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Recent advances in poly(amino acids), polypeptides, and their derivatives in drug delivery

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Poly(amino acids), polypeptides, and their derivatives have demonstrated significant potential as biodegradable biomaterials in the field of drug delivery. As degradable drug carriers, they can effectively load or conjugate drug molecules including small molecule drugs, nucleic acids, peptides, and protein-based drugs, enhancing the stability and targeting of the drugs *in vivo*. This strategy ultimately facilitates precise drug delivery and controlled release, thereby improving therapeutic efficacy and reducing side effects within the body. This review systematically describes the structural characteristics and preparation methods of poly(amino acids) and polypeptides, summarizes the advantages of poly(amino acids), polypeptides, and their derivatives in drug delivery, and detailedly introduces the latest advancements in this area. The review also discusses current challenges and opportunities associated with poly(amino acids), peptides, and their derivatives, and offers insights into the future directions for these biodegradable materials. This review aims to provide valuable references for scientific research and clinical translation of biodegradable biomaterials based on poly(amino acids) and peptides.

1. Introduction

Cancer, infectious diseases, and genetic diseases pose significant threats to human lives, the rapid development of therapeutic agents including small-molecule drugs,[1–4] therapeutic genes,[5–7] and protein/peptide agents,[8–10] has brought hope to patients. Nevertheless, these free drugs usually have poor stability and fail to effectively reach the lesion sites when administered orally or intravenously, resulting in extremely low bioavailability,[11] which in turn affects the therapeutic outcomes. Moreover, free small-molecule drugs such as chemotherapeutics, often reach normal tissues or organs, leading to severe side effects within the body.[12] Therefore, developing efficient and safe drug delivery systems is essential for enhancing drug efficacy and reducing side effects within the body.

The traditional drug delivery systems face numerous challenges in dealing with complicated pathological environments, particularly in improving drug stability,[13] enhancing targeting capability,[14] and increasing bioavailability.[15] Therefore, scien-

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tists are continuously exploring the development of novel drug carriers and delivery strategies to overcome these challenges.[16–19] For instance, Liu *et al.*[20] utilized fluorinated chitosan to deliver antibody drugs including anti-PD-1 and anti-CTLA-4 orally to tumor-bearing mice, effectively inhibiting tumor growth and extending the survival time of the mice. Additionally, Cheng *et al.*[21] used phenylboronic acid-modified polyamidoamine to achieve intracellular delivery of various protein drugs. Although these biomedical carriers can enhance the delivery efficiency and contribute to therapeutic effects of drugs, they are non-biodegradable in the body, leading to a significant burden and potential side effects.

Poly(amino acids) and polypeptide-based materials have become a research focus in drug delivery due to their excellent biodegradability and safety *in vivo*.[22] These materials are polymers or oligomers formed by the conjugation of amino acid monomers through peptide bonds. They are typically synthesized *via* various methods such as ring-opening polymerization of *N*-carboxyanhydride (NCA),[23] amide condensation reaction of amino acids,[24] microbial fermentation, and solid-phase peptide synthesis.[25] Such carriers not only exhibit good biocompatibility and biodegradability but can also be tailored in various shapes and functions through sequence design and chemical modification. For instance, by adjusting the types and ratios of hydrophilic and hydrophobic amino acids, polypeptide molecules can self-assemble into micelles,[26] fibers,[2] vesicles,[27] or hydrogels[28] for various disease treatments. Therefore, poly(amino acids) and polypeptides have broad clinical application prospects in the field of drug delivery (Fig. 1).

Typically, poly(amino acids) or polypeptides can encapsulate hydrophobic small molecule drugs to form nanoparticles (NPs) through hydrophobic interactions, which eventually enhances the delivery efficiency of small molecule drugs *in vivo*.[26] Additionally, hydrophilic small molecule drugs can be conjugated to poly(amino acids) or polypeptides *via* dynamic chemical bonds, thereby extending the circulation time of hydrophilic small molecule drugs in the bloodstream and their accumulation at target sites.[29] With the outbreak of the COVID-19 pandemic and the rapid development of gene editing technology, nucleic acid drugs have received widespread attention. However, nucleic acid drugs such as DNA, mRNA, or siRNA are usually unstable and easily degraded by nucleases in the body.[30] Furthermore, nucleic acids are often negatively charged, which hinders their uptake by target cells.[31] Poly(amino acids) or polypeptides can effectively load nucleic acids *via* electrostatic interactions and compress them into NPs, thus improving their stability and uptake efficiency by target cells *in vivo*.[32] Other macromolecular drugs, including protein and peptide drugs, generally have good bioactivity and are used to treat major diseases such as cancer and genetic disorders.[33] However, due to their large molecular weight and poor stability, these drugs are easily degraded by proteases or peptidases in the body, ultimately resulting in poor efficacy.[33] Poly(amino acid) or polypeptide carriers can effectively load protein or peptide drugs while maintaining their bioactivity, thereby enhancing the therapeutic efficacy of these macromolecular drugs *in vivo*.[16] Moreover, a search on Web of Science for publications over the past 10 years (2014–2024) on poly(amino acids) or polypeptides for drug delivery shows that the number of papers in this field has consistently exceeded 7000 per year, with a clear upward trend, which indicated that poly(amino acid)/polypeptide-based biomaterials holds great potential for addressing human health challenges and improving public health (Fig. 2).

Given the unique advantages of poly(amino acids) and polypeptides in drug delivery, this review systematically discusses the characteristics of these polymer carrier materials, common preparation methods, their applications in various diseases, and the progress of clinical research. In addition, we summarize the current challenges and opportunities faced by degradable polymer carrier materials, such as

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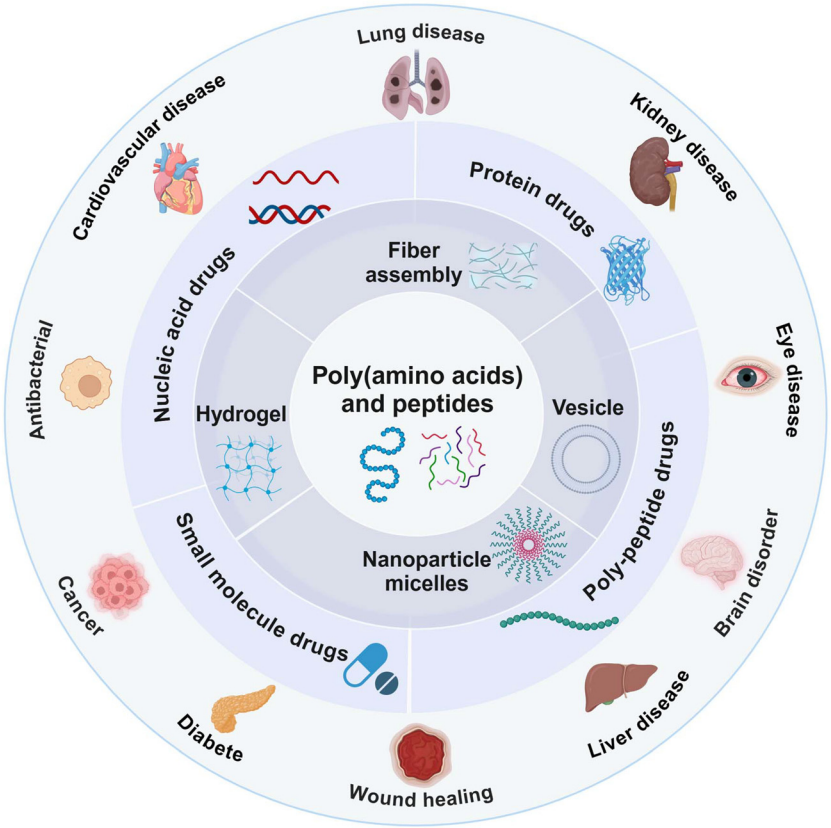

Fig. 1 Schematic illustration of drug delivery of poly(amino acids) and peptide carrier materials in different disease types.

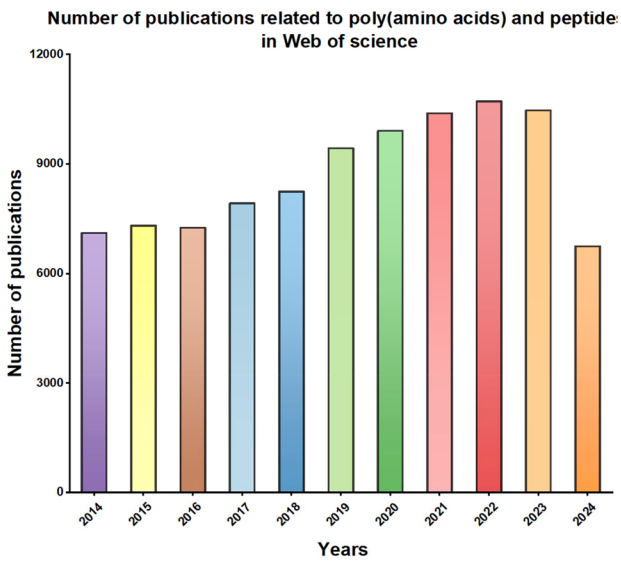

Fig. 2 Web of science statistical chart of the number of papers published on poly(amino acids) and peptides from 2014 to August, 2024.

poly(amino acids) and polypeptides, and propose future directions for the development of degradable biomedical carrier materials. This review aims to provide valuable insights for both fundamental research and clinical translation of poly(amino acid)- and polypeptide-based degradable biomaterials.

2. Structure of poly(amino acids) and peptides

According to the nomenclature recommended by the International Union of Pure and Applied Chemistry (IUPAC), a polypeptide is a molecule composed of more than 20 amino acids linked by peptide bonds,[34,35] and synthetic peptides are also referred to poly(amino acids). Amino acid materials exhibit excellent biocompatibility, biodegradability, and good self-assembly behavior, making them significant for biomedical applications,[36] as illustrated in Fig. 3, which presents for the common structural formula of poly(amino acids) and polypeptides. The structure of poly(amino acids) consists of linear or branched polymers formed by amino acid monomers through amide bonds, similar to peptide bonds. Each amino acid monomer features a basic structural unit that include an amino group, a carboxyl group, and a side chain (R group). Poly(amino acids) are linked by repeating peptide bonds (–NH–CO–), which create the poly(amino acid) backbone, typically in a linear configuration. The diverse side chains of amino acids impart a range of chemical and physical properties to poly(amino acids), including hydrophilicity, hydro-

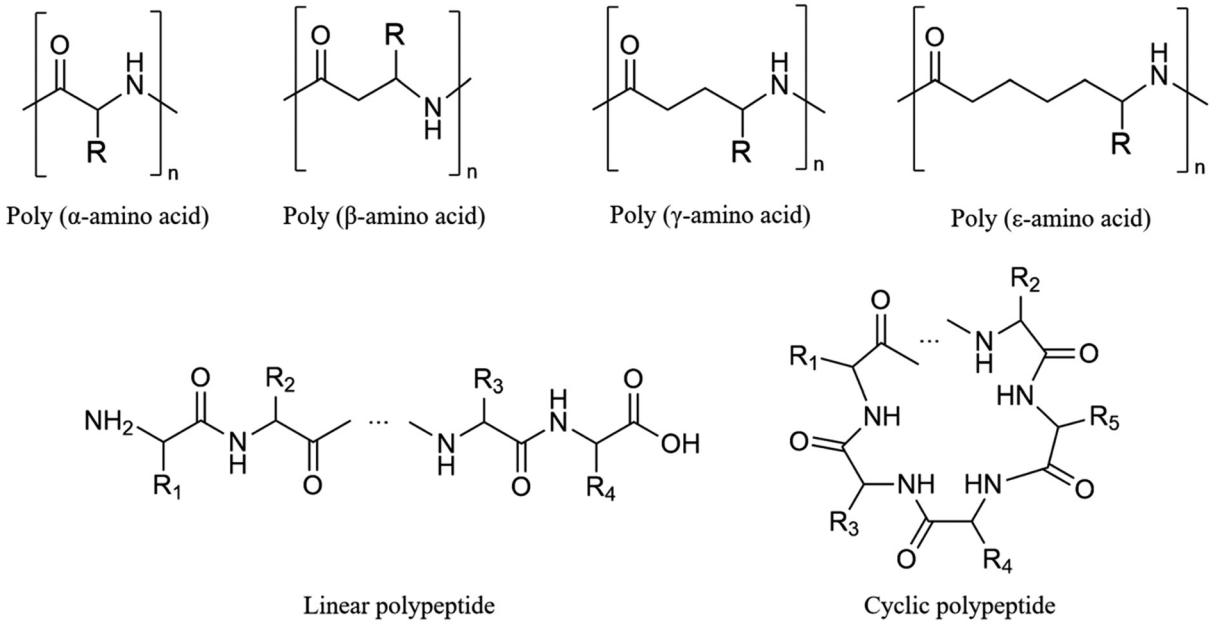

Fig. 3 Common structural formula for poly(amino acids) and polypeptides.

phobicity, acidity, and alkalinity. In addition, the side chains of poly(amino acid) contain numerous reactive functional groups, such as amino and carboxyl groups, which can be further modified with drugs or biologically active molecules to develop various nano-delivery systems.[37–39] Furthermore, the presence of secondary structures, such as α-helices and β-folds, allows poly(amino acids) to exhibit distinct properties compared to conventional macromolecules.[40]

3. Properties of poly(amino acids), peptides and their derivatives

3.1. High drug-carrying capacity

Poly(amino acids) and peptides can bind to drug molecules through covalent or non-covalent interactions and self-assemble to form nanomicelles and NPs. These structures are utilized to encapsulate and transport drugs, enhancing their drug solubility and stability.

NP systems, as modern drug delivery vehicles, significantly enhance drug solubility, improve drug stability, and promote the distribution of drugs within the body, thereby increasing clinical efficacy.[41] Amphiphilic polymers can adopt various morphologies, such as micelles or vesicles, through self-assembly in specific environments. These polymers can be physically encapsulated and chemically cross-linked to achieve controlled release of model drugs. In addition, drug-filled micelles are both thermodynamically and kinetically stable, exhibiting excellent properties such as durability, prolonged effects, and safety.[42] For example, glutamic acid-linked paclitaxel dimers (Glu–PTX2) can self-assemble into NPs (Glu–PTX2 NPs) in aqueous solutions, forming stable drug–polymer complexes that display a spherical morphology and good structural stability in aqueous media. These complexes exert potent cytotoxic effects in aqueous environments, thereby enhancing their bioavailability.[43]

3.2. Targeted delivery

Improving drug delivery to specific organs, cells, or even organelles to enhance therapeutic efficacy while minimizing dosage and side effects has long been a critical focus in the development of advanced drug delivery systems.[44] Targeted drug delivery leverages polymer chains to direct drug molecules to specific regions of interest. Poly(amino acids) and peptides are particularly effective in achieving this goal, as they can bind to specific molecular targets, increasing drug concentration within target tissues or organs while reducing systemic side effects.[45] Targeting peptides, in particular, exhibit strong specificity for their targets. For instance, peptides derived from cell surface protein, such as intercellular adhesion molecule-1 (ICAM-1), luteinizing hormone-releasing hormone (LHRH), bombesin, and LFA-1, have demonstrated high affinity for cell surface receptors, facilitating the selective delivery of therapeutic agents.[46]

Several widely utilized strategies for the targeted delivery of poly(amino acid)-based therapeutics include the following. (1) Ligand modification: poly(amino acids) can be surface-functionalized with specific ligands, enabling them to recognize and bind selectively to target cells or tissues.[47] This active targeting mechanism reduces non-specific cellular uptake, thereby decreasing off-target toxicity and enhancing therapeutic outcomes.[48] (2) Passive targeting: by exploiting the unique size and surface characteristics of NPs, poly(amino acid) carriers can passively accumulate in tumor tissue through the

enhanced permeability and retention (EPR) effect.[49,50] This effect arises from the abnormal vasculature of tumors, which is characterized by increased permeability and poor lymphatic drainage, allowing NPs to preferentially localize at the tumor site. (3) Environmental responsiveness: poly(amino acid) carriers can be engineered to respond to specific physiological conditions within the tumor microenvironment, triggering localized drug release.[51] For instance, polyglutamic acid NPs are designed to degrade in acidic environments, which are often found in tumors, thereby releasing their drug cargo precisely at the desired site.[52] (4) Cell-penetrating peptides: a strategy to enhance transcellular drug transport involves the use of CPPs (such as TAT peptides), which facilitate the internalization of therapeutic molecules across cellular barriers.[53] This approach significantly improves the delivery of small-molecule drugs into target cells, increasing intracellular drug concentrations and enhancing therapeutic efficacy.

These methods can be employed individually or in combination to enhance drug targeting and efficacy while minimizing side effects. For instance, an innovative mesoporous silica nanoparticle (MEMSN)-encapsulated cellular uptake shielding multifunctional system has demonstrated cancer-targeted triggered, triggered drug delivery to tumor cells.[54] The surface of the mesoporous NPs was modified with disulfide bonds that linked β-cyclodextrin, facilitating GSH-induced intracellular drug release. Subsequently, the NP surface was further modified with the RGD peptide sequence and the matrix metalloproteinase substrate peptide Pro–Leu–Gly–Val–Arg (PLGVR) through a host–guest interaction. The modification of the NP with pAsp resulted in MEMSN that could protect the target ligand. In addition, *in vitro* studies confirmed that MEMSN effectively blocked its uptake by normal cells. Upon reaching the tumor cells, the pAsp protective layer was removed through the hydrolysis of PLGVR in metalloproteinase-rich cancer cells. Consequently, the targeting properties were activated, allowing the cancer cells to readily uptake the drug-coated NPs, which subsequently facilitated GSH-induced intracellular drug release (Fig. 4).

3.3. Low toxicity and biocompatibility

Amino acids are fundamental building blocks of biological proteins, while peptides are composed of natural amino acids that share similarities with proteins found in living organisms. As a result, peptides typically exhibit high biocompatibility, minimizing immune reactions and toxicity. These materials generally do not provoke severe toxic responses in the body, as they can be recognized and processed by the organism.[38] Furthermore, peptides can be degraded into amino acids or

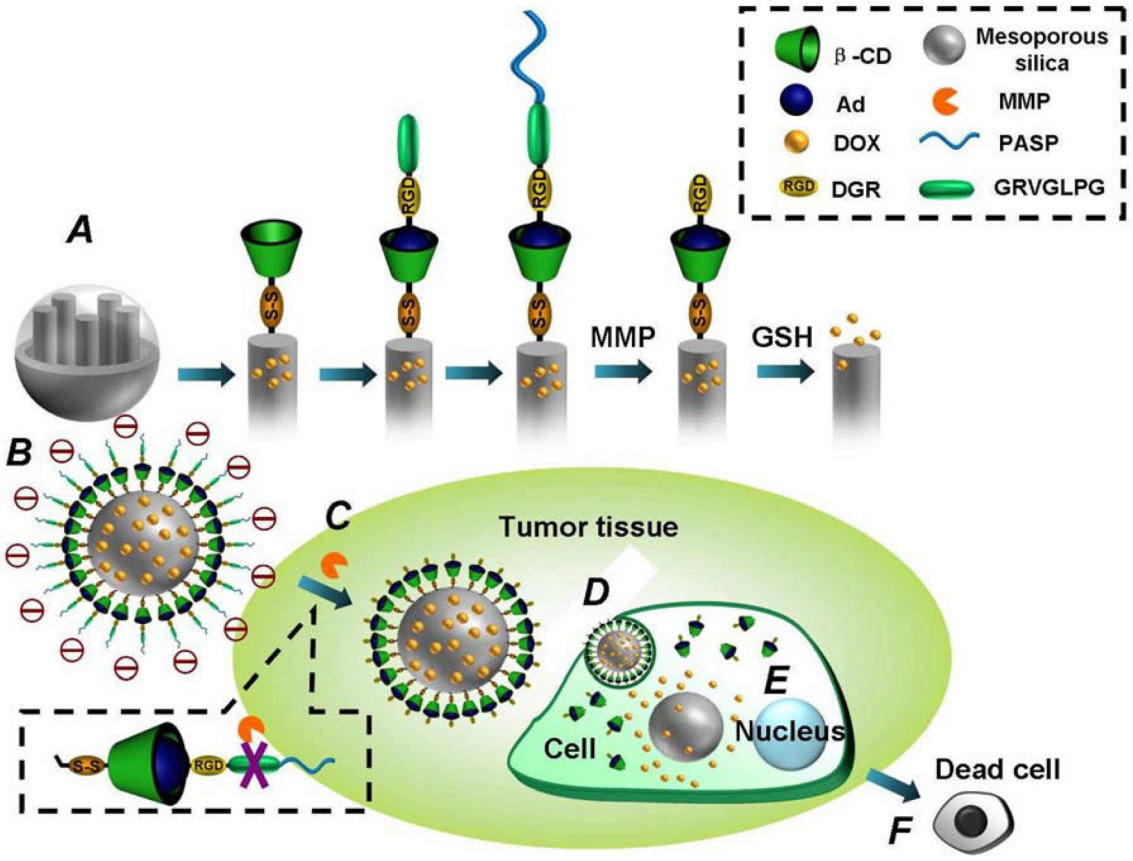

Fig. 4 Structure of multifunctional envelope-type mesoporous silica NP and tumor-triggered targeting drug delivery.[54] Copyright 2013, American Chemical Society.

Table 1 Advantages and disadvantages of other non-degradable biomaterials compared with poly(amino acids) and polypeptides and their derivatives

Polymers	Characteristic		Ref.
	Advantages	Disadvantages	
Polytetrafluoroethylene (PTFE)	Chemical inertness; low friction coefficient; non-adhesive properties; thermal stability; flexible and durable	Poor tissue integration; susceptibility to deformation; limited mechanical strength; biofilm formation risk; difficult to process; expensive	55
Polyethylene (PE)	Durability and mechanical strength; chemical inertness; low friction; cost-effectiveness	Non-biodegradable; poor tissue integration; wear debris; oxidative degradation	56 and 57
Silicone	Durability and stability; flexibility and elasticity; low immune response; non-degradable; wide range of medical applications	Lack of biodegradability; tissue integration; potential for foreign body reaction; limited bioactivity; rigid manufacturing process	58
Polyurethane (PU)	Mechanical properties; biostability; versatility in formulation; hemocompatibility; cost and availability	Non-biodegradability; potential for degradation products; limited biocompatibility; surface modification required	59
Polymethyl methacrylate (PMMA)	Mechanical strength and durability; stability and long-term use; non-immunogenic; ease of shaping and processing	Lack of biodegradability; potential for biofilm formation; exothermic polymerization; brittleness	60

small peptide fragments by enzymes within the body, ultimately being excreted through metabolic pathways. By modifying the amino acid sequence and structure of peptides, it is possible to design peptide materials with varying degradation rates, thereby allowing for controlled timing and rates of drug release. Table 1 presents a comparison of poly(amino acids) and polypeptides with other non-degradable biomaterials.

4. Synthesis of poly(amino acids) and peptides

The synthesis of poly(amino acids) and polypeptides include chemical polymerization methods,[61] enzymatic polymerization,[62] biosynthesis,[63] and self-assembly technology.[64] Each preparation method is suited to specific application scenarios. Chemical polymerization is ideal for producing poly(amino acids) and peptides with diverse structures, while biosynthesis is more appropriate for the creating biopolymers with specific sequences. In contrast, enzymatic polymerization and self-assembly techniques prioritize gentle and environmentally friendly preparation processes. The four methods are described in detail below.

4.1. Chemical polymerization

Chemical polymerization is one of the primary methods for the preparation of poly(amino acids) and currently serves as the predominant technique for both laboratory and industrial synthesis of polypeptides. This process primarily encompasses ring-opening polymerization, polycondensation reactions, and solid-phase synthesis. Among these methods, ring-opening polymerization can be conveniently conducted using α-amino acidic NCA monomers.[65] These macromolecules are typically synthesized through the ring-opening polymerization of various amino anhydrides, initiated by an amine. For example, Hu *et al.*[38] prepared triblock copolymers *via* ring-opening polymerization using mPEG–NH_2 as the initiator, while Zhang *et al.*[66] synthesized diblock copolymers using the macroinitiator PEG–NH_2. Since NCA is a cyclic derivative of amino acids, it readily undergoes ring-opening, facilitating participation in the polymerization reaction, which makes this method widely utilized. In addition, the polycondensation reaction is a condensation process involving the carboxyl group and the amino group of an amino acid, resulting in the formation of a poly (amino acid) and a by-product, such as water or another small molecule.[67] Poly(amino acids) with varying chain lengths can be produced by modifying reaction conditions, including temperature and the choice of catalyst. Furthermore, solid-phase synthesis is a stepwise technique for synthesizing poly (amino acids). This method involves immobilizing the carboxyl groups of amino acid monomers onto a solid-phase carrier, followed by the gradual addition of amino acid monomers, which are chemically linked to create a long chain. Once the reaction is complete, the poly(amino acids) are detached from the solid-phase carrier through cleavage.[68] This approach allows for precise control over the sequence and molecular weight of the resulting poly(amino acids), making it suitable for synthesizing poly(amino acids) with complex structures. However, it is important to note that this method can be costly and is typically employed for small-scale or laboratory preparations.

Chemical polymerization offers a degree of flexibility in the regulation of molecular weight.[69] For instance, in the case of NCA ring-opening polymerization, the molecular weight of poly(amino acids) or polypeptides can be effectively modulated by altering the ratio of initiator to monomer.[61] When the quantity of initiator is less than that of the monomer, each initiator molecule has increased opportunities for chain elongation, leading to the formation of polymers with higher molecular weights. Furthermore, the polymerization rate can be influenced by adjusting parameters such as reaction temperature and catalyst concentration, which in turn affects the molecular weight. Generally, elevated temperatures and higher catalyst concentrations accelerate the reaction rate, thereby

facilitating an increase in the polymer's molecular weight within an optimal range.[25] In addition, chemical polymerization allows for the introduction of specific stereochemical configurations through the selection of appropriate monomers and reaction conditions.[61] For example, during the synthesis of poly(amino acids), ring-opening polymerization can be conducted using NCA monomers derived from amino acids with defined chirality. The utilization of L-type amino acid NCA monomers enables the synthesis of optically active poly(amino acids), with the chiral structure preserved by carefully controlling the polymerization conditions to produce materials with targeted biological activities. Moreover, chemical polymerization can yield poly(amino acids) and polypeptides with varying stereochemical structures, resulting in a diverse array of stereochemical combinations, including isotactic, syndiotactic, and atactic configurations.

4.2. Enzymatic polymerization method

Enzymatic polymerization of polymers (amino acids) and peptides is carried out under mild conditions, such as normal temperature, atmospheric pressure and near neutral pH, using enzymes as catalysts.[70] In comparison to conventional chemical polymerization techniques, the utilization of mild reaction conditions necessitates less sophisticated equipment and does not require specialized reaction apparatus capable of withstanding elevated temperatures and pressures. For instance, the synthesis of poly(amino acids) and polypeptides through certain chemical polymerization methods often demands high temperatures (ranging from 100 to 200 °C) and the use of specific organic solvents. In contrast, enzymatic reactions circumvent these extreme conditions, thereby leading to reductions in production costs and energy consumption. The application of mild reaction conditions is advantageous for preserving the structural integrity of both reactants and products. Under harsh conditions, such as elevated temperatures or the presence of strong acids and bases, the structural integrity of amino acids and peptides may be compromised, resulting in denaturation or decomposition that adversely affects the quality and performance of the resulting polymer.[71] Enzymatic reactions facilitate precise catalysis of the polymerization process, ensuring that the synthesized poly(amino acids) and polypeptides exhibit the desired structural and functional characteristics.

In comparison to conventional chemical polymerization techniques, enzymatic polymerization exhibits notable advantages, particularly in terms of specificity. This method demonstrates a high degree of selectivity towards substrates during the synthesis of poly(amino acids) and polypeptides.[72] Certain enzymes are capable of selectively polymerizing specific amino acids. For example, γ-glutamyltranspeptidase is known to catalyze the polymerization of L-glutamine and its derivatives due to the strong complementarity of its active site with the structure of glutamine.[73] This allows the enzyme to accurately identify and bind glutamine molecules, thereby initiating the polymerization process. Similarly, papain preferentially recognizes peptides that contain particular amino acid sequences, as its active site can specifically bind to peptide bonds involving arginine or lysine residues. When these appropriate substrate peptides are present, papain facilitates the formation of peptide bonds, effectively linking smaller peptides into polypeptides.[74] Furthermore, enzymatic polymerization exhibits regional selectivity in the growth patterns of poly(amino acid) and polypeptide chains. Enzymes can precisely dictate whether polymerization occurs at the α-amino, α-carboxyl, or side chain functional groups of amino acids or peptides. In instances where amino acids or peptides possess multiple reactive sites, the enzyme can direct the polymerization to occur preferentially at specific sites.[75] For example, in the presence of lysine residues that contain multiple amino groups, the enzyme can select one amino group for polymerization based on its three-dimensional structure and the spatial orientation of its active site, resulting in a more structured poly(amino acid) or polypeptide product. Moreover, enzymatic polymerization demonstrates a pronounced ability to discriminate between stereoisomers of amino acids during the synthesis of poly(amino acids) and polypeptides.[25] Most enzymes exhibit activity solely towards L-type amino acids, showing little to no reactivity with D-type amino acids or exhibiting significantly reduced efficiency.[76] This selectivity arises from the specific three-dimensional spatial structure of the enzyme's active site, which closely aligns with the spatial conformation of L-amino acids. For instance, during peptide bond formation, the α-amino and α-carboxyl groups of the amino acids involved in the reaction must be precisely aligned with specific groups at the enzyme's active site. The atomic arrangement surrounding the chiral center of L-amino acids is ideally suited to meet the binding requirements of the enzyme, facilitating an efficient polymerization reaction. Conversely, D-type amino acids struggle to participate in polymerization due to the incompatibility of their spatial structure with the enzyme's active site. This stereoselectivity ultimately ensures that the resulting poly (amino acids) and polypeptides possess high optical purity and specific biological activity.

Currently, the utilization of certain enzymes for the synthesis of poly(amino acids) and polypeptides is hindered by their high cost, which contributes to elevated production expenses.[77] Furthermore, these enzymes exhibit limited stability and are prone to inactivation during both storage and application. For instance, certain proteases may lose their catalytic activity when subjected to elevated temperatures, extreme pH conditions, or prolonged exposure to inhibitors. To enhance enzyme stability, it may be necessary to implement specialized preservation techniques, such as low-temperature freezing or the incorporation of stabilizing agents. Consequently, the application of enzymatic polymerization in large-scale industrial production remains constrained.[78] In comparison to chemical polymerization methods, enzymatic polymerization often exhibits slower reaction rates, and maintaining uniform enzyme distribution and continuous functionality throughout large-scale reactions poses significant challenges.[79] In addition, the molecular weight of products generated through enzymatic polymerization may be restricted by

the specific enzyme employed and the prevailing reaction conditions, making it difficult to synthesize high molecular weight poly(amino acids) and polypeptides, as can be achieved with certain chemical polymerization techniques.

4.3. Biosynthetic methods

Biosynthesis refers to the process of synthesizing poly(amino acids) and polypeptides through the metabolic mechanisms inherent to an organism.[80] This technique predominantly depends on the transfer of genetic information and the cellular systems responsible for protein synthesis, which encompass transcription and translation processes. Within cells, the genes encoded in DNA are initially transcribed to produce mRNA. Subsequently, the mRNA facilitates the assembly of amino acids in accordance with the codon sequence it contains, utilizing tRNA as a mediator, with ribosomes catalyzing the formation of polypeptide chains.[81] This methodology is frequently employed for the synthesis of specific amino acid polymers and the production of naturally occurring peptides.

In this case, genetic engineering is applied to synthesize poly(amino acids) by introducing genes that encode the target poly(amino acids) into microorganisms, allowing for their expression within microbial cells.[62,82] The gene responsible for the target poly(amino acid) is integrated into the microorganism, facilitating the synthesis of the poly(amino acid). This process enables the production of specific sequences of poly (amino acids) that are both mass-producible and biocompatible. The method is frequently employed to synthesize biomedical materials, such as polyglutamic acid (γ-PGA).[83] Furthermore, in prokaryotic expression systems, such as *Escherichia coli*, the degradation of short peptides by intracellular proteases poses a challenge to production efficiency. This issue can be addressed by employing protease-deficient strains or by expressing peptides as insoluble inclusions.[84]

The synthesis of poly(amino acids) and polypeptides utilizing transgenic organisms involves several critical steps. Initially, the design and construction of the gene must be undertaken, with the corresponding gene sequence tailored to match that of the target polypeptide. This process necessitates careful consideration of codon usage, as different organisms exhibit varying frequencies of codon preference.[85] For instance, when designing genes intended for *Escherichia coli*, it is advisable to utilize codons that are favored by this organism to enhance the efficiency of gene expression. Subsequently, these gene fragments can be synthesized chemically or sourced from existing gene libraries, and then assembled into complete genes through genetic engineering methodologies. The constructed gene must then be introduced into a suitable host cell using an appropriate method. In the case of *E. coli*, a common approach is heat shock transformation, wherein the recombinant plasmid containing the target gene is mixed with *E. coli* cells at a low temperature, followed by a brief heat shock treatment to facilitate the uptake of the plasmid by the cells.[86] For yeast, electrical transformation techniques, such as the application of high-voltage pulses, can be employed to temporarily render the cell membrane permeable, thereby allowing the entry of foreign genes.[87] Finally, the expression and purification of the resultant product must be conducted. Within the host cell, the imported gene requires expression under optimal conditions. For certain genes regulated by inducible promoters, the addition of specific inducers is necessary. In *E. coli*, IPTG (isopropyl-beta-D-thiogalactoside) is frequently utilized to induce the expression of genes that are associated with lactose operon promoters.[88] The expressed peptides can subsequently be purified using various techniques, including affinity chromatography and ion exchange chromatography.

4.4. Self-assembly technology

Under specific conditions, certain amino acids or peptides can form polymers through self-assembly.[89] For instance, molecular self-assembly can lead to the formation of various types of NPs, including NPs, hydrogels, nanofibers, and nano micelles, depending on the chemical composition, molecular weight, and hydrophobicity ratio of the peptide (Fig. 5). This organization is driven by intramolecular and/or intermolecular interactions, such as hydrogen bonding, electrostatic interactions, and hydrophobic interactions, which may occur between the side functional groups of the amino acids in the peptide.[40] The β-sheets and α-helices formed by the polypeptides also significantly influence the morphology and size of the peptide-based NPs.[90] They are widely used in drug delivery, tissue engineering, combination therapy and other fields. Similar to NPs, nanotechnology in drug delivery can address the shortcomings of conventional methods by enabling cell-specific targeting, facilitating the transport of molecules to designated organelles, and enhancing intracellular transport. This method does not require complex chemical reactions and offer improved environmental adaptability and targeted drug delivery potential.

Researchers have designed specific peptide sequences to facilitate the self-assembly of peptides and enhance drug loading efficiency. Initially, Zhang *et al.* developed and synthesized an ion-complementary peptide, EAKl6, composed of 16 amino acid residues that self-assemble in aqueous solutions to form stable hydrogel membranes visible to the naked eye.[91] Furthermore, Ghadiri created a cyclic polypeptide consisting of eight amino acid residues. This peptide was self-assembled from a β-folded structure and was further highly integrated to form hollow peptide nanotubes.[92] Meanwhile, with an increasing understanding of sequence–structure relationships, Woolfson *et al.*[93] endeavored to design and explore more innovative assemblies to generate new protein functions based on α-helical helices, which are common domains in protein–protein interactions. This work opened up a potential new frontier in coiled-coil assemblies and α-helical barrels. Subsequently, Hu *et al.*[38] prepared a series of poly(amino acid) materials through ring-opening polymerization using mPEG113–NH_2 as the initiator. Cholesterol was grafted onto the side chain *via* an esterification reaction to render the macromolecules amphiphilic, allowing them to self-assemble in specific solvents to form NPs with distinct

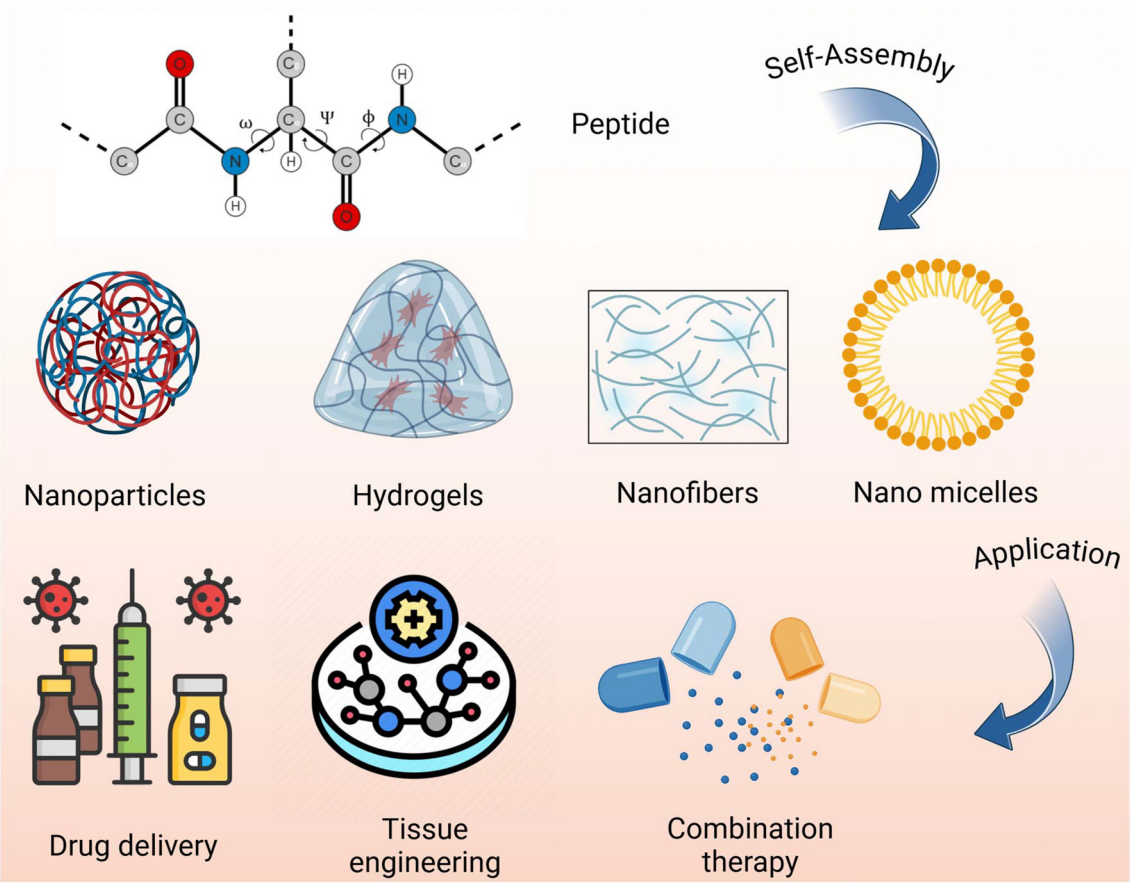

Fig. 5 Various types of NPs obtained from polypeptides and their derivatives and applications.

morphologies. At different pH values, these NPs exhibited different shapes, facilitating the study of their drug release properties.

So far, researchers have been combining peptide self-assembly with other functional materials to develop multifunctional systems for smart responses, precision drug delivery, and regenerative medicine. Some self-assembled peptide materials have entered preclinical studies and early clinical trials, demonstrating their promising applications. In addition, Table 2 compares the advantages and limitations of other non-degradable biomaterials with poly(amino acids) and polypeptides and their derivatives.

5. Optimization of poly(amino acids), polypeptides and their derivatives

Poly(amino acids) and peptides can be modified through derivatization *via* chemical alterations, binding to small molecules, or conjugation with antibodies, among other methods, to significantly enhance their functionality in drug delivery. These modifications and derivatives improve drug targeting, stability, controlled release, and biocompatibility, thereby increasing the efficiency and safety of drug delivery.

5.1. Optimize the sequence and composition of poly(amino acids) or polypeptides

Different amino acids exhibit distinct side chain structures and chemical properties, allowing for the optimization of specific characteristics through the careful selection of sequences and compositions in poly(amino acids) or polypeptides. Notably, modifying the amino acid composition to increase the proportion of rigid amino acids, such as phenylalanine and tyrosine, both of which possess large and relatively rigid side chains, can enhance the interactions between poly(amino acid) or polypeptide chains, thereby improving mechanical stability.[97] Furthermore, the incorporation of proline, which contains a pyrrolidine ring structure, can also contribute rigidity to the molecular chain; an appropriate increase in its content can further enhance the mechanical properties of the material.[98] In addition, the design of poly(amino acids) or polypeptides with repeating units or specific sequences that promote the formation of secondary structures, such as α-helices or β-sheets, can facilitate tighter molecular arrangements and strengthen intermolecular interactions, including hydrogen bonding. This, in turn, enhances the mechanical strength and stability of the material.[99] Moreover, the introduction of cross-linking groups or amino acids, such as cysteine with sulfhydryl groups, into the poly(amino acid) or

Table 2 Advantages and disadvantages of other non-degradable biomaterials compared with poly(amino acids) and polypeptides and their derivatives

	Characteristic		
Synthesis method	Advantages	Limitation	Ref.
Chemical polymerization	Simple operation; low cost; high molecular weight; synthetic diversity; controlled polymerization; one-step synthesis of functional clustering peptides	Difficult monomer purification; slow polymerization speed; high environmental sensitivity; difficult to control sequence selectivity; complex initiator design and preparation; initiator residue problems; molecular weight control and distribution problems; low functional group efficiency	61
Enzymatic polymerization method	Mild reaction conditions; high selectivity; controlled polymerization; biocompatibility; functionable modification; environmental friendliness; stimulus responsiveness	Molecular weight control; polymerization activity and controllability; low functional group efficiency; high substrate concentration requirement; reversible reaction; complex functional group introduction and modification steps	94
Biosynthetic methods	Easy to operate; narrow molecular weight distribution; environmentally friendly	Slow reaction speed; difficult to prepare large molecular weight polypeptides; cumbersome steps and high cost; difficult to achieve large quantities of preparation	95
Self-assembly technology	Simple structural design; low production cost; good biocompatibility; improved cell uptake; drug targeted release; versatility; high stability and low toxicity	Low stability; easy to be affected by pH; poor water solubility; low drug load; high production cost; poor mechanical and rheological properties; self-assembly process difficult to accurately control; sensitive to environmental factors	96

polypeptide chain can lead to the formation of covalent cross-linking networks through chemical or photocross-linking methods.[100] Such cross-linked structures can restrict the mobility of molecular chains and increase the material's resistance to deformation, significantly improving its mechanical stability.

To regulate the rate of drug release, it is possible to systematically adjust the ratio of hydrophilic to hydrophobic amino acids. An increase in the proportion of hydrophobic amino acids facilitates the formation of a denser structure in poly (amino acids) or polypeptides, thereby reducing the diffusion rate of the drug and extending the duration of drug release.[101] Conversely, a higher concentration of hydrophilic amino acids enhances the penetration of water molecules and the solubility of the drug, which accelerates its release. Additionally, the incorporation of chemical bonds or amino acid sequences that respond to specific stimuli, such as acid-sensitive hydrazone bonds or enzyme-sensitive peptide bonds, into the main or side chains of poly(amino acids) or polypeptides can also effectively modulate the drug release rate.[102] Upon reaching the target site, these sensitive bonds or sequences can be cleaved under appropriate stimulus conditions, leading to the structural disintegration of the poly(amino acid) or polypeptide and facilitating rapid drug release. For instance, in the acidic environment of tumors, poly(amino acid) drug carriers containing acid-sensitive hydrazone bonds can undergo rapid degradation and drug release due to the low pH.[103] Generally, carriers composed of poly(amino acids) or polypeptides with higher molecular weights and longer chain lengths exhibit a more compact structure, resulting in a longer diffusion pathway for the drug and a comparatively slower release rate.[104] Therefore, the molecular weight and chain length of poly(amino acids) or polypeptides can be manipulated by adjusting polymerization conditions to regulate the drug release rate.

To enhance the solubility of the material, one can increase the content of hydrophilic amino acids, decrease the amount of hydrophobic amino acids, introduce water-soluble groups, or modify the terminal ends of poly(amino acids) or polypeptides. For example, chemical modifications such as amination at the N-terminus or acetylation at the C-terminus can alter the charge distribution and polarity of the molecule, thereby improving its solubility in water and other solvents.[105]

5.2. Chemical modifications

Chemical modification involves altering the structure of a poly (amino acid) or peptide molecule to enhance its suitability for specific drug delivery applications.

5.2.1. Polyethylene glycolylation (PEGylation). Polyethylene glycolylation refers to the formation of PEGylated derivatives through the covalent attachment of polyethylene glycol (PEG) to poly(amino acids) or peptides. PEGylation diminishes immunogenicity by enhancing the molecular weight and altering the spatial configuration of proteins or peptides. The PEG molecules remain intact until they are eliminated from the body, and their protective barrier effectively shields protein molecules from proteolytic degradation *in vivo*. Additionally, PEGylation significantly decreases glomerular filtration during systemic administration, leading to reduced renal clearance and consequently lower urinary excretion.[106,107] Furthermore, it circumvents the clearance mechanisms of the reticuloendothelial system (RES), thereby substantially extending the plasma half-life and enhancing the release of therapeutic agents within the organism.[108] PEGylated peptides and poly (amino acids) are frequently utilized for anticancer drug delivery, as they prolong the retention of drugs in the bloodstream.

For example, Goserelin, a peptide drug empolyed in the treatment of prostate and breast cancer, can be PEGylated to prolong its duration of action and decrease the frequency of dosing.[109] Similarly, Leuprolide, a peptide hormone commonly used to treat sex hormone-dependent diseases, can be PEGylated to enhance its pharmacokinetic properties and extend its half-life.[110] Overall, PEGylation significantly improves the pharmacokinetic characteristics of these therapeutic agents. PEGylation has been shown to enhance the solubility of poly(amino acids) and polypeptides. The hydrophilic nature of polyethylene glycol contributes to an increased solubility of the resultant compounds in aqueous solutions when conjugated with poly(amino acids) or polypeptides.[111] This modification is particularly beneficial for highly hydrophobic poly(amino acids) or polypeptides, as PEGylation facilitates their dissolution under physiological conditions, thereby expanding their potential applications.

However, PEGylation also hinders the cellular uptake of the carriers, thereby limiting their therapeutic efficacy. Consequently, numerous peptide carriers with removable PEG shells have been developed in recent years.[112] Jiang *et al.*[113] prepared two cisplatin-loaded polyglutamic acid–lysine composite nanoformulations, featuring detachable polyethylene glycol grafted onto lysine fragments through two distinct bridging chemical bonds. These formulations are responsive to specific tumor tissue microenvironments, including low pH and matrix metalloproteinases. The nanoformulations containing PEG fragments circulated in the bloodstream for a prolonged period of time with increased drug accumulation in the tumor tissue compared to those without PEG fragments. Upon reaching the tumor tissue, the nanoformulations exhibited enhanced cellular uptake and cytotoxicity due to the cleavage of the bridging chemical bond between polyethylene glycol and polylysine (Fig. 6).

5.2.2. Lipid modifications. Lipid modification is the covalent bonding of lipid molecules to poly(amino acids) or polypeptides. Lipid molecules typically contain hydrophobic hydrocarbon chains and hydrophilic head groups, a structural property that allows them to change the properties of the polymer when combined with a poly(amino acid)/polypeptide.

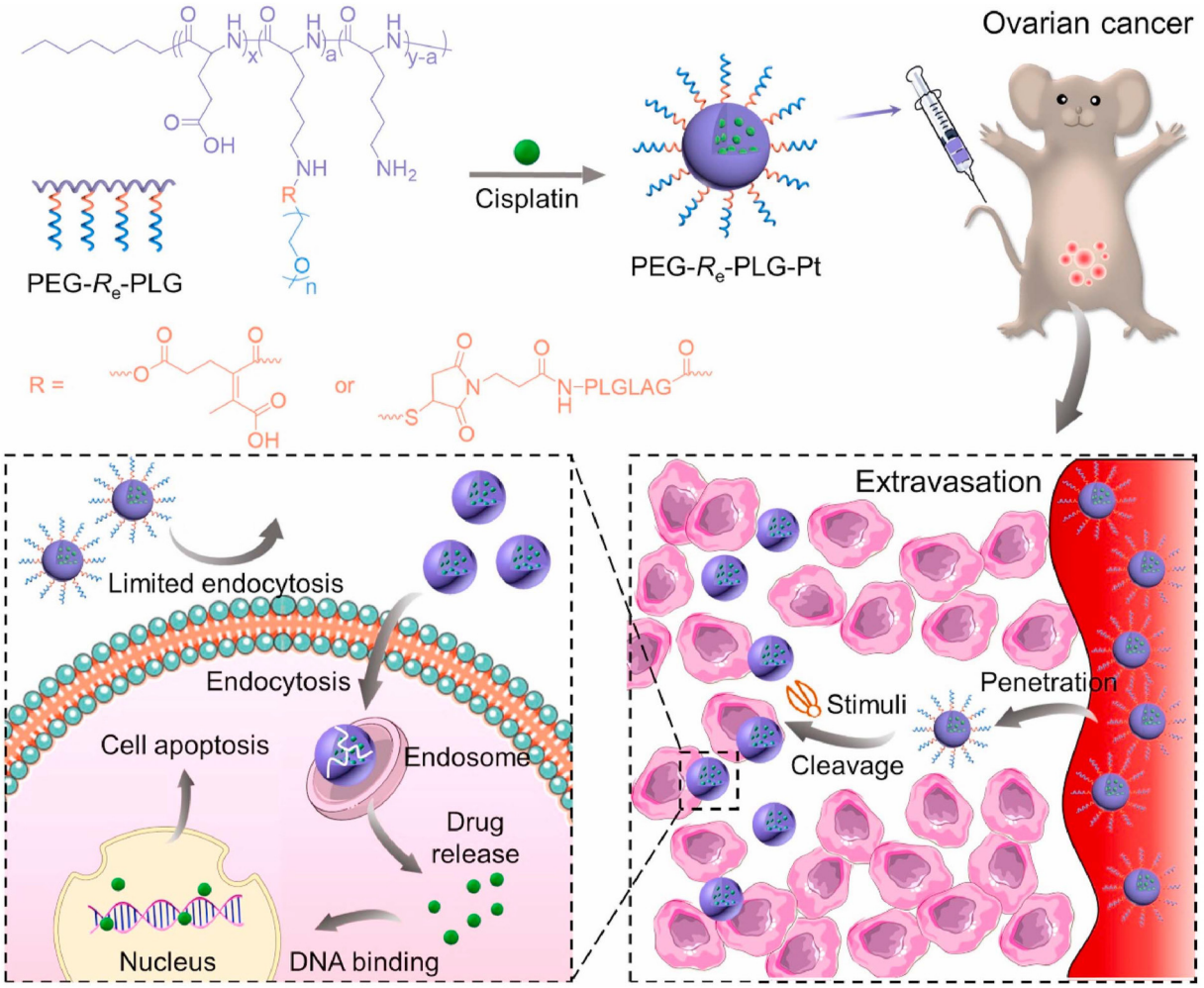

Fig. 6 Schematic representation of PLG–CDDP nano-formulations with separable polyethylene glycol response to the tumor microenvironment for enhanced treatment of peritoneal metastases from ovarian cancer.[113] Copyright 2021, Elsevier.

Through lipid modification, poly(amino acids) and polypeptides can be given some unique physical and chemical properties, such as the use of multi-functional biological materials to prepare liposomes, so that the liposomes respond to the target stimulation region and release the contents (Fig. 7).[114] Furthermore, the incorporation of lipid modifications has the potential to improve the stability of poly(amino acids) and polypeptides. In physiological conditions or during storage, these biomolecules may experience a decline in their functional activity or structural integrity as a result of enzymatic degradation or various physicochemical influences.[115] The attachment of lipid moieties can provide a degree of protection to poly(amino acids) and polypeptides against such detrimental factors.[116] For instance, attaching fatty acid chains to insulin can prolong its circulation time *in vivo*, thereby reducing the frequency of injections. Glycine insulin can form a long-release formulation of the drug through lipidation modification.[117] Furthermore, with respect to solubility, unmodified poly(amino acids) and polypeptides exhibit reduced solubility in lipid environments. However, following lipid modification, these compounds demonstrate enhanced interaction with lipids due to the incorporation of lipid moieties, which subsequently increases their solubility in lipid media.[4] For example, the modification of antimicrobial peptides with lipids such as palmitic acid can increase their lipophilicity and facilitate their penetration into cell membranes.[118] These peptides have enhanced lipophilicity and improved membrane penetration, which makes them promising for a wide range of applications in the biomedical field. Furthermore, peptide–drug conjugates can form NPs or micellar structures following lipid modification, which can enhance drug targeting and efficacy.

5.2.3. pH or temperature-responsive modifications. The modification of poly(amino acids), peptides, and their derivatives in response to pH or temperature is a significant technological advancement for the targeted and on-demand release of pharmaceuticals. By incorporating functional groups that are sensitive to specific pH levels or temperature changes into the structure of poly(amino acids) or polypeptides, it is possible to initiate drug release in particular environments, thereby leveraging the unique acidity or temperature characteristics of the tumor microenvironment. This approach enhances therapeutic targeting while minimizing adverse effects on healthy tissues.[120] For example, carboxyl or amino groups can be introduced through chemical synthesis. These functional groups undergo protonation or deprotonation reactions in varying pH conditions.[120] A decrease in pH typically results in the protonation of carboxyl groups, altering the charge state and hydrophobicity of the molecular chain. Conversely, an increase in pH may lead to the deprotonation of amino groups. Such alterations in charge and hydrophobicity can induce changes in the conformation and solubility of poly(amino acids) or polypeptides. Furthermore, fluctuations in pH can also result in the stretching or curling of the molecular chains. For example, polypeptides that contain a higher proportion of acidic amino acids, such as glutamic acid and aspartate, exhibit protonation of their carboxyl groups in acidic environments, which diminishes electrostatic repulsion between molecular chains and may lead to chain curling. In alkaline conditions, the deprotonation of carboxyl groups increases electrostatic repulsion, resulting in chain extension. In terms of temperature-responsive modifications, poly(*n*-isopropylacrylamide) (PNIPAM) serves as a prototypical temperature-sensitive polymer characterized by a low critical solution temperature (LCST) of approximately 32 °C. PNIPAM can be chemically conjugated with poly(amino acids) or polypeptides. Below the LCST, the PNIPAM segments exhibit hydrophilicity,

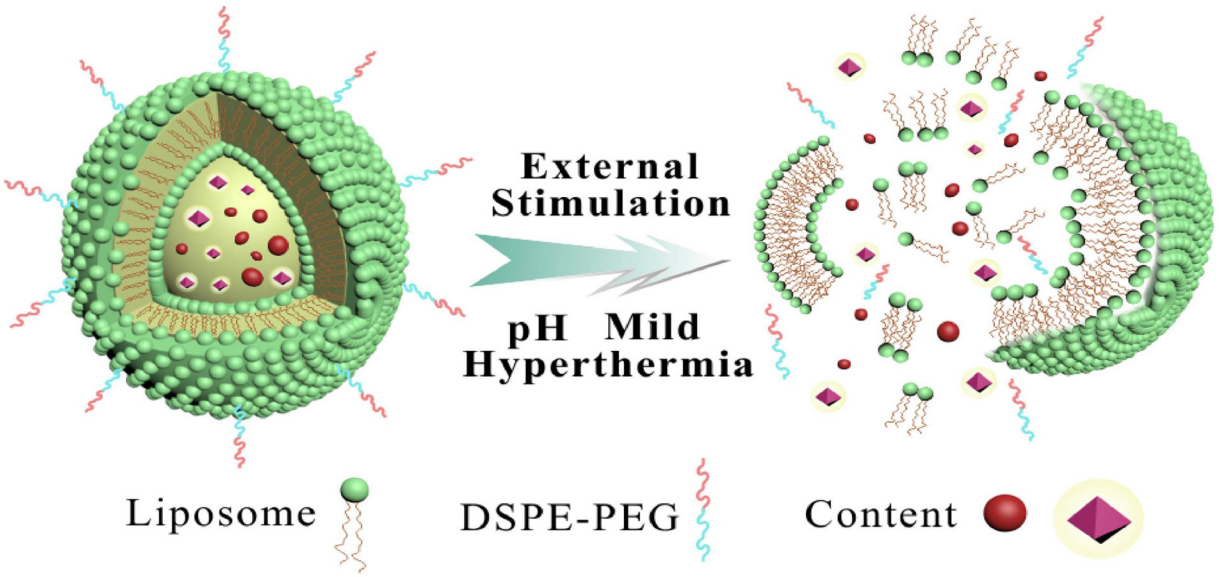

Fig. 7 Liposomes were prepared by multifunctional biomaterials, responding to external trigger around the stimulated zone, rapidly releasing its contents.[119] Copyright 2019, Elsevier.

leading to an extended molecular chain. Above the LCST, however, the PNIPAM segments become hydrophobic, causing the molecular chain to contract.[121] This temperature-induced transition also triggers phase behavior changes, affecting the overall properties of poly(amino acids) or polypeptides. In addition to alterations in hydrophilicity, such changes can influence solubility and aggregation states. For instance, in solutions containing temperature-responsive polypeptides, an increase in temperature beyond the LCST can result in the precipitation of polypeptides from the solution, forming aggregates. This aggregation behavior can be strategically utilized to regulate drug release or facilitate material self-assembly.[122]

pH or temperature-responsive modifications are often applied to smart drug delivery systems to build pH or temperature-responsive drug delivery vectors. The drug is wrapped in a responsive poly(amino acid) or polypeptide carrier to control the release of the drug by regulating the external pH or temperature. This smart drug delivery system can improve the targeting and therapeutic effect of drugs, and can also be used to make biosensors and prepare injectable tissue engineering scaffold materials.

5.2.4. Conjugated with bioactive molecules. The conjugation of poly(amino acids) and polypeptides with bioactive molecules represents a significant area of research within the domains of biomaterials and biomedicine, offering extensive application potential and substantial scientific relevance. Techniques such as amidation, esterification, and click chemistry facilitate the conjugation of poly(amino acids) or polypeptides with bioactive entities.[123] This includes the attachment of antibodies that specifically recognize antigens, peptide ligands that selectively bind to receptors on cell surfaces, and bioactive molecules, such as saccharides, which are crucial for cell recognition and intercellular communication. Such conjugation enables the targeted delivery of therapeutic agents to cells expressing the corresponding receptors. For instance, in the context of cancer therapy, many tumor cells exhibit high expression levels of specific antigens, such as the HER-2 antigen found on breast cancer cells. By conjugating anti-HER-2 antibodies with polypeptide drug carriers, it is possible to achieve targeted binding to HER-2 positive breast cancer cells, thereby facilitating precise drug delivery.[123] Furthermore, the conjugation of poly(amino acids) or polypeptides does not significantly increase the overall molecular size or complexity. This modification can mitigate the aggregation and precipitation of these polymers within the body, obstruct the access of enzymes to the poly(amino acids) or polypeptides, and, to some extent, protect bioactive molecules from enzymatic degradation and immune clearance.[124] For example, certain cyclic peptide ligands conjugated with polypeptides can enhance the stability and prolong the effective action of polypeptides *in vivo*, attributable to the inherent stability of their cyclic structure.[125]

5.2.5. Targeted ligand binding. Targeted ligand binding refers to the interaction of small molecule ligands with poly (amino acids) or through either covalent or non-covalent means.[126] This binding enhances the ability of poly(amino acid) or peptide carriers to specifically target certain cells or tissues, thereby facilitating the targeted delivery of drugs and minimizing systemic toxicity. Among these, peptide–drug couplers (PDCs) have emerged as a novel class of targeted therapeutics. Researchers have developed various targeted peptide couplers that integrate the specificity of peptides with the therapeutic advantages of drugs, resulting in complexes that exhibit targeted and highly effective therapeutic effects. Peptide–drug couplers (PDCs) are a type of drug delivery system (DDS) that typically consists of three components (Fig. 8). The first component is the carrier. In addition to aptamers and small molecules, various organisms, including peptides, proteins, and antibodies, have been extensively studied as carriers.[45] The second component is the payload, which targets the disease of interest by inducing specific biological functions, primarily through the use of cytotoxic drugs or radionuclides.[127] The third component is the linker, which connects the first two components to facilitate the controlled release of the drug. Cleavable linkers can be categorized into chemically cleavable and enzymatically cleavable linkers. Chemically cleavable linkers are activated in organelles with acidic environments, such as lysosomes and intranuclear vesicles, while enzymatically cleavable linkers are cleaved by histone proteases and enzymes associated with the tumor microenvironment.[128]

Recently, Li *et al.*[129] designed and synthesized a peptide–drug conjugate by linking the targeted transferrin receptor (TfR)-binding peptide analogue BP9a (CAHLHNRS) to doxorubicin (DOX) *via* *N*-succinimidyl-3-maleimidopropionate (SMP).[129] They concluded that BP9a could serve as a potential TfR-targeting peptide carrier for selective drug delivery. Subsequently, Yu *et al.*[130] investigated two new peptide–drug conjugates (PDCs), LT7-SS-DOX and DT7-SS-DOX, which were created by coupling peptide ligands composed of natural L-type amino acids. These ligands are known to suffer from issues related to enzymatic degradation and insufficient biostability. The new PDCs, LT7 (haiyprh) and its inverse analogue, DT7 (hrpyiah), were synthesized through disulfide bonding with DOX. They demonstrated enhanced serum stability, prolonged reduction-triggered drug release, and increased *in vitro* antiproliferative activity. Targeted peptide couplers exhibit significant potential for drug delivery and precision medicine due to their highly specific targeting capabilities and multifunctional design.

Furthermore, a pH and reducing dual-reactive peptide–dexamethasone (anti-inflammatory drug) conjugate (L-SS-DEX) was developed to improve the immunosuppressive tumor microenvironment for effective colorectal cancer therapy (Fig. 9).[131] This peptide–dexamethasone conjugation ensures the stability of the dexamethasone moiety and facilitates the rapid release of dexamethasone within the tumor microenvironment. Compared to free DEX, L-SSDEX was more effective in reducing pro-tumor inflammation by inhibiting cyclooxygenase-2 (COX-2) and resulted in enhanced tumor suppression through the infiltration of $CD8^+$ cytotoxic T cells. To achieve immune activation, a tumor-specific enhanced oxidative stress

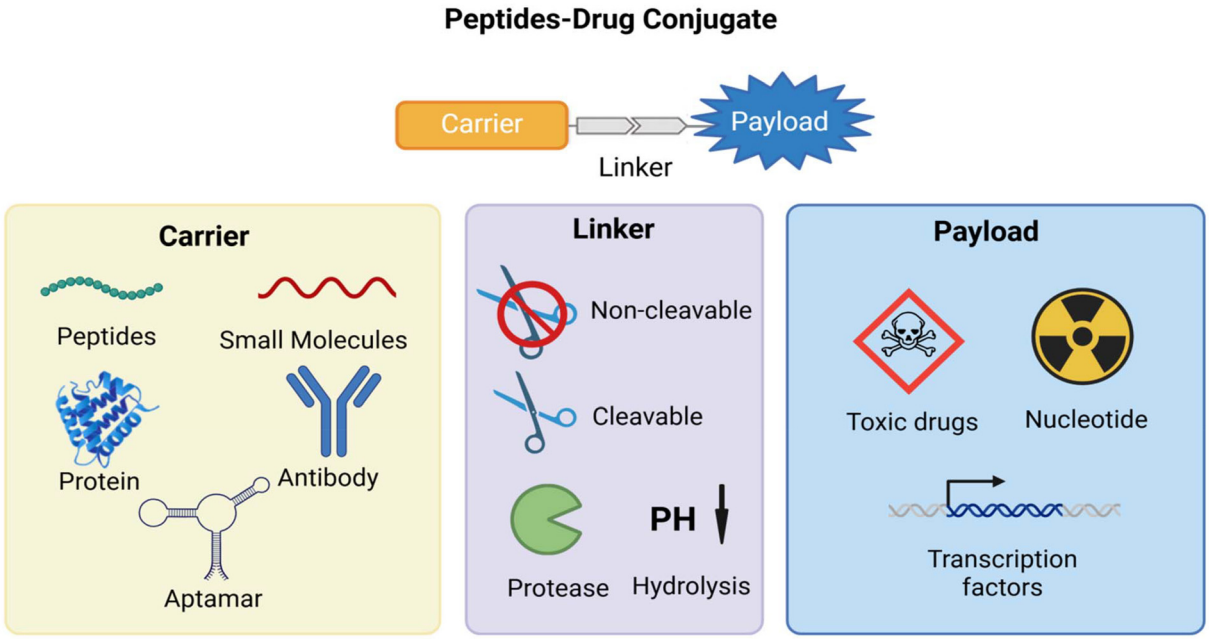

Fig. 8 Schematic structure of peptide–drug coupling.

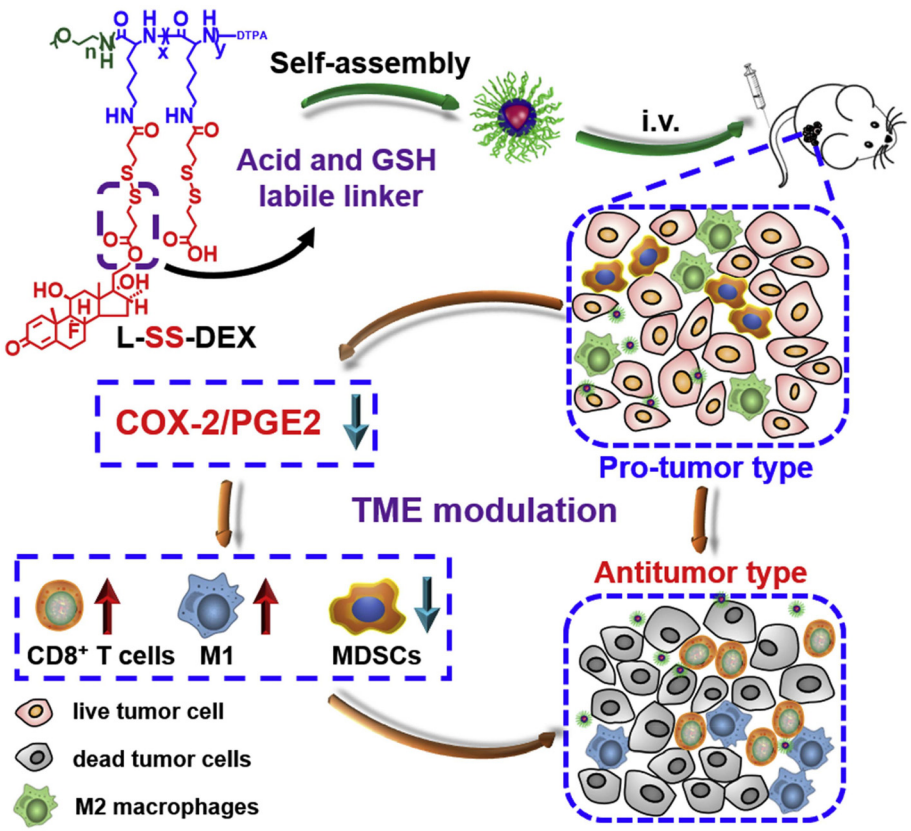

Fig. 9 Schematic illustration of L-SS-DEX for tumor microenvironment modulation. Reproduced with Reproduced with permission.[131] Copyright 2020, Elsevier.

polypeptide coupler (TSEOP) was synthesized by covalently linking an enhanced oxidative stress module to a long-circulating amphiphilic peptide, which induced immunogenic cell death (ICD) in tumor cells (Fig. 10).[132] In response to specific intra-tumor specific stimulation, TSEOP was degraded to generate cinnamaldehyde (CA) and the glutathione (GSH) eliminator quinone (QM). CA and QM synergistically increased oxidative stress and endoplasmic reticulum stress, leading to tumor cell death and enhanced anti-tumor immunity.

In addition, the use of poly(amino acids) and peptides in the modification of bispecific antibodies represents a cutting-edge technology aimed at enhancing therapeutic efficacy by improving the specificity and functionality of antibodies, particularly in the fields of cancer treatment and immunotherapy. Bispecific antibodies can simultaneously bind to two different antigens, allowing for more effective targeting of cancer cells or pathogens while minimizing damage to healthy tissues.[133] Modifying bispecific antibodies can result in several advantages, including increased stability of the antibody molecule, reduced immunogenicity, enhanced targeting capabilities, and prolonged half-life. This technology holds the potential to further improve the specificity and stability of antibody-based therapies, which is anticipated to lead to significant advancements in personalized medicine and targeted therapies in the future.

5.2.6. Cell-penetrating peptide (CPP) binding. This approach combines a CPP with a peptide or poly(amino acid), facilitating the crossing of the cell membrane. It enhances the

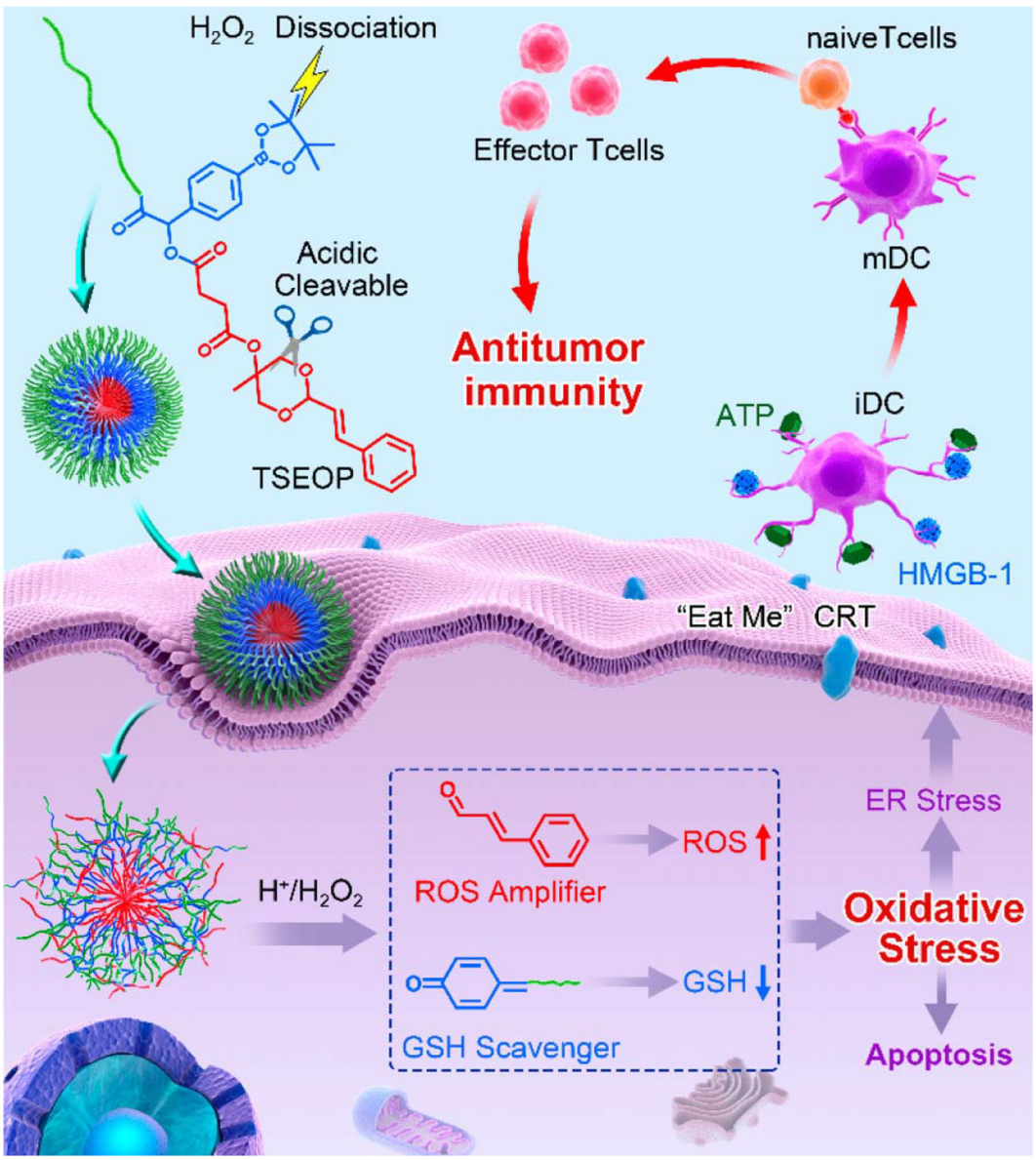

Fig. 10 Schematic illustration of TSEOP for inducing ICD and boosting antitumor immunity. Reproduced with permission.[132] Copyright 2020, American Chemical Society.

efficiency of intracellular drug delivery, allowing the drug to penetrate the cell membrane and enter the cell to exert its therapeutic effects. This method is particularly suitable for the delivery of large-molecule drugs and is frequently employed in the administration of nucleic acid therapeutics, such as siRNA and mRNA.[134] Additionally, it can be utilized to improve the efficacy of gene therapy.

The hydrophobicity of cell membranes and the blood–brain barrier selectively limits the cellular uptake of exogenous molecules larger than 500 Da. However, CPPs can efficiently penetrate cell membranes and have become essential tools for delivering nucleic acids, proteins, and small molecule drugs. There are two primary pathways for CPPs to enter cells: the cytosolic pathway and the direct penetration pathway. In the cytosolic pathway, the phospholipid bilayer first invaginates and encapsulates the penetrating peptide, forming small vesicles that facilitate entry into the cell. Alternatively, this pathway may involve the formation of reverse micelles to facilitate cellular entry. In contrast, the direct penetration pathway is more straightforward. One mechanism relies on the polarity of the penetrating peptide to directly perforate the phospholipid bilayer, while another mechanism involves the peptide spreading across the surface of the bilayer like a carpet and subsequently fusing with it to gain entry into the cell.[44]

Early HIV transactivator (Tat) proteins represent remarkable alternative strategies for penetrating the impermeable phospholipid bilayer of cell membranes and crossing biological barriers. After being secreted by HIV-infected cells, Tat translocates into neighboring cells, altering gene transcription and facilitating the spread of the virus.[135] Fawell at al. published the first report in 1994, demonstrating that Tat-derived peptides could deliver large proteins to various cell types and mammalian organs.[136] Subsequently, a research team led by Bernard Lebleu eventually identified the truncated polycationic peptide GRKKRRQRRR, which contains RNA-binding and nuclear localization signaling (NLS) motifs that are sufficient for efficient translocation into cells and tissues.[137] Following this, Gilles Divita's group designed a short peptide vector called MPG, the first non-covalent CPP for the delivery of nucleic acids into cultured cells.[138] This was closely followed by the development of Pep-1, a peptide carrier for the cellular transfer of peptides and proteins.[139] Later, peptide known as Xentry (LCLRPVG), derived from the N-terminal region of the hepatitis B virus protein X, represented a novel class of CPPs that entered cells exclusively through an energy-dependent endocytosis process, thereby expanding the range of cellular uptake pathways.[140] Recent studies have demonstrated that CPPs can be chemically modified and optimized in sequence to enhance their penetration efficiency and specificity. Furthermore, they can be combined with other functional molecules to develop multifunctional vectors for imaging, diagnostic, and therapeutic purposes. CPPs have shown significant potential for preclinical and clinical research, particularly in the fields of anticancer drug delivery and gene editing. The research history of CPPs illustrates a gradual evolution from early discoveries to mechanistic studies and widespread applications. The versatility and functionality of CPPs position them as promising candidates for critical applications in biomedicine.

5.3. Multifunctional complexes

Poly(amino acids) and peptides can be utilized to construct multifunctional delivery systems by simultaneously incorporating multiple functionalized groups. For instance, chemotherapeutic drugs and anti-tumor immune enhancers can be combined within the same peptide or poly(amino acid) carrier to achieve synergistic therapeutic effects.[141] In addition, by integrating nucleic acid drugs with chemotherapeutic drugs in poly(amino acid) carriers, the dual effects of gene silencing and chemotherapy on tumor cells can be realized concurrently.[142] This multifunctional delivery system, based on poly (amino acids) and peptides, integrates various features such as targeting, stabilization, and controlled release, thereby offering new possibilities for personalized therapy and the treatment of complex diseases. Furthermore, single-function gene delivery systems often fail to meet the demands of clinical applications, making the development of safe and effective multifunctional gene delivery systems particularly crucial. Multifunctional peptide-based gene vectors have shown significant promise in clinical gene therapy. In recent years, research has concentrated on the integration of peptides with diverse functions, including CPPs, targeting ligands, chimeric peptides, and nuclear localization signals. Compared to traditional vectors and single-function peptide vectors, multifunctional peptide gene vectors can markedly enhance gene transfection efficiency and therapeutic efficacy.

Researchers are developing multifunctional peptide NPs. Zhang *et al.*[143] prepared a series of multifunctional chimeric gene vectors composed of alkyl chains, R8 transmembrane peptides, and RGD peptides. The carboxy-terminal RGD sequence enhances the specificity of target recognition for integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$, which are overexpressed on tumor cells. The hydrophobic alkyl chain at the amino terminus was utilized to improve the encapsulation of DNA and enhance the stability of the vector/DNA complex. The results demonstrated that the multifunctional peptide vector significantly increased the transfection efficiency of cancer cells compared to the peptide or RGD-modified vector with R8. Subsequently, they constructed nuclear-targeting vectors by attaching the nuclear localization signal (NLS) PKKKRKV to the R8 peptide modified with hydrophobic stearic acid. The NLS ligand can be recognized by nuclear transporter proteins (importin-α and importin-β), which help overcome the nuclear membrane barrier and promote nuclear translocation. Although peptide-based gene vectors have successfully navigated many barriers and achieved significant positive outcomes in gene therapy, balancing the complex functionality of peptides remains a challenge. Future research will focus on optimizing and coordinating the structure and function of various functional peptide vectors for more efficient and stable gene delivery.

Multi-stimulus responsive peptides can react to two or more stimuli, including pH, temperature, enzymes, light, or

redox conditions. Consequently, it is feasible to develop innovative nanocarriers with enhanced responsiveness that leverage this multi-stimulus capability to efficiently release payloads at targeted sites.

5.4. Analytical technique

During the synthesis of poly(amino acids) and polypeptides, various side reactions and incomplete synthesis may occur, necessitating the development of diverse analytical techniques to characterize the resulting polymers, NMR spectroscopy serves as a valuable tool for elucidating the molecular structure of these polymers by exploiting the resonance of atomic nuclei within a magnetic field.[144] This technique can provide insights into the amino acid sequence, secondary structure, and intermolecular interactions. Notably, the detailed structures of polypeptides comprising fewer than 30 amino acids can be resolved using two-dimensional and three-dimensional NMR methodologies, which are crucial for understanding the spatial conformation of polypeptides and the nature of intramolecular hydrogen bonding.[145] Furthermore, by analyzing parameters such as chemical shifts and coupling constants, one can ascertain the types and sequences of amino acid residues, thereby confirming the primary structure of poly(amino acids) and polypeptides. In addition, FTIR can be used to identify various chemical bonds and functional groups in compounds, and then determine the molecular structure. In the study of poly (amino acid) and polypeptide, FTIR spectroscopy can be employed to identify various chemical bonds and functional groups within compounds based on their absorption characteristics in the infrared spectrum. In the context of poly (amino acids) and polypeptides, FTIR is primarily utilized to analyze secondary structures.[146] For example, the examination of the amide I band (1620–1690 cm^{-1}) allows for the determination of the relative proportions of α-helices, β-sheets, random coils, and turns. The overlapping vibrational peaks associated with these secondary structures within the amide I band can be distinguished through mathematical techniques such as deconvolution, facilitating the extraction of quantitative information regarding the secondary structure.

GPC serves as a method for determining molecular weight by utilizing a polymer solution that is introduced into a porous filler column. The retention times of molecules with varying molecular weights differ within the column, facilitating their separation and subsequent molecular weight assessment. This technique is particularly effective for poly(amino acids) and polypeptides, allowing for rapid and precise determination of their average molecular weight and molecular weight distribution. Such measurements are crucial for investigating the degree of polymerization, chain length, and molecular uniformity, making GPC a widely employed method for characterizing the molecular weight of polymeric materials.[147]

DSC and TGA are instrumental in evaluating molecular stability. DSC assesses the thermal transformation behavior of substances by measuring changes in heat flow during heating or cooling, thereby providing insights into properties such as melting point, glass transition temperature, and crystallization temperature.[148] For poly(amino acids) and polypeptides, DSC is utilized to evaluate thermal stability, phase transition behavior, and the strength of intermolecular interactions.[149] Conversely, TGA measures the mass of a sample in relation to temperature or time under programmed conditions, enabling the analysis of thermal decomposition processes, thermal stability, and the presence of moisture and volatile substances.[150] In the context of poly(amino acids) and polypeptides, TGA can ascertain decomposition temperatures and thermogravimetric conditions, thereby informing on their stability at elevated temperatures and offering guidance for material processing, storage, and usage conditions.[151]

Furthermore, mass spectrometry can be employed to determine the molecular weight, amino acid sequence, and modifications of poly(amino acids) and polypeptides by separating and detecting samples based on the mass-to-charge ratio of ions.[152] Circular dichroism chromatography is utilized to investigate the three-dimensional structure, reaction kinetics, and conformational changes of molecules in solution.[153] X-ray crystallography is a technique used to elucidate the structure of proteins, allowing for the precise determination of the spatial arrangement of atoms within crystals, thereby revealing the three-dimensional structures of proteins and peptides.[154] Additionally, ultraviolet spectroscopy can provide information regarding the solution conformation of biomacromolecules, requiring only that the samples be in a solution state for analysis.[155]

6. Application of poly(amino acids), peptides and their derivatives in drug delivery

Poly(amino acids), peptides, and their derivative drug delivery systems can significantly enhance the stability, bioavailability, and targeting of drugs, while also reducing side effects and improving therapeutic efficacy. Below are specific application cases of poly(amino acid), peptide, and their derivative in the delivery of small molecule drugs, nucleic acid drugs, peptide drugs, and protein drugs.

6.1. Types of drugs loaded

6.1.1. Anti-cancer drug delivery. Cancer is the second leading cause of death worldwide. The efficacy of many antineoplastic drugs is often diminished by rapid blood clearance, non-specific biodistribution, or inadequate accumulation and retention at the tumor site.[156] Targeted delivery of peptide anticancer drugs enhances the therapeutic efficacy of these agents while minimizing damage to healthy cells. This is achieved by conjugating the anticancer drug with a specific peptide that enables precise targeting of cancer cells. An ideal anticancer therapeutic agent should have the ability to selectively destroy cancer cells without harming normal tissues. Anti-cancer drug delivery refers to the precise administration of an anti-cancer drug to a tumor site through various technological means and carrier systems. This approach aims to

maximize the therapeutic efficacy of the drugs while minimizing the side effects on normal tissues. Specific methods for drug delivery include the efficient encapsulation of drugs using NPs and the targeted accumulation of these drugs at tumor sites through modification techniques. These methods encompass the use of vesicles composed of phospholipid bilayers for drug encapsulation, as well as polymer micelles that consist of both hydrophilic and hydrophobic chain segments to enhance the solubility and targeted delivery of poorly soluble drugs. Additionally, network-structured hydrogels with high water content can facilitate the localized slow release of drugs *via* injection. The photothermal effect of metal NPs can also be harnessed for tumor-targeted therapy, thereby enhancing the anticancer effect. Shen *et al*. analyzed the typical process of anticancer drug delivery using intravenously administered drug-carrying nanocarriers. They concluded that the delivery process consists of five steps: blood circulation, tumor-site accumulation, intra-tumor penetration, intracellular internalization, and intracellular drug release. This sequence is referred to as the CAPIR cascade (Fig. 11).[157]

The primary peptide–drug couplings utilized in anticancer therapy include peptide–DOX couplings, peptide–paclitaxel (PTX) conjugates, peptide–camptothecin (CPT) affixes, peptide–platinum (Pt) drug couplings, peptide–vessel-disrupting agent (VDA) affixes, peptide–protein conjugates, and peptide–gas molecule conjugates. For instance, Singer *et al*. designed poly(L-glutamic acid)–Gly–CPT conjugates to stabilize the reactive lactone form of CPT and enhance its water solubility. In addition, linking CPT to high molecular weight anionic polymers improved its solubility and increased tumor distribution through the EPR effect.[158] Klein's *et al*.[159] further developed the concept of PLG couplings of 20(S)–CPT to augment the aqueous solubility of CPT, which demonstrated stability in aqueous solutions at neutral pH and exhibited potent antitumor activity *in vivo*. They also assessed the correlation between PLG molecular weight, CPT loading, and solubility, as well as the relationship between CPT-equivalent dosage and the *in vivo* antitumor efficacy of various conjugates, ultimately identifying the optimal conjugate composition.

The RGD (Arg–Gly–Asp) peptide specifically recognizes and binds to integrin receptors on the surface of tumor cells. By combining the peptide with the anticancer drug paclitaxel (PTX), targeted delivery of the drug can be achieved, thereby improving therapeutic efficacy and reducing side effects on normal tissues. To address the limitations of PTX, such as solubility, membrane permeability, and non-selective cytotoxicity, Deng *et al*. synthesized a "smart" PDC[160] (peptide–drug coupling device) by linking PTX to a multifunctional peptide that consists of a tumor-targeting peptide (TTP) and a CPP. They constructed the TTP–CPP–PTX coupling, designated LTP-1, which intelligently delivers PTX to cells overexpressing the LHRH receptor. LTP-1 demonstrates a two-fold increase in

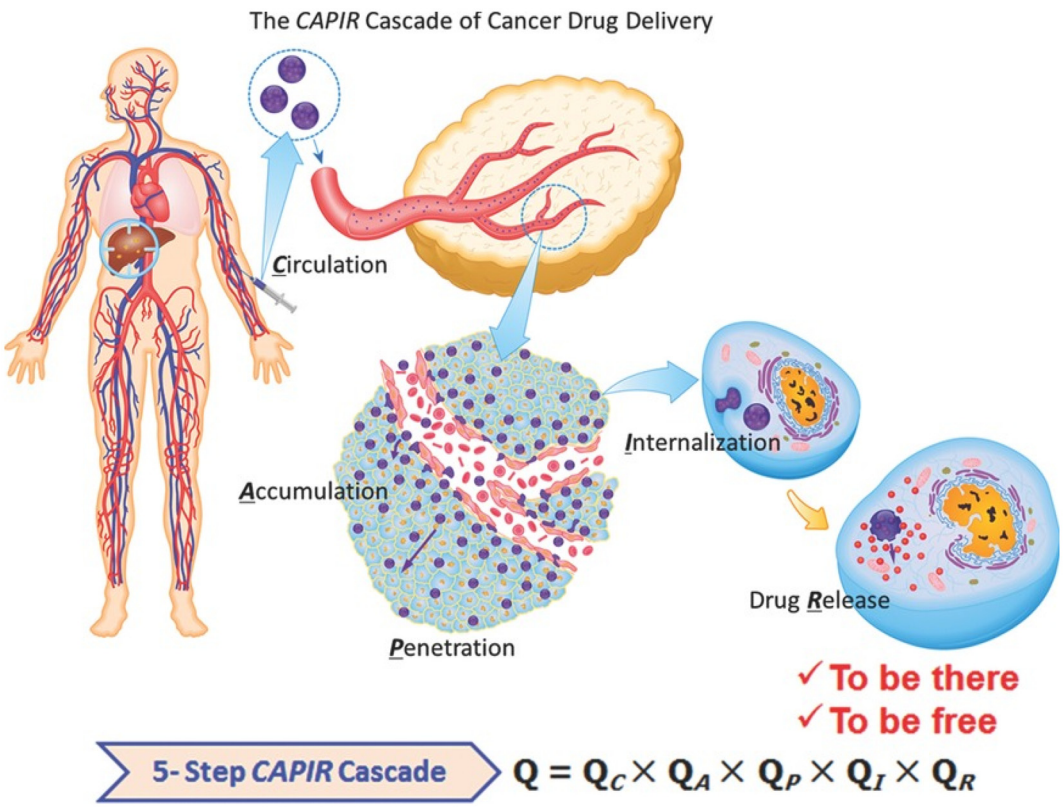

Fig. 11 Overview of the 2R2SP requirement for nanocarriers with high overall delivery efficiency and the 3S transition in the CAPIR cascade.[157] Copyright 2017, John Wiley and Sons.

cellular uptake compared to PTX and exhibits enhanced cytotoxicity, with an IC50 of 3.8 nM (compared to 6.6 nM for PTX). Additionally, LTP-1 shows reduced cytotoxicity to normal cells and has the capability to overcome PTX resistance. Furthermore, the *in vivo* antitumor efficacy of LTP-1 surpasses that of PTX, without significant toxicity. Zhang *et al.* synthesized an amphiphilic triblock copolymer methoxy poly (ethylene glycol)-*block*-poly(L-glutamic acid)-*block*-poly-(γ-propargyl-L-glutamate) (mPEG-*b*-PLGA-*b*-PPLG). The PLGA block of mPEG-*b*-PLGA-*b*-PPLG was modified with dopamine containing catechol groups through an amidation reaction, while the PPLG block was modified with a small tertiary amine molecule *via* a click reaction, resulting in a novel triblock copolymer, mPEG-*b*-PGCA-*b*-PGTA. This new copolymer could simultaneously load phenylboronic acid-modified ribonuclease A (RNase A) and hydrophobic DOX through pH-reversible phenylboronic acid–catechol linkages and hydrophobic interactions, respectively. The dual-drug-loaded NPs facilitate efficient delivery of both agents to tumor sites, exhibiting high systemic stability and enabling low-pH and ROS-triggered cooperative release of DOX and RNase A within tumor cells, leading to enhanced combined anticancer efficacy (Fig. 12).[161]

6.1.2. Antibiotic delivery. Using peptide NPs as carriers for antibiotics, the encapsulation of these drugs within peptide NPs through electrostatic interactions can significantly enhance their solubility and bioavailability. This method also protects the antibiotics from degradation by *in vivo* enzymes, thereby prolonging their half-life and improving drug stability. For instance, encapsulating vancomycin in peptide NPs can amplify its efficacy against drug-resistant bacteria.

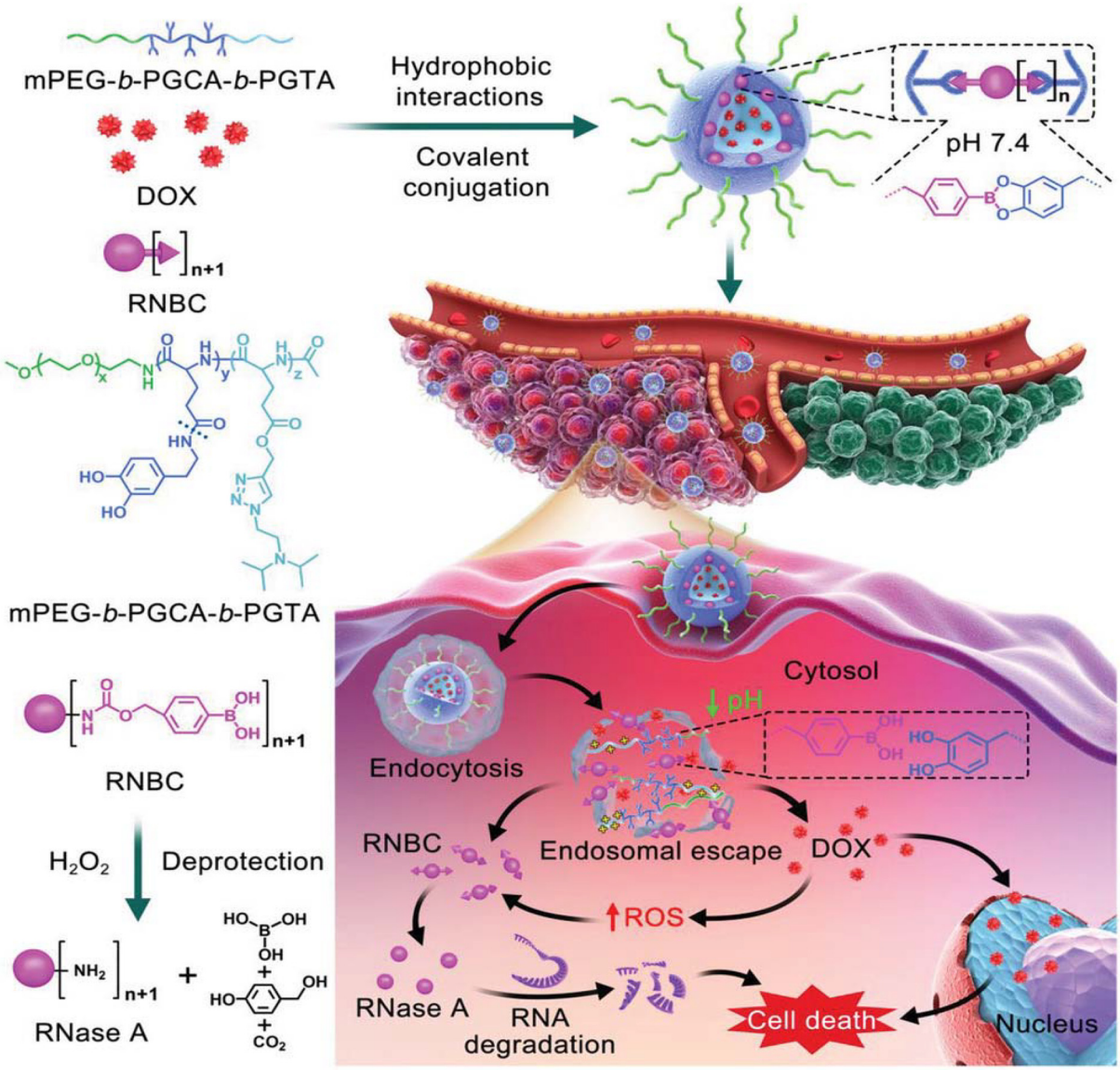

Fig. 12 Schematic illustration of mPEG-*b*-PGCA-*b*-PGTA mediated intracellular codelivery of RNase A and DOX for combination cancer therapy.[161] Copyright 2020, John Wiley and Sons.

The design of CPP-antibiotic couplers has primarily been based mainly on HIV–TAT peptides or their analogues, the usually arginine-rich peptides with short side chains.[162–164] These CPPs enter cells through a complex mechanism, which results in low membrane permeability and limited drug delivery efficacy. In response to these challenges, Jiang *et al.*[165] developed a class of structurally simple yet highly membrane-permeable CPPs that exhibited 100-fold greater membrane permeability than conventional CPPs, such as TAT and oligoarginine, as along with an unprecedented membrane penetration mechanism. Upon approaching the cell membrane, CPPs begin to adhere to the cell membrane through interactions between their peptide surface charges and the membrane's surface charge. They gradually reorient themselves orthogonally, allowing more charged side chains to make contact with the cell membrane. Following this initial adhesion, CPPs redistribute their surface charge to one side and penetrate the membrane orthogonally, with the exposed hydrophobic side facing the lipid interior. Given that the lengths of their side chains are comparable to the thickness of the lipid bilayer, and considering the driving force provided by the negatively charged inner leaflet, the side chains can facilitate further charge redistribution by tunneling their charged groups from the outer leaflet to the inner leaflet, ultimately resulting in complete membrane penetration.[165]

6.1.3. siRNA delivery. Over the past few decades, oligonucleotides, including small interfering RNA (siRNA), microRNA (miRNA), and short hairpin RNA (shRNA), have been introduced into cells with the goal of preventing, halting, or reversing diseases, such as cancer.[166] While siRNAs are promising tools for elucidating disease mechanisms and developing therapies, a fundamental challenge in gene delivery is the creation of safe and effective delivery systems.[167] Successful siRNA delivery necessitates an appropriate carrier system that facilitates the transport of siRNA, protects it from environmental degradation, and enhances intracellular uptake through controlled release.[168] Peptide NPs are widely utilized for siRNA delivery due to their effectiveness. Peptides can efficiently shield siRNA from nuclease degradation *in vivo* and promote its entry into target cells. For example, Saikat Biswas *et al.*[169] designed a novel pentapeptide (RΔFRGD) using a highly stable self-assembled dipeptide template that incorporated arginine-α,β-dehydrophenylalanine (*RΔF*) and the tripeptide arginine–glycine–aspartic acid (RGD), which played a crucial role in the cell adhesion process. Ultimately, this approach was characterized, yielding improved results.

6.1.4. Gene editing. Gene editing is a technology that enables precise modifications of genome sequences and is widely utilized in biomedical research, gene therapy, agriculture, and various other fields. This technology allows for the insertion, deletion, or replacement of specific DNA sequences to correct gene mutations, investigate gene function, or enhance biological traits. The efficient delivery of gene editing tools is crucial for successful outcomes. Traditional delivery methods often face limitations regarding safety and efficiency. Consequently, poly(amino acid) and peptide-based delivery systems have emerged as significant research avenues for the delivery of gene editing tools, owing to their unique advantages. For example, positively charged polylysine can form complexes with negatively charged nucleic acid drugs to protect the nucleic acid drugs, protecting them from degradation and facilitate their entry into cell interior for gene therapy and gene editing. These polymers can effectively safeguard nucleic acid molecules and promote their cellular uptake.

The application of peptides in gene editing is primarily demonstrated through their uses carriers for delivering gene editing tools into target cells. One significant application of peptide drug delivery systems in the delivery of the CRISPR/Cas9. Researchers utilize peptide NPs to transport the CRISPR/Cas9 components into target cells for gene editing. For example, the delivery of Cas9 nuclease and single guide RNA (sgRNA) using peptide-modified NPs has proven effective in modifying target genes for the treatment of genetic diseases. Ramakrishna *et al.*[170] demonstrated that a simple treatment involving recombinant Cas9 protein, coupled with CPP and CPP-complexed guide RNA, results in endogenous gene disruption in human cell lines. In this process, the Cas9 protein binds to the CPP *via* thioether bonding, while the guide RNA is complexed with the CPP to form cohesive, positively charged NPs.

Poly(L-lysine) (PLL) is one of the most widely utilized gene vectors. PLL is a polypeptide synthesized through ring-opening polymerization, using e-benzyloxycarbonyl-L-lysine *N*-carboxyanhydride (Lys(Z)–NCA) as the monomer.[171] Research demonstrated that exogenous DNA could be specifically delivered into hepatocellular carcinoma cells using an asialo–orosomucoid–PLL copolymer with transfection capabilities. However, the relatively high toxicity and low transfection efficiency of PLL, particularly due to its large molecular weight, have limited its applications. The incorporation of hydrophobic groups into PLL can enhance its cellular uptake. Clements *et al.* developed an amphiphilic vector by substituting PLL with palmitic acid (PLL–Pa). Compared to PLL, PLL–Pa exhibited significant intracellular transport in bone marrow stromal cells (BMSC) and demonstrated markedly higher transfection efficiency.[172] Kataoka *et al.* created redox-sensitive multi-ion complex micelles through spontaneous nuclear cross-linking of thio-PEG-*b*-PLL and antisense oligodeoxynucleotides (ODN). The stability of ODN against nuclease degradation within the micelles was significantly enhanced, allowing for efficient release in the intracellular environment.[173] Maruyama *et al.* showed that by grafting PEG onto the side chain of PLL increased circulatory lifespan and tumor without compromising siRNA activity.[174] On the one hand, PEGylation enhances the stability and circulation time of cationic NPs in the blood circulation. On the other hand, polyethylene glycolisation may also diminish cellular uptake and endosomal escape.[175] To address these challenges, Christie *et al.* modified PEG–PLL with cyclic arginine–glycine–glutamate peptide (cRGD), which binds to a variety of tumor-overexpressed integrin receptors, yielding improved results.[176]

Glioblastoma remains the most resistant malignant brain tumor due to the lack of effective therapeutic genes and drug delivery systems, particularly outside the tumor islands.[177,178] Y. S. Malik *et al.*[179] used non-viral agents, such as polylysine-modified polyethyleneimine (PEI–PLL) copolymers, to generate genetically engineered mesenchymal stem cells (MSCs) containing suicide genes, such as HSV-TK and TRAIL. This study suggested a promising non-viral approach for developing cell-based therapeutic treatments for gliomas. Furthermore, Lin *et al.*[180] employed methoxy polyethylene glycol (MPEG) conjugated to arginine-functionalized poly(L-lysine) dendrimer (PLLD–Arg) through a click reaction. They subsequently synthesized MPEG–PLLD–Arg interacting with α-cyclodextrin (α-CD) to form a supramolecular hydrogel *via* host–guest interactions. This hydrogel demonstrated highly efficient and sustained gene transfection of the cells tested, outperforming PEI-25k.

6.1.5. Peptide hormone delivery. Peptide hormones are susceptible to degradation by enzymes in the body, so employing peptide delivery systems can enhance their stability and bioavailability. In addition, these systems can minimize the distribution of peptide hormones to non-target tissues, thereby reducing systemic side effects. For example, hypoxia-sensitive PEG-*b*-P(Gln(Deta-NBCF)) micelles loaded with cytochrome C demonstrate a more pronounced cytotoxic effect on hepatocellular carcinoma cells (HepG2) under hypoxic conditions, attributed to the activation of the cell death pathway by cytochrome c (CC).[181] Furthermore, in diabetic mice, insulin encapsulated in the P(Glu-*co*-Gln(Ts))/PLys polyelectrolyte intercalation complex exhibited significant colonic permeability and effectively lowered glucose levels.[182]

6.1.6. Antimicrobial peptide delivery. Antimicrobial peptides (AMPs) are a class of peptides known for their broad-spectrum antimicrobial activity, which can be further enhanced through peptide drug delivery systems. For instance, AMP/CPP modulates autophagy, subsequently influencing the immune system's response. In addition, AMPs play a crucial role in establishing the microbiota, which is vital for various human behaviors and health aspects. Consequently, AMPs and CPPs are multifunctional peptides that regulate two critical components of our body that are essential for our health: autophagy and microbiota.[183]

Iudin and Vasilieva *et al.* demonstrated the suitability of PGlu-containing NPs for capturing positively charged peptide antibiotics, such as polymyxins, through electrostatic interactions or covalent coupling.[184] In all cases, the physically loaded antibiotics retained their antimicrobial properties at the free drug level, while the amide-linked conjugates exhibited reduced activity. Furthermore, all encapsulated forms of polymyxins displayed lower cytotoxicity against human embryonic kidney cells (HEK 293) compared to free cationic polymyxins. Liu *et al.*[185] selected the less cytotoxic antimicrobial peptide MP, coupled with the CPP Antp, and observed the changes in the cell-killing activity of the fusion peptide. They found that the uncoupled antimicrobial peptide MP and the CPP Antp did not exhibit any significant killing effect on the tumor cells. In contrast, the fusion peptide MPGA, which was formed by coupling the two peptides, demonstrated a strong tumor cell-killing effect by disrupting cell membranes. This suggests that coupling antimicrobial peptides with CPPs can significantly enhance their cytotoxicity, potentially providing a promising avenue for the development of novel antitumor drugs.

6.1.7. Enzyme delivery. Enzymes are vulnerable to inactivation or degradation within the body's environment. Peptide drug delivery systems can be employed to ensure stable delivery and maintenance of enzyme activity. These systems provide enhanced protection for enzymes against recognition and elimination by the body's immune system, thereby mitigating immune responses. For example, researchers have developed the use of peptide NPs to deliver enzyme drugs, such as superoxide dismutase (SOD), for the treatment of diseases related to oxidative stress.

6.1.8. Antibody drug delivery. Peptide NPs have also been utilized for antibody drug delivery. By conjugating peptide NPs with antibodies, the stability and targeting capabilities of the antibodies can be enhanced, while minimizing the distribution of antibody drugs in non-target tissues and reducing systemic side effects. For instance, the combination of an anti-HER2 antibody with peptide NPs can improve the targeting efficacy of the antibody on HER2-positive tumor cells. Li *et al.*[186] designed a recombinant protein, LP-scFv, which incorporates the single-chain variable region of the anti-human epidermal growth factor receptor-2 along with a novel non-oxygenated CPP as a lead peptide. The results indicated that the introduction of this lead peptide led to more than a two-fold increase in the internalization efficiency of single-chain antibodies.

6.1.9. Vaccine delivery. Poly(amino acid) materials have a wide range of applications in vaccine delivery. The use of poly (amino acid) materials as carriers allows for the efficient delivery of vaccine components to targeted sites in the body, thereby inducing an immune response. By carefully designing the structure of poly(amino acids), it is possible to achieve several advantages, including poly(amino acid) protection of vaccine components, controlled release, targeted delivery, biocompatibility, and degradability, all of which enhance the immune response.

Poly(amino acids), such as polyglutamic acid (PGA) and polytyrosine (PTY), have also been investigated for their potential use in vaccine delivery systems to enhance antigen stability and immunogenicity. Zhang *et al.*[187] explored the feasibility of NP-mediated antigenic peptides to efficiently induce cytotoxic T-lymphocyte responses to tumor-associated autoantigens in a C57BL/6 mouse model. They prepared biodegradable poly(D,L-lactide-*co*-glycolide) nanoparticle (PLGA-NP) carrying murine melanoma antigenic peptides, hgp100 and TRP, were prepared using a double-emulsification method, demonstrating the viability of NP-mediated antigen delivery for cancer immunotherapy. M. Chiba *et al.*[188] developed polymeric microspheres capable of controlled release of macromolecules over periods ranging from days to more than a month. These poly-

mers are particularly effective for the controlled delivery of vaccine antigens due to the incorporation of the immune adjuvant L-tyrosine into their backbone.

6.2. Enhance the therapeutic efficacy of drugs

6.2.1. Improving immunogenicity and biocompatibility. The immunogenicity and biocompatibility of vector systems are critical considerations in the design of drug delivery systems, as they significantly influence both safety and efficacy. Generally, poly(amino acids), polypeptides, and their derivatives exhibit favorable biocompatibility. The degradation products of these materials *in vivo* are typically non-toxic amino acids, which tend to elicit fewer adverse effects on the organism.[22] However, certain poly(amino acids) and polypeptides may themselves be immunogenic, especially if they are not modified or optimized. Furthermore, certain poly(amino acids) and polypeptides demonstrate relatively low immunogenicity due to their structural similarity to natural biological components.[189] T However, it is important to note that some poly(amino acids) and polypeptides may possess immunogenic properties, particularly if they are unmodified or inadequately optimized. Such materials may be identified by the immune system as foreign entities, thereby provoking an immune response. Consequently, the utilization of poly(amino acids) and polypeptides, along with their derivatives, is of paramount importance in drug delivery applications aimed at minimizing drug immunogenicity and enhancing biocompatibility. To achieve this, immunoinert groups are often incorporated into the design.[190] For instance, PEG chains are frequently conjugated to the surfaces of poly(amino acids) or polypeptide carriers, resulting in PEG-modified drug delivery systems. PEG is characterized by its excellent hydrophilicity and biocompatibility, forming an "invisible" protective layer that mitigates direct interactions between the drug and the immune system, thereby reducing immunogenicity.[191] Additionally, other immune-inert groups, such as methyl and ethyl alkyl groups, can be integrated into the molecular framework of poly(amino acids) or polypeptides.[192] These alkyl modifications can alter the surface characteristics of the molecules, as they possess smaller steric hindrance and lower polarity, which may diminish interactions with immune cells and enhance the stability of the carriers against enzymatic degradation. For example, methylated polyglutamate has been identified as an effective drug carrier with low immunogenicity and commendable biocompatibility.[193] Moreover, other polymers such as polyethylene oxide (PEO)[194] and poly(2-methyl-2-oxazoline) (PMeOx)[195,196] also exhibit favorable hydrophilicity and biocompatibility, contributing to the reduction of immunogenicity in poly(amino acid) or polypeptide carriers. Structural optimization of the carrier is another strategy to mitigate immunogenicity and enhance biocompatibility, which involves selecting poly(amino acids) or polypeptides with appropriate molecular weights and narrow molecular weight distributions. Generally, carriers with lower molecular weights and uniform distributions are more readily metabolized and cleared by the body, thus decreasing immunogenicity.[197] In Addition, the sequence of poly(amino acids) or polypeptides can be adjusted to create specific secondary structures, such as α-helices or β-sheets, which may exhibit reduced immunogenicity or influence drug release characteristics, ultimately leading to diminished immune responses and improved biocompatibility.[198]

6.2.2. Controlled release profiles and construction of stimulus reactivity. In conventional drug delivery systems, such as standard capsules or tablets, the mechanism of drug release predominantly relies on passive diffusion.[199] The release rate is primarily influenced by the concentration gradient of the drug and the physical characteristics of the dosage form. This method of release poses challenges in achieving precise targeting of specific sites, resulting in a relatively uniform distribution of the drug throughout the body. Consequently, this uniformity can lead to undesirable accumulation of the drug in non-target tissues, thereby increasing the likelihood of adverse side effects. Recent advancements in controlled polymerization chemistry have facilitated the seamless integration of polypeptides with other materials, enabling the synthesis of heterogeneous structures that exhibit improved functional self-assembly and controlled release properties.[200] Smart responsive peptide drug delivery systems can react to external environments such as low pH,[201] temperature, enzymes, and low oxygen concentrations,[202] thereby facilitating the controlled release of drugs. There are two primary strategies for constructing stimuli-responsive peptide nanocarriers.

The first advancement involves the introduction of blood-stable yet intracellularly unstable bonds in peptide–drug couplings. This includes acid-unstable bonds, such as hydrazine or benzoic acid imine, which respond to lysosomal acidity I as well as glutathione (GSH)-sensitive disulfide bonds[203,204] that react to elevated GSH levels in tumor cells.[205] Hoang *et al.* synthesized a ROS-responsive poly(ethylene glycol)-poly(methionine) and prepared micelles through the self-assembly of the hydrophobic pro-oxidant drug piperamide.[206] Increased ROS levels in cancer cells triggered a transition of the peptide from hydrophobic to hydrophilic, leading to the disassembly of the micelles and resulting in effective drug release and enhanced anticancer efficacy.

The second strategy involves the capacity of peptides and poly(amino acids) to facilitate targeted drug release in response to environmental changes or specific cellular markers. These advanced drug delivery systems can identify particular conditions within the disease microenvironment, allowing for precise drug release at the target site. This approach maximizes therapeutic efficacy while minimizing side effects.

6.2.2.1. Targeted release based on pH response. Poly(amino acids) and polypeptides are complex biological macromolecules comprised of amino acid residues, characterized by a diverse array of ionizable functional groups, including amino and carboxylic groups. The ionization state of these functional groups is influenced by the pH of the surrounding environment, which in turn affects the electrostatic interactions among molecular chains. Specifically, in polypeptides that

contain histidine residues, the imidazole side chain of histidine (with a pK_a of approximately 6.0) is partially protonated at physiological pH (7.4). As the ambient pH decreases towards the pK_a value, the degree of protonation of histidine residues increases, resulting in altered electrostatic repulsion or attraction between polypeptide chains, which subsequently modifies the structural configuration of the carrier.[207] variations in pH can induce significant structural transformations. Under acidic conditions, the protonation of amino groups enhances electrostatic repulsion between molecular chains, leading to an expansion of the carrier. Conversely, under alkaline conditions, this effect is reversed.[208] Such volumetric changes can influence the encapsulation and release dynamics of drugs. For instance, if a drug is physically embedded within the carrier, the expansion of the carrier may facilitate the opening of release channels, thereby promoting drug release. Additionally, fluctuations in pH can trigger depolymerization or polymerization processes in poly(amino acids) or polypeptides. Aggregates stabilized by hydrogen bonds or electrostatic interactions may be disrupted by changes in pH, resulting in depolymerization and subsequent drug release.[209] For example, poly(amino acid) complexes that rely on acid–base interactions may undergo depolymerization when the pH shifts, disrupting the acid–base equilibrium. Furthermore, certain pH-sensitive poly(amino acids) and polypeptides may experience hydrophilic transformations. In acidic environments, their hydrophilic properties are enhanced due to protonation, while in alkaline conditions, hydrophilicity diminishes and hydrophobicity increases. This alteration in hydrophilicity significantly impacts the interactions between the drug and the carrier, as well as the carrier's interactions with the surrounding environment, thereby regulating the drug release process.

Therefore, poly (amino acid) materials can be designed to be PH sensitive, allowing targeted release of drugs in specific acidic and alkaline environments. This responsiveness ensures that the drug is released at the desired site, thereby minimizing systemic side effects. For example, Shen *et al.*[210] developed a series of poly(amino acid) polymers that achieved an accelerated cumulative release rate of NPs as the pH decreased, demonstrating a significant targeted release effect. The synthesis of these materials and the preparation of NPs offer innovative approaches in the field of sustained drug release.

The primary disadvantage of conventional chemotherapy is its inability to selectively target cancer cells, resulting in damage to normal, healthy cells. The extracellular pH is 7.4, whereas in tumor cells, due to rapid and frequent cell proliferation and lactic acid accumulation lower the pH to between 5 and 6.[211] A pH-responsive carrier molecule that specifically responds to acidic conditions may provide an effective means of precisely delivering drugs within the tumor microenvironment, thereby enhancing cytotoxicity against cancer cells. This approach must also consider the varying pH levels in different regions of the gastrointestinal tract, including the stomach, duodenum, jejunum, ileum, and vagina, to ensure optimal drug uptake at the corresponding sites.[212–214] The introduction of aspartic acid (Asp) or glutamic acid (Glu) into peptides, with the pK_a of the side carboxyl group in the pH 4 region resulting in NPs capable of releasing drugs in the acidic extracellular environment of tumor tissue (pH 5.5–6.0).[215] Zhong *et al.* synthesized pH-responsive block polymers composed of polyethylene glycol (mPEG) and poly(asparagyl diisopropylethylenediamine-*co*-phenylalanine) (P(Asp(DIP)-*co*-Phe)), which can self-assemble into nanovesicles that encapsulate the hydrophilic drug tirapamine and the hydrophobic photosensitizer dihydroporphyrin.[216] Due to the pH sensitivity of the diisopropylethylenediamine fragments, these nanovesicles disintegrate after 24 hours of incubation in a 10 mM phosphate buffered saline (PBS) solution at pH 5.0, facilitating the release of the encapsulated active ingredients.

For example, Li *et al.* developed a multifunctional cationic hydrogel designed for synergistic antibacterial therapy that is responsive to cationic stimuli, pH changes, and near-infrared (NIR) light, specifically for the treatment of bacterial-infected wounds (Fig. 13).[217] The hydrogel matrix was created by crosslinking quaternary ammonium/boronic acid-modified poly (aspartic acid) (QPABA) and poly(vinyl alcohol) (PVA) with the RWRWRW–NH_2 peptide (MP196) linked to PDA NPs. The gelation time was recorded at 30 seconds. The incorporation of phenyl boronic ester bonds facilitated pH-triggered dissociation in the acidic environment characteristic of bacterial infections. The developed hydrogel demonstrated high *in vivo* antibacterial efficacy, achieving nearly 100% effectiveness when combined with NIR light exposure.

6.2.2.2. Targeted release based on temperature response. Some poly(amino acids) undergo phase transitions from hydrophobic to hydrophilic or from gel state to solution state over a certain temperature range. This phase transition can facilitate the release of the drug from the carrier. For example, when the temperature rises above a certain threshold, poly (amino acid) micelles, or NPs, break down, releasing encapsulated drugs. Furthermore, alterations in temperature can induce modifications in the molecular conformation of poly (amino acids) and polypeptides.[218] At lower temperatures, the molecular chains exhibit an extended configuration, creating sufficient internal space to accommodate drug molecules. As the temperature increases beyond a certain threshold, the molecular chains undergo contraction, resulting in a reduction of the internal space, which subsequently leads to the expulsion of the drug molecules.[219] Additionally, the conformational changes in the molecules can influence the hydrophilicity of the carrier, thereby modulating the interactions between the drug and the carrier, as well as between the carrier and its surrounding environment, ultimately governing the drug release process.[220]

Since tumor tissue tends to be slightly hotter than surrounding healthy tissue, typically displaying a 1–2 °C temperature difference, this variation provides a natural targeting advantage for temperature-responsive drug release. Drug delivery systems can be designed to respond to this slight temperature increase by selectively acting on the tumor site or by external means that can precisely control the temperature increase

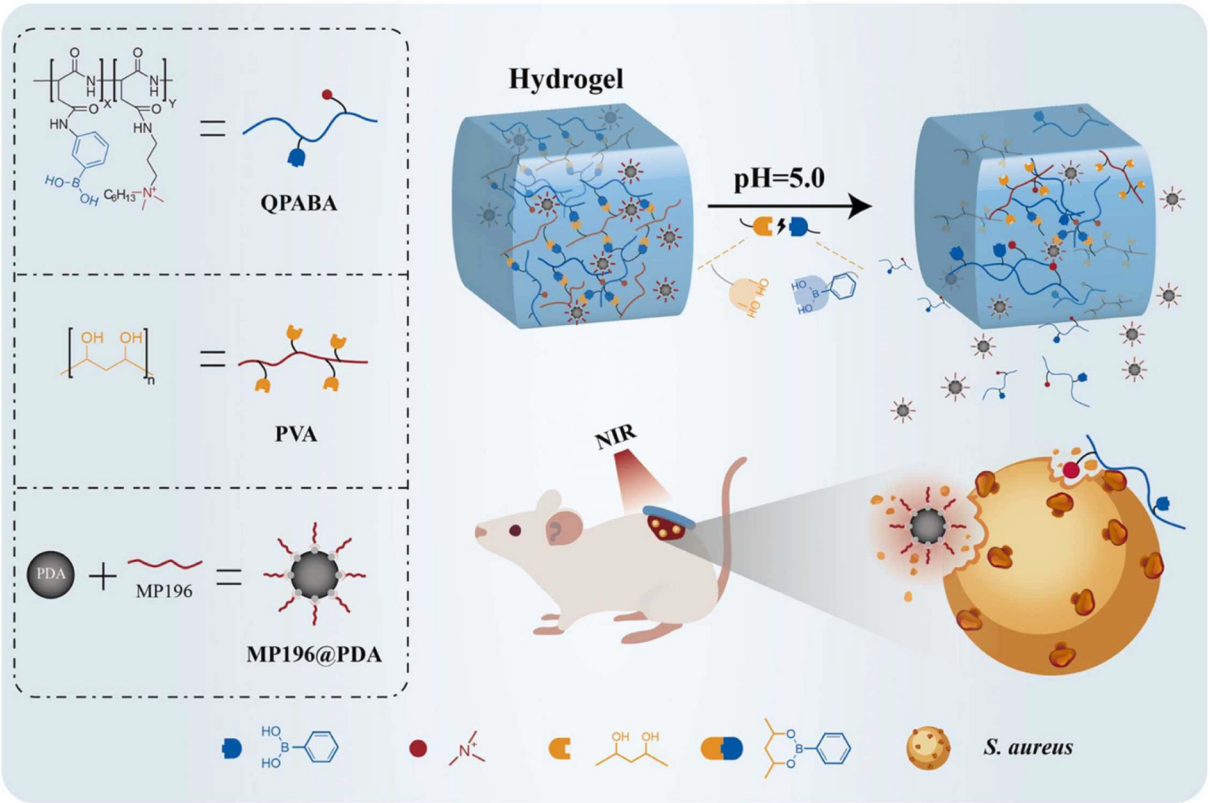

Fig. 13 Schematic representation of pH-responsive QPABA/PVA hydrogel with encapsulated NIRresponsive MP196@PDA NPs as a wound treatment material for antibacterial therapy. Reproduced with permission of Elsevier from ref. 217. Copyright 2023, Elsevier.

in the tumor area. A notable example is polyn-isopropylacrylamide (PNIPAM), a classic low-critical solution temperature (LCST) type temperature-responsive polymer that often binds to poly(amino acids) or peptides. When the temperature rises to the lowest temperature (usually around 32° C), PNIPAM changes from water soluble to hydrophobic, which triggers shrinkage or aggregation of the material, leading to drug release.[221] The realization of the temperature-sensitive mechanism includes physical mixing, in which drugs and temperature-sensitive poly(amino acids) or polypeptides are loaded into the carrier through physical mixing. Or the drug is chemically coupled to temperature-sensitive poly(amino acids) or polypeptides. This temperature-responsive property highlights their great potential for use in temperature-sensitive drug delivery against tumors.

6.2.2.3. Enzyme-based response release. The enzymatic sensitivity of poly(amino acids) and polypeptides is contingent upon the precise recognition between the enzyme and its substrate. *In vivo*, a diverse array of enzymes exists, each characterized by a unique recognition sequence and active site. For instance, matrix metalloproteinases (MMPs) possess the ability to identify and cleave specific amino acid sequences, such as glycine–proline–leucine–glycine–isoleucine–alanine (GPLGIA). When a poly(amino acid) or polypeptide drug carrier incorporates the recognition sequence of these enzymes, it becomes susceptible to enzymatic action. The enzymatic activity on poly (amino acids) or polypeptides primarily involves the hydrolysis of peptide bonds.[222] Upon recognition and cleavage of a specific peptide bond within the carrier, the molecular architecture of the carrier undergoes alteration. Such structural modifications may manifest as the fragmentation of molecular chains, conformational adjustments, or the emergence of new reactive termini. For example, when an enzyme cleaves a polypeptide carrier at a central location, the previously continuous molecular chain is divided into two segments, potentially resulting in the opening of the drug delivery channel encapsulated within or altering the interaction between the carrier and the drug, thereby facilitating drug release.[223] By leveraging the degradation of these polymers through specific enzymes present in the biological milieu, enzymatic reactivity governs the release of drugs from poly(amino acids). This mechanism ensures that drug release is preferentially activated at pathological sites enriched with enzymes, such as tumors or inflamed regions. This can be accomplished by integrating enzyme-sensitive segments into poly(amino acid) materials, such as polymers that contain specific amino acid sequences amenable to recognition and degradation by endogenous enzymes, thus enabling the release of encapsulated therapeutics.[224] Furthermore, the design of carrier architectures, including nanoparticles, micelles, or hydrogels, can enhance

the efficacy of drug release at sites of enzymatic degradation.[209]

In various pathological conditions, the expression and activity of enzymes in affected tissues exhibit significant deviations from those in healthy tissues. For instance, in the context of tumors, both tumor cells and adjacent stromal cells produce substantial quantities of MMPs, which facilitate the invasion and metastasis of cancer cells.[225] To enhance the targeted delivery of therapeutics to tumor tissues, enzyme-sensitive poly(amino acid) or polypeptide carriers are employed. These carriers are designed to release the drug upon cleavage by specific enzymes present in the tumor microenvironment, thereby improving the drug's utilization at the tumor site while minimizing adverse effects on normal tissues.[226] Within cellular compartments, various enzymes, such as acid hydrolases found in lysosomes, play a crucial role. Upon the endocytosis of the drug carrier, it is transported into endosomes and lysosomes. Enzyme-sensitive poly(amino acid) or polypeptide carriers can exploit lysosomal enzymes to facilitate drug release intracellularly. For example, a polypeptide carrier engineered to release drugs in response to lysosomal enzymes encapsulates gene therapeutics. When this carrier reaches the lysosome, it is cleaved by lysosomal enzymes, allowing the gene drug to be released, thus preventing excessive degradation by nucleases present in the lysosomal environment and enhancing the efficiency of gene transfection.[227] Considering the intricate enzymatic landscape within biological systems, some drug carriers are designed to be substrates for multiple enzymes.[228] This design strategy ensures that under varying physiological or pathological conditions, the presence and activity of any one of these enzymes can initiate drug release. Such a mechanism enhances the flexibility and adaptability of drug delivery systems.

6.2.2.4. Response release based on redox reactions. *In vivo*, different tissues, cells and cellular organelles have different redox environments. For example, the cytoplasm inside the cell is usually in a relatively reductive environment, while the extracellular environment and certain organelles (such as mitochondria) may have a high oxidation potential.[229] This difference in redox environment provides a physiological basis for designing redox sensitive drug delivery vectors.

The redox sensitivity of poly(amino acids) and polypeptide drug delivery carriers is mainly achieved by introducing specific redox sensitive groups. These groups can undergo biochemical structural changes under oxidation or reduction conditions, such as disulfide bonds (–S–S–). Disulfide bond is the most common redox sensitive group. In drug delivery vehicles, disulfide bonds can link molecular chains of poly(amino acids) or polypeptides, or they can be used to link drugs to carriers.[230] When the carrier enters the reducing environment within the cell (such as the cytoplasm), the disulfide bond is reduced and broken.[231] For example, a polypeptide nanocaller containing disulfide bonds encapsulates an anticancer drug. In the extracellular environment, the disulfide bonds remain stable and the drug is encapsulated inside the carrier. When the nanocarriers enter the cell through endocytosis, the disulfide bond breaks, the structure of the nanocarriers disintegrates, and the drug is released under the reducing environment of the cytoplasm. In addition, polyglutamic acid and polylysine containing disulfide bonds can be rapidly degraded in an intracellular reducing environment to release active drug molecules.[232] Hu *et al.*[233] developed a series of innovative poly (amino acid) materials that create drug-carrying NPs through physical encapsulation and chemical bonding.

Redox sensitive drug delivery vectors can effectively achieve intracellular targeted delivery. Due to the differences in the redox environment inside and outside the cell, this vector can remain stable outside the cell, while releasing drugs after entering the cell, especially in the reducing environment inside the cell. This is very important for drugs that need to play a role in the cell (such as gene drugs, protein drugs, *etc.*), which can improve the efficiency of drug release in the cell and avoid the drug being degraded or inactivated by early release outside the cell. The redox status of tumor tissues is also different from that of normal tissues. The redox balance within tumor cells is often disrupted and often has high levels of glutathione (GSH), an important reducing agent.[234] Taking advantage of this feature, redox sensitive poly(amino acids) or polypeptide carriers can target drug delivery into tumor cells for release. For example, drug carriers containing disulfide bonds are designed so that under the action of high concentration of GSH in tumor cells, disulfide bonds are broken and drugs are released, improving the killing effect of drugs on tumor cells while reducing the impact on normal tissues.[235]

6.2.3. Improve drug encapsulation efficiency and stability. Conventional drug delivery methods often provide inadequate protection for certain bioactive compounds. For instance, bioactive drugs, particularly proteins and nucleic acid-based therapeutics, are susceptible to enzymatic degradation within the body, which can occur prior to their arrival at the intended target cells. This degradation diminishes the therapeutic efficacy of these agents.[236] In contrast, poly(amino acids) and polypeptide carriers have been shown to enhance drug stability by either physically embedding the drugs or chemically binding to them.[104] The molecular architecture of these carriers offers a degree of protection against enzymatic degradation, thereby potentially improving the delivery and effectiveness of the therapeutic agents.

Poly(amino acids) and polypeptides possess the ability to self-assemble into nano-sized carriers, thereby enhancing the encapsulation capacity for pharmaceuticals.[237] By manipulating polymerization conditions or through strategic molecular design, the dimensions of these carriers can be accurately tailored. For instance, block copolymers derived from poly(L-glutamic acid) (PGA) and poly(L-lysine) (PLL) can spontaneously form nanomicelles in aqueous environments.[238] The nanomicelle's core is hydrophobic, allowing for the effective encapsulation of hydrophobic drugs, such as paclitaxel. Typically, these carriers exhibit particle sizes ranging from 10 to 1000 nm, which not only optimizes encapsulation efficiency but also facilitates the circulation and distribution of the carriers within biological systems. Furthermore, nano-sized

vectors can accumulate in tumor tissues due to the enhanced permeability and retention (EPR) effect.[239] The larger vascular endothelial spaces characteristic of tumor tissues enable these nanocarriers to infiltrate more readily and persist within the tumor environment, thereby facilitating localized drug release and indirectly enhancing the effective utilization of therapeutic agents.[240]

The incorporation of hydrophobic groups into poly(amino acids) or polypeptides has been shown to enhance the capacity for hydrophobic drug encapsulation.[241] Concurrently, the self-assembly properties of the carrier can be modified to further optimize drug encapsulation efficacy. For certain insoluble pharmaceuticals, such chemically modified carriers can significantly enhance both the solubility and stability of the drug.[242] For instance, the encapsulation of the poorly soluble anticancer agent camptothecin within a modified poly(amino acid) carrier not only increases the drug's encapsulation efficiency but also mitigates the rapid precipitation and degradation of the drug in aqueous environments.[243] In the case of protein therapeutics, a viable strategy involves conjugating them to polypeptide carriers *via* amide bonds. An illustrative example is the linkage of insulin to a poly(L-histidine)–poly(L-lysine) (PH–PLL) carrier through an amide bond, which not only safeguards insulin from proteolytic degradation but also facilitates its release in an appropriate milieu (such as an acidic intestinal environment or within cells) due to the pH-responsive characteristics of the carrier. This approach enhances both the encapsulation efficiency and stability of the drugs.

7. Pharmacological mechanisms of poly(amino acid) and peptide delivery systems

7.1. Mode of administration

Due to the specific structure of poly(amino acids) and peptides, their delivery must overcome the body's degradation and clearance mechanisms. Poly(amino acid) and peptide drug delivery systems can be utilized in various modes, including oral, intravenous, intramuscular, pulmonary, ocular, and transdermal routes. The most suitable delivery strategy is selected based on the type of disease types and therapeutic needs. The selection and optimization of each delivery method depend on the physicochemical properties of the drug as well as the requirements of the targeted therapy. When combined with the design of the carrier system, these factors can significantly enhance the drug's bioavailability and therapeutic efficacy.

7.1.1. Oral administration. Oral drug delivery is the most commonly utilized method and offers the best patient compliance. However, due to the biomacromolecular structure of poly(amino acids) and peptides, these compounds are prone to enzymatic degradation and are adversely affected by the acidic environment of the gastrointestinal tract, leading to low drug bioavailability (Fig. 14). Consequently, oral delivery presents significant challenges. Nevertheless, the stability and absorption of drugs can be enhanced through improved carrier design and delivery systems. Strategies include the use of

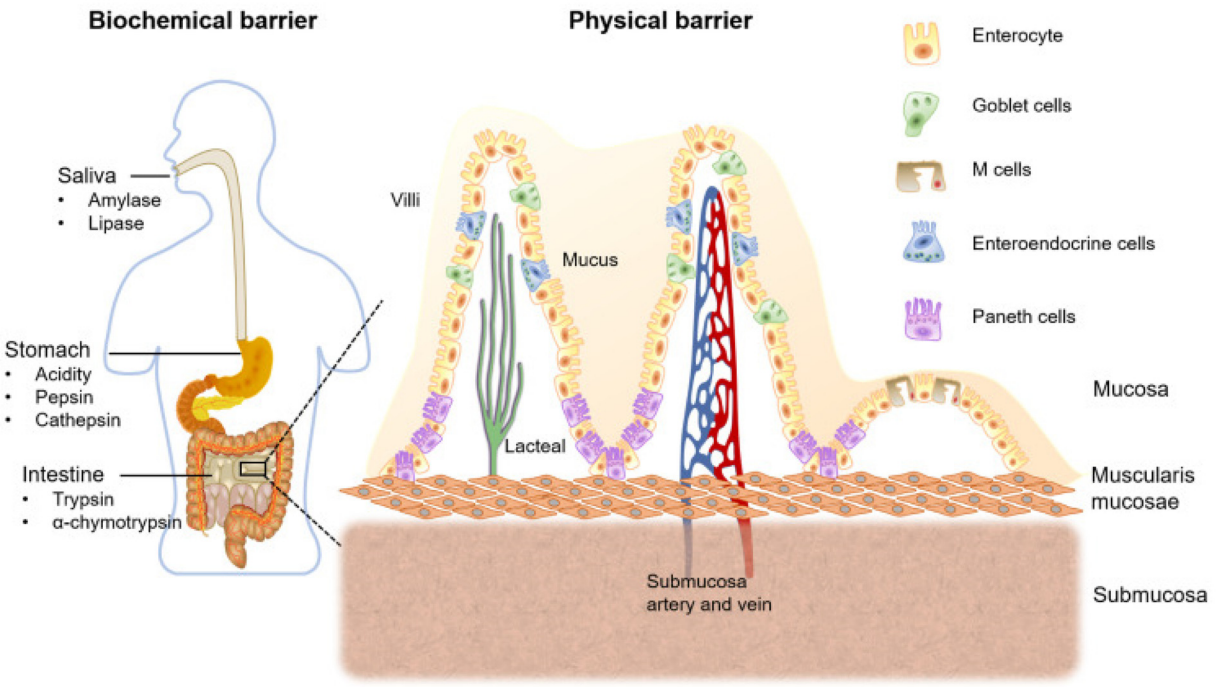

Fig. 14 Biochemical and physical barriers to oral drug delivery, and intestinal mucosal structures with major intestinal cell types.[248] Copyright 2022, Theranostics.

chemical modifications of poly(amino acids) and peptides to increase their resistance to enzymatic degradation, as well as encapsulating the drug within nanocarriers to shield it from degradation in the gastrointestinal tract.[244] Li *et al.*[245] developed a novel self-assembled core–shell nanosystem (CA-NP) aimed at addressing three significant challenges associated with oral drug delivery: gastric acid degradation, mucus clearance, and intestinal epithelial impermeability. The nanosystem features a shell composed of citric acid crosslinked carboxymethyl cellulose (CA-CMC) that encases the core nanoparticles (HA-NPs), thereby preserving the structural integrity of CA-NPs within the gastric environment. As the CA-NPs transit through the stomach, the CA-CMC shell undergoes gradual degradation, facilitating the release of the core HA-NPs in the intestinal tract. The HA-NPs, characterized by a high density of hydrophilic groups and mannose side chains, exhibit rapid penetration through the mucosal layer and effectively utilize transcellular glucose transporter (GLUT) mechanisms to mediate cellular transport. This process also induces a reversible opening of tight junctions (TJ) through the action of CA-CMC. In addition, the oral administration of nanoemulsions and intestinal absorption enhancers serves as effective strategies.[246] Currently, oral delivery systems for certain insulin derivatives have entered clinical trials, utilizing encapsulation with liposomes and NPs, and are demonstrating promising results.[247]

7.1.2. Intravenous administration (IV). Intravenous drug delivery is the most method of administration, effectively bypassing the first-pass metabolism of the gastrointestinal tract and liver, and introducing poly(amino acid) and peptide drugs directly into the systemic circulation. This route is particularly advantageous for delivering large molecules efficiently. However, the stability of the drug in the circulation and its clearance rate must also be taken into account. Consequently, common strategies for intravenous drug delivery include the design of long-acting sustained-release systems, such as the use of poly(amino acid) or peptide-modified liposomes and NPs, which aim to prolong the drug's half-life of the drug in the bloodstream and to reduce the rapid clearance by the kidney.[249] In addition, surface PEGylation (polyethylene glycolisation) is a widely used technique to enhance the blood stability of drugs. For instance, peptide drugs like Leuprolide, which is utilized in the treatment of prostate cancer and endometriosis, are administered through long-acting injections. The use of extended-release microsphere systems further contributes to prolonged drug efficacy.[250]

7.1.3. Intramuscular administration (IM). Intramuscular injection is a common route for administering poly(amino acids) and peptides, allowing for absorption into the bloodstream through the capillaries of muscle tissue. This method is typically employed for vaccines, hormones, and protein-based drugs. For example, growth hormone peptides are often administered intramuscularly to maintain stable blood levels and achieve long-lasting therapeutic effects. Compared to intravenous drug delivery, intramuscular injection is easier to perform and does not require specialized equipment. However, the absorption of the drug occurs at a slower rate. Consequently, drugs intended for intramuscular injection are usually formulated as long-acting preparations, where the release rate is regulated by a slow-release carrier or implant.[251] The release rate is controlled by a slow-release carrier or implant. Poly(amino acids) and peptides can be combined with biocompatible polymers, such as PLGA and PLA, to create microspheres or gel carriers that ensure a gradual release of the drug at the injection site.[252]

7.1.4. Pulmonary administration. Pulmonary drug delivery delivers involves administering drugs to the lungs *via* inhalation, facilitating rapid systemic absorption or localized treatment. Consequently, pulmonary delivery systems are typically designed as sprays, dry powder inhalers, or aerosols. These systems must ensure that the particle size is appropriate for alveolar absorption, generally within the range of 1–5 μm. This method of delivery circumvents gastrointestinal and hepatic degradation, making it a vital route for administering peptide and poly(amino acids) drugs, particularly in the management of respiratory diseases. The stability and absorption efficiency of drugs in the lungs can be enhanced by modifying peptides or poly(amino acids) or by employing protective carriers such as liposomes or NPs. For example, pulmonary delivery systems for specific peptides, such as insulin, have been developed in various dry powder inhaler formats for the non-invasive treatment of diabetes mellitus.[253]

7.1.5. Ocular administration. The treatment of ocular diseases typically necessitates topical administration, and the delivery of peptides and poly(amino acids) poses challenges due to the eye's barrier functions, including barriers. Ocular drug delivery can be achieved through corneal, conjunctival, or vitreous injection.[254] Poly(amino acids) and peptide drugs are primarily utilized for the treatment of retinopathy, macular degeneration, glaucoma, and other ophthalmic diseases. Vitreous injection involves administering drugs directly into the vitreous cavity of the eye, ensuring efficient delivery to the retinal area. To prolong the retention time of the drug within the vitreous cavity, slow-release microspheres or gels are often employed, facilitating long-lasting therapeutic effects.[255] Conversely, corneal injections are indicated for the treatment of corneal diseases or infections. To enhance drug absorption in the cornea, NPs, emulsions, or bioadhesive gels can be utilized to increase the drug's retention time of the drug on the corneal surface.[256] Peptide drugs, such as Ranibizumab (an anti-VEGF peptide), are commonly administered *via* vitreous injection to inhibit pathological angiogenesis associated with macular degeneration.[257]

7.1.6. Transdermal administration. Transdermal drug delivery offer distinct advantages, including high patient compliance and the ability to bypass the first-pass metabolism. Although peptides and poly(amino acids) are large molecules that inherently struggle to penetrate the skin barrier, the transdermal delivery of these molecules can be achieved through specific enhancement techniques, such as electroporation and ultrasound-mediated drug delivery.[258] By employing these techniques in combination with poly(amino acid) or peptide-

based therapeutics, the efficiency of subcutaneous delivery can be significantly improved. Clinical trials for insulin patches and growth factor-based peptide transdermal patches have further underscored the potential of transdermal drug delivery, highlighting its promise in advancing therapeutic options for patients.[259]

7.2. Cellular uptake mechanisms

In poly(amino acids) and peptide-based drug delivery systems, the cellular uptake mechanism is a pivotal factor influencing the overall effectiveness and therapeutic efficacy of drug delivery. The uptake mechanism of the drug carrier plays a key role in determining whether the drug can successfully enter target cells and thereby exert its intended therapeutic effect.

7.2.1. Receptor-mediated endocytosis. Receptor-mediated endocytosis is a widely utilized cellular uptake pathway for peptide and poly(amino acids) drug carriers. In this mechanism, peptide or poly(amino acids) NPs trigger endocytosis by binding surface-modified targeted peptides binding to specific cell surface receptors.[260] This pathway is characterized by its high specificity and plays a crucial role in targeted therapy. For example, RGD peptides can target integrin receptors,[261] while TSL peptides can target the folate receptors.[262] Upon receptor binding, the NPs enter the cell and are transported *via* vesicles or early endosomes. Fig. 15 shows the common absorption pathways of NP.

One of the common pathways for cellular uptake of external substances is cytophagy, which is mediated by lattice proteins. This process involves the formation of vesicles enveloped in lattice proteins at the cell membrane. Poly(amino acids) and peptide drug carriers can efficiently enter the cell through this pathway. When these drug carriers bind to receptors or membrane proteins on the cell surface, the lattice proteins aggregate to form membrane-coated vesicles, which are subsequently internalized into the cell.[263] After entering the cell, these endocytic vesicles are transported *via* endosomes and can eventually be degraded or translocated to other organelles. Grid protein-mediated cytophagy is highly selective and regulated, making it suitable for targeted drug delivery.[264] This mechanism is particularly effective in delivering therapies against cancer cells. By designing peptide or poly(amino acids) drug carriers that specifically bind to cancer cell receptors, efficient intracellular entry can be achieved.

7.2.2. Macrocystin effects. The entry of poly(amino acids) and peptides into cells *via* the megacytosis mechanism represents a significant uptake pathway in drug delivery systems, with a wide range of applications, particularly in cancer therapy, gene delivery, and the administration of macromol-

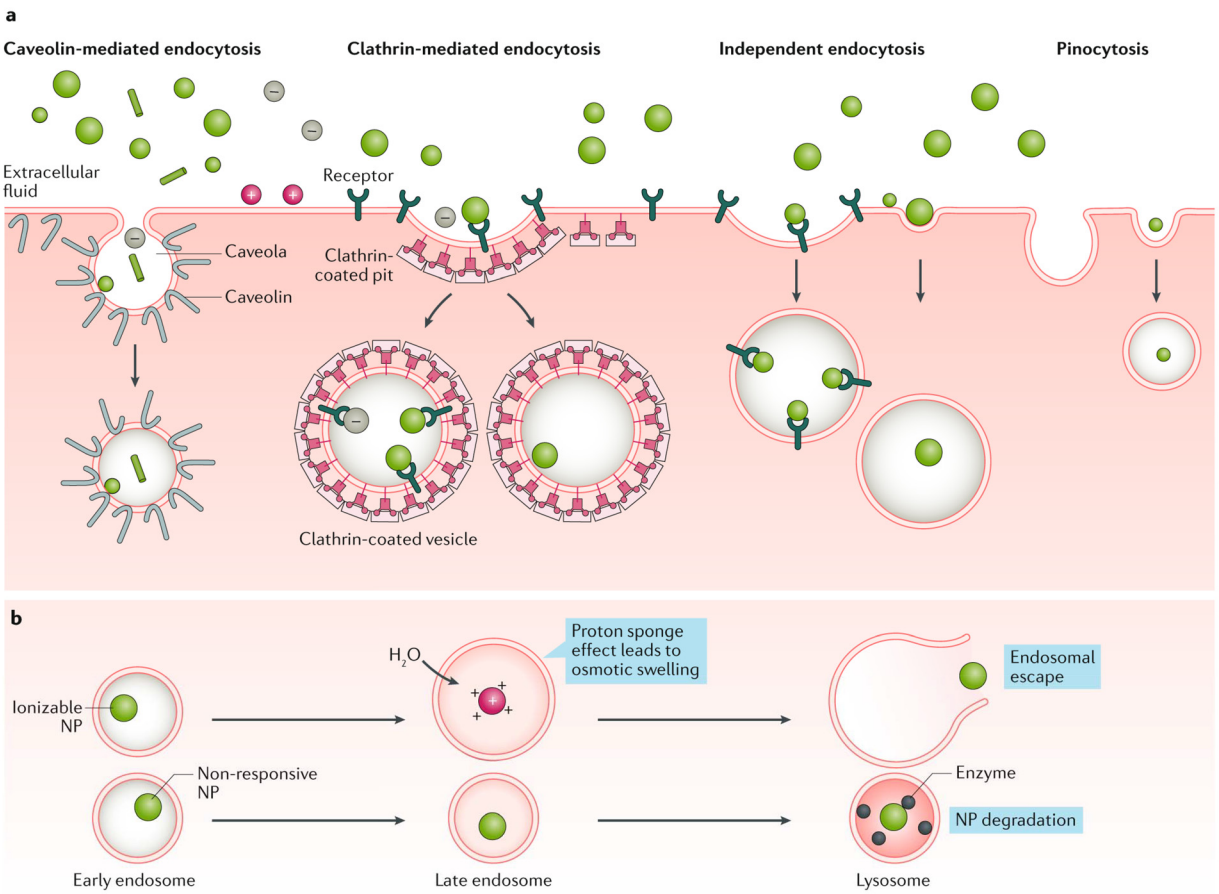

Fig. 15 Common uptake pathways of NP. (a) After interacting with the cell surface, NPs is absorbed through various endocytosis or pinocytosis. (b) Most NPs must escape from various vesicle cavities or endosomes before acidification.[265] Copyright 2021, Springer Nature.

ecular drugs. Megacytosis is a non-specific, energy-dependent uptake process, and the unique properties of poly(amino acids) and peptides enable them to effectively utilize this mechanism for delivery.[266] For example, poly(amino acids) and peptide molecules can induce rearrangements of cell membranes, leading to the formation of larger cytosolic vesicles due to their size and conformation. These large molecular structures can be efficiently recognized and internalized by the megacytic mechanism, especially in tumor cells, which exhibit a heightened uptake capacity. Furthermore, the surface charge of poly(amino acids) and peptides can influence their interaction with the cell membrane.[267] For example, cationized polypeptides enhance the interactions with negatively charged cell membranes, promoting cell membrane extension and facilitating giant cell drinking. In addition, the ratio of hydrophobicity to hydrophilicity in poly(amino acids) and polypeptides affects their uptake through the megacytic pathway.[268] Moderate hydrophobicity enhances binding to the cell membrane, which subsequently triggers membrane rearrangement. Moreover, poly(amino acid) and peptide delivery systems can also induce macrocytic drinking by activating cellular signaling pathways.[16]

7.2.3. Caveolae-mediated cytophagy. Caveolae-mediated cytophagy is the mechanism by which a drug carrier enters a cell by binding to a small depressions in the cell membrane known as caveolae.[269] These structures are rich in cholesterol and sphingolipids and are widely distributed in certain cell types, particularly endothelial cells.[270] The process of cytophagy is gentle and circumvents lysosomal degradation, making it suitable for delivering drugs that are sensitive to acidic environments or enzymatic degradation. Peptide drug delivery systems can effectively enter endothelial cells or other specific cell types by designing appropriate structures that bind to caveolae.

8. Progress in the clinical use of poly (amino acids) and peptides

The application of poly(amino acids) and peptides in clinical medicine is rapidly advancing and is increasingly recognized as a promising approach for treating a wide range of diseases.[271] The high specificity and low toxicity of these biomolecules demonstrate significant potential in drug development and therapeutic applications.

Antimicrobial peptides (AMPs) exhibit potent antimicrobial activity as integral components of the natural immune system.[272] In contrast to conventional antibiotics, antimicrobial peptides are less prone to drug resistance and can swiftly eliminate bacteria, fungi, and viruses.[273] In recent years, significant advancements have been made in the prevention and treatment of infections using antimicrobial peptides. For example, gramicidin[274] and polymyxin B[275] have been extensively utilized to treat ocular and dermal infections. Additionally, research is ongoing to develop novel antimicrobial peptides to address the challenges posed by multidrug resistant strains.

Progress has also been made in the application of peptides for metabolic diseases. Insulin, a classical peptide hormone, is widely in the treatment of diabetes. Ongoing research has led to the development of novel long-acting insulin analogs that enhance the stability of glycemic control and improve patient compliance.[259] GLP-1 receptor agonists, representing a new generation of antidiabetic medications, have demonstrated promising glucose-lowering effects as well as cardiovascular protective benefits.[276] In the treatment of neurological disorders, peptides have also shown significant potential. For example, neuropeptide Y (NPY) and its receptor antagonists are currently being investigated for their efficacy in treating anxiety and depression.[277] In addition, Aβ peptide vaccines and inhibitors for Alzheimer's disease are undergoing clinical trials aimed at slowing or halting the progression of the disease.[278]

In conclusion, advancements in the clinical application of poly(amino acids) and peptides indicate that these compounds are poised to become significant therapeutic options for a wide range of diseases.[279] Although challenges such as stability, delivery systems, and production costs remain, the clinical use of poly(amino acids) and peptide drugs is expected to become increasingly promising as technology progresses and research efforts intensify.

9. Outlook and conclusions

With the continuous advancement of biomedical and materials science, poly(amino acids), peptides, and their derivatives have demonstrated significant potential in the field of drug delivery. Future research will further explore the ability of these materials to enhance drug efficacy, reduce side effects, and achieve precision therapy.

One critical area of future development lies in improving the targeting specificity and controlled release capabilities of poly(amino acid) and peptide-based drug carriers. While these carriers have made progress in achieving targeted delivery and controlled release through the incorporation of targeting ligands and responsive design elements, there remains a need for greater precision and efficiency. By optimizing the selection of ligands and improving the structural design of the carriers, it is possible to develop more effective systems that achieve enhanced targeted delivery and more precise release profiles, thereby improving therapeutic outcomes.

Another promising direction for future research is the development of multifunctional carriers. These carriers not only transport and release drugs but also integrate additional functions such as diagnosis, imaging, and therapy. For instance, smart, responsive carriers can be designed to respond to physiological changes in the body, such as pH, temperature, or enzyme activity, allowing real-time monitoring of drug release and therapeutic efficacy. Additionally, these multifunctional systems may enable the combination of

various therapeutic modalities, such as photothermal therapy or immunotherapy, offering synergistic effects and improving treatment efficiency.

Despite the favorable biocompatibility of poly(amino acids) and peptides, their *in vivo* metabolic pathways and long-term safety profiles need further investigation. Ensuring that these drug delivery systems are non-toxic and exhibit low immunogenicity is crucial for their successful clinical application. Therefore, future studies should focus on understanding the impact of carrier degradation products on biological systems and on optimizing the chemical structure of these materials to ensure their safety and degradability *in vivo*.

Scaling up the production of poly(amino acid) and peptide-based carriers for clinical use presents another significant challenge. Much of the current research is still at the laboratory stage, and achieving large-scale production with standardized preparation methods will be essential for clinical translation. Developing efficient, cost-effective synthesis and production processes, while ensuring consistent and reproducible product quality, will be critical. Furthermore, extensive preclinical and clinical studies are needed to validate the safety and efficacy of these systems.

Personalized and precision medicine represents an exciting frontier for the application of poly(amino acids) and peptides in drug delivery. By integrating patient-specific genomic, proteomic, and other big data analyses, drug delivery systems can be tailored to meet individual patient needs, enabling precision therapies. This approach would allow customization of the type of drug carrier, dosage, and release method based on the patient's specific condition and physiological state, thus improving therapeutic outcomes and minimizing adverse effects. In addition to the current materials, there is a need to explore new materials and structures to meet the diverse requirements of drug delivery. Advances in nanotechnology, supramolecular chemistry, and biomaterials science provide novel ideas for designing and synthesizing drug carriers with unique properties. For example, self-assembly techniques and nanomaterials may be employed to construct carriers with higher drug-loading capacities and improved stability.[280,281]

Although significant progress has been made in the field of drug delivery, poly(amino acids), peptides, and their derivatives still face several challenges. Drug delivery systems must remain stable in complex *in vivo* environments and release their cargo under specific conditions, which presents a major challenge for the design and optimization of carriers. Additionally, overcoming biological barriers, such as the blood–brain barrier, is crucial for enhancing the effectiveness of drug delivery systems. Ensuring the long-term safety and minimizing the immune responses of these carriers is also a critical issue that requires further investigation.

In conclusion, while there are many challenges ahead, poly(amino acids), peptides, and their derivatives hold great promise for advancing drug delivery technologies. Continued research and innovation will help overcome existing technological barriers, leading to more efficient and safer drug delivery systems that can provide new solutions for clinical treatment. The future of this field will rely on interdisciplinary collaboration, with synergistic contributions from materials science, pharmacy, biology, and clinical medicine.

Author contributions

Huilin Yuan and Mingxia Jiang wrote the original draft, Huapan Fang and Huayu Tian designed the project, reviewed and revised the original manuscript. All authors have read and agreed to the published version of the manuscript.

Data availability

Data availability is not applicable to this article as no new data were created or analyzed in this study.

Conflicts of interest

The authors declare no conflict of interest.

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References

1 S. Hoelder, P. A. Clarke and P. Workman, *Mol. Oncol.*, 2012, **6**, 155–176.
2 H. Cui, M. J. Webber and S. I. Stupp, *Pept. Sci.*, 2010, **94**, 1–18.
3 M. Jiang, W. Chen, W. Yu, Z. Xu, X. Liu, Q. Jia, X. Guan and W. Zhang, *ACS Appl. Mater. Interfaces*, 2021, **13**, 43963–43974.
4 A. Sood and R. Panchagnula, *Chem. Rev.*, 2001, **101**, 3275–3304.
5 H. Li, Y. Yang, W. Hong, M. Huang, M. Wu and X. Zhao, *Signal Transduction Targeted Ther.*, 2020, **5**, 1.
6 H. Fang, Z. Guo, L. Lin, J. Chen, P. Sun, J. Wu, C. Xu, H. Tian and X. Chen, *J. Am. Chem. Soc.*, 2018, **140**, 11992–12000.
7 L. Xu, Z. Shao, X. Fang, Z. Xin, S. Zhao, H. Zhang, Y. Zhang, W. Zheng, X. Yu, Z. Zhang and L. Sun, *Exploration*, 2024, 20230165.

8 X. Liu, F. Wu, Y. Ji and L. Yin, *Bioconjugate Chem.*, 2018, **30**, 305–324.
9 H. Fang, L. Chen, Z. Deng, Y. Gao, Y. Yang, Q. Chen and Z. Liu, *ACS Nano*, 2023, **17**, 17595–17595.
10 H. Fang, Z. Guo, J. Chen, L. Lin, Y. Hu, Y. Li, H. Tian and X. Chen, *Nat. Commun.*, 2021, **12**, 6742.
11 S. Antimisiaris, A. Marazioti, M. Kannavou, E. Natsaridis, F. Gkartziou, G. Kogkos and S. Mourtas, *Adv. Drug Delivery Rev.*, 2021, **174**, 53–86.
12 S. Dragojevic, J. S. Ryu and D. Raucher, *Molecules*, 2015, **20**, 21750–21769.
13 R. Liu, C. Luo, Z. Pang, J. Zhang, S. Ruan, M. Wu, L. Wang, T. Sun, N. Li and L. Han, *Chin. Chem. Lett.*, 2023, **34**, 107518.
14 S. Hossen, M. K. Hossain, M. Basher, M. Mia, M. Rahman and M. J. Uddin, *J. Adv. Res.*, 2019, **15**, 1–18.
15 A. Akhtar, A. Andleeb, T. S. Waris, M. Bazzar, A.-R. Moradi, N. R. Awan and M. Yar, *J. Controlled Release*, 2021, **330**, 1152–1167.
16 H. Fang, Y. Wu, L. Chen, Z. Cao, Z. Deng, R. Zhao, L. Zhang, Y. Yang, Z. Liu and Q. Chen, *ACS Nano*, 2023, **17**, 4748–4763.
17 H. Fang, L. Chen, Z. Deng, Y. Gao, Y. Yang, Q. Chen and Z. Liu, *ACS Nano*, 2023, **17**, 1128–1143.
18 M. Jiang, W. Chen, Y. Sun, J. Zeng, L. Ma, J. Gong, X. Guan, K. Lu and W. Zhang, *Int. J. Biol. Macromol.*, 2023, **242**, 125223.
19 H. Fang, L. Zhang, Y. Wu, L. Chen, Z. Deng, Z. Zheng, Y. Wang, Y. Yang and Q. Chen, *Chem. Eng. J.*, 2024, **498**, 155781.
20 L. Liu, Y. Pan, C. Zhao, P. Huang, X. Chen and L. Rao, *ACS Nano*, 2023, **17**, 3225–3258.
21 J. Lv, C. Liu, K. Lv, H. Wang and Y. Cheng, *Sci. China Mater.*, 2020, **63**, 620–628.
22 J. V. González-Aramundiz, M. V. Lozano, A. Sousa-Herves, E. Fernandez-Megia and N. Csaba, *Expert Opin. Drug Delivery*, 2012, **9**, 183–201.
23 E. Liarou, S. Varlas, D. Skoulas, C. Tsimblouli, E. Sereti, K. Dimas and H. Iatrou, *Prog. Polym. Sci.*, 2018, **83**, 28–78.
24 J. Martin, A. Desfoux, J. Martinez, M. Amblard, A. Mehdi, L. Vezenkov and G. Subra, *Prog. Polym. Sci.*, 2021, **115**, 101377.
25 S. Mallakpour and M. Dinari, *J. Macromol. Sci., Part A:Pure Appl. Chem.*, 2011, **48**, 644–679.
26 H. Xu, Q. Yao, C. Cai, J. Gou, Y. Zhang, H. Zhong and X. Tang, *J. Controlled Release*, 2015, **199**, 84–97.
27 L. Zhao, N. Li, K. Wang, C. Shi, L. Zhang and Y. Luan, *Biomaterials*, 2014, **35**, 1284–1301.
28 M. J. Webber and E. T. Pashuck, *Adv. Drug Delivery Rev.*, 2021, **172**, 275–295.
29 A. W. Du and M. H. Stenzel, *Biomacromolecules*, 2014, **15**, 1097–1114.
30 B. B. Mendes, J. Conniot, A. Avital, D. Yao, X. Jiang, X. Zhou, N. Sharf-Pauker, Y. Xiao, O. Adir and H. Liang, *Nat. Rev. Methods Primers*, 2022, **2**, 24.
31 H. J. Vaughan, J. J. Green and S. Y. Tzeng, *Adv. Mater.*, 2020, **32**, 1901081.
32 Y. Liu, C.-F. Xu, S. Iqbal, X.-Z. Yang and J. Wang, *Adv. Drug Delivery Rev.*, 2017, **115**, 98–114.
33 T. D. Brown, K. A. Whitehead and S. Mitragotri, *Nat. Rev. Mater.*, 2020, **5**, 127–148.
34 H. B. Gdr, N. Sharon and E. Australia, *Pure Appl. Chem.*, 1984, **56**, 595–624.
35 R. Augustine, N. Kalva, H. A. Kim, Y. Zhang and I. Kim, *Molecules*, 2019, **24**, 2961.
36 K. Bauri, S. G. Roy, S. Pant and P. De, *Langmuir*, 2013, **29**, 2764–2774.
37 J. Rodríguez-Hernández and S. Lecommandoux, *J. Am. Chem. Soc.*, 2005, **127**, 2026–2027.
38 Z. Hu, J. Wang, S. Han, J. Hu and A. Reheman, *New J. Chem.*, 2022, **46**, 19888–19899.
39 P. Wu, H. Zhao, X. Gou, X. Wu, S. Zhang, G. Deng and Q. Chen, *Int. J. Nanomed.*, 2019, 4059–4069.
40 C. Bonduelle, *Polym. Chem.*, 2018, **9**, 1517–1529.
41 E. M. Cagil, *J. Mol. Struct.*, 2020, **1217**, 128382.
42 R.-S. Lee, K.-Y. Peng, S.-W. Wang and Y.-Z. Li, *Polym. J.*, 2014, **46**, 710–721.
43 Z. Wang, M. Zhuang, T. Sun, X. Wang and Z. Xie, *Bioorg. Med. Chem. Lett.*, 2017, **27**, 2493–2496.
44 Y.-J. Li, X.-F. Gong, D. Wang, Z. Yang, H. Cao and L. Wang, *Acta Polym. Sin.*, 2022, **53**, 445–456.
45 S. Guo, J. Wang, Q. Wang, J. Wang, S. Qin and W. Li, *Heliyon*, 2024, **10**, e26009.
46 S. Majumdar and T. J. Siahaan, *Med. Res. Rev.*, 2012, **32**, 637–658.
47 A. David, *Adv. Drug Delivery Rev.*, 2017, **119**, 120–142.
48 M. F. Attia, N. Anton, J. Wallyn, Z. Omran and T. F. Vandamme, *J. Pharm. Pharmacol.*, 2019, **71**, 1185–1198.
49 F. Danhier, O. Feron and V. Préat, *J. Controlled Release*, 2010, **148**, 135–146.
50 V. Torchilin, *Adv. Drug Delivery Rev.*, 2011, **63**, 131–135.
51 Y. Wang, T. Deng, X. Liu, X. Fang, Y. Mo, N. Xie, G. Nie, B. Zhang and X. Fan, *Int. J. Nanomed.*, 2024, 6253–6277.
52 P. Mi, *Theranostics*, 2020, **10**, 4557.
53 A. Komin, L. Russell, K. Hristova and P. Searson, *Adv. Drug Delivery Rev.*, 2017, **110**, 52–64.
54 J. Zhang, Z.-F. Yuan, Y. Wang, W.-H. Chen, G.-F. Luo, S.-X. Cheng, R.-X. Zhuo and X.-Z. Zhang, *J. Am. Chem. Soc.*, 2013, **135**, 5068–5073.
55 V. F. Cardoso, D. M. Correia, C. Ribeiro, M. M. Fernandes and S. Lanceros-Méndez, *Polymers*, 2018, **10**, 161.
56 M. Hussain, R. A. Naqvi, N. Abbas, S. M. Khan, S. Nawaz, A. Hussain, N. Zahra and M. W. Khalid, *Polymers*, 2020, **12**, 323.
57 N. C. Paxton, M. C. Allenby, P. M. Lewis and M. A. Woodruff, *Eur. Polym. J.*, 2019, **118**, 412–428.
58 M. Zare, E. R. Ghomi, P. D. Venkatraman and S. Ramakrishna, *J. Appl. Polym. Sci.*, 2021, **138**, 50969.
59 S. Wendels and L. Avérous, *Bioact. Mater.*, 2021, **6**, 1083–1106.

60 U. Ali, K. J. B. A. Karim and N. A. Buang, *Polym. Rev.*, 2015, **55**, 678–705.
61 A. R. Mazo, S. Allison-Logan, F. Karimi, N. J.-A. Chan, W. Qiu, W. Duan, N. M. O'Brien-Simpson and G. G. Qiao, *Chem. Soc. Rev.*, 2020, **49**, 4737–4834.
62 K. Numata, *Polym. J.*, 2015, **47**, 537–545.
63 M. Kunioka, *Appl. Microbiol. Biotechnol.*, 1997, **47**, 469–475.
64 Z. Li, Y. Zheng, J. Yan, Y. Yan, C. Peng, Z. Wang, H. Liu, Y. Liu, Y. Zhou and M. Ding, *ChemBioChem*, 2023, **24**, e202300132.
65 H. Lu, J. Wang, Z. Song, L. Yin, Y. Zhang, H. Tang, C. Tu, Y. Lin and J. Cheng, *Chem. Commun.*, 2014, **50**, 139–155.
66 S. Zhang, W. Fu and Z. Li, *Polym. Chem.*, 2014, **5**, 3346–3351.
67 S. S. Gupta, V. Mishra, M. D. Mukherjee, P. Saini and K. R. Ranjan, *Int. J. Biol. Macromol.*, 2021, **188**, 542–567.
68 J. Huang and A. Heise, *Chem. Soc. Rev.*, 2013, **42**, 7373–7390.
69 M. Thompson and C. Scholz, *Nanomaterials*, 2021, **11**, 1119.
70 A. Douka, S. Vouyiouka, L.-M. Papaspyridi and C. D. Papaspyrides, *Prog. Polym. Sci.*, 2018, **79**, 1–25.
71 S. P. Schwendeman, M. Cardamone, A. Klibanov, R. Langer and M. R. Brandon, in *Microparticulate systems for the delivery of proteins and vaccines*, CRC Press, 2020, pp. 1–49.
72 S. Kobayashi, H. Uyama and S. Kimura, *Chem. Rev.*, 2001, **101**, 3793–3818.
73 M. Saini, A. Kashyap, S. Bindal, K. Saini and R. Gupta, *Front. Microbiol.*, 2021, **12**, 641251.
74 K. Yazawa and K. Numata, *Molecules*, 2014, **19**, 13755–13774.
75 A. Boto, C. C. González, D. Hernández, I. Romero-Estudillo and C. J. Saavedra, *Org. Chem. Front.*, 2021, **8**, 6720–6759.
76 Y. Qiu, D. Xu, P. Lei, S. Li and H. Xu, *Trends Biotechnol.*, 2024, **42**, 310–325.
77 F. Guzmán, S. Barberis and A. Illanes, *Electron. J. Biotechnol.*, 2007, **10**, 279–314.
78 S. Thapa, H. Li, J. Ohair, S. Bhatti, F.-C. Chen, K. A. Nasr, T. Johnson and S. Zhou, *Mol. Biotechnol.*, 2019, **61**, 579–601.
79 J. Chapman, A. E. Ismail and C. Z. Dinu, *Catalysts*, 2018, **8**, 238.
80 F. B. Oppermann-Sanio and A. Steinbüchel, *Naturwissenschaften*, 2002, **89**, 11–22.
81 S. Chatterjee and S. Yadav, *Life*, 2019, **9**, 25.
82 M. Mindt, T. Walter, P. Kugler and V. F. Wendisch, *Biotechnol. J.*, 2020, **15**, 1900451.
83 Z. Luo, Y. Guo, J. Liu, H. Qiu, M. Zhao, W. Zou and S. Li, *Biotechnol. Biofuels*, 2016, **9**, 1–12.
84 D. Kour, K. L. Rana, S. Thakur, S. Sharma, N. Yadav, A. A. Rastegari, A. N. Yadav and A. K. Saxena, in *New and future developments in microbial biotechnology and bioengineering*, Elsevier, 2019, pp. 35–75.
85 H. Goodarzi, N. Torabi, H. S. Najafabadi and M. Archetti, *Gene*, 2008, **407**, 30–41.
86 A. Berlec and B. Štrukelj, *J. Ind. Microbiol. Biotechnol.*, 2013, **40**, 257–274.
87 T. Kotnik, W. Frey, M. Sack, S. H. Meglič, M. Peterka and D. Miklavčič, *Trends Biotechnol.*, 2015, **33**, 480–488.
88 D. G. Yansura and D. J. Henner, *Proc. Natl. Acad. Sci. U. S. A.*, 1984, **81**, 439–443.
89 N. Stephanopoulos, J. H. Ortony and S. I. Stupp, *Acta Mater.*, 2013, **61**, 912–930.
90 E. De Santis and M. G. Ryadnov, *Chem. Soc. Rev.*, 2015, **44**, 8288–8300.
91 S. Zhang, *Biotechnol. Adv.*, 2002, **20**, 321–339.
92 M. R. Ghadiri, J. R. Granja, R. A. Milligan, D. E. McRee and N. Khazanovich, *Nature*, 1993, **366**, 324–327.
93 D. N. Woolfson, G. J. Bartlett, M. Bruning and A. R. Thomson, *Curr. Opin. Struct. Biol.*, 2012, **22**, 432–441.
94 K. Tsuchiya and K. Numata, *Macromol. Biosci.*, 2017, **17**, 1700177.
95 S. B. Bankar, P. R. Nimbalkar, P. V. Chavan and R. S. Singhal, *Role of materials science in food bioengineering*, 2018, 381–412.
96 C. Cai, J. Lin, Y. Lu, Q. Zhang and L. Wang, *Chem. Soc. Rev.*, 2016, **45**, 5985–6012.
97 K. Tao, A. Levin, L. Adler-Abramovich and E. Gazit, *Chem. Soc. Rev.*, 2016, **45**, 3935–3953.
98 J. C. Jiménez-Cruz, R. Guzmán-Mejía, P. Navarro-Santos, S. García-Zavala, R. Herrera-Bucio, H. A. García-Gutiérrez and J. A. Aviña-Verduzco, *J. Mol. Struct.*, 2023, **1294**, 136354.
99 J. Shen, Y. Chen, X. Li, X. Zhou and Y. Ding, *Int. J. Biol. Macromol.*, 2024, **270**, 131758.
100 T. Miao, J. Wang, Y. Zeng, G. Liu and X. Chen, *Adv. Sci.*, 2018, **5**, 1700513.
101 N. Habibi, N. Kamaly, A. Memic and H. Shafiee, *Nano Today*, 2016, **11**, 41–60.
102 G. Liu, J. F. Lovell, L. Zhang and Y. Zhang, *Int. J. Mol. Sci.*, 2020, **21**, 6380.
103 M. Karimi, A. Ghasemi, P. S. Zangabad, R. Rahighi, S. M. M. Basri, H. Mirshekari, M. Amiri, Z. S. Pishabad, A. Aslani and M. Bozorgomid, *Chem. Soc. Rev.*, 2016, **45**, 1457–1501.
104 A. Varanko, S. Saha and A. Chilkoti, *Adv. Drug Delivery Rev.*, 2020, **156**, 133–187.
105 M. Frenkel-Pinter, M. Samanta, G. Ashkenasy and L. J. Leman, *Chem. Rev.*, 2020, **120**, 4707–4765.
106 J. S. Suk, Q. Xu, N. Kim, J. Hanes and L. M. Ensign, *Adv. Drug Delivery Rev.*, 2016, **99**, 28–51.
107 F. Oroojalian, F. Charbgoo, M. Hashemi, A. Amani, R. Yazdian-Robati, A. Mokhtarzadeh, M. Ramezani and M. R. Hamblin, *J. Controlled Release*, 2020, **321**, 442–462.
108 D. Agyei, K.-X. Tan, S. Pan, C. C. Udenigwe and M. K. Danquah, *in Peptide applications in biomedicine, biotechnology and bioengineering*, Elsevier, 2018, pp. 231–251.
109 L. Diao and B. Meibohm, *Clin. Pharmacokinet.*, 2013, **52**, 855–868.

110 J. Thundimadathil, *Drug Discovery*, 2019, 503–530.
111 I. W. Hamley, *Biomacromolecules*, 2014, **15**, 1543–1559.
112 Y. Wu, D. Zhong, Y. Li, H. Wu, H. Zhang, H. Mao, J. Yang, K. Luo, Q. Gong and Z. Gu, *Nanoscale*, 2021, **13**, 4887–4898.
113 Z. Jiang, X. Feng, H. Zou, W. Xu and X. Zhuang, *Bioact. Mater.*, 2021, **6**, 2688–2697.
114 S. Cavalli, F. Albericio and A. Kros, *Chem. Soc. Rev.*, 2010, **39**, 241–263.
115 Y. Shi, A. Lu, X. Wang, Z. Belhadj, J. Wang and Q. Zhang, *Acta Pharm. Sin. B*, 2021, **11**, 2396–2415.
116 D. Zhi, Y. Bai, J. Yang, S. Cui, Y. Zhao, H. Chen and S. Zhang, *Adv. Colloid Interface Sci.*, 2018, **253**, 117–140.
117 B. Yang, A. Gomes Dos Santos, S. Puri, A. Bak and L. Zhou, *Expert Opin. Drug Delivery*, 2022, **19**, 1233–1245.
118 W. Li, F. Separovic, N. M. O'Brien-Simpson and J. D. Wade, *Chem. Soc. Rev.*, 2021, **50**, 4932–4973.
119 H. Bi, J. Xue, H. Jiang, S. Gao, D. Yang, Y. Fang and K. Shi, *Asian J. Pharm. Sci.*, 2019, **14**, 365–379.
120 M. Kanamala, W. R. Wilson, M. Yang, B. D. Palmer and Z. Wu, *Biomaterials*, 2016, **85**, 152–167.
121 L. J. Abbott, A. K. Tucker and M. J. Stevens, *J. Phys. Chem. B*, 2015, **119**, 3837–3845.
122 M. Karimi, P. Sahandi Zangabad, A. Ghasemi, M. Amiri, M. Bahrami, H. Malekzad, H. Ghahramanzadeh Asl, Z. Mahdieh, M. Bozorgomid and A. Ghasemi, *ACS Appl. Mater. Interfaces*, 2016, **8**, 21107–21133.
123 Y. Zou, L. Zhang, L. Yang, F. Zhu, M. Ding, F. Lin, Z. Wang and Y. Li, *J. Controlled Release*, 2018, **273**, 160–179.
124 V. Sinha and A. Trehan, *J. Controlled Release*, 2003, **90**, 261–280.
125 R. He, B. Finan, J. P. Mayer and R. D. DiMarchi, *Molecules*, 2019, **24**, 1855.
126 T. Yang, J. Zhai, D. Hu, R. Yang, G. Wang, Y. Li and G. Liang, *Pharmaceutics*, 2022, **14**, 1919.
127 J. Yoo, C. Park, G. Yi, D. Lee and H. Koo, *Cancers*, 2019, **11**, 640.
128 J. D. Bargh, A. Isidro-Llobet, J. S. Parker and D. R. Spring, *Chem. Soc. Rev.*, 2019, **48**, 4361–4374.
129 S. Li, H. Zhao, Y. Fan, G. Zhao, R. Wang, F. Wen, J. Wang, X. Wang, Y. Wang and Y. Gao, *Chem. Biol. Drug Des.*, 2020, **95**, 58–65.
130 J. Yu, X. Mao, X. Yang, G. Zhao and S. Li, *Molecules*, 2024, **29**, 1758.
131 S. Ma, W. Song, Y. Xu, X. Si, D. Zhang, S. Lv, C. Yang, L. Ma, Z. Tang and X. Chen, *Biomaterials*, 2020, **232**, 119676.
132 S. Ma, W. Song, Y. Xu, X. Si, S. Lv, Y. Zhang, Z. Tang and X. Chen, *Nano Lett.*, 2020, **20**, 2514–2521.
133 J. M. Lambert and R. V. Chari, *J. Med. Chem.*, 2014, **57**, 6949–6964.
134 S. Ali, C. Dussouillez, B. Padilla, B. Frisch, A. J. Mason and A. Kichler, *J. Gene Med.*, 2022, **24**, e3401.
135 A. D. Frankel and C. O. Pabo, *Cell*, 1988, **55**, 1189–1193.
136 S. Fawell, J. Seery, Y. Daikh, C. Moore, L. L. Chen, B. Pepinsky and J. Barsoum, *Proc. Natl. Acad. Sci. U. S. A.*, 1994, **91**, 664–668.
137 E. Vives, P. Brodin and B. Lebleu, *J. Biol. Chem.*, 1997, **272**, 16010–16017.
138 M. C. Morris, P. Vidal, L. Chaloin, F. Heitz and G. Divita, *Nucleic Acids Res.*, 1997, **25**, 2730–2736.
139 M. C. Morris, J. Depollier, J. Mery, F. Heitz and G. Divita, *Nat. Biotechnol.*, 2001, **19**, 1173–1176.
140 K. Montrose, Y. Yang, X. Sun, S. Wiles and G. W. Krissansen, *Sci. Rep.*, 2013, **3**, 1661.
141 Y. Zhang, W. Song, Y. Lu, Y. Xu, C. Wang, D.-G. Yu and I. Kim, *Biomolecules*, 2022, **12**, 636.
142 C. Qiu, Y. Wu, Q. Shi, Q. Guo, J. Zhang, Y. Meng, C. Wang, F. Xia, J. Wang and C. Xu, *Int. J. Biol. Sci.*, 2023, **19**, 789.
143 J.-X. Chen, H.-Y. Wang, C.-Y. Quan, X.-D. Xu, X.-Z. Zhang and R.-X. Zhuo, *Org. Biomol. Chem.*, 2010, **8**, 3142–3148.
144 R. Fu and T. A. Cross, *Annu. Rev. Biophys. Biomol. Struct.*, 1999, **28**, 235–268.
145 S. P. Brown and H. W. Spiess, *Chem. Rev.*, 2001, **101**, 4125–4156.
146 H. Yang, S. Yang, J. Kong, A. Dong and S. Yu, *Nat. Protoc.*, 2015, **10**, 382–396.
147 H. Sun, F. Meng, A. A. Dias, M. Hendriks, J. Feijen and Z. Zhong, *Biomacromolecules*, 2011, **12**, 1937–1955.
148 A. Fortunato, in *Drug-biomembrane interaction studies*, Elsevier, 2013, pp. 169–212.
149 B. Mahesh, D. Kathyayani, D. C. Gowda, A. Sionkowska and S. Ramakrishna, *Mater. Chem. Phys.*, 2022, **281**, 125847.
150 N. Saadatkhah, A. Carillo Garcia, S. Ackermann, P. Leclerc, M. Latifi, S. Samih, G. S. Patience and J. Chaouki, *Can. J. Chem. Eng.*, 2020, **98**, 34–43.
151 I. M. Weiss, C. Muth, R. Drumm and H. O. Kirchner, *BMC Biophys.*, 2018, **11**, 1–15.
152 M. Kinter and N. E. Sherman, *Protein sequencing and identification using tandem mass spectrometry*, John Wiley & Sons, 2005.
153 B. Ranjbar and P. Gill, *Chem. Biol. Drug Des.*, 2009, **74**, 101–120.
154 L. Maveyraud and L. Mourey, *Molecules*, 2020, **25**, 1030.
155 K. Wang, D.-W. Sun, H. Pu and Q. Wei, *Trends Food Sci. Technol.*, 2017, **67**, 207–219.
156 S. Mura, J. Nicolas and P. Couvreur, *Nat. Mater.*, 2013, **12**, 991–1003.
157 Q. Sun, Z. Zhou, N. Qiu and Y. Shen, *Adv. Mater.*, 2017, **29**, 1606628.
158 J. W. Singer, R. Bhatt, J. Tulinsky, K. R. Buhler, E. Heasley, P. Klein and P. de Vries, *J. Controlled Release*, 2001, **74**, 243–247.
159 R. Bhatt, P. de Vries, J. Tulinsky, G. Bellamy, B. Baker, J. W. Singer and P. Klein, *J. Med. Chem.*, 2003, **46**, 190–193.
160 X. Deng, R. Mai, C. Zhang, D. Yu, Y. Ren, G. Li, B. Cheng, L. Li, Z. Yu and J. Chen, *Eur. J. Med. Chem.*, 2021, **213**, 113050.
161 P. Zhang, Y. Zhang, X. Ding, W. Shen, M. Li, E. Wagner, C. Xiao and X. Chen, *Adv. Mater.*, 2020, **32**, 2000013.

162 A. Brezden, M. F. Mohamed, M. Nepal, J. S. Harwood, J. Kuriakose, M. N. Seleem and J. Chmielewski, *J. Am. Chem. Soc.*, 2016, **138**, 10945–10949.
163 E. K. Lei, M. P. Pereira and S. O. Kelley, *Angew. Chem., Int. Ed.*, 2013, **52**, 9660–9663.
164 M. P. Pereira, J. Shi and S. O. Kelley, *ACS Infect. Dis.*, 2015, **1**, 586–592.
165 Y. Jiang, M. Han, Y. Bo, Y. Feng, W. Li, J. R. Wu, Z. Song, Z. Zhao, Z. Tan and Y. Chen, *ACS Cent. Sci.*, 2020, **6**, 2267–2276.
166 K. Bulaklak and C. A. Gersbach, *Nat. Commun.*, 2020, **11**, 1–4.
167 S. Mali, *Indian J. Hum. Genet.*, 2013, **19**, 3.
168 K. Tatiparti, S. Sau, S. K. Kashaw and A. K. Iyer, *Nanomaterials*, 2017, **7**, 77.
169 S. Biswas, N. Yadav, P. Juneja, A. K. Mourya, S. Kaur, D. M. Tripathi and V. S. Chauhan, *ACS Omega*, 2022, **7**, 36811–36824.
170 S. Ramakrishna, A.-B. K. Dad, J. Beloor, R. Gopalappa, S.-K. Lee and H. Kim, *Genome Res.*, 2014, **24**, 1020–1027.
171 J. Chen, X. Guan, Y. Hu, H. Tian and X. Chen, *Top. Curr. Chem.*, 2017, **375**, 32.
172 B. A. Clements, V. Incani, C. Kucharski, A. Lavasanifar, B. Ritchie and H. Uludağ, *Biomaterials*, 2007, **28**, 4693–4704.
173 Y. Kakizawa, A. Harada and K. Kataoka, *Biomacromolecules*, 2001, **2**, 491–497.
174 A. Kano, K. Moriyama, T. Yamano, I. Nakamura, N. Shimada and A. Maruyama, *J. Controlled Release*, 2011, **149**, 2–7.
175 H. Hatakeyama, H. Akita and H. Harashima, *Adv. Drug Delivery Rev.*, 2011, **63**, 152–160.
176 R. J. Christie, Y. Matsumoto, K. Miyata, T. Nomoto, S. Fukushima, K. Osada, J. Halnaut, F. Pittella, H. J. Kim and N. Nishiyama, *ACS Nano*, 2012, **6**, 5174–5189.
177 H. Fang, Y. Feng, J. Chen, H. Tian and X. Chen, *Mater. Today Chem.*, 2019, **11**, 269–282.
178 H. Fang, H. Tian and X. Chen, *J. Controlled Release*, 2017, **259**, e47.
179 Y. S. Malik, M. A. Sheikh, Z. Xing, Z. Guo, X. Zhu, H. Tian and X. Chen, *Acta Biomater.*, 2018, **80**, 144–153.
180 Q. Lin, Y. Yang, Q. Hu, Z. Guo, T. Liu, J. Xu, J. Wu, T. B. Kirk, D. Ma and W. Xue, *Acta Biomater.*, 2017, **49**, 456–471.
181 X. S. Sun, M.-S. Jang, Y. Fu, J. H. Lee, D. S. Lee, Y. Li and H. Y. Yang, *Mater. Sci. Eng., C*, 2020, **114**, 111069.
182 Y. Jeong, D. Lee, K. Choe, H. Ahn, P. Kim, J.-H. Park and Y.-C. Kim, *J. Ind. Eng. Chem.*, 2017, **48**, 79–87.
183 G. Del Rio, M. A. Trejo Perez and C. A. Brizuela, *Biosci. Rep.*, 2022, **42**, BSR20221789.
184 D. Iudin, M. Vasilieva, E. Knyazeva, V. Korzhikov-Vlakh, E. Demyanova, A. Lavrentieva, Y. Skorik and E. Korzhikova-Vlakh, *Int. J. Mol. Sci.*, 2022, **23**, 2771.
185 S. Liu, H. Yang, L. Wan, J. Cheng and X. Lu, *Cancer Biother. Radiopharm.*, 2013, **28**, 289–297.
186 J. Li, Y. Zhou, Z. Su, X. Li, L. Zhang and S. Li, *Molecules*, 2024, **29**, 1247.
187 Z. Zhang, S. Tongchusak, Y. Mizukami, Y. J. Kang, T. Ioji, M. Touma, B. Reinhold, D. B. Keskin, E. L. Reinherz and T. Sasada, *Biomaterials*, 2011, **32**, 3666–3678.
188 M. Chiba, J. Hanes and R. Langer, *Biomaterials*, 1997, **18**, 893–901.
189 S. H. Kelly, L. S. Shores, N. L. Votaw and J. H. Collier, *Adv. Drug Delivery Rev.*, 2017, **114**, 3–18.
190 L. Shi, J. Zhang, M. Zhao, S. Tang, X. Cheng, W. Zhang, W. Li, X. Liu, H. Peng and Q. Wang, *Nanoscale*, 2021, **13**, 10748–10764.
191 S. Y. Fam, C. F. Chee, C. Y. Yong, K. L. Ho, A. R. Mariatulqabtiah and W. S. Tan, *Nanomaterials*, 2020, **10**, 787.
192 P. Fabbri and M. Messori, in *Modification of polymer properties*, Elsevier, 2017, pp. 109–130.
193 R. J. Nevagi, Z. G. Khalil, W. M. Hussein, J. Powell, M. R. Batzloff, R. J. Capon, M. F. Good, M. Skwarczynski and I. Toth, *Acta Biomater.*, 2018, **80**, 278–287.
194 V. R. de la Rosa, *J. Mater. Sci.: Mater. Med.*, 2014, **25**, 1211–1225.
195 S. N. Mahand, S. Aliakbarzadeh, A. Moghaddam, A. S. Moghaddam, B. Kruppke, M. Nasrollahzadeh and H. A. Khonakdar, *Eur. Polym. J.*, 2022, **178**, 111484.
196 O. Sedlacek and R. Hoogenboom, *Adv. Ther.*, 2020, **3**, 1900168.
197 N. Škalko-Basnet, *Biol.: Targets Ther.*, 2014, 107–114.
198 N. Alharbi, M. Skwarczynski and I. Toth, *Biotechnol. Adv.*, 2022, **60**, 108029.
199 S. D'Souza, *Adv. Pharm.*, 2014, **2014**, 304757.
200 T. J. Deming, *Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol.*, 2014, **6**, 283–297.
201 Z. Li, J. Huang and J. Wu, *Biomater. Sci.*, 2021, **9**, 574–589.
202 P. Zhang, H. Yang, W. Shen, W. Liu, L. Chen and C. Xiao, *ACS Biomater. Sci. Eng.*, 2020, **6**, 2167–2174.
203 Y. Bae, S. Fukushima, A. Harada and K. Kataoka, *Angew. Chem.*, 2003, **115**, 4788–4791.
204 J. Li, L. Zhang, Y. Lin, H. Xiao, M. Zuo, D. Cheng and X. Shuai, *RSC Adv.*, 2016, **6**, 9160–9163.
205 W. Teng, F. Jia, H. Han, Z. Qin, Q. Jin and J. Ji, *Polym. Chem.*, 2017, **8**, 2490–2498.
206 Q. T. Hoang, D. Lee, D. G. Choi, Y.-C. Kim and M. S. Shim, *J. Ind. Eng. Chem.*, 2021, **95**, 101–108.
207 F. A. de Oliveira, L. J. Albuquerque, G. Delecourt, V. Bennevault, P. Guégan and F. C. Giacomelli, *Curr. Gene Ther.*, 2021, **21**, 431–451.
208 F. Ofridam, M. Tarhini, N. Lebaz, É. Gagnière, D. Mangin and A. Elaissari, *Polym. Adv. Technol.*, 2021, **32**, 1455–1484.
209 N. Kamaly, B. Yameen, J. Wu and O. C. Farokhzad, *Chem. Rev.*, 2016, **116**, 2602–2663.
210 C. Shen, J. Wang, X. Wu, J. Xu, J. Hu and A. Reheman, *J. Drug Delivery Sci. Technol.*, 2023, **87**, 104827.
211 T. Volk, E. Jähde, H. Fortmeyer, K. Glüsenkamp and M. Rajewsky, *Br. J. Cancer*, 1993, **68**, 492–500.
212 B. Singh, S. Maharjan, T. Jiang, S.-K. Kang, Y.-J. Choi and C.-S. Cho, *Biomaterials*, 2015, **59**, 144–159.

213 T. Woraphatphadung, W. Sajomsang, P. Gonil, S. Saesoo and P. Opanasopit, *Carbohydr. Polym.*, 2015, **121**, 99–106.
214 J. M. Knipe, F. Chen and N. A. Peppas, *Biomacromolecules*, 2015, **16**, 962–972.
215 C. R. Justus, L. Dong and L. V. Yang, *Front. Physiol.*, 2013, **4**, 354.
216 Y. Zhong, S. Huang, C. Zheng, J. Huang, B. Li, S. Han, H. Xiao, Y. Wang and X. Shuai, *Biomater. Sci.*, 2021, **9**, 5218–5226.
217 W. Li, J. Cai, W. Zhou, X. Zhao, M. Wang, X. Zhou and L. Ren, *Colloids Surf., B*, 2023, **221**, 112982.
218 S. Damodaran and K. L. Parkin, in *Fennema's food chemistry*, CRC Press, 2017, pp. 235–356.
219 Z. Kang, Y. Peng, L. Zhou, Z. Li, T. Wang, Z. Zhang, Q. Liao, J. Gao, Y. Li and Y. Zhang, *Mater. Chem. Front.*, 2018, **2**, 1609–1617.
220 K. Zhang, X. Tang, J. Zhang, W. Lu, X. Lin, Y. Zhang, B. Tian, H. Yang and H. He, *J. Controlled Release*, 2014, **183**, 77–86.
221 G. Pasparakis and C. Tsitsilianis, *Polymer*, 2020, **211**, 123146.
222 F. Rypáček, J. Pytela, R. Kotva, V. Škarda and I. Cífková, *Macromol. Symp.*, 2011, **123**, 9–24.
223 C. Ding, L. Tong, J. Feng and J. Fu, *Molecules*, 2016, **21**, 1715.
224 Y. Li, D. Maciel, J. Rodrigues, X. Shi and H. Tomás, *Chem. Rev.*, 2015, **115**, 8564–8608.
225 G. A. Conlon and G. I. Murray, *J. Pathol.*, 2019, **247**, 629–640.
226 H. Maeda, H. Nakamura and J. Fang, *Adv. Drug Delivery Rev.*, 2013, **65**, 71–79.
227 Y. Sun, Y. Sha, G. Cui, F. Meng and Z. Zhong, *Adv. Drug Delivery Rev.*, 2023, **192**, 114624.
228 M. Chourasia and S. Jain, *J. Pharm. Pharm. Sci.*, 2003, **6**, 33–66.
229 Y.-M. Go and D. P. Jones, *Biochim. Biophys. Acta, Gen. Subj.*, 2008, **1780**, 1273–1290.
230 M. Gongora-Benitez, J. Tulla-Puche and F. Albericio, *Chem. Rev.*, 2014, **114**, 901–926.
231 M. H. Lee, Z. Yang, C. W. Lim, Y. H. Lee, S. Dongbang, C. Kang and J. S. Kim, *Chem. Rev.*, 2013, **113**, 5071–5109.
232 D.-X. Ren, P.-C. Chen, P. Zheng and Z.-N. Xu, *Colloids Surf., A*, 2019, **577**, 412–420.
233 Z. Hu, G. Wang, R. Zhang, L. Wang, J. Wang, J. Hu and A. Reheman, *Colloids Surf., B*, 2023, **224**, 113232.
234 B. Niu, K. Liao, Y. Zhou, T. Wen, G. Quan, X. Pan and C. Wu, *Biomaterials*, 2021, **277**, 121110.
235 M. H. Lee, J. L. Sessler and J. S. Kim, *Acc. Chem. Res.*, 2015, **48**, 2935–2946.
236 K. K. Jain, *Drug Delivery Syst.*, 2020, 1–54.
237 M. Stepanova, A. Nikiforov, T. Tennikova and E. Korzhikova-Vlakh, *Pharmaceutics*, 2023, **15**, 2641.
238 M. Byrne, R. Murphy, A. Kapetanakis, J. Ramsey, S. A. Cryan and A. Heise, *Macromol. Rapid Commun.*, 2015, **36**, 1862–1876.
239 M. A. Subhan, F. Parveen, N. Filipczak, S. S. K. Yalamarty and V. P. Torchilin, *J. Pers. Med.*, 2023, **13**, 389.
240 J. L.-S. Au, B. Z. Yeung, M. G. Wientjes, Z. Lu and M. G. Wientjes, *Adv. Drug Delivery Rev.*, 2016, **97**, 280–301.
241 K. N. Sill, B. Sullivan, A. Carie and J. E. Semple, *Biomacromolecules*, 2017, **18**, 1874–1884.
242 K. T. Savjani, A. K. Gajjar and J. K. Savjani, *Int. Scholarly Res. Not.*, 2012, **2012**, 195727.
243 P. Botella and E. Rivero-Buceta, *J. Controlled Release*, 2017, **247**, 28–54.
244 M. Plaza-Oliver, M. J. Santander-Ortega and M. V. Lozano, *Drug Delivery Transl. Res.*, 2021, **11**, 471–497.
245 C. Li, L. Yuan, X. Zhang, A. Zhang, Y. Pan, Y. Wang, W. Qu, H. Hao, S. A. Algharib and D. Chen, *J. Controlled Release*, 2022, **352**, 540–555.
246 M. Azman, A. H. Sabri, Q. K. Anjani, M. F. Mustaffa and K. A. Hamid, *Pharmaceutics*, 2022, **15**, 975.
247 A. Abdel-Moneim and H. Ramadan, *Drug Dev. Res.*, 2022, **83**, 301–316.
248 G. Chen, W. Kang, W. Li, S. Chen and Y. Gao, *Theranostics*, 2022, **12**, 1419.
249 S.-J. Cao, Z.-Q. Lv, S. Guo, G.-P. Jiang and H.-L. Liu, *J. Drug Delivery Sci. Technol.*, 2021, **61**, 102124.
250 A. Gonella, S. Grizot, F. Liu, A. López Noriega and J. Richard, *Expert Opin. Drug Delivery*, 2022, **19**, 927–944.
251 W. Li, J. Tang, D. Lee, T. R. Tice, S. P. Schwendeman and M. R. Prausnitz, *Nat. Rev. Mater.*, 2022, **7**, 406–420.
252 B. Tyler, D. Gullotti, A. Mangraviti, T. Utsuki and H. Brem, *Adv. Drug Delivery Rev.*, 2016, **107**, 163–175.
253 H. Liu, X. Shan, J. Yu, X. Li and L. Hu, *Curr. Pharm. Biotechnol.*, 2020, **21**, 180–193.
254 R. Gaudana, H. K. Ananthula, A. Parenky and A. K. Mitra, *AAPS J.*, 2010, **12**, 348–360.
255 B. C. Ilochonwu, A. Urtti, W. E. Hennink and T. Vermonden, *J. Controlled Release*, 2020, **326**, 419–441.
256 B. Grassiri, Y. Zambito and A. Bernkop-Schnürch, *Adv. Colloid Interface Sci.*, 2021, **288**, 102342.
257 N. T. T. Nhàn, D. E. Maidana and K. H. Yamada, *Cells*, 2023, **12**, 1071.
258 R. Ruan, M. Chen, L. Zou, P. Wei, J. Liu, W. Ding and L. Wen, *Ther. Delivery*, 2016, **7**, 89–100.
259 H. Li, Y. Shi, X. Ding, C. Zhen, G. Lin, F. Wang, B. Tang and X. Li, *Int. J. Biol. Macromol.*, 2024, 133452.
260 E. Dahlén, N. Veitonmäki and P. Norlén, *Ther. Adv. Vaccines Immunother.*, 2018, **6**, 3–17.
261 S. Volpi, U. Cancelli, M. Neri and R. Corradini, *Pharmaceutics*, 2020, **14**, 14.
262 R. Kumar, D. S. Dkhar, R. Kumari, Divya, S. Mahapatra, A. Srivastava, V. K. Dubey and P. Chandra, *Biotechnol. Bioeng.*, 2022, **119**, 3022–3043.
263 H. Hillaireau and P. Couvreur, *Cell. Mol. Life Sci.*, 2009, **66**, 2873–2896.
264 M. Mettlen, P.-H. Chen, S. Srinivasan, G. Danuser and S. L. Schmid, *Annu. Rev. Biochem.*, 2018, **87**, 871–896.
265 M. J. Mitchell, M. M. Billingsley, R. M. Haley, M. E. Wechsler, N. A. Peppas and R. Langer, *Nat. Rev. Drug Discovery*, 2020, **20**, 101–124.

266 I. Szabó, M. A. Yousef, D. Soltész, C. Bató, G. Mező and Z. Bánóczi, *Pharmaceutics*, 2022, **14**, 907.
267 M. V. Chiarpotti, G. S. Longo and M. G. Del Pópolo, *Colloids Surf., B*, 2021, **197**, 111373.
268 Z. Zhao, Y. Wang, J. Han, K. Wang, D. Yang, Y. Yang, Q. Du, Y. Song and X. Yin, *Int. J. Nanomed.*, 2014, 5849–5862.
269 P. Lajoie and I. R. Nabi, *Int. Rev. Cell Mol. Biol.*, 2010, **282**, 135–163.
270 A. Filippini and A. D'Alessio, *Biomolecules*, 2020, **10**, 1218.
271 L. B. Vong, N.-T. Trinh and Y. Nagasaki, *J. Controlled Release*, 2020, **326**, 140–149.
272 R. Seyfi, F. A. Kahaki, T. Ebrahimi, S. Montazersaheb, S. Eyvazi, V. Babaeipour and V. Tarhriz, *Int. J. Pept. Res. Ther.*, 2020, **26**, 1451–1463.
273 J. Lei, L. Sun, S. Huang, C. Zhu, P. Li, J. He, V. Mackey, D. H. Coy and Q. He, *Am. J. Transl. Res.*, 2019, **11**, 3919.
274 K. Agam and D. K. Arya, *Int. J. Pharm. Prof. Res.*, 2023, **14**, 49–72.
275 M. H. Rigatto, D. R. Falci and A. P. Zavascki, *Polymyxin Antibiotics: From Laboratory Bench to Bedside*, 2019, pp. 197–218.
276 X. Ma, Z. Liu, I. Ilyas, P. J. Little, D. Kamato, A. Sahebka, Z. Chen, S. Luo, X. Zheng and J. Weng, *Int. J. Biol. Sci.*, 2021, **17**, 2050.
277 T. Rana, T. Behl, A. Sehgal, S. Singh, N. Sharma, A. Abdeen, S. F. Ibrahim, V. Mani, M. S. Iqbal and S. Bhatia, *Prog. Neuro-Psychopharmacol. Biol. Psychiatry*, 2022, **114**, 110478.
278 L. Pinheiro and C. Faustino, *Curr. Alzheimer Res.*, 2019, **16**, 418–452.
279 L. Chen, L. Zhang, R. Zhao, J. Shen, Y. Wang, J. Zhu, H. Fang, N. Liu, C. Wang and T. Wei, *Nano Today*, 2023, **50**, 101834.
280 M. Jiang, L. Zhao, X. Cui, X. Wu, Y. Zhang, X. Guan, J. Ma and W. Zhang, *J. Adv. Res.*, 2022, **35**, 49–60.
281 Q. Cao, H. Fang and H. Tian, *Biomaterials*, 2024, **310**, 122628.