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REVIEW

Self-assembled peptide nanomaterials for biomedical applications: promises and pitfalls

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Abstract: Over the last several decades, a great number of advances have been made in the area of self-assembled supramolecules for regenerative medicine. Such advances have involved the design, preparation, and characterization of brand new self-assembled peptide nanomaterials for a variety of applications. Among all biomolecules considered for self-assembly applications, peptides have attracted a great deal of attention as building blocks for bottom-up fabrication, due to their versatility, ease of manufacturing, low costs, tunable structures, and versatile properties. Herein, some of the more exciting new designs of self-assembled peptides and their associated unique features are reviewed and several promising applications of how self-assembled peptides are advancing drug delivery, tissue engineering, antibacterial therapy, and biosensor device applications are highlighted.

Keywords: self-assembly, peptides, biomedical applications, drug delivery, antibacterial therapy, biosensor devices

Introduction

Grounded in nature, self-assembled molecules, involving lipids, peptides, proteins, sugars, and nucleic acids, are the fundamental building blocks of life as they comprise cell membranes, cell cytoskeletal structures, and extracellular matrices.[1–3] In 1959, Feynman,[4] who described a process to form well-organized supramolecules by combining individual units through a bottom-up approach, first proposed the concept of self-assembled molecules. In the 1960s, Lehn[5,6] was the first one to introduce the term and concepts of supramolecular chemistry, and he had been awarded a Nobel Prize for his contribution to this area in 1987. Since then, efforts spanning a multitude of disciplines, involving mostly chemistry, physics, materials, mathematics, engineering, and biology, have contributed to this rapidly growing field to understand building block monomer structures, associated molecular assembly mechanisms, programmed architectures, controllable properties, and advanced functions.[1,7]

Just like higher hierarchical biomolecules in nature (such as proteins, DNA, and polysaccharides), most self-assembled molecules are formed by the interaction of individual monomers through weak, noncovalent interactions, including electrostatic interactions, hydrophobic interactions, hydrogen bonds, van der Waals interactions, and π–π stacking forces.[3,8] Even though individual noncovalent forces are very weak (eg, hydrophobic forces <10 kcal/mol, electrostatic forces =1–20 kcal/mol, hydrogen bonds =2–30 kcal/mol, and π–π aromatic stacking =0–10 kcal/mol), the combination of several noncovalent forces together can generate very stable and well-organized structures.[8] There are several advantages of developing self-assembled materials through noncovalent bonds compared to covalent forces. For example,

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due to strong covalent bonds prevalent in materials such as polymers, covalent bonds (eg, C–C bond =86 kcal/mol, C–H bond =103 kcal/mol, C–O bond =81 kcal/mol) are not reversible without adding catalysts or conducting extensive material processing. Compared with covalent bonding, these noncovalent forces present in numerous self-assembled materials are reversible and dynamic, providing for advanced functions, such as high synthetic convergence, error correction, programmed design, and self-organization.[9]

To maximize the formation of as many noncovalent forces as possible, the major component of self-assembled molecules has an amphiphilic structure with both hydrophobic and hydrophilic portions.[10] When responding to an aqueous environment, such molecules display hydrophobic or hydrophilic domains on the surface to match that of the solvent properties. Among all the self-assembling molecules designed today, peptides are particularly attractive building blocks in this respect since peptide sequences can be formulated to have both hydrophilic and hydrophobic domains in the same building block. Owing to such unique properties in self-assembled peptides, they have been used for numerous applications, including storing bioinformation via their various sequences, peptide folding, fast and easy synthesis, a variety of functionalization methods, and their programmed responses to external stimulations (such as pH value, ion concentration, and hydrophobicity).[8,11]

In terms of the higher hierarchical structure of self-assembled peptides, their stability depends on the monomer structure in terms of the length of the amino acid chain and the shape of the functional group.[12] At the same time, the external environment, including temperature, pH, ionic strength, and mechanical force, can affect or reverse the self-assembly process.[13] Therefore, based on these properties, scientists can design self-assembled molecules with an "intelligence" to be triggered by the changing environment, such as specific targeting, controlled release, and improved efficacy.[9]

To synthesize peptides and their hybrid structures, there are three main methods: solid-phase chemical synthesis, protein engineering, and ring-opening polymerization strategy.[14] Generally, solid-phase peptide synthesis is used for short-to-medium-length peptide sequences of highly precise structures. Although the yield of solid-phase peptide synthesis is >98% per step, the synthetic limitation of this method is in the range of 70 amino acids. To synthesize a long peptide sequence (>50 amino acids) with a defined structure such as silk and collagen molecules, researchers can utilize protein engineering to control genetic expression in bacteria. For the large-scale production of polypeptides,

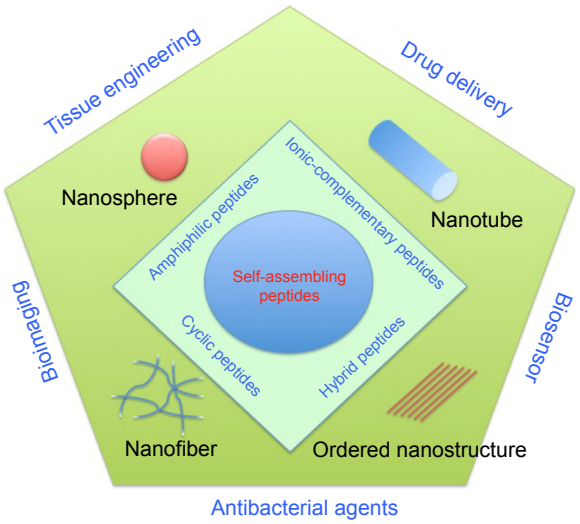

Figure 1 Schematic illustration of various nanostructures (nanosphere, nanotube, nanofiber, and ordered nanostructure) formed by self-assembling peptides (amphiphilic peptides, ionic-complementary peptides, cyclic peptides, and hybrid peptides) and their applications in tissue engineering, drug delivery, bioimaging, and biosensors.

it is recommended to use ring-opening polymerization, in which cyclic monomers are added to the chain end to form a long peptide. However, this method produces a peptide primary structure with a lower accuracy than that obtained during solid-phase synthesis.[14]

In this review, we would like to introduce self-assembled nanostructures (nanospheres, nanotubes, nanofibers, and other ordered nanostructures) with linear peptide monomers (amphiphilic peptides, ionic-complementary peptides, etc) and nonlinear peptide monomers (cyclic peptides and hybrid peptides) as building blocks and discuss some relevant recent applications of such molecules involved during drug delivery, tissue engineering, antimicrobial control, and electronic devices (Figure 1).

Self-assembled peptide types and structures

Amphiphilic peptides

Amphiphilic peptides involve pure linear peptides, ionic-complementary peptides, long-chain alkylated peptides, peptide phospholipids, and peptide-based block copolymers.[10]

Inspired by lipid molecules in cell membranes, pure peptides with hydrophobic tails and hydrophilic heads can self-assemble into nanotubes, nanovesicles, and micelles, depending both on their chemical properties (eg, peptide sequences, charges, concentration, etc) and physical properties (eg, size, shape, etc).[15–17] For the hydrophobic tail (which normally contains six residues), amino acids such as G, A, V, L, I, and F are good candidates; meanwhile, D, K, E, R, and H are used in the hydrophilic domain.[10,18,19] For example, similar

to surfactants, lipid-like peptides, such as A_6D, V_6D, G_4DD, G_6DD, G_8DD, A_6K, and KA_6 sequences, spontaneously form self-organized nanostructures (such as micelles, vesicles, and fibers) once reaching the critical aggregation concentration (CAC) (eg, a CAC of A_6D ≈1.6 mM and a CAC of A_6K ≈1.5 mM).[9,20,21] Moreover, due to their similarity to phospholipids, these peptides could stabilize membrane proteins, which denature and aggregate easily.

For example, Yeh et al[22] and Zhao et al[23] utilized lipid-like peptides (eg, A_6D and I_6K_2) to stabilize the functional forms of membrane proteins *Escherichia coli* glycerol-3-phosphate dehydrogenase and G protein-coupled receptor bovine rhodopsin, respectively. Compared to the common surfactants (eg, *n*-dodecyl-β-D-maltoside and octyl-D-glucoside) used for protein stabilization, these peptides have a higher efficiency to stabilize membrane proteins.[23]

Ionic-complementary self-assembling peptides

Inspired from the Z-DNA binding protein zuotin, in 1993, Zhang et al[24] discovered the first self-assembling peptide EAK16 (n-AEAEAKAKAEAEAKAK-c) that formed into nanofibers. Since then, the rapid development of this type of self-assembling peptide has fostered numerous applications, including three-dimensional (3D) cell culture, tissue engineering, regenerative medicine, and sensory devices.[25–27] This peptide has one side group with charged side chains and another side group with hydrophobic chains. In water, the charged side is exposed on the outside and the hydrophobic side forms a double sheet inside the nanofiber.[24,28] Meanwhile, the peptide sequences with periodically repeated positive and negative charges form the stable structure by ionic-complementary forces in a checkerboard-like pattern and then assemble typical beta-sheet structures and eventually form a hydrogel network of nanofibers.[13,29]

Owing to the combination of ionic force and hydrogen bonds inside the beta-sheet structures, these nanofibers are stable under a wide range of temperatures, pH values, and high concentrations of denaturing chemicals.[26] Since the hydrophobic interactions are not specific, these nanofibers may diffuse to minimize equilibrium energy.[13,30]

There are different types of ionic-complementary self-assembled peptides (Figure 2): 1) −+−+−+−+ (eg, peptide RADA16-I: Ac-RADARADARADARADA); 2) −−++−−++ (eg, peptide RADA16-II: Ac-RARADADARARADADA); 3) −−−+++; and 4) −−−−++++.[27,31,32] Among these peptides, the RADA16-I peptide can promote cell growth and tissue regeneration; therefore, it has been commercialized as a product termed PuraMatrix.[26,32]

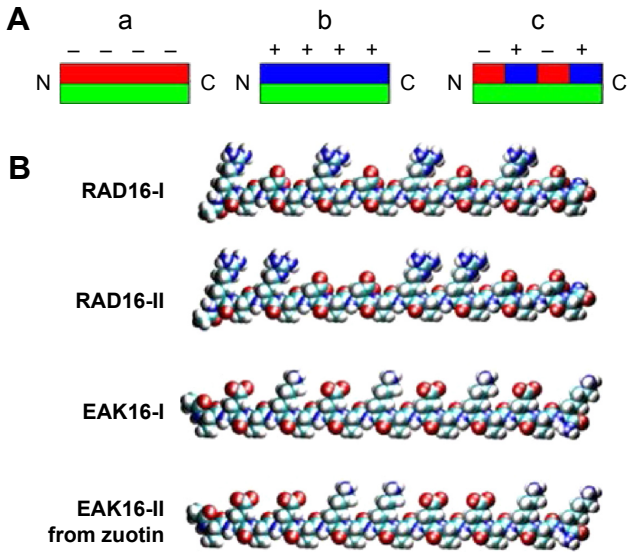

Figure 2 Different types of ionic-complementary peptides.
Notes: (**A**) The hydrophobic side of the peptide molecule is green, negative charges are red, and positive charges are blue. (a) The peptide structure has one hydrophobic side and one side with negative charges. (b) The peptide structure has one hydrophobic side and one side with positive charges. (c) The peptide structure has one hydrophobic side and one side with alternate positive and negative charges. (**B**) Four representative ionic peptides: RAD16-I, RAD16-II, EAK16-I, and EAK16-II. Reprinted from Nano Today, Volume 4, Edition 2, Yang Y, Khoe U, Wang X, Horii A, Yokoi H, Zhang S. Designer self-assembling peptide nanomaterials, pages 193–210, Copyright 2009 with permission from Elsevier.[27]

Once mixed with physiological fluids, amphiphilic peptides form a transparent hydrogel network within seconds. The hydrogel of these ionic-complementary self-assembled peptides is composed of 0.5%–1% peptides with 99.0%–99.5% water, and therefore, there are a large number of spaces between the nanofibers, which are enough for cells to grow and differentiate.[31,33] When used in 3D cell culture systems, this type of the self-assembled peptide scaffold provides mechanical strength to encapsulate cells in a desired location; has excellent nutrient, growth factor, and oxygen diffusion for cell growth; and can release therapeutic agents in a controlled or programmed fashion.[34,35] The ionic-complementary self-assembled peptides have been used to enhance various types of cell growth and differentiation, including bone, cartilage, vessel, heart, and neural systems.[34,36] For example, the self-assembled peptide scaffolds made of RADA16-I and RADA16-II peptides have been used to enhance neural cell attachment, differentiation, and neurite outgrowth during in vitro studies, as well as to promote active synapse formation during in vivo studies.[33] For example, Gelain et al studied the 3D cultures of mouse neural stem cells using the self-assembling peptide RADA16 with the functionalized sequences derived from neural cell adhesion motifs, bone marrow homing proteins, and collagen molecules. After 7 days of culturing, the bone marrow

homing peptides (SKPPGTSS and PFSSTKT)-conjugated RADA16 peptide scaffolds promoted the most neural stem cell growth and differentiation.[33]

Long-chain alkylated peptides

Long-chain alkylated peptides are composed of hydrophilic peptide sequences and hydrophobic alkyl chains. The alkyl chain can be conjugated on both the N- and C-termini of the peptide sequences. A representative amphiphilic peptide molecule has four domains: hydrophobic tail, beta-sheet forming segment, charged groups, and bioactive epitopes (Figure 3A).[37] In the aqueous solution, individual alkylated peptides could aggregate together to form cylinder nanofibers (Figure 3B and C).[11] Since the most of bioactive epitopes are hydrophilic, these functionalized peptides can be exposed on the surface of nanofibers to interact with cellular receptors, which consequently influence cell signaling pathways, gene expression, and cell functions.[11,37,38]

Yu et al[39] and Forns et al[40] demonstrated that the hydrocarbon chains can be used to stabilize peptide sequences and studied the folded structures. Self-assembled alkylated peptides have been utilized to functionalize carbon tubes via noncovalent bonds to enhance their solubility in water.[41]

To mimic cell-membrane proteins, peptides have been modified with phospholipids to form peptide–phospholipid conjugates. For example, Musiol et al[42] have synthesized lipopeptides with the sequence derived from human prion proteins. HeLa cell experiments using confocal fluorescence microscopy proved that the self-assembling lipid peptide micelles were transported into cells very quickly.

Cyclic peptide

As early as 1974, De Santis et al[43] determined theoretically that cyclic peptides with alternating D- and L-amino acids could self-assemble into nanotubes. However, it was not until 1993 that Ghadiri et al[44] synthesized the first type of self-assembling cyclic peptide, cyclo-(L-Gln-D-Ala-L-Glu-D-Ala)$_2$, based on De Santis' theory.

The self-assembling cyclic peptides are composed of stacking cyclic peptide monomers with a flat conformation structure, in which the carbonyl and amino groups are pointed perpendicular to the ring and the side chains present a pseudo-equatorial outward-pointing orientation.[45,46] Then the nanotubes were stabilized by hydrogen bonds between amide groups in neighboring peptide cycles (Figure 4). Typical cyclic peptide sequences include alternating D,L-α-amino acids, β-amino acids; alternating α,β-amino acids; and alternating α,γ-amino acids, δ-amino acids, and oligoureas.[46–49]

Compared to the other self-assembled peptide structures, cyclic peptide nanotubes have unique properties: 1) their precise diameter depends on the number of amino acids, chemical structure, and the side-chain size and 2) the functions of

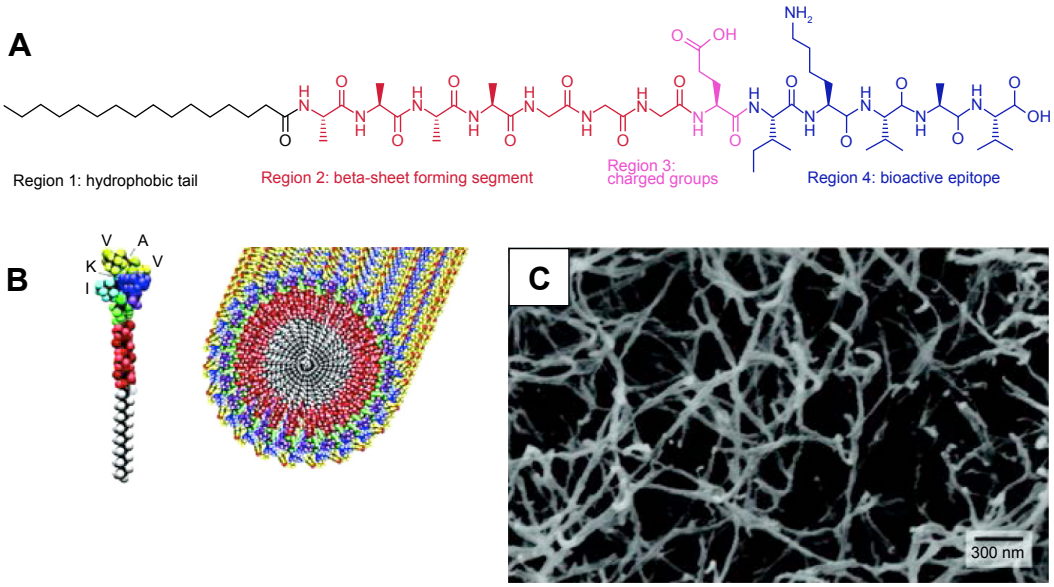

Figure 3 (**A**) Molecular structure of an alkylated peptide with four regions: hydrophobic tail, beta-sheet forming segment, charged groups, and bioactive epitope. Adapted from Cui H, Webber MJ, Stupp SI. Self-assembly of peptide amphiphiles: from molecules to nanostructures to biomaterials. *Biopolymers*. 2010;94(1):1–18. Copyright © 2010 Wiley Periodicals, Inc.[37] (**B**) Schematic illustration of an alkylated peptide (IKVAV) molecule and the nanofiber formed by IKVAV peptides. (**C**) TEM image of IKVAV nanofibers. From Silva GA, Czeisler C, Niece KL, et al. Selective differentiation of neural progenitor cells by high-epitope density nanofibers. *Science*. 2004;303(5662):1352–1355. Reprinted with permission from AAAS.[11]
Abbreviation: TEM, transmission electron microscopy.

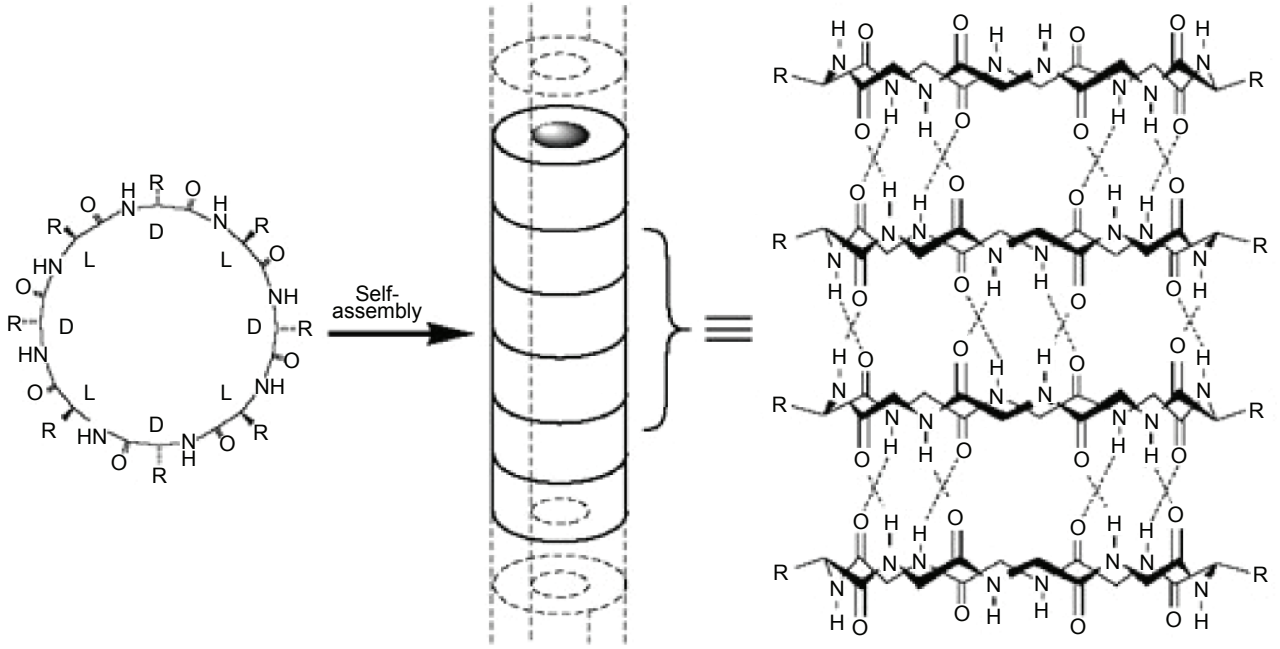

Figure 4 Schematic illustration of the cyclic peptide molecule and the nanotubular structure provided by cyclic peptides.
Notes: Reprinted with permission from Macmillan Publishers Ltd: Nature. Fernandez-Lopez S, Kim HS, Choi EC, et al. Antibacterial agents based on the cyclic D,L-alpha-peptide architecture. 2001;412(6845):452–455.[45]

the nanotubes can be tailored by varying the side chains.[46] For example, when the number of amino acids in the cyclic peptide ring increases from 4 to 12, the internal diameter increases from 2 Å to 13 Å.[46]

Applications in medicine

Drug delivery

The conventional administration of hydrophobic drugs suffers several disadvantages, including low water solubility, poor oral availability, quick biodegradation, nonspecific delivery, and serious side effects.[50,51] Therefore, drug delivery systems could encapsulate these therapeutic agents to increase drug efficacy.[52] Similarly, delivery vehicles can also improve the efficacy of certain hydrophilic drugs, which have unspecific targeting or short half-lives.[53] In order to protect highly sensitive biomolecules (such as peptides, proteins, DNAs, and RNAs) from biodegradation and concentrate and retain them in the target organ, it is necessary to use a delivery vehicle, for which self-assembled peptides are a good option.[54,55]

For drug delivery applications, the basic requirements for self-assembled nanomaterials are biocompatibility, noncytotoxicity, and biodegradablility.[56,57] For drug loading and release, the nanocarriers should encapsulate molecules at a high concentration, protect them from dilution and degradation, and release them in a controlled and prolonged manner (Figure 5).[58–61] For advanced functions, the self-assembled systems should have domains specific for targeting or sensory capability to be utilized for the intended application.[56,62]

As an example of the aforementioned application, Webber et al[63] conjugated the anti-inflammatory drug dexamethasone with the peptide amphiphile (C_{16}-$V_2A_2E_2$) via a hydrazine linkage to achieve long-term drug release in both in vitro and in vivo tests. In vitro studies showed that dexamethasone could be released over several weeks at physiological pH and temperature. Furthermore, the peptide nanofibers were mixed with polystyrene microparticles and injected into mice, and finally, a luminescence assay and histological staining were used to evaluate the inflammation after 3 days or 9 days. The results demonstrated that the localized drug-loaded nanofiber gel could promote immune suppression.[63]

Zhang et al[64] utilized the self-assembling Tat peptide to encapsulate the hydrophobic drug paclitaxel to facilitate drug delivery and efficacy. First, four sequences with different numbers of hydrophobic tails were tested, and only the four-tailed sequence qC_8-Tat self-assembled into nanotubes under aqueous solutions at a pH of 7.4 (Figure 6). After the coumarin-6-loaded nanofibers were delivered into KB-3-1 cervical cancer cells, confocal microscopy indicated that hydrophobic drugs were transported into cancer cells at high efficacy through the adsorptive-mediated pathway.[64]

For controlled drug releases, peptide hydrogels could encapsulate small molecules or proteins such as enzymes

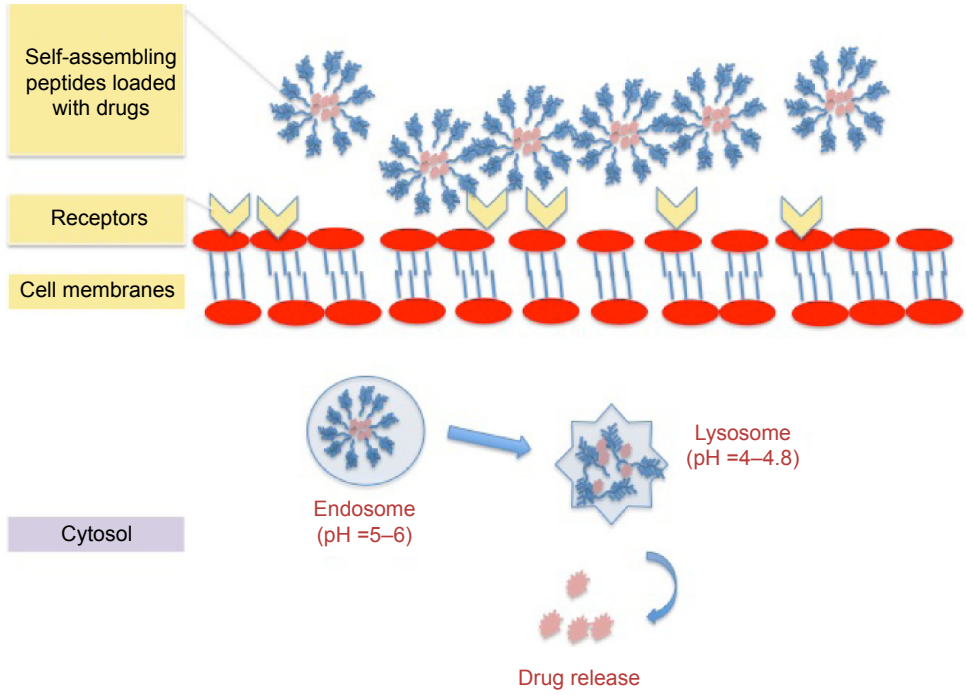

Figure 5 Schematic illustration of cell-membrane interactions and drug release of the drug-loaded self-assembling peptides.

and antibodies.[65,66] The release profiles are determined not only by the molecule size, charge, and hydrophobicity but also by the characteristics of the peptide hydrogel, including peptide sequences, concentration, and the hydrogel pore size.[59]

Tissue engineering

For tissue regeneration, self-assembled peptides with nanotubular or nanofibered structures could mimic the natural extracellular matrix of many tissues and localize drug release at the desired site.[11,58,67] Similar to the spherical shape of

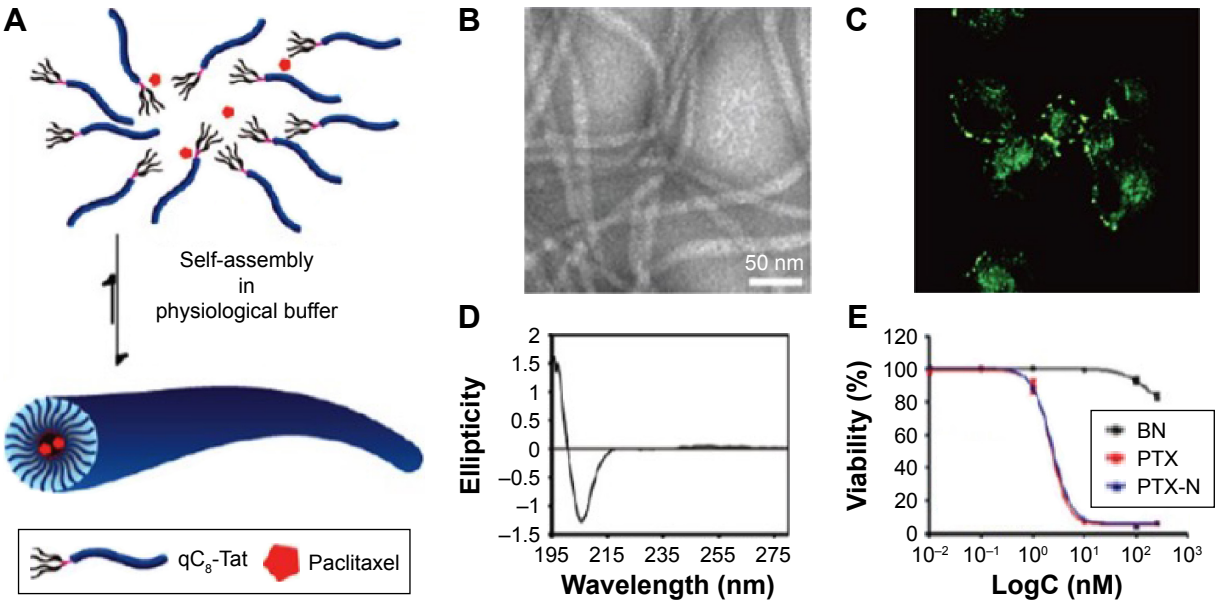

Figure 6 (**A**) Schematic illustration of paclitaxel-loaded Tat peptide nanofibers. (**B**) TEM image of paclitaxel-loaded Tat nanofibers in PBS. (**C**) Confocal image of the endocytosis pathway of drug release. (**D**) CD spectrum of the Tat peptide nanofibers. (**E**) KB-3-1 cervical cancer cell inhibition by the drug-loaded peptides. Reprinted with permission from Zhang P, Cheetham AG, Lin YA, Cui H. Self-assembled tat nanofibers as effective drug carrier and transporter. *Acs Nano*. 2013;7(7):5965–5977. Copyright 2013 American Chemical Society.[64]

Abbreviations: TEM, transmission electron microscopy; PBS, phosphate-buffered saline; CD, circular dichroism.

nanoparticles, nanotubes or fibers could not only display a high density of signals on the surface but also can carry multifunctional molecules to target specific cellular receptors, such as G protein-coupled receptors and kinase-linked receptors.[58,68,69] Different from micelles, nanofibers have a precise geometry that retains signals over long distances on the cell surface to benefit many applications in medicine that require directional growth, such as spinal cord regeneration, vessel growth, and cartilage and bone repair.[11,29,35,70,71] Moreover, several types of peptides could form a rigid network or filaments with good mechanical properties to serve as substrates or 3D scaffolds to support the growth, proliferation, differentiation, and function of cells.[34,72–74] To introduce some specific bioactive functions, it is generally a good idea to incorporate the functional peptide motifs on the C-termini of the peptide sequence with a GG spacer between the motifs and ionic-complementary sequence.[27] The functional motifs can then provide cells with more external stimuli than the 2D coating in terms of bioactive molecule concentration.[27,75]

Conventionally, a variety of polymers, such as poly(L-lactic acid) and poly(glycolic acid), and biopolymers, such as collagen and alginate, have been used as artificial scaffolds for various tissue engineering applications. Although these polymers are biodegradable and easy to fabricate, most of them lack bioactivity to direct cell growth and functions.[76] On the other hand, with their ability to self-assemble in the physiological environment, the peptide solution could be injected into the injured location and forms a scaffold to promote tissue regeneration.[34,72,77] With peptides derived from growth factor, nanofibers can expose these peptides on their surfaces to bind and activate cellular receptor for cell signaling and/or protein synthesis.[78,79] Furthermore, the scaffolds composed of peptides can encapsulate stem cells and induce their differentiation into normal cells through bioactive peptides at a high density on the scaffold surface.[76,78,80]

In a previous study, to improve cartilage regeneration, the hydrogels formed by the peptide KLD12 (KLDLKLDLKLDL) were cocultured with primary bovine chondrocytes, and the results showed that peptide hydrogel retained the chondrocyte phenotype to produce a cartilage-like extracellular matrix and type II collagen.[35] After 4 weeks of culturing, the material stiffness of the extracellular matrix increased, and therefore, it was suggested that the KLD12 self-assembling peptide scaffold was promising for cartilage tissue repair.[35]

Moreover, Stroumpoulis et al[81] studied cell responses on vesicles formed by $(C_{16})_2$-Glu-C_2-GRGDSP and $(C_{16})_2$-Glu-PEO-GRGDSP sequences. The sequence with PEO promoted the highest fibroblast cell adhesion and growth, which could be used to develop a membrane system to screen biological probes for cell adhesion and growth. Later on, Lin et al synthesized pH-sensitive ampiphilic peptides C_{16}GSH and C_{16}EOSH composed of histidine and serine with a single fatty acid tail and branched structure.[77] Using histidine as the switch, these peptides were viscoelastic liquids at a pH value <5.5. In a neutral solution, these peptides formed micelles. While in pH >6.5, the amphiphilic peptides assembled into nanofibers to form a hydrogel. Once cultured with cells, the hydrogel kept fibroblasts proliferating for 96 hours. This type of nanofibers is promising to develop a pH-sensitive cell scaffold to transfer the peptide solution into a hydrogel under physiological environment.[77]

In addition, mimicking the ligand–receptor interactions of extracellular matrix components, Lee et al[82] synthesized amphiphilic peptides with sequences specifically binding to heparin sulfate. Then the hybrid scaffold localized with the bone morphogenetic protein-2 to modulate the ligand–receptor signaling to promote bone regeneration.

In our group, anticancer drugs (eg, curcumin) were encapsulated into the self-assembled peptide $C_{18}GR_7$RGDS to inhibit selectively bone cancer cells (MG-63 osteosarcoma).[83] Fourier transform infrared and X-ray diffraction analyses proved that curcumin was encapsulated in the hydrophobic core of peptide nanoparticles. The cell studies showed that self-assembled peptides significantly improved the delivery efficiency. Moreover, compared to healthy bone cells, curcumin-loaded peptide nanoparticles demonstrated selective cytoxicity against bone cancer cells.[83]

Zhang et al[84] designed temperature-sensitive nanofibers ($V_3A_3E_3$) to form aligned cellular wires. Figure 7 demonstrates that the amphiphilic peptides could form a string-like hydrogel in a phosphate-buffered saline solution or can be mixed with a $CaCl_2$ solution after a temperature treatment (heated to 80°C for 30 minutes and then cooled down to 20°C). Accordingly, mechanical tests and scanning electron microscopy proved that the nanofibers organized along the length of the string. When incubated in the hydrogel, human mesenchymal stem cells grew both cell bodies and filopodia along the direction of the string formed by the peptides.[84]

Antibacterial agents

Over the past 3 decades, the antibacterial effects of various bioactive peptides have attracted extensive attention.[85,86] As a part of our innate defense systems, antimicrobial peptides were widely found in different types of organisms. With lengths of 10–40 amino acids, most antimicrobial peptides

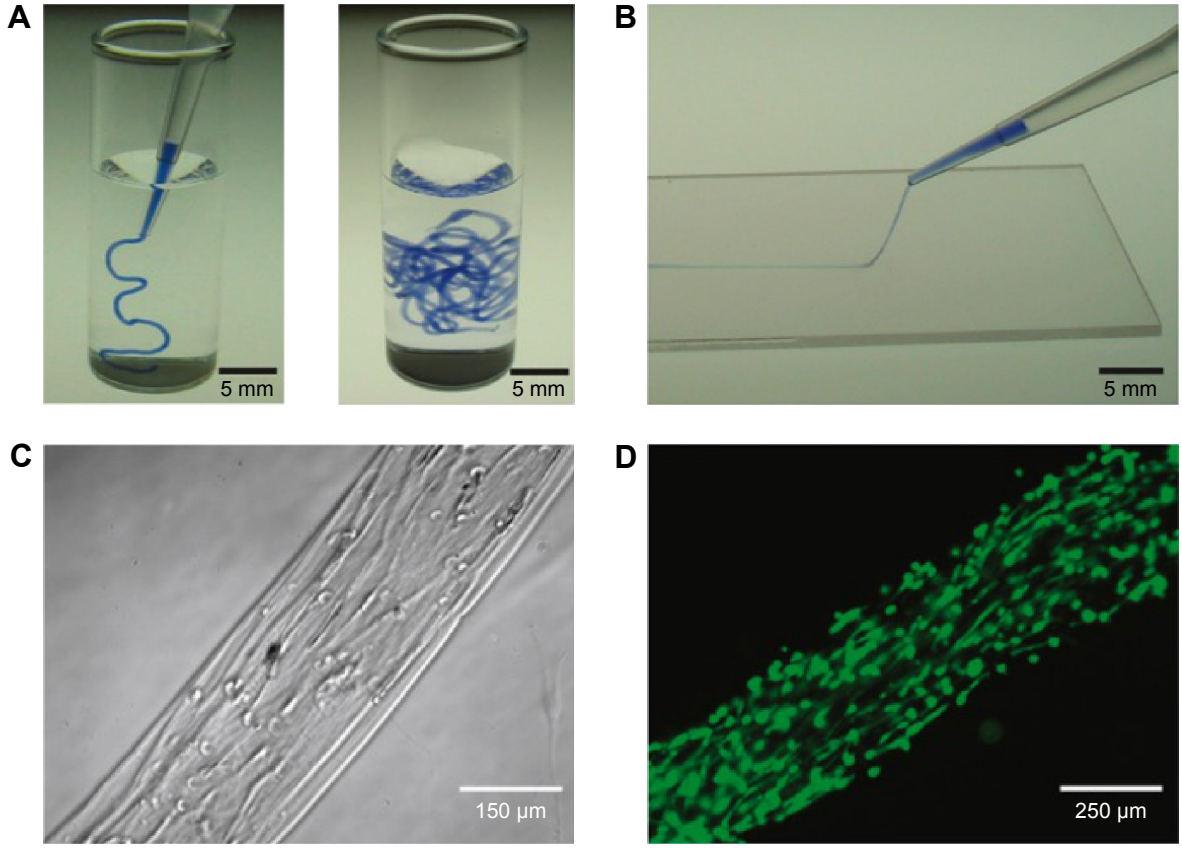

Figure 7 (**A**) After heating, amphiphilic peptides formed the hydrogel in a PBS solution. (**B**) After mixing with a $CaCl_2$ solution, the peptide solution formed a string-like hydrogel. (**C**) Mesenchymal stem cells grew and differentiated at along the direction aligned with the hydrogel string formed from the peptides. (**D**) Fluorescence image of calcein-labeled human mesenchymal stem cells in the peptide hydrogel. Reprinted by permission from Macmillan Publishers Ltd: Nature Materials. Zhang S, Greenfield MA, Mata A, et al. A self-assembly pathway to aligned monodomain gels. 2010;9(7):594–601., Copyright 2010.[84]
Abbreviation: PBS, phosphate-buffered saline.

are capable of interacting with negatively charged bacterial membranes to show a broad spectrum of ability. The mechanism of antimicrobial peptides is not only determined by the size, hydrophobicity, amphipathicity, net charge, and secondary structure of the peptide sequence but also affected by their interaction with bacterial membranes.[87–90]

For example, Veiga et al synthesized an antibacterial hydrogel using arginine-rich self-assembled peptides with different arginine contents (two, four, six, or eight Arg residues in the sequence). They investigated the viability of *E. coli* and *Staphylococcus aureus* (10^5 CFU/dm^2) on the peptide hydrogel surface with varying weight percentages (0.5, 1, 1.5, and 2) after 24 hours of culturing. Both increases in the number of arginine groups and the weight percent could enhance the antibacterial capability of the self-assembled hydrogels. For gels containing four, six, or eight arginine groups, both *E. coli* and *S. aureus* were completely inhibited after 24 hours, irrespective of the weight percentage of the peptides. However, the hydrogels with two arginine groups and containing 2 wt% peptides inhibited only 50% of *E. coli* growth. The antibacterial results indicated that the hydrogel formed by the self-assembled PEP6R peptides (VKVRVRVRV^{D}PPTRVRVRVKV) had the greatest antibacterial ability with a low cytotoxicity toward human erythrocytes and mesenchymal stem cells.[86]

Chu-Kung et al[91,92] studied the effect of the length of fatty acids of peptides on antimicrobial properties. In their study, the peptides YGAAKKAAKAAKKAAKAA (AKK) and LKKLLKLLKLLKL (LKK) were conjugated with fatty acids, and the results indicated that the increased length of the fatty tails conjugated to AKK peptides enhanced interactions between the peptides and membranes, which consequently increased the antibacterial activity (for *E. coli* and *S. epidermidis*).[91] However, once conjugated with a longer fatty acid tail and peptides start to assemble into nanofibers, the antibacterial activity was reduced.

As another example on the use of self-assembled peptides for antibacterial applications, bone fracture healing, and antimicrobial applications, KLD12 peptides (KLDLKLDLKLDL) with variable numbers of arginine residues at the N-terminus have been synthesized to introduce the antimicrobial properties while maintaining the β-sheet and self-assembled structures.[93]

The results of propidium iodide staining and scanning electron microscopy demonstrated that the increased arginine caused leaking of the *E. coli* membrane and death.

Chen et al[94] also assembled antimicrobial peptides (surfactin) on photoluminescent gold nanodots (SFT/DT-Au) to inhibit both normal and multidrug-resistant bacteria. In vitro studies indicated that the synergistic effect of peptides and gold dots enhanced anti-infection properties dramatically by the disruption of the membrane. Animal studies also demonstrated that SFT/DT-Au not only inhibited methicillin-resistant *S. aureus* infection but also promoted collagen production in wounded skin of rats infected by methicillin-resistant *S. aureus*.[94]

In previous studies, cyclic peptide nanotubes were mainly used for antibacterial applications.[45,95,96] With stacking peptide cycles, nanotubes are able to form transmembrane pores to mimic ion channels in membranes.[45,97,98] Mechanistic studies showed that the antibacterial activity is due to the enhancement of the membrane permeability.[96,97] Moreover, these cyclic peptides could also inhibit virus infections by blocking virus entry or endosome escape in mammalian cells. To inhibit methicillin-resistant bacteria, it is significant to choose a specific peptide sequence against bacterial selectively rather than mammalian cells.[99]

Khalfa and Tarek[98] simulated the interaction of self-assembling cyclic peptides with cytoplasmic membrane models using coarse-grained molecular dynamic simulations to study the mechanism of the antibacterial process. First, the cyclic peptide ([RRKWLWLW]) self-assembled on the top of the membrane formed by palmitoyl-oleoyl-phosphatidylethanolamine (POPE) and palmitoyl-oleoyl-phosphatidylglycerol (POPG) lipid bilayers. With the increased concentration of peptides on the surface, the lipids released from the POPE–POPG bilayers. Moreover, the mechanical properties of the membrane were affected by cyclic peptides. Figure 8 indicates that in the beginning of the interaction, cyclic peptides with a low concentration started to form short nanotubes and did not penetrate the membrane. At the 1/10 cyclic peptide/lipid ratio, lipid protrusion appeared. Once the cyclic peptide/lipid ratio increased to 1/5, the peptides aggregated with negative lipid molecules and caused large perturbations of the membrane.[98]

Nanosensors

Once targeting biomolecules or metallic nanoparticles into self-assembled peptides were incorporated, peptide nanotubes could act as biosensors with enhanced sensitivity and selectivity for mineralization, imaging probes, and electronic devices.[100–103]

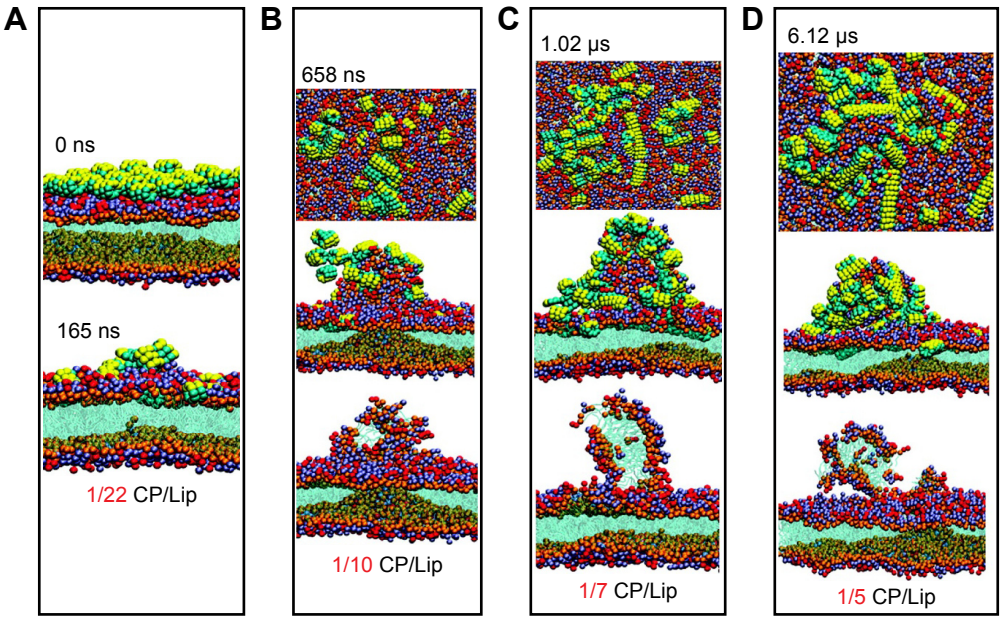

Figure 8 CG-MD images of the interaction of self-assembling cyclic peptides ([RRKWLWLW]) with POPE–POPG lipid membranes.
Notes: (**A**) With a ratio of 1/22 CP/Lip, the upper image is the initial interaction at 0 ns and the lower image is the final simulation omitting the peptides at 165 ns. (**B**) With the ratio of 1/10 CP/Lip at 658 ns, the top image is the top view, the middle image is the side view of the initial interaction, and the lower image is the final simulation omitting the peptides. (**C**) With the ratio of 1/7 CP/Lip at 1.02 μs, the top image is the top view, the middle image is the side view of the initial interaction, and the lower image is the final simulation omitting the peptides. (**D**) With the ratio of 1/5 CP/Lip at 6.12 μs, the top image is the top view, the middle image is the side view of the initial interaction, and the lower image is the final simulation omitting the peptides. Reprinted with permission from Khalfa A, Tarek M. On the antibacterial action of cyclic peptides: insights from coarse-grained MD simulations. *J Phys Chem*. 2010;114(8):2676–2684. Copyright 2010 American Chemical Society.[98]
Abbreviations: CG-MD, coarse-grained molecular dynamic; POPE, palmitoyl-oleoyl-phosphatidylethanolamine; POPG, palmitoyl-oleoyl-phosphatidylglycerol; CP/Lip, cyclic peptide/lipid.

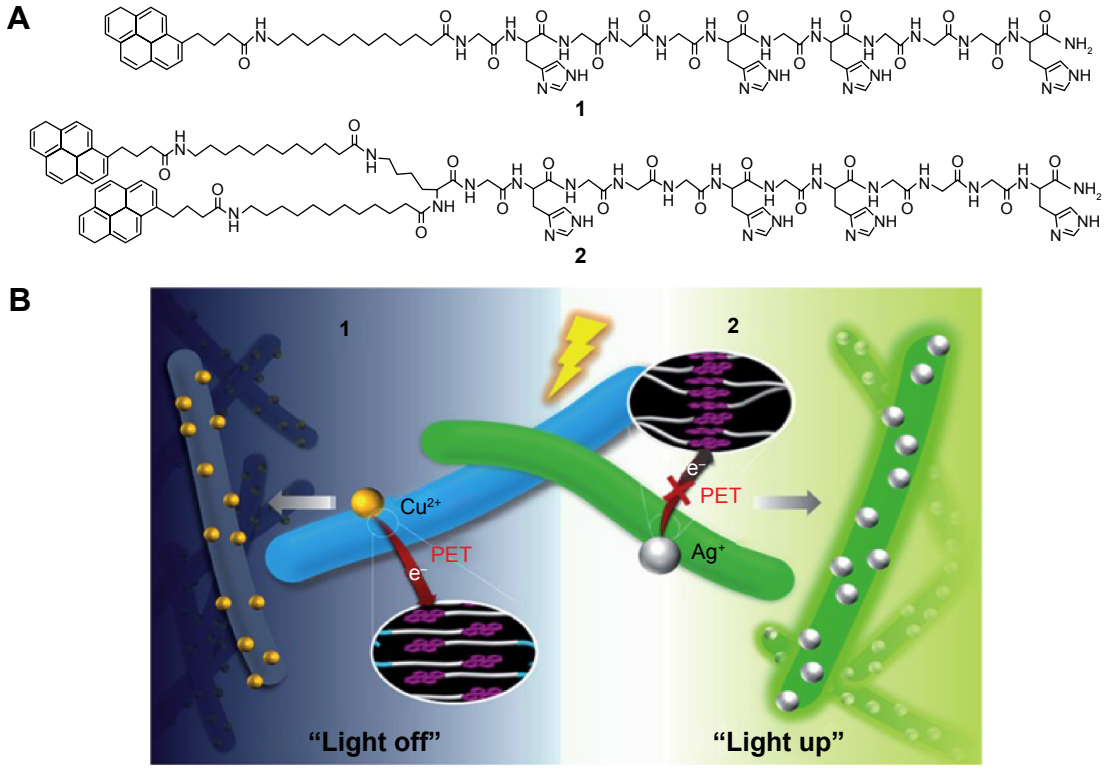

Figure 9 (**A**) The molecular structure of the pyrene-labeled peptide amphiphiles 1 and 2. (**B**) Schematic representation of the fluorescence light off and light up detections to copper and silver ions. Reproduced from Kim I, Jeong HH, Kim YJ, et al. A "light-up" 1D supramolecular nanoprobe for silver ions based on assembly of pyrene-labeled peptide amphiphiles: cell-imaging and antimicrobial activity. *J Mat Chem*. 2014;2(38):6478–6486, with permission of The Royal Society of Chemistry.[112]
Abbreviation: PET, photoinduced electron transfer.

Inspired from peptide sequences with a biomineralized capability in nature, peptide nanotubes can be conjugated with these sequences (such as peptide amphiphiles containing the RGD sequence) to nucleate metal/semiconductor materials on the surface. Several groups have utilized peptide nanomaterials to control mineralization for bone tissue regeneration.[104,105] This method could facilitate bone cell growth along the peptide fibers with a desired direction in the scale of nanometers.

For diagnostic imaging, peptide nanomaterials have been also conjugated with radiometals for in vivo tracking using the near-infrared fluorescence[106] or MRI.[107,108] Bull et al synthesized two Gd(III) conjugated amphiphilic peptides (DOTA-KK(K)K-LL-CCC-K-C_{16} and DOTA-KGRGDS(K)K-LLL-AAA-K-C_{16}) to form spherical and tubular structures. Since the self-assembling structure could increase the molecular weight of Gd, the rotational correlation time and consequently the relaxivity were enhanced. MRI tests demonstrated that these Gd functionalized peptides could increase T1 relaxation time for medical applications.[108]

With advantages of one-dimensional direction and a controllable size, peptide nanotubes are suitable to work as building blocks for nanoelectronic devices.[109] After nucleation, the electronic properties of the peptide nanotubes can be tuned by the size, shape, arrangement, and density of a mineralized coating. Additionally, the conditions (eg, pH, ion concentration, etc) of the extra environment also affected the mineralization process and the final conductivity.[109,110] Previous studies utilized such nanotubes to modify membranes to detect the conductivity change from the antigen–antibody interactions.[111]

For example, the self-assembled histidine-rich HGGGH-GHGGGHG (HG12) peptides with linear or branched alkyl chains were labeled by pyrene to detect metal ions (Figure 9).[112] Since the HG12 sequence bonded Cu^{2+} specifically, the fluorescence could be blocked. On the other hand, the Ag^{+} could "light up" the fluorescence response of the nanofibers with branched alkyl chains (Figure 9). Moreover, the nanofibers could work as templates for Ag nanoparticles for cell imaging and antibacterial effects.[112] Table 1 summarizes the highlighted medical applications of self-assembled peptides in this review.

Future directions and conclusion

Based on noncovalent forces, self-assembled molecules could construct ordered structures of defined shapes, various properties, and multiple functions. Examples from

Table 1 Highlighted medical applications of self-assembled peptide nanostructures

Applications	Peptides	Structures	References
Deliver anti-inflammatory drug dexamethasone	C_{16}-$V_2A_2E_2$	Nanofibers	63
Deliver hydrophobic drug paclitaxel to inhibit cervical cancer	qC_8-Tat (Tat peptide with four hydrophobic tails)	Nanofibers	64
Promote fibroblast cell adhesion and growth	$(C_{16})_2$-Glu-PEO-GRGDSP	Vesicles	81
Develop pH-sensitive cell scaffolds	C_{16}GSH and C_{16}EOSH	Micelles at pH =7 Nanofibers at pH >6.5	77
Promote bone regeneration	$C_{16}A_4G_3$LRKKLGKA	Nanofibers	82
Inhibit bone cancer cells	$C_{18}GR_7$RGDS	Micelles	83
Promote human mesenchymal stem cell growth and differentiation	$V_3A_3E_3$	Temperature-sensitive nanofibers	84
Improve chondrocyte growth and functions	KLDLKLDLKLDL (KLD12)	Nanofibers	35
Promote bone fracture healing and antimicrobial effect	KLDLKLDLKLDL (KLD12)	Nanofibers	93
Inhibit *E. coli* and *S. aureus* growth	VKVRVRVRV^{D}PPTRVRVRVKV	Nanofibers	86
Inhibit *E. coli* and *S. epidermidis*	YGAAKKAAKAAKKAAKAA	Nanofibers	91
Simulate the interaction of self-assembling cyclic peptides with cell-membrane models	The cyclic peptide ([RRKWLWLW])	Nanotubes	98
Increase T1 relaxation for MRI tests	DOTA-KK(K)K-LL-CCC-K-C_{16} and DOTA-KGRGDS(K)K-LLL-AAA-K-C_{16} coupled with Gd(III)	Nanofibers Micelles	108
Detect metal ions (Cu^{2+} and Ag^{+})	HGGGHGHGGGHG (HG12) with linear or branched alkyl chains was labeled by pyrene	Nanofibers	112

Abbreviations: *E. coli*, *Escherichia coli*; *S. aureus*, *Staphylococcus aureus*; *S. epidermidis*, *Staphylococcus epidermidis*.

recent studies have shown that self-assembled peptides with different structures have been used for a wide range of medical applications. However, in this field, scientists and researchers are still facing challenges to predict higher hierarchical structures, properties, and functions from the structure of the individual building molecule. With multidisciplinary efforts, self-assembled peptides will help us not only to study complex biological phenomena and create various applications but also to conquer the diseases and improve human health.

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Disclosure

The authors report no conflicts of interest in this work.

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