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Poly(α-L-lysine)-based nanomaterials for versatile biomedical applications: Current advances and perspectives

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ABSTRACT

Poly(α-L-lysine) (PLL) is a class of water-soluble, cationic biopolymer composed of α-L-lysine structural units. The previous decade witnessed tremendous progress in the synthesis and biomedical applications of PLL and its composites. PLL-based polymers and copolymers, till date, have been extensively explored in the contexts such as antibacterial agents, gene/drug/protein delivery systems, bio-sensing, bio-imaging, and tissue engineering. This review aims to summarize the recent advances in PLL-based nanomaterials in these biomedical fields over the last decade. The review first describes the synthesis of PLL and its derivatives, followed by the main text of their recent biomedical applications and translational studies. Finally, the challenges and perspectives of PLL-based nanomaterials in biomedical fields are addressed.

1. Introduction

Poly(α-L-lysine) (PLL) is a water-soluble cationic biopolymer containing the monomeric unit α-L-lysine. PLLs, possessing naturally inherent properties such as non-antigenicity, biocompatibility, and biodegradability, are widely utilized in various biomedical domains and pharmaceutical field. The monomer α-L-lysine, one of the 20 naturally occurring amino acids, is believed to be essential for eukaryotes and prokaryotes and plays critical roles in biological processes, including injury recovery and protein functions. Owing to these intrinsic characteristics, PLL-based polymers have attracted particular attention for a wide variety of biomedical applications.

The pK_a of the amino side chains of PLLs is around 9.0. Therefore, PLLs are positively charged under physiological conditions because of the protonation of primary amino groups. PLLs have been developed as functional biomedical materials where the activity primarily derives from this cationic property. Based on the electrostatic interactions between the positively charged PLL and the negatively charged guests, PLLs have been widely explored as nanocarriers, coating materials, and bacterial biofilm dispersal/membrane disruption candidates. However, interactions between cationic PLL and the anionic membranes of red blood cells and vascular endothelial cells can frequently cause hemolysis and cytotoxicity. High-molecular-weight PLLs are recognized as more cytotoxic than low-molecular-weight PLLs since high-molecular-weight PLLs are more deleterious to both mitochondrial oxidative phosphorylation and glycolytic activity, leading to significant intracellular ATP depletion and initiating necrotic-type cell death [1].

Unremitting efforts have been devoted to the structural modification of PLLs, aiming to alleviate their toxicity and diversify their functionality. The primary amino pendants across the side chains of PLLs can be readily derivatized via various facile strategies to afford a wide variety of multifunctional (*e.g.*, biocompatible, biodegradable, stimuli-responsive, targeting, and imaging) biomaterials that can be used in many biomedical contexts including antibacterial agents, delivery systems, bio-sensing, bio-imaging, and tissue engineering.

PLLs, because of their good water solubility, are traditionally

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recognized as hydrophilic biopolymers. Nevertheless, the amphiphilicity of PLLs resulting from the presence of alkyl groups in their side chains is indeed neglected. Conversely, PLLs can fold into different secondary structures such as α-helical, β-sheet, and random coil based on hydrogen bonding and electrostatic interactions among their backbones and side chains, which are frequently influenced by environmental stimuli including pH, temperature, solvent variations, and surfactants [2–4]. Therefore, different conformations of PLLs display varying hydrophobicity because the intramolecular hydrogen bonding influences their interactions with neighboring water molecules. Generally, the hydrophobicity of these three conformations gradually decreases in the order of β-sheet > α-helix > random coil. Much research has demonstrated that the secondary structure of PLLs plays a pivotal role in combining with phosphatidic membranes, accelerating delivery vectors to escape from lysosomes, promoting osteoblast adhesion and proliferation, as well as enhancing absorption on the surfaces of other substrates [3,5–7].

Thanks to the continuous efforts of many researchers from diverse fields, the last decade has witnessed considerable developments regarding promising biomedical applications of PLL-based polymers, and considerable exciting achievements have been reported. A swift review summarizing these recent vital advances is thus critically demanded. This review highlights the latest advances in the biomedical applications of PLL-based nanomaterials such as antibacterial agents, gene/drug/protein delivery, bio-sensing, bio-imaging, as well as tissue engineering. It begins with a brief illustration of the synthetic strategies for PLL-based polymers, followed by the main text of their recent biomedical applications and translational studies. Finally, a personal understanding of the perspectives of PLL-based nanomaterials for biomedical applications is presented.

2. Synthesis of PLL-based polymers

PLL, as its name implies, is a polymer composed of the monomeric unit α-L-lysine. PLLs are prepared by the polymerization of an α-L-lysine unit either in a step-by-step or successive manner. Traditionally, three polymerization approaches are employed: solid-phase peptide synthesis (SPPS), ring-opening polymerization (ROP), and chemo-enzymatic synthesis. The polymerization approach selected generally depends on the requirements of molecular weight, purity, productivity, and reaction time as well as environmental conditions.

2.1. Solid-phase peptide synthesis

SPPS is a conventionally employed method for the synthesis of polypeptides and is vital for research in drug discovery, biomedicine, and other biological fields. In general, SPPS synthesis involves the initial attachment of the first amino acid onto a solid support, usually resin, followed by appending another *N*-terminal protected amino acid via the amide bond formation between the carboxyl group and the amine group. The cycle is repeated until the desired sequence is obtained [8,9]. For example, penta-L-lysine-substituted cyclooctene was prepared via SPPS, which was then polymerized to prepare well-defined PLL brushes for investigating how a comb-like architecture of PLL affected complex coacervation and coacervate stability [10]. SPPS is well-recognized for its precise control of amino acid sequences of the synthesized peptide.

Nevertheless, SPPS is significantly limited to short peptides (<50 residues). Furthermore, SPPS is typically performed with the limitation of reaction yields, large excesses of reagents, long reaction times, and many repetitive washing steps. To address these problems, microwave heating-assisted SPPS was developed, which often accelerates the reaction rate and improves the purity of crude peptides [11]. High-efficiency SPPS was also explored by Collins et al., allowing for total cycle times of approximately 4 min along with a significant reduction (approximately 90%) in total chemical waste compared with traditional methods [12]. Conversely, increasing attempts have been directed to make SPPS more environmentally friendly in the context of "green chemistry" and "sustainable chemistry," which involves reducing the amount of material used or replacing hazardous materials for less hazardous ones [13,14].

2.2. Ring-opening polymerization

ROP is the most prevalently used technique for the synthesis of PLL-based polypeptides via the polymerization of α-amino acid *N*-carboxyanhydrides (NCAs) [15–17]. This NCA polymerization strategy provides polypeptides with high yields, predetermined composition, and narrow molecular weight distribution. Synthesis of PLL involves initial conversion of the ε-primary amine-protected α-L-lysine to the L-lysine NCA monomer. Generally, the strongly electrophilic NCAs display high reactivity toward various weak bases such as amines, water, and even alcohols. Therefore, molecules comprising amine and hydroxyl groups are widely used as effective initiators to mediate the ROP of α-L-lysine NCAs to produce PLLs. In these base-initiated NCA polymerizations, there are two well-accepted mechanisms, the "normal amine mechanism" generally initiated by a primary amine, and the "activated monomer mechanism" typically mediated by a tertiary amine. By associating primary (slow but controlled ROP) and tertiary (fast but uncontrolled ROP) amines in a single molecule, Hadjichristidis et al. proposed another mechanism, the "accelerated amine mechanism by monomer activation" mechanism [18], where an emerging initiator, triethylaminetriamine, including three primary amines and a sterically hindered tertiary amine, provided a fast yet substantially controlled polymerization of NCAs.

Conventional initiators, as previously mentioned, generally display a slow NCA polymerization, which takes several days to reach completion and lead to substantial side-reactions. Moreover, the polymerization process is significantly sensitive to moisture. To circumvent these drawbacks, various emerging strategies for controlled NCA polymerization have been explored. In recent years, increasing efforts have been devoted to exploring new and efficient initiators and catalysts for NCA polymerization. Diverse initiators such as hexamethyldisilazane, used by Cheng [19], lithium hexamethyldisilazide, used by Liu [20]; trimethylsilyl and trimethylstannyl sulfide, used by Lu [21,22]; ammonium salts, used by Schlaad [23,24]; frustrated Lewis pairs, used by Yang [25]; catalysts such as cobalt and nickel organometallic catalysts, used by Deming [26]; organophosphates catalysts used by Yang [27]; and 1,1,3,3-tetramethylguanidine used by Cabral [28] have been reported for the controlled synthesis of PLL and other polypeptides. Very recently, Dong et al. combined the inimer (initiator + monomer) ROP and photocaged chemistry to prepare hyperbranched and linear PLL. They applied a photocaged N^{ε}-(*o*-nitrobenzyloxycarbonyl)-L-lysine-NCA for the photo-triggered NCA polymerization without any initiator/catalyst addition [29]. Besides exploring emerging initiators and catalysts, reaction conditions such as vacuum, temperature, pressure, and solvent purity should also be carefully optimized for the controlled synthesis of PLL via ROP [30].

2.3. Chemo-enzymatic synthesis

Inspired by the concept of "green polymer chemistry," chemo-enzymatic peptide synthesis has been developed for the efficient synthesis of poly(amino acid)s with green catalysts, mild reaction conditions, and high yields [31,32]. Owing to their chemical and physical stability, amino acid ligases and proteases are generally used as catalysts for the chemo-enzymatic polymerization of amino acids [32]. Gross et al. explored protease-catalyzed (*e.g.*, bromelain, papain, α-chymotrypsin, and trypsin) L-lysine-ethyl ester oligomerization in an aqueous reaction medium, where bromelain was preferred over other protease catalysts because it provided the highest oligo(L-lysine) yields and optimal average chain lengths [33]. They further investigated papain-catalyzed oligomerization of N^{ε}-protected L-lysine-methyl ester

and revealed that the introduction of *tert*-butoxycarbonyl or benzyloxycarbonyl N^{ε}-protected groups afforded the products with high yield, simple purification, and narrow dispersion [34]. Numata et al. have been employing chemo-enzymatic peptide synthesis for the fabrication of functional and structural polypeptides [35]. Block and random oligo (L-lysine-*co*-L-alanine) were synthesized using activated papain as an enzymatic catalyst within 30 min and over 40% yield [36]. Adhesive peptides containing 3,4-dihydroxy-L-phenylalanine and L-lysine were also synthesized via two enzymatic reactions: chemo-enzymatic synthesis of co-polypeptides of L-tyrosine and L-lysine by papain, and enzymatic conversion from L-tyrosine to 3,4-dihydroxy-L-phenylalanine by tyrosinase, which demonstrated the benefit of combining multiple enzymatic reactions in obtaining new types of peptide-based materials [37]. They also reported the first example of chemo-enzymatic synthesis of branched PLLs as gene delivery agents synthesized via a one-pot reaction without using organic solvents or deprotection steps [38]. Chemo-enzymatic synthesis of oligopeptides including oligo(L-lysine) from a polylactide backbone was successfully initiated by a designed grafter (ethyl hept-6-enoylalaninate) with papain as the biocatalyst. The design of the grafter is essential for efficient enzymatic synthesis, and oligopeptides could be grafted in an easy, time-efficient, and environmental-friendly way [39]. Compared with other synthetic methods such as SPPS and ROP, chemo-enzymatic synthesis is cost-effective and environment friendly, requires mild reaction conditions, and exhibits relatively high productivity with purity. Nevertheless, chemo-enzymatic synthesis does show certain shortcomings, such as low molecular weights and difficulty in controlling amino acid sequences of the polypeptides.

3. Biomedical applications of PLL-based polymers

Biomedical applications are regarded as one of the most vital and widely pursued areas for multifunctional PLL-based polymers. What makes PLL-based polymers attract considerable attention in the biomedical fields is that PLL-based polymers not only possess excellent intrinsic properties such as biodegradability, biocompatibility, and stimuli-responsive conformation transition but also can be modified via facile reactions between the side amine groups and many other functional moieties, providing a unique platform for producing diverse hybrid polymers and copolymers with specific functionalities. The unique physicochemical properties of PLL-derived polymers are leading to ever-growing scientific research regarding their biomedical applications. The biomedical applications of PLL-based polymers are schematically depicted in Fig. 1.

3.1. PLL-based polymers as antibacterial agents

Bacterial infection is recognized to be an important factor in human diseases and deaths, raising a significant threat to worldwide human society and health. Thanks to the discovery of antibiotics, the morbidity and mortality caused by bacterial infections have been controlled over the last several decades. However, antibiotic resistance is emerging as a massive challenge because of the abuse of antibiotics worldwide [40], and there is a pressing demand for developing novel and efficient antibacterial agents. To this end, new generations of antibiotics [41–43] and versatile antibacterial materials [44–49] have been extensively explored to combat drug-resistant pathogens. Among diverse antibacterial materials, antimicrobial peptides (AMPs), initially discovered from the humoral immune system of silk moths in the early 1980s, have received ever-growing attention over the last decades. AMPs, which kill bacteria via well-received physical disruption of the bacterial cell membrane, have been developed to mitigate drug resistance and offer a potential therapeutic strategy against multiple microorganisms, including clinically important antibiotic-resistant pathogens [50–52]. AMPs are typically amphiphilic peptides composed of basic amino acids (such as lysine and arginine that carry positive charge under physiological pH)

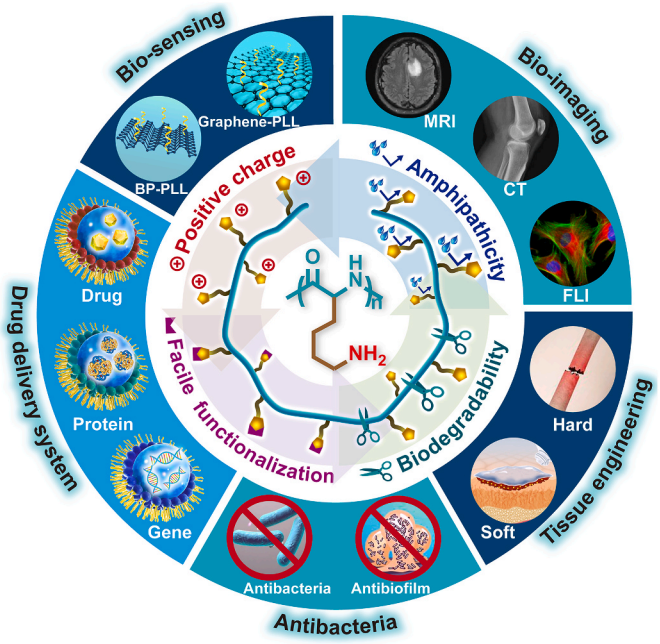

Fig. 1. Schematic illustration of structural characteristics and biomedical applications of PLL-based nanomaterials.

and hydrophobic amino acids (such as phenylalanine, leucine, and alanine). The cationic charges of AMPs can electrostatically combine with anionic charges on the surfaces of gram-positive (cell wall) or gram-negative (outer membrane) bacteria. At the same time, the hydrophobic segments are helpful for the AMPs to insert the bacterial membrane. Inspired by the structure of AMP, a plethora of PLL-based nanomaterials (antibacterial polymers mimicking AMP, antibacterial polymeric hydrogels, and antibacterial polymeric coatings) have been extensively explored for the treatment of bacterial infections.

PLL-derived polymers, being positively charged under physiological conditions, have been explored as antibacterial agents with promising antibacterial activity. PLLs are frequently conjugated with hydrophobic polypeptides or other hydrophobic blocks to balance the cationic/hydrophobic components for optimized antibacterial activity (Fig. 2). L-Phenylalanine, L-alanine, and L-leucine are representative hydrophobic amino acid residues which are employed to conjugate with PLLs for antibacterial applications [53–56]. These amphiphilic co-polypeptides could self-assemble into ordered supramolecular nano-sized structures like micelles and nanoparticles. The globally amphiphilic structure is favorable for increasing the local positive charge density and thus exhibits excellent antimicrobial activity against various gram-negative and gram-positive bacteria, as well as fungi while showing low cytotoxicity and hemolytic activity. The ratio and category of hydrophobic amino acids in these amphiphiles have a significant influence on water solubility, cytotoxicity, and antibacterial activity [54–56]. An alternative strategy to regulate the cationic/hydrophobic balance of PLL-based amphiphiles is the incorporation of hydrophobic hydrocarbons [57, 58], polycaprolactone (PCL) [59], or poly(2-hydroxypropyl methacrylate) [60], among others. The antibacterial activity of these types of cationic peptide amphiphiles depends on self-assembled supramolecular nanostructures (*e.g.*, micelles, vesicles, nanofibers, and ribbons) and the overall hydrophobicity. Notably, the PCL segment not only affords hydrophobic moieties, acting as the "weapon" to insert into the bacterial membrane and causing irreversible damage but also significantly offers better blood compatibility and lower cytotoxicity [59]. Partial PEGylation of PLL-based amphiphiles could alleviate cytotoxicity, induce and stabilize self-assembled nanoparticles, as well as elongate their circulation time *in vivo*. For instance, antibacterial cationic polypeptides

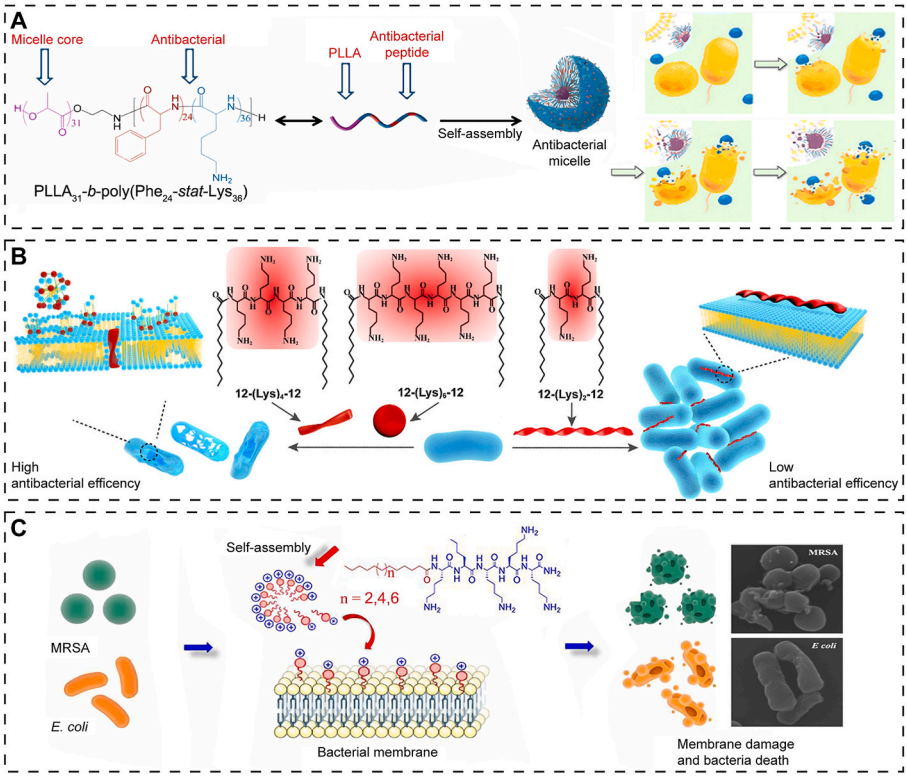

Fig. 2. Structures and mode of antibacterial action of peptide amphiphiles. (A) Self-assembly of poly(L-lactide)$_{31}$-*b*-poly(phenylalanine$_{24}$-*stat*-lysine$_{36}$)-based co-polypeptides into micelles with positive charges and amphipathic features, with PLLA segments aggregating to form the micellar core and with the polypeptide blocks serving as the corona of micelles. The cationic Lys units adhere to the bacterial membrane, and the hydrophobic Phe units enter and disrupt the bacterial membrane. Reproduced with permission from Ref. [53], copyright 2016, American Chemical Society. (B) C_{12}-(lysine)$_n$-C_{12} with diverse lysine spacer lengths form distinct supramolecular nanostructures: fibers (n = 2), rods (n = 4), and spherical aggregates (n = 6) that exhibit different antibacterial activities and action mechanisms. Reproduced with permission from Ref. [57], copyright 2018, American Chemical Society. (C) Micelle-forming cationic peptide amphiphiles, C_{n+11}-lysine$_5$ (n = 2, 4, 6), demonstrate excellent antibacterial activity against methicillin-resistant *Staphylococcus aureus* and *Escherichia coli*. Reproduced with permission from Ref. [58], copyright 2018, American Chemical Society.

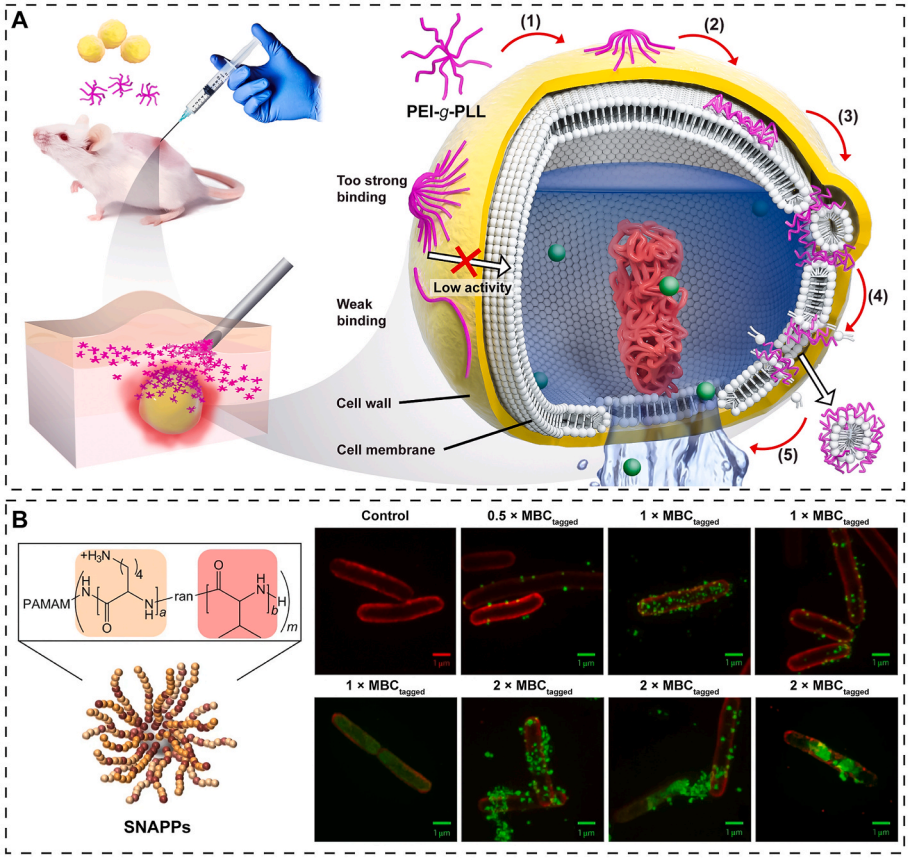

Fig. 3. Structural representations of two typical star-shaped, PLL-based, antibacterial polypeptides. (A) PEI-*g*-PLL. Reproduced with permission from Ref. [62], copyright 2019, Wiley. (B) PAMAM-*g*-poly(L-lysine-*ran*-DL-valine). Reproduced with permission from Ref. [63], copyright 2016, Springer Nature.

prepared from PEGylated poly(L-lysine-*co*-L-phenylalanine) form well-defined nanoparticles in solution, which exhibit broad-spectrum antimicrobial activity against clinically significant gram-positive and gram-negative bacteria with low hemolytic activity [61].

Regulating molecular conformation (linear, brush, and star shape) remarkably influences the antibacterial properties of PLL-based polymers. As indicated above, PLL-based amphiphilic co-polypeptides can self-assemble into ordered, supramolecular, nano-sized structures, such as micelles and nanoparticles, which exhibit significant antibacterial activity owing to their locally amplified density of cationic charges. Similarly, compared with the corresponding linear polypeptides, star-shaped PLL-based copolymers are also posited to have promising antibacterial activity owing to their substantially concentrated positive charges. We recently reported a series of PEI-*g*-PLL with a hyperbranched polyethyleneimine (PEI) core and PLL peripheral chains as a class of versatile molecular scaffolds for the development of star-shaped antibacterial peptides (Fig. 3A) [62]. Compared with their linear PLL counterpart, the star-shaped PEI-*g*-PLLs demonstrated unique characteristics of higher cationic charging density, increased surface electrostatic potential, and improved proteolytic stability. The alteration of PLL architecture from linear to star shape, as well as the absence of hydrophobic amino acid residues in PEI-*g*-PLL, imparts enhanced binding affinity and disruption of bacterial membranes, increased selectivity toward microbes over mammalian cells, and improved *in vitro* and *in vivo* antibacterial efficacy. A series of star-shaped peptide polymer nanoparticles were constructed from the ROP of lysine-NCAs and valine-NCAs initiated by a poly(amidoamine) (PAMAM) dendrimer [63–65]. These star-shaped nanoparticles, termed "structurally nanoengineered antimicrobial peptide polymers" (SNAPPs), demonstrated sub-micromolar activity against all gram-negative bacteria tested, including ESKAPE pathogens (Fig. 3B) [63]. Compared with the corresponding linear analogs, these SNAPPs showed enhanced microbicidal activity against *Staphylococcus aureus* and *E. coli*, with the minimum bactericidal concentration being at least 40-fold lower. Further study indicated that the presence of divalent cations (Ca^{2+} and Mg^{2+}) and serum proteins mediates an antagonistic effect on the antibacterial efficacy of SNAPPs. This is attributed to the divalent cation stabilization of the lipopolysaccharide layer, allowing serum proteins to adsorb onto SNAPP surface to reduce the free fraction of SNAPPs available for bacterial association or killing [65]. Similarly, Wang et al. prepared star-shaped poly(lysine-*ran*-valine) with a hyperbranched PAMAM core. The intrinsic aggregation-induced emission (AIE) effect of the PAMAM core allowed monitoring the bacterial concentration and visualizing the antimicrobial process in real time [66]. In another study, star-shaped PLL-based copolymers with three, four, six, or eight arms were used as antibacterial agents [67]. These star-shaped PLLs, partially modified with hydrophobic indole or benzyl groups, exhibited enhanced antibacterial activity mediated by anchoring of these groups onto the bilayer surface and inserting into lipid membranes as well as improved cytotoxic selectivity toward pathogens over mammalian cells without compromising their hemolytic activities. Cylindrical polypeptide brushes were also explored as antibacterial agents. Antimicrobial polypeptide brushes prepared from a β-polypeptide backbone and grafted with PLL side chains (α/β chimeric polypeptide brushes) exhibited potential *in vitro* antibacterial activity against all tested strains of methicillin-resistant *Staphylococcus aureus* (MRSA), even better than the typical antibiotics, such as vancomycin [68]. However, cytotoxicity studies indicated that these polymers displayed relatively high toxicity to mammalian cells owing to the high density of positive charges of PLLs.

Improving the structural affinity of PLLs toward the bacterial cell wall could enhance antibacterial activity. Inspired by the specific chemical components of the bacterial cell wall, which are composed of a peptidoglycan layer and carbohydrate receptors, increasing interest has been directed toward combining PLL with saccharides or polysaccharides to fabricate PLL-glycopolymer conjugates with enhanced affinity for bacterial membranes and carbohydrate receptors. Furthermore, these PLL-based glycopolymers exhibit hemocompatibility because of the excellent innate biocompatibility of saccharides and polysaccharides. Chan-Park et al. introduced various glycopolymers, such as chitosan [69,70], glucosamine [71], mannose, glucose, and galactose [72], to modify PLL for on-demand antibacterial conjugates. They developed a series of chitosan-*g*-PLLs that mimicked the structure of the peptidoglycan in bacterial cell wall [69]. These PLLs containing chitosan greatly improved antimicrobial activity against clinically significant gram- and gram-negative bacteria, as well as fungi, with low minimum inhibitory concentrations (MICs) (5–20 μg/mL). These also exhibited high hemocompatibility with HC_{50} (polymer concentration that causes 50% hemolysis of red blood cells) greater than 5000 μg/mL. Similarly, antimicrobial cationic peptidopolysaccharides prepared by conjugating poly(L-lysine-*ran*-L-phenylalanine) with dextran showed a potent, broad-spectrum antibacterial and antifungal activity with high selectivity [73]. Chan-Park et al. then designed antimicrobial star polymers that comprised a mixture of PLL and polyglucosamine-based arms, with PLL capable of inducing bacterial death. Likewise, the glycopolymer provides biocompatibility versus human cells as it is capable of infiltrating the peptidoglycan layer found only in bacteria [71]. Thus, by regulating the molar ratio of PLL to polyglucosamine-based arms, the antimicrobial activity and mammalian cell biocompatibility of these star polymers can be modulated. Recently, PLL was combined with a library of mannose-, glucose-, and galactose-based glycopolymers to form a four-armed star microstructure [72]. The two PLL arms confer bactericidal activity, whereas the other two glycopolymer arms moderate toxicity toward mammalian cells and enhance binding affinity for target bacteria. This study revealed that conjugates with shorter chains of mannose-based glycopolymer arms have an enhanced bactericidal efficacy against gram-negative and -positive bacteria. Interestingly, Wang et al. found that chitosan exhibited an AIE effect and excitation-dependent fluorescence, emitting colors from blue to red at diverse excitation wavelengths [74]. Accordingly, they fabricated chitosan-*g*-poly(lysine-*ran*-valine) by simulating the structure of peptidoglycan [75]. Such a peptidopolysaccharide structure not only promoted its passage through the cell wall and destroyed the bacterial structure but also enabled multi-color imaging and bacterial detection. These studies demonstrated that the combination of glycopolymers and PLL provides a new direction in the design of affinity-directed antimicrobial agents.

The hybridization of PLL with many other building blocks such as graphene, carbon nanotubes, and ethylenediaminetetraacetic acid (EDTA) has been investigated to promote antibacterial activity and biocompatibility. On account of slight bacterial resistance and tolerable cytotoxicity, graphene has been extensively investigated as a green broad-spectrum antimicrobial material which mainly produces antimicrobial action via physical damage of bacterial membranes with its sharp edges [76]. Typically, PLL is anchored to the surface of graphene via electrostatic interactions or covalent bonding to produce a series of novel composites [77]. Compared with either PLL or graphene alone, the dual-functional composites exhibit higher antibacterial activity and better biocompatibility. Single-walled carbon nanotubes are recognized as promising antibacterial agents that involve a combination of cell membrane damage and oxidative stress. Single-walled carbon nanotubes coated with PLL and poly(L-glutamic acid) (PLGA) via the layer-by-layer (LbL) technique profoundly diminish the activity of *E. coli* and *S. epidermidis* [78]. Synergistic inhibition of *E. coli* was achieved by combinational use of the antibacterial polymer PEG-*b*-PLL and the *E. coli* membrane disruptor EDTA [79]. The MICs of PEG-PLL-EDTA against *E. coli* were diminished to 1/4 of that of PEG-*b*-PLL. PEG-PLL-EDTA hybrids were less cytotoxic than PEG-*b*-PLL as a result of their reduced surface charges. The synergistic effects of an antibacterial polymer and small molecules (a bacterial membrane disruptor) with different antibacterial mechanisms may provide a versatile tool to design smart and robust supramolecular antibacterial materials.

PLL-based antibacterial gels (*e.g.*, hydrogels and cryogels) have demonstrated significant potential by virtue of their inherent antibacterial activity upon chemical and physical interactions between gels and bacteria [80]. The porous structures of gels with abundant cationic groups could adsorb anionic phospholipids from bacterial membranes, disrupting bacteria, and thus inducing bacterial death. Such gels were proposed as "anion sponges," which exhibit broad antibacterial activity. A synthetic polypeptide hydrogel with inherent antibacterial activity was constructed for cutaneous wound healing [81]. A series of polypeptides with varied hydrophobicity, poly(lysine)x(alanine)y, were synthesized and then crosslinked onto 6-arm PEG to form hydrogels that displayed significant antibacterial activity against *E. coli* and *S. aureus*. A novel peptide-based macroporous cryogel with inherent antimicrobial properties was prepared by crosslinking the amine residues of PLL-*b*-poly(L-valine) co-polypeptides with glutaraldehyde [82]. The fabricated macroporous cryogel exhibited promising antimicrobial activity against *E. coli*, which led to up to 95% reduction in viable cell counts within 1 h. Compared with the control hydrogel, the fabricated cryogel had macropores, which was vital to mediate the antimicrobial effect. The confined environment and increased antimicrobial surface area of the macropores might offer a "trap and kill" mechanism. A smart hydrogel based on L-lysine-modified cationic peptide amphiphiles with both pH-responsive and antibacterial properties has been reported [83]; in this hydrogel, sodium alginate has been introduced to enhance gelation. Hydrogelation under enzymatic catalysis to improve the crosslinking of cationic peptide amphiphiles has been employed to fabricate antibacterial hydrogels. For example, an enzyme-prompted, *in-situ*-forming PLL-*g*-4-hydroxyphenylacetic acid hydrogel was developed in the presence of horseradish peroxidase and hydrogen peroxide [84]. The hydrogels displayed good biological compatibility and shapeable properties. Such a PLL-containing hydrogel might also be used as an inherent antibacterial material for wound infection prevention.

Bacterial colonization and biofilm formation on the surfaces of biomedical devices or implants have been recognized as a serious challenge. Many antimicrobial and anti-biofilm surface coatings involving PLLs have been designed to counteract microbial contamination and biofilm-infected biomedical devices or implants [85]. There are two typical approaches for generating PLL-based antibacterial surfaces: covalent modification and noncovalent self-assembly. Schilardi et al. designed bactericidal surfaces by functionalizing titanium surfaces with PLL as a mediator for incorporating antimicrobial silver nanoparticles [86]. The physical coating of PLL on titanium surfaces not only offers intrinsic antibacterial properties but also promotes homogeneous distribution of silver nanoparticles. Antibacterial activity assays against gram-positive *S. aureus* and gram-negative *Pseudomonas aeruginosa* demonstrated that titanium surface functionalized with PLLs and silver nanoparticles had an enhanced antibacterial efficacy compared with surfaces modified with silver nanoparticles only [86]. This enhanced antibacterial activity was high enough to demonstrate bactericidal effect, which is often difficult to achieve on antimicrobial surfaces. Silicone is another widely used biomaterial that has excellent biocompatibility, superior flexibility, and bio-inertness. However, it is highly susceptible to microbial contamination and biofilm formation owing to its hydrophobicity. Ma et al. synthesized block copolymers of PEG-*b*-poly(L-lysine-*co*-L-phenylalanine) that were grafted onto the surface of silicone rubber via plasma/UV-induced surface polymerizations to form a bottlebrush-like coating [87]. These coatings demonstrated excellent antimicrobial activity against several pathogenic bacteria (*E. coli*, *P. aeruginosa*, and *S. aureus*) and effectively prevented biofilm formation. After the subcutaneous implantation of the materials in rats, the bottlebrush-like coating exhibited significant anti-infective activity *in vivo*. In another report, Ma et al. reported antimicrobial and antifouling polymer brush coatings obtained via the polymerization of methacrylate-ended poly(L-lysine-*co*-L-phenylalanine)/polysarcosine initiated under ultraviolet irradiation by a polydopamine layer [88]. This brush-like polymer coating exhibited antimicrobial efficacy against four pathogens (*S. aureus*, *E. coli*, *P. aeruginosa*, and *Candida albicans*) as well as antifouling activity. This dual-functional polymer brush coating can be immobilized on the surface of various materials and thus can be widely used for combating infections on biomedical materials of several classes. Very recently, Kang et al. fabricated tannic acid-based bifunctional brush coatings with antifouling and antimicrobial activities [89]. The naturally occurring tannic acid was dually functionalized with zwitterionic poly(2-methacryloyloxyethyl phosphorylcholine) and PLL, which self-assembled via coordination chelation with stainless steel surfaces in a simple "one-step" coating process. The functionalized stainless-steel surfaces exhibited significantly improved resistance to protein adsorption, bacterial adhesion, and microalgal attachment. Ji et al. fabricated mussel-inspired cationic PLL and mixed-charged poly(lysine-*co*-glutamic acid) catheter coatings using a dopamine-assisted co-deposition technique [90]. Although these two coatings displayed a similar antibacterial activity toward MRSA and *P. aeruginosa*, the mixed-charged polypeptide coating exhibited higher anti-biofilm efficiency, enhanced protein and platelet-resistance, and improved biocompatibility *in vitro*, as well as no inflammatory response *in vivo*.

3.2. PLL-based polymers as delivery systems

Delivery systems for therapeutic molecules have been investigated for many years. However, the fabrication of applicable vectors that can carry therapeutic molecules (*e.g.*, nucleic acids, proteins, or small-molecular drugs) across various barriers to the specific site of tissues or organs remains challenging. Among various delivery vectors, PLL-based polymers and PLL-coated nanoparticles as therapeutic nucleic acid/protein/drug delivery systems have attracted increasing attention because of their biodegradability, biocompatibility, low immunogenicity, high water solubility, and easy functionalization.

3.2.1. PLL-based polymers as gene delivery systems

The past decades have witnessed significant progress in the design and synthesis of cationic polymers as potential non-viral vectors for gene delivery [91,92]. Cationic polymers can condense nucleic acids, including small (*e.g.*, siRNA and miRNA) and large nucleic acids (*e.g.*, *p*DNA and mRNA) via electrostatic interactions to form polyplexes (in the order of 100 nm) that protect nucleic acids from degradation and enhance uptake via endocytosis. PLLs are one of the first cationic polymers to be used as transfection agents owing to their intrinsic biodegradability and high charge density, which are essential for effective nucleic acid complexation [93]. From a perspective of delivery progress, PLL-based nanomaterials must endure three successive stages *in vivo*: 1) vectors must maintain thermodynamic stability in the bloodstream, 2) vectors must accumulate at their target sites (generally tumor tissues), 3) vectors internalized by cells must escape from lysosomal or endosomal entrapment and then enter the nucleus for regulating the expression of the corresponding genes [94]. To this end, PLL-based polymers functionalized via a wide variety of strategies have been extensively explored in the field of gene delivery.

The modification of PLL with a hydrophobic segment is favorable for the amphiphilic molecules to self-assemble into well-defined micelles or vesicles, thus facilitating gene delivery and even alleviating cytotoxicity. Poly(L-leucine) (poly(L-Leu)), a hydrophobic polypeptide with facile α-helix formation, was prevalently conjugated with PLL to promote the self-assembly of co-polypeptides under aqueous conditions. PLL-poly(L-Leu) di- or tri-block co-polypeptides, including PLL-*b*-poly(L-Leu) [95], PLL-*b*-poly(L-Leu)-*b*-PLL [96,97], PEG-*b*-PLL-*b*-poly(L-Leu) [98,99], PEG-*b*-poly(L-Leu)-*b*-PLL [99], and PEG-*b*-(PLL-*co*-poly(L-Leu)) [99] have been fabricated to construct well-defined micelles or vesicles for gene delivery. The incorporation of poly(L-Leu) blocks not only allowed these synthetic polypeptides to self-assemble into micelles or vesicles with excellent colloidal stability, but also reduces the charge density, leading to an easier release of *p*DNA from the complexes, and thus a

higher transfection efficiency [96]. It revealed that the elongation of poly(L-Leu) chains improved the transfection efficiency via affording a rapid cellular uptake [95]. Very recently, Scholz et al. synthesized a series of PEG-*b*-PLL-*b*-poly(L-Leu), PEG-*b*-poly(L-Leu)-*b*-PLL, and PEG-*b*-(PLL-*co*-poly(L-Leu)) to investigate the effect of the spatial arrangement of PLL and poly(L-Leu) on the *p*DNA condensation capacity [99]. A more efficient *p*DNA complexation was observed when PLL and poly(L-Leu) formed individual blocks as opposed to them forming a random copolymer. PLL-*b*-poly(L-Leu)-*b*-PLL was also utilized as free cationic chains to promote the transfection efficiency of *p*DNA by a natural protamine. The hydrophobic middle poly(L-Leu) block was favorable for the insertion into the cell membrane, while the interactions between two PLL ends and the signal proteins facilitated the escape of the polyplexes from lysosomal entrapment. The combined use of a natural cationic protamine to condense anionic DNA and the polypeptides to promote different stages of the gene transfection process presented a more rational design and development of effective non-viral gene vectors [97]. Another hydrophobic segment of poly(α-amino palmitic acid) (PAPA) was introduced into chimeric liposomes constructed from an asymmetric PEG-*b*-PAPA-*b*-PLL tri-block copolymer as an ideal envelope-type carrier with improved gene packing and high stability against enzymes for efficient delivery of polo-like kinase 1 siRNA (siPLK1) to orthotopic human lung tumor xenografts *in vivo* [100]. It is worth noting that a higher density of PLL generally facilitates the gene condensing, the polyplex stability, and the cellular uptake. However, a higher density of positive charge deteriorates the intracellular release of *p*DNA cargoes. An interesting approach focused on a temperature-responsive "switchable" polymer segment between hydrophilicity and hydrophobicity, which indicated that hydrophilic/hydrophobic balance indeed affected polyplex formation and stability and thus, delivery performance [101]. Very recently, Miyata et al. incorporated a thermo-switchable block to an unimer poly-ion complex (uPIC) fabricated from siRNA and a tri-block copolymer comprising hydrophilic poly(2-ethyl-2-oxazoline), thermo-switchable poly(2-*n*-propyl-2-oxazoline), and cationic PLL to regulate the hydrophobicity of siRNA nanocarriers [102]. Hydrophobicity of the uPIC was improved at 37 °C since dehydration of poly(2-*n*-propyl-2-oxazoline) segment caused a hydrophilic-to-hydrophobic conversion, allowing for more potent hydrophobic association with the lipid layers of cell membranes, greater uPIC adsorptive endocytosis, and enhanced gene silencing effects of siRNA. In summary, the charge density and hydrophilic/hydrophobic balance can be carefully modulated to regulate the polyplex formation, the nucleic acids release, and the transfection efficiency [103].

PLLs exhibit moderate to high toxicity as a result of their positive charge under physiological conditions. To address this problem, the structure of PLL has been modified with PEG to provide PLL-*g*-PEG and PEG-*b*-PLL block copolymers, thus reducing the cytotoxicity of PLL and alleviating the nonspecific combination of PLL with serum proteins [104,105]. Furthermore, the PEGylation of PLL offers an opportunity to construct polyplex micelles with a core-shell structure, wherein *p*DNA molecules were packaged as a core surrounded by a PEG shell. Steric repulsion of the PEG shell provided a high shielding effect against nuclease and elongated the circulation time *in vivo*. Meanwhile, a peripheric multi-arm PEG on polycation vectors was demonstrated to effectively inhibit DNA condensation because of the steric repulsive effect among PEG arms at polyplex surfaces (*i.e.*, an intra-polyplex PEG crowding effect) [106]. The molecular weight, grafting density, and arm number of PEG on PLL-*g*-PEG were observed to affect the shape, size, and storage stability of the formed polyplexes, and eventually, the transfection efficiency [105,107,108]. Interestingly, Kataoka et al. determined the PEG crowdedness for PEGylated polyplexes comprising PEG-*b*-PLL and *p*DNA. Tethered PEG chains adopted a mushroom and even a squeezed conformation by modulating PEG crowdedness through PLL segment length. PEG crowdedness had a significant correlation with blood circulation profile; moreover, polyplex micelles with PEG assuming a squeezed conformation had better retention time in blood circulation than those assuming a mushroom conformation [109]. A similar study was also performed by Kono's group, who first reported a head-tail type polycation-based gene vector prepared from a PAMAM dendron head and a PLL tail [110]. They then introduced PEG chains to the periphery of the PAMAM dendron head to provide PLL with a terminally bearing multi-arm PEG [111]. Such PEGylation demonstrated effective tolerance to serum proteins and high efficiency of intracellular uptake. Furthermore, the morphological characteristics (*e.g.*, the aspect ratio of the polyplexes) could be controlled via the modulation of PEG crowdedness, which plays a vital role in dictating the *in vivo* circulation and transfection efficiency. Increased PEG crowdedness, resulting in an intra-polyplex PEG crowding effect, causes elongation of the polyplexes from spherical aggregates to nanorods and nanofibers as well as increased cell-free gene expression [108]. Aspect ratio-controlled polyplex formation was achieved via the mixing of *p*DNA with a polycation mixture of multi-arm PEG-PLL and PLL homopolypeptide at varying ratios (Fig. 4A) [112]. Increasing the multi-armed PEG-PLL content, i.e., the apparent increase in multi-armed PEG crowding, increased the aspect ratio of the polyplexes. Changing the aspect ratio of the polyplexes was observed to have opposing effects regarding cellular uptake and transcription, indicating that fine-tuning the aspect ratio is crucial for obtaining optimal gene expression *in vitro*. Another prominent strategy to improve the transfection efficiency and diminish the cytotoxicity of PLLs was explored by Chen's group, where a "molecular string" RT (*i.e.*, *p*-toluylsulfonyl [T] arginine [R]) was grafted onto the PLL backbone. The incorporation of the RT ring retained the amino groups but reduced the cationic density, resulting in low cytotoxicity (Fig. 4B) [113]. Notably, the introduction of an RT string contributed to the formation of multiple interactions between the polycationic gene carriers and cell membranes or DNA, as well as the adoption of an α-helix conformation, all of which would be beneficial for enhancing gene transfection.

The high strength of the complexation between PLL-based delivery systems and nucleic acids poses a dilemma, and a rather strong combination is required for efficient nucleic acid condensation. In contrast, easy intracellular escape of nucleic acids from the vector is also a requirement. To this end, pH-, redox-, and light-sensitive gene delivery systems have been explored for stimuli-responsive nucleic acid delivery. Notably, an efficient strategy to confer pH-responsive, charge-conversional properties was developed via optimizing the hybrid components of cationic PLL and anionic amino acids with distinct ionization behaviors. Thus, pH-responsive gene delivery systems were fabricated by introducing rapid charge-conversional zwitterionic co-polypeptides, PEI-*g*-PLL-*b*-poly(L-aspartic acid) or PEI-*g*-(PLL-*b*-PLGA), as shielding agents for the polyplexes formed using PEI and *p*DNA [114,115]. The zwitterionic co-polypeptides were negatively charged under physiological pH, thus shielding the positive charges of polyplexes. The acidic pH of the tumor environment resulted in the charge conversion of the shielding co-polypeptide and the restoration of the positive charges of the polyplex, leading to high cellular uptake of the polyplex. *In vitro* and *in vivo* studies demonstrated that the use of the zwitterionic co-polypeptides as the charge-conversional shielding systems for polycationic gene carriers improved the transfection efficiency. pH-responsive charge-conversional PEI-*g*-(PLL-*b*-PLGA) was also utilized as a direct gene delivery system [116]. The pH-responsive charge-conversional property was conferred to PEI-*g*-(PLL-*b*-PLGA) by tailoring the molar ratio between positively charged L-lysine units and negatively charged L-glutamic acid units. The PLL blocks condensed *p*DNA to form the core of polyplexes, which were shielded by PLGA segments. The charge conversion of PEI-*g*-(PLL-*b*-PLGA) from negative (under physiological pH) to positive (in acidic tumor environment) improved the cellular uptake of polyplexes, which resulted in efficient gene delivery. A three-layer core-shell nanoplatform comprising the PEI/siRNA polyplex as the inner core, citraconic anhydride-grafted PLL as the charge-conversional middle shielding shell, and

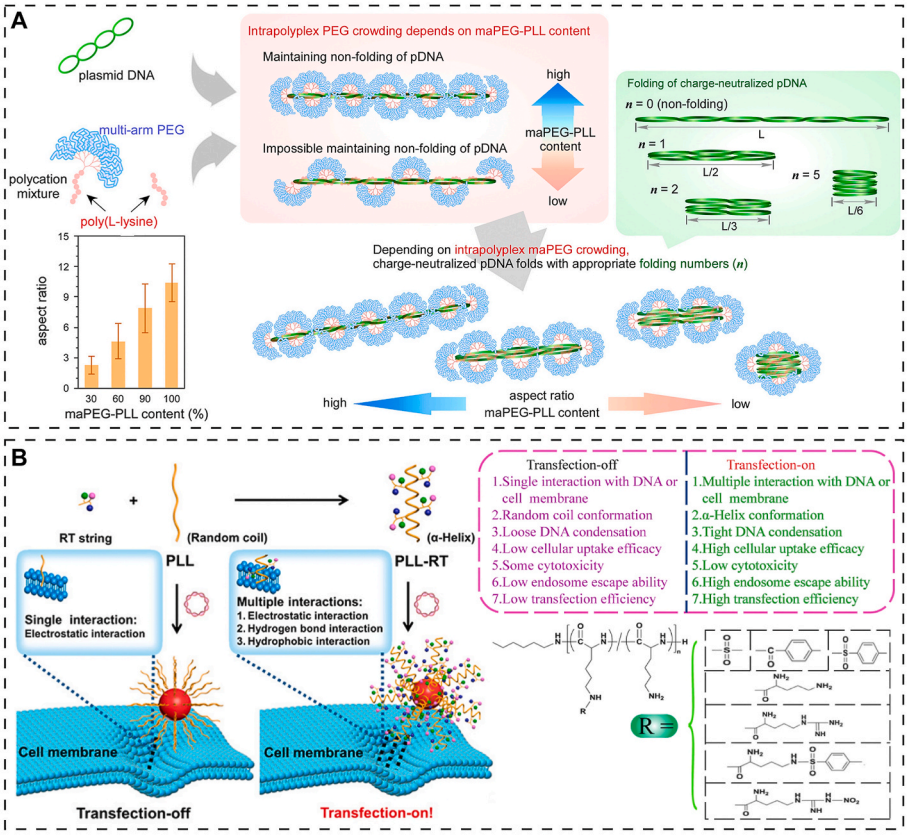

Fig. 4. Construction of gene vectors with high transfection efficiency and low cytotoxicity. (A) Aspect ratio-controlled (modulating PEG crowdedness) polyplex formation with multi-arm PEG-PLL. Reproduced with permission from Ref. [112], copyright 2019, American Chemical Society. (B) Introducing multiple interactions and α-helix conformation into PLL-based polycations via the incorporation of an RT ring in PLL. Reproduced with permission from Ref. [113], copyright 2018, American Chemical Society.

angiopep-2-coated red blood cell membranes as the biomimetic, immune-free homologous surface were prepared for programmatically delivering siRNA to glioblastomas [117]. The angiopep-2-coated red blood cell membrane not only protects siRNA from nonspecific clearance and immune responses, thereby enhancing both pharmacokinetics and pharmacodynamics, but also promotes blood-brain barrier penetration and then glioblastoma-targeted internalization. Citraconic anhydride was dissociated to restore the cationic amine groups once inside the acidic endo/lysosome of tumor cells, causing red blood cell membrane disruption and siRNA release for specific gene silencing and strong tumor suppression. Interestingly, Shi et al. constructed multistage delivery nanoparticles [118] and dual-locking nanoparticles [119] with the PEI-based copolymer/*p*DNA polyplex as their core and PLL-based copolymers as the shell for pH-sensitive and pH/ROS dual-sensitive delivery of CRISPR/dCas9 and CRISPR/Cas13a, respectively. PLL-based shell was dissociated to expose the cationic core in the tumor microenvironment with both low pH and high H_2O_2 concentration, thus leading to the accumulation of the cationic polyplex at tumor sites. The reductive conditions, particularly increased glutathione (GSH) concentration in diseased tissues, could be utilized for designing reduction-sensitive gene nanocarriers. Post-crosslinking polyplexes formed between PEG-*b*-PLL and *p*DNA via disulfide bonds could mitigate the unfavorable effects of shear stress during blood circulation; this mitigation probably alters the stability and integrity of polyplexes and might afford redox-responsive release of the loaded *p*DNA cargoes [120]. Huh et al. developed an AB_2 mikto-arm polymeric gene carrier comprising one PEG block and two PLL blocks, with the PLL blocks conjugated to the PEG block with or without bio-reducible disulfide linkers. The bio-reducible nanocomplexes formed with *p*DNA showed lower cytotoxicity and higher gene expression in cancer cells than the non-reducible nanocomplexes [121]. Caruso et al. explored redox-sensitive PEG-PLL nanoporous particles for the delivery of siRNA targeting the anti-apoptotic factor, survivin, in prostate cancer cells [122]. PEG-PLL nanoporous particles, containing PEG and PLL blocks crosslinked via the disulfide bond, were prepared using mesoporous silica particles as sacrificial templates. These engineered nanoparticles were redox responsive and highly loaded depots that allowed effective silencing of the target gene in prostate cancer cells. Light, a potential external stimulus for photo-responsive delivery systems, has unique advantages, including spatiotemporal precision, relative safety, and minimal cross-reaction with cellular signaling networks, enabling site-specific payload release by precisely controlling light irradiation site, dose, and duration [123]. Typically, photochemical internalization (PCI)-mediated gene transduction via the systemic administration of a multi-compartmentalized nanocarrier was first designed by Kataoka et al. The ABC-type tri-block copolymer nanocarrier had a PLL core compartment for stable encapsulation of *p*DNA, an intermediate compartment for the incorporation of photosensitizer to induce PCI, and an outer PEG compartment that formed a shield. The polyplex micelles displayed >100-fold photo-enhanced gene expression in cultured cells and exhibited light-induced *in vivo* gene transfer in solid tumors following systemic administration [124]. A PLL-based siRNA delivery vehicle functionalized with melanin, which has an excellent photothermal conversion efficiency, was reported to confer the vehicle with easy rupturing of endosomal membranes and to facilitate siRNA delivery into the cytoplasm upon near-infrared (NIR) light irritation [125]. Notably, Kataoka et al. designed a pH and disulfide-reduction, dual stimuli-responsive delivery vehicle to transport *p*DNA into cell cytoplasm [126]. The thiolated PLLs formed polyplexes with *p*DNA via electrostatic interactions, and these polyplexes were then crosslinked through disulfide formation to enhance the stability in the extracellular environment. A ternary structure was fabricated by coating this binary polyplex with a pH-sensitive PEGylated polyanion, which could be degraded at acidic pH to generate a membrane-active polymer. This regenerative polymer was active toward endosomal or lysosomal membranes and could thus assist in the endosomal escape of *p*DNA

following endocytosis. The ternary crosslinked polyplex appreciably enhanced gene transfection efficiency without associated cytotoxicity by featuring reversible stability, endosome-disrupting functionality, and PEGylation.

PLLs can be readily functionalized with various targeting ligands, including folic acid [127,128], cRGD [129], CAGW peptide (Cys-Ala--Gly-Trp) [130], galactose [131], and glucose [132], to improve the selectivity and cellular internalization of the polyplex toward tumor cells or tissues or to enhance transport across the blood-brain barrier. Folic acid receptors are often overexpressed in tumor cells but are rarely found in healthy tissues, and folic acid is the most commonly used targeting ligand in tumor-targeting delivery systems. For example, Shuai et al. used folate-coated PEG-PLL-*g*-PEI for gene delivery. They found that the folate-PEG-PLL-*g*-PEI/*p*DNA complex exhibited higher transgene activity than its nontargeting counterpart for both reporter and therapeutic genes in folate receptor-positive cells [127]. The RGD tripeptide is widely used as a tumor homing ligand to selectively bind to integrin αvβ3 receptors, which are overexpressed in cancer cells and tumor blood vessels. Kataoka et al. investigated the targeted systemic delivery of siRNA to a cervical cancer model using cRGD-functionalized uPIC-assembled gold (Au) nanoparticles, where the uPIC comprised one PEG-*b*-PLL catiomer and a single molecule of therapeutic siRNA [129]. The targetability of cRGD allowed enhanced cellular internalization in cultured cells and induced more efficient E6 oncogene silencing in the cervical cancer model. Feng et al. used CAGW functionalized charge-conversional PLLs as the shielding polymers to coat binary gene complexes [130]. The CAGW targeting peptide promoted cellular uptake, improved gene delivery efficiency, and enhanced human umbilical vein endothelial cell proliferation and migration. Galactose, a specific gene-targeting ligand of the asialoglycoprotein receptor in hepatocytes, was employed to modify PEG-*b*-PLL to produce galactose-PEG-*b*-PLL. Galactose-PEG-*b*-PLL can form a complex with *p*DNA and serve as an effective gene delivery carrier with lower cytotoxicity than PLL. A transfection study showed that galactose-PEG-*b*-PLL effectively delivered DNA into hepatoma cells *in vitro* [131]. Recently, Kataoka et al. fabricated an antisense oligonucleotide-loaded, glucosylated poly-ion complex micelle from antisense oligonucleotides with a mixture of glucose-PEG-PLLs and PEG-PLLs. PIC micelles are capable of crossing the blood-brain barrier via the specific binding of glucose with glucose transporter-1 expressed on brain capillary endothelial cells, enabling noninvasive antisense oligonucleotide administration to brain parenchyma for treating central nervous system disorders [132]. Numata et al. prepared a novel exogenous gene delivery carrier in the form of maleimide-conjugated tetra(ethylene glycol)-*b*-PLL (Mal-TEG-PLL) [133]. Upon condensation with *p*DNA, Mal-TEG-*b*-PLL adopted a micellar structure with maleimide groups on the surface, allowing for post-modification with cysteine-containing targeting moieties such as the cell-penetrating peptide BP100. Such peptide post-modifications could avoid undesired interactions, and the gene delivery efficiency conferred by such modifications is highly dependent on the amount of BP100 on the micelle surface.

Native PLLs have relatively low transfection efficiency owing to complete protonation of the pendent primary amino groups, implying the absence of buffering capacity (*i.e.*, the lack of a "proton sponge" or "pH-buffering" effect) at a pH below 8. Once entrapped within endosomes, PLLs hardly buffer the acidification and escape from unfavorable environments under time-efficient modes to avoid enzymatic degradation in lysosomes [134]. The modification of PLLs with protonatable polyamine-containing polymers at acidic endosomal pH could enhance Cl^- accumulation as well as osmotic swelling or lysis; this modification can also improve the endosomal escape of polyplexes via the so-called "proton sponge" effect, thus increasing transfection efficiency [135]. PEI, PAMAM, and poly(L-histidine), among others, containing polyamines (such as tertiary amines) with low pK_a and thus demonstrating excellent buffering capacity in acidic environments [134], have been the most-investigated polymers for modifying PLLs for gene delivery. Shuai et al. grafted linear PEI onto block copolymer PEG-*b*-PLL to produce the ternary copolymer PEG-*b*-PLL-*g*-PEI for gene delivery. In this copolymer, PLL, PEI, and PEG were expected to render nucleic acid condensation, proton buffering capacity, and potentially long circulation time *in vivo*, respectively [127]. Minko et al. fabricated PAMAM-*b*-PEG-*b*-PLL tri-block nanocarrier for the delivery of siRNA. The tertiary amine groups in the PAMAM dendrimer functioned as a proton sponge and played a vital role in endosomal escape [136]. Sasaki used mixtures of PLL and poly(L-histidine) as gene delivery vectors and demonstrated that the addition of poly(L-histidine) to PLL complexes promoted endosomal escape by enhancing the pH-buffering effect [137]. Bae et al. prepared two types of histidine-grafted PLLs: a stacking form of poly(L-histidine) (PLL-*g*-PHis) and a mono-L-histidine (PLL-*g*-mHis) with the same number of imidazole groups. Both mHis and PHis grafting enhanced the buffering capacity of PLL, and PLL-*g*-PHis containing imidazole rings in polymer form had significantly stronger endosomolytic activity and showed enhanced gene transfection compared with PLL-*g*-mHis [138].

In addition to regulating the composition of cationic polymers, modulating their spatial structure also has a significant influence on their transfection efficiency and cytotoxicity. Star-shaped PLLs present unique opportunities for potential cargo encapsulation, cargo binding, cell targeting, stimuli-responsive release, and enhanced biocompatibility [139]. Notably, star-shaped or brush-like PLLs with well-defined spatial conformation and reasonable chain flexibility can be possibly combined with *p*DNAs to form stable nanoparticles. Pan et al. prepared star-shaped PAMAM-*g*-PLL by grafting PLL on the surface of the PAMAM dendrimer. PAMAM-*g*-PLL could condense *p*DNA to form spherical complexes with a diameter ranging from 100 to 200 nm. The unique radial charge distribution of PAMAM-*g*-PLL resulted in higher transfection efficiency *in vitro* than the PAMAM or the PLL counterpart, as well as significant inhibition of restenosis via local gene delivery in injured rabbit vessels [140]. Klok et al. systemically investigated the influence of molecular weight and architecture of linear, hyperbranched, and dendritic PLLs on their transfection properties [141]. At a comparable polycation molecular weight, using hyperbranched PLLs provided higher transfection efficiency than using linear and dendritic PLLs. This was likely attributed to higher affinity binding to external cell membranes, a more substantial fraction of free cation polymer, and higher pH-buffering capacity of hyperbranched PLLs. Cryan's group prepared star-shaped PPI-*g*-PLL copolymers by covalently grafting a range of polypropyleneimine (PPI) dendrimer generations with varying lengths of divergent PLL chains. The star-shaped polypeptides were capable of condensing both *p*DNA and siRNA, unlike linear PLLs. All star-shaped polypeptide-based polyplexes adopted a discrete spherical shape, whereas linear PLLs formed elongated, irregularly shaped complexes. This difference in morphology may help explain the 300-fold increase in luciferase expression demonstrated by star-shaped PLL polyplexes compared with that demonstrated by linear PLL polyplexes in epithelial cells [142]. Star-shaped PPI-*g*-PLLs served as particularly efficient vectors that could deliver both reporter genes and the therapeutic transgenes bone morphogenetic protein-2 and vascular endothelial growth factor to mesenchymal stem cells (MSCs), a cell type often refractory to transfection. This composition facilitated a 1000-fold increase in transgene expression in MSCs compared with its linear PLL analog [143]. Cylindrical co-polypeptide brushes in the form of PLLF-*g*-(PLF-*b*-PLL), comprising poly(L-lysine-*co*-L-phenylalanine) (PLLF) as the backbone and poly(L-phenylalanine)-*b*-poly(L-lysine) (PLF-*b*-PLL) amphiphilic block co-polypeptides as side chains, have been synthesized and evaluated as gene carriers by our group [144]. The synthesized polymer brushes adopted cylindrical morphology and resembled unimolecular micelles with a hydrophobic oligo(L-phenylalanine) core and a hydrophilic PLL shell. These co-polypeptides with brush-like PLL arms demonstrated low cytotoxicity and strong complexation capacity with *p*DNA. The *in vitro* transfection study revealed that these co-polypeptide brushes could mediate efficient gene transfection in HEK293 and HeLa cells.

PLL-inorganic nanoparticle hybrids with integrated advantages have garnered considerable attention in the field of gene delivery. Large-pore mesoporous silica nanoparticles functionalized with PLLs were designed as a new carrier material for gene delivery by Qiao et al. PLLs were conjugated to large-pore mesoporous silica nanoparticles via 3-glycidoxypropyl trimethoxysilane, avoiding reduction in silica particle porosity and pore blockage. PLL-functionalized nanoparticles exhibited high binding capacity and efficient delivery of functional siRNA against minibrain-related kinase and polo-like kinase 1 in osteosarcoma cancer cells [145]. A PLL epilayer covalently attached to an Au nanoshell was used to capture antisense ssDNA or siRNA molecules. The controlled release of the captured therapeutic oligonucleotides was accomplished by continuous NIR laser irradiation. This PLL nanoshell complex was successfully used as a non-viral delivery vector characterized by laser-triggered release of antisense ssDNA or siRNA to silence the target reporter gene and downregulate protein expression *in vitro* [146]. Lee et al. prepared a bidentate dithiol-mediated stable coating of PLL on Au surfaces for loading and releasing ssDNA [147]. They used a mixture of PLL molecules with different chain lengths that provided an optimized platform for loading ssDNA and for thermal-responsive release. Kataoka et al. developed uPIC-assembled Au nanoparticles (AuNPs) for the controlled release of siRNA to tumors (Fig. 5) [148]. The nanoarchitecture was constructed by generating uPIC from a charge-matched PEG-*b*-PLL copolymer and a single siRNA, which was then attached to the Au nanoparticle via a thiol-Au bond. The uPIC-AuNPs with a uniform size of 38 nm were stable in serum-containing media and more resistant to heparin-induced counter-polyanion exchange. At the same time, glutathione substantially compromised its stability and triggered the release of siRNA. The uPIC-AuNPs delivered siRNAs into cultured cancer cells, facilitating significant sequence-specific gene silencing without cytotoxicity. The uPIC-AuNPs efficiently accumulated in a subcutaneously inoculated luciferase-expressing cervical cancer model and achieved significant luciferase gene silencing in the tumor tissue. Equipping the surface of uPIC-AuNPs with cRGD or glucose further allowed targeted siRNA delivery to cervical cancer cells or glucose transporter 1-overexpressing breast cancer stem-like cells [129,149].

3.2.2. PLL-based polymers as drug delivery systems

Specific and efficient intracellular delivery of chemotherapeutic agents across various biological barriers remains a prominent challenge. The unintended delivery of chemotherapeutic agents to unaffected body parts could result in systemic toxicity or immunogenic reactions [150]. Thus, drug delivery systems that could efficiently encapsulate chemotherapeutic agents, alleviate premature drug leakage, and target specific tissues are considerably potential for improving the treatment of diseases through enhancing therapeutic efficacy and minimizing nonspecific toxicity [151,152]. PLL serves as a superior candidate as a drug delivery system and has been extensively explored as various formulations such as nanocapsules, micelles, and hydrogels. Drug molecules can be chemically conjugated to PLL-derived polymers with labile linkages or physically entrapped into the interior of the delivery systems.

Polymeric nano-sized capsules and vesicles hold promise for high drug-loading capacity and versatile encapsulation. Du et al. reported poly(L-lysine-*stat*-L-phenylalanine)-grafted chitosan-based nanocapsules with an excellent antibacterial efficacy and blood compatibility as a drug carrier (Fig. 6A). Nanocapsules could simultaneously deliver anticancer drugs such as doxorubicin (DOX) and antiepileptics such as dilantin and showed an enzyme-triggered release profile [153]. Later, they used copolymer PCL-*b*-poly[L-lysine-*stat*-L-phenylalanine-*stat*-(L-lysine-folic acid)] to fabricate antibacterial, biocompatible, biodegradable, and tumor-targeted vesicles for the delivery of DOX [154]. Yang et al. reported a co-assembly method to prepare pH-sensitive polymeric vesicles from cholate-grafted PLL and a PEG-DOX conjugate with a low-pH labile benzoic imine linkage. The pH sensitivity led to enhanced uptake of the vesicles by MCF-7 cancer cells under conditions resembling the extracellular environment of solid tumors (pH 6.5) and subsequent efficient endosome escape after endocytosis [155]. A class of PIC vesicles with the external surface-grafted glycopolypeptides were fabricated from oppositely charged hydrophilic block copolymers of glycopolypeptide-*b*-PLL and PEG-*b*-poly-(L-glutamate), such vesicles could be used as carriers for the versatile encapsulation of hydrophilic drugs with sustained release [156].

Polymeric micelles formed from amphiphilic block copolymers have attracted remarkable attention as drug nanocarriers because of their easy fabrication, structural diversity, tailorable drug-loading capacity, and improved stability relative to traditional micelles formed from surfactant molecules. Generally, polymeric micelles have an appropriate size in the order of tens of nanometers, which facilitates increased accumulation in tumor tissues or organs owing to the so-called enhanced permeability and retention (EPR) effect [159]. Many PLL-based polymers have been employed to construct polymeric micelles for drug delivery. Drug molecules can be physically solubilized in the core of polymeric micelles for efficient encapsulation. For example, Chen et al. synthesized poly(L-aspartic acid(lysine))-*b*-poly(L-lysine(Z)), a novel amphiphilic block polypeptide with key structures of protein, as a nano-drug vehicle for the delivery of doxorubicin (DOX). The polypeptide self-assembled into micelles in aqueous solution with a zwitterionic shell, demonstrating an excellent antifouling property and biocompatibility. The loaded DOX showed low cell uptake because of

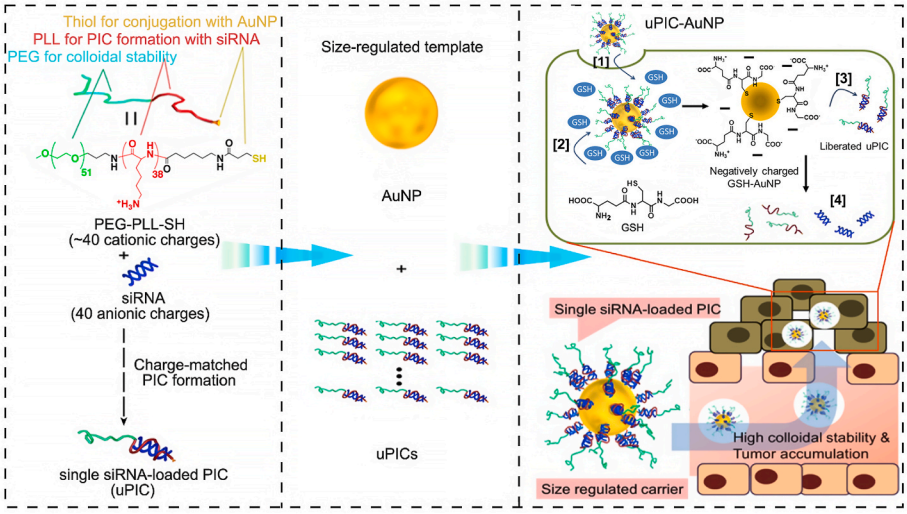

Fig. 5. Illustration of the nanoconstruction of uPIC-AuNPs, where a single pair of positively charged PEG-PLL combined with negatively charged siRNA to form uPIC via charge-matched polyionic complexation. The uPICs were used as monodispersed building blocks for fabricating smart uPIC-AuNP nanoarchitecture. The uPIC-AuNP nanoarchitecture exhibited high colloidal stability, tumor accumulation, and redox-responsive release of siRNA in the presence of GSH. Reproduced with permission from Ref. [148], copyright 2014, American Chemical Society.

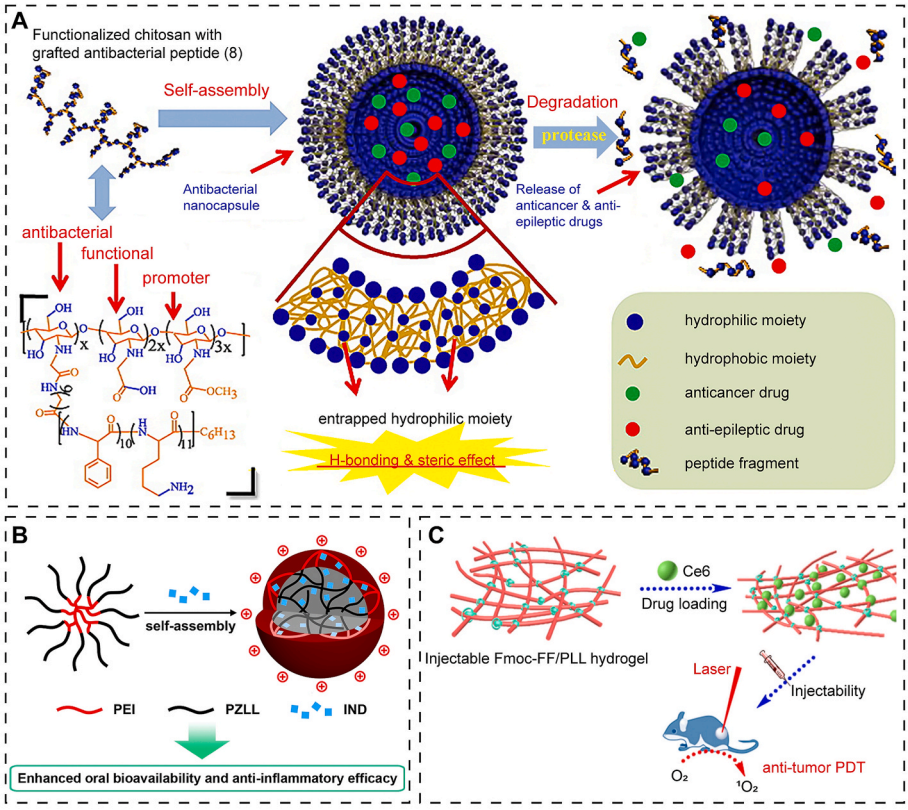

Fig. 6. Construction of diverse PLL-based drug delivery systems. (A) Antibacterial polypeptides-grafted chitosan-based nanocapsules as "armed" drug carriers. Reproduced with permission from Ref. [153], copyright 2013, American Chemical Society. (B) Surface-crosslinked polymeric micelles from amphiphilic star-block copolymers for indomethacin (IND) encapsulation. Reproduced with permission from Ref. [157], copyright 2017, Elsevier. (C) Injectable polypeptide-based fibrous hydrogels for the delivery of chlorin e6. Reproduced with permission from Ref. [158], copyright 2018, American Chemical Society.

the zwitterionic polypeptide shell, thus prolonging the circulation time [160]. L-Phenylalanine-grafted PLL was used to prepare a capacious nanoemulsion for the encapsulation of squalene that served as a potential adjuvant for the influenza vaccine [161]. The nanoemulsion exhibited a high squalene-loading efficiency, good biocompatibility, and excellent colloidal stability. The squalene-loaded nanoencapsulation could induce effective humoral and cellular immunity against the influenza virus. Recently, Han et al. fabricated mucoadhesive cationic polypeptide micelles from poly(L-lysine-*ran*-L-phenylalanine) for sustained cabozantinib release and inhibition of corneal neovascularization [162]. The cationic characteristics of PLL present on polypeptide micelles facilitated their penetration through the hydrophilic tear film as well as their adhesion to the mucosal surface of the cornea via electrostatic interactions. This resulted in prolonged retention time and improved bioavailability of cabozantinib. Therefore, these cabozantinib-loaded micelles exhibited superior anti-angiogenic effects without apparent side-effects.

Another strategy for drug encapsulation in polymeric micelles is to employ polymer-drug conjugates. Chemical bonding between drug molecules and the amine residues of PLL via labile linkages can maintain the integrity of the micellar structure by avoiding undesirable disassembly during bloodstream circulation. This can also ensure triggered release of drug molecules in the cytosol. Ma et al. prepared DOX-conjugated polymeric micelles based on the 4-carboxy benzaldehyde-grafted PLL-*b*-poly (methacryloyloxyethyl phosphorylcholine) copolymer by conjugating DOX and electronegative 4-carboxy benzaldehyde to the PLL block via an imine linkage [163]. The drug-loaded micelles exhibited pH-triggered charge conversion and accelerated drug release in acidic tumor microenvironments, resulting in excellent *in vitro* and *in vivo* antitumor efficiency. Ding et al. prepared a self-programmed pH-sensitive prodrug micelle from protocatechualdehyde and bortezomib grafted PEG-*b*-PLL with imine bonds. Compared with protocatechualdehyde or bortezomib alone, the micelles showed synergistic cellular growth inhibition and apoptosis. The pH-sensitive micelles significantly inhibited tumor growth following intravenous injection into nude mice bearing a subcutaneous liver tumor [164].

Polymeric micelles exist in a dynamic equilibrium between micelles and individual polymer chains in infinite dilute environments, such as after introduction into the bloodstream. Once the concentration drops below the critical micelle concentration, the disruption of micellar structures leads to a burst release of entrapped drugs. Micellar stability can be improved by covalent crosslinking of preformed polymeric micelles either in the core, through the waist, or on the corona [165]. Jan et al. prepared core crosslinked PIC micelles from poly(diethylenetriaminepentaacetic acid-*co*-cystamine) and PEG-*b*-PLL using genipin as the cross-linker. The stabilized polymeric micelles were used to encapsulate DOX and afforded noticeable pH- and GSH-responsive releasing behavior [166]. Core crosslinked PIC micelles were also developed from PEG-*b*-poly(lysine-*co*-lysine-phenylboronic acid) for the co-encapsulation of DOX and (-)-epigallocatechin-3-O-gallate (EGCG) [167]. Electrostatic interactions and phenylboronic acid-catechol interactions between PEG-*b*-poly(lysine-*co*-lysine-phenylboronic acid) and EGCG allowed the construction of core crosslinked PIC micelles with high stability under physiological conditions. Liu et al. prepared polymeric micelles from the amphiphilic tri-block copolymer PEG-*b*-PLL-*b*-PLF, which were then crosslinked through the waist using the disulfide-containing crosslinking agent 3,3′-dithiobis(sulfosuccinimidylpropionate). Crosslinking improved the stability of paclitaxel (PTX) [168] or DOX [169] -loaded micelles and alleviated premature drug leakage, and therefore, significantly suppressed tumor cell viability, proliferation, and migration *in vitro*, and also significantly inhibited tumor growth and prolonged survival in tumor-bearing mice without detectable side-effects. We have synthesized star-block copolymers PEI-*g*-PZLL with a branched PEI core and multiple grafted poly (ε-benzyloxycarbonyl-L-lysine) (PZLL) peripheral chains [157]. In aqueous solutions, the amphiphilic star-block copolymer (hydrophilic PEI and hydrophobic PZLL) enabled spontaneous self-assembly into well-defined nano-sized polymeric micelles with inverted core-shell

structures. This micellar structure was stabilized by the partial surface crosslinking of PEI. The surface-crosslinked polymeric micelles demonstrated high loading capacity and loading efficiency for indomethacin (IND) (Fig. 6B). The utilization of PEI-*g*-PZLL as a carrier significantly enhanced the oral bioavailability of IND and improved its protective effect on renal ischemia-reperfusion injury, as evidenced by *in vivo* pharmacokinetic and pharmacodynamic studies [157]. We further fabricated super-amphiphiles in the form of PEG-crosslinked multi--armed PEI-*g*-PZLL as curcumin carriers. The curcumin-loaded crosslinked polymeric micelles demonstrated high thermodynamic stability, high loading capacity, and improved cellular uptake, thus displaying strong inhibitory effects on the growth of human hepatoma (HepG2) cells [170].

An alternative strategy to improve the stability of polymeric micelles is the employment of unimolecular polymeric micelles generally developed from dendritic polymers such as dendrimers and hyperbranched polymers. Such unimolecular polymeric micelles exhibit excellent stability against dilution because of covalent linkages rather than the utilization of intermolecular forces. These unimolecular micelles can be designed to encapsulate not only hydrophobic drugs but also hydrophilic guest molecules through various host-guest interactions, including hydrophobic interactions, electrostatic interactions, hydrogen bonding, or their combinations. Conversely, drug molecules can also be chemically conjugated to the interior of unimolecular micelles via labile linkages, therefore, enabling responsive drug release upon stimulation such as pH, a reducing agent, or temperature. The peripheries of these unimolecular micelles could also be decorated with various functionalized groups with targeting and imaging properties to prepare multifunctional drug nanocarriers [171]. Since 2009, our group has been working on the design, synthesis, and evaluation of star-shaped and brush-like block copolymers as unimolecular polymeric micelles for the delivery of various drugs. Star-block copolymers PEI-*g*-(PLL-*b*-PEG) with a branched PEI core, a PLL inner shell, and a PEG outer shell were synthesized and evaluated as potential nanocarriers for hydrophilic anionic drugs. Anionic drug diclofenac sodium can be encapsulated efficiently by PEI-*g*-(PLL-*b*-PEG), and the entrapped drugs demonstrated pH-responsive release [172]. The incorporation of poly (L-phenylalanine) (PLF) hydrophobic blocks to PEI-*g*-(PLL-*b*-PEG) gave rise to the star-block quadripolymers PEI-*g*-(PLF-*b*-PLL-*b*-PEG), which can simultaneously encapsulate hydrophobic and hydrophilic drugs in the site-isolated state [173]. The replacement of PLL with PZLL blocks in PEI-*g*-(PLL-*b*-PEG) could convert the anionic drug carrier to hydrophobic drug delivery vectors. Such unimolecular polymeric micelles demonstrate high loading capacity toward poorly water-soluble drugs [174]. We also developed a type of cylindrical co-polypeptide brush, consisting of a poly(L-lysine-*co*-L-phenylalanine) (PLLF) backbone and PLF-*b*-PLL amphiphilic block co-polypeptide side chains, which resembled unimolecular polymeric micelles with a hydrophobic PLF core and a hydrophilic PLL periphery. The amphiphilic cylindrical co-polypeptide brush was demonstrated to be a versatile nanocarrier to solubilize hydrophobic drugs in the PLF core and to entrap hydrophilic molecules through the PLL periphery simultaneously [175]. Another PLL-based amphiphilic brush copolymer, PLL-*g*-(PZLL-*b*-PEG), with a PLL backbone and amphiphilic PZLL-*b*-PEG side chains from our previous report served as promising unimolecular nanocontainers to encapsulate hydrophobic drug molecules with high loading capacity [176].

Hydrogels comprise three-dimensional network structures with unique properties such as porosity, strength, degradability, and swelling in aqueous environments that can be tuned over a wide range of parameters, making them ideal for the application in drug delivery systems [177]. Spontaneous hydrogelation occurs for a PLL-based amphiphilic block or star-shaped polypeptides owing to the noncovalent interactions, including hydrophobic interactions or hydrogen bonding [178,179]. PLL-based hydrogels have been explored as an emerging drug delivery platform where drug molecules can be entrapped in the hydrogel via physical capture, hydrogen bonding, or hydrophobic interactions. Injectable *N*-fluorenylmethoxycarbonyl diphenylalanine/PLL hydrogels, capable of encapsulating the photosensitive drug chlorin e6, were explored for photodynamic antitumor therapy (Fig. 6C) [158]. Benefiting from local injection and sustained drug delivery at the tumor site, the strategy of "once injection, multiple-treatments" was realized for the therapy of superficial tumors. *In vivo* therapeutic results showed that the tumor growth could be efficiently inhibited, while no detectable toxicity or damage to healthy organs was observed. Genipin crosslinked hydrogels composed of PLL-*b*-poly(L-alanine) or PLL-*b*-polyglycine with good cell compatibility and enzyme degradability entrapped DOX efficiently. At the same time, the drug release kinetics could be tailored by modulating the structure and concentration of hydrogel composition and the crosslinking ratio [180]. A hydrogel composed of equally mixed L-glutamic acid and L-lysine residues in poly (L-glutamic acid-*co*-L-lysine) was prepared via an amidation reaction. This hydrogel showed ultra-low protein fouling and good biocompatibility. When DOX and diclofenac were loaded as model drugs in the hydrogel, it showed responsive drug release to both pH and trypsin [181]. A disulfide-crosslinked mucoadhesive polypeptide nanogel of PLL-*b*-poly(L-phenylalanine-*co*-L-cystine) was developed to deliver 10-hydroxycamptothecin (HCPT) for the treatment of orthotopic bladder cancer [182]. The reduction-responsive polypeptide nanogel accurately and rapidly delivered HCPT in bladder cancer cells. The HCPT-loaded nanogel significantly inhibited the proliferation of human bladder cancer cells *in vitro* and enhanced antitumor activity in an orthotopic rat bladder cancer model *in vivo*.

Despite the advantages such as intrinsic biodegradability and bioabsorbability of PLLs, their cytotoxicity as a result of the high density of cationic charges cannot be neglected for these applications in drug delivery vectors. Surface shielding and charge conversion modification were recognized as well-established strategies to improve the biocompatibility of PLL. Surface decoration with a PEG chain afforded drug delivery vectors excellent water solubility, low cytotoxicity, nonspecific interaction with blood components, and elongated systemic circulation [183]. Self-assembled polymeric micelles of poly(L-methionine-*b*-L-lysine)-PLGLAG-PEG were fabricated for delivering DOX, in which the PEG shell afforded non-toxicity [184]. The protease-sensitive short amino acid linkers PLGLAG can be cleaved by excess matrix metalloproteinase-2 enzyme around the tumor tissues, resulting in the exposure of cell-permeable PLLs. Such DOX-loaded polymeric micelles exhibited long retention and outstanding tumor inhibition capability compared with free DOX. A hypoxia-responsive polymeric micelle, comprising a PCL core and a mixed shell of PEG and 4-nitrobenzyl chloroformate (NBCF) modified PLL, was also developed as a DOX nanocarrier [185]. The neutral micellar PEG shell offered low cytotoxicity during blood circulation. However, the hypoxic tumor microenvironment triggered NBCF degradation that recovered the amine groups of PLL, leading to a remarkable change in the surface to a positively charged one that enabled the penetration of the nanocarrier into the tumor. The *in vitro* and *in vivo* studies confirmed that DOX-loaded hypoxia-responsive micelles exhibited better penetration and improved inhibitory efficacy on tumor tissues. In addition, Shen et al. demonstrated a PLL-based pH-triggered charge-reversal drug conjugate for the nuclear delivery of the anticancer drug camptothecin (CPT) [186]. A cleavable intracellular disulfide bond conjugated CPT to PLL, and its primary amines were amidized as acid-labile β-carboxylic amides, whose negative charge endowed very low toxicity and low interaction with cells. The acid-labile amides hydrolyzed into primary amines inside the acidic lysosomes of cancer cells, resulting in the escape of PLL from the lysosomes into the nucleus. In the polypeptide-based amphiphilic micelles fabricated by Chen et al., the zwitterionic poly(L-aspartic acid(lysine)) shell formed an excellent coating resistant to nonspecific protein adsorption, demonstrating excellent antifouling properties and biocompatibility [160]. Similarly, DOX-PLL conjugated polymeric micelles with poly(2-methacryloyloxyethyl phosphorylcholine) zwitterionic shells demonstrated outstanding biocompatibility and fruitful

resistance to protein adsorption [163].

The surface modification of PLL-based drug delivery vehicles with various targeting ligands or cellular uptake-enhancing ligands provides selectivity and efficacy toward targeted tissues. For example, poly(lactic acid) (PLA)-*b*-PLL polymeric micelles with surface-tethered folate demonstrated tumor targeting with enhanced cellular internalization and antitumor efficiency [187]. Self-assembled PEGylated PLL-*b*-poly(L-leucine) micelles were prepared by Tian et al. to encapsulate dimeric CPT. The decoration of polymeric micelles with RGD significantly enhanced their internalization and inhibitory effects against breast cancer cells [188]. Wang et al. reported peptide Cys-Ile-Gln-Pro-Phe-Tyr-Pro (CP7) conjugated PEG-*b*-PLL for the delivery of DOX [189]. CP7 was recognized as a high-affinity peptide that could specifically bind to vascular endothelial growth factor receptor 3 (VEGFR-3). The decoration of CP7 on the periphery of DOX-loaded micelles improved chemotherapeutic efficacy *in vitro* in the A549 cell line and enhanced *in vivo* antitumor effects in A549 tumor-bearing nude mice. Polymeric micelles self-assembled from PLA-*b*-PEG-*b*-PLL with the surface coupling of the VEGF antibody as the targeting ligand were prepared by Zhang et al. for the delivery of CPT [190]. The functionalization with the VEGF antibody significantly increased the cellular uptake of polymeric micelles and enhanced antitumor effects *in vitro* and *in vivo*. Liu et al. prepared dehydroascorbic acid-modified PEG-*b*-PLL-*b*-PLF polymeric micelles as PTX nanocarriers [168]. Dehydroascorbic acid was employed to functionalize the polymeric micelles for active tumor targeting as it has a high affinity for glucose transport protein 1, which is overexpressed on hepatic carcinoma and colon cancer cell surface. PTX-loaded polymer micelles significantly suppressed tumor cell viability, proliferation, and migration *in vitro*, and significantly inhibited tumor growth and prolonged survival in tumor-bearing mice.

The fabrication of PLL-based drug delivery vehicles that can realize controlled drug release in response to intracellular stimuli (*e.g.*, pH, redox, and light, among others) have been extensively investigated. Introduction of stimuli-cleavable chemical bonds into polymer-drug conjugates or polymeric containers represents the typical strategy for creating stimuli-sensitive drug delivery systems. Among them, pH-triggered chemical bond cleavage for drug delivery is recognized as the most commonly employed method. Several pH-responsive chemical bonds such as imine, hydrazone, boronate, oxime, amide, ether, and orthoester bonds have been incorporated into drug delivery systems to facilitate rapid bond breakage and accelerated drug release at tumor pH [191]. A self-programmed pH-sensitive prodrug micelle was prepared from PEG-*b*-PLL, where protocatechualdehyde and bortezomib were conjugated to the side chains of PLL via imine bonds to impart pH-responsive drug release [164]. DOX and 4-carboxy benzaldehyde were conjugated to the PLL block via imine linkages across the copolymer PLL-*b*-poly (methacryloyloxyethyl phosphorylcholine), and the self-assembled drug-loaded micelles were thermodynamically stable and negatively charged under physiological conditions. The breaking of the imine bonds at lower tumor pH restored the positive charges of PLL, enhancing the cellular uptake of drug-loaded micelles, and accelerated the release of DOX into the cytoplasm (Fig. 7A) [163]. Phenylboronic acid-modified cholesterol was attached to catechol-pending PEG-*b*-PLL, inducing the formation of micellar nano-constructs capable of encapsulating poorly water-soluble drugs [192]. Owing to the acid-labile feature of the boronate linkage, a reduction in environmental pH could trigger the dissociation of polymeric micelles, which in turn could accelerate the liberation of entrapped drugs. pH-modulated electrostatic interactions can also be employed in polymeric containers to construct a stimuli-responsive drug delivery system. An adjustable pH-sensitive carrier was fabricated by mixing PLL and poly(L-glutamate)-modified mesoporous silica nanoparticles (MSNs) (PLL-MSNs and poly(L-glutamate)-MSNs) [193]. These two polypeptides selected as the nanovalves

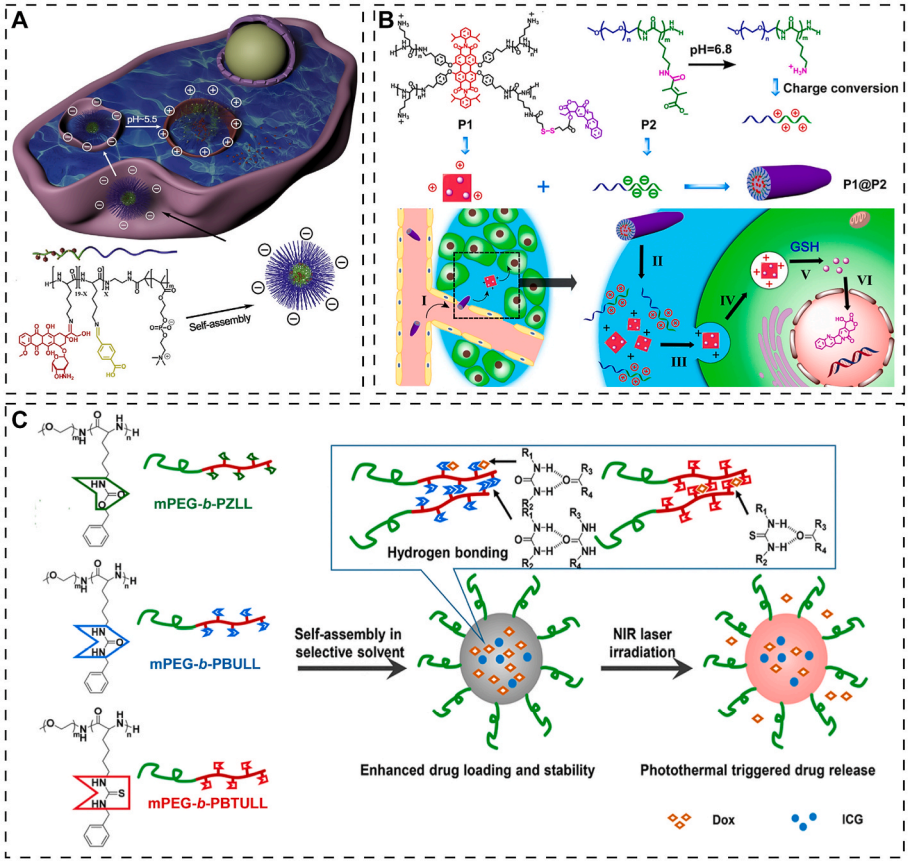

Fig. 7. Construction of diverse smart PLL-based drug delivery systems. (A) The prodrug micelles with their pH-triggered charge conversion and drug delivery. Reproduced with permission from Ref. [163], copyright 2018, Elsevier. (B) Programmable delivery of CPT with charge conversion and reduction-responsive drug release. Reproduced with permission from Ref. [199], copyright 2017, American Chemical Society. (C) Hydrogen bonding-enhanced micelles for encapsulation and NIR-triggered release of DOX. Reproduced with permission from Ref. [200], copyright 2018, American Chemical Society.

of MSNs could self-assemble at pH 7.4 via electrostatic interactions for pore blocking and could be disassembled by pH changes for pore opening. The release study showed that the controlled release of entrapped cargoes could be realized at different pH by adjusting the ratio of PLL-MSNs to poly(L-glutamate)-MSNs. pH-Triggered charge-reversal polymers have considerable potential for developing drug delivery systems, especially to tumor tissues and cells or bacterial biofilms [194]. A tumor-acidity-activated charge-conversional PIC nanoparticle system for the delivery of DOX was developed by simply mixing anionic PEG-*b*-(PLGA-*co*-PLF) and cationic PEG-*b*-(PLL-*co*-PLF) [195]. The nanoparticles, with loaded DOX, could stay negatively charged under normal physiological pH and reverse the surface charge to positive in the extracellular tumor environment, thus enhancing cellular uptake, intracellular drug release, and *in vivo* antitumor activity. Dual pH-sensitive charge-reversal polypeptide micelles consisting of succinyl chloride modified nuclear targeting peptide (Tat) and 2,3-dimethylmaleic anhydride decorated amphiphilic di-block co-polypeptides PLL-poly(L-Leu) were fabricated by Zhang et al. for tumor-targeting cellular uptake and nuclei internalization of encapsulated DOX [196]. 2,3-dimethylmaleic and succinyl amide hydrolyzed in a stepwise manner when exposed to mildly acidic tumor extracellular environments and more acidic intracellular endo/lysosomes, respectively, resulting in a negative-to-positive charge transition to facilitate internalization by tumor cells and then reactivation of Tat targeting function to promote DOX delivery into nuclei. For the treatment of biofilm-associated chronic lung infections, a series of size and charge adaptive azithromycin-conjugated clustered nanoparticles formed from azithromycin-conjugated PAMAM (AZM-PAMAM), and 2,3-dimethyl anhydride-modified PEG-*b*-PLL (PEG-*b*-PLL-DA) were prepared as long-term anti-biofilm agents [197]. The cleavage of amide bonds between DA and PLL in acidic biofilm microenvironments caused the release of AZM-PAMAM from the nanoparticles, and as a result, achieved large-to-small size transition and anionic-to-cationic charge conversion. The cationic and small-sized AZM-PAMAM was beneficial for improved penetration and long-term retention inside biofilms, enhanced bacterial membrane permeabilization, and effective AZM internalization, thereby achieving great anti-biofilm efficiency. pH-Triggered conformational changes and subsequent re-assembly of nanostructures provide a new strategy in nanomedicine for controlled drug release and enhanced therapy. For example, an L-lysine-rich peptide amphiphile could undergo a conformational transition of its secondary structure from β-sheet to α-helix with decreased pH, leading to simultaneous evolution of the self-assembled structure from the nanosphere to a nanofiber which could promote assembly retention and then release drugs in the cytoplasm of tumor cells [198].

Besides pH-sensitive drug delivery systems, reduction-responsive PLL-based nanocarriers have also been explored. Disulfide-crosslinked PEG-*b*-(PLL-*co*-poly(L-tyrosine)) nanoparticles were prepared, aiming to both improve the loading capacity of DOX and offer GSH-responsive release [201]. Another GSH-responsive nanoparticles based on a multifunctional PEG-*b*-PLL drug conjugate were fabricated, in which CPT was anchored to the residual lysine amines in PEG-*b*-PLL via disulfide bonds and thus could be released once inside tumor cells with an elevated GSH concentration [202].

Several studies attempted to construct pH/GSH dual-responsive PLL-based complex micelles or nanoparticles for drug delivery [199, 203–208]. Four-armed PLLs with conjugated CPT through a reduction-responsive disulfide bond were encapsulated by PEG-*b*-PLL, in which the side chains of the PLL block were modified with charge-conversional citraconic anhydride to form poly-ion nanorods (Fig. 7B) [199]. Programmed drug delivery was achieved by a dual response of the extracellular acid and intracellular reductive environment. The supramolecular system demonstrated enhanced tumor suppression compared with that of the cationic prodrug and free CPT treatment at a low concentration. Shi et al. fabricated a novel kind of pH/GSH dual-responsive drug-loaded complex micelles via direct subcomponent self-assembly of the block copolymer PEG-*b*-PLL, 2-formylbenzeneboronic acid (2-FPBA) and hydrophilic 1,2-diol-containing drugs (*e.g.*, capecitabine) under physiological pH based on the synergistic formation of an iminoboronate structure [203]. The complex micelles were stable under neutral physiological conditions but could be destroyed for drug release in response to the stimuli of acidic physiological conditions or GSH. Another example of a pH/GSH dual-responsive complex nanoparticle was designed by Tang et al. [204]. 3,3′-Dithiodipropionic acid-modified paclitaxel (DTPA-PTX) and 2,3-dimethylmalefic anhydride (DMMA) were conjugated to the amino groups of PEG-*b*-PLL. The surface charge of the obtained PEG-*b*-(PLL-*co*-(PLL-DMMA)-*co*-(PLL-DTPA-PTX)) nanoparticles could change from negative at blood pH to positive at tumor extracellular pH, affording elongated blood circulation times and enhanced cell uptake at the tumor site. The polymeric prodrug nanoparticles exhibited active cellular uptake, rapid intracellular drug release, and significantly increased cytotoxicity against MCF-7 tumor cells, owing to the tumor-relevant pH and reductive conditions. Chen et al. fabricated shell stacked nanoparticles with dimethylmaleic anhydride-modified PEG-PLL as the shell and a disulfide-crosslinked polypeptide copolymer as the core for the delivery of DOX with a transformable size and surface *in vivo* [208]. These nanoparticles maintained a specific size (145 nm) and negative surface charge in the bloodstream while reduced to a smaller size (40 nm) and switched to positive charges at tumor sites because of the pH-sensitive linkage between dimethylmaleic anhydride and PLL. Under the conditions of elevated GSH, the disulfide-cross-linkage further disrupted to release the loaded DOX. Very recently, Chen et al. also synthesized pH/GSH dual-sensitive PLL-dexamethasone conjugates to explore the use of anti-inflammatory drugs for cancer therapy [205]. Dexamethasone was anchored to the side chains of PLL in PEG-*b*-PLL via disulfide and ester bonds, and can, therefore, be released in response to elevated GSH and a lower pH inside the tumor microenvironment.

Light-induced drug release represents an emerging strategy of drug delivery systems. Recently, Chen et al. reported photothermal effect-triggered drug release from hydrogen bonding-enhanced polymeric micelles. PLL in PEG-*b*-PLL was modified with benzyl isocyanate/isothiocyanate for developing amphiphilic block copolymers bearing different hydrogen bonding donors (e.g., urethane, urea, and thiourea groups), which self-assembled into polymeric micelles for encapsulating DOX and a photothermal agent (*e.g.*, indocyanine green). Upon NIR laser irradiation, destabilization of hydrogen bonding through a photothermal effect triggered fast and controlled drug release. This dramatically promoted the aggregation of drug molecules in the nuclei, resulting in enhanced anticancer activity (Fig. 7C) [200]. Liu et al. developed DOX-loaded nanoparticles from hydrophilic PEG- and hydrophobic 2-diazo-1,2-naphthoquinones (DNQ)-grafted PLL brush copolymers [209]. The NIR laser-responsive hydrophobic DNQ could be transformed into hydrophilic DNQ following two-photon laser irradiation, leading to fast DOX release. Kataoka et al. explored the first example of *in vivo* light-activated chemotherapy by combining systemically injected reduction-sensitive polymeric micelles and the photosensitizer photofrin [210]. The polymeric micelles were self-assembled after conjugating CPT to the PLL block of PEG-*b*-PLL copolymers via a disulfide linker. The escape of the CPT-loaded micelles from the endocytic vesicles into the cytosol was observed only in the irradiated tissues, which accelerated drug release and enhanced antitumor efficacy without toxicity.

3.2.3. PLL-based polymers as protein delivery systems

Protein drugs have emerged as potent medicines for various types of human diseases and have drawn significant interest owing to their high specificity and activity at relatively low concentrations compared with small molecule drugs. However, the use of protein-based biotherapeutics faces several challenges, such as rapid elimination from the circulation, poor bioavailability, low cell permeability, and inefficient endosomal escape. The future success of proteins as therapeutic agents is critically dependent on the development of appropriate delivery

systems. An ideal protein carrier should have a reasonably high protein encapsulation efficiency, loading capacity, retained bioactivity, and targeted and sustained release of the loaded protein drugs [211,212].

Among numerous protein delivery carriers, PIC micelles have attracted considerable attention in the past decades owing to their facile encapsulation and high loading efficiency of protein therapeutics. The nano-sized PIC micelles are usually constructed from a block copolymer of a neutral hydrophilic block (typically PEG) and an ionic block with oppositely charged protein molecules. These protein molecules combine with the ionic block of the block copolymer via electrostatic interactions to form the core of PIC micelles, which is stabilized and protected by the hydrophilic block (shell). The protein molecules can be encapsulated into PIC micelles with high loading efficiency under mild conditions, which prevents the use of organic solvents. The entrapped protein molecules can be well protected from rapid degradation and excretion *in vivo* [213–215]. PLLs are positively charged at physiological pH and thus can encapsulate protein molecules with negative charges in aqueous conditions by forming PIC micelles via electrostatic interactions. Pispas et al. used PEG-*b*-PLL to encapsulate negatively charged insulin by forming PIC micelles. The physicochemical and structural characteristics of the complexes were largely determined by protein concentration and solution ionic strength [216]. Ramsey et al. employed a library of PLL-*g*-PEG to entrap a model protein bovine serum albumin [217]. Encapsulation of bovine serum albumin into PIC micelles was strongly dependent on the copolymer-to-protein mass ratio, PEG grafting density, and PLL molecular weight.

Despite the numerous advantages presented by PIC micelles as protein nanocarriers, the loading stability of protein molecules remains challenging. The stability of PIC micelles is strongly affected by medium ionic strength, and high ionic strength might result in the destabilization of PIC micelles because of the electrostatic shielding effects. Several strategies, such as incorporating hydrophobic motifs to the ionic block of the copolymer, employing star-shaped unimolecular PLL copolymers, utilizing brush-like PLL to improve the cationic charging density, or cross-crosslinking the preformed micelles, have been employed to enhance the stability of PLL-based PIC micelles under increased ionic strength and temperature conditions. The incorporation of hydrophobic blocks in the host copolymers is favorable for the formation and stabilization of PIC micelles. The self-assembled PEG-*b*-PLL-*b*-poly(L-Leu) polypeptide micelles could spontaneously encapsulate ovalbumin antigens with high efficiency and excellent stability owing to the presence of a poly(L-Leu) core. These polypeptide micelles dramatically enhance vaccine-induced antibody production [218]. We reported a star-block copolymer PEI-*g*-(PLL-*b*-PEG) as a potential unimolecular protein nanocarrier [219]. Model protein insulin can be efficiently encapsulated, forming so-called unimolecular PIC micelles. The encapsulated insulin retained its chemical integrity and immunogenicity. To strengthen the electrostatic interactions between PLL and the encapsulated protein molecules, thus the stability of the formed PIC micelles, the brush-like PLL-based block copolymer PEG-*b*-poly((2-aminoethyl-L-glutamate)-*g*-PLL was developed by our group for the delivery of protein drugs such as insulin and TNF-α [220–222]. The employment of a PLL brush instead of a linear PLL enhanced the loading capacity and loading stability of model protein drugs, afforded sustained release, and enhanced bioavailability.

The stability of PIC micelles could also be improved by crosslinking preformed micelles with susceptible bonds, which simultaneously provided a stimuli-responsive release of the encapsulated protein drugs. Murthy et al. designed functionalized PEG-*b*-PLL block copolymers, where the PLL segment was modified with cross-linkable dithiopyridine groups, to encapsulate ovalbumin via the formation of PIC micelles, followed by crosslinking with disulfide linkages to improve the stability in serum and to provide a GSH-responsive intracellular release property [223]. These PIC micelles were also employed as long-circulating enzyme carriers that maintained the enzymatic activity of catalase. pH-Responsive myoglobin-loaded PIC micelles were prepared via concurrent ion complexation and pH-cleavable amide crosslinking between the myoglobin guest and carboxy-dimethyl maleic anhydride-modified PEG-*b*-PLL host [224]. These micelles were stable under physiological pH with enhanced circulation time *in vivo*, while rapidly dissociated at pH 6.5 with the release of an intact protein payload. Both pH- and sugar-sensitive PIC micelles self-assembled from PEG-*b*-poly(glutamic acid-*co*-glutamicamidophenylboronic acid) and PEG-*b*-poly(L-lysine-*co*-ε-3,4-dihydroxyphenylcarboxyl-L-lysine) copolymers with a boronate ester crosslinked core were developed by Shi et al. for intracellular protein delivery (Fig. 8) [225]. The crosslinked micelles displayed superior physiological stability compared with their non-crosslinked counterparts while swelling and disassembling in the presence of excess fructose or at endosomal pH. Notably, either negatively or positively charged proteins can be encapsulated into micelles efficiently under mild conditions. The cytochrome C-loaded micelles could efficiently deliver proteins into HepG2 cells and exhibited enhanced apoptotic ability.

Besides PIC micelles, PLL-based nanoparticles, nanocapsules, and hydrogels were employed for the delivery of protein drugs. PEG-*b*-PLL-derived copolymers, with the side-chain amine groups of lysine residues modified to contain ortho-amine-substituted phenylboronic acid with improved pH-responsive diol-binding properties, were prepared by Deming et al. [226]. These block copolymers complex with glycosylated proteins to form core-shell nanoparticles that are stable at physiological pH while dissociating and releasing the glycoproteins under acidic conditions (pH 5.0). A water-soluble conjugated polymer brush, consisting of a relatively low portion of the rigid poly(*p*-phenyleneethynylene) backbone and a large amount of PLL pendants was fabricated for ovalbumin delivery [227]. The designed polymer benefited from the brush-like PLL and demonstrated high antigen-loading capacity to form nanoparticles. The polymer/-ovalbumin nanoparticles showed high cellular uptake by dendritic cells and induced cell maturation and cytokines release. As a dendritic cell vaccine for cancer immunotherapy, the nanoparticle-pulsed dendritic cells effectively induced a robust immune response *in vivo*. Another polyelectrolyte complex nanoparticle as a biomimetic proteoglycan nanocarrier, prepared from dermatan sulfate-PLL or gum tragacanth-PLL mixtures, showed excellent immobilization and controlled release of growth factors as a result of the proteoglycan/-protein complexation [228]. Polypeptide nanocapsules represent a promising class of packaging structures that could be utilized for protein delivery. For example, polypeptide nanocapsules could be constructed from self-assembly of poly(L-lysine-*co*-L-tyrosine) and subsequently

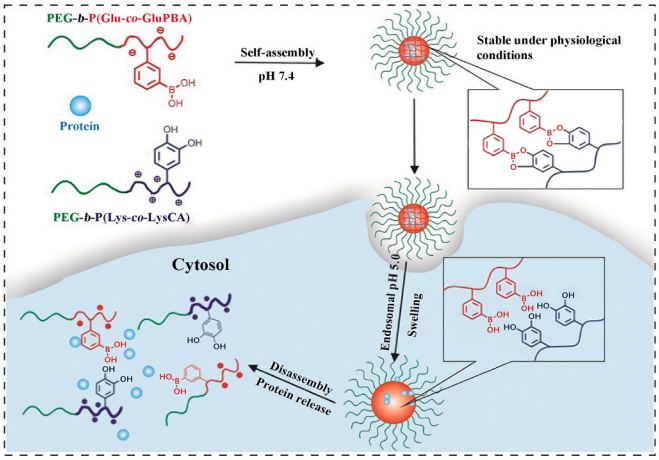

Fig. 8. Schematic illustration of the construction of protein-loaded core-crosslinked PIC micelles and intracellular protein release from the PIC micelles triggered via endosomal pH. Reproduced with permission from Ref. [225], copyright 2013, American Chemical Society.

crosslinked by either UV irradiation or chloroaurate reduction. Protein molecules (*e.g.*, myoglobin) could be readily encapsulated with retained biological functions via facile electrostatic interactions [229]. Jeong et al. prepared temperature-sensitive nanogels from ionic complexes of positively charged PEG-*b*-PLL-*b*-poly(L-alanine) and negatively charged hyaluronic acid as intracellular delivery vehicles of bovine serum albumin [230]. The nanogel could be tightened by heat-induced shrinkage, and the internalization efficiency of the nanocarrier could be controlled by modulating the size and zeta potential of the nanogel.

3.2.4. PLL-based polymers as co-delivery systems

Combination therapy, the co-administration of two or more therapeutic agents including chemotherapeutics, genes, and proteins to achieve a therapeutic efficiency better than that achieved with each treatment, has been a cornerstone of treatment of many diseases, including cancer. Combination therapy often involves the employment of multi-drug-loaded nanoparticles such as polymeric micelles, silica/carbon/metal-based nanoparticles, and hydrogels as the co-delivery vehicles [231,232]. Multifunctional co-delivery vectors derived from PLL-based polymers have emerged over the past decade.

Traditionally, cancer treatment regimens have combined the use of two or more chemotherapeutic drugs to achieve a synergistic or additive effect, with drugs acting through different mechanisms. To address this need, nano preparations that combine bioactive payloads within a single carrier have been constructed. Protocatechualdehyde and bortezomib with different anticancer mechanisms were successively conjugated to the side chains of PLL in the block copolymer PEG-*b*-PLL to construct a self-programmed pH-sensitive prodrug micelle wherein the drugs were released at lower pH in the tumor tissue and intracellular microenvironment [164]. Compared with protocatechualdehyde or bortezomib alone, the micelle exhibited higher cellular growth inhibition and enhanced antitumor efficacy *in vivo*. Haam et al. prepared multi-prodrug nanoparticles from PTX-conjugated cationic PLL and gemcitabine-conjugated anionic hyaluronic acid via electrostatic interactions for CD44-targeted synergistic biliary cancer therapy (Fig. 9) [233]. These nanoparticles facilely transferred into target cancer cells through CD44-mediated endocytosis and released PTX and gemcitabine through hydrolysis of the ester linkage in acidified endosomes, thereby realizing synergistic cancer therapeutic efficacy. Redox-responsive and high drug-loaded nanoparticles were synthesized by Shen et al. from PEG-*b*-PLL for enhanced cancer therapy [202]. The hydrophobic drug CPT was anchored to the residual lysine amines in PEG-*b*-PLL via disulfide bonds. The conjugate self-assembled into nanoparticles of around 100 nm with hydrophobic CPT moieties forming the core. The nanoparticle could be used as a nanocarrier further to encapsulate other drugs such as DOX for combined chemotherapy. The pH/Redox sensitive, dual drug-loaded nanoparticles were prepared from PEG-*b*-PLL to improve cancer therapy [234]. Platinum (IV) and *cis*-aconitic anhydride doxorubicin (CAD) were anchored to residual amine groups of PLL to form polymer prodrug conjugates, which then self-assembled into nanoparticles with hydrophobic platinum (IV) prodrug and CAD as the core. The drugs demonstrated accelerated release under acidic and reductive conditions with synergistic antitumor effects.

Combination therapy may also involve the co-administration of multiple nanocarriers that contain single agents. Matsumura et al. devised a micelle-hydrogel composite as a general carrier for the switchable and controlled release of dual drugs. Two different di-block polypeptides, PLL-*b*-PLF and PLGA-*b*-PLF, were employed to fabricate distinct self-assembling micellar systems that were loaded with curcumin and amphotericin B, respectively. The drug-loaded micellar mixture was crosslinked using genipin to yield a micelle-hydrogel composite. This composite allowed for controlled multiphasic drug release and could be effectively tuned to moderate the pace and amount of drug release and be easily regulated to switch the drug release kinetics over a range of simple factors such as a change in pH, crosslinking density, and composition [235]. Mainly, novel pH-sensitive PIC micelles prepared from PEG-*b*-(PLL-*g*-Ce6) (chlorin e6 grafted poly(ethylene glycol)-poly(L-lysine)) and PEG-*b*-(PLL-*g*-DMA)-*b*-PLA (2,3-dimethylmaleic anhydride-grafted poly(ethylene glycol)-poly(L-lysine)-poly(lactic acid)) were employed to encapsulate DOX, where Ce6 and DOX were localized in a site-isolated state to overcome antagonistic interactions between chemo-drugs and photosensitizers in photo-chemo combination cancer therapy [236].

Cancer treatment with chemotherapy is often limited by multi-drug resistance (MDR), which is commonly associated with the overexpression of drug transporter proteins in cancer cells. A combination therapy strategy such as co-delivery of anticancer drugs and nucleic acid using multi-functionalized nanocarriers has been employed to overcome MDR by combining modulators of drug efflux pumps like P-glycoprotein. The co-delivery of drugs and genes could also achieve a synergistic or additive therapeutic effect at lower doses [237]. Zhu et al. designed an enzyme-triggered drug and gene co-delivery system combining hollow mesoporous silica with enzyme degradable PLL coatings [238]. Hollow mesoporous silica with a drug payload was coated with a

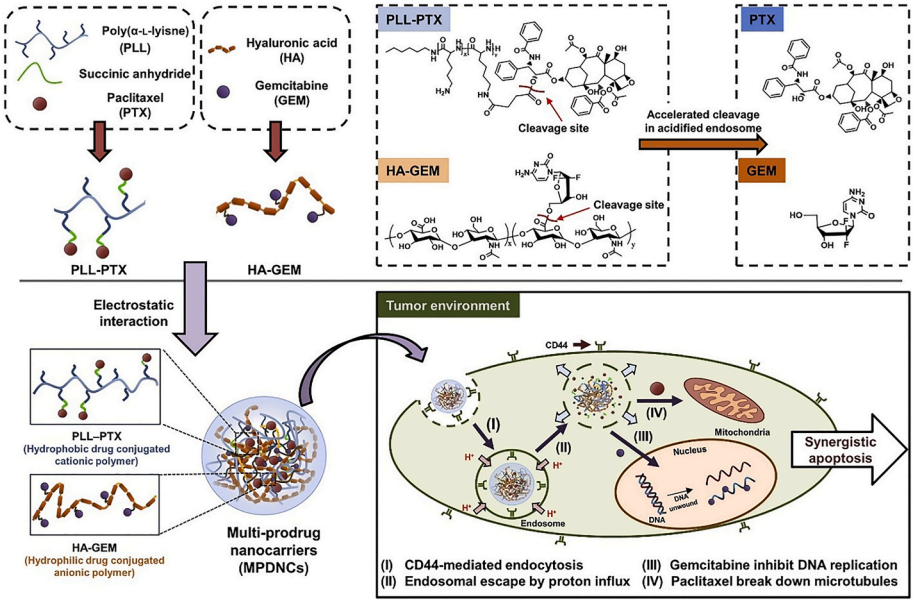

Fig. 9. Preparation of multi-prodrug nanocarriers with the activated release behavior of PTX and gemcitabine in targeted cancer cells. Multi-prodrug nanocarriers were constructed via electrostatic interactions between PLL-PTX and hyaluronic acid-gemcitabine and taken up by CD44-mediated endocytosis, followed by hydrolyzing ester linkage of the prodrug conjugates in acidified endosomes, thereby leading to synergistic apoptosis. Reproduced with permission from Ref. [233], copyright 2015, Elsevier.

negatively charged gene and positively charged PLL via the LbL technique to achieve co-encapsulation. The dual-loaded silica nanoparticles exhibited an *in vitro* enzyme-triggered release of the drug and gene, which could be modulated by varying the enzyme concentration. Co-delivery of DOX and p53, which could reduce drug resistance of tumor cells through the inhibition of P-glycoprotein expression, by biodegradable micellar carriers based on PZLL-grafted chitosan was explored by Wang et al. [239]. PZLL-grafted chitosan self-assembled in an aqueous solution into polymeric micelles with the PZLL core for the encapsulation of DOX and the chitosan shell for complexation with a p53 plasmid. DOX and p53 could be efficiently transported into HeLa tumor cells simultaneously and exhibited improved inhibitory effect versus DOX or p53 used individually. Co-delivery of DOX and p53 by a pH-sensitive charge-conversion system was developed by Li et al. [240]. The pH-sensitive charge-reversal co-delivery system was obtained by the adsorption of negatively charged *cis*-aconityl-DOX and p53 gene on positively charged PEI to form the drug and gene co-loaded complex, followed by the shielding of the complex with pH-sensitive charge--conversional PEI-*g*-(PLL-*co*-PLGA). The co-delivery system produced marked apoptosis in tumor cells *in vitro*, and *in vivo* imaging in subcutaneous xenograft and *situ* tumor models demonstrated that the co-delivery system could accumulate efficiently in tumors, suggesting significant potential applications of this co-delivery system in cancer therapy. Besides cancer treatment, co-delivery of osteogenic genes and drugs using functionalized MSNs was explored by He et al. for enhanced osteo differentiation [241]. PEI-*g*-PLL copolymers were anchored onto the surfaces of MSNs to construct a dual-factor delivery system, which allowed the surface adsorption of an osteogenic gene and osteogenic dexamethasone loading in the mesopores of nanoparticles. The dual-factor delivery system could quickly release *p*DNA while releasing dexamethasone in a sustained manner. With the simultaneous delivery of dexamethasone and the bone morphogenetic protein-2 gene, this dual-factor delivery system could significantly enhance the level of osteogenic differentiation of bone MSCs.

Several siRNA-based treatments that target genes involved in key cellular processes such as apoptosis and drug resistance have been utilized for cancer therapy to date, and many of these treatments have also been evaluated in combination with conventional anticancer drugs. Simultaneous co-delivery of chemotherapeutics and siRNA in a single-vehicle in cancer chemotherapy is more effective than treatment with either chemotherapeutics or siRNA. He et al. developed a pH- and redox-responsive co-delivery system, through the capping of PEI-*g*-PLL copolymers onto the surfaces of MSNs via disulfide bonds, for simultaneous delivery of anticancer drug and siRNA to achieve enhanced chemotherapeutic efficacy [242]. The nanocarrier was capable of not only loading the anticancer drug DOX into the mesoporous channels of MSNs but also simultaneously complexing Bcl-2 siRNA with its positively charged PEI-*g*-PLL. The release of Bcl-2 siRNA downregulated the Bcl-2 protein expression, and co-delivery of DOX and Bcl-2 siRNA in MDA-MB-231 breast cancer cells could induce marked cell apoptosis. Self-assembled polymeric micelles of *N*-succinyl chitosan-*g*-(PLL-*b*-palmitic acid) were fabricated by Zhang et al. to co-deliver DOX and siRNA-P-glycoprotein [243]. The *N*-succinyl chitosan micellar surface afforded good biocompatibility while the PLL and palmitic acid blocks were responsible for the condensation of siRNA and encapsulation of DOX, respectively. The *in vitro* antitumor efficacy of DOX-siRNA-micelles against HepG2/ADM cells significantly increased because of the downregulation of P-glycoprotein. The DOX-siRNA-micelles accumulated in the tumor region beyond 24 h post-injection, and the co-delivery system significantly inhibited tumor growth with synergistic effects *in vivo*.

MicroRNAs (miRNAs) are short endogenous non-coding RNAs recognized as playing a crucial role in regulating tumor initiation, progression, and metastasis, as well as drug resistance. Combinational therapy of chemotherapeutics and miRNA might be a promising strategy to improve their therapeutic efficacy. Self-crosslinked redox-responsive nanoparticles with the capability of co-delivering DOX and miR-129-5p were fabricated by Ma et al., using PEG-*b*-PLL-*b*-poly(L-cysteine) co-polypeptide, to overcome MDR in MCF-7/ADR cancer cells [244]. The co-delivery of miR-129-5p and DOX with these nanoparticles effectively elevated miR-129-5p in MCF-7/ADR cells, which reversed drug resistance by targeting P-glycoprotein, thereby robustly enhancing the cytotoxicity of DOX. DOX combined with miR-129-5p synergistically induced both S-phase reduction and G2-phase cell cycle arrest, thus enhancing chemosensitivity of MCF-7/ADR cells to DOX. Kim et al. utilized a polymeric LbL assembly of silica-supported mesoporous titania nanoparticles (MTN) to co-deliver PTX and miR708 for simultaneous chemotherapy and c-FLIP (cellular Fas-associated protein with death domain-like interleukin-1β-converting enzyme-inhibitory protein) suppression in colorectal carcinoma [245]. PTX was loaded into the silica-supported MTN carriers, followed by surface grafting of PLL, the subsequent miR708 loading via electrostatic interactions, and the final capping with PEG-*b*-poly(L-aspartic acid). These nanotherapeutic agents showed excellent *in vitro* cytotoxicity against HCT-116 and DLD-1 colorectal carcinoma cells, superior to those of free PTX or PTX-loaded nanoparticles. When administered intravenously into DLD-1 xenograft tumor-bearing mice, the nanoparticles showed substantial accumulation in tumors and synergistically inhibited tumor growth.

There has been considerable interest in using protein therapeutics, such as monoclonal antibodies, cytokines, and enzymes to enhance the cytotoxicity of chemotherapeutic agents. Cheng et al. developed well-defined injectable nanogel-crosslinked physical and chemical composite gels that could simultaneously deliver IL-2, IFN-γ, and DOX to tumor regions for combined chemo-protein therapy [246]. The composite gels were fabricated using quick gelation induced by ionic crosslinking of 4-arm PEG-*b*-PLGA and hydroxypropyl chitosan/4-arm PEG-*b*-PLL, followed by the formation of covalent bonds via a Schiff-base reaction of the oxidized, cholesterol-bearing dextran nanogels with hydroxypropyl chitosan/4-arm PEG-*b*-PLL. This double-network formulation loaded with DOX, IL-2, and IFN-γ exhibited a synergistic anticancer efficacy in xenograft tumor-bearing mice. The pH-responsive self-assembled polymeric micelles, composed of PEG-*b*-(PLL-*g*-2,3-dimethyl maleic anhydride)-*b*-PLA tri-block copolymer, were developed for the pro-apoptotic peptide D-(KLAKLAK)$_2$ and DOX co-delivery [247]. DOX and D-(KLAKLAK)$_2$ were encapsulated with the micellar core via hydrophobic interactions and the inner shell via electrostatic interactions, respectively. The charge reversal of the PLL block from negative to positive led to the release of membrane-lytic D-(KLAKLAK)$_2$, accelerating the disruption of the endo-lysosomal membrane. Nanoparticles with dual payloads displayed enhanced anticancer activities both *in vitro* and *in vivo*, arising from strong synergism.

3.3. PLL-based polymers for bio-sensing

Over the past decade, various nanomaterials have been explored to prepare highly efficient biosensors for the detection of analyte biomolecules [248–251]. Generally, biosensors for biomedical applications require chemical functionalization to bestow their surfaces with desired properties such as specific molecular recognition and antifouling properties. PLL serves as a potential candidate for the surface coating of biosensors because it can strongly adsorb onto a variety of anionic material surfaces via multivalent electrostatic interactions, or it can be easily modified with different functional groups upon the pedant primary amine in its side chain. Typically, PLL-based biosensors were manufactured by coating PLL on the surface of other functional materials such as graphene, black phosphorus (BP), liquid crystal (LC) droplets, among other materials.

3.3.1. Graphene-PLL hybrids for bio-sensing

Graphene and its oxygenated derivatives (*e.g.*, graphene oxide and reduced graphene oxide) have been used widely to construct electrochemical biosensors owing to their exceptional electrical conductivity,

optical transparency, and high specific surface area. Moreover, they can serve as electrode-modifying materials to amplify signals or as a platform to immobilize biomolecules for electrochemical detection. However, pristine graphene displays poor solubility in most solvents and easily stacks to form graphite due to the robust and cohesive van der Waals energy of the π-stacked layers. To address this problem and to improve its functionality, graphene is frequently coated with specific materials via covalent or noncovalent modification for the detection of biomolecules [252,253]. PLL, by virtue of its flexible molecular backbone, good biocompatibility, and water solubility, has been widely used to functionalize graphene to obtain water-soluble graphene sheets and biocompatible surfaces [252]. Plentiful free positively charged active amine groups in the structural framework of PLL can not only adsorb anionic molecules, which allows direct electrochemical detection but also adsorbs or chemically anchors functional biomacromolecules such as enzymes, improving the selectivity of electrochemical detection. PLL can be grafted onto the surface of graphene by electropolymerization process for solid attachment (Fig. 10A). Alternatively, the strong physical adhesion of PLL to graphene surfaces via synergistic hydrophobic interactions, cation-π interactions, and electrostatic interactions also affords stable PLL-graphene hybrids in aqueous solution [254].

A graphene-coated glass carbon electrode with PLL surface modification could combine with small molecules such as bergenin [255], quercetin [256], dopamine and uric acid [257], or bisphenol A [258] via hydrogen bonding for electrochemical detection using cyclic voltammetry or differential pulse voltammetry. The three-dimensionally stable PLL coatings provide more active sites and strong interactions with analyte molecules, thus offering high detection sensitivity, wide linear range, good reproducibility, and long-term stability. A biocompatible film assembled by combining graphene and PLL on glass carbon electrodes for adhesion and electrochemical impedance detection of leukemia K562 cells was proposed by He et al. [259]. The use of positively charged PLL promoted cell adhesion and preserved the activity of the immobilized living cells. The immobilized K562 cells were directly monitored with electrochemical impedance spectroscopy in the presence of $[Fe(CN)_6]^{3-/4-}$ as redox probes. Notably, the proposed method was used to express the viability of cells and to evaluate the effectiveness of antitumor drug Nilotinib on K562 cells.

The aforementioned examples were based on nonspecific hydrogen bonding or electrostatic interactions between PLL and the analyte molecules. A graphene/PLL hybrid could also be employed to immobilize redox enzymes (*e.g.*, glucose oxidase and arylsulfatase) to improve the selectivity of detection. Xia et al. employed a graphene-PLL hybrid-coated glass carbon electrode to immobilize hemoglobin for the electrochemical detection of hydrogen peroxide (H_2O_2) [254]. Hemoglobin, carrying negative charges under physiological conditions, could be easily immobilized on the hybrid via electrostatic interactions. The immobilized protein retained its native structure and exhibited reversible direct electrochemistry. The fabricated enzymatic electrochemical biosensor demonstrated excellent catalytic activity toward its substrate H_2O_2. A PLL-coated reduced graphene oxide-zirconium oxide (or manganese dioxide) composite was explored by Chen et al. to immobilize glucose oxidase as a glucose biosensor [260,261]. The incorporation of PLL could not only provide more active sites for the electrostatic adsorption of glucose oxidase but also plays a crucial role in promoting electrical communication between the modified electrode surface and glucose oxidase. The modified electrode exhibited excellent electrocatalytic ability toward the determination of glucose with good analytical parameters. Kato et al. used a hybrid of graphene oxide with PLL-containing hydroxyapatite (HA) particles to achieve the immobilization of glucose oxidase for the detection of glucose [262]. HA particles were employed because of their strong affinity for various proteins. PLL-containing HA composites were demonstrated as enzyme immobilization agents with high stability and as biosensors with high sensitivity.

3.3.2. *BP-PLL hybrids for bio-sensing*

BP was first mechanically exfoliated from bulk black phosphorus in

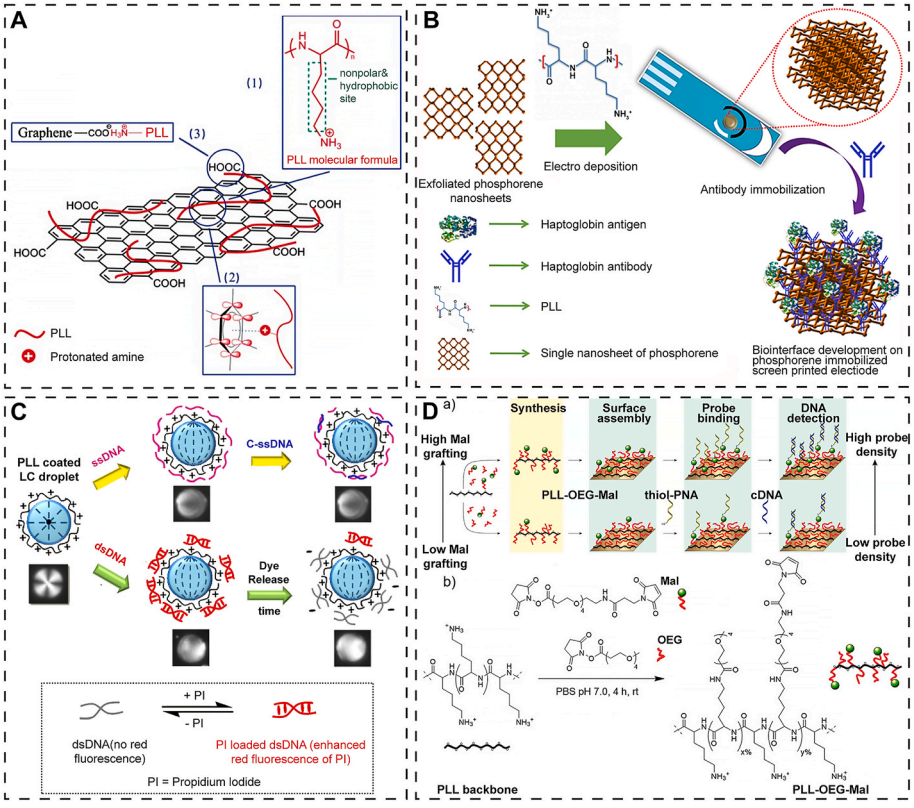

Fig. 10. Various PLL-coated nanomaterials for biosensing. (A) The self-assembly of PLL onto graphene surfaces via hydrophobic interaction, protonated amine-π interaction, and electrostatic interaction. Reproduced with permission from Ref. [254], copyright 2013, The Royal Society of Chemistry. (B) Attachment of 2D phosphorene on the working surface of screen-printed electrodes (SPEs) by electrodeposition in the presence of PLL. The SPEs were bio-modified with haptoglobin antibodies to develop a haptoglobin detecting device. Reproduced with permission from Ref. [263], copyright 2018, IOP Publishing Ltd.. (C) PLL-coated LC droplets for sensitive detection of DNA. Reproduced with permission from Ref. [264], copyright 2017, American Chemical Society. (D) Construction of distinct maleimide density of PLL-OEG-Mal polymers immobilized on substrates for sensitive detection of cDNA. Reproduced with permission from Ref. [265], copyright 2018, American Chemical Society.

2014. In BP, each P atom forms covalent bonds with three adjacent P atoms to give a puckered honeycomb orthorhombic lattice. Such a unique structure endows BP with fascinating electronic properties such as high electron mobility, anisotropic electrical and thermal conductivity, as well as excellent optical response [266,267]. BP has attracted increasing attention in recent years in various biomedical fields, including phototherapy, drug delivery, bio-sensing, bio-imaging, and theranostics, among others [266,267]. Owing to its excellent conductivity and intrinsic redox properties, the use of BP to construct electrochemical biosensors are emerging. To this end, the modification of PLL on the surfaces of BP not only reserves BP's originally puckered honeycomb structure and thus good conductivity but also enhances BP's stability and biocompatibility in aqueous solution, facilitating bio-sensing applications. A PLL-BP hybrid, fabricated by the adhesion of PLL to the surface of BP via synergistic hydrophobic and electrostatic interactions, was proposed by Zhao et al. as an ideal matrix for hemoglobin (Hb) immobilization mediated by electrostatic interactions with PLL, aiming to construct a biosensor Hb-PLL-BP for the electrochemical detection of H_2O_2 [268]. The noncovalent functionalization of PLL improved BP's stability while maintaining its good conductivity. The Hb-PLL-BP-based enzymatic electrochemical biosensor displayed excellent catalytic activity toward the reduction of H_2O_2 with a wide detection range. PLL-BP hybrid not only functioned as an excellent electrical conductor but also served as a suitable biocompatible matrix that provided a favorable microenvironment for the immobilized protein to retain its biological activity and achieve fast direct electron transfer.

Sabherwal et al. reported the electrochemical detection of the redox-active cardiac biomarker myoglobin (Mb) by measuring direct electron transfer using aptamer-functionalized PLL-BP electrodes [269]. The surface modification of BP with PLL facilitated binding with generated anti-Mb DNA aptamers on nanostructured electrodes. This label-free aptasensor platform exhibited a low detection limit, high specificity, and sensitivity toward Mb in serum samples. Similarly, Tuteja and Neethirajan reported on the development of an antibody-functionalized interface based on PLL-BP for the label-free electrochemical immuno-sensing of haptoglobin (Hp) biomarker, a clinical marker of severe inflammation (Fig. 10B) [263]. Hp antibodies were immobilized via electrostatic interactions with PLL. The as-prepared electrochemical immunosensor platform demonstrated remarkable sensitivity and specificity with a low detection limit. Such a strategy represents avenues to point-of-care and on-farm livestock disease monitoring technologies for multiplexed diagnosis in complex biological samples such as serum.

3.3.3. LC-PLL hybrids for bio-sensing

LC droplets have been regarded as an essential type of functional platform for the detection of biomolecules by virtue of their large surface area and tunable director configuration [270,271]. In particular, LC droplets offer a direction for developing a novel bio-sensing system, which provides an increased spatial resolution in the range of micrometers with very high sensitivity. The adsorption and reaction of chemical and biological species at the surface of LC droplets may induce the director configuration transition of the LC droplets that can be easily monitored with a polarizing optical microscope [272]. Polymer-stabilized LC droplets in aqueous solutions have been developed as a simple and label-free optical probe for the detection of chemical and biological species. Recently, Pal's group explored the use of PLL-coated LC droplets for the bio-sensing of the cell, DNA, and anionic proteins such as fibronectin, bovine serum albumin, concanavalin A and cathepsin D, based on the transition in the LC director induced by interfacial intermolecular interactions between PLL and the anionic biomolecules [264,273–275]. The LC droplets were formed from 4-cyano-4′-pentylbiphenyl (5CB) and coated with alternative cationic PLL and anionic poly (styrene sulfonate) via the LbL technique. Notably, dsDNA-adsorbed PLL droplets have been found to effectively displace propidium iodide (a model drug) encapsulated within dsDNA over time. This observation suggested the potential for a label-free droplet-based LC detection system that may provide a simple method to develop DNA-based drug nanocarriers (Fig. 10C) [264]. Overall, these studies provided new pathways utilizing ordering transition in LC droplets to understand biomolecular interactions for various interfacial and sensing applications. LCs of 5CB formed a bright fan shape (planar orientation at the water-LC interface) when in contact with a mixture of 1,2-dioleoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol) (DOPG) and PLL. The LCs took on a dark cross appearance (homeotropic orientation) in a DOPG-PLL mixture preincubated with the protease trypsin. Such interactions between PLL and phospholipid monolayer could be utilized to construct a promising biosensor for trypsin [276]. PLL-LC hybrids provided emerging biosensors with high sensitivity and easy detection; however, challenges remain in the exploration of functionalized PLL-LC droplets as bio-sensing systems with improved selectivity.

3.3.4. Other PLL-derived hybrids for bio-sensing

The strategy to equip surfaces with suitably modified PLL is a promising approach because the material surface properties can be tailored by customizing the PLL with desired functional groups, potentially with reactive, binding, or release properties, thus offering broad applicability in bio-sensing, among others. Owing to electrostatic interactions, modified PLL polymers can be applied as a passivation or functionalization layer on virtually any surface such as metals, metal oxides, or polymers. A new surface functionalization scheme for nano-bio field-effect transistors using biocompatible PLL-*g*-oligo(ethylene glycol) (OEG) thin films was developed by Duan et al. [277]. The surface modification of Si nanowires (Si-NWs) with PLL-*g*-OEG was driven by electrostatic interactions between the positively charged PLL and the negatively charged Si/SiO_2 surface, resulting in a facile, stable, uniform, and antifouling nanoscale thin film. The reactive groups of the polymer chains can be further functionalized with other chemical groups (*e.g.*, biotin) in specific stoichiometry for biomolecules detection (*e.g.*, streptavidin). Such assemblies of the polymers together with the bound analytes could be removed with pH stimulation, resulting in the regeneration of a bare sensor surface without compromising the integrity and performance of the Si-NWs. Huskens et al. reported modified PLL polymers with appended OEG and thiol-reactive maleimide moieties (PLL-*g*-OEG-Mal) as versatile surface platforms for bio-sensing (Fig. 10D) [265]. PLL-OEG-Mal self-assembles on the negatively charged silica- or Au-coated sensors, then conjugates with thiol-modified bio-sensing probes, whose density could be precisely controlled by the design and synthesis of PLL-*g*-OEG-Mal. Using peptide nucleic acid as the probe, the specific detection of cDNA was carried out by employing a quartz crystal microbalance with dissipation. Molecular imprinting is a well-known fabrication technique for designing artificial receptors and molecular sensors. Bisphenol A-imprinted polypeptide gel layers based on cyclodextrin-modified PLL (CD-PLL) on a polyterthiophene-modified quartz crystal microbalance (QCM) sensor chips were prepared by Advincula et al. for the selective and sensitive detection of bisphenol A using QCM [278]. The bisphenol A-imprinted CD-PLL gel layer chip exhibited a much higher QCM response than the non-imprinted gel layer chip in an aqueous bisphenol A solution.

In conclusion, PLL-based nanomaterials have received increasing attention in the field of bio-sensing. However, challenges remain in increasing the selectivity of detection. A promising strategy to handle this tissue relies on the functionalization of PLL with one or more specific probes, aptamers, antibodies, or enzymes on the surface of these PLL-based nanomaterials. The stability of these bio-sensing nanomaterials also deserves systematic investigation.

3.4. PLL-based polymers for bio-imaging

Bio-imaging, including magnetic imaging (*e.g.*, magnetic resonance imaging [MRI] and chemical exchange saturation transfer imaging [CEST]), optical imaging (*e.g.*, photoacoustic imaging [PAI] and

fluorescence imaging [FLI]), and radionuclide-based imaging (*e.g.*, computed tomography [CT]) has played an irreplaceable role in the diagnosis of various diseases. Bio-imaging often involves the use of contrast agents to improve the imaging effects, and the contrast agents are frequently modified with organic components (*e.g.*, small molecules, polymers, and biomolecules) to increase solubility, improve biocompatibility, prolong circulation time *in vivo*, offer targeting properties, and accomplish theragnostic effects [279]. PLLs can be used as imaging agents themselves or serve as coating materials because of their biodegradability, biocompatibility, cationic charging state, and easy post-functionalization.

3.4.1. PLL-based polymers for MRI

MRI is a noninvasive imaging modality for diagnosis with high temporal and spatial resolution. In the presence of an external magnetic field used for MRI, the magnetic nuclei align with the field, resulting in resonance through a radiofrequency pulse. The water hydrogen nuclei are regarded as the most abundant magnetic nuclei in the body, thus generally used as the analytic subject in MRI. In comparison with manipulated pulse sequences alone to achieve contrast images of tissues, using contrast agents more readily shows the pathologic and anatomic features of a region of interest [280]. A variety of contrast agents, including Gd (III) complexes, Mn (II) complex, and superparamagnetic iron-oxide nanoparticles (SPIONs) have been used to enhance the diagnostic sensitivity and specificity of MRI. To shorten the T_1 relaxation time, elongate the circulation time *in vivo*, and alleviate extravasation from blood vessels, MRI contrast agents are generally carried or modified with nano-sized vehicles [281,282], including PLL-based scaffolds. The chelate group, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), was attached to the primary amino groups of the block copolymer PEG-*b*-PLL, which could complex with Gd (III) ion to form core-shell polymeric micelles as MRI contrast agents [283–285]. These polymeric micelle-based MRI contrast agents exhibit stable blood circulation and selective accumulation at the tumor tissues because of the EPR effect, thus substantially enhancing the signal intensity of MR images at the tumor. We have used star-shaped PLLs as the carriers for diethylene triamine pentaacetic acid (DTPA)-Gd (III) (Fig. 11A) [286]. The use of star-shaped PLLs provided a large number of primary amine groups with high density, thus favorable for the formation of Gd (III) complex clusters. A star-shaped PLL scaffold could enhance the T_1 MR contrast *in vitro* and *in vivo*, reduce the cytotoxicity of the contrasting agents, and elongate the circulation time in the blood. Positively charged PLLs could be coated on the surfaces of negatively charged MRI contrast agents (typically surface-decorated SPIONs) to improve biocompatibility, stability, and water solubility. Shen et al. prepared PLL-coated SPIONs and evaluated their performance on MSCs [287]. However, the results indicated that a high iron dose and long incubation period could lead to potential cytotoxicity.

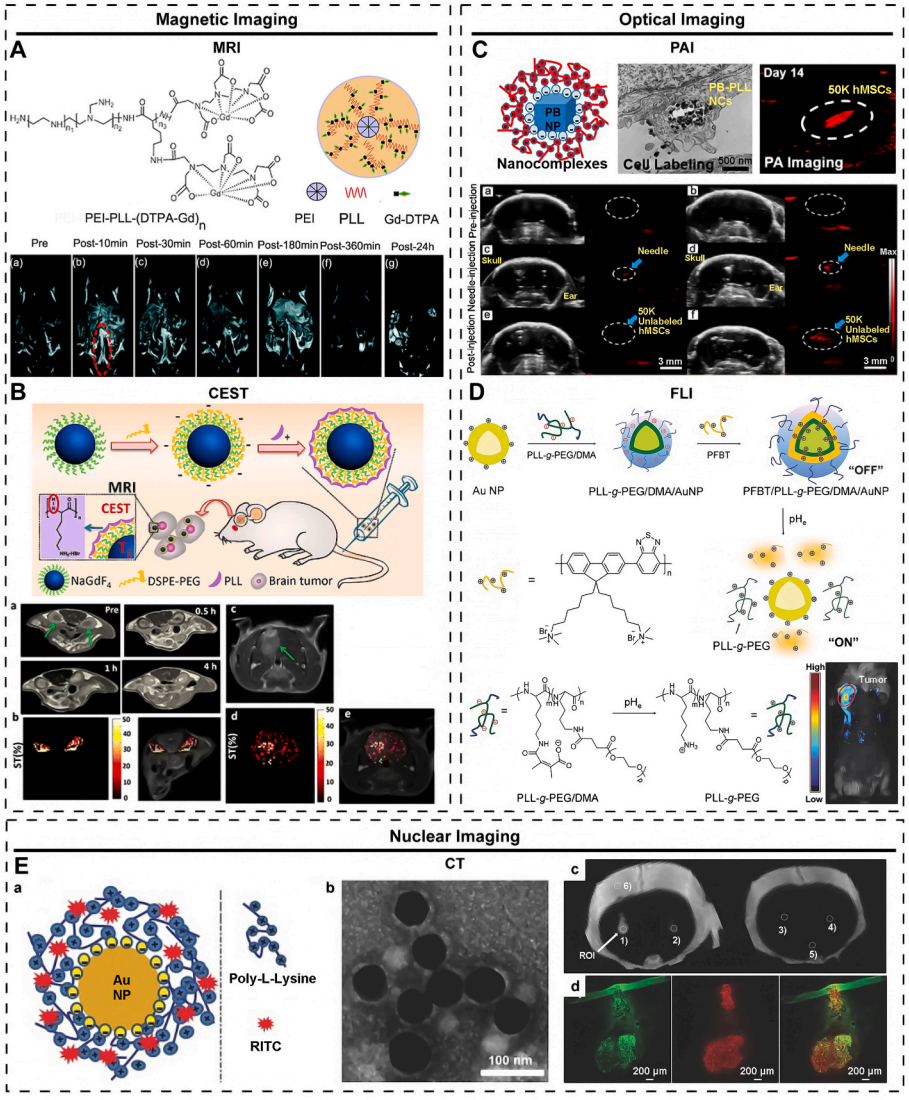

Fig. 11. Examples of PLL-based nanomaterials for various *in vivo* bio-imaging applications. (A) Synthesis of star-shaped PEI-PLL-DTPA-Gd^{3+} for T_1-weighted MR imaging. Reproduced with permission from Ref. [286], copyright 2018, The Royal Society of Chemistry. (B) The synthesis of $NaGdF_4$@PLL nanodots for T_1-weighted and CEST MR imaging. Reproduced with permission from Ref. [295], copyright 2016, American Chemical Society. (C) Prussian blue-PLL nanocomplexes for photoacoustic stem cell imaging. Reproduced with permission from Ref. [296], copyright 2017, American Chemical Society. (D) Fabrication of PFBT/PLL-*g*-PEG/DMA/AuNP and its tumor-acidity-activated fluorescence activation. Reproduced with permission from Ref. [299], copyright 2014, Wiley. (E) Transmission electron microscopic structure of AuNP-PLL-RITC complexes and their *in vivo* micro-CT images. Reproduced with permission from Ref. [303], copyright 2017, Wiley.

PLL-coated MRI contrast agents have the potential for post-modification to construct multifunctional MRI systems. Roque et al. explored the neutravidin-iminobiotin pH-dependent affinity interaction to develop an affinity-triggered multilayered magnetic nanoparticle-based MRI nanoprobe for preferential labeling of tumor cells [288]. The multilayer system was assembled from a *meso*-2,3-dimercaptosuccinic acid-coated iron-oxide magnetic nanoparticle core, the electrostatically adsorbed PLL inner layer, and a pH-responsive neutravidin-iminobiotin interconnected PEG outer layer. In the acidic tumor microenvironment, the outer layer tended to dissociate from the nanoprobe, rendering cell-magnetic nanoparticle interactions more favorable, and cancer cells visible by MRI. Surface modification of MRI contrast agents with PLL could integrate imaging properties and delivery capacity to achieve theranostics. Heise et al. fabricated fully water-dispersible PLL-*ran*-(glycosylated poly(propargyl-L-glutamate))-grafted SPIONs, offering synergistic effects of imaging properties and siRNA loading without a loss of colloidal stability [289]. Jeong et al. evaluated the ability of a SPION-loaded micellar system to safely and efficiently transfer *p*DNA into cells. The micellar system PLL-HA-SPION was prepared by loading oleic acid-coated SPIONs into hyaluronic acid-5β-cholanic acid conjugates, followed by the electrostatic surface coating with PLL. The PLL-hyaluronic acid-SPION micelles have the potential to be utilized in MRI-guided gene therapy [290]. Haam et al. prepared magnetic nanoparticles for the efficient delivery of therapeutic siRNA and the simultaneous detection of cancer using MRI [291]. Magnetic nanoparticles were constructed by anchoring PLL-*g*-imidazole onto the surfaces of manganese ferrite nanoparticles, ensuring high water solubility, enhanced MR contrast effects because of their clustering effect, and endosome-disrupting functionality in the presence of cationic imidazole. The synthesized nano vectors were then complexed with siRNA via electrostatic interactions with PLL for specific gene silencing. Anker et al. reported magnetic nanocapsules for magnetically assisted delivery, pH-triggered drug release, and MRI imaging [292]. These magnetic nanocapsules consisted of an iron nanocore, a mesoporous silica shell, and a surface coating with multiple polyelectrolyte layers of PLL and sodium alginate. The iron core provided a high saturation magnetization, the hollow space between the iron core and mesoporous silica shell was used to load the anticancer drug DOX and a T_1-weighted MRI contrast agent Gd-DTPA, while the surface coating allowed pH-responsive drug release.

CEST imaging is an emerging MRI contrast approach in which the contrast agents containing either exchangeable protons or exchangeable molecules are selectively saturated and, after the transfer of this saturation, detected indirectly through the water signal with enhanced sensitivity [293]. PLL, a molecule that contains exchangeable protons (amide NH and amine NH_2), which resonate at a chemical shift different from the bulk water signal, could be employed as a CEST contrast agent. McMahon et al. demonstrated the use of "multi-color" mixtures of liposomes loaded with proton exchange agents such as glycogen, PLL, and L-arginine, in which the different chemical shifts of the exchangeable protons were assigned different colors analogous to optical imaging [294]. Being nonmetallic and bioorganic, these diamagnetic CEST liposomes formed an attractive novel platform for multi-color imaging *in vivo*. Bu et al. prepared ultra-small $NaGdF_4$@PLL nanodots as a novel class of MRI contrast agents (Fig. 11B) [295]. In such a structure, the $NaGdF_4$ core was demonstrated to be feasible for T_1-weighted MRI as it exhibited much higher longitude relaxivity than clinically applied Gd-chelates. In contrast, the PLL shell demonstrated a sensitive CEST effect responding to the pH of the lesions. *In vivo* animal experiments showed the feasibility of NaGdF4@PLL nanodots as contrast agents for efficient kidney and brain tumor diagnosis and pH mapping.

3.4.2. PLL-based polymers for optical imaging

PAI captures the strengths of acoustic and optical imaging via introducing (laser) light pulses into samples and then receiving acoustic information in the form of ultrasound to create images. Optical absorption in biological tissues can be attributed to endogenous molecules such as hemoglobin and melanin, or exogenously delivered contrast agents such as Prussian blue nanoparticles (PBNPs) [282]. PBNPs were regarded as the emerging photoacoustic contrast agents because of their strong absorption in the NIR region [296]. To reduce the negative charges of PBNPs and accelerate their cellular internalization, PBNPs were coated with cationic PLL to prepare PB-PLL nanocomplexes as biocompatible and efficient photoacoustic contrast agents that can be used for labeling and tracking of hMSCs (Fig. 11C) [296]. The hMSCs were efficiently labeled when PLL was complexed to the PBNPs, whereas there was hardly any uptake of PBNPs without PLL modification. The PB-PLL nanocomplexes exhibited low cytotoxicity and excellent colloidal stability in aqueous media. Melanin, a type of endogenous molecular absorber, was explored as a photoacoustic agent because of its innate NIR absorption and photothermal transduction properties [297]. Very recently, a PAI-guided tumor therapy approach based on PLL-functionalized melanin nanoparticles (MNP-PLL) was introduced by Zhang et al. to treat laryngeal squamous cell carcinoma (LSCC) [298]. In this study, the PLL polymer, helping condense nucleic acids into nano-sized complexes, was grafted on the surfaces of melanin nanoparticles to achieve a multimodality therapy. As expected, the MNP-PLL/miR-145-5p NPs displayed a potent anticancer effect against primary tumors via photothermal ablation and significantly inhibited tumor progression of LSCC through the upregulation of miR-145-5p, indicating the significance of integrating thermo-gene therapies into the theranostic nanoplatform.

FLI is an essential optical analytical technique that is widely applied in biological and medical research. This imaging technology offers not only high sensitivity but also high spatial resolution. A tumor-responsive nanoprobe based on a conjugated polyelectrolyte, a pH-triggered charge-reversible PLL and AuNP hybrid, was designed to respond to the low-pH extracellular microenvironment of tumors with light-up fluorescence (Fig. 11D) [299]. The AuNPs with positive surface charges were sequentially coated with a pH-responsive charge-reversible PLL-*g*-PEG/2,3-dimethyl maleic anhydride and a cationic conjugated polyelectrolyte poly[9,9-bis(6′-*N*, *N*,*N*-trimethylammonium) hexyl)fluorene-*alt*-4,7-(2,1,3-benzothiadiazole) dibromide] (PFBT) through electrostatic interactions. The hybrid probe was almost non-fluorescent under physiological conditions because of the super-quenching of PFBT by AuNPs. However, when exposed to acidic extracellular microenvironments in tumors, the acid-labile amides hydrolyzed into primary amines, resulting in strong electrostatic repulsion between PFBT and AuNPs and the subsequent recovery of PFBT fluorescence. The nano-sized structure was suitable for efficient accumulation and fluorescence activation at tumor sites when intravenously administrated. Deoxycholic acid (DOCA), PEG, and cyanine 5.5 were conjugated to the amine group of PLL to prepare PLL-*g*-(DOCA-PEG-cy5.5), which self-assembled into polymeric micelles capable of encapsulating curcumin. The incorporation of cyanine 5.5 provided successful biodistribution in fluorescent images *in vivo* [300].

3.4.3. PLL-based polymers for nuclear imaging

CT is one of the most widely used clinical imaging modalities. To increase the sensitivity of CT, iodinated nanomaterials, as well as nanoparticles based on heavy atoms such as Au, lanthanides, and tantalum have been utilized as CT contrast agents [301]. AuNPs are of particular interest because of their high atomic number and efficient X-ray attenuation properties [302]. In general, high cellular uptake of AuNPs is a prerequisite for labeling cells to enable *in vivo* visualization using CT. Bulte et al. presented a simple method to render hMSCs sufficiently radiopaque by complexing 40 nm citrate-stabilized AuNPs with PLL (Fig. 11E) [303]. hMSCs could be efficiently labeled when PLL was complexed to AuNPs, in contrast to naked citrate-stabilized AuNPs that showed almost no uptake. Labeled hMSCs can be visualized *in vitro* and *in vivo* with a micro-CT scanner. Very recently, Yu et al. reported chitosan-*g*-PLL (CPL) dendron-assisted facile self-assembly of AuNPs for

enhanced X-ray CT imaging and precise MMP-9 plasmid shRNA delivery [304]. Biocompatible CPL dendrons were synthesized for the facile preparation of Au@CPL nanoclusters in one step. The obtained Au@CPL nanoclusters exhibited enhanced CT imaging properties compared with clinically used iohexol (Omnipaque) and displayed high pMMP-9 delivery efficiency.

3.5. PLL-based polymers for tissue engineering

Tissue engineering, including hard tissue (*e.g.*, bone and teeth) and soft tissue (*e.g.*, tendons, skin, fibrous tissues, nerves, among others.) restoration and regeneration, has been extensively exploited and rapidly developed in regenerative medicine [305,306]. Bioinspired materials play a pivotal role in providing a template and extracellular environment to support regenerative cells and promote tissue regeneration [307,308]. As a polycation under physiological conditions, PLL can promote attachment of negatively charged cells to its surfaces, which makes it possible to be used as a scaffold material. However, PLL cannot be used alone as the scaffold because of its poor mechanical properties. Two strategies are generally employed to address this problem. One strategy is a coating of solid inorganic substrates, including HA, silica, and rutile (TiO_2), among others, with PLL, which makes it an essential part of composite materials for tissue engineering. This strategy has been extensively explored in biomedical fields for hard tissue engineering applications. Another strategy generally involves the construction of hybrid organic biomaterials, including hydrogels, polyelectrolyte multilayer films, among others, from PLL with many other biopolymers or biomolecules for soft tissue engineering.

3.5.1. PLL-based polymers for hard tissue engineering

Hard tissues are calcified to varying degrees and contain different amounts of minerals, in contrast with soft tissues. There is a growing demand for developing hard tissue repair technology because most hard tissues lack the ability of self-repair [309]. Bone tissue engineering is an exciting approach to repair bone defects directly or engineer bone tissue for transplantation. In general, the scaffolds, cells, and signaling cues are regarded as the essential elements for bone tissue engineering. Scaffolds are served as substitutes for natural extracellular matrixes (ECM), which can match the size range of the integral parts of natural bone and affect seeded cells, including cell adhesion, proliferation, migration, and differentiation [310]. On account of the cationic amine groups and the secondary structure of PLL that are demonstrated to enhance adhesion and proliferation of osteoblasts, PLL has been widely explored as an orthopedic coating and bone scaffold fabrication. For instance, Zheng et al. demonstrated a simple method for modifying porous poly(lactic acid-glycolic acid)/HA scaffolds with poly(γ-benzyl-L-glutamate) (PBLG) and PLL to promote cell growth and osteogenic differentiation [311]. The surface coatings of scaffolds with PLL improved the hydrophilicity and promoted MC3T3-E1 cell adhesion, proliferation, and bone repair *in vivo*. Likewise, Kouhi et al. prepared PLL-anchored poly (3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV)-hydroxyapatite/-bredigite (HABR) nanofibrous scaffolds toward bone tissue engineering (Fig. 12A) [312]. The results indicated that human fetal osteoblast cells seeded on PLL-modified PHBV-HABR display enhanced cell adhesion, proliferation, and osteogenic differentiation. To increase PLL adhesion to inorganic particles and surfaces, the chemical modification of PLL with 3,4-dihydroxybenzylaldehyde (DHBA) was introduced by Zhitomirsky et al. [313]. The catechol groups of PLL-DHBA allowed for the co-deposition with HA and rutile (TiO_2), combining the bioactivity of HA with the biocompatibility and stability of TiO_2. The nanocomposite films exhibited dual nano- and micro-scale topography with a high wettability and enhanced osteoblast adhesion and proliferation.

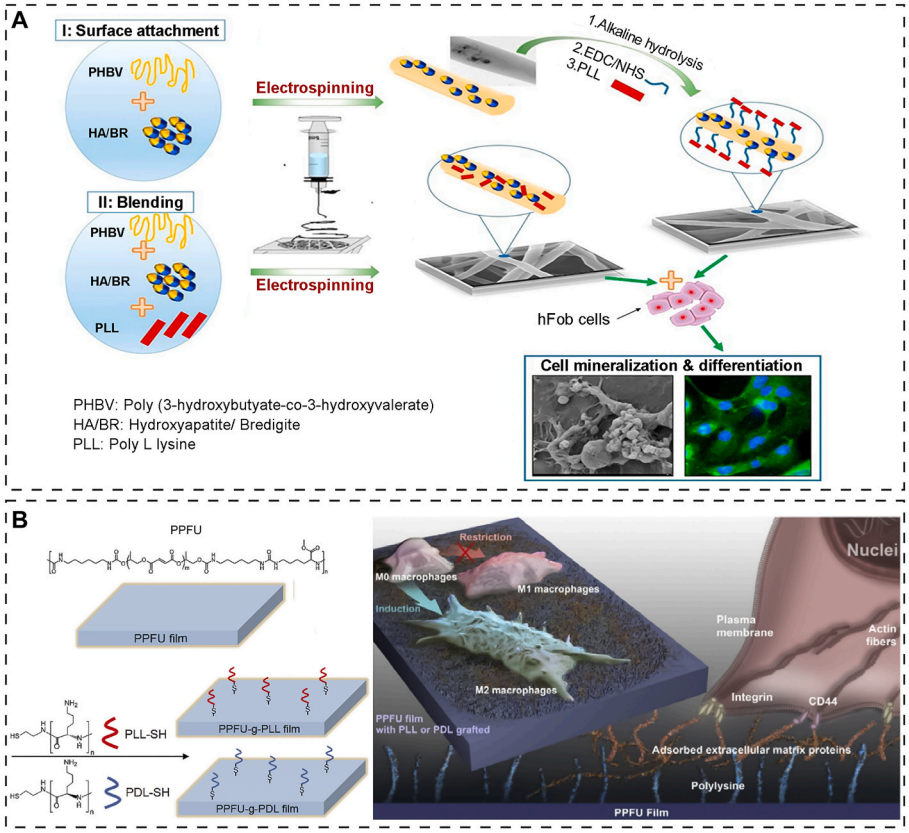

Fig. 12. (A) A schematic illustration of the construction of PLL-modified PHBV-HABR nanofibrous scaffolds for bone tissue engineering. Reproduced with permission from Ref. [312], copyright 2018, Elsevier. (B) PLL or poly(D-lysine)-grafted poly(propylene fumarate) polyurethane films selectively promote M2 polarization of macrophages. Reproduced with permission from Ref. [320], copyright 2020, Elsevier.

Hyaluronic acid, a component of ECM, plays an essential role in improving cell growth, differentiation, and migration, but it is poorly adhesive to cells. The composite multilayers of PLL and hyaluronic acid with integrated advantages on the surfaces of bioactive silica could induce the osteogenic proliferation and differentiation of hMSCs and human adipose-derived stem cells [314,315].

In addition to the capability to connect with cells and stimulate desired cell functions for bone repair and regeneration, scaffolds can also be used for the simultaneous delivery of bioactive signal cues, such as growth factors, hormones, nucleic acids, and anti-inflammatory drugs, to induce and accelerate bone regeneration [310,316,317]. These biomimetic drug delivery systems could provide control over the location, timing, and release kinetics of the signaling molecules according to the drug's physiochemical properties and specific biological mechanisms. A poly(lactide-*co*-glycolide) scaffold containing fibrin gel, MSCs, and PEG-*b*-PLL/*p*DNA-TGF-β1 complexes was fabricated for *in vivo* synchronized regeneration of cartilage and subchondral bone by Gao et al. [318]. The incorporation of PEG-*b*-PLL could condense *p*DNA-TGF-β1 and then transfect MSCs to produce TGF-β1 *in situ*, which induced the chondrogenesis of MSCs and improved the restoration of the full-thickness cartilage defects. Wang et al. prepared a porous HA scaffold that was functionalized by a PLL/polydopamine hybrid coating [319]. The PLL/polydopamine coating could anchor bone morphogenic protein-2 (BMP2) to the HA scaffold via catechol chemistry under a mild condition and could also release BMP2 in a tunable and sustainable manner as the PLL degrades in the physiological environment. The BMP2-entrapped PLL/PDA coating on the HA scaffold promoted osteogenic differentiation of bone marrow stromal cells *in vitro* and efficiently induced ectopic bone formation *in vivo*.

Regarding other hard tissues, dental caries is believed to be the most prevalent disease in humans. Caries are caused by acidogenic bacterial fermentation of carbohydrates to produce acids, which leads to mineral loss [321]. The conventional approaches for treating tooth diseases involve the fabrication of biomaterials and constructs for enhancing dental and periodontal regeneration, inhibiting acid-producing bacteria, and responding to pH to protect tooth structures [322]. To the best of our knowledge, there are only a few publications regarding the applications of PLL in dental treatment. Very recently, given that peri-implantitis is caused by bacterial inflammation at the percutaneous abutment region, van den Beucken et al. established a tetracycline-releasing system based on LbL-deposited poly(acrylic acid) and PLL coatings on Ti [323]. Tetracycline exhibited a significant burst release upon incubation in acidic conditions to diminish *Porphyromonas gingivalis* colonies.

3.5.2. PLL-based polymers for soft tissue engineering

Soft tissue, regarded as a kind of complex fiber-reinforced construction with high water content, is recognized to be invaded via disease, surgery, aging, and trauma, which often leads to large scale soft tissue loss [324]. Various types of PLL-based nanomaterials have been investigated for soft tissue regeneration and reconstruction. Hydrogels are characterized regarding their structural similarity to natural ECMs, high water content, porous frameworks for cell proliferation and transplantation, ability to match irregular defects, among other properties, which make them attractive as scaffolds in cartilage and other soft tissue engineering [325,326]. Interestingly, the employment of PLL could not only enable the influence of swelling and degradation behavior of hydrogels but also enhance the cell absorption and proliferation because of its pendent primary cationic amine groups. Mikos et al. fabricated oligo(PEG fumarate) (OPF)-based hydrogels loaded with PLL [327]. The incorporation of PLL into synthetic OPF hydrogels dynamically influenced their swelling behavior, which might be leveraged for the development of constructs with desirable dynamic swelling properties for cartilage tissue engineering applications. Furthermore, PLL could function as an inductive factor that primes the cellular microenvironment for early chondrogenic gene expression and chondrogenesis of MSCs. Wang et al. presented a novel photopolymerizable PLL with one end-capped allyl group and used it to modify polyethylene glycol diacrylate (PEGDA) hydrogels for creating a superior and permissive nerve cell niche [328]. Compared with their neutral counterparts, these PLL-grafted hydrogels greatly enhanced pheochromocytoma cell survival in encapsulation, proliferation, and neurite growth and promoted neural progenitor cell proliferation and differentiation capacity. Covalent immobilization of PLL in PEGDA hydrogels represented promising injectable materials for nerve repair and regeneration. A biodegradable co-polypeptide, poly(L-glutamic acid-*co*-L-lysine) hydrogel was synthesized by He et al. and utilized as both a wound dressing and therapeutic prodrug [329]. The hydrogel could degrade in the presence of elevated degradative enzymes in a wound environment, releasing therapeutic amino acids to enhance wound healing. *In vivo* results demonstrated that the hydrogel could effectively promote wound regeneration in both macroscopic and microscopic scales. Further investigation revealed that the wound-healing effects of the hydrogel were highly attributed to its enhanced impact on angiogenesis, cell proliferation, and re-epithelialization of the wound.

PLL-coated fibrous constructs were also employed for soft tissue repair and regeneration. A braided bio-scaffold with flexible and elastic poly(L-lactide-*co*-caprolactone) (PLCL) was developed by Liu et al. for ligament tissue engineering [330]. The fibrous scaffold was coated with PLL and hyaluronic acid via the LbL technique to combine the cell adhesion properties of PLL and enhanced cell growth, differentiation, and migration of hyaluronic acid. The surface modification of the PLCL scaffold retained its mechanical properties and improved its biocompatibility. Good metabolic activity and increased chemotaxis of MSCs were observed on surface-coated PLCL braided fibers. In a recent study by Sharma et al., a nanostructured LbL adhesive coating composed of dopamine-modified hyaluronic acid and PLL was designed to tenaciously adhere to the polycarbonate urethane, a type of biomaterial employed in meniscus replacement [331]. The introduction of the nanostructured LbL adhesive coating could reestablish boundary lubrication between cartilage and biomaterial as a result of effective recruitment of lubricious protein by dopamine-modified hyaluronic acid, thereby drastically reducing cartilage wear and further avoiding pain and tissue degradation. PLL-grafted natural protein zein was employed to prepare nanofibrous membranes as an efficient scaffold for nerve repair and regeneration [332]. The PLL chain length affected the hydrophilicity of fibrous membranes as well as the cell viability, adhesion, proliferation, and differentiation of neural stem cells (NSCs). The tailoring of PLL content could direct the differentiation of NSCs toward mature neurons with extensive neurite formation and astrocytes rather than oligodendrocytes. Gao et al. prepared PLL or poly(D-lysine)-grafted poly(propylene fumarate) polyurethane films to modulate the macrophage functions [320]. The decoration of polylysine on the poly(propylene fumarate) polyurethane surface could selectively promote the polarization of macrophage toward the M2 phenotype and restrict the M1 polarization to a great extent *in vitro* and *in vivo* (Fig. 12B). Polylysine coating was demonstrated as an effective method to regulate the immune response of biomaterials, and thus could be applied widely in regenerative medicine.

4. Translational studies on PLL-based nanomaterials

PLLs represent suitable candidates for clinical treatment purposes because (1) PLLs are composed of natural amino acids along with their inherent biocompatibility and biodegradability, (2) they provide a myriad of biomedical opportunities due to their facile and controllable synthesis, diverse architecture with tunable properties, including but not limited to net charge, size, shape, self-assembly, conformation, and bioresponsiveness, and (3) their side chains can be modified and conjugated to therapeutic drugs to achieve preclinical and even clinical translation. Preclinical studies performed in mouse models have

exemplified the great potential of PLLs for future clinical applications, although up to now, only a limited number of PLL-based nanomedicine products have been either investigated or undergone clinical trials [333].

Polyinosinic: polycytidylic acid stabilized with PLLs in carboxymethyl-cellulose (poly-ICLC, Hiltonol®) is being developed by Oncovir, Inc. (Washington DC) for use as a synthetic double-stranded RNA viral mimic that serves as a pathogen-associated molecular pattern or "danger signal" that binds to the endosomal toll-like receptor 3, the cytosolic helicase, and other pathogen receptors to activate dendritic cells and subsequently trigger natural killer cells to kill tumor cells [334]. In a completed phase I clinical trial, poly-ICLC has been demonstrated to be safe and well tolerated when administered subcutaneously or intranasally at a single dose of 1.6 mg to healthy volunteers. Preliminary innate immune response data demonstrated marked upregulation of gene expression (mainly interferon-stimulated genes) in whole peripheral blood mononuclear cells and activation of various blood cell types, including dendritic cells and monocytes, following subcutaneous injection of poly-ICLC. Notably, changes in gene expression were homogeneous among the 8 volunteers who received poly-ICLC treatment [335]. In an evaluation of poly-ICLC in melanoma and head and neck cancer patients, upregulation of genes associated with chemokine activity, T cell activation, and antigen presentation, as well as increased cluster of differentiation 4, cluster of differentiation 8, programmed cell death protein 1, and programmed cell death ligand 1 levels were observed [336]. Poly-ICLC administered to solid tumor patients was well-tolerated with minimal toxicity and generated local and systemic immune responses. Notably, it is currently being explored in a multicenter phase II clinical trial (NCT02423863) for the treatment of solid tumors.

Kataoka et al. employed a Y-shaped block catiomer, comprising a cationic PLL chain and two PEG arms, for the delivery of oligonucleotide drugs. The spontaneous ion-pair interactions between the PLL chain and the oligonucleotide generated a uPIC that conferred dynamic stability to oligonucleotide drugs in the bloodstream. The uPIC was able to efficiently deliver oligonucleotides into mice, bearing pancreatic and brain tumors, due to the significant longevity in the bloodstream and appreciably small size (~18 nm) [337]. In another pancreatic cancer mouse model, the Y-shaped block catiomer was utilized for delivering DNA-chimeric siRNAs, allowing for high transfection efficiency and protection from kidney filtration [338]. In 2020, this Y-shaped block catiomer has been directed to a phase I clinical trial for the treatment of triple negative breast cancer by delivering siRNA against positive regulatory domain-containing protein 14 [339].

Starpharma Holdings Lt. (Melbourne, Australia) has explored PLL-based dendrimer products for pharmaceutical, life science, and other applications. It is worth noting that the macromolecular dendrimer itself could be an active pharmaceutical agent [340]. SPL7013 (VivaGel®), a topically administered PLL dendrimer-based microbicide, received market approval in Australia and the European Union for treatment of bacterial and viral (HIV-1 and HSV-2) infections. Excitingly, a recent research demonstrated that SPL7013 showed significant activity against SARS-CoV-2 (coronavirus) [341]. Starpharma's dendrimers can also be used to enhance the properties of other drugs, known as DEP®. Starpharma's DEP® drug delivery platform has demonstrated reproducible preclinical benefits across multiple internal and partnered DEP® programs, including improved efficacy, safety, and survival. Starpharma has three internal DEP® products, DEP® docetaxel (phase II), DEP® cabazitaxel (phase II), and DEP® irinotecan (phase I/II), and one partnered DEP® product, DEP®-Bcl2/xL conjugates (phase I), in clinical development in patients with solid tumors [342]. For all of these PLL dendrimer-drug conjugates, preliminary clinical trial results have demonstrated enhanced safety, greater overall drug exposure, lower peak blood concentrations, longer plasma half-life and reduced side effects compared to conventional free drugs.

5. Summary and perspective

In summary, this review provides an overview of the recent advances of PLL-based nanomaterials in biomedical applications. The intrinsic biodegradability, cationic charging properties, and facile functionalization make PLL an ideal candidate for use as a biomedical polymer, and the hybrid constructs of PLL with many other functional blocks have found tremendous applications in diverse biomedical fields such as antibacterial agents, delivery systems, bio-sensing, bio-imaging, and tissue engineering.

Many biomedical applications of PLL-based nanomaterials, including antimicrobial agents, gene delivery, protein delivery, and biosensors, are derived primarily from their cationic charging properties. Nevertheless, the positive charge of PLL poses a dilemma in which the cationic charge is correlated with high cytotoxicity and fast systemic clearance. Strategies to address this predicament, for example, employing hybrids with other building blocks, modulating the spatial conformation of PLL, and stimuli-responsive shielding of PLL, will certainly direct the future design and biomedical applications of PLL-based nanomaterials.

Conversely, the cationic charge on PLL might be favorable for its selective binding to tumor cells owing to the higher negative charge of the glycocalyx on tumor cell surface [343]. In other words, PLLs could serve as promising antitumoral agents that behave differently from traditional small chemotherapeutic agents such as PTX, CPT, and DOX. Early reports demonstrated that PLL could cause rapid membrane damage in tumor cell lines and subsequent mitochondrial-mediated apoptosis [344,345]. Similar to the mechanism of antibacterial action, the antitumor activity of PLL, which is based on membrane penetration or disruption, might avoid MDR and display relatively selective toxicity toward cancer cells versus healthy cells. A recent study by Chatterjee et al. revealed that PLL induced *in vitro* cytotoxic effect in K562, A549, and U937 cancer cell lines and *in vivo* tumor growth inhibition in Ehrlich ascites carcinoma and Sarcoma-180 tumors via an apoptotic pathway and tumor angiogenesis suppression [346]. Jan et al. reported that cationic, one-dimensional fibril assemblies formed from coil-sheet PLL-*b*-poly(L-threonine) block co-polypeptides that exhibited potent anticancer activity by enhancing membranolysis [347]. The fibril assemblies could efficiently interact with negatively charged cellular and mitochondrial membranes via electrostatic interactions, leading to necrosis via membrane lysis and apoptosis via a mitochondrial lytic effect. Moreover, the fibril assemblies exhibited low hemolytic activity and selective cytotoxicity toward cancer cells. The PLL-based nanomaterials have demonstrated promising anticancer potential. However, this emerging area remains largely unexplored. Increasing efforts could be devoted to the structural modulation, functional modification, stimuli-responsiveness, passive and active targeting decoration of PLL to shed new light on the antitumor applications of PLL-derived nanomaterials.

The diverse biomedical applications of PLL-based nanomaterials are primarily dependent on the interdisciplinary understanding and efforts from the areas of chemistry, materials, biology, and medical sciences, from which the functionalization of PLL, as well as the combination of PLL with many other building segments, could be rationalized. To this end, the integration of the versatile biomedical applications (*i.e.*, antibacterial agents, delivery systems, bio-sensing, bio-imaging, tissue engineering, and many other new applications) of PLL-based nanomaterials are of great interest. The actual biomedical applications of PLL-derived nanomaterials may require the combination of drug delivery with the antibacterial capacity, the incorporation of bio-imaging ability to drug delivery vectors, as well as the integration of scaffolds, drug delivery, and antibacterial properties in tissue engineering, to name a few.

Great efforts are also needed for converting the "potential" application of PLL-based nanomaterials to "practical" applications. Till date, the concerned investigations are limited to *in vitro* or preliminary animal experiments. Some critical issues, including mutagenicity,

teratogenicity, reproductive toxicity, and genetic toxicity, need to be evaluated extensively to further improve the clinal applications of PLL-based nanomaterials.

Author contributions

Maochao Zheng: Conceptualization, Investigation, Writing - original draft, Writing - review & editing. **Miao Pan**: Writing - review & editing. **Wancong Zhang**: Writing - review & editing. **Huanchang Lin**: Writing - review & editing. **Shenlang Wu**: Writing - review & editing. **Chao Lu**: Writing - review & editing, Funding acquisition. **Shijie Tang**: Writing - review & editing, Funding acquisition. **Daojun Liu**: Writing - review & editing, Supervision, Funding acquisition. **Jianfeng Cai**: Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare no conflict of interest.

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Abbreviations

AIE	Aggregation-induced emission
AMP	Antimicrobial peptide
AuNP	Gold nanoparticle
BP	Black phosphorus
Ce6	Chlorin e6
CEST	Chemical exchange saturation transfer
CMC	Critical micelle concentration
CPT	Camptothecin
CT	Computed tomography
DOX	Doxorubicin
ECM	Extracellular matrixes
EDTA	Ethylenediaminetetraacetic acid
EPR	Enhanced permeability and retention
FLI	Fluorescence imaging
GSH	Glutathione
HA	Hydroxyapatite
HC_{50}	Median hemolytic concentration
hMSCs	Human mesenchymal stem cells
IND	Indomethacin
LbL	Layer-by-layer
LC	Liquid crystal
MDR	Multi-drug resistance
MIC	Minimum inhibition concentration
MRI	Magnetic resonance imaging
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSCs	Mesenchymal stem cells
MSN	Mesoporous silica nanoparticle
NCA	*N*-carboxyanhydride
NIR	Near-infrared
PAI	Photoacoustic imaging
PAMAM	Poly(amido amine)
PCI	Photochemical internalization
PCL	Polycaprolactone
PEG	Polyethylene glycol
PEI	Polyethyleneimine
PIC	Poly-ion complex
PLA	Poly(lactic acid)
PLF	Poly(L-phenylalanine)
PLGA	Poly(L-glutamic acid)
PLL	Poly(α-L-lysine)
PLLF	Poly(L-lysine-*co*-L-phenylalanine)
Poly(L-Leu)	Poly(L-leucine)
PPI	Polypropyleneimine
PTX	Paclitaxel
PZLL	Poly(ε-benzyloxycarbonyl-L-lysine)
QCM	Quartz crystal microbalance
ROP	Ring-opening polymerization
SPPS	Solid-phase peptide synthesis
SPIONs	Superparamagnetic iron oxide nanoparticles
uPIC	Unimer polyion complex
VEGF	Vascular endothelial growth factor

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