Polymer-Assisted Magnetic Nanoparticle Assemblies for Biomedical Applications

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ABSTRACT: Magnetic nanoparticle (MNP) assemblies have demonstrated great potential in biomedical applications due to their controllable magnetic properties and collective functions. Among the versatile approaches to obtaining MNP assemblies, polymer-assisted assembling methods offer unique advances, by which the assembled nanostructures could exert the merits of both the inorganic magnetic nanoparticles and organic polymeric materials to realize combined advantages for medical diagnosis and treatment. Precise control over the interactions among different building moieties and spatial organization of magnetic nanoparticles with the aid of polymers provides promising strategies in manipulating the physical, chemical, and biological properties of nanoassemblies for biomedical applications. In this review, we summarize the recent progress of polymer-assisted MNP assemblies, which include the mutual interactions between building blocks, architectural diversity of the assemblies, and their synthetic strategies along with biomedical applications. The current review provides a comprehensive insight into controlled and intelligent nanomedicines, which shall facilitate the development of next-generation high-performance theranostic agents based on MNP assemblies.

KEYWORDS: magnetic nanoparticle assembly, polymer, interaction force, architecture, biomedical application

1. INTRODUCTION

Magnetic nanoparticles (MNPs) have received growing attention for their promising applications in disease diagnosis and therapy.1,2 Recent advances in chemical synthesis and characterization allow the production of MNPs with controlled shape, size distribution, and magnetism, while surface engineering strategies endow particles with water dispersibility, colloidal stability, and biocompatibility in biological environments.3–5 Currently, the manipulation of the assembling/disassembling process has been demonstrated to be an effective and facile approach to alter the physicochemical properties of MNPs,6 paving the way for breakthroughs in various research fields including drug delivery and targeted biological imaging.8–10 For instance, compared with isolated magnetic nanoparticles, the MNP assemblies with higher magnetic moment afford a considerable enhancement in the detection sensitivity of magnetic resonance imaging (MRI). Moreover, the conjugation of therapeutics or imaging agents mediated by polymers or coassembling with MNPs enables the simultaneous implementation of therapeutics and/or multiple imaging modalities in one system. Especially, the responsive assembly/disassembly of MNPs upon specific endogenous conditions or externally applied stimulus could impart a typical T2/T1 relaxation switching or significant T2 enhancement through the tailoring of interparticle distance and interaction11–13 or controlled drug release at the targeted diseased sites with minimized systemic side effects. Consequently, improved performance with increased signal-to-noise (S/N) ratios through the targeted delivery and amplified MRI signals or controlled drug release at the disease lesions can be achieved for precision medicine and theranostic applications.14,15

Recently, a number of techniques have been reported for the constructions of assembly structures through self-organization, directed-, or templated-assisted conformation arrangement by introduced external fields,16 and various moieties including small molecules,17 biomolecules,18 inorganic materials,25,26 as well as organic polymers.25,26 Precise spatial organization of nanoparticles (NPs) in the assemblies can lead to multiple structures including spherical, worm-like, and other template-guided structures (Scheme 1).27–30 Among these assembly moieties, polymer-based materials present a huge potential in...
terms of facilitating various biomedical applications since their properties can be well controlled by manipulating the construction including chemical constitution, molecular weight, chain length, and electric charge, as well as the ability to accommodate various ingredients. The diverse and easy manipulation of polymers endows the MNPs with possibilities to be precisely modified or structurally rearranged in the assemblies to meet the rapidly rising demands for preclinical studies and clinical applications.

Through a typical encapsulation method, MNPs could easily coassemble with amphiphilic block copolymers into a broad range of hierarchically complex nanostructures. The spatial distribution of MNPs in the polymeric assemblies is tunable through the adjustment of the hydrophobic and hydrophilic block ratio of the copolymers, capping ligands and grafting density on the surface of inorganic MNPs, solubility of the assembly blocks in the solvents, etc. Nowadays, many efforts have been dedicated to controlling the fundamental properties of polymers and to designing the functionally responsive polymers using different polymerization techniques or modification methods, which makes polymers highly attractive for nanoengineering. To date, the development of polymeric science in versatile designing and chemical synthesis methods for polymers, together with the recent advances in the controlled synthesis of MNPs, and the various strategies in encapsulating inorganic NPs into the block copolymer matrixes, place great promise for preparation and application of the polymer-assisted MNP assemblies in the biomedical field. In this review, we summarize the methods and principles of polymer-assisted MNP assemblies, and then, we focus on the recent progress of MNP assemblies in medical imaging, drug/gene delivery, and therapy. Finally, we will provide a brief overview of the challenges and potential opportunities for future MNPs research in the biomedical field.

2. ASSEMBLY OF MAGNETIC NANOSTRUCTURE DIRECTED BY POLYMERS

The controlled assembly of MNPs directed by polymers is a dynamical process of recognition and arrangement of structural units to form multilevel architectures, resulting in complex hierarchical constructions via different interactions of the building blocks (Scheme 1).

2.1. Interactions between the Building Blocks. Since most MNPs are synthesized in organic media, the original hydrophobic ligands limit their dispersion in aqueous solution for direct biological applications. Although the early generation of water-soluble MNPs could be achieved through coprecipitation method, the as-synthesized MNPs are often polydisperse and unstable, resulting in the form of aggregates. In spite of different methods used to disperse aggregates or avoid the reaggregation, the obtained nanoparticles are not satisfactory in clinical applications. It is noted that particle aggregation is mainly induced by the imbalance of attractive and repulsive colloidal forces acting between particles. Therefore, the tight control over the colloidal forces, which mainly include van der Waals forces, electrical double-layer steric interactions, hydrophobic and solvation forces for particle stabilization, is highly demanded. Commonly, it
is well-accepted that one of the most practical methods for stabilizing the nanoparticle dispersion in aqueous solution is to use polymeric surfactants during or after the synthesis of MNPs for finely balancing the colloidal forces through the minimization of Gibbs free energy. In addition, polymeric coating can also prevent the formation of large aggregates when exposed to the biological system. Among the reported methods up to now, the ligand exchange and physical assembly/encapsulation approaches are often considered to be effective ways for the surface coating of nanoparticles with functional polymers. To date, a number of biocompatible copolymers such as poly (ethylene glycol) (PEG), poly (ethylene glycol)-poly (acrylic acid) (PEG-PAA), polyvinylpyrrolidone (PVP), or poly(vinyl alcohol) (PVA) have been widely used as coating materials for MNPs in aqueous suspension. In addition, amphiphilic block copolymers with multifarious compositions such as poly(acrylic acid)-b-poly(styrene) (PAA-b-PS), poly(ethylene oxide)-b-poly(styrene) (PEO-b-PS), poly(4-vinylpyridine)-b-poly(styrene) (P4VP-b-PS), poly(e-caprolactone)-b-poly(ethylene glycol) (PCL-b-PEG), or Pluronic F68 (PF68) are also frequently used as coating materials. Notably, these amphiphilic block copolymers attracted great attention especially for their ability to coassemble with MNPs into complex nanocomposites for biological applications, where rational modification of functional groups in the copolymers supplies extra fascinating biological functionalities. At this point, more representative examples for the polymers used as coating materials or assembling modules can be referred to previously published reviews.

On the basis of the above principles, Qiao et al. utilized dibromomaleimide (DBM)-terminated poly(oligoethylene glycol) methyl ether acrylate (poly(OEGA)) homopolymer for the surface modification of MNPs to achieve enhanced colloidal stability and biocompatibility for the fluorescence imaging detection. The polymer shell, constituted of stabilizing dispersants and solubilizers, effectively decreased the high surface energy and alleviated aggregation of the individual nanoparticles, making the polymer-modified MNPs or their assemblies counterparts much more stable compared to the bare ones in aqueous media. Moreover, a mussel-inspired multiple-interaction ligand (MIL) was developed by Ling and co-workers for the improvement of NP stability in aqueous media. The MIL was composed of PEG, polyethylenimine (PEI), and poly(l-3,4-dihydroxyphenylalanine) (polyDOPA) moieties to stabilize various metals and metal oxide nanoparticles via multibinding reactions including coordinate binding between catechol and amine groups, micelle formation via hydrophobic interactions, and electrostatic interaction between positively charged moieties of the ligands and the negatively charged nanoparticle surface (Figure 1). Among the three synthesized ligands with different L-DOPA ratios (MIL0, MIL1, MIL2), MIL2 exhibited the best performance for stabilizing nanoparticles of Fe3O4, MnO, and Au in various harsh aqueous environments, especially in highly acidic and basic media, concentrated NaCl solutions, and even in boiling water.

Currently, many contemporary studies on the MNPs are focusing on the manipulation and organization of individual components into new nanoscale hierarchical architectures with novel and fascinating functions. Compared to the chaotic aggregation, the MNP assemblies with a controlled and ordered spatial arrangement of MNPs are much more imperative in bioapplications, since the as-constructed nanocomposites with well-defined sizes, shapes, or conformations can not only increase their in vivo circulation time, but also change the interactions between the individual MNP in the nanoassemblies, which will in turn improve the magnetization and MRI performance. It is known that the introduced external magnetic field could guide or control the assembling processes, sizes, morphologies, and structures of...
the MNP assemblies via magnetostatic dipole–dipole interaction, which plays a vital role as the predominant driving force in the course of self-assembling and enables the long-range organization of the MNPs.61,62 Although it is possible to form magnetic assemblies with the help of an external magnetic field, the biocompatible materials, such as silica and polymer, are often required in the formation of the MNP assemblies for biomedical applications.62,63 In this regard, the assemblies of MNPs assisted by polymers render more opportunities to the facial fabrication with desired and diverse functionalities.

Since the assembling behavior of NPs with copolymers in aqueous solvents is a process for balancing the enthalpic and entropic contributions,31 the assembly construction can be achieved by altering the interaction forces between the blocking units or with the solvents. The inherent forces in the assembling process can mainly refer to the hydrophobic interaction, hydrogen bonding, electrostatic interaction, and other strong chemical reactions between the building blocks (Scheme 1).16,64 By means of the aforementioned colloidal forces, desired multilevel structures with nanoscopically confined geometries could be obtained. For example, the most common magnetic core–shell structures could be easily achieved via hydrophobic interactions between the hydrophobic components in amphiphilic polymer segments and organic capping ligands on the surface of MNPs.65,66 Moreover, the ordinarily electrostatic attractive forces between the ligands capping on the surface of MNPs and the segments with opposite electric charges in the polymer also assist MNPs assembling. For instance, the integration of super-paramagnetic iron oxide (SPIO) clusters assisted by polysaccharide polymer was initiated by the electrostatic interaction between ammonium cations of glycol chitosan/acyrl/biotin and carboxylic anions on the surface of SPIO.67

Besides, other strong chemical bonds inspired from the stimuli-responsive polymers could also offer a high degree of control to direct the assembly process.68 Instead of summarizing all achievements in the interactions between the MNPs and polymers, we next focus on the formation of the multilevel structured MNP assemblies assisted by polymers.

2.2. Architectural Diversity of Magnetic Nanoparticle Assemblies. The architecture plays an important role in regulating the characteristics and performance of the polymer-based hybrid materials. For example, the detection sensitivity of MNP assemblies in MRI depends not only on the magnetism of individual MNP but also on their spatial organization. On the basis of the above principles, the magnetically assembled systems with architectural diversity offer more possibilities and unique properties, which makes them extremely attractive as building blocks for nanomedicine.5 Some typical hybrid superstructures prepared by operating the localization of MNPs in the polymer matrix are shown in Scheme 1, including spherical structures, 1D worm/ring-like structures, and 2D arrays. These nanocomposites can be constructed through two representative methods; one is in situ method based on the template via chemical reactions, while the other one is ex situ method, which is referring to the coassembling or encapsulating the MNPs and copolymers by coprecipitation, emulsion, heating–cooling and other methods.31 Herein, we first introduce the fabrication of polymeric MNP assemblies based on the ex situ method for that it provides facile approaches for precise control over the size or position of MNPs in the copolymer matrix.

By virtue of the ex situ method, multiple magnetic spherical structures, including magnetic polymersomes,14 magneto-micelles,69 and condensed clusters70,71 could be obtained through regulating the hydrophobic/hydrophilic ratio of amphiphilic copolymer chains, the amount and species of the MNPs assemblies via magnetostatic dipole–dipole interaction, which plays a vital role as the predominant driving force in the course of self-assembling and enables the long-range organization of the MNPs.61,62 Although it is possible to form magnetic assemblies with the help of an external magnetic field, the biocompatible materials, such as silica and polymer, are often required in the formation of the MNP assemblies for biomedical applications.62,63 In this regard, the assemblies of MNPs assisted by polymers render more opportunities to the facial fabrication with desired and diverse functionalities.

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capping groups on the surface of MNPs, solvent composition and immiscibility, as well as the temperature/pH of the solution in the polymer-assisted assembling process. On top of that, through the fine-tuning of the interactions between the selected solvent with copolymer, or copolymer with nanoparticle, different assembly architectures can be obtained. For instance, three distinct structures were achieved through encapsulating MNPs with diblock copolymer poly(acrylic acid)-b-polystyrene (PAA-b-PS) (Figure 2), in which the position of the MNPs is (1) locating between the interface of the polymer core and shell (magneto-core-shell) (Figure 2a); (2) homogeneously distributing across the polymer matrix (magneto-micelles) (Figure 2b); (3) aggregating in the polymersome wall (magneto-polymersomes) (Figure 2c).29

Among all the three assemblies, magneto-polymersomes showed the highest transverse relaxivity rate ($r_2$) due to high MNPs loading density and water molecules accessibility. Besides, the magneto-core—shell assemblies also showed a significantly higher $r_2$ relaxivity rate than typical magneto-micelles for the close MNPs packing density and water accessibility. Therefore, precise control over a desired spatial arrangement of MNPs within the polymers can not only improve the biological stability of MNPs, but also endow the assemblies with enhanced collective properties for further in vivo applications. Furthermore, they also confirmed that the heterogeneous structures could be drastically changed as long as the relative volume ratio between the hydrophobic and the hydrophilic block of the polymers be finely tuned during the assembling process (Figure 2d). When a long hydrophobic block was used, the nanoparticles could self-assemble into vesicles, while tubular assemblies could be generated with shorter hydrophobic blocks. Besides, the dissolution properties of block copolymers and nanoparticles in the solvents as well as the nanoparticle size and concentrations also have impacts on their magnetic relaxation properties and could also be directed to optimize the MRI performance.72 Condensed cluster is another main structure of the MNP assemblies assisted by polymers via electrostatics and compensation,71,74 or other chemical interactions.75 The monodispersed magnetite colloidal clusters composed of small primary nanocrystals can be tuned precisely from ~30 to ~180 nm and rendered high magnetization and water-dispersion by the surface-tethered PAA chains.6 The morphology and MNP’s arrangement of the condensed clusters are multiple, and the loading quantity of MNPs may range from several to hundreds. Moreover, the MNP’s interparticle spacing can be precisely adjusted by varying the composition or length of grafted polymers.

Apart from the spherical structure, there is a limited number of examples for the successful preparation of nonspherical ordered structures of MNP assemblies because controlled clustering of the nanoparticles and polymers during ex situ coassembling or encapsulation process is exceptionally challenging. Herein, the in situ method based on the polymer template provides opportunities for directing the formation of the nonspherical magnetic polymeric hybrid materials, where the architecture of the resulting magnetic assemblies primarily relies on the template structure. A typical example reported by Lee et al.71 is the utilization of hyaluronic acid-graft-catechol as a 1D template to precisely control the preparation of metallic-nanoparticle assemblies. The length of the 1D nanochain is tunable when adjusting the contour lengths. Except for the nanochain structure, other architectures such as worm-78 and ring-like82 nanostructures are reported in other literature studies. In addition to these 1D linear structures, MNPs could also form 2D arrays83 and gels84 mainly based on the species of polymer templates. Through optimizing the assembly parameters, such as nanoparticle size, concentration, surface grafted ligands or the relative ratio between the hydrophobic block and the hydrophilic block of the polymer media, etc., MNPs could be localized in different domains of assemblies, and the obtained nanocomposites exhibit rich composition and structural diversity.85-87

Generally, such a variety of structures not only allows the combination of properties from the individual nanoparticle but also takes advantage of the interactions between neighboring nanoparticles, which can result in new properties and applications ranging from photonics, separation and detection, to multimodal imaging, energy storage and transformation, and catalysis.75,88-90 Besides, the size, morphology, and structure of the magnetic polymeric nanoassemblies are emerging as important modulators for their in vivo destiny, such as the blood circulation time,91 the extent of cell internalization and metabolism,92,93 as well as drug delivery capability for subsequent therapeutic efficacy.94-96

3. SYNTHETIC STRATEGIES OF POLYMER-ASSISTED MAGNETIC NANOPIRLE ASSEMBLIES

Compared to the individual MNP, assembled MNPs produce a collective behavior, especially the modified magnetic performance induced by coupling the magnetic moments.27 Among the various techniques reported for the construction of assembly structures, methods based on polymers assisted assembling have been well developed to pursue the enhancement and regulation of the performance and properties of MNP assemblies to meet the need for improved colloidal stability, biocompatibility, and specific biological applications in the complicated biological environments.98-99

In this section, we provide an introduction of the fundamental techniques that can be used to construct assembly structures assisted by polymers, which include template-free and template-based methods.100,101

3.1. Template-free Assembly. To date, several strategies have been demonstrated for the assembly of MNPs.5,102 The relatively flexible way for controlled fabricating of stable MNP assemblies mainly mediated by the molecular interactions or chemical bonds is called the template-free assembly, commonly including the hydrophobic interaction, hydrogen bonding, or electrostatic interaction.8

3.1.1. Hydrophobic Reaction Directed Assembly. Hydrophobic reaction directed assembly is a typical method to construct the hierarchical nanostructures through the assembling of MNPs and amphiphilic copolymers. Since the hydrophobic segments of an amphiphilic polymer could drive the assembly of MNPs via hydrophobic—hydrophobic interactions with similar hydrophobic ligands on the surface of MNPs and provide the nanocomposite structure with enhanced mechanical properties in the integrity, namely, not easily broken down, which in turn grant them better adaptability and biocompatibility in biological systems.99,103-105

The coprecipitation method is a representative approach that has been widely used to transfer the preformed NPs into the copolymer matrix and form a typical core—shell nanostructure in the selected solvent based on the hydrophobic—hydrophobic interactions. The resulting nanoassem-
blies could be obtained through dialysis with further purification process. For example, Ling and co-workers\textsuperscript{9} reported the fabrication of magnetic nanoassemblies based on this method. Briefly, amphiphilic copolymer ligands were dissolved in dimethylsulfoxide (DMSO) and slowly added into the chloroform solution containing oleic acid-coated extremely small iron oxide nanoparticles (ESIONs) to form a homogeneous mixture. Then the chloroform was evaporated from the system and the water was supplied. The DMSO (good solvent) was completely removed and substituted with water (selective solvent) through the subsequent dialysis process, leading to the aggregation of ESIONs into the hydrophobic regions in the nanoassemblies. Besides, Gao et al.\textsuperscript{50} added tetrahydrofuran (THF) solution which contained poly(ε-caprolactone)-b-poly(ethylene glycol) (PCL-PEG) and hydrophobic MNPs into aqueous solution under sonication, the resulting solution was stirred to allow the evaporation of THF to obtain the MNP assemblies dispersed in water. In this process, therapeutic drug doxorubicin could be loaded into the hydrophobic inner center of the micelle structure simultaneously for further therapeutic applications.

Emulsification is another typical method that is particularly advantageous for designing assembled structures utilizing amphiphilic block copolymers to accommodate MNPs within a complex nanostructure through the interfacial instability of emulsion droplets\textsuperscript{106} endowing the magnetic assemblies with ordered structures and high water-soluble characteristics. For instance, amphiphilic poly(isoprene)-b-poly(ethylene glycol) diblock copolymer (PI-b-PEG) and iron oxide nanoparticles (IONPs) were injected into water and formed micellar encapsulation of MNPs via hydrophobic interaction between the hydrophobic segments of polymers chains and oleic acids anchored on the surface of IONPs.\textsuperscript{7} Then the magnetic micelle was further stabilized by cross-linking polystyrene (PS) shell via emulsion polymerization process. The resulting MNP clusters ranging from the nano- to the microscale showed enhanced magnetic properties, and the $r_2$ reflexivity value increased along with the increasing of cluster size in a certain dimension range, neither obtainable from the singly encapsulated nor from the bulk material. Moreover, MNP assemblies mediated by polymers upon physical emulsification have structural flexibility and collectively functional adjustability, which shows great potential for fabricating multifunctional magnetic nanoparticle-based nanomedicine toward various applications.\textsuperscript{107} Besides, another advantage of the emulsification-based method is the ability to generate worm-like 1D hybrid micelle structures. For instance, Bae et al. dissolved PEO\textsubscript{6.4k}-PS\textsubscript{19k} with hydrophobic IONPs in chloroform, and the mixture was then injected into water containing a surfactant of poly(vinyl alcohol) (PVOH) and stirred in an open atmosphere for further emulsion and micelles-formation process. With the evaporation of chloroform, a worm-like 1-D hybrid micelle structure was exclusively formed, showing promise for further biological applications.\textsuperscript{107} Moreover, various structures ranging from spherical, cylindrical, to lamellar can be easily achieved through tuning the composition of the copolymer, which is rather difficult to obtain through the aforementioned coprecipitation method.\textsuperscript{108}

3.1.2. Hydrogen-bonding Interaction Directed Assembly. Apart from the hydrophobic interactions, the integration of the MNPs with organic components could also be achieved dependent on the formation of hydrogen bonds. For instance, a magnetic $\textit{cis}$-diamminedichloroplatinum(II) ($\textit{cis}$platin, CDDP)-encapsulated nanocapsule (CDDP-PEA-NC) was prepared using a double emulsion method through ultrasonication to form a core–shell structure with CDDP-PEA core and PVA/SPION shell. The hydrogen bonds between PAA and PVA facilitated the formation of a nanoshell and further incorporation of hydrophobic SPIONs into the hydrophobic organic layer. Moreover, the encapsulation of CDDP was facilitated by the formation of coordination bonds between the platinum(II) atoms of $\textit{cis}$platin and the carboxylate groups of PAA. Both in vitro and in vivo results clearly showed that the magnetic CDDP-PEA-NCs significantly reduced toxicity and exhibited high anticancer activity.\textsuperscript{109} Another similar hydrogen bonding network involving PVA was exploited by Huang and co-workers.\textsuperscript{110} Highly thermosensitive MNP assemblies composed of poly(ethylene oxide)-b-poly(p-phenylene oxide)-b-poly(ethylene oxide) (PEO-PPO-PEO) (F127) triblock-copolymer and PVA was prepared using a mini-emulsion method (Figure 3).

Within the nanoemulsion, amphiphilic polymer PVA was employed as the H-bond provider to react with the PEO block of F127 to form an ultrathin nanoshell for the stabilization of the cargo and avoiding the undesired drug leakage before reaching the targeting sites.

3.1.3. Electrostatic Interactions Directed Assembly. Electrostatic interactions also show their potential as a simple and versatile route to assemble MNPs into well-defined superstructures at room temperature and atmospheric pressure, and it displays a much higher yield as to allowing scaling up this process toward industry.\textsuperscript{111,112} For instance, Yan et al.\textsuperscript{113} presented a useful approach to control the electrostatic interaction during the self-assembly process, in which the negatively charged poly(acrylic acid) coated maghemite NPs and homopolyelectrolytes [poly(diallyldimethylammonium chloride) (PDADMAC) or PEI] were directly mixed at an appropriate ionic strength in an aqueous medium to address the controllability over the shape and morphology. This aggregation process was slowed down to the time scale of
hours and indicated a clarity of the growth mechanism with Phase I of clustering of individual NP with the assistance of cationic polyelectrolytes and two different Phase II mechanisms, one is a random directional link and accretion of the precursor, and the other is subwires-like aggregation with the presence of external magnetic field induced by the magnetic dipolar interactions. Via the specific electrostatic absorption, MNPs could be selectively deposited at the desired site in the polymeric matrix, providing another opportunity for the precise arrangement of MNPs in the assemblies.

3.1.4. Other Interactions Directed Assembly. In addition to the interactions mentioned above, several other methods are also studied for the controlled construction of MNP assemblies. For example, Liu et al. reported the cross-linked PEG-coated Fe₃O₄ NPs bearing surface reactive carbonyl groups with poly-l-lysine (PLL) via molecular dipole reaction between the carbonyl moieties and the amine group in PLL repeated units to obtain the magnetic aggregates. On this basis, further targeted modification created another opportunity for the application of the MNP aggregates that could result in the signal amplification effect in a lateral flow immunochromatographic assay (LFIA) (Figure 4).

On top of that, Lee and co-workers introduced Dox and four different molecular building blocks, including adamantane (Ad)-grafted polyamidoamine dendrimers (Ad-PAMAM), β-cyclodextrin-grafted branched polyethyleneimine (CD-PEI), Ad-functionalized polyethylene glycol (Ad-PEG), and Ad-grafted Zn₀.₄Fe₂.₆O₄ magnetic nanoparticles (Ad-MNP), to obtain multifunctional MNP assemblies as a unique on-demand drug delivery system (Figure 5). The self-assembly process initiated through a multivalent molecular recognition between Ad and CD motifs in order to facilitate the control over the size, surface chemistry, and payloads of MNP vectors for a wide range of diagnostic and therapeutic applications. Meanwhile, the incorporated Ad-MNPs served as a transformer that could convert radio frequency external alternating magnetic field (AMF) into heat, triggering the burst release of DOX molecules from the magnetothermally responsive assemblies.

As compared to a stable integrity in which the individual elemental constituents are strongly chemically bonded, these nanoparticle assemblies are usually weakly interacted since the nanoparticle subunits are acting through hydrophobic interaction, hydrogen bond, or electric/magnetic dipole interactions; however, by virtue of the weakly interacting forces, it is feasible to construct multifarious flexible dynamic nanostructures for “on-demand” signal amplification and targeted drug delivery.

3.2. Template-Based Assembly. Template-based assembly has become an attractive fabrication approach with effective guidance for the formation of specific structures and morphologies. The templates can be divided into inorganic rigid templates and organic soft templates according to their physical properties, where the mesoporous silica nanoparticles, block copolymers, biological materials such as DNA scaffolds, viruses, peptides, can all be applied for the controlled assembly of MNPs.

The catechol group is an adhesive molecule that can actively interact with a variety of organic/inorganic substrates. On the basis, a bioinspired polymeric template, hyaluronic acid–catechol was reported and introduced into the fabrication process of the MNP assemblies and showcased the abilities for synthesizing 1D assembled nanochains, in which the morphology and properties of the assemblies were demonstrated to be determined by the nanoparticles solution concentration (Figure 6a). Another interesting study is to utilize the incompatible feature between polymers and inorganic nanoparticles to prepare monolayer- or multilayer-nanoparticles coated polymer beads. With the increased concentration of polymers during the evaporation process, nanoparticles were expelled and accumulated on the surfaces of the polymer beads (Figure 6b), where the number of nanoparticle layers is tunable by adjusting the polymer/nanoparticle ratio in the oil droplet phase. Besides, Newland and co-workers adopted a photo-cross-linkable ethylene glycol dimethacrylate (EGDMA) homopolymer to make polymeric nanotubes within the pores of an anodized aluminum oxide template. Then the magnetically controllable nanotubes could be formed based on ferric oxide (Fe₃O₄) filled polymeric nanotubes, which could be directed by an external magnetic field for targeted drug delivery when loaded with anticancer drugs. Yet another novel preparation method applied to fabricate 3D porous Cu₂O–Fe₃O₄@carbon nanocomposites (Cu₂O–Fe₃O₄@CN) was using natural polymer-based hydrogel as a template to immobilize cupric ion and ferrous ion by chemical complexation and adsorption, which showed distinct photocatalytic performance for organic contaminant degradation under visible light irradiation.

In the above-mentioned nanoassemblies, the polymers not only act as glue, matrix, or template for the assembling with NPs, but also provide fascinating opportunities and possibilities for the accommodation of drugs or other functional moieties. Recently, a variety of functional polymers have emerged and been used to construct “smart” MNP assemblies with responsiveness to specific stimuli, such as pH, redox gradient, temperature, glutathione, and some enzymes, enabling promising biomedical applications such as precise diseases diagnosis, controlled and targeted drug delivery, as well as therapeutics.

4. BIOMEDICAL APPLICATIONS

Compared to the individually dispersed nanoparticles, the assembly of MNPs assisted by polymers could have novel collective properties and multifunctionalities, simultaneously maintaining stability in the physiological environment.
Furthermore, MNPs coassembling with stimuli-responsive ligands have also attracted considerable attention to targeted drug delivery or signal amplification triggered by the specific endogenous or exogenous conditions, facilitating their potential applications in biomedical imaging, drug delivery, and diseases therapeutics, thus provide intriguing strategies and possibilities for further clinical uses.

4.1. Biomedical Imaging Platform. It is well-known that MNPs are excellent contrast agents for MRI. The enhanced contrast properties are driven from the proton relaxation rate affected by the MNPs local field and can be tuned through the altering of particle size, shape, surface modification, and spatial arrangement. Normally, small-sized MNPs provide a higher number of surface-exposed magnetic metal ions for water proton coordination and chemical exchange, exhibiting a decreased magnetization and can be used as $T_1$ contrast agents presumably through the spin-canting effect. On the contrary, larger-sized MNP assemblies display unconventionally enhanced $T_2$ contrast effect due to the higher $r_2$ value which highly depends on the size of the particle. Moreover, the number and arrangement of MNPs in the assemblies also affect the biomedical imaging performance. On the basis of the above principles, Schmidtke et al. improved the $T_2$ MR contrast performance of amphiphilic PI-b-PEG diblock copolymer-based magnetic nanoclusters by elevating the SPIONs loading density and increasing the cluster size in the assemblies. Similar effect was also observed in a triblock copolymer PEI-b-PCL-b-PEG encapsulated FeO$_x$-NPs and PLGA immobilized SPIONs systems, which can further be utilized for the construction of $T_2$ MR contrast agents.

Figure 5. Self-assembled synthetic strategy of DoxCMNPs and the magneto-thermally triggered release of Dox. The embedded Ad-MNP served as a built-in heat transformer that triggered the burst release of Dox molecules by the magneto-thermal stimuli under an alternating magnetic field (AMF). Reproduced with permission from ref 115. Copyright 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Figure 6. Schematic illustration of the fabrication process of the 1D assembled nanochains on the bioinspired polymeric template (a) and polymer-template assisted clustering followed by phase segregation (b). (a) is reproduced with permission from ref 77. Copyright 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (b) is reproduced with permission from ref 122. Copyright 2009 American Chemical Society.
Especially, some smart MR contrast agents that possess biological stimulus groups shed lights on the selective amplification of S/N ratio in the specific lesion sites. For example, MNP assemblies were prepared and served as a novel...
A novel type of pH-responsive biodegradable copolymer was developed based on the methyloxy-poly(ethylene glycol)-b-poly[dopamine-2-(dibutylamino) ethylamine-L-glutamate] (mPEG-b-P(DPA-DE)LG), which contains amine-terminated dopamine groups and pH-sensitive moieties. The assemblies conferred high loading efficacy of SPIONs and enhanced negative contrast revealed the ability as a $T_2$ contrast agent and could maintain stable status at physiological pH ($\sim$7.4), while quickly protonated under the targeted acidic pathological environment to trigger the release of SPIONs. The effective integration of SPIONs in the biological systems resulted in amplified $T_2$-magnetic resonance contrast signals at 24 h post-injection (Figure 7b).

On the other hand, $T_1$ contrast agents are usually preferred for better clarity due to the bright signal presented at target tissues especially in a clinical situation, such as NaGdF$_4$-based nanoparticles,$^{12,136}$ rather than the dark signal produced by $T_2$ contrast agents. Additionally, the generated dark signals may be confused with other internal conditions, such as bleeding or...
calcification. Recent studies have suggested that IONPs with core size below 5 nm are optimal to form $T_1$ or dual $T_1/T_2$ contrast agents.\textsuperscript{137,138} For instance, ultrasmall super-paramagnetic iron oxide nanoparticles (USNPs) were anchored by mussel-inspired multidentate block copolymers (MDBC) via pendant catechol groups, fabricating aqueous Cat-MDBC/USNP colloids with a diameter of $\sim 20$ nm through a biphasic ligand exchange process (Figure 8a).\textsuperscript{75} They provided brighter $T_1$-weighted imaging at the concentration of $0.38$−$0.57$ mM Fe, which is significantly lower than that ($0.9$−$1.9$ mM Fe) for the corresponding MDBC with pendant carboxylates.\textsuperscript{139,140} At the same time, the Cat-MDRC/USNP colloids fabricated by the copolymer with the random distribution of pendant catechol groups, yielding a mixture of cluster-like structure with a diameter of 42.6 nm and large aggregates (diameter $> 1$ $\mu$m) in aqueous solution, presenting a transverse relaxivity rate close to the typical values of negative MRI contrast agents. This result suggests that the Cat-MDRC/USNP colloids with aggregate-like structure are suitable for $T_2$ contrast enhancement, but not for $T_1$ MR imaging (Figure 8b). Hence, not only the particle size, but also the nanoparticle arrangement in the obtained assemblies based on the polymer design will ultimately decide their imaging behaviors.

Figure 10. Formation and in vivo behavior of the PMNs. (a) Design of PMNs for tumor pH activation based on the ligand-assisted self-assembly of ESIONs; (b) schematic representation of pH-dependent structural transformation and related magnetic/photoactivity change in PMNs in vivo $T_1$-weighted MR images (c) and fluorescent images (d) of tumor sites after injection of PMNs or InS-NPs into nude mice bearing HCT116 tumors; (e) Ce6 uptake by tumor cells in tumor tissue of nude mice after intravenous injection of PMNs. Reproduced with permission from ref 9. Copyright 2014 American Chemical Society.
However, with the urgent requirement for accurate clinical diagnosis, single-phase contrast enhancement of current results are not that satisfying for the early detection and diagnosis of some debilitating diseases.13,14 As a consequence, an evolved versatile MRI platform based on the stimuli-responsive assembly disassembly characteristic of the MNP assemblies as a dual-phase images transformation agent from $T_2$ to $T_1$ pattern was investigated by Lu and co-workers. The ultrasmall iron oxide nanoclusters (USIONCs) with an excellent ability for $T_1$ MRI contrast enhancement were first synthesized through a typical thermal-deposition method and further covered with anchor DNAs. Then USIONCs were cross-linked by the pH-responsive linkers composed of i-motif DNAs and their partially complementary strands (Figure 9a,b). The obtained nanocluster assemblies based on the hybridization of oligonucleotides took less than 40 s ($t_{1/2} = 12.14$ s) to transform into individually dispersed USIONCs upon the pH value changing from 7.4 to 5.5 (Figure 9c). They also exhibited a significant drop in the $r_2/r_1$ ratio when the pH value changed from neutral to acidic, consequently led to the transformation of the assemblies from a $T_2$ contrast agent in the neutral environment to a $T_1$ contrast agent in acidic conditions (Figure 9d,e). The in vivo application of the pH-responsive assemblies on orthotopic hepatocellular carcinomas (HCC) animal model further verified the inverse contrast enhancement at the liver region. The tumor region was significantly brightened by the pH-responsive disassembling behavior after a two-hour injection while the surrounding normal liver tissues were darkened for significantly enhanced imaging contrast (Figure 9f).

Recently, an increasing amount of efforts have been dedicated to acquiring a more sensitive, accurate and clearer diagnoses imaging.142,143 thus combing different diagnoses imaging techniques in one platform becomes a promising prospect.144 The multimodal imaging system could substantially enhance the versatility for the biomedical applications, especially on the conditions where more than one stimulus-responsiveness can be easily achieved by using polymers containing various functional groups. As in the early stage of disease diagnosis, MRI could be combined with other imaging patterns for a more accurate clinical diagnosis result, including optical, ultrasound, and X-ray-based CT imaging, etc.1,145–147 Ling et al. utilized self-assembled IONPs with pH-responsive ligands to construct the pH-sensitive magnetic nanogranades (PMNs) as an early stage tumor diagnosis probe, which would switch the surface charge to a positive state for enhanced tumor cell internalization once exposed to the acidic tumor microenvironment. Then they disassembled into a highly active state and turned on the unique $T_1$ MR contrast and fluorescence signal when reached upon the more acidic lysosomes (Figure 10a,b). Compared to the pH-insensitive nanoparticle assemblies (Ins-NPs), PMNs showed significant $T_1$ enhancement and high-resolution fluorescent imaging of ultrasmall HCT116 tumors after intravenous injection (Figure 10c–e). Moreover, PMNs exhibited long blood half-life and provided opportunities for enhanced tumor accumulation.

In another case, Yang et al. reported redox-responsive magnetic nanovectors (RMNs) through self-assembling of SPIONs and polymeric ligands that contain disulfide bonds, while Cy5.5 labeled HSA was loaded into the magneto-core-shell structures at the same time. The redox-sensitive disassembly behavior of the RMNs facilitated substantial accumulation of SPIONs at the target tissue, which was serving as an enhanced negative $T_2$ contrast agent. The resulting RMNs-HSA-Cy5.5 system not only showed MR imaging capacity, but also simultaneously presented near-infrared fluorescence imaging feature once reached the redox environment in the tumor. The in vivo results revealed its excellent function as a dual-modal imaging probe for early stage cancer diagnosis.

4.2. Drug Delivery. Conventional drug molecules without the delivery system often suffer from the harsh internal environment with metabolic instability, nonspecific toxicity, and poor treatment effects. As one of the most extensively explored classes of nanosystems suitable for drug delivery, MNPs can both enhance the drug delivery effect and also monitor the treatment’s efficacy via imaging techniques supplied by the magnetic cores or the polymers and loadings, with improved stability and structural integrity in the biological environment without significant detrimental effects.149–151

Normally, MNPs are widely used in the drug delivery system based on the covalent and the noncovalent methods of drug loading mediated by polymers. With the external stimuli or changes in physiological conditions, MNPs display diverse drug release behaviors at the targeted locations. For example, Kim and co-workers reported a one-step fabrication of spontaneous self-assembly of the water-insoluble prodrug $\mu$-oxo-bis(N,N’-ethylenediamine (salicylideniminato)iron) [Fe(salen)] (magnetic core) and polypyrrole (PPy)-b-polycaprolactone (PCL) smart diblock copolymers, where PCL acted as a heat-responsive core scaffold, and PPy served as an electronic core-size controller and pH-responsive shell. Moreover, the as-synthesized core-shell magnetic nanocomposite had a high-loading capacity (~90%) and also exhibited high colloidal stability, biocompatibility, and thermo-stability for effective drug delivery. Another multifunctional core-shell magnetic polymer nanocomposite consisting of a closely packed magnetic Fe$_3$O$_4$ “nanocore”, an acid hydrolysis PLGA “nanoshell”, a PVP stabilizer and a Herceptin targeting ligand, were rational designed to serve as a novel drug delivery system in terms of the targeted delivery and sustained release of therapeutic agent to the breast cancer cells. The as-synthesized nanocomposites exhibited pH-dependent feature that could switch between the intact and activated conditions in blood circulation (“Off” state) and tumors (“On” state) to release the loading drugs at the desired sites.

However, as delivery vehicles, not merely the loading and protection capability of various therapeutic agents, but also the metabolic stability suffering from the harsh internal environment and the targeting efficiency to the specific cell and tissue should be emphasized. Therefore, another reported approach to increase the drug accumulation in the target tissue is to utilize MNPs under the assistance of an external magnetic field to provide direct guidance, in which MNPs could be appealed to the targeted site when the magnetic force exceeds the hydrodynamic drag forces exerted by the blood flow in vivo. Nevertheless, heavy dependency on the external magnetic field of MNPs hinders their practically clinical applications for field-directed targeting behavior.

Recently, Zhang et al. reported a nonviral and magnetic field-independent gene transfection approach using magnetosome-like ferrimagnetic iron oxide nanochains (MFIONs) to genetically engineer mesenchymal stem cells (MSCs) for highly efficient poststroke recovery. The compositions of MFIONs are cubic ferrimagnetic iron oxide nanocubes
(FIONs) but not conventionally spherical SPIONs. In aqueous solution, hydrophilic polymer and the permanent magnetic dipole interactions devoted to spontaneous self-assembly into 1D nanochains, which combined pDNA under the assistance of the branch positive charge PEI through electrostatic interactions. The facilitated cellular internalization and successfully overexpression of brain-derived neurotrophic factor (BDNF) were detectable, and the gene delivery capability of MFION was confirmed in the genetic engineering of MSCs. It is the first publication reported to dramatically enhance the therapeutic performance in ischemic stroke recovery using MFION-engineered MSCs (Figure 11).

In order to cater for the needs in clinical applications, multifunctional magnetic polymer nanocomposites with adjustable MNPs/drug loading capabilities and drug release rates are gaining much more important status for the safety and practical use in the delivering systems. Thus, the fabrication of a multifunctional magneto-vesicles (MVs) comprising tunable layers of densely packed SPIONs in membranes and therapeutic agents encapsulated in the hollow cavity of MVs were described by Yang and co-workers (Figure 12a). The release of payload doxorubicin could be tuned by varying the membrane thickness of nanovesicles based on the poly(styrene)-b-poly(acrylic acid) (PS-b-PAA). Besides, due to the high packing density of SPIONs, the multilayered MVs (MuMVs) showed the highest magnetization and $r_2$ in MRI compared to the monolayered MVs, double-layered MVs and individual SPIONs. Under the synergetic effect of magnetic and active targeting, doxorubicin-loaded MuMVs conjugated with RGD peptides could be effectively enriched within tumor sites after intravenous injection (Figure 12b). The magnetic-field enhanced accumulation of MuMVs was more significant than individual SPIONs. Moreover, the group of RGD-Dox-MuMVs (magnet + ) exhibited the strongest fluorescence of Dox in tumors tissues among all the groups and a 1.6-, 1.3-, 11.8-fold increase for the groups of Dox-MuMVs (magnet + ),
RGD-Dox-MuMVs (magnet − ), and RGD-Dox-MuMVs (magnet + ), respectively, indicating the enhanced delivery efficacy thanks to a synergetic magnetic and active targeting strategy (Figure 12c,d).

4.3. Therapeutic Platform. Another branch of applications concerning polymer-assisted MNP assemblies that being widely investigated is their inherent therapeutic efficiency. Utilizing the intrinsic property of IONPs, which could generate heat when exposed to an alternating external magnetic field (AMF), is referred to as magnetic hyperthermia. Consequently, the calorific nanoparticles could increase the temperature of the surrounding tissues, which could be utilized to induce the cell death of tumor tissues. The magnetic hyperthermia therapy, namely noninvasive and selective for cancer treatment, has been realized to be one of the most promising therapeutic tools. However, the conventional IONPs-mediated magnetic hyperthermia is currently limited to the treatment of localized and relatively accessible cancer tumors because the required therapeutic temperatures above 40 °C can only be achieved by direct intratumoral injection. Hence, the novel nanomaterials which could guarantee sufficient intratumoral temperatures and accumulation efficiency at tumor sites following systemic administration under AMF are highly desirable. Recently, an efficient magnetic nanocluster composed of cobalt- and manganese-doped, hexagon-shaped IONPs (CoMn-IONP) and poly(ethylene glycol)-b-poly(ε-caprolactone) (PEG–PCL) was developed to fit the need of high heating efficiency via systemically delivery.157 Encouragingly, compared to the 38.5 °C caused by spherical IONPs, the intratumoral temperature of the CoMn-IONP nanoclusters reached 44 °C after 12 h postintravenous injection. Furthermore, magnetic hyperthermia mediated by CoMn-IONP nanoclusters significantly inhibited the growth of subcutaneous ovarian tumors under a safe AMF frequency (Figure 13). The nanoclusters and their capability to achieve therapeutically relevant temperatures provide a new possibility for tumor treatment by magnetic hyperthermia, either alone or in combination with...
other therapeutic modes including radiation, chemotherapy, or immunotherapy.

In addition, the development of magnetic nanoclusters (MNCs) by encapsulating Mn$_{x}$Zn$_{1-x}$Fe$_2$O$_4$ magnetic nanoparticles into amphiphilic block copolymer matrix for magnetic fluid hyperthermia (MFH) in vitro exhibited superparamagnetic characteristics, high specific absorption rate (SAR), large saturation magnetization ($M_s$), excellent stability, and good biocompatibility. It is clearly shown in these studies that the cancer cell death rate could be reached up to 90% within 15 min after the treatments of MnFe$_2$O$_4$/MNC and Mn$_{0.6}$Zn$_{0.4}$Fe$_2$O$_4$/MNC under optimized conditions of AMF. Moreover, the apoptosis mechanism of cell death was also investigated. Since the efficiency of magnetic hyperthermia is significantly affected by the size, composition, and structures of the MNP assemblies, further optimized processes may need to be taken into account to develop novel MNP assemblies for more future applications.

From another perspective, as a critical component of MNP assemblies, polymers present strong controllability and diversity that could synergistically combine with other treatment modalities such as chemotherapy, radiation therapy, gene therapy and photodynamic therapy, etc. For instance, the treatment pattern of MNP assemblies can be turned into photodynamic therapy (PDT), which is also a minimally invasive therapeutic procedure for cancer treatment. The exerting of PDT relies on cytotoxic singlet oxygen generation (SOG) produced by photosensitizers under irradiation, while the indiscriminating damage to the normal tissues was seen as troubling to the treatment. Recent compelling evidence reported by Yang et al. was a hierarchical tumor acidity-responsive magnetic nanobomb (termed HTAMN) developed for PDT and diagnostic imaging. The chlorin e6 (Ce6), which was employed as a photosensitizer, methoxy poly(ethylene glycol)-b-poly(dopamine-ethylenediamine-2,3-dimethylmaleic anhydride)-l-glutamate-Ce6 [mPEG-b-P(Dopa-Ethy-DMMA)-LG-Ce6] and SPIONs were incorporated into the HTAMN via self-assembly. An improved tumor accumulation and enhanced tumor cellular internalization via pH-induced surface charge switching were achieved, and then HTAMN was further disassembled in more acidic intracellular compartments, which “turn on” the near-infrared fluorescence (NIRF) and initiated SOG generation triggered by the photosensitizer. In comparison to pH-insensitive magnetic nanoparticles, HTAMNs revealed more distinct clarity via a $T_2$-weighted MR/NIRF imaging, enhanced tumor retention and superior in vivo antitumor efficiency (Figure 14).

Another interesting case exhibited the dual-mode heating thermal treatment, including magnetothermal and photothermal therapy via polymer-assisted self-assembled super nanoparticles were consist of superparamagnetic Fe$_3$O$_4$ nanoparticles and photoluminescent PbS/CdS quantum dots (Figure 15). The proposed self-assembled Fe$_3$O$_4$ and PbS/CdS (II-BW) super nanoparticles [SASNs (II-BW)] presented outstanding detectable photoluminescence through a 14 mm tissue, and significantly enhanced $r_2$ relaxivity, which was 4 times higher than the individually dispersed Fe$_3$O$_4$ nanoparticles. Besides, the dual-mode heating studies with SASNs (II-BW) as heating agents showed an extremely efficient heating output at the local site from the pork tissue, demonstrating the potential use for deep-tissue dual-mode (magnetic resonance and photoluminescence) in vivo imaging, while could simultaneously provide the possibility of SASNs (II-BW)-mediated amplified dual-mode heating treatment for cancer therapy. Therefore, the combination of favorable MNP assemblies-based thermal treatment, as well as other effective therapeutic methods, may allow simultaneous tumor diagnosis
and therapy toward personalized precise cancer treatment in the future.

5. CONCLUSIONS AND FUTURE PERSPECTIVES

The assembly of MNPs opens up new avenues to achieve the desired and favorable physicochemical properties for various biomedical applications. In contrast to individually dispersed MNPs, assembling of MNPs offers distinct constructions, physicochemical characteristics, as well as different in vivo circulation, distribution, and elimination pathways. Manipulation of MNP assemblies can enable target-amplified imaging and responsive drug release for local, on-demand applications with reduced side effects and enhanced treatment efficacy. Although some progress in the design and synthesis of MNP assemblies has been achieved, the precise control of the assemblies is still in the initial stage, such as the architecture, size distribution, the location of NPs in the assemblies and their stability, etc.

The utilization of polymers has been demonstrated to be a powerful tool to assist assembly of MNPs with improved precision of control, biocompatibility, and multifunctionality, in which the delicate interaction forces between MNPs with polymers, polymers with solvent, as well as MNPs with MNPs direct the assembling process offering facile control over self-assembly to achieve various sizes and shapes. This review provides an overview of recent progress in MNP assemblies mediated by polymers, we briefly introduce the interactions between the blocking units, assembly methods as well as their biomedical applications. The state-of-the-art assembly strategies including template-free and template-based approaches have been highlighted. Important intrinsic characteristics, such as magnetic properties and magnetothermal effect, which

Figure 14. Design and utilization of the hierarchical tumor acidity-responsive magnetic nanobomb for PDT and diagnostic imaging. (a) The formation and mechanism of pH-induced surface charge switch and pH-dependent disassembling process of HTAMN in the extracellular and intracellular of tumor cells; in vivo T2-weighted MRI (b) and NIRF (c) tumor images at 4 and 24 h after intravenous injection of HTAMN or non-HTAMN into tumor models; (d) tumor weights after the treatments of PBS (controlled), free Ce6, non-HTAMN, and HTAMN at the end of the in vivo PDT study. Reproduced with permission from ref 163. Copyright 2019 Elsevier B.V.
present deep insight into the collective functions of the assembled structures have been discussed. Furthermore, we have highlighted the recent cutting-edge research of MNP assemblies based biomedical applications such as biomedical imaging, drug delivery, therapy, and theranostics.

To date, various polymer-assisted MNP assemblies have been continuously developed and applied in the biomedical field, which has achieved great progress in multifunctional and intellectual nanostructures such as stimuli-responsive MNP assemblies. However, it should be noted that the development of these assemblies always demands knowledge from both inorganic chemistry and polymer science. The complexity of the assembling/disassembling process is a challenge, both for most of the up-to-date synthetic and analysis techniques, and for the control of the interactions between two phases to obtain precisely designed materials with attractive and desired functions. Given that the synthesis methods of MNPs are relatively mature, well-defined polymer construction especially for the synthesis or purification of stimuli-responsive copolymers or other functional triblock/multiblock polymeric ligands for MNPs surface coating, as well as the synergic combination of two phases remains challenging for the control over the polymer/MNP interfaces. On top of that, the reproducibility of as-synthesized assemblies from different batches should not be ignored.

Besides, despite the clinical trials with early generations of MNPs, e.g., SPION and USPION, the clinical translation of updated MNP assemblies is still hindered by the variations between different batches, the lack of information in their in vivo toxicological and pharmacokinetic profiles, as well as host immune responses. Moreover, understanding the interactions between the MNP assemblies and diverse biomolecules in the living system is currently limited, and the in vivo behaviors of the assemblies still need further in-depth investigation. In this consideration, polymers and MNPs may need to be evaluated separately in order to provide a full picture of the toxicity profile of MNP assemblies, which is especially a concern for MNP assemblies encompassing both organic and inorganic materials.

In summary, polymer-assisted MNP assemblies are witnessing more applications in precision medicines because of their improved imaging performance with enhanced S/N ratio at lesions and effective disease treatments. Although challenges still remain to be addressed, advances in this field provide a new opportunity for developing novel nanomaterials with controlled responsive behaviors and tunable functionalities, opening a new frontier for biomedical imaging/or drug delivery applications.

Figure 15. Schematic illustration of the formation (a) and utilization (b) of self-assembled Fe₃O₄ and PbS/CdS (II-BW) supernanoparticles; (c) Ex vivo NIR images of SASNs (I-BW) and SASNs (II-BW) through different thicknesses of pork tissue; (d) relaxation rate ($T_2^*$) of SASNs (II-BW) and free Fe₃O₄ NPs; (e) in vivo T₂-weighted MRI of a tumor position taken at preinjection and 12 h intravenous postinjection of SASNs (II-BW); (f) time-dependent temperature curves of different concentrations SASNs (II-BW) under three types of modulation; (g) optical image of pork tissue used for ex vivo dual-modal heating and their thermal images at 3 min postinjection of SASNs (II-BW) under three types of modulations. Reproduced with permission from ref 164. Copyright 2019 American Chemical Society.
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Notes

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