement of the two networks in case of double network gels can result in more resilient and load-bearing hydrogels.

Recently, photoinitiated chemical reactions were used to initiate the formation of a noncovalently crosslinked network by establishing chemical bonds between the crosslinking moieties, or to reinforce an already formed noncovalent network by introducing additional covalent crosslinks. For instance, Feng et al.^[71] first prepared supramolecular gelatin macromers by establishing host–guest complexation between freely diffusing photo-crosslinkable acrylated β -CDs and aromatic moieties of gelatin (e.g., phenylalanine, tryptophan, and tyrosine). Thereafter, a photoinitiated reaction established covalent bonds between the acrylated β -CDs resulting in the formation of a self-healing gel which was noncovalently crosslinked based on reversible host–guest interactions. The biological studies

Figure 5. Common strategies for the synthesis of self-healing biomedical hydrogels using the combinations of noncovalent and covalent interactions.

suggested that the dynamic nature of these self-healing gels assisted cell infiltration and migration into their matrix and the in vivo results confirmed the capability of the materials to facilitate regeneration of bone defects. Similarly, host-guest macromers have been developed using hyaluronic acid functionalized with adamantane and preassembled with monoacrylated β -CDs.^[72] Thereafter, photoinitiated reaction between the acrylates led to the formation of an interpenetrating network that was self-healing and could assist cartilage regeneration. In another study, Yao et al.^[73] prepared photo-crosslinkable macromers by chemical modification of two polypeptides with polyethylene glycol diacrylate groups. The polypeptides were then assembled to form supramolecular macromers based on coiled coil interactions, and gel formation could be induced by photoinitiated reaction between the acrylate arms. These hydrogels exhibited self-healing capability after cutting and displayed a strong potential for use as synthetic extracellular matrices. Several studies have used photoinitiated covalent crosslinks to reinforce noncovalently crosslinked hydrogels. In one study,^[74] noncovalently crosslinked hydrogels were made using recombinant suckerin-19 which self-assembled to supramolecular networks through multiple hydrogen bonding and hydrophobic interactions within β -sheets. Thereafter, covalent crosslinks were introduced by forming dityrosine in the presence of ruthenium complex and UV light, which could be achieved due to the large tyrosine content of suckerins (7–16 mol%). These hydrogels could self-heal after shear-induced failure most likely due to breakage and reassembly of β -sheets. Burdick and co-workers have developed two self-healing hyaluronic acid-based hydrogels which were subjected to secondary photo-crosslinking. In one study,^[61b] injectable and self-healing gels were prepared based on molecular recognition interactions between peptide-modified hyaluronic acid and recombinant polypeptides, which could undergo secondary covalent crosslinking through a photoinitiated reaction between methacrylate groups incorporated at the polypeptides or hyaluronic acid components of the gel. In another study,^[75] a similar photo-crosslinking strategy was used for post-printing stabilization of self-healing gels formed by host-guest interactions between β -CD and adamantane moieties linked to hyaluronic acid. This UV-induced covalent crosslinking strategy has also been used by Ossipov and co-workers for post-printing reinforcement of hyaluronic-based hydrogels formed based on bisphosphonate- Ca^{2+} coordination bonds.^[47]

Heilshorn and co-workers have also investigated the development of self-healing hydrogels with dual crosslinking mechanisms. In one study,^[76] shear-thinning and self-healing hydrogels were prepared based on the combination of dynamic covalent crosslinks and temperature-induced noncovalent crosslinks. In this system, dynamic covalent hydrazone bonds were formed ex situ between aldehyde-modified hyaluronic acid and hydrazine-modified elastin-like protein (ELP). Thereafter, the ELP underwent a thermal phase transition in situ (at 37 °C), providing additional noncovalent crosslinking, thereby reinforcing the hydrogels and slowing down their erosion rate. In another study,^[77] a weak hydrogel was formed through peptide-based molecular recognition interactions between engineered recombinant proteins and star-shaped peptide-PEG copolymers, enabling ex situ encapsulation of cells. Thereafter, a second

network was formed in situ through temperature-induced phase transition of PNIPAM motifs which were conjugated to the PEG copolymer. Both of these self-healing systems could provide mechanical protection for encapsulated cells during injections as well as prolonged cell retention, which might be of great benefit for applications in regenerative medicine.

An interesting hydrogel system has been developed mimicking the sliding filament theory of muscle contraction through the dynamic sliding of noncovalent crosslinks within an otherwise covalently crosslinked network.^[78] In this system, a covalent gel network was formed based on carbodiimide crosslinking between lysine and poly (γ -glutamic acid). Small organic molecules (dihydric alcohol and polyol) were introduced into the hydrogels as a “movable” interstitial phase. These molecules could form hydrogen bonds with adjacent polymer chains of the main network, thereby acting as reversible crosslinks endowing the hydrogels with self-healing capability.

Finally, Rodell et al.^[79] have used combinations of host-guest noncovalent crosslinking and Michael-addition covalent crosslinking to develop dual cross-linked or double network hydrogels. In one study,^[79a] hyaluronic acid polymers were modified with adamantane and thiol motifs or β -CD and methacrylate motifs. Consequently, a dual crosslinked hydrogel was formed based on the combination of the two polymer types, which could be used as an injectable hydrogel for treatment of myocardial infarctions in a rat model. In another study,^[79b] a noncovalently crosslinked network was first formed between hyaluronic acid polymers modified with β -CD or adamantane, after which a second network was formed based on reaction of methacrylated hyaluronic acid chains with dithiothreitol, resulting in double network hydrogels which were tough, injectable, and self-healing.

4.1.4. Particle-Based and Particle-Containing Hydrogels

Self-healing hydrogels can also be developed by using (nano) particles as building blocks or as an addition to continuous polymer matrices. The gel formation in these hydrogel systems is mainly based on noncovalent interactions between the particles (colloidal gels) or between polymer chains and particles (polymer-particle gels). Finally, particles can be also used to reinforce or functionalize self-healing hydrogel matrices (hydrogel-particle mixtures). **Figure 6** illustrates common strategies used for the production of these types of self-healing biomedical hydrogels.

Colloidal Gels: Colloidal gels are assembled solely from particles that form 3D percolating networks dispersed in a liquid phase.^[80] Recently, these gels have gained increasing interest in the biomedical field. In addition to their attractive viscoelastic properties, these materials can be rendered self-healing by the use of reversible interparticle bonds. Moreover, various particle types and combinations can be used as building blocks for the development of colloidal gels. These building blocks can also be loaded or functionalized with different types of drugs or biomolecules. The properties of colloidal gels depend on interparticle forces, particles/gel volume fraction, colloidal network structure as well as the size, morphology, and mechanical properties of their particulate building blocks.

Figure 6. Common strategies for the synthesis of particle-based and particle-containing biomedical hydrogels with self-healing capability.

Initial efforts to develop self-healing colloidal gels were focused on fully organic systems.^[81] So far, the most common approach for the preparation of these gels has focused on combining oppositely charged (nano)particles, leading to self-assembly of colloidal gel networks based on attractive electrostatic forces between these building blocks. To the best of our knowledge, Arimura et al.^[82] were the first to report the preparation of biodegradable colloidal gels as biomaterials in 2004. Nevertheless, this study did not investigate the self-healing potential of these gels that were composed of oppositely charged polylactide-based microspheres. In 2005, Van Tomme et al.^[83] reported the reversible character of a colloidal gel made of oppositely charged dextran microspheres by means of creep experiments. In 2008, Berklund and co-workers^[81a] reported that biodegradable and cytocompatible colloidal gels self-assembled from positively charged poly(vinylamine) (PVA_m)-coated and negatively charged poly(ethylene-co-maleic acid) -coated poly(D,L-lactic-co-glycolic acid) (PLGA) nanoparticles were self-healing. Nevertheless, the self-healing potential of these gels was only studied by evaluating the viscosity of the gels during several cycles of ascending/descending shear-rate. In a follow-up study,^[84] it was shown that these moldable and injectable gels could afford the controlled release of a glucocorticoid, i.e., dexamethasone, and were able to stimulate bone regeneration upon 4 weeks of implantation in rat cranial bone defects. To prevent potential cytotoxicity caused by the charged nanospheres, the same team also developed a similar colloidal gel system^[85] by coating the PLGA nanoparticles with biocompatible cationic chitosan or anionic alginate, which resulted in colloidal gels. Moreover, in a recent study,

Zhang et al.^[86] coated PLGA nanoparticles with red blood cells membranes producing biofunctional nanoparticles which were negatively charged. Subsequently, by mixing these nanoparticles with positively charged chitosan-coated PLGA nanoparticles, a colloidal gel was formed which exhibited shear-thinning behavior when subjected to an ascending shear stress and recovered its viscosity once the high shear stress was removed. Furthermore, this colloidal gel network could enhance the retention of cell-membrane coated nanoparticles *in vivo* and exhibited a remarkable antibacterial effect when injected subcutaneously in a mouse model with Group A *Streptococcus* infection. Overall, these studies suggest that surface modification of nanoparticles is a promising strategy for the preparation of building blocks that can be assembled into colloidal gel networks. Nevertheless, it can be expected that by the degradation of these surface layers, the nanoparticles lose their function, the gel network lose its integrity and, consequently, the nanoparticles leak out of the treatment site. One strategy that can overcome this potential problem is the use of biodegradable particulate building blocks which self-assemble into colloidal gel network without the need of surface coatings and present specific biological cues.^[81b,87] A particularly promising material toward this end is gelatin, since this natural polymer not only contains cell binding motifs, but is available in both cationic (from porcine skin) and anionic (from bovine skin) forms. Self-healing colloidal gels were easily assembled from oppositely charged gelatin nanoparticles.^[81b] The particle size of these colloidal gels strongly influenced the functional properties of resulting colloidal gels as gels made of gelatin microspheres were less elastic, less cohesive, less injectable and

non-self-healing, as opposed to colloidal gels prepared from gelatin nanoparticles.^[88] In vitro investigations showed that the nanostructured gelatin-based colloidal gels facilitated controlled release of multiple growth factors^[88,89] as well as antibiotics.^[90] In vivo investigations^[89,91] revealed that these colloidal gelatin gels allowed controlled delivery of angiogenic basic fibroblast growth factor and osteogenic bone morphogenetic protein-2 for the regeneration of bone defects.

Although the above-described polymeric colloidal gels exhibited a great potential for biomedical applications, their weak mechanical properties (maximum storage modulus achieved was ≈ 10 kPa) limit their applicability. To overcome this limitation, a composite colloidal gel was developed using oppositely charged gelatin nanospheres and stiff needle-like hydroxyapatite nanoparticles.^[92] Although the incorporation of inorganic hydroxyapatite nanoparticles effectively improved the mechanical properties of the gels, this reinforcement effect compromised the self-healing ability of the colloidal gels. A similar approach was employed by Wang et al.^[93] who mixed oppositely charged chitosan-coated PLGA and hydroxyapatite nanoparticles to synthesize a colloidal composite gel. The resulting composite gel showed, however, mechanical properties comparable to the previously reported PLGA-based colloidal gels.^[81a]

It is recognized that the assembly process, composition, and microstructure of colloidal gel networks strongly affect their properties.^[80] Therefore, these factors were recently controlled to enable the formation of composites which combined mechanical robustness with self-healing behavior.^[94] By controlling the self-assembly process, gelatin/silica ratio, and total particle volume fraction, composite colloidal gels were synthesized which were highly elastic and self-healing. These colloidal gels could withstand substantial tensile and compressive loads and self-healed completely after both shear-induced and cutting-induced failure. The shear-induced structural change of colloidal gels determined the degree of their self-healing. These structural changes improved the elasticity of the gels during several cycles of destruction and recovery, resulting in self-healing efficiencies of more than 100%. In a follow-up study,^[95] colloidal composites were assembled from bioactive glass and gelatin particles to facilitate regeneration of osteoporotic bone defects. To this end, bioactive silicate glass particles (45S5) were used as inorganic building blocks, and gelatin nanoparticles were functionalized with bisphosphonate groups to (i) enhance their affinity toward the bioactive glass particles and (ii) induce antiresorptive medicinal effects. By optimization of the ratio between bioactive glass and gelatin, self-healing composite gels were produced that could be injected into femoral condyle defects of osteoporotic rats, resulting in remarkable bone regeneration inside the defects and enhancement of bone density around the defects within eight weeks of implantation.

An alternative strategy for the production of composite colloidal gels is the use of composite particles as the building blocks. In a recent study, Hou et al.^[96] prepared oppositely charged hydroxyapatite- and dexamethasone-loaded PU microspheres which self-assembled as colloidal gels. The resulting hydrogels were injectable, highly elastic (G' up to 200 kPa), and could recover around 70% of their initial storage modulus after shear-induced network destruction (1000% strain for 1 min). Both in vitro and in vivo evaluations of these hydrogels

suggested their high potential for application in bone regeneration through minimally invasive methods.

Other types of building blocks which have been used for the preparation of self-healing colloidal gels include a.o. graphene nanosheets and cellulose nanocrystals and silicate nanoplatelets. Chen et al.^[97] developed a graphene-based double network hydrogel by hydrothermal reduction of suspensions of graphene oxide nanosheets to synthesize a graphene-based network followed by polymerization of *N,N*-dimethylacrylamide within the graphene hydrogel to establish a second network. While the first network contained β -CD groups that enabled loading and controlled release of anticancer drugs, the second network endowed the hydrogels with self-healing ability after cutting. In another study, Khabibullin et al.^[98] developed a self-healing colloidal gel by combination of cellulose nanocrystals and graphene quantum dots as injectable fluorescent hydrogel for 3D printing applications. Interestingly, although cellulose nanocrystals and graphene quantum dots were both negatively charged, the authors reported that hydrophobic interactions and hydrogen bonding could overcome the interparticle electrostatic repulsions and yield the formation of hydrogels. Nevertheless, the self-healing of this gel was evaluated after applying destructive-shearing up to 50% strain only.

Generally, it can be concluded that self-healing colloidal gels exhibit a strong potential for the biomedical field, especially considering the fact that only a limited number of colloidal building blocks have been used so far for biomedical applications.

Polymer-Particle Gels: Reversible interactions between polymer chains and particles have been exploited to produce self-healing biomedical hydrogels. In these systems, polymer chains bridge the surfaces of particles leading to bridging flocculation of particles and formation of percolated hydrogel networks. Important parameters which determine the properties of these hydrogels are the binding affinity between particles and polymer chains, the size of particles, the persistence length of polymers, the polymer concentration, and the crosslinking density of the gel network, i.e., the number of particles per gel volume. So far, hydrophobic,^[99] electrostatic,^[100] coordination interactions^[101] as well as molecular recognition motifs^[102] have been exploited to prepare this class of self-healing biomaterials.

Recent studies have shown that bisphosphonate functionalization of hyaluronic acid renders this polymer highly adhesive to calcium phosphate,^[101b] calcium silicates,^[101a] and magnesium silicate^[101c] particles. Consequently, by exploiting these strong yet reversible coordination bonds between polymers and particles, injectable and self-healing composite hydrogels have been synthesized which display promising results with respect to bone regeneration^[101a,b] and drug delivery^[101c] applications. In general, bisphosphonates exhibit a strong binding affinity for the mineral inorganic phase of bone, i.e., hydroxyapatite.^[46,103] In a recent study, we found that bisphosphonate-functionalized hyaluronic acid exhibits significantly stronger binding affinity for a commonly used calcium silicate phase (45S5 bioactive glass) than for hydroxyapatite, leading to the formation of more elastic and self-healing composite hydrogels. In addition, the formation of hydrogels by combination of this polymer with magnesium silicate nanoparticles^[101c] suggests that this strategy can be extended to a wide range of

divalent cation-containing inorganic phases including glasses doped with therapeutic ions^[104] to produce self-healing biomedical hydrogels. Moreover, other types of coordination-type interactions can be used to develop these self-healing hydrogel systems. For instance, Holten-Andersen et al.^[105] recently developed a self-healing hydrogel by exploiting coordination bonds between a catechol-terminated PEG and iron oxide nanoparticles. In an effort to improve the mechanical properties and in vivo cohesion of this class of hydrogels for bone regeneration, Shi et al.^[101d] recently developed a dual crosslinked hydrogel by functionalizing hyaluronic acid with both bisphosphonate and acrylamide groups. Injectable and self-healing hydrogels could be formed by mixing the functionalized polymer with calcium phosphate-coated silk microfibers. The results of this study revealed that the mechanical properties and cohesion of the hydrogels could be significantly improved by photo-polymerization of acrylamide groups that were linked to the backbone of hyaluronic acid macromolecules.

Hydrophobic interactions between particles and polymers have also been utilized to form self-healing hydrogels. Rao et al.^[99b] developed a self-healing hydrogel using cholesterol-end capped PEG (Chol-PEG-Chol) polymer chains and liposome particles. In this study, an interconnected gel network was self-assembled by embedding cholesterol groups of Chol-PEG-Chol into the bilayer of liposomes through hydrophobic interactions, leading to bridging flocculation of liposome particles. In another study, Appel et al.^[99a] utilized hydrophobic forces between hydrophobically modified cellulose derivatives and poly(ethylene glycol)-block-poly(l-lactic acid) (PEG-*b*-PLA) nanoparticles to produce self-healing hydrogels for drug delivery applications. The self-healing feature of these hydrogels enabled their minimally invasive implantation in vivo via direct injection through a needle for the differential release of multiple drugs in vivo. In another study, Dennis et al.^[106] developed a composite gel by mixing hydroxyapatite nanoparticles and glycosaminoglycans (chondroitin sulfate or hyaluronic acid). The obtained results suggested that particle-polymer bridging played a substantial role in the formation of the self-healing gel. However, both polymer chains and nanoparticles were highly negatively charged and the nature of these particle-polymer interactions was not identified.

Electrostatic interactions between oppositely charged nanoparticles and polymer chains have also been exploited for the synthesis of self-healing hydrogels. Roux et al.^[13] reported that a hydrogel could be synthesized by mixing a dispersion of negatively charged PLA nanoparticles and a solution of positively charged chitosan. The resulting hydrogel was highly elastic (elastic moduli of ≈ 300 kPa), injectable, and recovered its elasticity after shear-induced network destruction. Nevertheless, the gel network was very brittle (yield strain $\approx 3\%$) and consequently only a 50% oscillatory strain was applied to evaluate the self-healing.

Another possibility for preparation of this class of hydrogels is to use intermediate molecules that can act as a linkage between the particles and polymeric macromolecules. Appel et al.^[100] used a positively charged surfactant, i.e., cetyltrimethylammonium bromide, to establish a self-healing gel network between negatively charged polymers (hyaluronic acid or carboxymethylcellulose) and hydrophobic PEG-*b*-PLA

nanoparticles. Rheological tests showed that the hyaluronic acid-based gels exhibited faster self-healing as compared to the gels made using carboxymethylcellulose, which was attributed to the greater flexibility of hyaluronic acid chains. Moreover, the amount of surfactant directly affected the rheological properties and the recovery kinetics of hydrogels when the polymer with a higher negative charge density was used (i.e., carboxymethylcellulose). Accordingly, the strategy used in this study can be applied to a wide range of polymer-particle systems to develop self-healing biomedical hydrogels using appropriate intermediate molecules (e.g., surfactants). Yet, special attention should be paid to the selection of surfactants in term of their cytocompatibility.^[107] For example, Parisi-Amon et al.^[102] recently developed a self-healing hydrogel system using a hydroxyapatite-binding peptide to establish a gel network between hydroxyapatite nanoparticles and recombinant proteins, which could be successfully used to deliver adipose derived stem cells in vivo for bone regeneration.

Finally, in a recent study, Peak et al.^[108] used silicate nanoparticles (Laponite) in combination with PEG to produce self-healing colloidal bioinks. Nevertheless, suspensions of Laponite nanoparticles alone are able to form a weak gel with a “house-of-cards” structure based on electrostatic interaction between positively charged edges and negatively charged faces of the Laponite platelets.^[109]

Hydrogel-Particle Mixtures: In addition to their use as colloidal building blocks or crosslinkers, nanoparticles can also be incorporated into polymeric hydrogels for mechanical reinforcement or other types of functionalization without affecting the self-healing behavior of the polymeric hydrogel.^[110] This approach has been employed by Gantar et al.^[111] to produce injectable and self-healing gels for bone regeneration. This study showed that clusters of bioactive glass nanoparticles could effectively reinforce a dynamic hydrogel matrix composed of gold-containing 4-arm thiol-terminated PEG, resulting in the formation of composite gels that were bioactive (apatite-forming) and self-healing. In another study, Zeng et al.^[112] prepared an in situ forming self-healing hydrogel by incorporating bioactive glass particles into an ELP matrix. In this system, the in situ release of alkaline ions from bioactive glass particles locally raised the pH of solution which triggered a Schiff base reaction leading to the formation of cross-links between aldehyde- and amine-functionalized polymer chains. Lin et al.^[113] incorporated micelle-like amphiphilic chitosan-based nanoparticles as carrier for a model hydrophobic drug (retinoic acid) into an alginate hydrogel matrix. The resulting composite gel was injectable, self-healing, cytocompatible, and enabled local delivery of the hydrophobic drug. The strategy used in this study can be used to locally deliver insoluble or sensitive drugs and biomolecules using various self-healing hydrogel systems.

4.2. Nonhydrated Biomaterials

Nonhydrated biomaterial systems include organic, inorganic, or composite biomaterials which, in contrast to the above-discussed hydrogels, do not contain large amounts of water. In particular, this section focuses on nonhydrated organic biomaterials which have been developed based on dynamic

bonds and possess an intrinsic ability to self-heal at low temperatures. Although self-healing has been reported in inorganic (hydroxyapatite) coatings,^[114] the self-healing concept in these systems is nonautonomous and requires hydrothermal treatment at high temperatures, which restricts their applicability as an implantable biomaterial. Consequently, these systems will not be discussed further in this review.

4.2.1. Films and Membranes

During the past decade, the development of polymeric biomaterials with self-healing capability based on reversible covalent or noncovalent bonds has gained considerable interest. The self-healing capability of these materials is usually assessed by processing them into films and evaluating their ability to self-heal after cutting-induced failure. Besides their application as films, these self-healing polymeric systems can often also be used to produce 3D constructs such as scaffolds for tissue engineering. Elastomers such as PU and poly(urethane urea) (PUU) are particularly interesting materials for various biomedical purposes.^[115]

Xia et al.^[116] synthesized a hyperbranched PU containing a high amount of catechol-B³⁺ crosslinks and a low amount of hydrophilic quaternary ammonium salts, which self-healed underwater after cutting. In this polymer, the quaternary ammonium salts enhanced the hydrophilicity and facilitated the reformation of catechol-B³⁺ bonds by promoting the mobility of dangling polymer chains at fractured surfaces. Moreover, the quaternary ammonium cations enhanced the stability of boronic esters in water, allowing for self-healing of this polymer at a wider pH range (7–9). In another study, Liu et al.^[117] developed self-healing and biodegradable PUU elastomers by exploiting reversible hydrogen bonds between urea groups. These biodegradable PUUs were synthesized from poly(ϵ -caprolactone) (PCL)-based soft and L-lysine-based hard segments, and were solvent-cast into films which self-healed when their cut sections were kept in contact at 37 °C for 30 min. By increasing the amount of hard segments in this co-polymer, the density of hydrogen bonds increased, thereby improving the self-healing capacity of these films. Ying and co-workers^[118] synthesized polyureas and PUUs that could self-heal at 37 °C by attaching bulky side groups to the nitrogen in urea groups resulting in the formation of hindered urea bonds. The simplicity and catalyst-free nature of this strategy can potentially enable development of polymeric biomaterials with self-healing capability and adjustable mechanical properties. Recently, Aida and co-workers reported^[119] that poly(ether-thioureas) can form mechanically robust amorphous structures that self-heal upon compression of their fractured surfaces. The self-healing in this system was enabled by (i) thiourea moieties that formed nonlinear arrays due to hydrogen bonding, and (ii) a structural element (triethylene glycol spacer) that facilitated the reformation of hydrogen-bonded thiourea pairs. This healing mechanism allowed self-repair at room and body temperature, which might enable future application of this material in the biomedical field.

Several studies^[120] have been carried out focusing on the development of polymeric biomaterials using UPy-functionalized

polymers such as PCL and PEG. This strategy not only allows modular assembly of supramolecular biomaterials,^[120a,121] but can also produce biomaterials with self-healing capability^[121,122] owing to the reversibility of UPy interactions. Van Gemert et al.^[122b] developed several self-healing polymers by UPy-functionalization of different biocompatible polymers. This study showed that self-healing of solvent-cast films only occurred at high temperatures (150 °C) when short polymer chains were end-functionalized with UPy moieties. In chain-extended polymers containing UPy in the polymer backbone, however, self-healing of cut strips occurred at room temperature resulting in 80% self-healing efficiency after 2 h and near complete self-healing after 16 h. Wu and co-workers^[122a] developed self-healing supramolecular bioelastomers that were biodegradable and biocompatible by grafting UPy moieties to the backbone of poly(glycerol sebacate). The solvent-cast films were able to self-heal at 37 °C after cutting-induced failure, while the self-healing efficiency of the films was enhanced at an elevated temperature of 75 °C by assisting reformation of UPy–UPy bonds. Interestingly, the self-healing capability of these biomaterials could be utilized to develop a modular coculture system by establishing contact (at 37 °C for 1 min) between films that were cultured with different cell types.

Polymeric biomaterial films which self-heal based on electrostatic forces have also been developed. Daemi et al.^[123] developed biocompatible and biodegradable alginate-based polyurethane elastomers with self-healing capability for applications in tissue engineering. An isocyanate (NCO)-endcapped PCL-urethane polymer was reinforced through crosslinking with alginate, using NCO-hydroxyl chemistry. The cationic element *N*-methyldiethanolamine, incorporated in the PCL-urethane, interacted with the anionic carboxylic acid groups on the alginate, improving the mechanical properties of the material. Self-healing, attributed solely to ionic interactions, was assessed by measuring the mechanical properties after cutting solvent-cast films in half and subsequently re-uniting the two halves for 30 seconds. A healing efficiency of 70% was reported after three healing cycles, whereas the healing efficiency dropped to 47% after 5 cycles and 19% after 8 cycles.

Interestingly, PLGA films were shown to self-heal pores created by microneedles.^[124] The mechanism of self-healing in these films is based on viscous flow of PLGA above its glass-transition temperature ($T_g = 38$ °C at dry state), which is driven by the stress field originating from the surface tension and the curvature of the pores.^[125] The self-healing process in these materials, which is interesting for encapsulation of drugs, was accelerated by rendering them more hydrophobic due to enhancement of the surface tension-based driving force. Moreover, hydrated films could self-heal at lower temperatures as compared to dry films, owing to the decrease of T_g of PLGA upon hydration.^[124]

Finally, the properties and functionalities of self-healing polymeric materials can be altered by incorporation of functional particles. Tee et al.^[126] incorporated electroconductive nanostructured nickel microparticles into a supramolecular polymeric hydrogen-bonding matrix to develop a self-healing electronic skin. In this system, the supramolecular polymer matrix provided self-healing, while the nickel microparticles rendered these materials electroconductive as well as flexion- and pressure-sensitive. Consequently, upon repeated rupture,

the composite films could recover up to $\approx 90\%$ of their initial electroconductivity within 15 s, and their mechanical properties recovered completely after ≈ 10 min at ambient conditions.

4.2.2. Coatings

Several polymeric coatings have been developed with self-healing capability which allow recovery from surface damage. In the biomedical field, such self-healing coatings have been used for corrosion protection,^[127] drug delivery,^[128] and antifouling^[129] applications. The most common strategy for the production of biomedical self-healing coatings is the layer-by-layer (LbL) approach. This approach is based on self-assembly of alternating layers of polymers that are usually oppositely charged.^[10c] Generally, self-healing in polymeric coatings can be more challenging than in free-standing films or hydrogels since the binding of the coating with the underlying substrate can limit the healing process after damage.^[129]

Jia et al.^[127] developed self-healing coatings to control the degradation behavior of highly degradable magnesium-based alloys. Initially, a micro/nanoporous layer was developed on the surface of a magnesium alloy, acting as an adhesive bridge between the alloy and the subsequent polymeric coating. Thereafter, a LbL technique was used to coat the substrate with chitosan multilayers containing trivalent cerium nanoparticles which could form complexation interactions with the chitosan matrix through Ce-NH₂ coordination. The self-healing mechanism in this system was triggered by the local increase of

pH upon enhanced degradation of the underlying Mg alloy when the coating was damaged. As shown in **Figure 7a**, this pH change resulted in the swelling of the chitosan matrix which in turn assisted the flexible chitosan chains to close the crack and neutralize the pH. At the same time, Ce³⁺ ions precipitated on the damaged surface forming highly insoluble Ce₂O₃ deposits which could inhibit the corrosion process.

With respect to the application of self-healing coatings for drug encapsulation, Chen et al.^[128,130] developed porous poly(ethylenimine)/poly(acrylic acid) multilayer coatings through a LbL strategy followed by acid treatment and freeze-drying. The pores within these polyelectrolyte multilayer coatings were loaded with various drug types which could be encapsulated through self-healing closure of the pores upon exposure to high humidity. This strategy is particularly interesting for the encapsulation and local delivery of hydrophobic drugs.

Several studies^[129,131,132] have focused on the development of self-healing antifouling coatings that can eliminate undesired attachment of biomolecules and cells on their surface. The self-healing capability in these coatings provides an additional tool to maintain the antifouling properties in demanding physiological conditions. Sun and co-workers^[129] used a LbL approach to fabricate antifouling coatings consisting of poly(ethylene imine)-poly(ethylene glycol) copolymer and hyaluronic acid based on electrostatic interactions between the two components. Owing to the dynamic electrostatic and hydrogen bonds, self-healing in these coatings could be established for cuts with a depth and width of several tens of micrometers. Nevertheless, this self-healing process was only effective in the presence

Figure 7. Examples of self-healing mechanisms in coatings made for A) corrosion protection and B) antifouling applications. A) Reproduced with permission.^[127] Copyright 2016, The Royal Society of Chemistry. B) Adapted with permission.^[131] Copyright 2013, John Wiley and Sons.

of water.^[133] In an effort to produce antifouling coatings with longer durability, Kuroki et al.^[131] used a 3D polymer-grafting strategy in which PEG brushes were grafted through the entire thickness of poly(2-vinylpyridine) coatings. In this system, as shown in Figure 7b, new PEG brushes were exposed on the surface recovering the antifouling properties of the coatings upon damage or degradation of the coatings. A similar reorganization of polymer chains was reported for highly protein-repellent fluoropolymer brushes, although self-healing was only achieved at elevated temperatures.^[134] Finally, an interesting self-healing system that can be applied as an antifouling coating is the so-called “slippery liquid-infused porous surface(s)” developed by Aizenberg and co-workers.^[135] Inspired by pitcher plants, these surfaces were prepared by infiltrating a liquid phase of low surface tension (e.g., a perfluorinated oil) into a porous layer (e.g., Teflon). In this system, the low-surface-tension liquid phase endows the surface with the ability to repel fluids such as blood, while the porous layer immobilizes the liquid phase at the material surface. Furthermore, following a damage, the liquid phase can refill the damaged areas of the porous layer based on capillary action, thereby enabling rapid self-healing of the antifouling and slippery features of the surface.

4.2.3. Particles and Capsules

Particles and capsules that can self-heal their surface pores are interesting biomaterials for controlled release of drugs and biomolecules. Nevertheless, only a limited number of studies have been reported on this approach. Koole et al.^[7] prepared radiopaque polymeric capsules based on a radical copolymerization of two methacrylate monomers, which were loaded with concentrated solutions of a cytostatic drug. The drug-releasing capsules could be refilled with drug solutions through injection using a sharp syringe needle while being implanted in vivo. After injection, the punctured holes could self-heal themselves, thereby preventing an excessively fast drug release. PLGA microparticles have also been shown to be able to self-heal their pores at physiological temperature.^[125,136] Reinhold et al.^[136a,b] exploited self-healing of pores in PLGA microparticles to encapsulate sensitive biomolecules for controlled release applications. Porous PLGA microparticles with pores ranging from 30 to 3000 nm in diameter were prepared via an emulsion-based method using a leachable sugar. While the pores were open below the glass transition temperature of hydrated PLGA ($T_g \approx 30^\circ\text{C}$), they could self-heal once the temperature was raised to above 30°C . Consequently, this principle could be utilized for the encapsulation of biomolecules with sizes up to 2 MDa, thereby avoiding the use of organic solvents or processing conditions that can reduce the biological activity of biomolecules. A similar principle was exploited by Bailey et al.^[136c] for the encapsulation of protein antigens. The antigens encapsulated using this method were highly effective for immunization of mice and therefore this strategy can be a promising method for the development of various vaccination platforms. The mechanism of pore healing in PLGA microparticles is conceptually similar to the self-healing mechanism in PLGA films which was studied and modeled by Mazzara et al.^[124] and Huang et al.,^[125] respectively, as described in the previous section.

Finally, vault particles are cytoplasmic organelles that are found in various kinds of eukaryotic cells. Although their cellular function is not clear yet, these protein-based particles are considered interesting nanovehicles for drug delivery applications.^[137] Llauro et al.^[138] recently found that vault particles display a remarkable self-healing ability upon fracture. The authors proposed that this self-repair mechanism, which occurs in aqueous solutions at room temperature, can be attributed to reversible ionic and hydrogen bonds as well as the geometry of the vault particles imposing a direct contact between fractured surfaces. The self-healing features of these nanovehicles might allow penetration of the nuclear pore complexes of cells by adapting to geometric constraints.

5. Perspective

During the past few years, research interest in self-healing biomaterials has increased tremendously and opened up a wide range of potential biomedical applications. Generally, the field of self-healing biomaterials is still in its infancy and practical applications of self-healing biomaterials are still scarce, since the field of self-healing biomaterials research emerged ≈ 10 years later than the initiation of self-healing materials research for nonbiomedical applications. In addition, the physicochemical demands for the application of biomaterials are often more complex than other fields of engineering due to the highly challenging physiological in vivo conditions. Nevertheless, current literature indicates that self-healing can be advantageous for biomaterials in view of their processing, handling, and clinical performance. Regarding their processing, biomedical constructs can be manufactured from self-healing biomaterials using potentially destructive techniques such as injection molding or extrusion-based 3D-printing. Moreover, these biomaterials can self-heal defects created during or after their production. For example, Wang et al.^[139] demonstrated that the self-healing feature of a hyaluronic acid-based hydrogel enabled the fabrication of hydrogel constructs using 3D-printing by allowing the recovery of the mechanical integrity of the hydrogel after extrusion. In addition, the adapting feature of these biomaterials can provide a suitable environment for culturing of cells in vitro, allowing the production of cell-laden constructs. For instance, McKinnon et al.^[140] showed that adaptable hydrogels were suitable scaffolds for in vitro culture of myoblasts, whereas non-adaptable hydrogels constrained the cells and prevented their cytoskeletal extension and rearrangement. With respect to their handling properties, self-healing biomaterials can be applied via minimally invasive treatments or (bio)printing by means of injection through narrow syringes which tend to shear biomaterials destructively. As an example, Appel et al.^[99a] demonstrated that a self-healing hydrogel could be directly implanted in vivo using a minimally invasive injection through a needle. Moreover, this self-healing capability allows surgeons to cut and fuse sections of biomaterials repeatedly without compromising the final mechanical performance of these implants. For instance, cut pieces of self-healing colloidal gels were shown^[94] to establish mechanically stable bonds within few minutes when put into direct contact at room temperature. Finally, the clinical performance of self-healing biomaterials can be superior to

their non-self-healing counterparts due to their improved durability and increased biological suitability for tissue regeneration. A clinically relevant example of such improved durability can be observed in yttria-stabilized zirconia ceramics which are frequently used as crowns and bridges in restorative dentistry. In yttria-stabilized zirconia ceramics, the stress fields around propagating cracks can induce a phase transformation from metastable tetragonal to stable monoclinic phases which is accompanied with expansion and thus closure of crack tips.^[141] Furthermore, the dynamic and adapting nature of self-healing biomaterials can provide a suitable microenvironment for tissue ingrowth and cellular migration, potentially resulting in a stronger tissue-regenerative capability for these novel materials.^[39b,140,142] For instance, self-healing hydrogels have been shown to allow the migration, differentiation and proliferation of cells as well as tissue infiltration into their inner area without losing their integrity.^[95,142] In addition, it has recently been shown by Heilshorn and co-workers that active adaptation of biomaterials can have other biological benefits such as maintenance of stemness of stem cells.^[39a] Nevertheless, more systematic studies using different cell types are required to fully understand the effects of the dynamic, adapting nature of self-healing biomaterials on biological processes such as cell migration and differentiation. Such studies can be performed using both in vitro and in vivo models that monitor the migration, proliferation, and differentiation of cells as function of the self-healing capability of biomaterials.

Generally, self-healing concepts and requirements of biomaterials for biomedical applications can be completely different from self-healing concepts in traditional engineering applications. For instance, novel self-healing biomaterials can be developed by using stem cells as their building blocks, whereas such a concept is not at all applicable for traditional engineering applications. In general, materials often have a passive function in traditional fields of engineering, whereas biomaterials in biomedical engineering are increasingly expected to promote biological processes such as regeneration of damaged tissues. This implies that biomaterial properties may change in the course of tissue regeneration. Consequently, self-healing biomaterials do not necessarily match the mechanical properties of natural tissues (e.g., bone) after implantation. Instead, these self-healing biomaterials should be injectable, bioactive, shape-stable during complex physiological processes, and replaceable by regenerating tissues. To this end, principles from biomimetic mineralization may be implemented into new self-healing biomaterials.^[10a,143] Despite the high potential of biomineralization principles for the development of self-healing biomaterials for, e.g., hard tissue repair,^[144] this area has not been sufficiently explored yet.

The field of self-healing biomaterials urgently needs standard protocols for reliable and comparative quantification of self-healing properties. Nevertheless, these protocols cannot be established without understanding the specific self-healing mechanism of each type of biomaterial as a function of the type of damage. This understanding can only be achieved by combining different experimental techniques such as microscopy and mechanical tests. Such tests should be preferably carried out in situ to allow for real-time evaluation of structure and properties of biomaterials during (repeated) cycles of damage

and recovery. Some of the experimental methods that can allow such in situ measurements are optical micromechanical mapping,^[145] confocal microscopy,^[94] scanning electrochemical microscopy,^[66] Raman spectroscopic imaging^[146] as well as small-angle X-ray,^[147] small-angle neutron,^[147] and wide-angle X-ray^[148] scattering techniques.

Concluding, it is anticipated that standard protocols, together with improved understanding of self-healing mechanisms and their role in biological processes, will facilitate (pre)clinical evaluation of self-healing biomaterials. These translational developments will advance the field of self-healing biomaterials research, thereby creating new opportunities for commercialization of self-healing biomaterials with a clear added value compared to conventional biomaterials.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

biomaterials, extrinsic, intrinsic, self-healing, self-repair

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