



## Polymeric nanostructured materials for biomedical applications



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### ARTICLE INFO

#### Article history:

Received 12 May 2015

Received in revised form 21 May 2016

Accepted 31 May 2016

Available online 11 June 2016

### ABSTRACT

Polymeric nanostructured materials (PNMs), which are polymeric materials in nanoscale or polymer composites containing nanomaterials, have become increasingly useful for biomedical applications. In specific, advances in polymer-related nanoscience and nanotechnology have brought a revolutionary change to produce new biomaterials with tailored properties and functionalities for targeted biomedical applications. These

**Abbreviations:** 3D, three-dimensional; ACA, alkylcyanoacrylates; AETMAC, 2-acryloxyethyltrimethylammonium chloride; ATRP, atom transfer radical polymerization; AuNPs, gold nanoparticles; bFGF, basic fibroblast growth factor; BM-MSCs, bone marrow-derived mesenchymal stem cells; CD44, cluster determinant 44; CPA, collagen-mimetic peptide amphiphile; CPT, camptothecin; CR-CAs, cancer-recognizable MRI contrast agents; CS, chitosan; CT, X-ray computed tomography; CTA, chain transfer agent; DEXlactateHEMA, dextran-lactate-2-hydroxyethyl methacrylate; DEX-PCL, dextran-block-poly( $\epsilon$ -caprolactone); DexS, dextran sulfate; DLC, drug loading capacity; DLE, drug loading efficiency; DMIPM, 2,3-dimethylmaleic imidopropyl methacrylate; DNA, deoxyribonucleic acid; DOTMA, N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride; DOX, doxorubicin; DTT, 1,4-dithio- $\beta$ -threitol; ECMs, extracellular matrices; EGDMA, ethylene glycol dimethacrylate; EPR effect, enhanced permeation retention effect; FDA, US Food and Drug Administration; FDG, fluorodeoxyglucose; FI, fluorescence imaging; FITC, fluorescein isothiocyanate; GA, glutaraldehyde or glycolide; GSH, glutathione; HEA, 2-hydroxyethyl acrylate; HF, hydrofluoric acid; HPMMA, N-(2-hydroxypropyl) methacrylamide; LA, lactide; LBL, layer-by-layer; LCST, lower critical solution temperature; L-Cys NCA, L-cysteine N-carboxyanhydride; LMW, low molecular weight; MBA, N,N'-methylenebisacrylamide; MMA, methyl methacrylate; MMC, mitomycin C; mPEG-PDLLA, monomethoxy poly(ethylene glycol)-block-poly(D,L-lactide); MRI, magnetic resonance imaging; MTX, methotrexate; NIPAM, N-isopropylacrylamide; NK, natural killer; O/W, oil-in-water; OEG-A, oligoethylene glycol acrylate; PAA, poly(L-aspartic acid); PACA, poly(alkylcyanoacrylate); PAMAM, polyamidoamine; PCL, poly( $\epsilon$ -caprolactone); PDLA, poly(D,L-lactide); PDLA, poly(D,L-lactide); PEG, poly(ethylene glycol); PEG-b-PCL, poly(ethylene glycol)-b-poly( $\epsilon$ -caprolactone); PEG-b-PLA, poly(ethylene glycol)-b-polylactide; PEG-b-PLGA, poly(ethylene glycol)-b-poly(lactide-co-glycolide); PEGDA, poly(ethylene glycol) diacrylate; PEG-p(L-His), methoxy poly(ethylene glycol)-b-poly(L-histidine); PEG-p(L-LA)-DTPA-Gd, methoxy poly(ethylene glycol)-b-poly(L-lactic acid)-diethylenetriaminopentaacetic acid dianhydride-gadolinium chelate; PEO-PAA-PNIPAM, poly(ethylene oxide)-b-poly(acrylic acid)-b-poly(N-isopropylacrylamide); PEG-PAC, acid-functionalized poly(carbonate) and poly(ethylene glycol) diblock copolymer; PEG-PCL, poly(ethylene glycol)-block-poly( $\epsilon$ -caprolactone); PEG-PCL-PEG, poly(ethylene glycol)-poly( $\epsilon$ -caprolactone)-poly(ethylene glycol); PEG-PUC, urea-functionalized poly(carbonate) and poly(ethylene glycol) diblock copolymer; PEI, polyethylenimine; PEO, poly(ethylene oxide); PEO-PBLA, poly(ethylene oxide)-poly( $\beta$ -benzyl L-aspartate); PET, positron emission tomography; PGA, poly(L-glutamic acid); PiPrOx-P(Asp), poly(2-isopropyl-2-oxazoline)-b-poly(aspartic acid); PiPrOx-P(Lys), poly(2-isopropyl-2-oxazoline)-b-poly(L-lysine); PK1, HPMA copolymer-DOX conjugate; PLA, poly(L-lactic acid); PLGA, poly(DL-lactide-co-glycolide); PLL, poly(L-lysine); PLLA, poly(L-lactide); PMMA, poly(methyl methacrylamide); PMPA, poly[(3-morpholinopropyl) aspartamide]; POEGMA, poly(oligo(ethylene glycol) methacrylate); poly(S-co-MAA), poly(styrene-co-methacrylic acid); PTX, paclitaxel; PU, polyurethane; PVA, poly(vinyl alcohol); PVAc, poly(vinyl acetate); QDs, quantum dots; RAFT, reversible addition-fragmentation chain transfer; RGD, Arg-Gly-Asp; RHAMM, receptor for hyaluronate-mediated motility; RNA, ribonucleic acid; ROP, ring-opening polymerization; S/O/O, solid-in-oil-in-oil; SCK, shell crosslinked; SI-ATRP, surface initiated atom transfer radical polymerization; siRNA, short interfering RNA; SPECT, single-photo emission computed tomography; SPIO, super paramagnetic iron oxide; tBMA, *tert*-butyl methacrylate; TPT, tetraaniline-polylactide-tetraaniline; USPIO, ultra-small super paramagnetic iron oxide; VEGF, vascular endothelial growth factor; W/O, water-in-oil; W/O/O, water-in-oil-in-oil; W/O/W, water-in-oil-in-water;  $\beta$ -CD-S, thiol- $\beta$ -cyclodextrin.

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**Keywords:**  
 Polymer  
 Nanostructure  
 Biomaterials  
 Nanocarriers  
 Biomedical applications

materials, including micelles, polymersomes, nanoparticles, nanocapsules, nanogels, nanofibers, dendrimers and nanocomposites, have been widely used in drug delivery, gene therapy, bioimage, tissue engineering and regenerative medicine. This review presents a comprehensive overview on the various types of PNMs, their fabrication methods and biomedical applications, as well as the challenges in research and development of future PNMs.

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## 1. Introduction

Polymeric nanostructured materials (PNMs) have been playing an increasingly important role to revolutionize the diagnoses and treatments of diseases [1–3]. Through the development of PNMs as new biomaterials, significant improvement in the quality of health care can be achieved, due to the better accuracy and reliability in diagnostics, more effective targeting of therapeutic agents, and improved usability of scaffolds for tissue engineering and regenerative medicine, just to name a few [1–16].

PNMs (Fig. 1), including micelles, polymersomes, nanoparticles, nanocapsules, nanogels, nanofibers, dendrimers, brush polymers and nanocomposites, can be prepared in a variety of pathways. Their properties, such as stability, size, shape, surface charge, surface chemistry, mechanical strength, porosity, and so on, can be tailored toward the specific functionalities that are required to meet the needs of the targeted biomedical application. As a result, the development of biomedical PNMs has attracted a great deal of research interests in the field, where a very large number of recent publications can be found in the literature [17–26]. In general, PNMs for biomedical applications should have: (a) significant water solubility or dispersibility, (b) well-controlled nanoparticle dimension to avoid fast clearance (10–200 nm) and to achieve preferred biodistribution, (c) biodegradability to minimize side effects (residue with MW < 45K or hydrodynamic size <10 nm for complete clearance from circulation), (d) functionality to link with prodrug, targeting component, or

imaging element, etc. (e) responsivity to release therapeutic loading under triggered conditions.

One unique property of PNMs, in particular those intended for nanocarrier usage such as contrast agents or therapeutic agents in cancer diagnosis or cancer therapy, is related to their prolonged blood circulation time, which allows the agent to have more opportunity to accumulate in the tumor cells via either the “passively” or “actively” route [5,27–29]. This is in contrast with small molecules agents or drugs that often extravasate from vascular structures rapidly, where more than half of the injected dose can be gone from the blood circulation in just one or two passes of the cardiac cycle. Thus, for bioimaging applications, the contrasting agents based on larger polymeric nanoparticles not only could remain in the vasculature to give higher angiography contrast, but the tumor assessment could also be less hindered by the rapidly changing arterial input function [30]. For therapeutic applications, PNMs also offer better efficacy and lower systemic toxicity because of the enhanced targeting and sustained release ability of polymeric nano-drugs [8,31,32]. For these reasons and other considerations such as high tunability, good biocompatibility and diversity, PNMs shall play a more vital role in the future for biomedical applications, especially for the development of new controlled-released drugs.

Due to the increasing interests and great potentials of PNMs for biomedical applications, this review is intended to give an overview of the current state-of-the-art fabrication methods to produce PNMs and their intended biomedical applications. The challenges and outlook of developing new PNMs for future applications will be also discussed.

## 2. Fabrication of polymeric nanostructured materials

### 2.1. Micelles

Polymeric micelle is a sub-microscopic aggregate of polymeric molecules dispersed in liquid, forming a colloidal system. It typically has a core–shell architecture, where the inner core is composed of the hydrophobic part of the amphiphiles, and the core region, surrounded by a corona, is composed of the hydrophilic part of the amphiphiles [33]. The micelle formation is a process of the force balance between the attractive and repulsive interactions. There are different types of attractive forces, which govern the segregation of the core segment from the aqueous phase. The forces include hydrophobic interactions, electrostatic interactions, and complexation [34]. The repulsive forces can be caused by different sources such as electrostatic repulsion, hydration, and steric interactions. These forces prevent the unlimited growth of the micelles [35,36]. Similar to low molecular weight

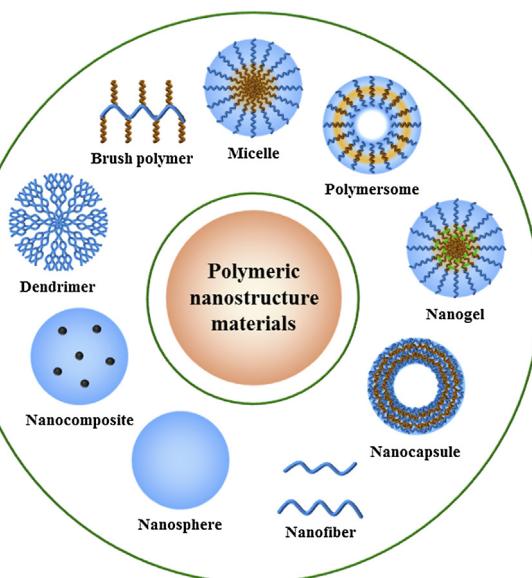


Fig. 1. Polymeric nanostructured materials for biomedical applications.

**Table 1**

Selective methods to prepare polymeric micelles with different compositions.

Preparation method	Composition	Driving force	Refs.
Direct dissolution	Pluronic block copolymer	Hydrophobic interaction	[42,43]
	Poly(2-isopropyl-2-oxazoline)- <i>b</i> -poly(L-lysine);	Electrostatic interaction	[44]
	Poly(2-isopropyl-2-oxazoline)- <i>b</i> -poly(aspartic acid);		
	Methoxy poly(ethylene glycol)- <i>b</i> -poly(L-glutamic acid);	Electrostatic interaction	[45]
	Doxorubicin hydrochloride		
Film casting	Poly(ethylene glycol)-poly(glutamic acid) + cisplatin	Complexation	[46]
	Methoxy poly(ethylene glycol)- <i>b</i> -block-poly( $\delta$ -valerolactone)	Hydrophobic interaction	[47]
	Methoxy poly(ethylene glycol)- <i>b</i> -block-poly(D,L-lactide)	Hydrophobic interaction	[48]
	Acid-functionalized poly(carbonate) and poly(ethylene glycol) diblock copolymer;	Hydrophobic and electrostatic interaction	[49]
	Urea-functionalized poly(carbonate) and poly(ethylene glycol) diblock copolymer;		
	Thioridazine hydrochloride		
	Methoxy poly(ethylene glycol)- <i>b</i> -block-decyllamine-grafted poly(L-aspartic acid);	Hydrophobic interaction	[50]
	Cypate		
Dialysis	Poly(ethylene oxide)-poly( $\beta$ -benzyl L-aspartate)	Hydrophobic interaction	[51]
	Poly(2-(4-vinylbenzyl)oxy)-N,N-diethylnicotinamide);	Hydrophobic interaction	[52]
	Paclitaxel		
	Poly(ethylene oxide)- <i>b</i> -poly( $\epsilon$ -caprolactone)	Hydrophobic interaction	[53]
	PEG-dendritic polylysine-camptothecin	Hydrophobic interaction	[54]
	poly(ethylene glycol)- <i>b</i> -poly( $\epsilon$ -caprolactone);	Hydrophobic and electrostatic interaction	[55]
Oil-in-water emulsion	Poly( $\epsilon$ -caprolactone)- <i>b</i> -poly(2-aminoethyl ethylene phosphate); siRNA		
	Poly(ethylene glycol)- <i>b</i> -block-poly(alkyl acrylate-co-methacrylic acid)s;	Hydrophobic interaction	[56]
	Fenofibrate		
	Poly(ethylene glycol)- <i>b</i> -poly(alkyl acrylate-co-t-butyl methacrylate);	Hydrophobic interaction	[57]
	Fenofibrate		
	Methoxy poly(ethylene glycol)- <i>b</i> -block-poly(D,L-lactide);	Hydrophobic interaction	[4]
	Poly(D,L-lactide);		
	Docetaxel		

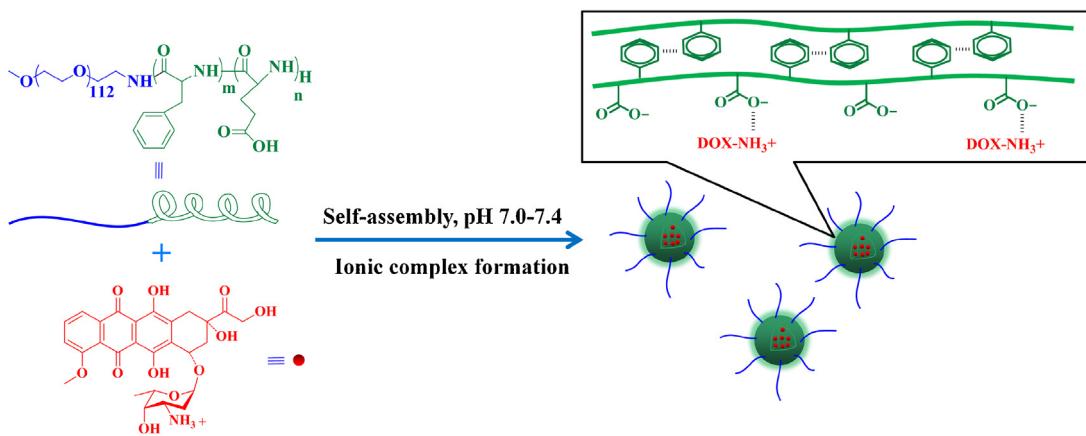
surfactants, amphiphilic copolymers also possess a critical micelle concentration (CMC) in the process of micelle formation. At concentrations below CMC, the copolymers only exist as individual molecules in solution. When the concentration is above CMC, the amphiphilic copolymer chains can associate and form micelles in a way that the hydrophobic part of the copolymer would avoid direct contact with the aqueous media [35]. Polymeric micelles are generally composed of several hundred molecules, where the corresponding diameter typically ranges from 10 to 100 nm [34]. The micellization process is entropy-driven and has an endothermic enthalpy [37,38]. Based on light scattering, sedimentation velocity and small-angle X-ray scattering studies, the aggregated polymeric molecules in the micelle are in a dynamic equilibrium with the unimers in the bulk phase [33,39–41]. The fabrication methods to prepare polymeric micelle can be classified into four categories: direct dissolution, dialysis, film casting and oil-in-water (O/W) emulsion, which are described below. The selection of the proper method must consider the solubility of the polymers in water. Some representative examples of polymeric micelles and their compositions are illustrated in Table 1.

### 2.1.1. Direct dissolution

The direct dissolution method is usually employed to prepare micelles from block or graft copolymers with moderate solubility in water. The method simply involves the addition of copolymers to water or other aqueous

medium such as phosphate buffer saline without using any organic solvents and surfactants. In some cases, copolymer and water are mixed at elevated temperatures to promote micellization [42,58]. The direct dissolution method is routinely used for the preparation of Pluronic (block copolymer of ethylene oxide and propylene oxide) micelles [43]. It can also be used for the fabrication of micelles from poly(ethylene glycol)-polyester copolymers. For example, Qian et al. demonstrated the preparation of blank micelles by directly dissolving poly(ethylene glycol)-poly( $\epsilon$ -caprolactone)-poly(ethylene glycol) (PEG-PCL-PEG) in distilled water at 50 °C. The drug-loaded micelles were subsequently prepared by this method assisted by ultrasonication [58].

The direct dissolution method can also be used to fabricate micelles formed by electrostatic interactions or metal complexation. Kataoka et al. reported the preparation of polyion complex micelles by direct mixing the solutions of poly(2-isopropyl-2-oxazoline)-*b*-poly(L-lysine) [PiPrOx-P(Lys)] and poly(2-isopropyl-2-oxazoline)-*b*-poly(aspartic acid) [PiPrOx-P(Asp)] in a Tris-HCl buffered solution [44]. Similarly, doxorubicin-loaded micelles could be prepared by direct mixing the aqueous solutions of doxorubicin and carboxyl-containing polymers, where the preparation scheme is illustrated in Fig. 2 [45,59]. Polymeric micelles containing platinum-based anticancer drugs were also demonstrated through the polymer–metal complex formation by mixing the aqueous solution of polypeptide and drug [46,60–68].



**Fig. 2.** Schematic illustration of the preparation of the DOX-NPs. [59]. Copyright 2013. Reproduced with permission from Elsevier Ltd.

Although the direct dissolution method is simple, it requires that the hydrophobic segments can be swollen quite extensively in water and reach the equilibrium within a reasonable time period during micelle preparation. However, as most polymeric micelles form very rapidly in water and become kinetically ‘frozen’ once they reach certain size [69,70], the direct dissolution method sometimes becomes inapplicable for the preparation of polymeric micelles with desired structure and properties, therefore, other methods are necessary.

#### 2.1.2. Film casting

The film casting method is often referred to as the dry-down method or the solution casting method, which involves the dissolution of copolymer (or copolymer and drug mixtures) in a volatile organic solvent. In this method, the solvent is first evaporated to create a thin film. Subsequently, a warm buffer solution or water is added under agitation to dissolve the polymer film. The hydrophobic part forms the core of the micelle, while the hydrophilic block becomes hydrated and dissolves to form the shell (corona) [33,71]. Allen et al. reported the micelle preparation scheme by hydrating the amphiphilic copolymer film in a phosphate buffer saline at 60 °C [47,72]. With this method, cancer drug loaded micelles could be prepared by the hydration of paclitaxel containing monomethoxy poly(ethylene glycol)-block-poly(D,L-lactide) (mPEG-PDLLA) films in water [48]. Similarly, thioridazine-loaded micelles were prepared by the hydration of thioridazine containing lipid film based on acid-functionalized poly(carbonate) and poly(ethylene glycol) diblock copolymer (PEG-PAC) and urea-functionalized poly(carbonate) and poly(ethylene glycol) diblock copolymer (PEG-PUC) with phosphate buffered saline (PBS 7.4) at 50 °C [49].

#### 2.1.3. Dialysis

The dialysis method can be utilized for micelle preparation if the copolymer has low solubility in water. In this method, the micelle-forming copolymer is first dissolved in a water miscible organic solvent, such as ethanol [57],

acetone [73], dimethylsulfoxide [53,55], dimethylformamide [53,54], N,N-dimethylacetamide [49,53], tetrahydrofuran [53], acetonitrile [52], where the dialysis is carried out subsequently against the aqueous media to remove the solvent. The solvent selection in this method can dramatically affect the stability of the polymeric micelles [51]. For example, using dimethylsulfoxide as the initial solvent to dissolve poly(ethylene oxide)-poly(β-benzyl L-aspartate) (PEO-PBLA) and followed by the dialysis against water, only 6 wt% of the total amount of PEO-PBLA could form micelles, where a considerable amount of secondary aggregates was also present. However, when N,N-dimethylacetamide was used as the initial solvent, the micelles were obtained in a very high yield (87 wt%) [51]. Compared to the direct dialysis of polymer solution in the dialysis bag against water, the rapid addition of the organic solution in water and vice versa prior to the dialysis can produce micelles with a more reproducible and narrower size-distribution [53].

#### 2.1.4. Oil-in-water emulsion

The oil-in-water (O/W) emulsion method is usually used for the preparation of drug-loaded micelles, involving the dissolution of the drug and copolymer in a volatile, non-water-miscible organic solvent, such as dichloromethane [56,57,74], ethyl acetate [4,75] and chloroform [76], first. The O/W emulsion is subsequently formed in an aqueous medium by vortexing and sonicating, which is followed by the evaporation of the organ solvent. The O/W emulsion method has an advantage over the dialysis method, where the drug is present in the inner organic droplets of the emulsion that are stabilized by the copolymer. Upon evaporation of the organic solvent, the drug will remain in the core of the micelles, thereby increasing the drug loading capacity. This is contrasted to the dialysis method, in which the drug may precipitate before being incorporated in the micelles if the solvent diffuses rapidly from micelle core, or if the drug molecules interact with each other more favorably than with the copolymer. Sant et al. observed at least 1.5-fold rise in the incorporation efficiency of drug loading by the O/W emulsion method, when compared to the dialysis method [57].

## 2.2. Polymersomes

Polymersomes (also referred to as polymer vesicles) are a class of artificial vesicles made from amphiphilic polymers. Similar to liposomes, typical polymersomes are hollow assemblies that contain an aqueous solution in the core surrounded by a bi-layer membrane shell. However, as a result of their significantly higher molecular weight, polymersomes exhibit enhanced mechanical stability and reduced permeability, possessing an impermeable physical barrier capable of isolating the encapsulated functional materials from the environment. The bi-layer membrane is composed of hydrated hydrophilic coronas, located at both the inside and outside of the hydrophobic part of the membrane. The aqueous core can be utilized to encapsulate therapeutic molecules, such as low molecular weight drugs, proteins and deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) fragments. Therefore, polymersomes have attracted a great deal of research interests recently and have been highlighted for a number of biomedical applications [77–84].

Similar to micelles, hydrophobic interactions are usually the mainly driving force in the polymersome formation. Many techniques can be used to prepare the polymersomes by self-assemble of amphiphilic block copolymers, and the recent advancement in polymer synthesis has made it possible to prepare small to giant polymersomes spanning from nano- to microscales [85], including stimuli-sensitive polymersomes that can respond to an external or internal stimulus [19,86,87], chimaeric polymersomes that contain distinct interior environments separated from the outside by an asymmetric membrane [88], porous polymersomes with tailored permeability [89], and biomimetic and targeting polymersomes that selectively deliver drugs, proteins, and/or imaging probes to the action sites [90,91]. The most important preparation methods to prepare polymersome are generally classified into two groups: the organic solvent based method (by the solvent-switch technique) and the solvent free method (by the polymer rehydration technique). In the following sub-section, these two methods were discussed in detail.

### 2.2.1. Organic solvent-based method

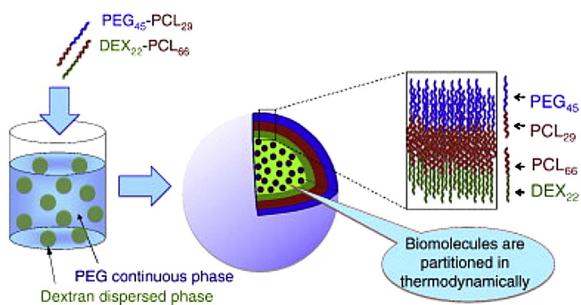
In the organic solvent-based method, polymersomes are formed by first dissolving amphiphilic block copolymers in an organic solvent, which is a good solvent for all the block segments, followed by hydration of the solution. The hydration can be done by either slowly adding water to the organic polymer solution or by injecting the organic solution into water. This procedure renders the hydrophobic blocks insoluble, triggering copolymer self-assembly into polymersomes as a result of increasing interfacial tension between the hydrophobic blocks and water. Therefore, this technique is also called “solvent-switch” or “phase inversion” technique.

Amphiphilic block copolymers can self-assemble into various morphologies like vesicles, spherical and cylindrical micelles, determined by the interfacial area, chain length and volume of the hydrophobic part of the polymer [92]. In the classical description, for PEG based block

copolymers possessing high interaction parameter with water, the vesicular structures are favored when the mass or volume fraction of the PEG ( $f_{\text{PEG}}$ ) is between 10 and 40% (at  $f_{\text{PEG}} = 45\text{--}55\%$ , cylindrical micelles tend to form, and at  $f_{\text{PEG}} = 55\text{--}70\%$ , spherical micelles are predominantly formed) [78]. The polymer architecture can have significant influence on the PEG volume fraction for the formation of polymersomes. For example, Bae and coworkers prepared a series of AB<sub>2</sub> type 3-miktoarm PEG-*b*-(PLLA)<sub>2</sub> copolymers, which mimic the natural structure of phospholipids, and these copolymers showed much broader PEG volume fraction range (0.2–0.7) than their linear diblock counterparts (0.2–0.4) [93]. The vesicular formation can also be affected by the preparation pathway and conditions like polymer concentration, the type of organic solvent and the volume ratio of solvent to water. The size and size distribution of the polymersomes can also be manipulated by selecting different organic solvents or adjusting the stirring rate [94].

Another polymersomes formation method based on organic solvents involves the use of “water-in-oil-in-water” (W/O/W) double emulsions as templates. In this method, W/O/W double emulsions with a core-shell structure are first prepared in capillary microfluidic devices. Amphiphilic diblock copolymers were dissolved in a volatile organic solvent and used as the middle phase, where the subsequent solvent evaporation would lead to the polymersome formation [95]. Weitz and coworkers investigated the evolution of structure during solvent evaporation, and found that the initially homogeneous organic phase underwent a wetting transition, which they called it “dewetting”. They proposed that it is important to consider the concentration of diblock copolymer in the middle phase of the double emulsion, and the presence of excess polymer could result in polymer shells with inhomogeneous thickness [96]. Later, they modified this method by adopting microfluidic devices for fabricating monodisperse polymersomes with biocompatible and biodegradable diblock copolymer poly(ethylene-glycol)-*b*-poly(lactic acid) for efficient encapsulation actives [97]. Furthermore, they fabricated non-spherical multi-compartment polymersomes from W/O/W double emulsions with different morphology as templates using glass capillary microfluidics [98].

There are some other ways to prepare polymersomes. Hauschild et al. reported a novel technique for the preparation of nano-sized, unilamellar polymersomes having a narrow size distribution by inject printers. In this technique, organic solutions of copolymers were injected drop-wise into water using the same technology used by inkjet printer, and the size could be controlled via the amphiphile concentration and cartridge type [99]. In addition, Maglio et al. reported a W/O emulsion-melting-sonication technique, which produced self-organized vesicles from PEO-PCL copolymer [100]. Zhang et al. also developed a unique polymersome system having asymmetric bilayer membrane formed by phase-guided assembly. By adding two diblock copolymers, poly(ethylene glycol)-block-poly( $\epsilon$ -caprolactone) (PEG-PCL) and dextran-block-poly( $\epsilon$ -caprolactone) (DEX-PCL), into a dextran/PEG aqueous two-phase system, DEX-PCL formed the inner leaflet around the dispersed dextran phase and PEG-PCL formed the outer leaflet



**Fig. 3.** Schematic description of the preparation procedure and structure of polymersomes of asymmetrical bilayer membrane. Left diagram: preparation procedure by adding two block copolymers, DEX<sub>22</sub>-PCL<sub>66</sub> and PEG<sub>45</sub>-PCL<sub>29</sub>, into a dextran/PEG aqueous two-phase system; right diagram: polymersome structure consisting of a dextran core in which bio-macromolecules are packed by preferential partition and an asymmetric block copolymer bilayer shell with the dextran block facing the core and PEG block facing the PEG continuous phase. [88]. Copyright 2010. Reproduced with permission from Elsevier Ltd.

with the PEG block facing the PEG continuous phase (Fig. 3) [88]. Finally, Holowka et al. prepared stable vesicles based on amphiphilic poly(L-lysine)-*b*-poly(L-leucine) block copolypeptides that formed stable vesicles in a aqueous solution, driven by the packing of the  $\alpha$ -helical poly(L-leucine) blocks [101].

Despite the fact that the organic solvent based method is the most commonly used method, the residual organic solvent can induce biological toxicity. Furthermore, as traces of organic solvent can reduce the colloidal stability of polymersomes, considerably, extensive dialysis against water is often carried out to ensure the complete removal of organic solvent. However, this process would restrict the biomedical application of polymersomes using this method. In this perspective, the solvent free method has become increasingly more attractive.

#### 2.2.2. Solvent free method

The solvent free method is based on the hydration of amphiphilic block copolymer films to induce self-assembly. In this method, polymers are first dissolved in an organic solvent and then a thin film is produced by evaporation of the organic solvent. Subsequently, the film is hydrated by the addition of water (this method is also called the “polymer rehydration” technique). The critical steps in the formation of polymersomes by the hydration procedure include: water permeation through defects in the polymer layers driven by hydration forces, inflation of polymer layers, and formation of bulges that finally yield vesicles upon separation from the surface [102]. In order to ensure the occurrence of mutual diffusion, an external energy source is required. The most common method used for overcoming the kinetic barrier is mechanical stirring. An alternative method is by using the electroformation of liposomes which is based on the hydration of the amphiphilic film under an a.c. electrical field that enhances water diffusion across the bulk copolymer [103]. Typically, the polymer rehydration technique produces polymersomes with a broad size distribution. Therefore, the final polymersomes are often size-regulated by sequential extrusion

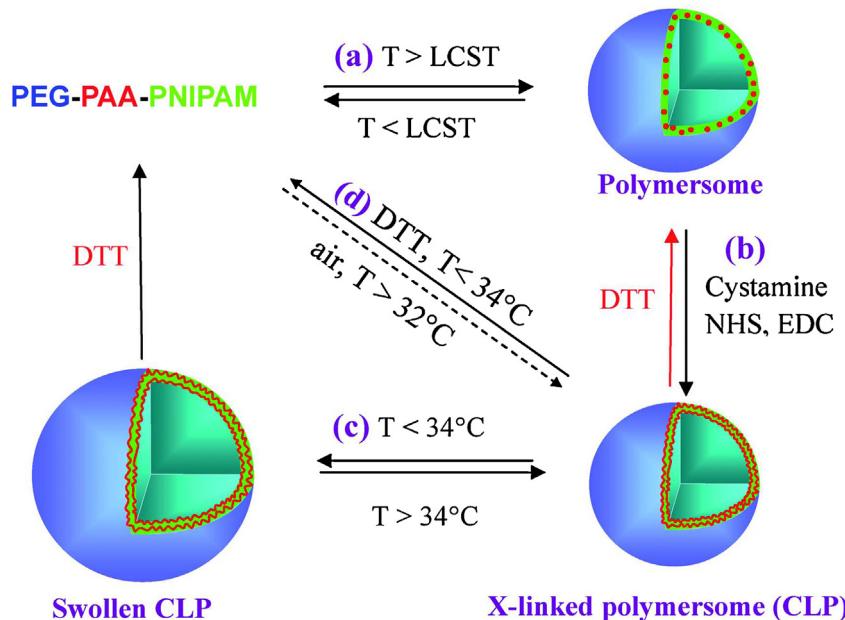
through filters with different pore dimensions under high pressure [104,105].

As mentioned above, the size distribution of polymersomes produced by this method is polydisperse. Recently, Howse and coworkers have reported a method for the production of giant polymersomes having controlled size distributions by combining photolithography with bulk phase dewetting. This enables the spontaneous creation of unilamellar polymersomes with a narrow size distribution, regulated by the pre-patterned substrate [106]. An alternative direct rehydration process was also proposed by O’Neil and coworkers [107]. They demonstrated that polymersomes could be effectively formed by direct hydration of polymers composed of poly(ethylene glycol)-*b*-poly(propylene sulfide) (PEG-PPS) AB diblock copolymers blended with poly(ethylene glycol) dimethyl ether ( $M_w$  500 Da, or PEG 500 DME). This method leads to high encapsulation efficiency when compared to that typically observed by the thin film hydration method. Reversibly crosslinked temperature responsive polymersomes was also prepared through the self-assembly of triblock copolymers poly(ethylene oxide)-*b*-poly(acrylic acid)-*b*-poly(*N*-isopropylacrylamide) (PEO-PAA-PNIPAM) followed by crosslinked at the interface using cysteamine [108]. The crosslinked polymersomes showed remarkable stability against high salt conditions and change of temperature in water, but they could be rapidly dissociated under reductive conditions mimicking the intracellular environment (Fig. 4). In summary, the direct hydration method is normally limited to polymers with relatively high chain flexibility, thus it cannot be used for glassy and crystalline copolymers because of the kinetic restriction.

#### 2.3. Nanoparticles

In this section, nanoparticle refers to homogeneous nanosized solid particle. Nanoparticles are widely used as drug carriers, in which drugs can be either entrapped inside or adsorbed on the surface. Compared to other drug carriers, nanoparticles are stable and tight, and can be easily prepared and piloted. Drug-loaded nanoparticles have been applied for subcutaneous or intravenous injection, oral administration and so on.

For the preparation of nanoparticles, most methods are carried out in the emulsified system, involving two steps: preparation of an emulsified system, and the formation of nanoparticles by precipitation/gelation of a polymer or by polymerization of monomers [109]. A few other methods, which do not require the preparation of an emulsion during nanoparticle fabrication, are also demonstrated. For example, the microfabrication process has been used to make nano/microparticles with a monodisperse size distribution [110], where solid templates were used to make particles. Different than the solid template approach, Park and coworkers developed a hydrogel template approach and demonstrated that this simple process could prepare nano/microparticles at a fast rate, suitable for scale-up production [111]. In addition, nanoparticles can be fabricated through an inter/intramolecular crosslinking process [112,113]. However, the emulsification and emulsion polymerization pathways remain to be the most commonly



**Fig. 4.** Illustration of reversibly crosslinked temperature-responsive nano-sized polymersomes. (a) Formation of polymersomes by simply increasing temperature; (b) stabilization of the vesicular structure via crosslinking at the interface between the membrane and hydrophilic PEG layer; (c) formation of swollen polymersomes by decreasing temperature below its LCST; (d) destabilization of polymersomes by decreasing temperature below its LCST in the presence of 10 mM 1,4-dithio-*D,L*-threitol (DTT). [108], Copyright 2009. Reproduced with permission from the Royal Society of Chemistry.

used methods for the preparation of polymeric nanoparticles [114,115].

### 2.3.1. Emulsification

The first step of the emulsification method is the preparation of an emulsified system. Typically, this step is accomplished by a high-energy emulsification technique such as mechanical shearing, creating emulsions with droplets of uniform size [116]. The droplet size and the distribution of emulsions can significantly affect the physical, physicochemical and organoleptic properties of the emulsified system. Narrowly-dispersed emulsion can be made by using the techniques such as capillary [117], microchannel emulsification [118], straight-through microchannel emulsification [119] and microfluidic approach [120]. The size-controlled narrowly-dispersed emulsion can be formulated by the membrane emulsification method [121–123].

Apart from the simple O/W emulsion system [124], novel emulsions having multiple components such as W/O/W [125,126], water-in-oil-in-oil (W/O/O) [127] and solid-in-oil-in-oil (S/O/O) [128] were demonstrated, and they can lead to higher emulsion stability and drug loading efficiency. Furthermore, the applications of mini-emulsions, nano-emulsions and micro-emulsions instead of the classical emulsions were also demonstrated [129–132].

The second step of the emulsification method to fabricate polymeric nanoparticles usually involves a precipitation method. In this step, solvent of emulsions can be removed from the organic phase by various pathways such as solvent evaporation [133,134], fast diffusion after dilution [135], or salting out [136] process.

Polymeric nanoparticles can also be produced by the gelation of droplets in the emulsion [137,138].

### 2.3.2. Emulsion polymerization

The emulsion polymerization method is another well-known technique to fabricate polymeric nanoparticles. However, in most cases, the polymerization process can lead to non-biodegradable nanoparticles, unsuitable for medical applications in the human body. So far, only a few monomers are suitable to produce biodegradable nanoparticles for in vivo applications, where the most notable system is alkylcyanoacrylates (ACA) and their derivatives [139,140]. The ACA monomers are very reactive, and the anionic polymerization process is usually spontaneously and can be quickly initiated by small amounts of a weak base such as hydroxyl ions of water (the traditional radical emulsion polymerization is not applicable to ACA). Thus, poly(alkylcyanoacrylate) (PACA) nanoparticles can be prepared by anionic emulsion polymerization of monomers initiated by not only the hydroxyl groups in water, but also any types of nucleophilic groups in the molecules dissolved in the polymerization system. To slow down the polymerization rate, the polymerization process is often performed in the acid conditions to enable the formation of nanoparticles instead of polymer aggregates [141]. In addition, suitable nucleophilic components that can initiate the polymerization of alkylcyanoacrylates are often embedded in the polymer structure to facilitate the nanoparticle formation. Other emulsion polymerization methods, such as redox radical emulsion polymerization and mini-emulsion polymerization, can also be used to prepare PACA nanoparticles [131,142–146].

The main problem for the emulsion polymerization method is that some unreacted monomer or oligomer may be present in the formed nanoparticles, while this may cause toxic in clinical use.

#### 2.4. Nanocapsules formed on sacrificial templates

Nanocapsules are hollow spherical structures with dimensions in the sub-micrometer region. Typical nanocapsules are composed of a polymer shell and a hollow inner space. Numerous approaches have been developed to fabricate nanocapsules. In order to distinguish them from polymersomes, we will focus on the nanocapsules that are formed on sacrificial templates in this sub-section. Generally, polymer shell is formed around a pre-formed template particle that can be subsequently removed, resulting in the formation of an empty polymeric shell [147].

##### 2.4.1. Layer-by-layer self-assembly

A popular method to fabricate polymeric shell based on template particles is through the charged surfaces of polyelectrolyte self-assemblies [147–149]. In this approach, oppositely charged polyelectrolytes are used to apply layer-by-layer (LbL) deposition steps, resulting in the formation of multilayered polyelectrolyte shells. The thickness of the polymeric shell can be controlled by the number of polyelectrolyte layer [150]. Briefly, solid particles with charged surface are used as the templates, followed by immersing the template particles into the solution of polyelectrolytes carrying opposite charges. Consequently, a polymeric layer is formed on the surface through electrostatic interactions. As the surface charge of the polymer-coated particles converses, the free ionic groups can adsorb another layer of polyelectrolytes with opposite charges.

A class of commonly used templates for LbL electrostatic self-assembly is functionalized polystyrene latex particles bearing surface charges. For instance, sulfonated polystyrene beads were good templates, where chitosan and polyglutamic acid with low molecular weights could be alternately assembled onto the particles [151]. The template polystyrene core was subsequently removed by dissolving the nanoparticles in tetrahydrofuran. The sizes of the nanocapsules obtained were found to vary from 110 to 990 nm, depending on the size of the polystyrene cores used.

Silica nanoparticles and mesoporous silica particles, which are slightly negatively charged, are another type of templates suitable for polyelectrolyte self-assembly. Itoh et al. developed nanocapsules composed of chitosan (CS) and dextran sulfate (DexS) through the LbL approach [152]. Cationic CS was first adsorbed onto the surface of the template particles, and anionic DexS was then absorbed to form the next layer. After repeated LbL deposition of CS and DexS and etching of the silica cores using hydrofluoric acid (HF), biodegradable hollow capsules were obtained. Mesoporous silica particles can offer some unique advantages. For example, a model drug, fluorescein isothiocyanate (FITC) labeled albumin, could be first loaded into the pores of mesoporous silica particles [153], where the

nanocapsules were formed by the LbL assembly of CS and DexS. As a result, after removal of the silica nanoparticles, drug-loaded hollow CS/DexS particles were formed.

Besides the nanocapsules composed of oppositely-charged polyelectrolytes, non-ionic polymeric shells can also be fabricated via stereo-complexation. Kida et al. [154] reported a hollow sphere composed of poly(methyl methacrylamide) (PMMA). The PMMA stereo-complex, a double-stranded helical assembly formed between isotactic (it) and syndiotactic (st) PMMA units, was used as the component of porous membranes for artificial dialysis. These membranes contained hollow capsules made of PMMA stereocomplex multilayered shells using the alternating LbL assembly approach involving it- and st-PMMA based on the silica template. Van Der Waals interactions are believed to be responsible for the LbL deposition.

##### 2.4.2. Surface initiated *in situ* polymerization

The surface-initiated controlled or “living” radical polymerization method is an effective approach for preparation of inorganic–organic core–shell hybrid nanoparticles having controllable shell structure and thickness. Accordingly, the nanocapsules were achieved by removing the core templates from the core–shell hybrid nanoparticles. In this method, silica nanoparticles are commonly used templates because they can be easily modified and etched. In particular, the hydroxyl groups on the silica surface template can be modified and used as initiators for polymerization of suitable monomers. In “living” polymerization processes, such as surface initiated atom transfer radical polymerization (SI-ATRP), block polymers can be synthesized by the addition of different monomers sequentially. Different from the LbL self-assembly method, the polymer shells that are formed via *in situ* polymerization at the particle surface usually need to be stabilized by crosslinking before the removal of the core templates.

Some examples are as follows. Mu et al. [155] developed a pH-responsive nanocapsule using the SI-ATRP method. First, the bromoacetamide groups were conjugated onto the surface of the silica nanoparticles. The atom transfer radical polymerization (ATRP) of *t*-butyl acrylate and styrene was subsequently initiated to occur at the nanoparticle surface. The polystyrene outer layer was crosslinked by directly exposing the core–shell particles to UV radiation (365 nm). After etching the silica templates with HF and deprotection of the *t*-butyl alcohol side groups, the carboxyl-functionalized crosslinked polystyrene nanocapsules were obtained. When the co-monomers were changed into *t*-butyl acrylate, *N*-isopropylacrylamide (NIPAM) and *N,N*-methylenebisacrylamide (MBA), crosslinked nanocapsules with dual pH- and temperature-sensitive shell were fabricated via a similar approach [156]. In addition, magnetic molecules such as Fe<sub>3</sub>O<sub>4</sub> could be mixed into the silica cores [157]. After SI-ATRP and etching of the silica cores, nanocapsules containing magnetic particles were constructed.

In addition to ATRP, reversible addition-fragmentation chain transfer (RAFT) polymerization is another “living” polymerization process that can be used in this method. Huang et al. [158] reported the fabrication of nanocapsules having size between 450 and 900 nm using the

RAFT polymerization approach. Amino silica nanoparticles were first functionalized by aminosilane agents, and then the dithiocarbonate chain transfer agent (CTA) was conjugated to the amino-functionalized surface of silica nanoparticles. Block copolymers were obtained at the particle surface by copolymerization of *tert*-butyl methacrylate (tBMA) and 2,3-dimethylmaleic imidopropyl methacrylate (DMIPM), followed by successive initiation of 2-hydroxypropyl methacrylamide. With UV crosslinking of the polymer shells and subsequent dissolution of the silica cores in an NH<sub>4</sub>F/HF buffer solution, hollow nanocapsules were obtained.

#### 2.4.3. Polymer monolayers formed by facial adsorption

Besides the electrostatic interactions in the LbL assembly, other forces were also used to induce the adsorption of polymers on the surfaces of nano-sized sacrificial templates. Different from the multilayered hybrid nanocapsules formed via the LbL self-assembly method, the adsorbed polymer monolayers need to be crosslinked in order to gain stability. Some examples are as follows.

Gold nanoparticles (AuNPs) were found to be good sacrificial templates. To be specific, gold nanoparticles can enable the chemical interactions with certain functional groups, such as thiol or dithiocarbonate groups. In a study by Boyer et al. [159], when oligoethylene glycol acrylate (OEG-A) and maleic anhydride block polymers were synthesized via RAFT polymerization, the chain transfer agent at the end of maleic chain could be adsorbed onto AuNPs. The polymer layer, which surrounded the nanoparticles, could be subsequently crosslinked using a small diamine molecule. The removal of the gold cores using aqua regia thus led to the formation of stable, biocompatible, and antifouling hollow polymer nanocapsules. Similarly, AuNPs can bind with thiol-containing polymers, which can also be assembled into polymer shells. For example, Sun et al. [160] demonstrated the fabrication of nanocapsules composed of polycyclodextrin. In this study, thiol- $\beta$ -cyclodextrin ( $\beta$ -CD-S) was first adsorbed onto AuNPs, where the aqueous suspension containing  $\beta$ -CD-S/Au nanoparticles was then added to the dilute solution of excess I<sub>2</sub> in aqueous KI for further oxidation. During oxidation, the Au–S bonds were cleaved while the S–S bonds were formed simultaneously, resulting in the formation of hollow polycyclodextrin nanocapsules.

The Au(0) surface has also been shown to adsorb terminal alkyne groups, forming relatively densely packed and stable monolayer. Zhang et al. [161] demonstrated that AuNPs could be used not only as the template for formation of polymer shell, but also as the catalyst for cross-linking reaction. In their study, linear polymers carrying pendant propargyl ether groups were adsorbed onto the surfaces of AuNPs, where the crosslinking reaction occurred between the alkyne groups (Fig. 5). No additional crosslinking reagents or synthetic operations were required. Clearly, the gold(0)-catalyzed crosslinking reaction is a facile tool to synthesize nanopods.

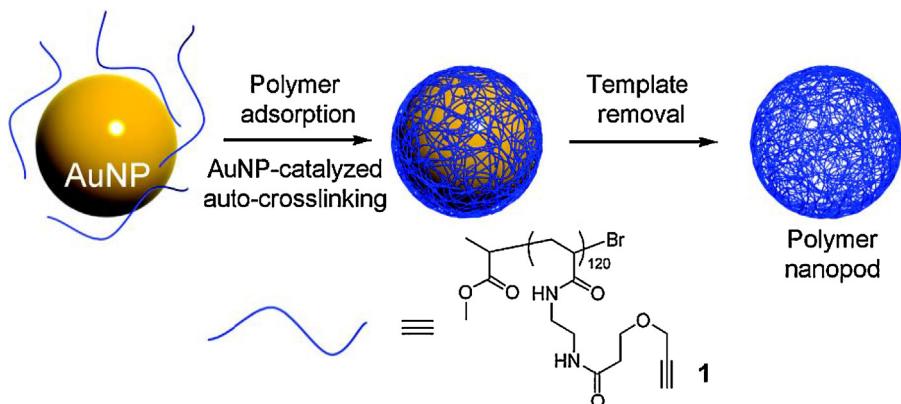
Ionic polymeric nanoparticles have also been used as sacrificial templates. For example, polyacrylic acid or polymethacrylic acid can form solid cores with polystyrene, and polycations can be adsorbed onto them accordingly. In the

study by Liu et al. [162], they demonstrated the fabrication of chitosan nanocapsules by covering CS around the poly(styrene-co-methacrylic acid) (poly(S-co-MAA)) particles. After crosslinking of the polymer shells and etching of the cores, nanocapsules could be obtained. In addition, cationic functional molecules can be trapped by the negatively charged polymer monolayer. For example, Du et al. [163] synthesized Fe<sub>3</sub>O<sub>4</sub> nanoparticles at the surface of the poly(styrene-co-acrylic acid) templates, followed by the coverage of CS monolayer. After crosslinking of the shells and removal of the cores, magnetic particles could be entrapped in the nanocapsule shells.

#### 2.4.4. Self-templating

Recently, a “self-templating” method has also been developed to fabricate nanocapsules. In this method, both the template cores and the polymer shells are formed from the same polymeric materials. One example study was demonstrated by Liu et al. [164]. In this study, poly(vinyl acetate) (PVAc) lattices were prepared by emulsion polymerization. The surface segments of PVAc lattices were hydrolyzed into poly(vinyl alcohol) (PVA) segments. Subsequently, the surface PVA segments were crosslinked using glutaraldehyde (GA). The crosslinked polymeric nanocapsules were achieved after the PVAc molecules encapsulated within the crosslinked polymer shells were removed by dissolution in methanol. A similar method was reported by Dong et al. [165]. In their study, poly(tBA-co-NIPAm-co-AA) terpolymer nanoparticles were synthesized via soapless emulsion polymerization. The surface carboxyl groups of the terpolymer nanoparticles were then crosslinked with calcium ions. Subsequently, the cores were removed with acetone, rendering the formation of hollow nanocapsules.

Moreover, nanocapsules have been designed and prepared by partial removal of the polymeric self-assembled nanostructures or even unimolecular polymeric templates, rendering the shell-crosslinked hollow nanostructures. Wooley and co-workers developed an amphiphilic shell cross-linked knedel-like (SCK) nanostructures as the precursors for preparing nanocapsules, which are formed by self-assembly of the poly(isoprene-*b*-acrylic acid) diblock copolymer in aqueous solution, followed by crosslinking of the poly(acrylic acid) shell with diamino crosslinkers [166]. The nanocapsules were then prepared by selective removing the polyisoprene cores by oxidative scission with ozone, followed by reduction with sodium sulfite. The diameter of the nanocapsules was found to be affected by the length of the diamino crosslinker, which increased from 75 ± 10 nm to 130 ± 35 nm based on TEM measurements by replacing diamino ethylene glycol with diamino poly(ethylene glycol) as the crosslinker. In a subsequent study of the same group, a similar strategy was applied to fabricate a nanocapsule based on a unimolecular brush copolymer [167]. Following the peripheral crosslinking of the poly(acrylic acid) segments of the brush copolymer, the polyisoprene-based core of the brush copolymer was selectively degraded with ozone, resulting in the formation of poly(acrylic acid)-based nanocapsule. In addition, unimolecular dendritic macromolecules containing an inner porphyrin template were developed through a “unimolecular imprinting” approach [168]. The shell-crosslinked



**Fig. 5.** Synthesis of polyvalent propargyl ether nanopods. [161]. Copyright (2010). Reprinted with permission from the American Chemical Society.

dendrimers were prepared by covalent attaching vinyl-containing dendrons to a porphyrin core, and then by crosslinking of the vinyl end-groups. After removing the porphyrin template core through hydrolysis, nanocapsules were obtained as potential synthetic host molecules with nearly homogeneous binding sites.

## 2.5. Nanogels

Polymeric nanogels are swellable nanosized hydrogel dispersions fabricated by physical or chemical crosslinking of hydrophilic or amphiphilic polymer chains [169,170]. Various approaches have been reported for the preparation of polymeric nanogels, such as emulsion polymerization, precipitation polymerization, self-assembly, and “one-step” polymerization in a homogenous solution.

### 2.5.1. Emulsion polymerization

The emulsion polymerization method has been widely used to fabricate nanogels. With this method, polymeric nanogels can be prepared by radical copolymerization of hydrophilic or water-soluble monomers in the presence of difunctional or multifunctional crosslinkers. For example, DeSimone and coworkers have synthesized nanogels by copolymerization of 2-acryloxyethyltrimethylammonium chloride (AETMAC) and 2-hydroxyethylacrylate (HEA) using poly(ethylene glycol) diacrylate (PEGDA) as a crosslinker for cellular gene and antisense delivery [171]. The size of the nanogels was found to be independent of the concentration of the crosslinker in heptane due to the poor solubility of these polymers. Nanogels containing charged monomer showed higher swelling ratios than the nonionic nanogels, and a higher crosslinking density could result in a lower swelling ratio. Although positive charge emerged in the nanogel surface, acceptable *in vitro* biocompatibility was found after the incubation with the HeLa cell line. The ionic nanogels were found to be resistant to aggregation and exhibited good stability in water, making these materials suitable for biomedical applications such as gene and antisense delivery. Smedt and coworkers have reported dextran-based cationic nanogels through copolymerization of dextran hydroxyethyl methacrylate and cationic methacrylate [172]. These nanogels could entrap

short interfering RNA (siRNA) with a high loading efficiency due to electrostatic interactions. These nanogels are biodegradable due to the presence of ester bonds in the side chains of polymethacrylate derivatives, allowing for the sustained release of encapsulated siRNA and the efficient gene silencing over several days. As the degradation kinetics of nanogels can be easily tailored, these materials show great potential for the intracellular controlled release of siRNA.

Various functionalities can be introduced into nanogels through the incorporation of different monomers. For example, Nagasaki and coworkers have synthesized a series of nanogel, containing a crosslinked PEAMA core and a PEG shell, by using emulsion copolymerization [173–175]. Gold nanoparticles (GNPs) could be effectively incorporated into the core at low pH through the self-reduction of chloroaurate ions without the need for reducing agents. The GNPs incorporated nanogels exhibited photothermal efficiency, which could not only trigger the release of the payload, but also induce cell apoptosis due to the irradiation-induced thermal energy. Notably, after the modification with FITC-labeled DEVD peptides, nanogels exhibited less fluorescence in the normal cells due to the fluorescence resonance energy transfer process between GNPs and FITC, whereas marked fluorescence signals were observed in apoptotic cells because of the cleavage of DEVD peptide by caspase-3 and the release of FITC [176]. The fluorescence quenching nanoprobe can provide an effective means in monitoring the early response of cancer cells for therapeutic treatment as well as in high-throughput testing of anticancer drugs using the cell culture approach. In addition, Berkland and coworkers have reported an acid-sensitive nanogel system by polymerization of *N*-vinylformamide using 2-bis[2,2'-di(*N*-vinylformamido) ethoxy]propane as the crosslinker [177]. This nanogel system could be dissociated at low pH due to the cleavage of acid-labile ketal structure, leading to an acid-accelerated release profile of the payload. After hydrolysis of the aldehyde groups, these nanogels formed complexes with plasmid DNA in the presence of primary amines. The acid-labile nanogels having low charges showed more sustained gene transfection and low cytotoxicity compared to the highly charged nanogels.

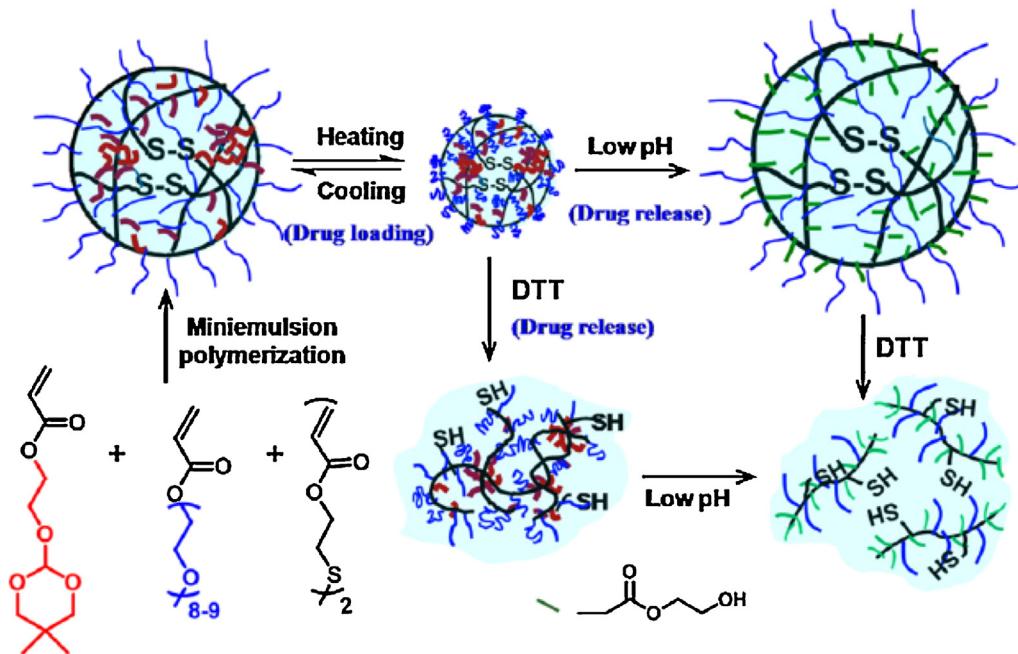


Fig. 6. Synthesis and stimuli-responsive properties of the nanogels. [183]. Copyright 2011. Reproduced with permission from Elsevier Ltd.

NIPAM has been widely used as a monomer to prepare thermosensitive nanogels, of which the size could be finely tuned by controlling the temperature [178–180]. For example, thermosensitive and hydrolytically degradable nanogels were prepared by emulsion polymerization of NIPAM and dextran-lactate-2-hydroxyethyl methacrylate (DEXlactateHEMA) [181]. It was found that this nanogel system became smaller and more rigid with degradation. Liu and coworkers have reported thermosensitive nanogels based on P(NIPAM-co-NPTUA) for selective detection of  $\text{Hg}^{2+}$  through emulsion polymerization [182]. The NPTUA-labeled nanogels could react with  $\text{Hg}^{2+}$ , resulting in a prominent blue shift of the fluorescence emission peak. The nanogels showed high selectivity and sensitivity for  $\text{Hg}^{2+}$  with a detection limit at the nanomolar level at room temperature, which was enhanced due to the collapse of the nanogels at elevated temperatures. Monomethyl oligo(ethylene glycol) acrylate has also been a popular monomer for the preparation of thermosensitive nanogels. Li and coworkers prepared thermosensitive nanogels by copolymerization of monomers containing oligo(ethylene glycol), ortho ester and disulfide linkage [183]. At temperatures above the phase transition temperature, the nanogels exhibited a hydrophobic nature, resulting in an enhanced drug loading capacity. It was shown that the drug-loaded nanogels had a good stability in the normal condition while the drug release could be triggered under bio-related stimuli, due to the existence of acid-labile ortho ester and reduction-cleavable disulfide linkage (Fig. 6).

The controlled/living radical polymerization techniques, such as ATRP, have also been used in mini-emulsion for the preparation of nanogels with well-controlled polymer segments. For example, Matyjaszewski and coworkers have prepared well-defined nanogels using the inverse

mini-emulsion ATRP method [184–186]. The two-step sequential emulsion copolymerization approach resulted in the formation of a new type of hairy nanogels through one-pot synthesis [187]. In their studies, uniform nanogels were prepared by the micro-emulsion ATRP method using methyl methacrylate (MMA) and ethylene glycol dimethacrylate (EGDMA) as the monomer and cross-linker, respectively. The micro-emulsion system were converted into an emulsion polymerization by addition of a second monomer, where linear arms grew from the remained initiating sites in the nanogels, rendering the formation of hairy nanoparticles in situ. Based on this approach, this research group used reduction-sensitive cross-linkers to construct nanogels for intracellular drug delivery, where various water-soluble biomolecules including anticancer drugs, carbohydrates and proteins were successfully loaded in the nanogels [188,189]. They demonstrated that the colloidal stability, swelling ratios, controlled degradability of the nanogels prepared by the ATRP method are superior to their counterparts prepared by the conventional free radical polymerization approach in inverted mini-emulsion.

### 2.5.2. Precipitation polymerization

Precipitation polymerization is another commonly used approach for preparation of nanogels, especially for thermosensitive nanogels (e.g., NIPAM based nanogels). When the polymerization temperature is above the lower critical solution temperature (LCST) of the resulting NIPAM-based polymers, the growing NIPAM chains would collapse when they reach a critical length. This process can lead to the formation of precursor particles [190]. Nanogels can be subsequently formed from precursor particles through three different mechanisms, including the continuous growth of the precursor particles by addition of monomers

or macroradicals, deposition of the polymer chains on the surface of existing polymer particles, and aggregation of the precursor particles to form a large colloidally stable polymer particle. During precipitation polymerization, the charges originated from the initiator fragments as well as considerable water may be incorporated into the collapsed polymer chains. This differs from classical emulsion polymerization of water-insoluble monomers, in which the particles containing a compact structure are formed. After the polymerization process is completed and the temperature is reduced below the LCST, the formed nanogels can swell and incorporate abundant water.

Since the first example demonstrated by Pelton et al. [191], the preparation of PNIPAM-based nanogels by the precipitation polymerization method has been extensively investigated [192–194]. Li et al. have prepared a series of core–shell nanogels with a thermo-responsive core and a pH-responsive shell via this method [195]. Recently, multifunctional nanogels have received increasing attention for their potentials in biomedical and pharmaceutical applications [196–198]. Yang and coworkers have synthesized a new class of temperature-sensitive nanogels based on poly(*N*-isopropylacrylamide-*co*-butyl methacrylate) for this purpose [180]. These nanogels have potential applications as a blood-vessel-embolic material in the interventional therapy of liver tumors. In addition to the PNIPAM system, other temperature-responsive polymers were also demonstrated. For example, Wu et al. developed pH and temperature dual responsive nanogels, composed of hydroxypropylcellulose and poly(acrylic acid) with CdSe quantum dots (QDs) in the interior through an in situ immobilization method [199]. The hybrid nanogels displayed good potential as a stimuli-responsive platform for sensing physicochemical environment, cell imaging and pH-triggered anti-cancer drug delivery. Nie and coworkers also demonstrated a multifunctional nanogel for anti-tumor drug delivery application by the free radical precipitation polymerization approach [200]. They found that surface modification of the nanogels with iRGD could lead to enhanced intracellular uptake of nanoparticles by both vein endothelial cells (HUECs) and extravascular tumor cells (B16). Moreover, the combined diagnostic and therapeutic features could be achieved by incorporation of bovine serum albumin – gold nanoclusters to the nanogels.

### 2.5.3. Self-assembly

The self-assembly method has also been extensively used to prepare nanogels. In this method, controlled aggregation of hydrophilic or amphiphilic polymers is formed by physicochemical interactions, such as hydrophobic interactions, electrostatic interactions, hydrogen bonding, stereocomplexation or supramolecular chemistry. The preparation of nanogels via self-assembly is usually carried out in aqueous media under mild conditions. Akiyoshi et al. demonstrated nano-sized hydrogels by the hydrophobic association of cholesterol-modified pullulan in the presence of insulin [201]. The nanogels were formed in a narrow range of cholesterol/sugar units ratio (1:40–1:100) with a diameter of 20–30 nm and they contained up to five insulin molecules per particle. Bronich and coworkers also demonstrated functional polymeric nanogels with an ionic core

for delivery of chemotherapeutic agents [202]. Reductively labile disulfide bonds were incorporated in the ionic cores by using cystamine as a biodegradable cross-linker through electrostatic interactions.

Besides the formation of physical interactions, covalent cross-linking of amphiphilic polymers chains were also used in the preparation of nanogels, especially for the nanogels with a core–shell structure [170]. The cross-linkers display marked influence on the swelling behavior, pore size and morphology of the nanogels, and therefore the release kinetics of the entrapped drug molecules. To achieve the controlled delivery feature, various stimuli-responsive moieties, cleavable under pH change, redox environment, light, enzymes and etc., were introduced into the polymer networks. For example, Zhao and coworkers prepared thermal- and photo-responsive core–shell nanogels by temperature-sensitive self-assembly of a double-hydrophilic block copolymer in aqueous solution, followed by reversible photo-dimerization and photo-cleavage of coumarin moieties [203]. Chen and coworkers also prepared pH-responsive biodegradable polypeptide nanogels as potential drug carrier by photo-crosslinking [169]. The drug-loaded nanogels exhibited the release profiles dependent on the external pH value as a result of the pH-responsive units in the polymer. Due to the marked difference in the redox potential between the extracellular and intracellular spaces, the nanogels crosslinked by reduction-responsive linkages, especially the disulfide bonds, that can be selectively decrosslinked in the intracellular space, have received a great deal interest for intracellular drug delivery. Zhong and coworkers developed the reduction-responsive dextran nanoparticles by the self-assembly of lipoic acid-modified dextran in aqueous media, followed by the formation of intermolecular disulfide linkages between the lipoic acid units and dithiothreitol (DTT) [204]. The reduction-triggered release of the doxorubicin-loaded nanoparticles was observed in an *in vitro* study. Thayumanavan and coworkers also synthesized a polymethacrylate derivative containing oligo(ethylene glycol)(OEG) and pyridyldisulfide [205]. The disulfide-crosslinked nanogels could be prepared by the formation of nanoaggregates in water via self-assembly, followed by the formation of intermolecular disulfide linkages via thiol-disulfide exchange reaction.

### 2.5.4. “One-step” polymerization in homogenous solution

Fabrication of nanogels via “one-step” polymerization (ROP) in homogenous media has attracted a great deal of interest recently [206]. This approach often involves the use of difunctional or multi-functional monomers. For instance, the microscopic polyacrylate networks were synthesized by atom transfer radical copolymerization of acrylate derivatives and diacrylate comonomers [207,208]. To avoid the formation of macroscopic gelation, highly diluted monomer solutions were used. Nanogels could also be prepared via “one-step” ring-opening polymerization (ROP) in homogenous media, where difunctional ROP monomers are initiated by using a macro-initiator with or without other comonomers [209]. For example, Wang and coworkers synthesized various difunctional phosphate monomers, and nanogels having PEG as the shell and

polyphosphoester as the crosslinked core was obtained by ROP of the difunctional phosphate monomers [210,211]. Reduction-responsiveness could be incorporated in the nanogels by the introduction of disulfide linkages to the difunctional phosphate monomers [211]. The release of vancomycin from the nanogels was markedly accelerated by the addition of alkaline phosphatase or DTT. In two separate studies, a difunctional L-cystine N-carboxyanhydride (L-Cys NCA) containing a disulfide bond was synthesized [212,213]. Disulfide core-crosslinked nanogels with the PEG shell and the crosslinked polypeptide core were prepared by ROP of L-Cys NCA and comonomers using amino-terminated PEG as a macro-initiator. The enhanced drug release rates were observed in the presence of intracellular reducing agent, glutathione (GSH). The pH-responsiveness could also be introduced into the nanogels by copolymerization of amino acids containing ionizable side groups [214]. In this case, ionizable anti-cancer drug, e.g., DOX, could be loaded into the nanogels having oppositely charged core through electrostatic interactions, resulting in nanogels with high drug loading capacity (DLC) and drug loading efficiency (DLE).

## 2.6. Nanofibers

Nanofibers are generally defined as fibers with diameters equal to or less than 100 nm. This definition is sometimes extended to include fibers with diameters measured less than 1000 nm [215]. Conventional fiber-spinning technologies cannot produce robust fibers with diameter smaller than 2  $\mu\text{m}$  because of the physical limitations in the process. In contrast, polymeric nanofibers for biomedical applications can be fabricated by a range of techniques including electrospinning, phase separation, membrane templating, self-assembly, and etc. [215,216].

### 2.6.1. Electrospinning

Electrospinning is the most versatile method for the fabrication of polymeric nanofiber. Electrospun polymeric nanofibers are formed by stretching a jet using electrostatic forces from a liquid polymer solution or melt [217–221]. The parameters affecting the electrospinning process include molecular weight, molecular-weight distribution, polymer architecture, surface tension, viscosity, solvent, solution conductivity, voltage, feed rate, temperature, diameter of the spinneret, distance between the tip and collector, humidity, and etc. By varying the above parameters, polymeric nanofibers with diameters ranging from a few nanometers to several micrometers can be obtained from a variety of polymers [222–225]. Despite the great potential of this technique, the conventional electrospinning process of polymer solution suffers from a number of drawbacks including the low production rate (up to 300 mg per hour per spinneret); need of solvent extraction; lack of suitable solutions for certain polymers at the ambient temperature; and environmental concerns when hazardous solvents are used [216]. In comparison, the melt electrospinning process is free from the use of solvent and has undeniable advantage in terms of environment friendliness [226–228]. Nanofibers of biodegradable polymers including poly(lactic acid) (PLA), poly( $\epsilon$ -caprolactone)

(PCL), and PEG-PCL have been fabricated using the melt electrospinning technique [229–231]. Generally speaking, much less studies have been carried out by the melt electrospinning technique than the solution electrospinning technique. This is because of the former requires much higher viscosity from the molten polymer, where the degradation of certain polymers of interest at high temperatures can be an issue [216]. To increase the productivity of solution electrospinning, several approaches including the increase in the number of spinnerets were used. Multiple-jet electrospinning and needleless electrospinning have found to be able to provide high production rate of polymeric nanofibers [217,232–237]. For the creation of polymeric nanofiber with unique micro-structure, such as core-sheath nanofibers, modification of the spinneret and/or the type of solution was also applied (e.g., coaxial and emulsion electrospinning) [238–243].

### 2.6.2. Phase separation

The phase separation method can also be used to fabricate a nanofibrous matrix. This method would involve a series of preparation steps: dissolution of polymer, gelation of polymer, extraction of solvent with water, and freeze-drying of the polymer scaffold [244–246]. The parameters affecting the morphology of the final nanofibers include polymer concentration, crystallization ability of polymer, annealing temperature, gelation temperature, and freezing temperature. For example, Ma et al. reported the creation of electroactive biodegradable nanofibrous biomimetic scaffolds from the blends of polylactide and electroactive degradable tetraaniline–polylactide–tetraaniline (TPT) copolymer via the thermally induced phase separation technique. The diameters of the resulting fibers could be controlled between 200 and 500 nm through the adjustment of polymer concentration, phase separation temperature and TPT content in the blends [247]. Although the phase separation technique requires simple equipment in lab scale, it is difficult to scale up due to the requirement of multiple and relatively complex steps as well as the limitation of specific polymer that can be used [248].

### 2.6.3. Membrane templating

The membrane templating method can be used to create nanofibers within the cavity (i.e., nanochannels) of nanoporous membranes (with 5–50 mm thickness) [244]. Because the nanochannels in the membranes are very uniform in size, the diameter and the aspect ratio of the nanofibers fabricated by the membrane template approach can be precisely controlled [249]. With this method, Xu et al. reported the fabrication of nanofibers from biocompatible and biodegradable polymer PCL using an aluminum oxide membrane as the template. The nanostructure morphology can be controlled by the processing parameters and template design [250,251]. The membrane templating method has several advantages. For one, a wide range of polymers can be used to fabricate nanofibers. Second, polymeric nanofibers with different diameters and lengths can be prepared using different templates. However, this technique is also difficult to scale up and thus with limited commercial value [216].

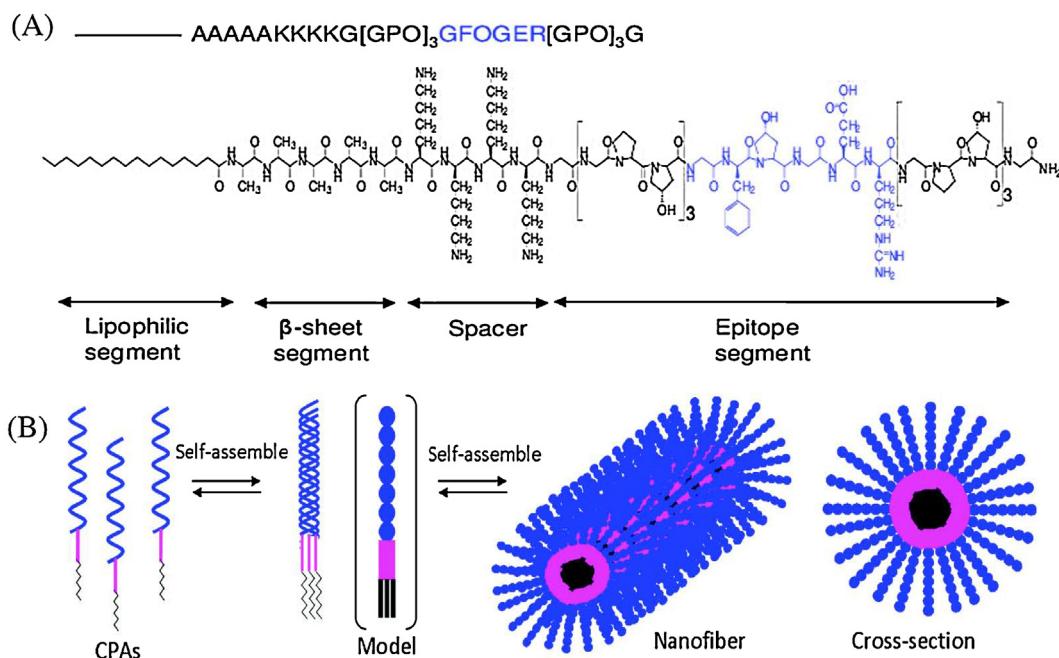
#### 2.6.4. Self-assembly

Self-assembly is an autonomous process, in which the disordered system of pre-existing components can organize and arrange themselves into ordered patterns or structures through non-covalent forces, such as hydrogen bonding, hydrophobic forces, and electrostatic interactions [248,252], without human intervention. Peptide amphiphiles are most commonly used by the self-assembly method to produce nanofibers [253,254]. This is because peptide amphiphile is a triblock polymer having three segments: a hydrophobic tail that can provide the hydrophobic driving force for self-assembly thus forming the core of the nanofiber, a region of beta-sheet forming amino acids that can stabilize the self-assembled nanofiber structure, and a peptide epitope that can enhance bioactivity and solubility of the molecule in water. As a result, nanofibers having diameters around 5–25 nm can be formed by the self-assembly process [248]. Based on this method, Tong et al. [255] reported a collagen-mimetic peptide amphiphile (CPA) system that could be self-assemble into nanofibers with structure and biological functions similar to native collagen fibers (Fig. 7). The parameters that can affect the morphology of peptide amphiphile nanofibers include the sequence and length of the peptide molecules, the geometrical constraints of the peptide molecules, and the interplay between the hydrophilicity and hydrophobicity [254]. Besides the peptide amphiphiles, other collagen-mimetic peptides, diblock copolymers, triblock copolymers, dendrimers, chitin, and bolaform of glucosamide and its deacetylated derivative can also be used to fabricate nanofibers through

self-assembly method [244,256–258]. Self-assembly has some unique advantages such as controlled physical and biochemical properties of nanofibers through the control of composition and chemistry of amphiphilic peptides, easy incorporation of cells during the fiber formation, 3-dimensional pore arrangement, and injectable for *in vivo* assembly. However, it is a laboratory scale process that has limitations including the complex process, limited polymer structures, and limited fiber diameter (~2 to 30 nm) and length (~10 μm) [244,248].

#### 2.7. Others

Dendrimers [259–261], brush polymers [262] and polymer nanocomposites [23] have also been considered as promising nanostructured biomaterials. Two distinct synthetic routes have been demonstrated for the preparation of dendrimers: the divergent and the convergent methods. In the divergent method, the molecular growth is initiated from the core and proceeds radially outward toward the dendrimer periphery. In the convergent method, the molecular growth starts at what will become the periphery of the dendrimer and thus proceeds inward [259,263]. Both methods are stepwise, tedious and time-consuming processes as they include several protection-deprotection and extensive purification steps. The divergent method is more suitable for the syntheses at a larger scale and the preparation of higher generation dendrimers. Nevertheless, incomplete growth steps and side reactions could lead to the production of slightly imperfect samples. The convergent syntheses usually require only two simultaneous



**Fig. 7.** (A) Molecular structure of CPA1 that contains four segments: lipophilic, β-sheet, spacer, and epitope segments. A bioactive GFOGER sequence is inserted within the repeating structural unit GPO as the epitope segment. (B) CPA1 self-assembly process: three collagen-mimetic peptide head groups self-assemble into a triple helix, while the hydrophobic tails and β-sheet-type hydrogen bonding drive and guide the assembly of triple-helical CPA1 into nanofibers. The peptide portion is exposed on the periphery of the nanofiber. [255], Copyright (2011). Reprinted with permission from the American Chemical Society.

reactions for any generation-adding step, therefore making the purification of dendrimers relatively simple. However, the number of steps required to fabricate a large structure by the convergent method is the same as that for the divergent method, but more starting material is required for the former [259,264].

Brush polymers, composed of densely grafted side chains along a polymeric backbone, can form spherical, cylindrical, or worm-like structures by varying the composition and chain length of either backbone or side chains [265]. Three main strategies have been established for the preparation of brush polymers: “grafting through” (the end-group polymerization of macromonomers) [265,266], “grafting onto” (the construction of functionalized backbone and side chains separately followed by coupling reactions) [267], and “grafting from” (the polymerization of monomers from presynthesized backbones with multi-initiating sites) [268]. Within each strategy, various polymerization techniques such as anionic polymerization [269], ring-opening polymerization [270,271], ring-opening metathesis polymerization [272–275], controlled radical polymerizations [271,276,277], and various coupling reactions (“click chemistry”) [270,272,278] have been employed. An extensive review on the preparation of brush polymers can be found elsewhere [262,279].

Polymer nanocomposites are another class of nanostructured materials, consisting of inorganic/organic fillers with nanometer scale dimensions (i.e., at least one dimension is within the range of 1–100 nm) and a polymer matrix. For biomedical applications, the demonstrated nanofillers include, but are not limited to, clays, hydroxyapatite, carbon nanotubes, silver, gold, titanium oxide, silica, nanocalcium carbonate, and nanocellulose crystals, whereas the biopolymer matrices include polysaccharides, aliphatic polyesters, polypeptides, proteins, and polynucleic acids [280–282]. These polymer nanocomposites can be prepared by techniques such as in situ polymerization, solution casting, melt processing, electrospinning, or supercritical fluid methods. The preparation schemes of polymer nanocomposites have been extensively reviewed elsewhere [282–284].

### 3. Biomedical applications of polymeric nanostructured materials

#### 3.1. Drug delivery

In the past decade, various polymeric nanostructured materials have been used for the controlled delivery of a diverse range of bioactive molecules, including low molecular weight (LMW) drugs and peptides/proteins. The delivery of drugs by polymeric nanovehicles has exhibited significant advantages over the delivery of parent free drugs. These advantages include the improved water solubility, increased bioavailability, minimized deactivation potential, reduced antigenic activity, decreased systemic toxicity, and enhanced acceleration ability in the lesion site.

##### 3.1.1. Polymer-drug conjugates

In 1975, Ringsdorf first proposed the concept of “polymeric prodrug”, which provided the theoretical bases for

the development of covalently bound polymer-drug conjugates [285]. In Ringsdorf’s model, three components can be conjugated with the biocompatible polymeric backbone, i.e., (1) the hydrophilic segment, to ensure the solubility of the system in an aqueous environment; (2) the bioactive molecule, to play a role in the pharmacodynamics; and (3) the targeting agent, to specifically recognize the desired physiological destination. Based on the rational design of the pharmacologically active system, various polymer-drug conjugates have been exploited. In recent years, some of polymeric prodrugs have entered the clinical trial stage (Table 2) [286].

Many natural and synthetic polymers having different topologies, compositions and functional groups were used to construct polymer-drug conjugates, which could be spontaneously self-assembled into micelles or micelle-like nanoparticles (Fig. 8). These polymers include natural polysaccharides (e.g., hyaluronic acid, dextran, chitosan and heparin), linear hydrophilic synthetic polymers (e.g., *N*-(2-hydroxypropyl) methacrylamide (HPMA) copolymers, poly(ethylene glycol) (PEG), poly(*L*-glutamic acid) (PGA), poly(*L*-aspartic acid) (PAA)), dendrimers, and some amphiphilic block copolymers (e.g., poly(ethylene glycol)-*b*-polylactide (PEG-*b*-PLA), poly(ethylene glycol)-*b*-poly(lactide-co-glycolide) (PEG-*b*-PLGA) and poly(ethylene glycol)-*b*-poly( $\epsilon$ -caprolactone) (PEG-*b*-PCL) copolymers). Some specific examples are as follows.

**3.1.1.1. Polysaccharides.** Polysaccharides are an important class of natural polymers which have been widely used for the development of polymeric prodrugs. The unique properties of these polymers include the excellent biodegradability, biocompatibility, non-toxicity, non-immunogenicity and even some biological activities (e.g., targetability, and antiviral and antitumor activities) [310]. The pharmacokinetics of the corresponding prodrugs are greatly influenced by their electric charges, molecular weight, polydispersity, chemical modification and topology [311]. The chemical structures of polysaccharides typically used for conjugating antitumor drugs are illustrated in Fig. 8A.

In these polymers, hyaluronic acid is a linear anionic polysaccharide composed of alternating D-glucuronic acid and *N*-acetyl-D-glucosamine linked with the  $\beta$  (1→4) interglycosidic linkage. As a main component in the extracellular matrix, hyaluronic acid plays a critical role in the cellular growth, proliferation, differentiation, migration and even malignant metastasis [312,313]. The elevation of hyaluronic acid is considered to be a reliable disease progression index in some types of malignant tumors (e.g., bladder cancer) [310]. Due to its strong affinity with cell-specific markers (e.g., cluster determinant 44 (CD44) [314,315]) and receptor for hyaluronate-mediated motility (RHAMM, overexpressed in many malignant cells and stem cells [316,317]) that is directly correlated with the invasive properties and metastatic processes of aforementioned cells, hyaluronic acid has often been used for targeted drug delivery without any additional ligands. The hydroxyl and carboxylic groups in the hyaluronate backbone provide the appropriate sites for conjugating antitumor drugs, such as

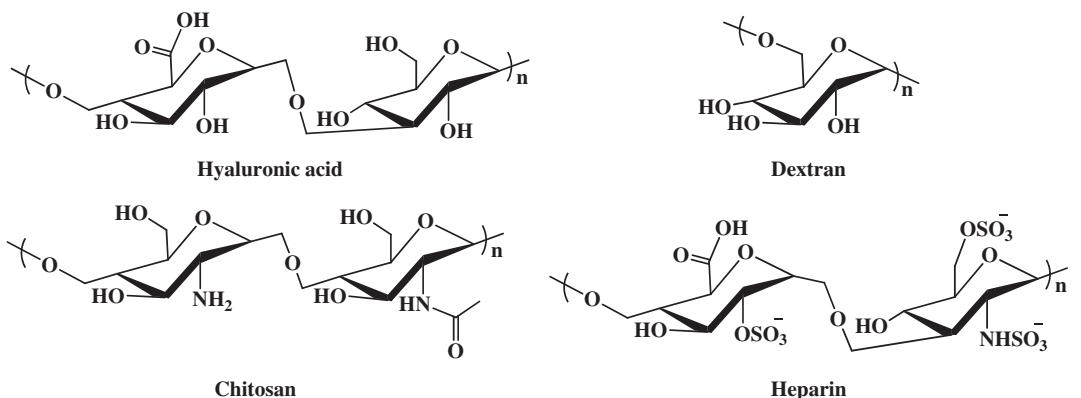
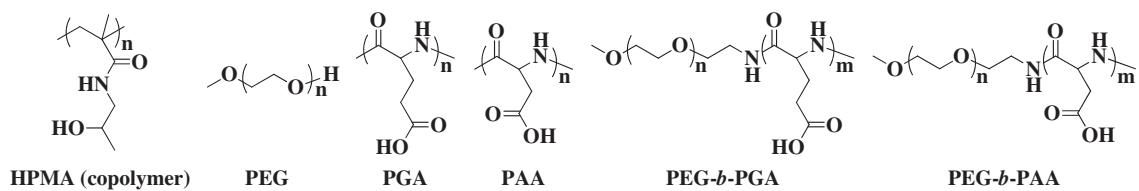
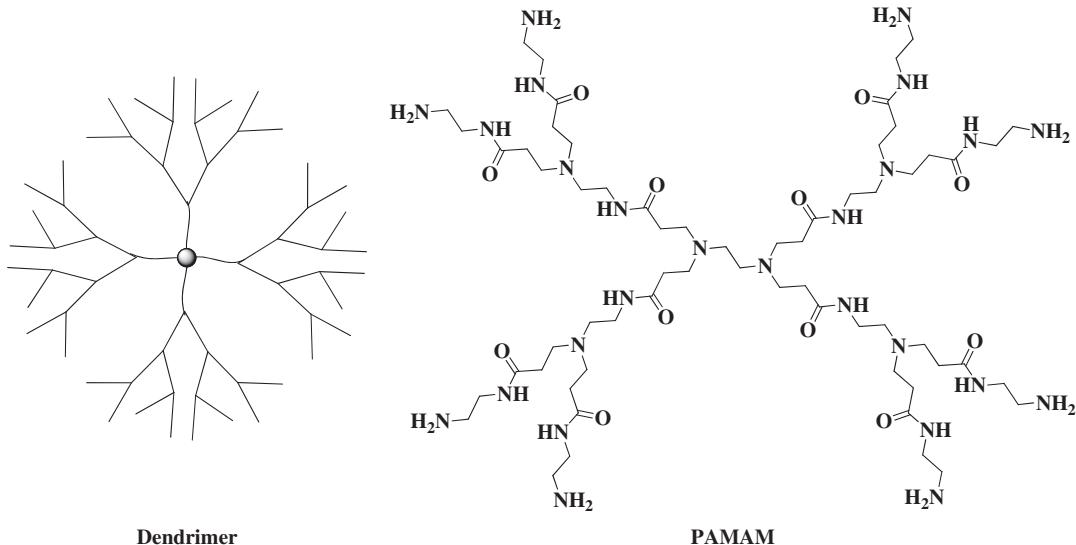
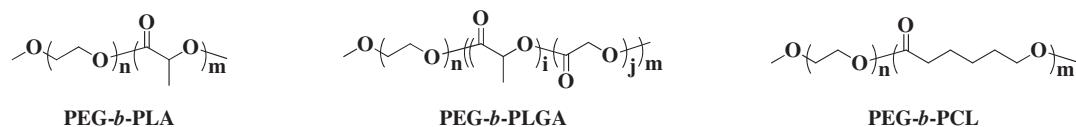
**Table 2**

Selective polymer-drug conjugates under clinical trials.

Trade name	Excipient	LMW drug	Diameter (nm)	Drug loading content (wt.%)	Indications	Status	Refs.
AP5280	HPMA copolymer	Carboplatin palmitate malonato-platinante	— <sup>a</sup>	8.5 (7)	Various cancers	Phase I/II (Netherlands)	[287]
MAG-CPT, PNU166148 AP5346, ProLindac™	HPMA copolymer HPMA copolymer	CPT DACH platinante	— <sup>a</sup> — <sup>a</sup>	10 — <sup>a</sup>	Various cancers Various cancers, particularly ovarian and colorectal cancers	Phase I (UK, discontinued) Phase II (France)	[288,289] [290]
PK1, FCE28068	HPMA copolymer	DOX	— <sup>a</sup>	8.5	Various cancers, particularly lung and breast cancer	Phase II (UK)	[291]
PK2, FCE28069	HPMA copolymer-galactosamine	DOX	8.4	7.5	Particularly hepatocellular carcinoma	Phase I/II (UK, discontinued)	[292]
PNU166945 Pegamotecan, Prothecan™	HPMA copolymer PEG	PTX CPT	— <sup>a</sup> — <sup>a</sup>	5 1.7	Various cancers Various cancers	Phase I (Netherlands, discontinued) Phase II (USA discontinued)	[293] [294]
NKTR-105	PEG	DTX	— <sup>a</sup>	— <sup>a</sup>	Various cancers	Phase I (USA)	[295]
NKTR-102	PEG	Irinotecan	— <sup>a</sup>	— <sup>a</sup>	Particularly ovarian and colorectal cancers	Phase III (USA)	[296]
NKTR-118 (oral)	PEG	Naloxone	— <sup>a</sup>	— <sup>a</sup>	Opioid-induced constipation	Phase II (USA)	[297]
EZN-2208	PEG	SN-38	— <sup>a</sup>	— <sup>a</sup>	Various cancers	Phase I (USA)	[298]
CT-2106	PGA	CPT	— <sup>a</sup>	33–35	Various cancers, particularly lung, ovarian and colorectal cancers	Phase I/II (USA)	[299]
CT-2103, Xyotax™, Opaxio®	PGA	PTX	— <sup>a</sup>	37	Various cancers, particularly NSCLC, ovarian cancer as a single agent or in combination therapy	Phase III (USA)	[300,301]
DE-310	Carboxymethyldextran	Exatecan	— <sup>a</sup>	6.6	Various cancers	Phase I (Netherlands)	[302]
AD-70, DOX-OXD	Dextran	DOX	— <sup>a</sup>	— <sup>a</sup>	Various cancers	Phase I (Germany)	[303]
XMT-1001, PHF-CPT	Polyacetal	CPT	— <sup>a</sup>	5–7	Various cancers	Phase I (USA)	[304,305]
NK012	PEG- <i>b</i> -PGA	SN-38	20	20.0	Colorectal, advanced metastatic triple negative breast cancer, relapsed small cell lung cancer and SCLC	Phase II (Japan and USA)	[306,307]
NK911	PEG- <i>b</i> -PAA	DOX	40	— <sup>a</sup>	Metastatic pancreatic cancer	Phase II (Japan)	[308,309]

DACH, diaminocyclohexane; SN-38, 7-ethyl-10-hydroxycamptothecin; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer.

<sup>a</sup> Not available.

**A) Polysaccharides****B) Linear hydrophilic synthetic polymers****C) Dendrimers****D) Amphiphilic block copolymers****Fig. 8.** Chemical structures of natural and synthetic polymers used for constructions of polymer-drug conjugates.

doxorubicin (DOX), paclitaxel (PTX), camptothecin (CPT), sodium butyrate and curcumin [310].

Dextran is another type of polysaccharide synthesized by certain lactic acid bacteria or dextranase, which consists of the  $\beta$  (1 $\rightarrow$ 6) linked D-glucose main chain with

various ratios of linkages and branches. More than three dextran derivatives, including carboxymethyl dextran, oxidized dextran and amino dextran, have been used to conjugate different antitumor drugs, such as DOX, CPT, mitomycin C (MMC) and methotrexate (MTX) [318–321].

Chitosan is a linear polysaccharide containing  $\beta$  (1 $\rightarrow$ 4) linked 2-amino-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucose, which is the N-deacetylated derivative of chitin. Like hyaluronic acid, chitosan plays a remarkable role in malignant biology, which have been shown to inhibit tumor angiogenesis [322]. In addition to the chitosan molecules, several derivatives including glycol chitosan, N-succinyl chitosan and carboxymethyl chitosan have also been used to chemically conjugate with DOX, PTX and MMC.

Heparin is one kind of mucopolysaccharide polysulfuric esters having alternating units of sulfated glucuronic acid and structurally diverse glucosamine derivative jointed by the  $\alpha$  (1 $\rightarrow$ 4) linkage. It has been shown that the heparin molecules, especially LMW heparin, can inhibit malignant cell adhesion, deactivate heparanase, activate the attack by natural killer (NK) cells in the immune system, and interfere with the activities of some growth factors, such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) [44]. Through the above approaches, the presence of heparin can prevent tumor angiogenesis, exacerbation and metastasis. Similar to the abovementioned polysaccharides, the heparin molecules are also capable of conjugating with various biological molecules (e.g., PTX).

**3.1.1.2. Linear hydrophilic synthetic polymers.** Many polymeric prodrugs have been synthesized utilizing various linear hydrophilic synthetic polymers. As shown in Fig. 8B, HPMA copolymers, PEG, PGA, PAA, and their copolymers (PEG-*b*-PGA and PEG-*b*-PAA) are used to prepare the drug conjugates.

HPMA copolymers, which were first exploited by Duncan and coworkers, are widely used as hydrophilic and biocompatible matrices for drug carriers [323]. Diverse modifications of HPMA copolymers have been carried out by copolymerization with various functional monomers, where different antitumor drugs were combined with HPMA copolymers through facile synthesis techniques. In this system, the HPMA copolymer-DOX conjugate (PK1) was the first polymeric prodrug, which entered clinical trials in 1994 [324]. PK1 represents the first milestone for the development of polymer-drug conjugates. PEG (also known as poly(ethylene oxide) or (PEO)) having different molecular weight, low polydispersity and activation form, is another kind of widely used and versatile linear hydrophilic synthetic polymer for biomedical applications. PEG has been approved by the US Food and Drug Administration (FDA) as an excipient in various pharmaceutical formulations due to its negligible immunogenicity, antigenicity and toxicity. For example, PEG has been routinely used to modify different bioactive molecules (e.g. DOX) [325].

Synthetic poly(amino acid)s represents another kind of biocompatible and biodegradable polymers with many important applications. These polymers exhibit precise secondary conformations, and have been widely utilized in different biomedical realms, including drug and gene delivery, as well as tissue engineering [169,326–329]. Among these polymers, PGA and PAA are the most promising candidates for drug conjugating ascribed to the respective

pendent carboxyl groups of each L-glutamic acid and L-aspartic acid.

**3.1.1.3. Dendrimers.** Dendrimers (or dendritic polymers) are three-dimensional, highly ordered star-like oligomeric and polymeric macromolecules (Fig. 8C). The physicochemical properties, such as solubility, stability, functionality and charge density, are determined by the chemical structure of the monomer unit(s) in the core and surface. In 1985, Tomalia and coworkers first demonstrated the preparation of polyamidoamine (PAMAM) dendrimers with precisely defined structures in a stepwise fashion [330,331]. Since then, many chemists have reported the different synthetic routes to prepare a wide range of dendrimers.

Compared with most of the synthetic polymers, the major advantage of using dendrimers for biomedical applications is their monodispersed chemical structures usually having a polydispersity less than 1.05. Due to the abundant and dense functional groups on the surface of the dendrimer, a large number of therapeutics could be linked to the dendrimers, thus maximizing their ability for biological interactions [286]. However, the poor biocompatibility and biodegradability of the dendrimers, and the relative difficult and costly syntheses are also noted in this system. These challenges need to be overcome before the successful implementation of clinical translation.

**3.1.1.4. Amphiphilic block copolymers.** The amphiphilic block copolymers are another kind of polymer system that can effectively incorporate and deliver anti-tumor drugs [312,332–334]. In this system, PEG is the most commonly used hydrophilic block because of its minimal immunogenicity, high water-solubility, high hydration and flexibility, where biocompatible and biodegradable aliphatic polyesters and their copolymers, such as PLA, PLGA and PCL, are often chosen as the hydrophobic blocks (Fig. 8D). The selection of the hydrophobic moieties can notably change the properties of prodrug micelle, such as size, stability and drug release profile.

The system with hydrophobic linear aliphatic polyesters (e.g., PLA, PLGA and PCL) as the core-forming blocks has been approved by FDA for therapeutic applications. Generally, PLA can be synthesized by condensation polymerization of lactic acid or by ROP of lactide (LA). PLA has three different stereoisomeric forms: poly(L-lactide)(PLLA), poly(D-lactide)(PDLLA) and poly(D,L-lactide) (PDLLA), due to the chiral nature of lactic acid. PLGA is a random copolymer of lactic acid and glycolic acid linked by ester linkage, which can be synthesized by ROP of LA and GA. PLGA exists in several distinct forms that can be controlled through the ratio of LA and GA. PCL is also a biodegradable polyester that can be prepared by ROP of CL. In an aqueous media, the amphiphilic block copolymers containing PEG and aliphatic polyesters can self-assemble into micelles or micelle-like aggregations, which is ideal to serve as drug carriers. A great amount of drugs, especially antitumor drugs (e.g. PTX, DTX, DOX and CPT), have been conjugated to the hydrophobic aliphatic polyester blocks [335–339], which moieties can adjust the characteristics

of prodrug nanoparticles, such as the size and stability for clinical applications.

**3.1.1.5. Others.** In addition to the above-described systems, various drugs, such as PTX, DOX, and CPT were conjugated to hydrophobic PLA through the ROP of LA initiated by the hydroxyl group on the drug molecule [340–342]. The PLA-drug conjugates could form uniform nanoparticle through nanoprecipitation, which would be further encapsulated by amphiphilic copolymers, such as PEG-*b*-PLGA. The obtained prodrug nanoparticles exhibited sustained drug release in the long-term detection (i.e., 14 days). In addition, the amphiphilic brush polymer-drug conjugates have been synthesized by ring-opening metathesis copolymerization and post-polymerization azide-alkyne click reaction [270,273]. The developed prodrugs also exhibited controlled drug release, and effective endocytosis and cytotoxicity. Furthermore, there are other types of polymer-drug conjugates attracted the wide attention of researchers, such as peptide-drug and antibody-drug conjugates, and so on [343,344]. However, this is not the focus of this review.

### 3.1.2. Encapsulation of low molecular weight drugs

In addition to the chemical conjugation, physical entrapment is another vital approach to incorporate drugs into nanocarriers that can reduce the side effects and enhance the efficacy. We will briefly review the use of this approach to fabricate nanovehicles, including micelles, polymersomes, nanogels, to deliver bioactive LMW drugs.

**3.1.2.1. Polymeric micelles.** Polymeric micelles are nanoscale colloids that can be formed by self-assembly of amphiphilic copolymers in an aqueous environment, where the formed micelles consist of a hydrophilic shell and a hydrophobic core. Various hydrophilic flexible polymers have been used as the shell-forming moieties. For intravenous drug delivery applications, the hydrophilic blocks of the amphiphilic copolymers are often composed

of zwitterionic materials or PEG, which can resist the nonspecific protein adsorption (i.e. non-fouling property) and prolong the circulation time of nanoparticles in the complex *in vivo* environment [214,345–347]. The most commonly chosen hydrophobic blocks include poly(propylene oxide) (PPO), biodegradable aliphatic polyesters (PLA and PLGA), and poly(amino acid)s (e.g. PGA and PAA), which can segregate and form micellar cores in the aqueous environment, where the segregation process is driven by a combination of physical interactions, including hydrophobic and electrostatic interactions, metal complexation, and hydrogen bonding [66,326,328,346,348–355].

The hydrophobic core can serve as the sustained release reservoir of bioactive LMW drugs (e.g., antitumor drugs), whereas the hydrophilic shell can stabilize the hydrophobic core and make the micelle a stable vehicle for i.v. administration. The typical polymeric micelles have an average diameter between 10 and 200 nm but a narrow size distribution. These micellar sizes are similar with those of natural mesoscale vehicles (i.e., viruses and lipoproteins), thus possessing enhanced permeation retention (EPR) capability [90,356].

Based on the promising results from clinical trials, polymeric micelles appear to be the most advanced platform for drug delivery. Table 3 lists six formulations to fabricate polymeric micelles that can encapsulate various LMW anti-tumor drugs such as PTX, DTX, DOX, oxaliplatin and cisplatin. The encapsulation process involves the physical entrapment or coordinate bonding of drug molecules with the hydrophobic segment in the micellar core. These formulations are in different stages of clinical trials for the treatments of various malignancies, especially for some advanced or metastatic cancers [31,349,356]. It is encouraged to learn that Genexol®-PM, composed of PEG-*b*-PDLLA and PTX, has been approved for the treatment of non-small cell lung cancer (NSCLC), and ovarian, breast and gastric cancers in Korea [356–358]. In addition, BIND-014, the first prostate-specific membrane antigen targeted PEG-*b*-PLA

**Table 3**  
Selective polymeric micelle formulations for cancer therapeutics under clinical trials.

Trade name	Excipient	LMW drug	Loading mode	Diameter (nm)	Drug loading content (wt.%)	Indications	Status	Refs.
SP1049C	Pluronic® L61, F127	DTX	Physical entrapment	30	8.2	Advanced gastric and esophageal cancer	Phase II (Canada)	[359]
Genexol®-PM	PEG- <i>b</i> -PDLLA	PTX	Physical entrapment	<50	16.7	NSCLC, ovarian, breast and gastric cancers	Approved (Korea) and Phase II (USA and Russia)	[357,358]
BIND-014	PEG- <i>b</i> -PDLLA or PEG- <i>b</i> -PLGA	DTX	Physical entrapment	100	10	Advanced or metastatic solid cancers	Phase II (USA)	[360]
NC-4016	PEG- <i>b</i> -PGA	Oxaliplatin	Coordinate bonding	40	— <sup>a</sup>	Various solid cancers	Phase I (Japan)	[361]
NC-6004	PEG- <i>b</i> -PGA	Cisplatin	Coordinate bonding	30	39.0	Advance solid cancers	Phase II/III (East Asia)	[362]
NK105	PEG- <i>b</i> -PPBA	PTX	Physical entrapment	85	23.0	Advanced stomach cancer	Phase III (Japan)	[363]

PPBA, polyaspartate modified with 4-phenyl-butanol.

<sup>a</sup> Not available.

and PEG-*b*-PLGA mixed micelles, containing DTX, has also been approved for the phase II clinical trials in the treatment of advanced or metastatic solid cancers [4]. These polymeric micelle formulations exhibited some notable advantages, including the reduced side effects, rational drug metabolism, and improved efficacy and tolerance in the course of current clinical trials.

However, despite the significant progress made in the past decades, the therapeutic efficacy of the varying polymeric micelle prescriptions is still far from our expectation. In specific, the desired performance for this system is the programmable “on-demand” drug delivery capability, which is yet to be realized completely [364,365]. To achieve this goal, tremendous efforts have recently been made to develop “intelligent” micelles with the response ability to intracellular stimuli, i.e., pH, redox potentials and enzyme, for the purpose of efficient intracellular drug delivery [366,367]. One of the most promising means involves the incorporating stimuli-responsive cleavable linkages in the polymer structures. Once triggered by the intracellular microenvironment, the chemical structures of such macromolecules can be changed leading to the disintegration of the assemblies and the lease of payloads.

**3.1.2.2. Polymersomes.** Polymersomes can be considered as an artificial reservoir-like platform, composed of self-assembled amphiphilic copolymers. Typical polymersomes are hollow nanoparticles, similar to the assembly of natural phospholipids, having sizes from about 10 nm to 1  $\mu\text{m}$  range but with a very narrow distribution [90,368]. In this system, the hydrophobic blocks of the copolymers can aggregate under the aqueous environment, while the hydrophilic blocks would directly confront the inside and outside aqueous solutions, creating two interfaces as the typical bilayered membrane in vesicles produced by micro-phase separation. The vesicle properties, such as size and membrane wall thickness, can be controlled by the copolymer moiety, composition and molecular weight for specific biomedical applications [350]. Hydrophilic drugs can be encapsulated in their aqueous cavities, whereas the hydrophobic component of the membrane can also incorporate hydrophobic drugs. Thus, the format of polymersomes has the ability to deliver hydrophilic and hydrophobic drugs simultaneously to generate synergistic effects to treat tumors.

Recently, polymersomes have attracted a great deal of attention as versatile carriers for biomedical application due to their colloidal stability, tunable membrane thickness and permeability, and ability to encapsulate a broad range of drugs. The relatively long blood circulation times can also be achieved by the introduction of a hydrophilic non-fouling surface layer (e.g., zwitterionic materials or PEG). Additionally, the diverse biodegradable and stimuli-responsive polymersomes can be fabricated from the copolymers that are responsive to pH, temperature, redox, light, magnetic field, ionic strength and concentration of glucose [369].

**3.1.2.3. Polymeric nanogels.** Polymeric nanogels are another promising drug delivery vehicles with unique features including tunable chemical and three-dimensional

physical structure, good stability, excellent drug loading capability, and responsiveness to the environmental factors, such as pH, temperature, redox and ionic strength [213,214,370–372].

Various LMW biological agents can be loaded into polymeric nanogels through physical interactions, e.g., electrostatic and hydrophobic interactions, between the agents and the polymer matrices. Aggregations and rapid in vivo clearances of nanogels can be prevented by the coating of biocompatible hydrophilic polymers (e.g., PEG) on the surface of drug-loaded nanogels, which forms a protective hydrophilic layer to prevent phase separation. A diverse range of polymeric nanogels have been developed that can deliver their payloads across the different biological barriers and inside the cells [170,213,370,371]. Such nanogels generally exhibit high stability and can protect the LMW drugs from degradation due to the metabolic systems of cells.

**3.1.2.4. Others.** Polymeric nanoparticles, nanocapsules and nanofibers are other commonly exploited nanoscale platforms that can enable the controlled delivery of LMW drugs. Nanoparticles and nanocapsules are both stable solid colloidal systems with diameters in the range of 10–1000 nm [369]. Nanoparticles are matrix-like vehicles, in which the LMW drugs are dispersed between the polymer chains. Nanocapsule is a vesicular system, which is formed by a drug-containing aqueous core or a lipophilic liquid core surrounded by the polymeric membrane. Polymeric nanoparticles and nanocapsules offer a very wide range of possibilities for modification (e.g. through composition and surface) that can modulate the circulation time, drug loading content and release behavior. The therapeutic applications of nanoparticles and nanocapsules are thus diverse, including cancer therapeutics, antimicrobial action and vaccine delivery, just to name a few. In addition, the polymeric nanoparticles and nanocapsules tend to accumulate in tumors, inflammatory or infectious sites, thereby exhibit the EPR effect on the vasculature [90,358].

Polymeric nanofibers are referred to polymeric fibers with diameters in the range of 1–1000 nm. The nanoscale diameters of fibers bestow them with unusual properties, including very high surface areas, and enhanced physical, chemical and mechanical properties [373]. Several techniques have been demonstrated that can fabricate polymeric nanofibers, such as electrospinning, self-assembly, template synthesis, and phase separation. Similar to nanoparticles, the polymer matrix in nanofibers can also incorporate LMW drugs, where the delivery can be accomplished from the fibrous scaffold with high surface area, good mechanical strength and adjustable porosity. Several nanofiber-based drug delivery systems have been demonstrated for the delivery of anticancer, antibiotics, antifungal, antimicrobial and antihypertensive drugs [373–376].

### 3.1.3. Delivery of peptides/proteins

Recently, peptides/proteins have emerged as promising agents for the therapeutics of various diseases, including cancers, diabetes, anemia, heart attacks, strokes, cystic

fibrosis, hemophilia and etc., due to their specific modes of actions and relatively low doses for therapeutic effects [377]. Diverse routes of administration, such as oral, buccal, transdermal, nasal, pulmonary and intravenous pathways, have been demonstrated for the delivery of therapeutic peptides and proteins. However, the controlled delivery of these agents to the targeting sites are often challenged by some unfavorable properties, including large molecular size, susceptibility to denaturation and degradation, short half-life, and poor bioavailability [377,378]. To solve the above problems, two types of strategies, i.e., chemical conjugation and physical entrapment, have been demonstrated for the efficient delivery of peptides and proteins.

**3.1.3.1. Chemical conjugation.** Chemical modifications of peptides/proteins with hydrophilic polymers, such as PEG and poly(oligo(ethylene glycol) methacrylate) (POEGMA), have proven effective in enhancing the enzymatic stability, minimizing immunogenicity, as well as improving the circulation time, pharmacokinetics and pharmacodynamics [378]. The PEGylated peptides/proteins are usually prepared by the integration of various functional groups in PEG toward primary  $\epsilon$ -amines in the lysine residues, thiolates in the reduced cysteine residues, or carboxylic acids in the glutamic and aspartic acid residues of the

peptide and protein molecules. To simplify the purification of PEG-peptide/protein conjugates and enhance the modification process, polymerization of POEGMA with predefined length is often performed from the functionalized N or C-terminus of a peptide/protein [379,380]. This approach has proven to be an efficient means for clinical applications, where several bioactive enzymes, cytokines, hormones and growth factors have been translated to the market or approved for clinical trials (Table 4).

**3.1.3.2. Physical entrapment.** Physical entrapment is another way to combine therapeutic peptides/proteins with polymeric nanocarriers, such as micelles, vesicles, nanogels, nanoparticles and nanocapsules. This approach has also been proven to be an efficient and secure means for the controlled delivery of peptides and proteins [377], as polymeric nanocarriers can be fine tuned regarding the surface properties and nanoscale structures for the targeted applications. Generally, upon administration, the polymeric nanocarriers should protect the peptide/protein molecules under degradative conditions, such as the presence of enzymes and acidic pH. In addition, the viable peptide/protein formulation should be considered not only on the stability, but also on the capability to maintain the native structure and activity during the preparation,

**Table 4**  
Selective polymer-peptide/protein conjugates in the market or under clinical trials.

Trade name	Excipient	Peptide/protein	Administration	Indications	Status	Refs.
Adagen®	PEG	Adenosine deaminase	Intramuscular injection	Severe combined immunodeficiency disease	Market	[381]
Oncaspar®	PEG	L-Asparaginase	Intramuscular injection	Acute lymphoblastic leukemia	Market	[382]
Neulasta™	PEG	G-CSF	Subcutaneous injection	Neutropenia	Market	[383]
PEG-Asys®	PEG	Interferon $\alpha$ -2a	Subcutaneous injection	Hepatitis B and C Melanoma, chronic myeloid leukemia and renal-cell carcinoma	Market Phase I/II	[384] [385]
PEG-Intron™	PEG	Interferon $\alpha$ -2b	Subcutaneous injection	Hepatitis C Melanoma, multiple myeloma and renal-cell carcinoma	Market Phase I/II	[384] [385]
Somavert®	PEG	HGH antagonist	Intraperitoneal injections	Acromegaly	Market	[386]
Cimzia®, CD870 Pegvisomant	PEG	Anti-TNF- $\alpha$ -Fab Human growth hormone	Subcutaneous injection Subcutaneous injection	Crohn's disease, arthritis Acromegaly	Market	[387]
Hemospan®	PEG	Hemoglobin	Intravenous infusions	Delivery of CO and O <sub>2</sub> in trauma patients	Phase II (Swedish)	[389]
ADI-PEG20	PEG	Arginine deiminase	Intramuscular injection	Hepatocellular carcinoma	Phase II	[390]
PEG-PGA and DON	PEG	Glutaminase combined with DON	Intravenous infusions	Various cancers	Phase IIA (Germany)	[391]
Zinostatin Stimalamer®	Styrene maleic anhydride	Neocarzinostatin	Intra-arterial infusions	Hepatocellular carcinoma	Market	[392]

G-CSF, granulocyte colony-stimulating factor; Anti-TNF- $\alpha$ -Fab, antibody fragments against tumor necrosis factor  $\alpha$ ; DON, 6-diazo-5-oxo-L-norleucine.

storage, administration and delivery. Overall, the application of polymeric nanocarriers for peptide/protein delivery is an emerging field with increasing importance for clinical applications.

### 3.2. Gene carriers

Gene therapy holds great promise for the prevention or treatment of certain diseases and genetic disorders by delivering therapeutic nucleic acids into the defective cells, thereby adjusting and controlling the corresponding cellular processes and responses [393–395] (Fig. 9). The delivery of therapeutic nucleic acids into cells is one of the major hurdles for the successful gene therapy [396,397]. In other words, therapeutic nucleic acids (*p*DNA or siRNA) need to be shuttled and successfully transferred into the defective cells by gene carriers [398]. One way to deal with this problem is through viral based gene carriers. Although viral gene carriers are efficient, the clinical application based on this approach is restricted by several safety concerns, including immunogenicity, carcinogenicity, immune response, and virus replication [399,400]. Some of these shortcomings may be overcome by applying non-viral carriers [401,402]. Polymeric gene carriers have exhibited some unique advantages, such as safety, physiological stability and suitable for large-scale production. However, the transfection efficiency of the non-viral carriers is usually lower than that of the viral carriers. Generally, the complexes that the non-viral carriers form with nucleic acids for gene therapy can be classified into four categories: polyplex, lipoplex, micelleplex and others [403,404].

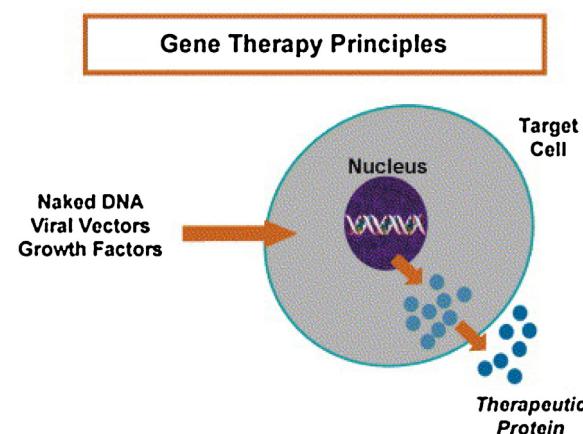
#### 3.2.1. Polyplex based gene carriers

Polyplexes are formed by electrostatic interactions between cationic polymers and anionic nucleic acids. The most often used cationic polymers for gene therapy include polyethylenimine (PEI), poly(L-lysine) (PLL), polyamidoamine (PAMAM) dendrimer, chitosan,

poly(beta-amino esters), helical polypeptides and cationic aliphatic polyesters. Among these polymers, linear and branched PEIs have received the most attention [398,400,405,406]. For example, PEI25k ( $M_w = 25,000$ ) has become the gold standard of polymeric gene carriers, which exhibited high transgene efficacy, but its application is also hampered by its relatively high toxicity. Although the low molecular weight PEI (OEI) shows low cytotoxicity, its utility is also limited by the low transgene efficacy [402]. To solve the above problems, Wen and Dong reported polyplexes formed by biodegradable OEI-grafted-polypeptide derivatives and nucleic acids. The *in vitro* study showed the system exhibited low toxicity but good transgene efficiency [407,408]. The modification of PEI25k with biocompatible molecules could also improve the transgene efficacy and biocompatibility [398]. In other cationic polymers, PLL was the first cationic polypeptide used for gene therapy [409]. The complexes formed by PLL and nucleic acids with sizes around 100 nm could be easily endocytosed by cells [410]. However, the application of PLL has been limited due to its poor biocompatibility and low transfection ability. To overcome this problem, Kataoka et al. demonstrated PEG-*b*-PLL block copolymers having the improved transfection efficiency due to the incorporation of PEG [411]. To enhance the endosome escape of therapeutic genes during gene transfection, Kataoka et al. also designed an A-B-C triblock copolymer system [412], in which each block possesses a different effect: the PEG block to reduce the toxicity, the low  $pK_a$  amine block based on poly[(3-morpholinopropyl) aspartamide] (PMPA) to enhance the endosome escape, and the high  $pK_a$  block of PLL to enable DNA binding. Other cationic polymers demonstrated for gene therapy include PAMAM dendrimer due to its relatively high transfection efficacy [413], and polysaccharide, especially chitosan, for its low toxicity as viable non-viral gene carriers for gene therapy [414]. Recently, some relatively new types of biodegradable cationic polymers such as poly( $\beta$ -amino esters), helical polypeptides and cationic aliphatic polyesters have been drawn more attention. Poly( $\beta$ -amino esters) can condense plasmid DNA into smaller and stable nanoparticles and help to promote cellular uptake and endosomal escape [415–417]. Gene carriers based on  $\alpha$ -helical polypeptides having the similar properties like cell-penetrating peptides, can effectively deliver genes [418–420]. Aliphatic polyesters representing a new type of biodegradable cationic block copolymer, which has well defined structure and tertiary amine-based cationic groups can effective deliver gene despite high levels of serum [421].

#### 3.2.2. Lipoplex based gene carriers

Lipoplexes are widely used for nucleic acid delivery due to their effectiveness and safety [422,423]. Lipoplexes can be divided into several categories: cationic lipoplexes, anionic lipoplexes and neutral lipoplexes, according to the type of the charge. Cationic lipoplexes are formed by electrostatic interactions of cationic lipid and anionic molecules (e.g., anionic lipid or anionic nucleic acid). The cationic lipids are usually consisted of a cationic head, linker and hydrophobic segment. In this



**Fig. 9.** Schematic diagram of the principles of gene therapy. Therapeutic genes of interest or growth factors that influence cellular function can be placed in viral or nonviral vectors that enter a targeted cell to significantly alter its function. [395]. Copyright 2006. Reproduced with permission from Elsevier Ltd.

system, the cationic lipid *N*-[1-(2,3-dioleyloxy)propyl]-*N,N,N*-trimethylammonium chloride (DOTMA) was first introduced for gene delivery by Felgner et al. in 1987, where the mixture of DOTMA and neutral lipid dioleoylphosphatidethanolamine (DOPE) was used as the gene carrier [423]. Since then, a large variety of cationic lipids have been designed and synthesized as candidates for effective gene therapy [424]. To achieve successful *in vivo* applications, the lipophilic cationic system must have the capacity to remain in circulation for a prolonged period of time, thus enhancing the opportunity for lipoplexes to reach the targeted tissues. However, their potential toxicity is a major obstacle for gene delivery. In order to overcome the toxicity issue, PEG segments are often incorporated into lipoplexes, where PEG can cover the positive charged surface thus improving the biocompatibility [425]. It has been found that the delivery of siRNA may yield a faster result than the delivery of plasmid DNA because the faster diffusion of siRNA in cytosol. We note that DOTMA was first used as a cationic lipid carrier for the RNA delivery [426]. Since then, many cationic RNAi lipoplexes were developed and some are under clinical trials [427]. As the single use of anionic or neutral lipids is not so effective for gene therapy in human [428], neutral lipids are used as assistant transgene enhancers in the cationic lipoplex formation.

### 3.2.3. Micelleplex based gene carriers

Micelleplexes are formed by polyion complexation between the phosphate groups in anionic nucleic acids and cationic copolymers with segments that are both cationic and hydrophilic [429,430]. For example, micelleplexes can be formed by self-assembly of amphiphilic copolymers containing PEG blocks [430]. Micelleplexes are promising candidates for the controlled delivery of nucleic acids because of their tunable characteristics, especially the particle size [430]. Kunath et al. demonstrated that the typical size of micelleplex particles formed by PEGylated copolymers are smaller than those formed by copolymers containing shorter blocks [431]. Recently, to improve the site-specific gene delivery capability, micelleplexes equipped with target moieties were developed, e.g., lactose-equipped micelleplexes for hepatocyte targeting [412]. Recently, micelleplexes for co-delivery of antitumor drugs and therapeutic nucleic acids have also been demonstrated [432–438]. For example, Zheng reported that polypeptide cationic micelles can be used for synergistic tumor therapy by co-delivery of docetaxel and therapeutic siRNA [432]. Wang built cationic core-shell nanoparticles using biodegradable amphiphilic copolymer as carrier for co-delivery of paclitaxel with DNA or RNA in therapy of breast cancer *in vitro* and *in vivo* [439]. Garbuzenko prepared nanoscale-based delivery system containing doxorubicin and mRNA to treat lung cancer by using inhalation delivery method to enhance accumulation of therapeutic drug in the lungs [440]. Chen designed biodegradable cationic polylactide nanocapsules to achieve drug-gene co-delivery for prostate cancer by overcoming multidrug resistance [441].

### 3.2.4. Others

Other promising polymer based nanostructured materials for biomedical applications include polymer nanoparticles, cationic nanogels and polymer-modified inorganic particles [193,442–444]. PLA and PLGA are commonly used to fabricate non-ionic nanoparticles because of their good biocompatibility and degradability. The nucleic acids can be encapsulated in these nanoparticles (PLA or PLGA) by the solvent evaporation or spray-drying method [442]. Nanogels can be surface-functionalized to achieve high loading capacity and tumor-specific targeted delivery due to their high porosity [193]. Taking into account of all the characteristics mentioned earlier, nanogels are very suitable for utilization as gene carriers. The polymer-modified inorganic nanoparticles have also been used in gene therapy for their unique characteristics, such as good biocompatibility, tumor-specific targeting capability, rich functionality and good storage stability [443]. Among this system, the most notable platform is polymer-modified gold nanoparticle [444–446].

Although polymeric non-viral carriers have exhibited great potential for gene delivery, their high toxicity and low transgene expression need to be further improved for clinical applications.

## 3.3. Bioimaging

Bioimaging is a powerful technique that can directly “observe” normal and abnormal biological processes in individual patients. Many bioimaging modalities have been developed, tested and utilized in preclinical and clinical applications in the past two decades. However, the applications of this technique are often hampered by the poor sensitivity, specificity, and targeting ability of the available and suitable bioimaging probes. The typical polymeric nanostructured bioimaging probes are nanoassemblies consisting of a bioimaging core and a polymer coating as shell. The polymer coating not only protects the loaded probes from the environment, but also improves the pharmacokinetics and bio-distribution of the probes, thus significantly amplifying the diagnostic imaging signals. The developed modalities include contrast agents for magnetic resonance imaging (MRI), X-ray computed tomography (CT), fluorescence imaging (FI), single-photo emission computed tomography (SPECT) and positron emission tomography (PET), which will be discussed as follows.

### 3.3.1. Magnetic resonance imaging

Magnetic resonance imaging (MRI) is a powerful technology to visualize the internal structures of the body (e.g. brain, muscle, heart, and cancer regions) using magnetic fields and radio waves. This technology generally provides superior contrasts between the different soft tissues of the body compared to other imaging modalities. However, the inherent contrast in some parts of the body is often insufficient for clear differentiation, e.g., detection of small tumors. To overcome this problem, one must rely on the use of MRI contrast enhancing agents. The most commonly used MRI contrast agents are low molecular weight chelates of gadolinium ( $Gd^{3+}$ ) or iron oxide particles. Often,

the clinical applications of these MRI contrast agents are limited by the short imaging time window, low signal to noise ratio (as a result of the short transient tissue retention time), toxicity, and unfavorable pharmacokinetic profiles. Polymer based nanotechnology has exhibited great potentials to reduce the toxicity of metal ions, prolong the blood circulation time of the agent/probe, and improve the contrast. This is because the suitable polymer system can connect the specific ligands on the probe.

Biocompatible polymers such as polylysine, poly(L-glutamic acid)-cystamine, poly(ethylene glycol), poly(lactic acid)-poly(ethylene glycol)-poly(L-lysine), poly(ethylene glycol)-*b*-poly(*N*-(*N'*-(2-aminoethyl)-2-aminoethyl)aspartamide), polysilsesquioxane, dextran, and L-cystine bisamide copolymer have been used for the preparation of MRI contrast enhancing agents through conjugation with low-molecular weight Gd chelates [447–450]. Bock et al. reported that the use of Gd-DTPA-polylysine could increase the signal intensity by 118% in pulmonary arteries of healthy lungs and by 121% in damaged lungs [451]. The PEGylation strategy in PLL has also been used to modulate the pharmacokinetic properties of the MRI contrast agents. For example, Yokoyama et al. found that the usage of PEG-*b*-poly(L-lysine) could significantly prolong the circulation time of gadolinium ion in blood. A considerable amount of PEG-P(Lys-DOTA-Gd) micelles was found to accumulate in solid tumors 24 h after intravenous injection due to the EPR effect. As a result, the MRI signal intensity of the tumor was enhanced 2.0-fold by the use of this polymeric micelle contrast agent [452]. Na and coworkers also reported a cancer-recognizable MRI contrast agents (CR-CAs), consisting of methoxy poly(ethylene glycol)-*b*-poly(L-histidine) (PEG-p(L-His)) and methoxy poly(ethylene glycol)-*b*-poly(L-lactic acid)-diethylenetriaminopentaacetic acid dianhydride-gadolinium chelate (PEG-p(L-LA)-DTPA-Gd) [453]. The CR-CAs had a spherical shape with a uniform size of ~40 nm at the physiological pH (pH = 7.4) level. Under the acidic tumoral environment (pH = 6.5), the CR-CAs disintegrated into positively charged water-soluble polymers due to the protonation of the imidazole groups of p(L-His) segments. As a result, the CR-CAs exhibit highly effective T1 MR contrast enhancement in the tumor region, which enabled the detection of small tumors of ~3 mm<sup>3</sup> *in vivo* at 1.5 T within a few minutes (Fig. 10).

Dendrimers such as Gadomer, PAMAM and polypropylenimine tetrahexacontaamine, have also been used for the conjugation with low-molecular weight Gd chelates [454–459]. The unique advantages of using dendrimers include the uniform surface chemistry, monodisperse molecular weight and shape. In particular, dendrimers have a large number of surface amino groups in a compact spherical space. With these groups, one can significantly enhance the MRI signals by attaching low molecular weight based chelates of gadolinium, such as Ga-DTPA. For example, Kobayashi et al. demonstrated that the conjugates of DAB-G5 and PAMAM-G8 with chelate of Ga could successfully be used to identify lymph nodes and lymphatic vessels, respectively [454].

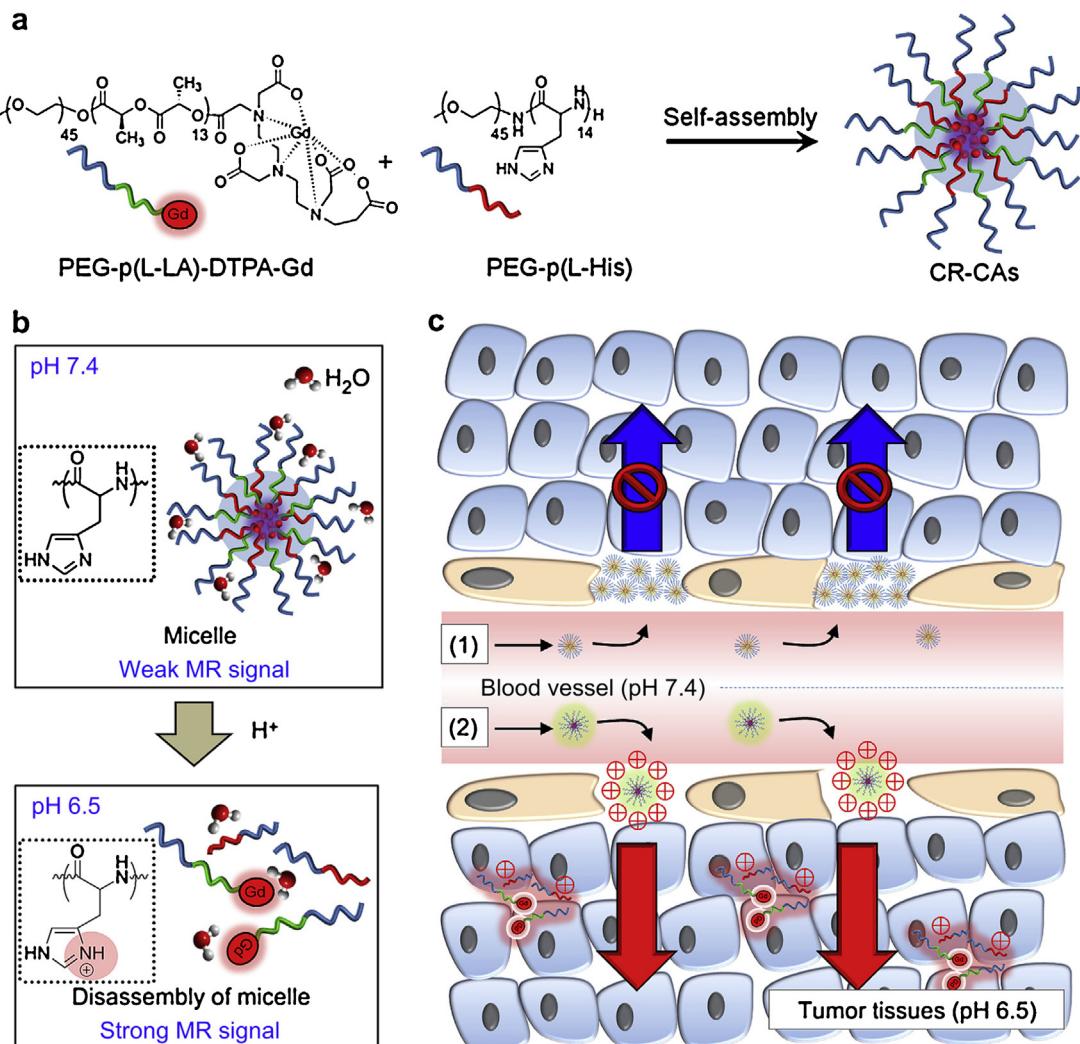
In the past two decades, the use of superparamagnetic iron oxide nanoparticles for medical imaging has advanced

notably. Based on the particle size, iron oxide nanoparticles for MRI can be classified into superparamagnetic iron oxide (SPIO, diameter > 50 nm) and ultrasmall superparamagnetic iron oxide (USPIO, diameter < 50 nm). SPIO agents can be used to image tumors in the liver and spleen, while USPIO agents are often used to image of lymphography and angiography [447,460,461]. Superparamagnetic iron oxide nanoparticles can enhance the negative contrast in MRI by causing tissues to appear darker than their surroundings because of their very large T2 relaxation and negligible T1 relaxation. However, as natural iron oxide particles have the following problems: easy aggregation, change in magnetic properties, rapid degradation under physiological conditions, and toxicity, they cannot be directly used in clinical applications. To overcome these problems, SPIO and USPIO contrast agents in clinical and preclinical applications are usually coated with polymers [462], such as dextran [463,464], dextran derivatives [463,464], silicone [463], oxidized-starch [464], PVA [465], or PEG [466]. Briley-Saebo et al. investigated the effects of the particle size and the coating material based on the various iron oxide nanoparticles on the rate of liver clearance in rat. They found that materials with similar coating but different sizes exhibited similar rates of liver clearance, but the coating material could significantly alter the rate of iron oxide clearance in rat liver. For example, the half-life of iron oxide nanoparticles in rat liver was 8 days for dextran-coated materials, 10 days for carboxydextrans materials, 14 days for unformulated oxidized-starch, and 29 days for formulated oxidized-starch [464]. Several long-circulating dextran or carboxydextrans coated iron oxide nanoparticles, including Feridex/Endorem (Ferumoxide), Resovist/Cliavist (Ferucarbotran), and Combidex/Sinerem (Ferumoxtran) have already been approved for clinical usage [467].

### 3.3.2. X-ray computed tomography

X-ray computed tomography (CT) has become a common tool in the arsenal of modern diagnostic medicine [468,469]. Unenhanced X-ray imaging provides superior visualization of bone structures due to the inherent contrast between electron-dense bones and permeable surrounding soft tissues. However, the native contrast between the different soft tissues is so small that unenhanced X-ray imaging cannot differentiate between them. To enable better delineation of soft tissue regions such as the cardiovascular system, CT contrast agents were introduced [470,471]. Contrast agents used in CT contain atoms that are electron dense, such as barium [472], iodine [473], bismuth [474,475], lanthanide and gold [476,477]. The CT contrast is linearly proportional to the total amount of the high-Z molecules in a voxel. Thus, in order to induce sufficient contrast in the desired organ, a much larger amount of contrast agent molecules is needed [471].

Small molecular weight iodine compounds are dominant CT contrast agents in the present clinical applications. However these iodinated molecules have very short imaging times due to the rapid clearance by the kidneys [471], which could significantly restrict the applications of CT in target-specific imaging and angiography [469]. In addition, a large dose of iodines, which are often used to enhance the



**Fig. 10.** (a) Schematic representation of the preparation of the cancer-recognizable MRI contrast agents (CR-CAs); amphiphilic block copolymers (i.e., PEG-p(L-LA)-DTPA-Gd and PEG-p(L-His)) self-assemble into micelles in an aqueous solution at pH 7.4. (b) Schematic representation of pH-dependent structural transformation and related MR signal change in CR-CAs. Inset: Chemical structural representation of the protonation of imidazole groups in PEG-p(L-His) at acidic pH. (c) Schematic representation of the tumor-accumulation behavior of (1) conventional micelle-based CAs and (2) CR-CAs. [453]. Copyright 2014. Reproduced with permission from Elsevier Ltd.

imaging because of their low contrast efficacy, can lead to serious adverse effects (e.g., some patients are hypersensitive to iodine [469]). To address these problems, functional polymeric nanoparticles have been developed [478–480]. For example, Seo and coworkers reported that lipiodol-encapsulated Pluronic/PEG-crosslinked nanocapsules had stable structure at high concentrations and with a safety profile similar to or better than that of lopromide. These polymeric nanoparticles exhibited a longer circulation time than the commercial iodinated system [481]. However, the contrast efficacy of the iodinated nanocapsules is still relatively low.

To enhance the contrast efficacy, a variety of metal-based nanoparticulate contrast agents have been explored, besides iodinated agents [477]. Among these, gold nanoparticles (AuNPs) have received the most interest as

CT contrast agents. This is because they can be readily functionalized with small molecules [482], PEG [483] or targeted proteins [484]. For example, Aurovist®, a commercial product of AuNPs with thioglucose coating, has recently been approved in Europe for imaging applications [485]. Generally, functionlized AuNPs show a prolonged blood circulation time, low toxicity, and comparable or better efficacy against iodinated agents [486–489]. Lu et al. also reported a binary contrast agent BaYbF<sub>5</sub>@SiO<sub>2</sub>@PEG that exhibited a long retention time in vasculature, remarkably low cytotoxicity and much higher contrast efficacy than lobitridol and NaYbF<sub>4</sub>@PEG with a single contrast element [490]. The general challenges for the development of new and effective CT contrast agents include the route of functionalization, in vivo targeting, efficiency, long-term stability, toxicology and excretion [469].

### 3.3.3. Fluorescence imaging

Fluorescence imaging has been widely used for non-invasive studies of fundamental mechanisms and processes at the organ, tissue, cellular, and molecular levels. Because tissue is relatively transparent for near-infrared (NIR) light, the ideal near-infrared fluorescent (NIRF) agents for *in vivo* imaging should have a peak fluorescence in the range of 700–900 nm. In addition, the characteristics of high quantum yield, a narrow excitation/emission spectrum, high stability, non-toxicity, available functionality for conjugation, good biocompatibility, good biodegradability and good excretability are important for NIRF imaging agents [491–493]. Organic NIRF agents such as indocyanine green (ICG) and inorganic fluorescent agents such as quantum dots (QDs) fit some of the above specifications. However, they also have some limitations, such as a lack of specificity for their targeted cells, tissues or organs, rapid aggregation, and short blood circulation time [447]. Polymeric fluorescent agents can prevent rapid aggregation, prolong the blood circulation time, improve stability, enhance targeting capabilities and reduce background signals. A variety of polymer-based protease-activatable or targeted imaging probes have been developed and demonstrated good fluorescence imaging capability [447,494–497]. Some examples are as follows.

It is well known that PLL backbone is cleavable by protease such as cathepsin B and trypsin [498,499]. A polymer-based protease-activatable imaging probe ( $\text{Cy}5.5)_{11}\text{-PLL-mPEG}_{92}$  has been successfully developed. In  $(\text{Cy}5.5)_{11}\text{-PLL-mPEG}_{92}$ , the PLL backbone contained an average of 44 unmodified lysines, 92 methoxy polyethylene glycol side chains and 11 molecules of the near-infrared fluorochrome Cy5.5. The existence of unmodified lysine groups renders the polymeric imaging probe cleavable by cathepsin B and trypsin [500]. The *in vitro* evaluation of this system showed that the enzyme-activated form of  $(\text{Cy}5.5)_{11}\text{-PLL-mPEG}_{92}$  exhibited 12-fold higher NIR fluorescence signals than the unactivated probes [498]. Such polymer-based protease-activatable imaging probes can be used for the *in vivo* detection of cathepsin B-overexpressed diseases such as cancer [501,502], rheumatoid arthritis [503], and atherosclerosis [504]. Using the peptides as protease-activatable linkers grafted on the side groups of polymers, a variety of polymer-based protease-activatable imaging probes could be obtained. The demonstrated proteases include cathepsins [505], matrix metalloproteinases [494], thrombin [506], factor XIIIa [507], caspases [508], urokinase-type plasminogen activator [509], and HIV protease. These polymer-based protease-activatable imaging probes have great potential to be used as effective tools to target a variety of protease [447,510].

Compared with organic fluorophores, quantum dots (QDs) have special advantages for fluorescence imaging, including easy control of excitation and emission wavelengths, high quantum yield, easy modification, and no fluorescent bleaching [447]. However, the high toxicity *in vivo* and low solubility in water have hindered the clinical applications of QDs [511,512]. To address the toxicity and solubility problems, QDs were encapsulated by amphiphilic copolymers [513–516]. The improved stability

and targeting ability, reduced toxicity, and enhanced solubility in aqueous solutions have been achieved through polymer coating [125,517–520], a process that has been discussed earlier.

### 3.3.4. Nuclear imaging

Nuclear imaging is a technique for producing images of various body parts using radioactive agents that can be traced in the body using a gamma ray camera. The acquired information is not only useful for diagnostic purposes, such as detection of functional abnormalities or early identification of tumors, but also helpful in therapy planning and follow-up procedures. Positron emission tomography (PET) and single photon emission computed tomography (SPECT) are two most commonly used nuclear imaging techniques.

PET is an imaging technique that detects pairs of gamma rays emitted indirectly by a positron-emitting radionuclide introduced into the body. It has been clinically used for oncology, neurology, cardiology and pharmacology. As compared with other molecular imaging modalities, PET has the advantages of high sensitivity (the level of detection can approach  $10^{-11} \text{ M}$  of tracer) and isotropism (i.e., the ability to detect expression accurately, regardless of tissue depth) [521]. At present, the most commonly used radiotracer in clinical PET scanning is fluorodeoxyglucose (also called FDG or fludeoxyglucose). However, FDG does not have the specific targeting ability as some newer bioimaging probes [447].  $^{64}\text{Cu}$  is another popular radionuclide for PET imaging due to its suitable half-life ( $t_{1/2} = 12.7 \text{ h}$ ), appropriate positron emission energy (0.65 MeV), and relatively convenient radiolabeling ability via coordination with specially designed chelators [522]. Several demonstrated schemes to improve of the specific activity of radiopharmaceuticals have allowed the  $^{64}\text{Cu}$ -based PET system to achieve high quality images at low dosage [523,524]. One very promising scheme is to encapsulate or conjugate  $^{64}\text{Cu}$  chelating agents with nanocarriers [525–527]. With this approach, Wooley et al. developed shell crosslinked (SCK) nanoparticles termed  $^{64}\text{Cu}$ -complexed pre-DOTAlysine-SCK, based on the copolymer of poly(acrylic acid)-*b*-polystyrene grafted with DOTAlysines. The SCK nanoparticles showed impressive specific activity (ca.  $400 \mu\text{Ci } \mu\text{g}^{-1}$ ), suggesting that these nanoparticles might be used for the development of highly sensitive *in vivo* PET tracers at low administering dosage [521].

Similar to PET, SPECT uses radiotracer (that emits gamma radiation) and scanner/detector to construct two- or three-dimensional images of the body part of interest. Generally, SPECT is cheaper than PET and the technique has also been clinically used for tumor imaging, infection imaging, thyroid imaging and bone scintigraphy. Gamma emitters, such as  $^{99}\text{Tc}$ ,  $^{111}\text{In}$ , or  $^{166}\text{Ho}$ , are common radiotracers [528–530]. The low molecular weight radiotracers have short circulation times, which often do not allow the agents to successfully penetrate into the targeted tissue. As a result, the sensitivity of nuclear imaging can be greatly compromised by the existing radiotracers. However, this problem can be overcome by using tumor targeting ligand decorated nanoparticles with long circulation times as the carriers of radionuclide. For example, Li et al. reported

that PC-3M tumors could be clearly visualized by SPECT after intravenous administration of  $^{111}\text{In}$ -labeled core-crosslinked polymeric micelle nanoparticles with EphB4 binding peptide [531].

### 3.4. Tissue engineering and regenerative medicine

Nanotechnology and nanoengineering are effective means in the design, preparation, characterization and applications of nanoscale devices, which consist of functional organizations with at least one dimension in the range from several to hundreds nanometers. Recently, the synergy between nanoscience and tissue engineering has led to great developments in biomedical research as well as clinical practices, including the realms of bone and cartilage regeneration, blood vessel tissue engineering, wound dressing, and so on.

#### 3.4.1. Bone tissue engineering

Bone tissue engineering is an important branch of tissue engineering with aims to repair and/or regenerate bone tissue using cell-based therapies and/or growth supplements based on functional scaffolds. This technology is usually used to restore the skeleton function in the process of orthopedic or oral-maxillofacial surgery [532]. In this approach, scaffolds are considered as the fundamental element for the success of engineered constructs, which are developed to mimic the biophysical structures of nature extracellular matrices (ECMs) and to provide an appropriate microenvironment for the host-cell colonization. These scaffolds are often porous biodegradable three-dimensional (3D) structures that can meet some fundamental properties of ECMs such as biocompatibility, osteoconductivity, osteoinductivity, osteogenicity and mechanical match between the implanted scaffold and surrounding tissues [533,534].

Various scaffolds have been prepared using recently developed nanofabrication techniques that can endow us the capability to modulate aperture configuration and nanostructures. In bone, the organic matrix (that amounts to about 85% of the mass) is made of native collagen type I fibrils, which are approximately 50 nm in diameter. The aligned or irregular patterns of these fibrils induce the distinguishing characteristics of lamellar or woven bone [535,536]. In view of the above characteristics, polymeric nanofibers of similar diameters can be uniquely fabricated to imitate the fibrous nature of bone ECMs in bone engineering.

Several in vitro and in vivo studies have confirmed the possibility of using polymeric nanofibers as scaffolds for bone regeneration. Three specific approaches, i.e., electrospinning [228,537], phase separation [538] and self-assembly [539], are commonly used to produce polymeric nanofibers for tissue scaffolding [535]. As a typical example for bone tissue engineering, PCL scaffolds containing electrospun nanofibers with dimensions from 20 nm to 5 mm have been employed to support the in vitro mineralization and differentiation of bone marrow-derived mesenchymal stem cells (BM-MSCs) from rat [540]. It was found that the PCL scaffold with nanofiber diameter  $\sim$ 370 nm was particularly effective, where the scaffold could

facilitate both adhesion and proliferation of MSCs and create higher levels of alkaline phosphatase activity, mineralization, and osteocalcin and osteopontin productions. The effectiveness of the PCL nanofibrous scaffold was further evaluated through an in vivo study. In this study, the scaffold was seeded with MSCs and subsequently implanted in the omenta of rat for 4 weeks [541], where the cells were found to successfully differentiate and infiltrate into the scaffolds [542]. In addition, nanofibers based on self-assembled peptide-amphiphiles (e.g., Arg-Gly-Asp (RGD) and phosphoserine) were found to be able to promote the cellular binding and mineralization [543]. Composite polymeric nanofibers containing osteoinductive factors have also been prepared [535,544] for bone engineering. For example, nanofibers consisting of silk, PEG, hydroxyapatite nanoparticles and bone morphogenetic protein 2 (BMP-2) were electrospun to create composite scaffolds. In these scaffolds, the silk/PEG nanofibers were found to be capable of supporting the osteogenic differentiation of human MSCs (hMSCs), where the presences of BMP-2 and hydroxyapatite nanoparticles could significantly enhance the bone formation *in vitro* [545].

Polymeric nanoparticles have also been used for bone tissue engineering. These materials are mainly to entrap and deliver biomolecules (growth and differentiation factors), bone morphogenetic proteins and genetic materials [546,547]. As polymeric micelles, polymersomes, nanogels, nanoparticles, nanocapsules and dendrimers are all possible vehicles for controlled delivery, their usage for bone regeneration requires some specific considerations. In general, the solid, hollow or porous nanoparticles are suitable for bone applications. These materials can be prepared through self-assembly, nanomanipulation, bioaggregation and photochemical patterning [547–549]. The scaffolds for bone tissue engineering containing bioactive polymeric nanoparticles can exhibit several advantages over traditional monolithic scaffolds: (1) enhanced control over sustained delivery of therapeutic agents; (2) acting as porogen or reinforcement phase to introduce porosity and/or improve the mechanical properties of bulk scaffolds; (3) acting as compartmentalized microreactors for dedicated biochemical processes; (4) acting as cell delivery vehicles; and (5) imbedding injectable or moldable formulations to be applied in minimally invasive surgery [550].

#### 3.4.2. Cartilage tissue engineering

Articular cartilage covers the articular surface and protects the underlying bone. The tissue in articular cartilage consists of chondrocytes, collagen (primarily type II), proteoglycans and water, where the ECMs play an essential role in both biological and mechanical functions in this tissue [532]. Thus, a well-designed scaffold that can mimic the structure and functions of native ECMs is highly desired for the development of engineered cartilage. Most ECMs are in the form of a mesh structure containing fibrils that provides tensile mechanical properties and also traps bioactive molecules in the fibrous network [535,551]. This fibrous structure of polymer nanofibers thus becomes an ideal scaffold for engineered articular cartilage.

In cartilage tissue engineering, polymeric nanofibrous scaffolds were found to be able to prevent

de-differentiation and promote re-differentiation of chondrocytes. For example, when fetal bovine chondrocytes were cultured in a chondrogenic growth media on the PCL nanofibrous scaffold (fiber diameter about 700 nm), higher levels of proliferated and expressed cartilage-associated genes were achieved than those cultured on the polystyrene platform typically used in tissue culture [552]. In addition, bovine articular chondrocytes were found to be capable of infiltrating the PCL nanofibrous scaffolds having diameters ranging from 400 to 1400 nm [553]. These results indicated the great potential of using polymeric nanofibrous constructs as scaffolds for the support of chondrocytes in vitro and in vivo. Polymeric nanofibrous scaffolds have also been exploited to sustain the chondrogenesis of progenitor cells. For instance, electrospun PCL scaffolds with fiber diameters of approximately 700 nm were shown to crutch multilineage differentiation of BM-hMSCs [554]. The level of chondrogenesis on the nanofiber scaffolds was shown to be equivalent and even higher than the gold standard pellet cultures in some cases. In addition, polymeric nanofiber scaffolds could improve mechanical properties, making them a viable option for in vivo transplantation [555]. Recently, an in vivo study demonstrated that the implantation of hMSCs in the nanofibrous PCL scaffolds to a swine model could induce the formation of hyaline-like cartilage with a smooth cartilage surface [535].

Similar to bone tissue engineering, polymeric nanoparticles have also been used to deliver growth factors in cartilage regeneration therapies. Although polymeric nanoparticles can be shaped into different platforms to control the release of growth factors, most of the nanoparticle systems for cartilage regeneration are designed for local delivery, often through the pathways of hydrogels and composite constructs [547,554,556]. The incorporation of polymeric nanoparticles as dispersing agents in tissue scaffolds would often induce nanostructured features on the scaffold surface, which can improve cellular adhesion and other cellular behaviors.

### 3.4.3. Vascular tissue engineering

Vascular tissue engineering is a means to substitute large-scale blood vessels with diameters greater than 6 mm, where the procedure would induce microvasculature or neovascularization processes inside or near the implanted scaffolds. To achieve good blood vessel regeneration, various vascular tissue scaffolds, including nanoscale porous membranes (e.g., electrospun polymeric nanofibrous scaffolds) have been designed and fabricated for different types of blood vessels. Some specific considerations for the fabrication of these scaffolds are: (1) they should exhibit proper mechanical strength and elasticity; (2) they should maintain endothelial coverage to control the diverse physiological signals; and (3) the remodeling of blood vessel should be able to respond stimulatory cues (Fig. 11) [557].

In the past decades, notable efforts have been made to produce vascular scaffolds with nanoscale properties, aiming to replicate the ECMs architecture. ECMs consist of nanofibers with diameters in the range of 5–500 nm, and compositions including collagens, elastins and nanoscale adhesive proteins (e.g. laminin and fibronectin) [558,559].

Thus, the typical engineered scaffolds are 3D constructs having porous interwoven structures. For example, Zhang and coworkers have demonstrated a nanostructured polymeric scaffold (with the feature dimension between 200 and 400 nm), which exhibited excellent mechanical properties and stable tube-like networks for cell seeding [560].

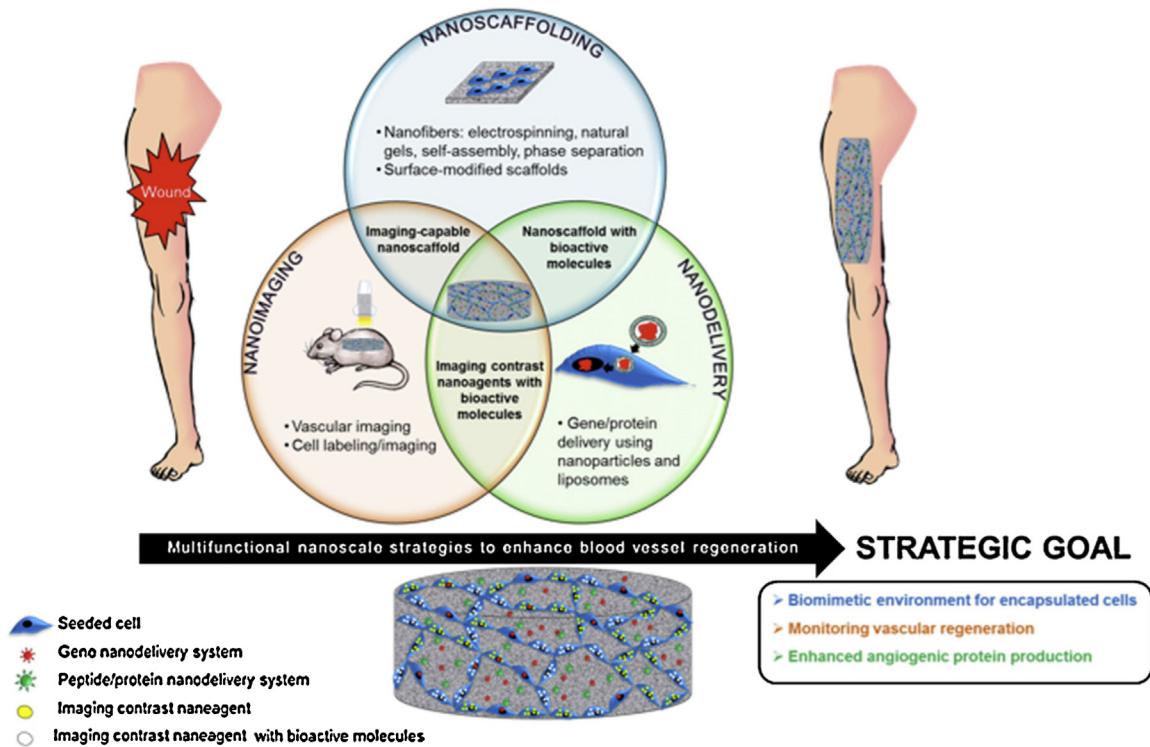
The incorporation of bioactive polymeric nanoparticles into the scaffolds is a logical strategy to enhance the regenerative capacity of tissue-engineering devices. In blood vessel regeneration, polymeric nanoparticles can be used for this purpose and deliver bioactive biomolecules such as adhesion molecules, growth factors, extracellular matrices, tight junction proteins and signaling molecules. These molecules can penetrate into the microvasculature inside the tissue scaffolds, cells or cell nuclei [557,561] and accelerate the regeneration process.

### 3.4.4. Wound dressing

The wound dressing is developed to prevent further harm, promote healing and achieve the best esthetic repair [562]. Electrospun polymeric nanofibers are among the most advanced and efficient wound dressing materials with performance that can surpass other existing wound dressing materials such as hydrocolloids, hydrogels, and alginates. This is because the bandage made of non-woven polymeric nanofibers is a natural 3D porous architecture with high surface area. The porous nature of nanofibrous scaffolds enable excellent ability to absorb wound exudates, prevent the moisture loss around the wound, allow oxygen permeation, protect the wound from bacterial infection, and exhibit good conformability [562,563]. A diverse range of synthetic and natural polymers, including polyurethane (PU), PLA, PCL, PLGA, polyvinyl alcohol, dextran, chitin, chitosan, cellulose acetate, gelatin and collagen, have been exploited as candidates for dressing materials, where bioactive agents such as anti-inflammatory drugs and tissue growth agents were also incorporated in the polymeric nanofibers for controlled delivery [562,564–567].

### 3.4.5. Others

Polymeric nanostructured materials, especially polymeric nanofibers, have also been used in other areas of tissue engineering, such as tendon and ligament, neural, and cardiovascular tissue repair [535,562]. In tendon and ligament tissue engineering, the scaffolds based on aligned polymeric nanofibers have been considered as a promising platform because the scaffold structure has the anisotropy similar to that of native tissue [568]. In addition, polymeric nanofibers are ideal to develop effective neural guidance conduits, suitable for bridging gaps in damaged peripheral or central neurons. Again, this is because the nanofibrous scaffolds have a structure very similar to that in neural ECMs. As a result polymeric nanofibrous conduits can also be used to direct axon sprouting and deliver neurotrophic factors to the site of injury [535]. For cardiac tissue engineering, the polymeric nanofibers can mimic the fiber-like feature of natural tissue architecture, where the nanofibrous scaffold was found to be effective in inducing elongated and aligned cardiomyocytes [569].



**Fig. 11.** Multifunctional nanoscale strategies, including scaffolding, imaging, and bioactive molecule delivery systems for vascular tissue engineering. [557], Copyright 2012. Reproduced with permission from Elsevier Ltd.

#### 4. Conclusions and future challenges

In conclusion, efforts to develop polymeric nanostructured materials have attracted a great deal of attention due to the increasingly importance of these materials in biomedical applications. Recent and extensive reports on the development of fabrication methods that can prepare various polymeric nanostructured materials, including micelle, polymersome, nanoparticle, nanocapsule, nanogel, nanofiber, dendrimer and nanocomposite, have been reviewed. Internal material properties, such as solubility, interactions between polymer and payload, polymer chain flexibility, surface charge, stereochemistry, surface chemistry, molecular weight, and crystallization ability, etc. should be taken into consideration for the selection of appropriate preparation method and suitable processing conditions.

Polymeric nanostructured materials (PNM) can provide great value for accurate diagnosis and effective treatment of diseases. For instance, in controlled delivery of bioactive molecules (e.g., low molecular weight drugs and genes), PNMs can be used to enhance the *in vivo* stability, increase the target specific delivery of drugs and genes, optimize the pharmacokinetics and biodistribution of the payload, reduce the side effects, and improve the efficacy of the system. For bioimaging, polymeric nanostructured contrast agents can exhibit prolonged blood circulation time, thus enhancing the targetability and reducing the adverse effects of toxic probe. Polymer-based nanotechnology can

also provide opportunities for personalized diagnosis and treatment by combining therapeutic and imaging contrast agents together. In addition, for tissue engineering and regenerative medicine, polymeric nanofibrous scaffolds can offer the unique advantage of imitating the 3D fibrous porous structure of natural ECMs.

However, though polymeric nanostructured materials have shown great potential to revolutionize the diagnosis and treatment of disease, there are also great challenges for the successful translation of basic research to clinical applications. For example, the issue of large scale production of PNMs must be carefully addressed, especially for those PNMs that require many steps to complete. The toxicity of the materials for cells, tissues and organs are another important issue to consider, especially for those PNMs that contain non-biodegradable or inorganic components. In addition, PNMs and the loaded therapeutic and imaging agents usually have a pharmacokinetics pattern different from the currently used low molecular weight drugs and imaging agents, which must also be investigated. In spite of these challenges, PNMs offer new possibilities to complement or replace the existing systems. We certainly believe that PNMs will become a major diagnostic and therapeutic tool for biomedical applications in the future.

#### Acknowledgments

The authors thank Wantong Song, Xiaoye Gao, Yuce Li, Jie Chen, Xiaofeng Song for the help in the review. This

work was supported by National Natural Science Foundation of China (Projects 51173184, 51373168, 21174142, 51222307, 51390484, 51233004 and 51321062), Ministry of Science and Technology of China (International Cooperation and Communication Program 2011DFR51090), Jilin Province Science and Technology Development Program (20130206058GX and 20130521011JH). B.H. acknowledges the support from the National Science Foundation (DMR-1409507) in the U.S.

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