FEATURE ARTICLE

Magnetic Hydrogels and Their Potential Biomedical Applications

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Hydrogels find widespread applications in biomedical engineering due to their hydrated environment and tunable properties (e.g., mechanical, chemical, biocompatible) similar to the native extracellular matrix (ECM). However, challenges still exist regarding utilizing hydrogels in applications such as engineering 3D tissue constructs and active targeting in drug delivery, due to the lack of controllability, actuation, and quick-response properties. Recently, magnetic hydrogels have emerged as a novel biocomposite for their active response properties and extended applications. In this review, the state-of-the-art methods for magnetic hydrogel preparation are presented and their advantages and drawbacks in applications are discussed. The applications of magnetic hydrogels in biomedical engineering are also reviewed, including tissue engineering, drug delivery and release, enzyme immobilization, cancer therapy, and soft actuators. Concluding remarks and perspectives for the future development of magnetic hydrogels are addressed.

1. Introduction

Hydrogels have emerged as 3D, water-swollen, soft polymer materials with tunable properties (e.g., mechanical, chemical, biocompatible),[1] and, as a kind of smart biomaterial, find widespread applications in biomedical engineering, such as in tissue engineering and cell/drug delivery.[2–4] Various biomimetic hydrogels have been employed to mimic the native hydrated microenvironment and to engineer thin or avascular tissues such as skins, cartilages, and bladders.[5–7] In addition, hydrogels with pores and/or microchannels have also been developed for delivering drugs and cells, and for providing a 3D cell microenvironment that preserves tissue volume and supports cell interactions, as well as delivers biological agents to cells.[8–11] Despite the superior performances of hydrogels, there are still several limitations in current hydrogel-based systems, mainly due to their poor controllability, actuation, and response properties.[12,13] For example, due to the lack of microarchitecture control in hydrogels, it is challenging for conventional hydrogel-based tissue-engineering systems to provide 3D tissue constructs with complex architectures and hierarchical vascular networks to mimic the native tissue microenvironment.[14,15] In addition, for drug and cell delivery, most biological agents and drugs are released from porous hydrogels through passive mechanisms including molecular diffusion, hydrogel degradation and cell migration. However, the controlled delivery of agents and drugs is highly demanded to improve the efficiency and safety of the agents and drugs.[8] Furthermore, the passive diffusion process may induce hydrogel deformation, leading to reduced hydrogel volume and pore size.[16] Therefore, hydrogels with improved controllability, actuation and response properties are urgently needed.

Recently, significant advances have been achieved in the development of magnetic hydrogels (i.e., the combination of hydrogels with micro- and/or nanomagnetic particles (e.g.,

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γ-Fe_2O_3, Fe_3O_4, $CoFe_2O_4$)) that can quickly respond to an external magnetic field (MF), enabling their enhanced controllability.[17–23] Here, we focus on hydrogels containing magnetic nanoparticles (MNPs) that have been demonstrated to be more suitable than those with microsized particles for biomedical applications, due to their super-paramagnetic and responsive properties.[24–26] For example, alginate hydrogels have been embedded with MNPs (iron oxide) to control drug and cell release both in vitro and in vivo by inducing large deformation and volume changes (over 70%) using an external MF.[27] Microscale hydrogels (i.e., microgels) encapsulated with MNPs and cells have also been developed as building blocks, and assembled to fabricate 3D multilayer cellular constructs.[28] In addition, hydrogels with super-paramagnetic iron oxide nanocrystals have been used to raise the temperature of different drug-target systems by magnetic coupling between the magnetic moment of the nanoparticles (NPs) and the alternating MF, which may be used for cancer hyperthermia treatments.[29–31] Thus, the development of magnetic hydrogels holds great potential applications in tissue engineering and cell/drug delivery.

In this review, we present state-of-the-art methods for preparing magnetic hydrogels and examples of their use in biomedical applications. In section 2, various methods for preparing magnetic hydrogels are discussed. In section 3, current applications of magnetic hydrogels in tissue engineering, drug delivery, and immobilization of enzymes, as well as in cancer therapy and as soft actuators are presented. Finally, concluding remarks and future perspectives for the development of magnetic hydrogels are given.

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2. Methods for the Preparation of Magnetic Hydrogels

Magnetic hydrogels generally consist of a polymer matrix and a magnetic component embedded in the matrix. The properties of magnetic hydrogels (e.g., magnetic response) rely on several factors, including the type of hydrogel and MNP used, the hydrogel and MNP concentration, and the size and distribution of the MNPs within the hydrogels.[32] Various methods have been developed to fabricate magnetic hydrogels, including a blending method,[33] an in situ precipitation method[34] and the grafting-onto method,[35] as summarized in **Figure 1** and **Table 1**.

2.1. The Blending Method

In the blending method, MNPs and hydrogels are prepared separately in a sequential order (Figure 1A). MNPs (e.g., Fe_3O_4) are mostly prepared using a conventional coprecipitation method. The prepared MNP sediment is then dispersed in an aqueous or oil phase (i.e., a ferrofluid) to avoid oxidization and aggregation. Finally, the mixture of the ferrofluid and the hydrogel precursor solution are crosslinked, resulting in encapsulation of the MNPs in the hydrogel. Tong and co-workers[36] fabricated poly(*N*-isopropylarcylamide) (PNIPAAm)/Fe_3O_4 magnetic hydrogels using the blending method. The prepared ferrofluid was added to *N*-isopropylacrylamide (NIPAAm) solution and hydrogels were formed by a suspension-polymerization method, which were readily moved and collected through an external MF. Jonas and co-workers[37] prepared magnetic dextran hydrogels by mixing different types of Fe_xO_y NPs (e.g., Fe_3O_4, γ-Fe_2O_3, and both Fe_3O_4 and γ-Fe_2O_3) and dextran solution followed by photo-crosslinking of the hydrogels. Magnetic alginate hydrogels for triggered drug release were prepared by entrapped magnetic fluid (maghemite NPs with a spinel structure) synthesis by Massart's procedure in transparent alginate microbeads with the size of 50 μm.[18] It was found that the magnetic microbeads were easily aligned when applying an external MF.

With the blending method, MNPs with uniform particle size in hydrogels over a wide range from nanometers to micrometers can be achieved by adjusting the concentration of the reactants, the stirring speed, and the preparation cycle. Moreover, the method is easy to process since MNP preparation and MNP encapsulation are performed separately. However, it is challenging to achieve a uniform MNP distribution within the hydrogels, and MNPs may diffuse out of the magnetic hydrogels when immersed in a liquid solution.

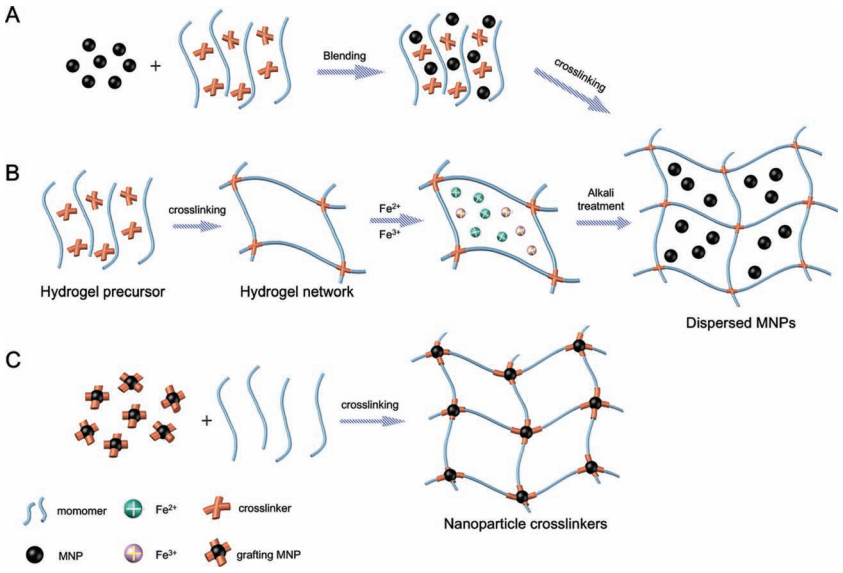

Figure 1. Schematic of preparation methods of magnetic hydrogels. A) The blending method: the prepared MNPs mix with a hydrogel precursor solution at a certain molar ratio and crosslink hydrogels to entrap the MNPs. B) The in situ precipitation method: MNPs are fabricated via an in situ precipitation reaction in the network of the polymer hydrogels after the crosslinking reaction. C) The grafting-onto method: grafting several functional groups onto the surface of the MNPs as nano-crosslinkers, leading to the crosslinking reaction.

2.2. In Situ Precipitation Method

In the in situ precipitation method, the hydrogel networks work as a chemical reactor, within which an inorganic salt with iron ions swollen in hydrogels react with precipitating agents (e.g., NaOH, $NH_3 \cdot H_2O$)[38,39] to generate MNPs. A typical procedure for the in situ precipitation method is shown in Figure 1B. Firstly, hydrogels are fabricated via temperature change, radical polymerization, or a photo-crosslinking reaction. Secondly, the hydrogels are placed into a concentrated aqueous solution containing Fe^{2+} and Fe^{3+}, and the ferrous ions are taken at a molar ratio of 1:2 until swelling equilibrium is reached. Finally, the swollen hydrogels with absorbed Fe^{2+} and Fe^{3+} are immersed into an alkali solution for MNP precipitation. MNPs are fabricated into the hydrogel network according to the following reaction:

$$Fe^{2+} + 2Fe^{3+} + 8OH^- \rightarrow Fe_3O_4(\downarrow) + 4H_2O \quad (1)$$

The yield of this reaction in the hydrogel phase depends on the concentration of the iron salts and of the alkali, and on the properties of the swollen hydrogel networks.[40] Nagireddy et al.[41] prepared magnetic hydrogels by in situ precipitation of MNPs in polyacrylamide-gum acacia (PAAm-GA) hydrogels, with uniform distributed MNPs in hydrogels. Hernandez and Mijangos[42] fabricated semi-interpenetrating alginate and PNIPAAm hydrogels with Fe_3O_4 NPs, prepared through in situ precipitation, showing increased porosity with the introduction of iron oxide NPs. The increased porosity enhanced the hydrophobic interactions between the isopropyl groups and improved the deswelling rate of the hydrogel network compared with pure PNIPAAm. Based on the chelation capacity

Table 1. Different methods for preparing magnetic hydrogels.

Method	Hydrogels	MNPs	MNP concentration [wt%]/Distribution	Capability for drug and cell encapsulation	Stability	Reference
In situ precipitation	Chitosan	Fe_3O_4	≈6.0–15.0%/	Low due to alkali processing	-	[43–46]
	Alginate/PNIPAAm	γ-Fe_2O_3	dispersed uniformly			[42]
	PAAm-GA					[41]
	PAAm					[118]
Blending method	Fibrin	Fe_3O_4/γ-Fe_2O_3	all sizes of NPs/easy to aggregate	Easy to achieve through the hydrogel encapsulation or MNP bonding	Low due to escape of MNPs from the hydrogel network	[59,119]
	Dextran	$CoFe_2O_4$				[37]
	Alginate	FePt				[63,120]
	PNIPAAm	CoPt				[36]
	PEG/GelMA					[28,71]
	PVA					[82,111]
Grafting onto method	NIPAAm	Fe_3O_4/γ-Fe_2O_3	≈0.75–12.5%/well dispersed	High	High due to covalent coupling	[121]
	PAAm	$CoFe_2O_4$				[49]
	CMC					[20]

PNIPAAm = poly(*N*-isopropylarcylamide); NIPAAm = *N*-isopropylarcylamide; PAAm-GA = polyacrylamide-gum acacia; PAAm = polyacrylamide; PEG = poly(ethylene glycol); GelMA = gelatin methacrylate; PVA = poly(vinyl alcohol); CMC = carboxymethylcellulose.

of the amino group in chitosan, Wang and co-workers[43,44] proposed a chitosan/iron ions complex (CS/Fe(II,III)) as a precursor of magnetite NPs to synthesize magnetic chitosan hydrogels. Ferric and ferrous ions were chelated by the amino groups of chitosan to form the CS–Fe(II, III) precursor. When the precursor encountered hydroxide ions, the chelation of the amino groups with iron ions provides a nucleation site for the magnetite crystals. The chelation effect limits the diffusion of iron ions and avoids the abnormal crystal growth of magnetite. Therefore, magnetite NPs with a size of about 16 nm were uniformly dispersed in the chitosan hydrogel through chelating with the –OH and $–NH_2$ groups of chitosan. Whilst the methods as discussed above are stepwise in hydrogel formation and MNP synthesis, Li et al.[45] reported an in situ hybridization method to prepare magnetic chitosan hydrogels, in which hydrogel gelation process and MNPs synthesis were simultaneous.

The in situ precipitation method has several advantages in the fabrication of magnetic hydrogels. Firstly, a large number of inorganic particles can be introduced into the hydrogel network, ensuring that colloidal-sized particles can be well dispersed in the matrix. Secondly, the preparation process is simple and low-cost. However, the in situ precipitation method is only suitable for specific hydrogels with a stable network, otherwise the hydrogel network may be destroyed by alkali solution during the preparation process.[46] In addition, the yield of Fe_3O_4 MNPs may be low in hydrogels that have negatively charged functional ligands (like $–COO^-$), since these ligands can react with iron Fe^{2+} and Fe^{3+} in salt solution to form complex compounds.[47] Moreover, the use of an alkali solution may limit the applications of magnetic hydrogels for cell encapsulation.

2.3. The Grafting-Onto Method

For both in situ precipitation and the blending method, there are no covalent bonds between the MNPs and the hydrogel networks. Thus, the stability of MNPs dispersed within the hydrogels cannot be guaranteed.[48] With the grafting-onto method, covalent bonds are formed between hydrogel network and the MNPs by grafting several functional groups onto the surface of the MNPs, which work as nano-crosslinkers to form a covalent coupling with the monomers when polymerized, Figure 1C. Atrei and co-workers[20] prepared magnetic hybrid hydrogels with functionalized $CoFe_2O_4$ MNPs covalently bonded to a carboxymethylcellulose (CMC) polymer. The $CoFe_2O_4$ MNPs were modified with an aminopropyl silane to introduce amino groups onto the surface of metal oxide NPs as nano-crosslinkers, which were also bound to the carboxylic groups of the CMC polymer via amide bonds. Schmidt and co-workers[49] reported the incorporation of magnetic $CoFe_2O_4$ NPs onto polyacylamide (PAAm) hydrogel networks. The $CoFe_2O_4$ NPs were surface-functionalized with siloxane-based unsaturated methacrylic groups, which were capable of forming a covalent coupling with the PAAm-based hydrogel matrix during the polymerization process. A significant feature of the grafting-onto method is that the direct covalent coupling between the hydrogel matrix and the MNPs enables the MNPs to be entrapped in the hydrogel without seeping out (Table 1). Nevertheless, the complex fabrication process, long preparation cycle, and high cost associated with the method limit its broad applications.

The properties of magnetic hydrogels, including their biocompatibility, biodegradability, magnetic response, and mechanical properties, depend on both the constituent hydrogel network and the magnetic component (embedded MNPs). Both natural and synthetic polymers can be employed to fabricate magnetic hydrogels. Natural polymers, including proteins (e.g., collagen, gelatin, and fibrin) and polysaccharides (e.g., chitosan, alginate, and dextran) possess important biological functions as scaffolds for cell growth and adhesion (see Table 1). However, natural polymers may not be suitable for fabricating magnetic hydrogels with grafted MNPs due to the lack of active sites for MNPs. Another limitation for magnetic hydrogels made of natural polymers is their weak mechanical properties, which may limit their applications in areas such as tissue engineering. In contrast, magnetic hydrogels fabricated from synthetic polymers (e.g., poly(ethylene glycol) (PEG), poly(vinyl alcohol) (PVA), and PAAm) enable rational control of the chemical compositions, which may benefit the in situ precipitation of MNPs in hydrogels, the grafting of MNPs onto hydrogel networks, and the regulation of biodegradable and mechanical properties.[50] Moreover, magnetic composites (e.g., MNPs) also play a pivotal influence on the magnetic response behavior of magnetic hydrogels. The magnetization of magnetic hydrogels is proportional to the concentration and saturation magnetization of MNPs, which can be described by:[51]

$$M = \phi_m M_s \left(\coth \xi - \frac{1}{\xi} \right) \quad (2)$$

where φ_m is the volume fraction of MNPs in hydrogel, M_s is the saturation magnetization, and ξ is defined as $\xi = mH/k_B T$, with m, H, k_B and T representing the giant magnetic moment of the MNPs, the external MF, the Boltzmann constant, and the temperature, respectively.

3. Magnetic Hydrogels for Biomedical Applications

3.1. Applications in Tissue Engineering

In the past two decades, tissue engineering has succeeded in engineering thin or avascular tissues, such as skin,[52] bladder,[53] and cartilage.[54] However, the fabrication of complex and large functional tissues such as liver, heart, and kidney[55] still faces challenges. One of the main challenges is the need to prevascularize the engineered tissues in vitro to accomplish cell growth at a high density and at metabolic requirements.[56,57] Another challenge is to reload the engineered constructs with biological agents after implantation.[58] Magnetic hydrogels, as building blocks (e.g., microgels), can be assembled to form complex tissue constructs at a controlled manner via a MF. In addition, magnetic hydrogels in vivo can attract and take up cells, growth factors, and other biological agents bound to MNPs. In this section, we will focus on the applications of magnetic hydrogels in tissue engineering as scaffolds and building blocks. Moreover, a brief review of recent 3D tissue culture based on magnetic hydrogels will be also presented.

3.1.1. Magnetic Hydrogels as Scaffolds for Tissue Engineering

As a template for cell growth and tissue formation, a porous scaffold is one of the most important components for tissue engineering. However, whilst a variety of synthetic polymers (e.g., poly(L-lactide) (PLLA), poly(lactic-*co*-glycolic acid) (PLGA)), and natural polymers (e.g., collagen, alginate, agarose) have been used to fabricate the porous scaffolds, several challenges remain, including the limited available cell density and the active controllability of the cell growth. Magnetic-hydrogel-based scaffolds have the potential to address these challenges. Firstly, magnetic scaffolds are able to stabilize and occupy growth factors or other biological agents bound to MNPs through an external MF,[59,60] providing a nutritious environment for initial cell seeding and further cell proliferation. Secondly, magnetic scaffolds can respond to physical cues such as mechanical stimulus through interactions between MNPs and an alternating magnetic field (AMF), which may be employed to control cell biological behavior such as the angiogenesis process in vitro.[61,62]

Margel and co-workers[59,60] fabricated a series of magnetic fibrin hydrogel scaffolds with fluorescent rhodamine γ-Fe_2O_3 NPs conjugated by various growth factors, including basic fibroblast growth factor (bFGF), beta nerve growth factor (β-NGF), and glial-cell-derived neurotrophic factor (GDNF). They demonstrated that the bFGF-conjugated NPs significantly improved the growth of nasal olfactory mucosa (NOM) cells seeded in the magnetic scaffold (**Figure 2**A). This type of magnetic hydrogel may be investigated as a potential nanocomposite implant for tissue engineering. However, controlling the discharge of MNPs with the degradation of the natural material is difficult. Goranov and co-workers[62] developed a magnetic scaffold made of collagen gels (self-assembly of collagen fibers) embedded with nano-hydroxyapatite and MNPs as a bone-graft substitute for bone-tissue engineering. They observed that the new magnetic scaffold had the ability to support cell adhesion and proliferation in vitro. Polyak and co-workers[63] found that aortic endothelial cells in magnetite-impregnated alginate scaffolds (Figure 2B) exhibited elevated metabolic activity and organized into early capillary-like structures when exposed to an AMF for a total of 14 days in vitro culture (Figure 2C). However, despite advances of the above magnetic-hydrogel-based scaffolds, the understanding of how to control cellular organization and vascularization precisely in complex tissue constructs is still at a preliminary stage.

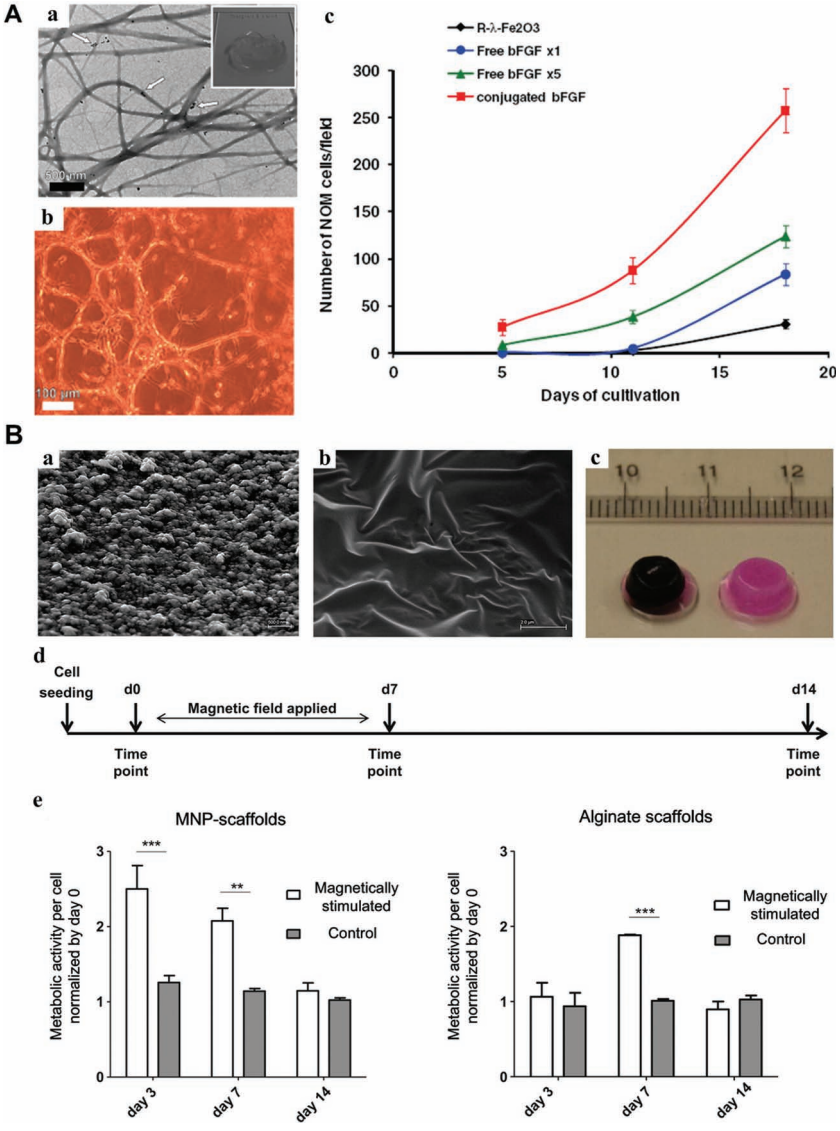

Figure 2. Magnetic hydrogels as biological scaffolds for tissue engineering. A) a) Typical TEM image of magnetic fibrin hydrogel scaffold in which the arrows point to the γ-Fe_3O_4 NPs used for scaffold fabrication (scale bar: 500 nm); b) NOM cells cultivated for 30 days in a magnetic hydrogel scaffold containing bFGF-conjugated NPs (scale bar: 100 μm); c) Quantitative analysis of the grown NOM cells which have migrated from the magnetic scaffolds treated with free factor or non-conjugated or bFGF-conjugated NPs of different concentrations. B) a,b) Scaffold morphology of magnetic alginate hydrogels: 1.2% (w/v) MNP-alginate (a) (scale bar = 500 nm) and non-magnetic alginate scaffolds (b) (scale bar = 2 μm); c) Macroscopic view of different scaffolds hydrated with culture medium for 24 h; d) Experimental time scale for cell culture with magnetic hydrogel scaffolds; e) Metabolic activity per cell in different scaffolds. A) Reproduced.[60] B) Reproduced with permission.[63] Copyright 2012, Elsevier.

3.1.2. 3D Assembly of Magnetic Microscale Hydrogels

The lack of spatiotemporal control for 3D cellular microarchitectures and extracellular matrix (ECM) distribution in large tissue constructs is another challenge for conventional tissue-engineering approaches.[15,28,64,65] Bottom-up methods hold great potential to address this challenge, which involve assembling microscale building blocks (e.g., cell-encapsulating

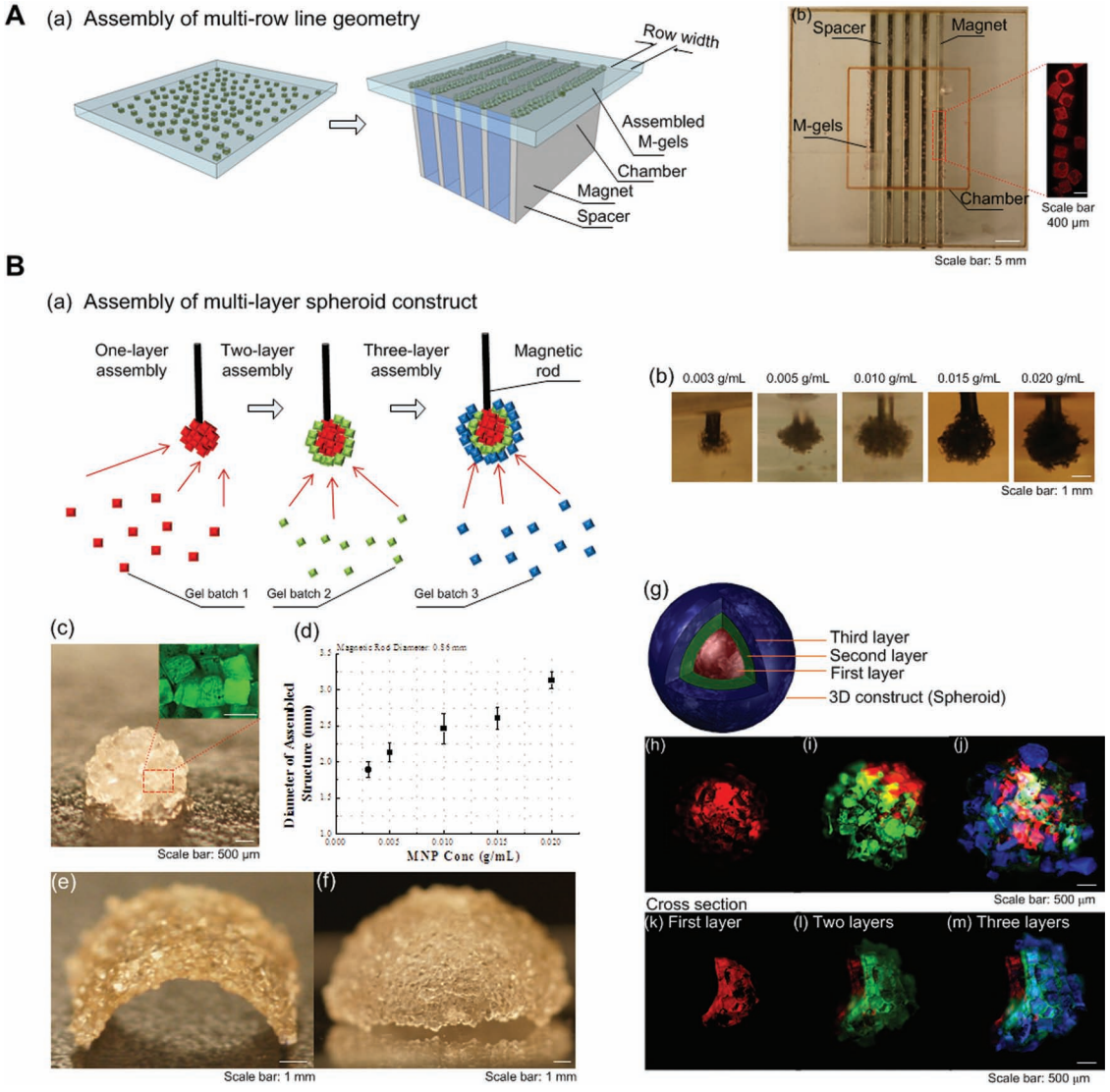

Figure 3. Three-dimensional assembly of magnetic microscale hydrogels by a MF. A) Schematic of magnetic-directed assembly of magnetic microscale hydrogels (M-gels): a) M-gels in a fluidic chamber with magnets are assembled into rows and arrays of constructs; b) Image of the magnetic assembler. B) Multilayer spherical assembly of M-gels: a) M-gels are assembled to fabricate multilayer spherical constructs via an external MF; b) Images of assembled single-layer spheroids using different MNP concentrations; c) Magnified image of the assembled single-layer construct; d) Maximum 3D assembly size as a function of MNP concentration; e,f) Images of arc- and dome-shaped constructs through a flexible surface and magnetic assembly; g–m) Merged fluorescent images of three-layer spheroids. First-layer gels are stained with rhodamine-B (h); Second-layer gels are stained with fluorescein isothiocyanate (FITC)-dextran (i); Third-layer gels are stained with 1,1,4,4-tetraphenyl-1,3-butadiene (TPB) (j); Cross sections of the layers obtained by cutting the assembled construct into two hemispheres (k–m). Reproduced.[28]

microscale hydrogels) into complex tissue constructs with control over the features (e.g., shape and composition) of the individual building blocks.[66,67] Various assembling approaches including those based on microfluidics,[68] nanotextured surfaces,[69] acoustic fields,[65] and surface tension[70] have been investigated.

Recently, we developed a novel assembly approach by utilizing a MF to assemble cell-encapsulating microscale magnetic hydrogels (M-gels) (**Figure 3**).[28] In our approach, M-gels in an assembly chamber were subjected to a MF by placing sheet magnets in parallel. These M-gels assembled into multirow patterns and retained their shape on exposure to an external MF, and remained intact after the MF was removed. 3D multilayer spherical constructs were obtained by assembling M-gels onto the tip of a magnetic rod layer by layer. Other complex 3D constructs were also fabricated, including flexible 3D surfaces such as dome and arc shapes. We later evaluated the release of encapsulated MNPs from photo-crosslinkable gelatin methacrylate (GelMA) hydrogels as the hydrogel network underwent biodegradation using inductively coupled plasma atomic emission spectroscopy.[71] We found that MNP release was linearly correlated with hydrogel biodegradation rate with correlation factors of 0.96 ± 0.03, and 0.99 ± 0.01 for MNP concentration of 1 wt% and 5 wt%, respectively. The effect of the MNPs on the hydrogel mechanical properties, porosity, and swelling behavior, as well

as the viability and growth of encapsulated fibroblasts in M-gels have also been studied.[71]

One of the potential drawbacks of this method is that the MNP distribution in M-gels may differ due to the aggregation of MNPs in prepolymer solutions, which may reduce the affinity of M-gels to MF.[43,72] Another potential problem with this method is that a certain amount of heat generated by MNPs exposure to an alternating current (AC)-based MF (magnetic hyperthermia), which may induce a negative effect on cells.

3.1.3. 3D Tissue Culture Based on Magnetic Hydrogels

Most of the in vitro cell-/tissue-culture systems are based on 2D substrates (e.g., Petri dish, multiwell plates), which are quite different from the 3D microenvironment cells experience in vivo. Several methods, such as protein-based hydrogel systems and rotational/agitation-based bioreactors have been utilized to culture cells in a 3D microenvironment.[12,73,74] Recently, a novel 3D cell-culture system based on magnetic levitation of cells has been developed, using magnetic hydrogels in the presence of filamentous bacteriophages and gold NPs.[75] It was demonstrated that control of the tissue shape and in situ monitor of interaction in a confrontation assay can be achieved (**Figure 4**). As the study of this method is at preliminary stage, more long-term goals for engineering 3D in vivo-like chemical and mechanical microenvironment should be further investigated.

3.2. Applications in Drug Delivery and Enzyme Immobilization

The emergence of pulsatile release systems capable of mimicking the release profile of specific peptides or hormones in the body may lead to optimal drug delivery and achieve the ideal goal of zero-order release of drugs over a prolonged period of time.[76,77] The feasibility of using magnetic hydrogels to control the pulsatile release of drugs remotely has been verified.[78,79] For example, Langer and co-workers[80] demonstrated on-demand controlled insulin release from magnetic ethylene vinylacetate hydrogels by using a low-frequency oscillating MF. De Paoli and co-workers[81] reported the enhancement of dextran release from magnetic collagen hydrogels by applying a low-frequency oscillating MF. Chen and co-workers[82] fabricated an intelligent Fe_3O_4 MNP PVA hydrogel with controlled drug release by fine-tuning the switching duration time. Specifically, in the beginning, the magnetic moments existing in the magnetic hydrogels are non-oriented without a MF applied and the drug-release profile displays a normal diffusion mode due to the zero magnetization of magnetic hydrogels. Upon applying a MF, the particles in the hydrogels aggregate together instantly and produce a bulk magnetic moment, leading to a rapid reduction in the porosity of the hydrogel. In this state, the hydrogel is characterized as a "close" configuration and the rate of drug release is at a lower level. On the contrary, a higher level will be achieved when the MF is instantly switched "off " (**Figure 5**A).

Moreover, thermosensitive hydrogels have also been integrated with MNPs to achieve remotely controlled pulsatile release of drugs, where MNPs are used to induce a change of the hydrogel temperature. In this method, negative-temperature-responsive hydrogels (e.g., NIPAAm-based hydrogels) with a lower critical solution temperature (LCST) between 30 °C and 35 °C, and a high-frequency AMF were both used.[83,84] When the AMF was applied to the negative-temperature-responsive hydrogels, heat was generated by hysteresis of the MNPs in the hydrogel. Thus, AMFs can be used to adjust the temperature of a hydrogel network, driving the swelling transition of hydrogels remotely. When the temperature is increased above the LCST, magnetic hydrogels will collapse, resulting in expulsion of drugs. Satarkar and Hilt.[85] demonstrated that magnetic NIPAAm hydrogels can be used as remotely actuated ON-OFF type modes, where the release of vitamin B_{12} was turned ON using an AMF. Magnetic alginate, which can undergo a large deformation with a volume change of over 70% through a moderate MF, has also been developed (Figure 5B).[27] The controlled release of various drugs, including plasmid DNA, mitoxantrone, and chemokine, were demonstrated. Moreover, the controlled release of mouse mesenchymal stem cells in vivo by various parameters, including peptide density of the modified magnetic hydrogel, the strength and frequency of the external MF, and the number of magnetic cycles, were also observed.[27]

Although magnetic hydrogels can control pulsatile release of drugs remotely via an external MF, it is currently difficult to delivery drugs to regions within deep tissues as the targeting efficiency depends on the distance between the tumor and the magnets. In addition, the cytotoxicity and biodegradability of magnetic hydrogels and the long-term fate of embedded MNPs in vivo also need to be considered. Even though there is no universal criteria to predict this important aspect of magnetic hydrogels due to their different physicochemical properties, two steps should take place during the clearance of the embedded MNPs. Firstly, the release of MNPs embedded in hydrogels can be achieved as the hydrogels biodegrade and the cells secrete their own ECM.[71] Secondly, the elimination of MNPs out of the body is also required. The United States Food and Drug Administration (FDA) has approved the use of MNPs in several clinical applications such as the magnetic resonance imaging (MRI), and these should be rapidly eliminated through the known pathways for Fe metabolism by the reticuloendothelial system (RES), particularly by the liver.[86,87] In addition, the clearance of chemotherapeutical iron oxide NPs for oral drug delivery depends on the particle size. For instance, particles smaller than 5.5 nm can be eliminated rapidly renally,[88] particles up to 500 nm can be cleared by the liver,[89] and particles up to 5 μm in diameter can be removed through lymphatic drainage.[90] However, further studies of the biodistribution and elimination of metallic (e.g., cobalt and nickel) and bimetallic (e.g., platinum) MNPs in vivo are needed for more clinical applications.

Magnetic hydrogels can also be used for immobilization and release of various enzymes (e.g., yeast alcohol dehydrogenase,[91] glucoamylase,[92] and L-asparaginase[93]) with rapid aggregation/separation and adjustable heating properties through an AMF. The immobilization process is generally achieved by chemical modification including covalent bonding between proteins and polymers or MNPs (e.g., carbodiimide method[94]), or physical interactions (e.g., absorption[91] and encapsulation method[93]). The release of enzymes can be accomplished through regulating the swelling-deswelling transition of hydrogels with an adjustable temperature, as discussed above for drug-release systems. Magnetic hydrogels can have multipoint interactions with immobilized enzymes, which may enhance the thermal stability of the

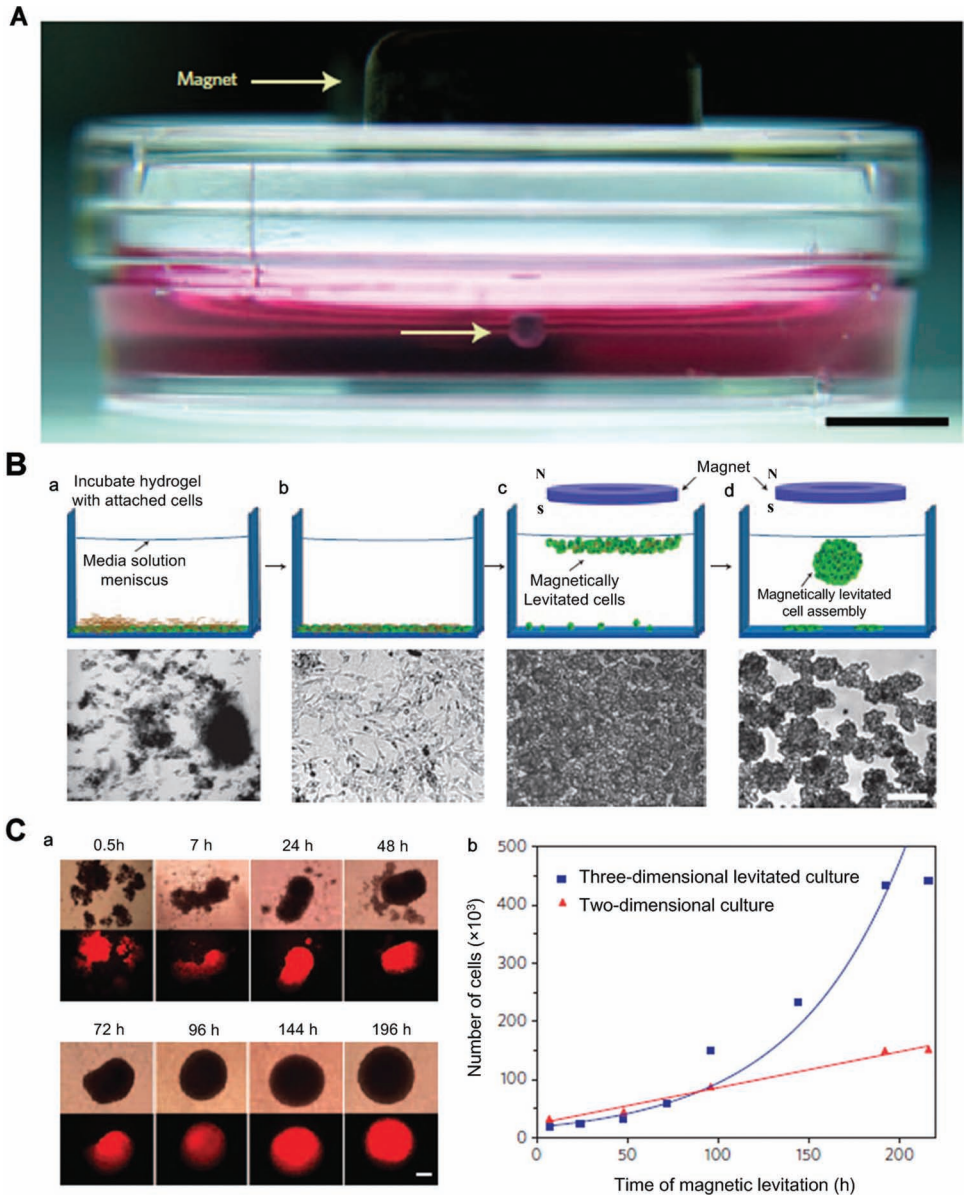

Figure 4. Three-dimensional cell culture based on magnetic levitation. A) Magnetic iron oxide-containing hydrogels. Human glioblastoma cells (lower arrow) treated with magnetic iron oxide (MIO)-containing hydrogels held at the air-medium interface by a magnet (scale bar = 5 mm). B) a–d) The top row shows a schematic of the general strategy of magnetic cell levitation and the bottom row shows the corresponding optical micrographs of neural stem cells at each stage (scale bar = 30 μm). C) a) Phase-contrast and fluorescence images of levitated human glioblastoma cells monitored over eight days (scale bar = 200 μm); b) Number of cells as a function of time for the levitated cell cultures (blue squares) and representative 2D cultures (red triangles). The line fits indicate an exponential trend for the levitated cells (blue line) and a linear trend for the surface-attached cells (red line). Reproduced with permission.[75] Copyright 2010, Nature Publishing Group.

enzymes and restrict the conformational change of the enzyme molecules during heating compared with those in the free form (without immobilization).[92]

3.3. Applications in Cancer Therapy

Hyperthermia cancer therapy (i.e., the heating of certain cancer organs or tissues to temperature between 41 °C and 46 °C), which may be combined with well-developed therapeutics such as chemotherapy and irradiation, has been proven to be an effective way to treat many refractory cancers, such as glioblastoma and pancreatic cancer.[95,96] However, precise temperature control within, as well as outside, the target region is still challenging.[97]

Recently, magnetic hydrogels have been applied to heat up target tumors remotely through an external MF, together with controlled release of an anticancer drug from the hydrogel. Lao and Ramanujan[98] fabricated a PVA-Fe_3O_4 hydrogel that can

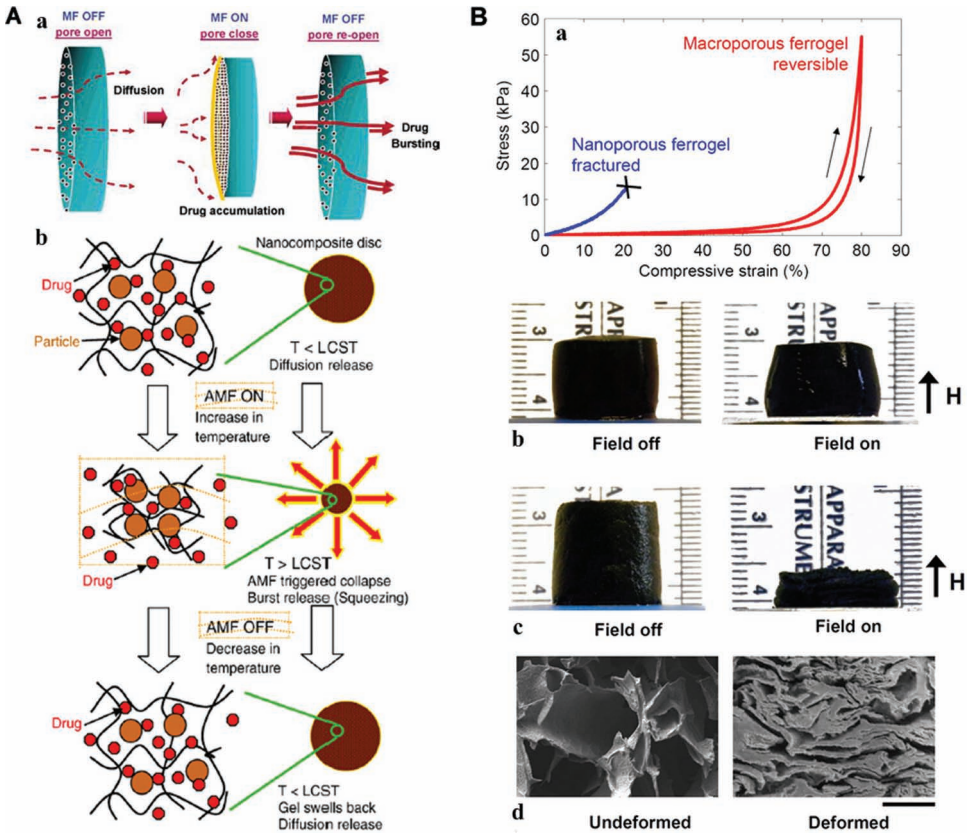

Figure 5. Magnetic hydrogels for drug delivery and release**.** A) a) Schematic of "close" configuration mechanism of magnetic hydrogel: aggregation of Fe_3O_4 NPs when applying a MF ("on") causes the porosity of the hydrogel to decrease; b) Effect of the ON-OFF cycles of an AMF on magnetic NIPAAm hydrogels: the AMF triggers collapse and resultant burst drug release due to the squeezing effect.[85] B) The active hydrogel scaffold undergoes a large deformation and volume change via a moderate MF. a) Compressive stress–strain curves for a nanoporous hydrogel and a macroporous hydrogel; b) A cylinder of nanoporous hydrogel reduced to ≈5% of its height when subjected to a vertical MF gradient of ≈38 A m^{-2}; c) The corresponding macroporous hydrogel deformed ≈70% under the same MF; d) SEM images of a free-dried macroporous hydrogel in the undeformed and deformed states. Scale bars = 500 µm. A) a) Reproduced with permission.[82] Copyright 2006 American Chemical Society. b) Reproduced with permission.[85] Copyright 2008, Elsevier. B) Reproduced with permission.[27] Copyright 2011, the National Academy of Sciences.

steadily reach a maximum temperature ranging from 43 °C to 47 °C within 5–6 min under an AMF (357 kHz). The results indicated that the amount of generated heat depended on the concentration of Fe_3O_4 and the MF amplitude. They also synthesized temperature-sensitive PNIPAAm hydrogels with microsized iron oxide particles.[99] The maximum temperature of the magnetic hydrogels can be adjusted by the MF strength and the concentration of MNPs. Anderson and co-workers[13] fabricated a series of PEG-based magnetic hydrogels, capable of heating to both hyperthermia (41–44 °C) and thermoablative temperatures (61–64 °C). This kind of magnetic hydrogel can kill M059K glioblastoma cells in vitro at the thermoablative temperature. These authors also prepared a poly(*β*-amino ester) (PBAE) biodegradable hydrogel composed of PEG diacrylate (PEGDA) with isobutylamine (IBA) for hyperthermia cancer therapy, which can be used as a drug-delivery vehicle, and which enabled remote heating when an AMF was applied (**Figure 6**).[100]

An injectable hydrogel system is an important strategy for the delivery of various cells and therapeutic agents for tissue repair and cancer therapy due to the ability to encapsulate cells or drugs homogeneously and target diseased sites with minimal invasiveness.[101–103] Various molecules and cells can be incorporated by mixing with a polymer precursor, which will experience a solution-to-gelation (sol-gel) phase transition after injection, through chemical or physical cross-linking including thermal gelation, pH-induced gelation, and ionic interactions.[104] Magnetic hydrogels hold great potential to be used as an injectable hydrogel system, especially for cancer therapy, due to their local hyperthermia ability. For instance, several polymers, including thermosensitive (e.g., chitosan and poloxamer) and ionic-response polymers (e.g., sodium alginate), embedded with MNPs have been investigated as injectable hydrogels for cancer therapy.[105] The polymer solutions containing MNPs undergo a sol-gel transition at a higher temperature or a higher calcium-ion concentration after injection to form magnetic hydrogels, and then the embedded MNPs allow a local heating under the influence of an AMF. However, several challenges exist: i) to increase the content of MNPs in these hydrogels, which is required to generate sufficient heat content; ii) to enhance the viscosity of magnetic hydrogels after injection, which may avoid undesired migration of MNPs; and iii) to clear MNPs from the body after injection.

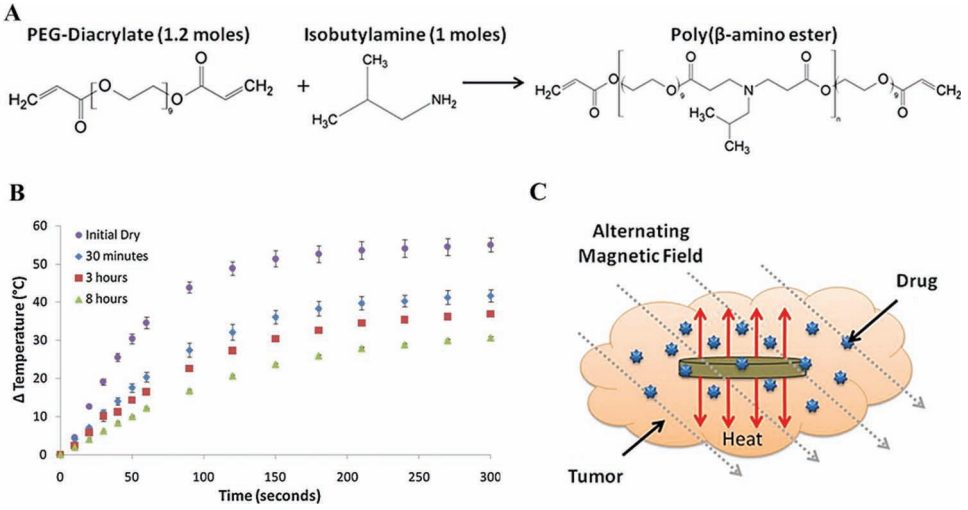

Figure 6. Magnetic hydrogels for hyperthermia cancer therapy. A) Schematic for synthesis steps of PBAE hydrogels composed of PEGDA, with isobutylamine resulting in a diacrylate-terminated structure. B) Analysis temperature change in magnetic hydrogels over time under an AMF. C) Schematic of magnetic hydrogels that are able to be heated remotely upon exposure to an AMF and provide a drug-delivery vehicle. Reproduced with permission.[100] Copyright 2012, Elsevier.

In addition, various polymer NPs (e.g., micelles and liposomes) have been used as drug carriers through passive or active targeting. The passive targeting results from the enhanced permeability and retention (EPR) effect, depending on the "leaky" vasculature structures of particular pathologies (e.g., tumors). Active targeting usually contains a specific conjugation of targeting molecules (e.g., antigen-antibody).[106–108] Different from these approaches, various chemotherapeutic agents embedded in magnetic hydrogels can target pathological sites via magnetic drug targeting (MDT). This process includes several steps: i) encapsulation of drugs in magnetic hydrogels; ii) intravenous injection of magnetic hydrogels in the colloidal form; iii) targeting to the pathological site via an applied MF gradient; and iv) release of drugs from magnetic hydrogels. MDT can also be combined with the active targeting method to enhance the targeting specificity, and thus potential side effects in surrounding tissues caused by non-specific targeting of chemotherapeutic agents may be avoided.

3.4. Magnetic Hydrogels as Soft Actuators

There have been many attempts to fabricate artificial muscles and soft actuators, which are sensitive to external stimuli, ranging from robot-like metallic actuators to more-advanced soft actuators.[109,110] Sensitive to an external MF, the mechanical behavior of magnetic hydrogels, including shape deformation and the swelling/deswelling process can be controlled through noncontact triggering using the external MF.[111–113] Because of this unique feature, various synthesized magnetic hydrogels have been applied to construct artificial muscles and soft actuators.

Szabó and Zrinyi[114] prepared a magnetic PVA hydrogel with MNPs dispersed in hydrogel networks using the blending method. The shape distortion of magnetic hydrogels occurred instantaneously and disappeared abruptly when the external MF was applied and removed, respectively. When the magnetic hydrogels were situated in a spatially non-uniform MF, strong interactions between the particles and polymer chains occurred due to the magnetic forces acting on the MNPs. Thus, the MNPs and polymer chains moved together as a single unit. Ramanujan and Lao[111] introduced iron oxide particles into a PVA hydrogel, which was then subjected to a MF of 40 mT. They found that the magnetic hydrogel showed instantaneous deflection and recovery upon application and removal of the MF, respectively, and observed a finger-like motion by encapsulating the magnetic hydrogel in a rigid transparent plastic material and then exposing it to a static MF. Kasi and co-workers[115] developed physically crosslinked hierarchical hydrogels through the swelling of a series of polymer hydrogels composed of the amphiphilic liquid-crystalline (LC) pentablock copolymer PB20-C5MA$_x$-Aa$_y$ and Fe_3O_4-based MNPs (**Figure 7**). These magnetic hydrogels featured tunable swelling degrees, and good mechanical and super-paramagnetic properties, and thus may be good candidates for artificial muscles and advanced soft actuators.

4. Conclusions and Future Perspectives

Various smart hydrogels that are responsive to external stimuli (e.g., temperature, pH, light, charge, pressure) in a physiological range have great potential in biomedical applications such as drug delivery and release. However, two main limitations exist: 1) the response time of these stimuli-sensitive hydrogels is long; and 2) hydrogels with more biocompatibility and biodegradability are required.[92]

Benefitting from the super-paramagnetic and heating ability when exposed to an AMF, magnetic hydrogels exhibit quick response properties in changing the deformation degree,[27] swelling state,[116] and degradation rate[117] by adjusting the external MF (e.g., intensity and frequency). Because this quick response is only caused by MNPs and polymers that are more biocompatible and biodegradable can be used to form hydrogels (e.g., fibrin and GelMA). Thus, magnetic hydrogels may

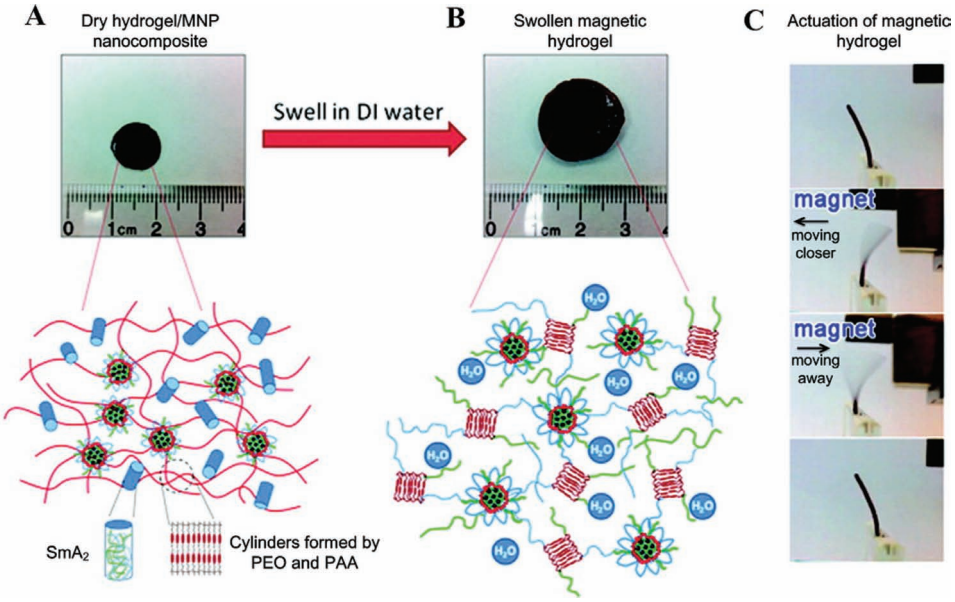

Figure 7. Magnetic hydrogels for soft actuator application. A) Schematic of a dry hydrogel/MNP nanocomposite with a hierarchical structure dispersed in a polymer matrix. B) Schematic of a swollen magnetic hydrogel with a network structure connected by LC domains and MNP clusters. C) Actuation of magnetic hydrogels using a magnet. reproduced with permission.[115] Copyright 2012, American Chemical Society.

be employed to engineer 3D complex tissue constructs through bottom-up assembly approaches, fabricate soft actuators, control temperature, and target to the tumor region in cancer therapy.

However, there are still several challenges that need to be addressed for these applications. Firstly, to fabricate 3D large tissue constructs with precisely controlled architectures, the ability to assemble cell-laden microscale magnetic hydrogels (M-gels) should be further extended meanwhile avoiding overheating, which may counteract the assembly process and induce a loss of the encapsulated cell viability. Secondly, as most current research for drug delivery with magnetic hydrogels is concerned with only in vitro investigations, more in vivo testing including controllable release of MNPs and targeting deep tissue needs to be evaluated. Finally, the long-term fate of embedded MNPs in vivo needs to be further studied before clinical applications. Despite all these challenges, the emergence of magnetic hydrogels with good response properties and controllability will greatly promote the development of biomedical engineering.

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