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CRITICAL REVIEW

Designer nanomaterials using chiral self-assembling peptide systems and their emerging benefit for society

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Chirality is absolutely central in chemistry and biology. The recent findings of chiral self-assembling peptides' remarkable chemical complementarity and structural compatibility make it one of the most inspired designer materials and structures in nanobiotechnology. The emerging field of designer chemistry and biology further explores biological and medical applications of these simple D,L- amino acids through producing marvellous nanostructures under physiological conditions. These self-assembled structures include well-ordered nanofibers, nanotubes and nanovesicles. These structures have been used for 3-dimensional tissue cultures of primary cells and stem cells, sustained release of small molecules, growth factors and monoclonal antibodies, accelerated wound-healing in reparative and regenerative medicine as well as tissue engineering. Recent advances in molecular designs have also led to the development of 3D fine-tuned bioactive tissue culture scaffolds. They are also used to stabilize membrane proteins including difficult G-protein coupled receptors for designing nanobiodevices. One of the self-assembling peptides has been used in human clinical trials for accelerated wound-healings. It is our hope that these peptide materials will open doors for more and diverse clinical uses. The field of chiral self-assembling peptide nanobiotechnology is growing in a number of directions that has led to many surprises in areas of novel materials, synthetic biology, clinical medicine and beyond.

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1 Introduction

A large number of complex and sophisticated small-molecule drugs including palytoxin and taxol have been synthesized with remarkable chiral precision. Likewise design and fabrication of

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functional chiral biological materials have also made great improvements that meet the needs of scientific, technological and medical challenges. These achievements showed that chemical and materials scientists have made significant advances in major frontiers of chemical constructions.

Nature has not only selected and evolved numerous and diverse chemical and molecular building blocks, but also defined structural motifs through billions of years of molecular selection and evolution. These basic building blocks possess extraordinary capacities to form complex, active and specific functional motifs and sophisticated nanomachines including ribosomes, photosystems, membrane transporters, ion channels and ATP synthesis complex that work at astonishing speed, exquisite selectivity, and marvellous efficiency with the finest controls.

We are learning the basic molecular engineering principles for milli-, micro-, and nano-meter scale to precisely position these motifs with the molecular self-assembly phenomena in the way that nature does. We are just at the beginning and we are moving forward steadily. One of our ultimate goals is to carry out programmable assembly of matters just as nature does.

1.1 Molecular self-assembly

Molecular self-assembly is the spontaneous association of individual components into well-organized structures with a fine-tuned balance between numerous noncovalent weak interactions. It is not only well-studied chemistry but also essential in all chemical and life sciences. The key elements in molecular self-assembly are chemical complementarity and structural compatibility through numerous non-covalent weak interactions.[1,2] These include: (1) hydrogen bonds, (2) electrostatic interactions, (3) hydrophobic interactions, (4) van der Waals forces and (5) water-mediated hydrogen bonds. Each interaction in isolation is rather weak, but collectively they exert strong molecular forces to facilitate the formation of molecular structures and maintain the stability of these structures. In analogy to these interactions, as Chinese wisdom best puts: "easy to break one chopstick, it is difficult to break 10-bundled chopsticks".

The weak interactions and self-assembly are one of the principal means that nature uses to achieve complexity and its sophistication, that includes DNA double helix, ribosomes and light-harvesting photosystems, shell and tooth growth.

Self-assembly has recently emerged as a new approach in chemical synthesis, nanobiotechnology, polymer science, materials and engineering. Molecular self-assembly is a rather broad and fast-moving field; it is impossible to fully cover the entire spectrum, so here we solely focus on the chiral self-assembling peptide systems.

1.2 Discovery of the first self-assembling peptide

While working on yeast genetics and protein chemistry and trying to understand a left-handed Z-DNA structure at Alexander Rich laboratory at Massachusetts Institute of Technology in 1989, one of us (Shuguang Zhang), identified a protein *Zuotin* for its ability to bind to left-handed Z-DNA in the presence of 400-fold excess of sheared salmon DNA that contains ubiquitous right-handed B-DNA and other form DNA structures, so he named EAK16 (AEAEAKAKAEAEAKAK) for its amino acid composition.

Initial computer modelling of this EAK16 sequence showed the structure to be a α-helix: its lysines and glutamic acids on the side-chains with i, $i + 3$ and i, $i + 4$ arrangements could form potential ionic bonds. Alexander Rich then supported Zhang to synthesize the actual EAK16 peptide. When the EAK16 peptide was studied following the reported method using circular dichroism spectroscopy, an unexpected result occurred, instead of showing the expected α-helix, the peptide formed an exceedingly stable β-sheet structure. Zhang later met Francis Crick and told him about the discovery. Crick suggested Zhang to look under a scanning electron microscope (SEM), so Zhang did. It took Zhang more than one year to understand how the seemingly soluble short peptides underwent self-assembly to form well-ordered nanofibers and scaffolds and to form naked-eye visible materials. MIT filed an U. S. patent application in 1992 (issued in 1997). Zhang and his colleagues published the yeast *Zuotin* where the first self-assembling peptide was discovered;[1] since then self-assembling peptides have been growing in several directions in the past two decades (Fig. 1).

2. Chiral amino acid system to peptide nanoscaffolds

Chirality plays an absolutely central role in all life forms.[3–5] Amino acids are available with D- and L-forms, but all proteins are made of only L-amino acids. There is an intense and wide spread interest in understanding the origin and selection of chirality in prebiotic molecular evolution and biology. For example, the chirality of life is asymmetric and nature only uses L-amino acid and D-sugars.

In the past few decades, much effort has been focused on the study of various self-assembling peptides and their relevance in biology, protein self-assembly and their applications in biotechnology and nanobiotechnology. Not surprisingly, most of this work has been carried out using the naturally occurring L-form amino acid (Fig. 2). Symmetries are common in nature and in man-made structures: leaf, butterfly wings, virus structure, animal and human body structures, the pyramids in Egypt, the Imperial Palace, as well as to the chiral self-assembling peptide molecules[3–5] (Fig. 2).

2.1 L-Amino acid self-assembling peptide systems

2.1.1 Complementary strategies of the self-assembling peptide systems. Designer materials that are self-assembled molecule by molecule (or atom by atom) to produce novel supramolecular architectures belong to the "bottom-up" instead of the "top-down" approach, and likely become an integral part of materials manufacture. This approach requires a deep understanding of individual molecular building blocks, their structures and dynamically assembly properties.[6,7]

These self-assembling peptides have alternating hydrophobic, *e.g.*, alanine, valine, leucine, isoleucine, and phenylalanine, and hydrophilic sides, which include positively charged lysine, arginine, histidine, and negatively charged aspartic acids and glutamic acids.

The complementary ionic sides have been classified into modulus I, II, III, IV and mixed moduli. This classification is based on the hydrophilic surface of the molecules that have alternating + and − charged amino acid residues, either alternating by 1, 2, 3, 4 and so on. For example, charge

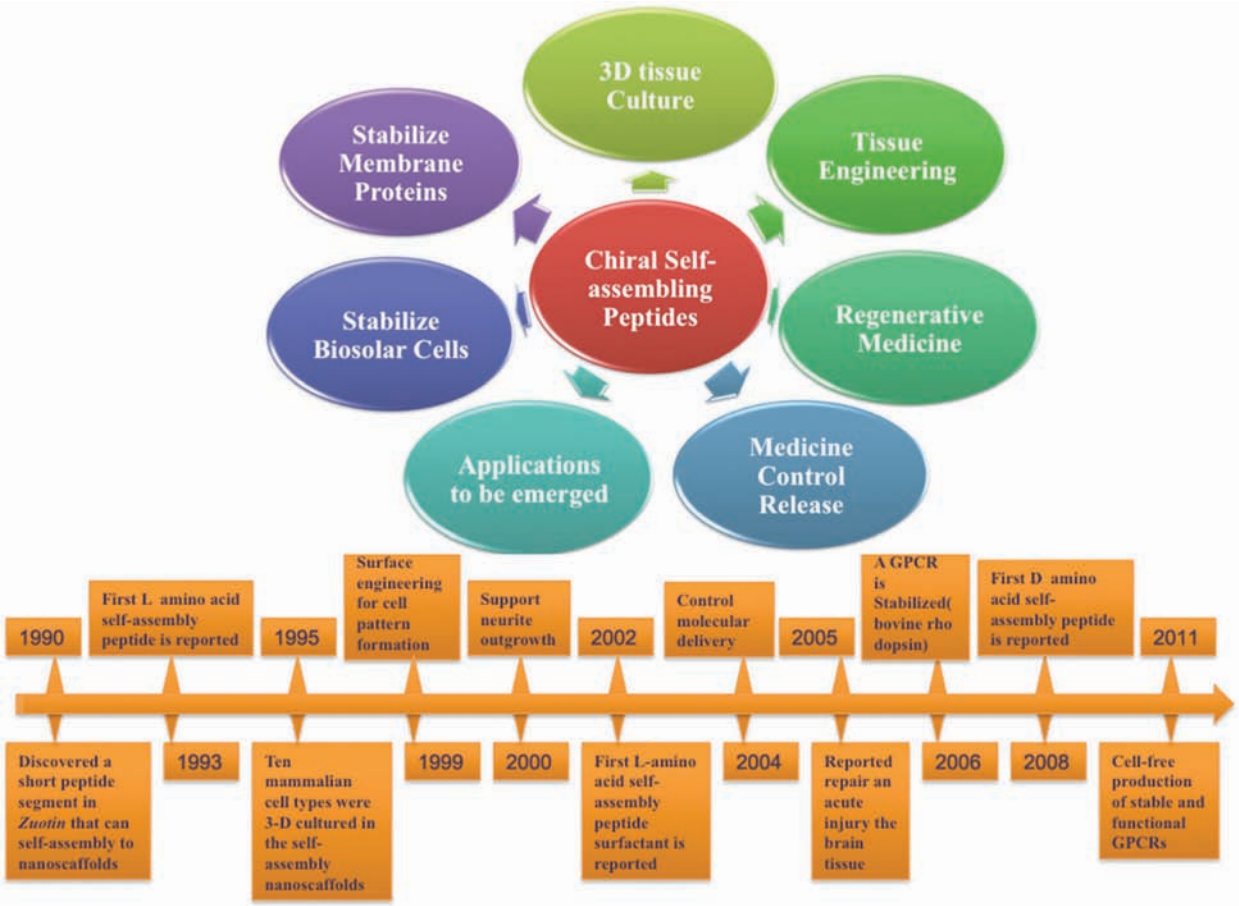

Fig. 1 The timeline of development process and diverse applications of designer chiral self-assembling peptides. It spans over 20 years from the curiosity-driven research and serendipitous discovery of the first self-assembling peptide EAK16-II in 1990 to successful human clinical trials in 2011.

arrangements for the different moduli are as follows: modulus I, − + − + − + − +; modulus II, − − + + − − + + ; modulus III, − − − + + +; and modulus IV, − − − − + + + + (Fig. 3A).[1,2] The charge orientation can also be designed in reverse orientations that yield entirely different molecules with distinct molecular behaviors.[8–10] These well-defined sequences allow them to undergo ordered self-assembly, resembling some situations found in well-studied polymer assemblies. This simple idea is considered to be the cornerstone of the self-assembling peptide building blocks.

2.1.2 Basic chemical properties of the self-assembling peptide systems. The peptide synthesis has become more and more affordable that uses conventional mature solid phase or solution peptide synthesis chemistry. The peptide production cost directly correlates with the motifs length, purity of peptides, skill of the manufacturers and the chirality of amino acids. Most of the self-assembling peptides are readily soluble in water since their amino acid molecules consist of alternating hydrophilic and hydrophobic moieties that contain 50% charged residues with distinct polar and non-polar surfaces and periodic repeats of 2–4 times. The self-assembly is accelerated by millimolar salt concentration under the physiological pH conditions to form ordered-nanostructure such as nanofiber, nanotube and nanovesicle.[1,6,11–13]

For example, RADA16-I and RADA16-II with arginine and aspartate residues replacing lysine and glutamate have been designed. The alanines form overlapping hydrophobic interactions in water, both positive Arg and negative Asp charges are packed together through intermolecular ionic interactions in a checkerboard-like manner. They self-assemble to form nanofibers ~10 nm in diameter, and these nanofibers interweave into scaffolds that retain extremely high hydration, > 99% in water (1 mg–10 mg ml^{-1}, w/v) (Fig. 3B).[14,15]

The formation of the scaffold and its mechanical properties are influenced by several factors: (1) amino acid sequence, (2) the level of hydrophobicity, (3) length of the peptides, and (4) self-assembling time. For example, to the extent of the hydrophobic residues, Ala, Val, Ile, Leu, Tyr, Phe, Trp (or single letter code, A, V, I, L, Y, P, W) can significantly influence the mechanical properties of the scaffolds and the speed of their self-assembly. The higher the content of hydrophobicity, the easier it is for scaffold formation and the better for their mechanical properties.[16]

2.1.3 General self-assembling peptide systems. The first self-assembling peptide l-EAK16, the lipid-like A_6D,[20–22] and a D-EAK16,[17,18] are general peptides without active motifs. Here, the RADA16 is presented as a progress milestone in development. RADA16 has become a commercial product (BD Biosciences), and it has been successfully used in various medical applications.[23–35]

The peptides can form stable secondary structures, including α-helix, β-sheet and random-coil. But some peptide

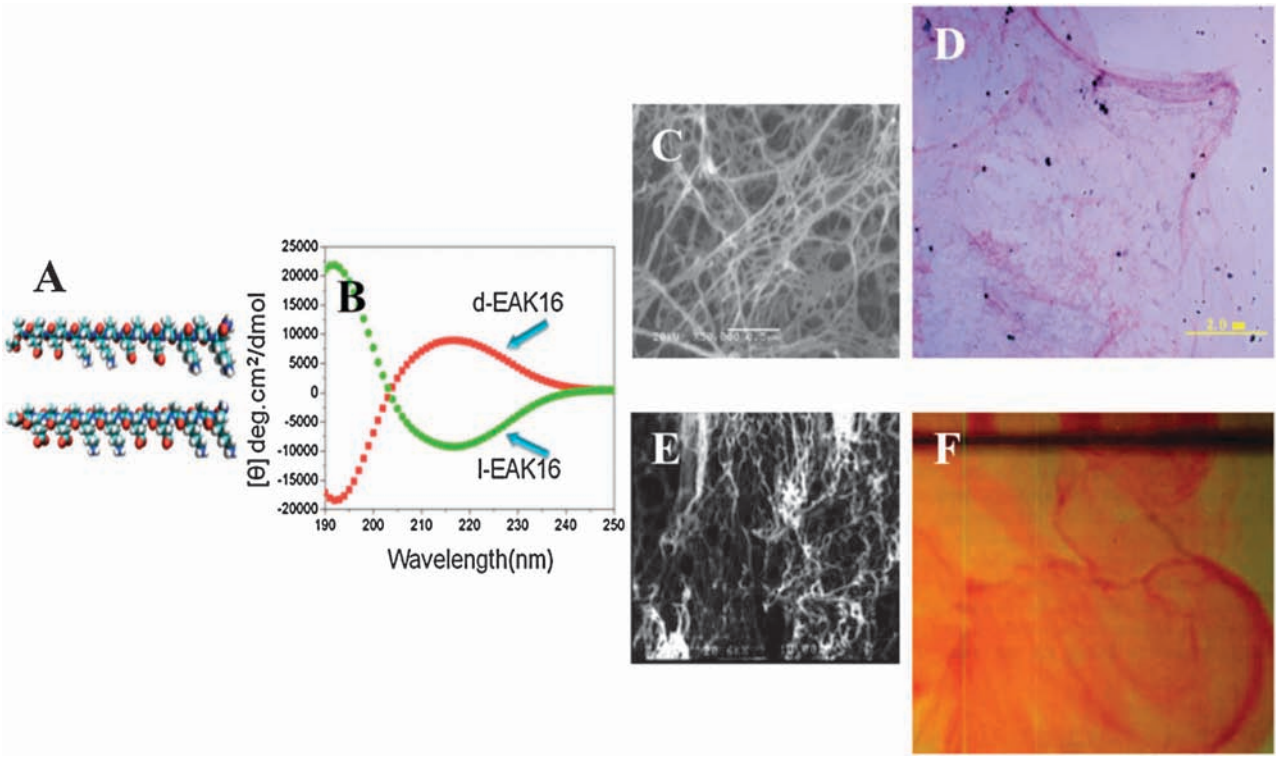

Fig. 2 The typical chiral self-assembling peptides. (A) Molecular models of D-EAK16 and L-EAK16;[17,18] (B) circular dichroism spectra of the peptides at 37 °C. D-EAK16 and L-EAK16 are a pair of chiral molecules which are a 3D mirror image to each other;[18] (C) SEM photograph of D-EAK16, and the nanofiber structure is similar to that of L-EAK16. The diameter of the nanofibers is about 10 nm, and the pores of the scaffold are about 20–500 nm;[19] (D) a colorless membranous structure was formed in PBS and transferred to a glass slide and can then be seen by naked eyes. The image is stained bright red with Congo red;[19] (E) SEM photograph of L-EAK16, and the diameter of the nanofibers of is about 10 nm[1]; (F) the photograph of L-EAK16 was stained Congo red as well.[14]

secondary structures are more dynamic under various environmental factors: (i) the amino acid sequence arrangements (even with the identical composition), (ii) the molecular size of the peptide, (iii) concentration, (iv) pH of the solution, (v) temperature, (vi) the medium composition, such as solvent or substrate, (vii) ionic strength, and (viii) the presence of denaturation agents, such as sodium dodecyl sulfate (SDS), urea and guanidium hydrochloride. These factors can significantly influence the dynamic behaviours of peptide secondary structures and also affect the process of self-assembly.

For L-amino acid peptides, both EAK12-d and DAR16-IV*, an increase in temperature results in an abrupt structural transition from β-sheet to α-helix. EAK12-d exhibits a small loss in β-sheet content upon heating to 60 °C, and the beginnings of a structural conversion is observed due to α-helical characteristics at 70 °C. A profile was obtained for the transition from β-sheet and α-helix at 85 °C. By 90 °C, the structural conversion is complete.[36] Likewise, the peptide DAR16-IV* underwent a similar transformation in the two distinct spectra at 25 °C and 90 °C.[36]

These peptides can be used as model systems for studying protein conformational diseases, and aid in studying the structural dynamics and plasticity of proteins. Similarly the pH changes can also have drastic effects on EAK12-d and DAR16-IV*. Much of this work has been carried out using these peptides.[37]

Another important class of peptides are the lipid-like peptide surfactants, these peptides are similar to that of natural phospholipids, with tuneable hydrophobic tails to various degrees of hydrophobic amino acids such as alanine, valine or leucine, a hydrophilic head with either negatively charged aspartic and glutamic acids or positively charged histidine, lysine or arginine, they undergo self-assembly to form well-ordered nanostructures including nanotubes, nanovesicles and micelles (Fig. 3C).[20,21,38–43]

2.1.4 Designer specific self-assembling peptide scaffolds. In order to fully understand how cells behave such as the 3-dimensional (3D) microenvironment, 3D gradient diffusion, 3D cell migration and 3D cell–cell contact interactions and in tissue engineering and regenerative medicine, it is important to develop a well-controlled 3D tissue culture system where every single ingredient is known.

In order to achieve fine-tuning and control, we have designed tailor-made peptides to suit the specific individual needs of studies or applications through appending specific active motifs onto the basic peptide, such as RADA16-I or EAK16-II.

From a synthetic organic chemistry aspect, both of peptides C- or N-termini could be attached to the modified motifs. However, the functional motifs are always located on the C-termini because solid phase peptide synthesis initiates synthesis from the C-termini and proceeds toward the N-terminus. The longer the peptide sequence made, the more probable the coupling error would occur. Thus in order to avoid peptide synthesis errors, the active sequence motifs should always be at the C-terminus without exception (Fig. 3A).

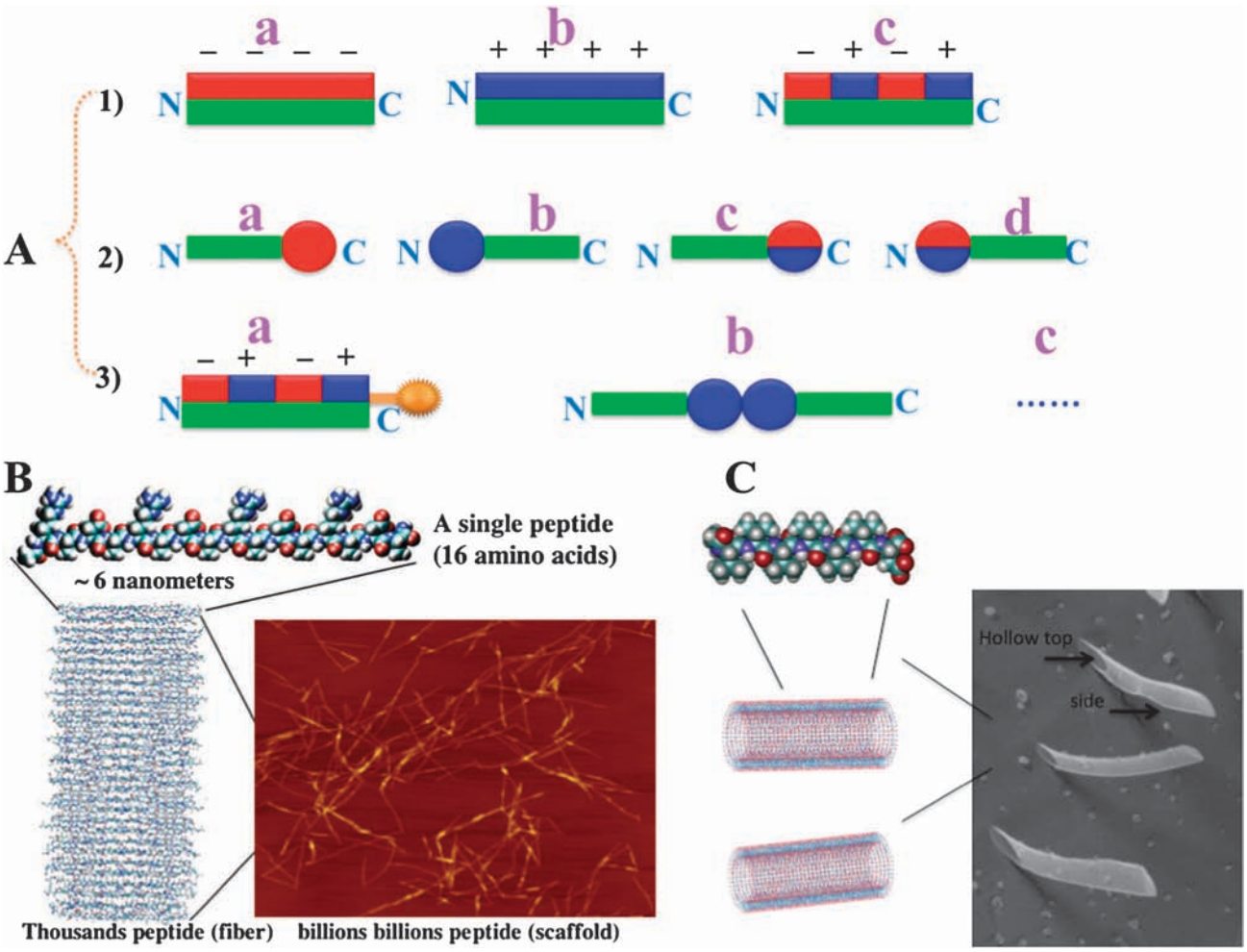

Fig. 3 The simple and molecular models of the designer chiral self-assembling peptides that form well-ordered nanostructures. (A) (1) These peptides have two distinctive sides, one hydrophobic and the other hydrophilic. The hydrophobic side forms a double sheet inside of the fiber and the hydrophilic side forms the outside of the nanofibers that interact with water molecules, forming an extremely high water content hydrogel that contains as high as 99.9% water. At least three types of molecules can be made with −, +, −/+ on the hydrophilic side. They undergo self-assembly to form nanofibers with the nonpolar residues inside (green) and + (blue) and − (red) charged residues form complementary ionic interactions;[1,2,7,44] (2) lipid-like peptides, ~2 nm in size, which have a distinct head group, either positively charged or negatively charged, and a hydrophobic tail consisting of six hydrophobic amino acids. They can self-assemble into nanotubes and nanovesicles with a diameter of ~30–50 nm. These nanotubes go on to form an inter-connected network, which has also been observed in other nanotubes;[20,22] (3) modified peptides from C-terminus like biological motif,[45] or double head-double tail lipid-like peptide and more; More details see Fig. 7 and Fig. 10; (B) Self-assembling peptide RADA16-I nanofibers scaffold. Amino acid sequence of RADA16-I, molecular model of a single RADA16-I nanofibers, the calculated peptide dimensions are B6 nm long depending on end capping, 1.3 nm wide and 0.8 nm thick; tens and hundreds of thousands of individual peptides self assemble into a nanofibers; suggesting that the nanofibers formation is independent of the air-drying process (AFM);[14,15] (C) self-assembling peptide V_6D nanotubes. Amino acid sequence of V_6D, molecular model of a single V_6D self-assembling to thousands of nanotubes; tens and hundreds of thousands of individual peptides self assemble into nanotubes (TEM).[22]

We attached several functional motifs including cell adhesion, differentiation and bone marrow homing motifs on the C-termini. We used them to study neural stem cells, and these functionalized peptides underwent self-assembly into nanofiber structures as well.[45] More functionalized self-assembling peptides have been shown to promote specific cellular responses and cell survival, proliferation, migration, and morphological differentiation,[46–48] but these peptides are expensive for most applications.

Designer self-assembling peptide scaffolds have also shown interesting interactions with functional proteins for the study of molecular releases since the release kinetics results suggested that protein diffusion through nanofiber scaffolds primarily depended on the size of proteins.[49]

In addition, some modified peptide labelling of Au or Ag nanoparticles,[50] DNA,[51] or use in the orthogonal Cu(I)-catalyzed azide-alkyne and photoinitiated thiolene reactions, through the conjugation of biotin followed by streptavidin-nanogold particles, or rhodamine, have been shown for fibers harbouring this approach, which allows for the development of multi-component functionalized systems.[52]

For lipid-like peptide surfactants, some are expanded to 2–4 amino acids, which have useful applications.[53,54] Another way is from primary sequence geometry angles[55] to design cone-shaped,

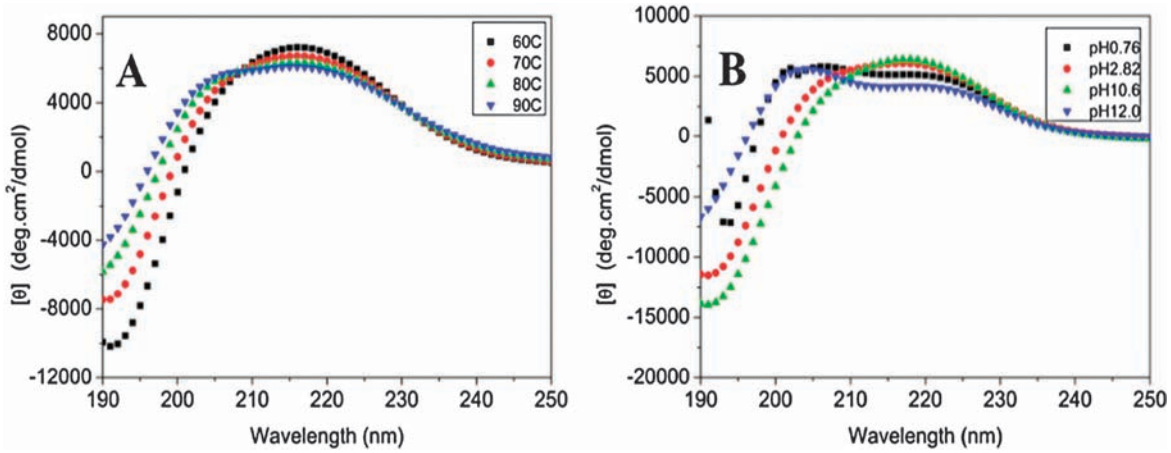

Fig. 4 The effects of temperature and pH values to the D-form peptide D-EAK16. (A) D-EAK16 exhibits three distinctive structures at 60 °C, 80 °C and 90 °C; (B) the D-EAK16 spectra suggest four variations of structures at pH 0.76, pH 2.8, and pH 10.5 and pH 12.0.[17]

Ac-GAVILRR-NH_2, which has a hydrophilic head with two positive charges and a relatively large head size, a hydrophobic tail with decreasing hydrophobicity and a side-chain size with a cone shape, which can self-assemble into interesting nanodonut structures.[56]

2.2 D-Amino acid peptide system

Peptides and proteins made of only D-amino acids are more stable since naturally occurring proteases can readily degrade L-form peptide bonds, but cannot degrade D-form peptide bonds.[17,19,57] Likewise, ribosomes can incorporate L-form amino acids but not D-form.[58–62] Nature has a remarkable ability for chiral selectivity.[63–66] It has been shown that a HIV protease made of all D-amino acids could only catalyze D-substrates.[67]

In August 2005, we lively discussed some tantalizing questions, such as why the chirality of life is asymmetric and why nature only uses L-amino acids and D-sugars. We then decided to design several self-assembling peptide using (i) D-amino acids only instead of L-amino acids and (ii) a combination of both in an alternating manner.

One of us (Zhongli Luo) filed a patent application in 2007 and published the paper in 2008.[17] D-EAK16 is influenced by environmental factors including temperature, pH and various salts. For example, D-EAK16 peptide displayed a typical β-sheet spectrum at 25 °C. Upon increase of the temperature above 80 °C, the D-EAK16 peptide is transformed into a typical α-helix CD spectrum without going through a detectable random-coil intermediate (Fig. 4A). D-EAK16 formed a α-helical conformation at pH 0.76 and pH 12 but formed a β-sheet at neutral pH (Fig. 4B) and the self-assembling process can be influenced by high temperature and extreme pH (Fig. 5).

D-EAK16 can also form nanofibers as function of time (Fig. 6). More importantly, D-form peptides are resistant to natural L-enzyme degradation.[19] Because a higher concentration of D-EAK16 in solution causes the self-assembling of a higher density of nanofibers, these highly hydrated nanofibers with numerous lysine and glutamic acids on the surface can perhaps organize water molecules to form numerous nanopores, like its counterpart L-peptide RADA16,[15] it formed hydrogel (Fig. 2D).

This hydration phenomenon is similar to that found in jellyfish, which itself is highly hydrated, containing > 95% water.

2.3 D,L-amino acid hybrid peptide system

Most of the D,L-amino acid hybrid peptides were found in antibiotic peptides[68,69] and antimicrobial peptides.[70,71] They involve in inactivation of membrane proteins,[72] suppression of human prostate tumour growth,[73] potential anti-melanomic drugs,[74] cell-penetrating,[75] inhibited T-cell activation,[76] or for an immunosensor.[77]

In the self-assembling peptide system we used the hybrid peptides with identical sequences. We discovered that their secondary structures undergo drastic changes.[18] For example, the four peptides, (i) all D-amino acids in D-EAK16, (ii) all L-amino acids in L-EAK16, (iii) EA*K16, only A* is D-alanine and E and K are L-amino acids, (iv) E*AK*16, Ala is L-amino acid and E* and K* are D-amino acids.

EA*K16 has a maximal ellipticity at 208 nm and a minimal ellipticity at 199 nm. Likewise, E*AK*16, which is an enantiomer of EA*K16, has a minimal ellipticity at 208 nm and a maximal ellipticity at 199 nm. Their structures are almost symmetrical to each other in water. The secondary structures of EA*K16 and E*AK*16 are neither a typical β-sheet nor a α-helix formation and seem to be uncharacterized, as they appear closer to "random coil" CD spectra. The mixed chiral amino acids in the peptides indeed drastically alter the secondary structures, especially for the D-amino acid substitutions that disrupted the β-sheet structure in either all L- or all D-form peptides, and changed the content of secondary structure completely. Moreover, they showed poor self-assembling properties to form the ordered-nanostructures,[18] thus we can use D- or L-isomer to fine-control the structures of self-assembling nanofibers.[78]

2.4 Other self-assembling peptide systems

Charlotte A. E. Hauser and her colleagues reported several ultra-small peptides with only 3–4 residues that could undergo self-assembly to form hydrogels with remarkable mechanical strength (Fig. 7A).[79,80] Two of the 6-residue peptides formed the shortest α-helixes, since they cannot form 2 helical turns (720°).

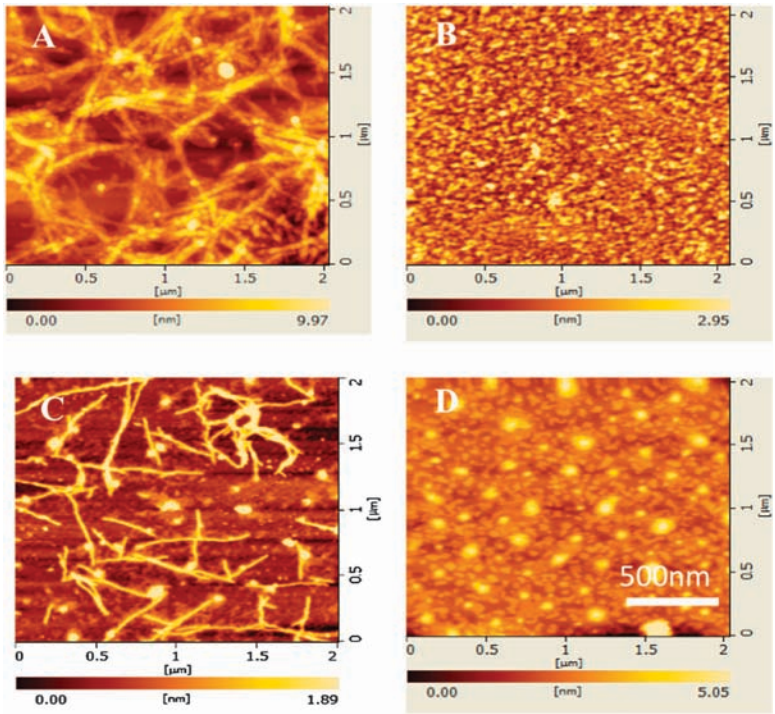

Fig. 5 The AFM images of D-EAK16 (1 mg ml^{-1}, 0.1%) under repeated cycles of thermal treatment and pH sensitivity. (A) D-EAK16 solution was incubated at 100 °C for 4 h, and PBS was added to allow self-assembly for 2 days. The AFM images of D-EAK16 (100 mM) are displayed under (B) pH 1, (C) pH 10.6 and (D) pH 12.8.[17] Reproduced with permission from ref. 17.

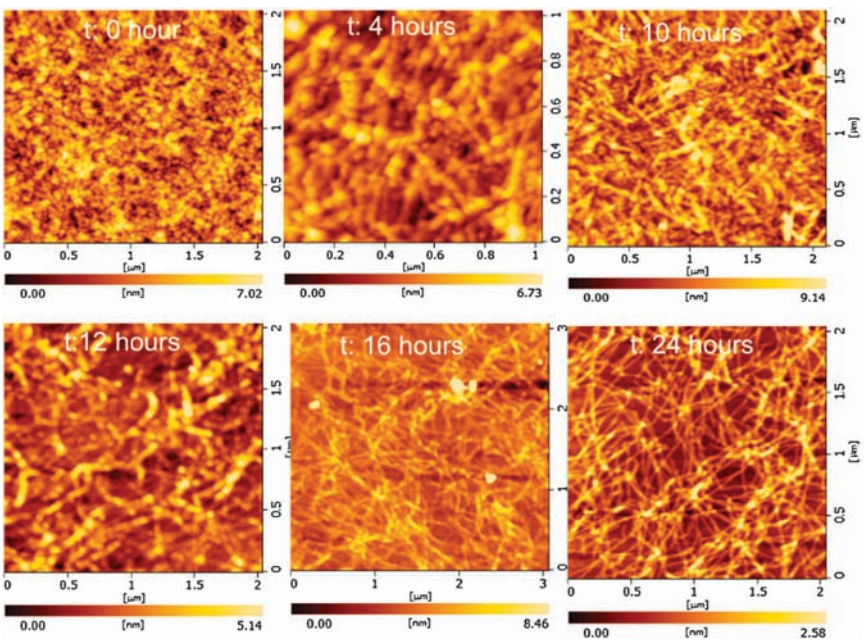

Fig. 6 The time dependent self-assembly process of d-EAK16 to nanofibers. AFM images show the progressing differences at 0, 4, 10, 12, 16 and 24 h.[18] Reproduced from with permission.[18]

They also studied two peptides, A_6D and A_6K, which co-assemble into the nanostructures. They also surprisingly observed that a peptide A_6K formed a stable α-helix in SDS, and it could transition to β-sheet as a function of time over many hours.[80–84]

Brian Lin and Matthew Tirrell presented a platform method for controlling the self-assembly of biofunctional PAs into spherical 50 nm particles using dendrimers as shape-directing scaffolds. This templating approach results in biocompatible, stable protein-like assemblies displaying peptides with native secondary structures and biofunctionality (Fig. 7B).[85]

Bengt Nordén and colleagues expanded the single-tail theme of the surfactant-mimics to choose hetero-dimers to ordered-nanostructures (Fig. 7C),[86] synthesized tryptophane

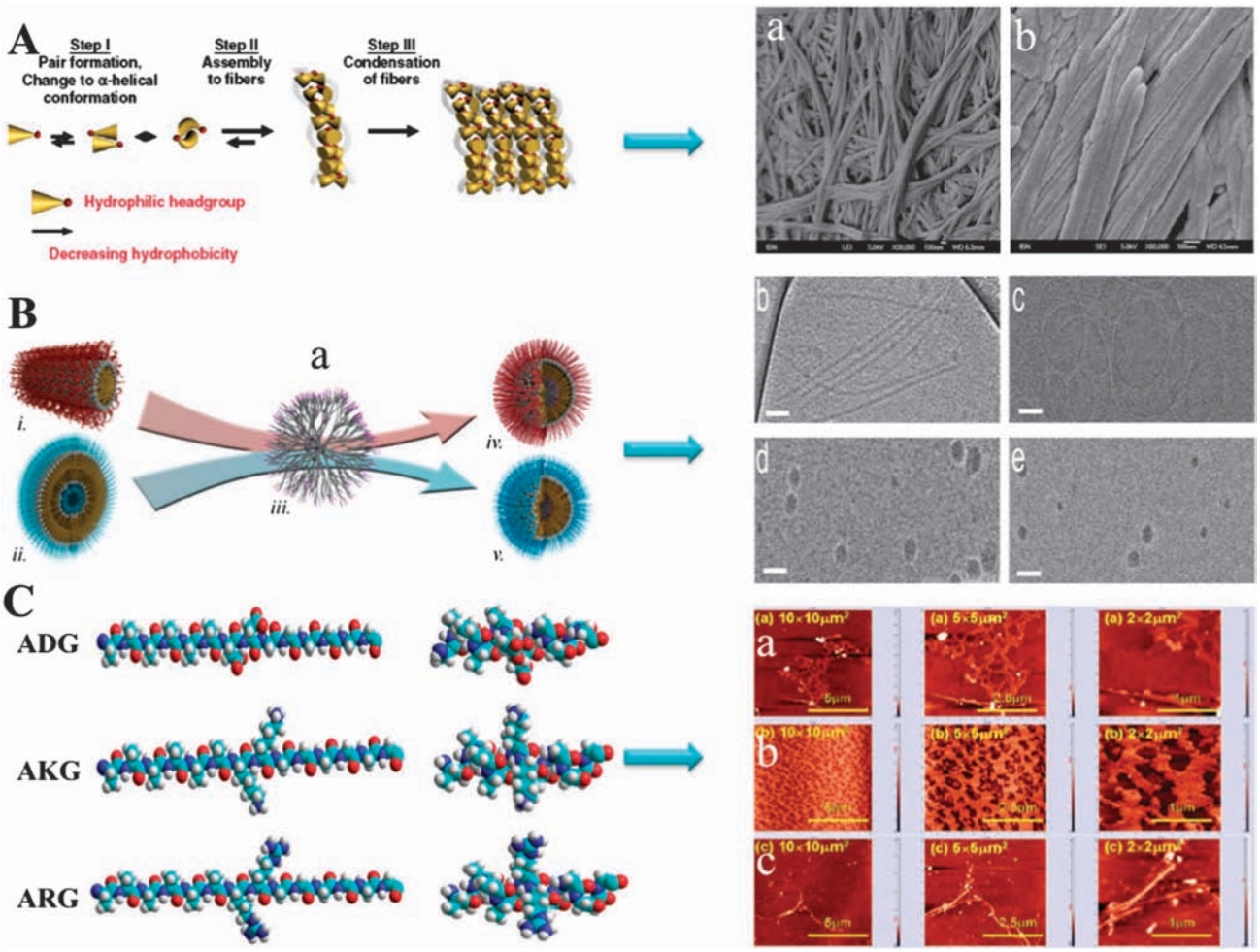

Fig. 7 Examples of several self-assembling peptides. (A) The proposed model of self-assembly from peptide monomers to supramolecular networks of condensed fibers. Self-assembly is initiated with antiparallel pairing of two peptide monomers by changing to an α-helical conformation. Subsequently, peptide pairs assemble to fibers and nanostructures and condense to fibrils resulting in hydrogel formation; morphological and structural characterization of the peptide scaffolds by FESEM. Condensed fibers of Ac-ID3 hydrogels of 15 mg ml^{-1} (A-a) and 20 mg ml^{-1} (A-b) (images reprinted with permission);[80] (B) The dendrimers template micelle-forming and vesicle-forming PAs into spherical PRTNs. (a) Schematic of templating process; (b) bZip PA cylindrical micelles, (c) NLS PA vesicles, (d) spherical bZip PRTNs, (e) spherical NLS PRTNs. Scale bars are 100 nm; (the figures are courtesy of Brian Lin and Matthew Tirrell);[85] (C) Proposed β-sheet (left) and α-helix (right) conformations of each of the three peptide-heterodimers, AFM-images of the structures formed by (a) ADG, (b) AKG, (c) ARG in 0.1M NaCl, at different degrees of magnification at 25 °C.[86]

zipper-forming,[87] or used the amino acid hybrid of the chemical compounds,[88] enriched the area to more nanostructures and more applications.

3 Proposed mechanisms of chiral self-assembling peptides

3.1 The proposed mechanism of nanofiber formations

The detailed mechanism of how self-assembling peptides self-organize themselves in water at such low concentrations still remains poorly understood at present, although various peptides as biological materials have produced diverse applications.

Several plausible ideas for different nanostructures have been proposed. First, for nanofibers, a molecular model to interpret the formation of EAK16 and RADA16 was proposed.[15] These two peptides are representative of a class of peptides that can undergo self-assembly into ordered-nanofibers:[15] (1) the contribution from the intermolecular hydrogen bonds in conventional β-sheets on the peptide backbone, (2) the side chains of positively and negatively charged residues form intermolecular ionic bonds, (3) hydrophobic interactions from the amino acid residues, (4) alternating polar and nonpolar surface interaction.[16] It is known that ions facilitate the self-assembly, however, it is not yet clear whether the monovalent ions coordinate the charged residues in a higher order of geometry.

Second, using another peptide KFE12, the self-assembly occurs when the solution conditions reduce intermolecular electrical double-layer repulsion below the van der Waals attraction in accordance with the DLVO theory.[89] The theory further predicts that self-assembly should occur when the peptide is electrically neutral, even in the absence of an exogenous salt.[90] The result suggested that increasing the number of repeats yields

a biphasic dependence – first decreasing then increasing the critical salt concentration, it is likely due to unequal competition between a greater hydrophobic (favourable) effect and a greater entropic (unfavourable) effect as the peptide length is increased.[91]

Computer simulation suggested that these left-handed helical ribbons are comprised of a double β-sheet and that the experimentally measured dimensions correspond to a local energy minimum.[91,92] Side chain interactions are found to be critical in determining the stability and curvature of the helix.[8] Hydrophobic interactions orient the peptides so as to minimize the solvent accessible surface area, and the dimer structures become trapped in energetically unfavourable conformations.[93]

3.2 The mechanism of nanotube and nanovesicle formations

The lipid-like self-assembling peptides can form the ordered-nanotubes and nanovesicles,[20,21,94] but the mechanism is not clear. A plausible path from the monomer state to the assemblies, is that two peptides form a dimer tail-to-tail packing to form a bilayer, monomeric peptides form small segments of the bilayer ring, with hydrophobic tails packing together to avoid water and hydrophilic heads exposed to water on the inner and outer portion of the tube, subsequently stacking through non-covalent interactions to form longer nanotubes.[22] Further study of the mechanism is of great importance because these lipid-like peptides have successfully stabilized diverse membrane proteins including GPCRs.[53,54]

4 Diverse applications of chiral self-assembling peptide nanomaterial systems

4.1 Applications of self-assembling peptide nanofibers

4.1.1 Designed 3D tissue cell cultures. The designer self-assembling peptide nanofiber scaffolds have been used to culture diverse types of tissue cells (Fig. 8A–D). Ten mammalian tissue type cells cultured in 2 peptide matrices, RADA16-I and EAK16-II, demonstrated that the cells are attached on the nanofiber scaffolds (Fig. 8E).[14] Several biologically active and functional motifs including cell adhesion, differentiation and bone marrow homing motifs, have been appended on the C-terminus of RADA16 directly through peptide synthesis, thus it provided a promising controlled 3D culture system for diverse tissue cells (Fig. 8E–F).[45–48] Self-assembling peptide monolayers

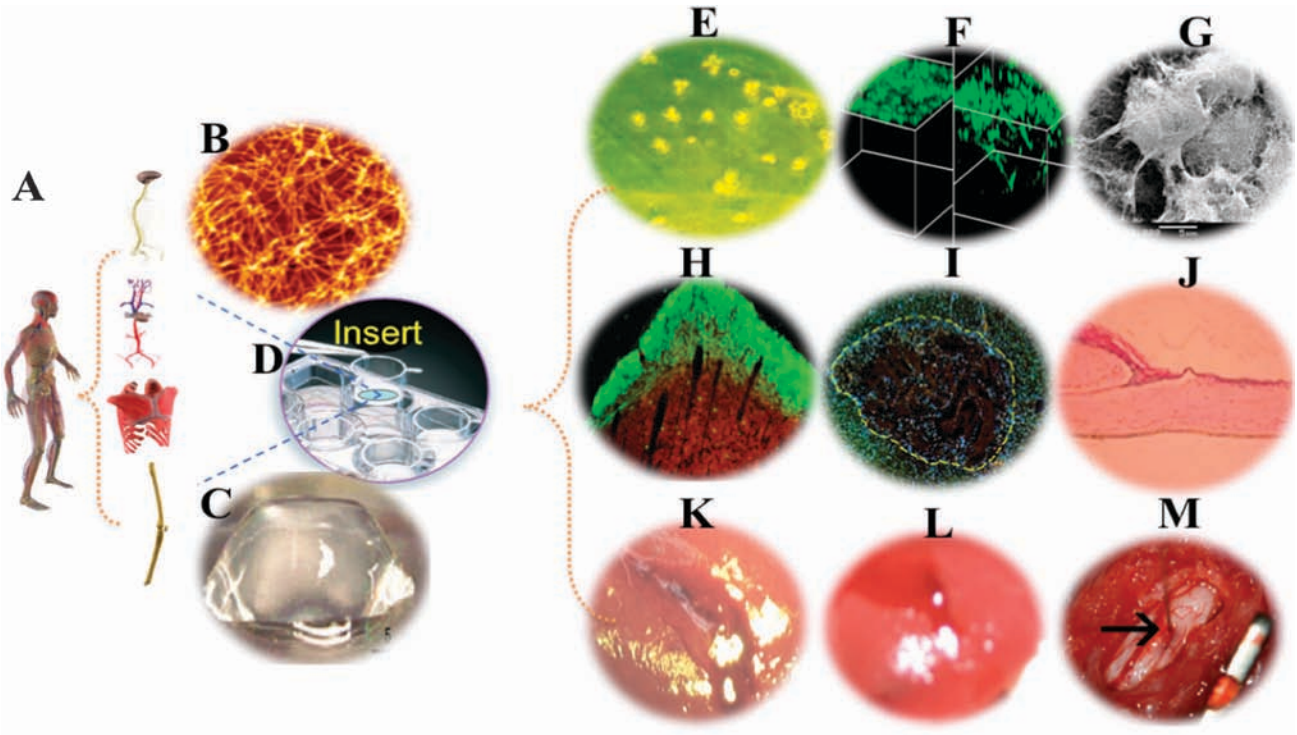

Fig. 8 Chiral self-assembling peptides used in cell engineering, tissue engineering and regenerative medicine. (A) The model of the body including the nervous system and spinal cord, cardiovascular, muscle and ossature. Human clinical trials have been launched for cardiovascular surgeries, skin wound healing and other diseases and injuries, for more information see the section 5; (B) the chiral self-assembling peptide nanofibers (D-EAK16) and peptide hydrogels of L-RADA (C); (D) cultured in 3-dementional cell insert; (E) first used the self-assembling peptide to cell-culture;[14] (F) Reconstructed image of 3D confocal microscope image of culturing on the different scaffolds consisting of different mix ratios of RADA16 1% (w/v) and PRG 1% (w/v) using calcein-AM staining;[100] (G) SEM images of adult mouse neural stem cells (NSC) embedded in designer peptide nanofiber scaffold RADA16-BMHP1 (1% v/w) after 14 day *in vitro* cultures;[45] (H) dark-field photo of a parasagittal section from the brain of an 8-month-old hamster treated with SAPNS at the time of surgery (at age 8 weeks) in the lesion site. The axons, shown by green fluorescence, have grown through the site of the lesion and are reinnervating the SC at ~82% of normal density;[116] (I) lesion sites with different treatments 6 weeks after surgery, the cavity formed by the trauma was filled with SAPNS, which integrated very well with the host tissue with no obvious gaps.[118] (J) Hematoxylin and Eosin staining of the wound healing after 48 h;[111] (K) D-EAK16 used in rapid hemostasis and (L) EA*K stopped bleeding very slowly;[18] (M) delivery of the peptide biomaterial into the damaged rat spinal cord.[119] These preclinical studies of repairing or regenerative tissues or body parts suggest that the chiral peptides have great potential in clinical medicine.

combining with microcontact printing also showed to either support or inhibit cell adhesion, thus they are capable of aligning cells in a well-defined manner, leading to specific cell arrays and pattern formations.[76,95]

Additional experiments showed that when a matrix metalloproteins-2 cleavage site, PVGLIG, was incorporated into one of the peptides, RADA, it showed that the scaffold could be cleaved, thus changing its property for cell and tissue remodeling.[96]

We have shown that the scaffolds are good materials to support neuronal cell attachment, differentiation as well as extensive neurite outgrowth.[12] Along the line of tissue engineering, the peptide scaffolds have also been used for cartilage repair and cartilage tissue engineering.[96] Injection of the peptide-encapsulated cells into the free-wall of the left ventricle can recruit cells to the microenvironment that form functional vascular structures.[97] When combining with other biomaterials, the self-assembling peptide RADA16 facilitated osteoblast growth and differentiation.[98]

Other experiments showed using a biotin sandwich method for targeting IGF-1 to the self-assembling peptide RADA16-II.[99] This biotin sandwich method allowed binding of IGF-1, but did not prevent the self-assembly of the peptides into nanofibers within the myocardium and improved the systolic function after experimental myocardial infarction.[99]

Moreover, addition of the biologically active motifs through direct coupling to different general self-assembling peptides showed that these functionalized designer peptide scaffolds have greater benefits for promoting cell growth, dedifferentiation, migration, and tubulogenesis (Fig. 8F).[48,100,101] These studies demonstrated broad applications for diverse tissues and tissue regeneration (Fig. 8H–J).[47,102–109]

4.1.2 Tissue engineering and regenerative medicine. The self-assembling peptides are easy to use in tissue engineering, wound healing, addressing chronic wound problems, and regenerative medicine (Fig. 8H–J).[110–115] Ellis-Behnke and colleagues used RADA16-I to repair injured rat and mouse brain structures, and their results showed that the peptide scaffold hydrogel was excellent, not only for axons to regenerate through the site of an acute brain injury, but also to knit the injured brain tissue together seamlessly. This work represents enabling nanobiomedical technology for brain tissue repair and restoration (Fig. 8H).[116,117]

Xiaojun Zhao's laboratory also used peptide scaffolds to repair the deep second degree burns in rats, and it can shorten the time of eschar healing by 3–5 days, and speed up wound contraction by ~25% compared with control groups, the healed eschar without obvious edema.[123] These results suggest that the self-assembling peptide scaffolds may be promising simple, effective and affordable tools for burn victim and skin lesion treatments.

The self-assembling peptides seem to be useful in the area of emergent trauma. Ellis-Behnke and colleagues found that the peptide scaffold hydrogel instantly stopped bleeding in a few seconds during the procedure of repairing the injured brain. They then expanded their study to the haemostasis of the brain, spinal cord, femoral artery, and the liver of rats.[124,125] Their early work has been followed up by others, expanding experiments to different kinds of animals. RADA16 functionalized with biologically active motifs also induced favourable reparative injured spinal cords (Fig. 8I and M).[118,119] Song *et al.* utilized an injury model to evaluate the haemostatic efficacy of peptide RAD16 in rat kidney.[126]

In order to better understand the individual molecular and material building blocks, their structures, assembly properties, dynamic behaviours and applications for rapid haemostasis, we reported that using D-amino acids, the chiral self-assembling peptide D-EAK16 also forms a 3-dimensional nanofiber scaffold, but E*A*K16 or EA*K16 is rather poor for self-assembly.[18] 1% D-EAK16 for the liver wound haemostasis took ~20 s, but using 1% of E*A*K16 and EA*K16 that have alternating chiral D- and L-amino acids took ~70 and ~80 s, respectively (Fig. 8K and L). This study not only provided insights for understanding the chiral assembly properties for rapid haemostasis, but also to aid in the further design of self-assembling D-form peptide scaffolds for clinical trauma emergencies.[18]

4.1.3 Controlled drug delivery for molecular medicine. Since the self-assembling peptides form the nanofiber scaffolds and its process is dynamic over time, it is possible to use it for the controlled drug delivery of molecular medicine, for small molecules, large proteins and nucleic acids (Fig. 9A).[120,127–131]

Using EAK16 II, RAD16-II and RAD16-I as a model for pyrene slow release, results showed that these types of self-assembling peptide scaffolds encapsulated hydrophobic drugs (Fig. 9B).[121,132,133] Various dye molecules including phenol red, bromophenol blue, 8-hydroxypyrene-1, 3, 6-trisulfonic acid trisodium salt, 1,3,6,8-pyrenetetrasulfonic acid tetrasodium salt, and Coomassie Brilliant Blue G-250 could also be released through RADA16 hydrogels, providing an alternate route for controlled release of small molecules (Fig. 9C).[120] Furthermore, nanofiber encapsulated camptothecin or ellipticine have been confirmed to inhibit tumour growth.[134–136]

The scaffolds have also been used for the sustained release of active cytokines βFGF, TGF, VEGF and BDNF (Fig. 9D).[122,137] Furthermore, proteins including lysozyme, trypsin inhibitor, BSA, MMP-13 and monoclonal antibody IgG have been loaded into the scaffolds and then slowly released into the environment (Fig. 9E).[49,138–140] In addition, they can be used for sustained release, ranging from a few days to over 100 days (>3 months) when the experiments were terminated.[141] It may be possible to have sustained-release for much longer. Future experiments will be carried out to test these ideas.

These results not only provide evidence for long-term sustained molecular release from self-assembling peptide scaffolds, but also inspire others to design more self-assembling peptides to control molecular release for clinical applications.

4.2 Application of designer self-assembling peptide nanotubes and nanovesicles

4.2.1 A class of lipid-like self-assembling peptides. The lipid-like peptides is a new class of short peptides. Cationic, anionic, and zwitterionic peptide detergents were designed. These lipid-like peptides are a class of molecules with properties similar to surfactants. They have hydrophilic heads comprised of 1–2 residues, and hydrophobic tails 3–6 residues long. They are about 2–3 nm in length, and their ionic character

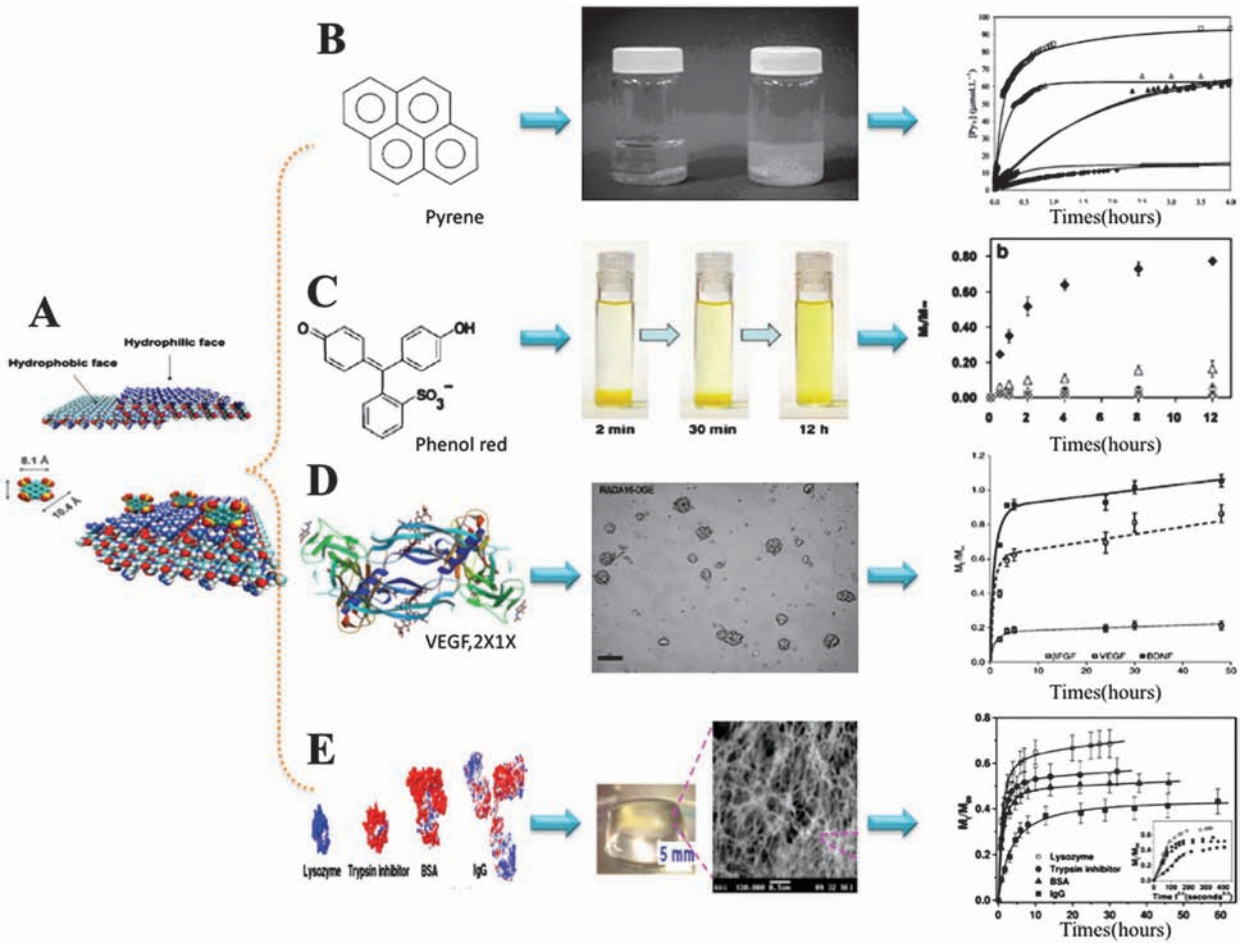

Fig. 9 Chiral self-assembling peptides are used in controled drug delivery for molecular medicines. (A) Illustration of the molecular modeling results for self-assembling RADA16 peptide nanofibers;[120] (B) pyrene molecular structure, pyrene in water and with EAK in aqueous solution (right) after stirring both solutions, and the fluorescence *versus* time profiles for the release of molecular pyrene from pyrene microcrystals encapsulated in an EAK coating into a solution of EPC liposomes (images courtesy of P. Chenl, Copyright permission from JACS);[121] (C) Molecular structure of the dye molecules: phenol red, and the typical experiment using 0.5% w/v RADA16 peptide hydrogel and phenol red as a diffusing molecule 2, 30 min and 12 h. The color intensity of the bulk solution increased with time as a result of phenol red release, and the measured release kinetics of the dye molecules through the hydrogel cumulative dye release results for first 12 h; (D) molecular structure of VEGF (*2X1X*) from the RCSB (www.pdb.org), and proliferation effect of released β-FGF over neural stem cells, and cytokine release profiles from hydrogel scaffolds composed of RADA16-PFS. β-FGF, VEGF and BDNF were released from the RADA16-PFS hydrogel scaffold for up to 48 h. VEGF was released slower than the other protein systems, whereas almost all BDNF was released. β-FGF release was slightly impeded in comparison to BDNF;[122] (E) graphical representation of lysozyme, trypsin inhibitor, BSA, and IgG, the Ac-$(RADA)_4$-$CONH_2$ peptide monomer, and of the peptide nanofiber. Color scheme for proteins and peptides: blue, positively charged; red, negatively charged; light blue, hydrophobic. The release profiles for lysozyme, trypsin inhibitor, BSA, and IgG through the self-assembling peptide hydrogel in PBS (pH 7.4) at room temperature. Data points represent the average of 4 or 8 samples with calculated SD values that are $<12\%$. (Inset) Protein release plotted as a function of the square root of time showing a biphasic diffusion mechanism. The initial linear part of the plots represent simple diffusion of the proteins through the peptide hydrogel.[49]

and strength can be controlled by selecting appropriate amino acids or by capping the termini. Lysine or aspartic acid was used for the hydrophilic head. To control the detergent ionic nature, each peptide was capped by acetylation at the N-terminus, or selective amidation at the C-terminus when required. Alanine, valine, leucine, and isoleucine were used for the hydrophobic tails (Fig. 10).

These lipid-like peptides behave comparably to traditional detergents, but offer several advantages over other novel detergents. Their chemical properties are similar to commonly used detergents, they can be systematically designed and economically produced at high purity, and they remain stable for long periods of time.

4.2.2 Stabilized membrane proteins. Membrane proteins play vital roles in all living systems. They are involved in energy conversions, cell–cell and cell–environment communications and sensing, specific ion channels and pumps, transporters, and all sorts of transports. Membrane proteins are also essential for our 5 senses: sight, hearing, smell, taste, touch, and also temperature sensing. G-protein coupled receptors (GPCRs) are crucial in learning, memory, stem cell renewal and differentiation, body-plan development, the immune system, aging and more. However, our understanding of their structures and functions falls far behind that of soluble proteins.

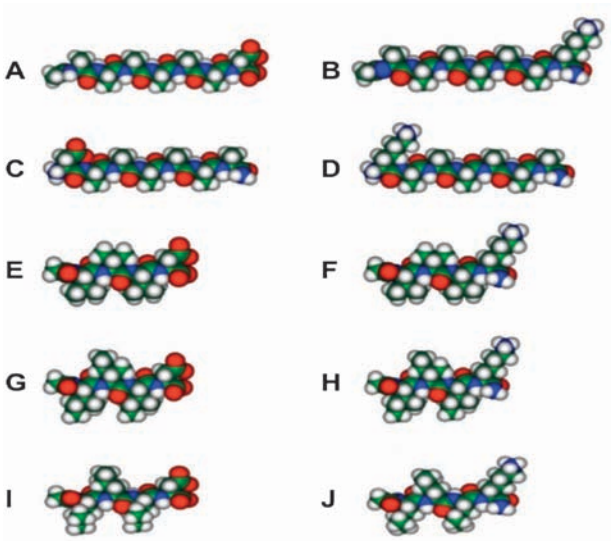

Fig. 10 Molecular models of peptide detergents at neutral pH. (A) Ac–AAAAAAD–COOH. (B) Ac–AAAAAAK–$CONH_2$. (C) DAAAAAA–$CONH_2$. (D) KAAAAAA–$CONH_2$. (E) Ac–VVVD–COOH. (F) Ac–VVVK–$CONH_2$. (G) Ac–IIID–COOH. (H) Ac–IIIK–$CONH_2$. (I) Ac–LLLD–COOH. (J) Ac–LLLK–$CONH_2$. Aspartic acid (D) is negatively charged and lysine (K) is positively charged. The hydrophobic tails of the peptide detergents consist of alanine (A), valine (V), isoleucine (I) and leucine (L). Each peptide is ~2–2.5 nm long, similar in size to biological phospholipids. Color code: teal, carbon; red, oxygen; blue, nitrogen and white, hydrogen.[151]

Over the past few years, our teams have gradually overcome some of the obstacles of membrane protein production and purification.[20–22,142–146] We solubilized and stabilized several classes of membrane proteins using the lipid-like peptides,[20–22] including *E. coli*, glycerol-3-phosphate dehydrogenase,[147] the multi-domain protein complex photosystem-I (PSI) on the surface in dry form and in aqueous solution,[148,149] G-protein coupled receptor-bovine rhodopsin[150] (Fig. 11) and many olfactory receptors.[151]

4.2.3 Lipid-like peptides used in cell-free productions of high yield membrane proteins. Cell-free production of proteins is a robust method and capable for molecular fabrications quickly, often in a few hours.[152–166] It can easily be industrially standardized with rigorous quality controls. The technology is simple and versatile, an unskilled person can be trained in a few hours to perform the task.[167,168] Various functional proteins including membrane proteins,[169–175] particularly difficult G-protein coupled receptors and other 7-transmembrane proteins (7TM), have been made for a variety of studies and for biotechnology and nanobiotechnology applications.[166,176–188]

Recently, cell-free protein productions have become cost effective and competitive. Grams amount of functional proteins and materials can be made in a few hours instead of weeks. Cell-free system can be integrated into other devices because it's simplicity and robustness.

Selecting the right surfactant is thus crucial because challenges in elucidating the structure and function of membrane proteins are the drawback of producing large quantities of functional receptors. Various self-assembling peptide surfactants in commercial *E.coli* cell-free systems can rapidly produce milligram quantities of soluble G-protein coupled receptors (GPCRs) that include the human formyl peptide receptor (FPR), human trace amine-associated receptor (TAAR), vomeronasal type 1 receptor 1 (VNR),[189,190] and other olfactory receptors.[53,142,143,191,192]

Furthermore, using short, designer lipid-like peptides as surfactants, 12 unique mammalian olfactory receptors have

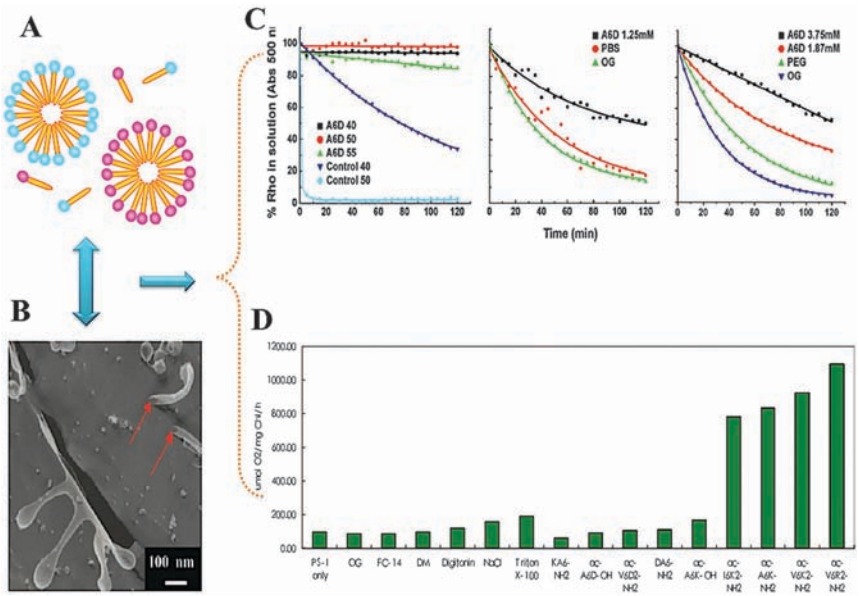

Fig. 11 Designer self-assembling peptides stabilize membrane proteins. (A) Proposed model peptide surfactants; (B) quick-freeze/deep-etch TEM image of peptide surfactant (V_6D) dissolved in water;[22] stability kinetics of rhodopsin (Rho) in different surfactants;[150] (C) Kinetics of bovine rhodopsin under different conditions. Stability of rhodopsin in the absence of OG at different temperatures. Half-life of rhodopsin was as follows: not available in 2.5 mM A_6D at 40 °C, 50 °C, and 55 °C (left); (D) decay of A500 in delipidated rhodopsin in the absence of OG. Half-life of rhodopsin was as follows: 122 min in 1.25 mM A_6D, 47 min in PBS, and 27 min 1% OG (middle); Stability of delipidated rhodopsin at 40 °C (right); lipid-like peptides stabilize the functional photosystem I.[149]

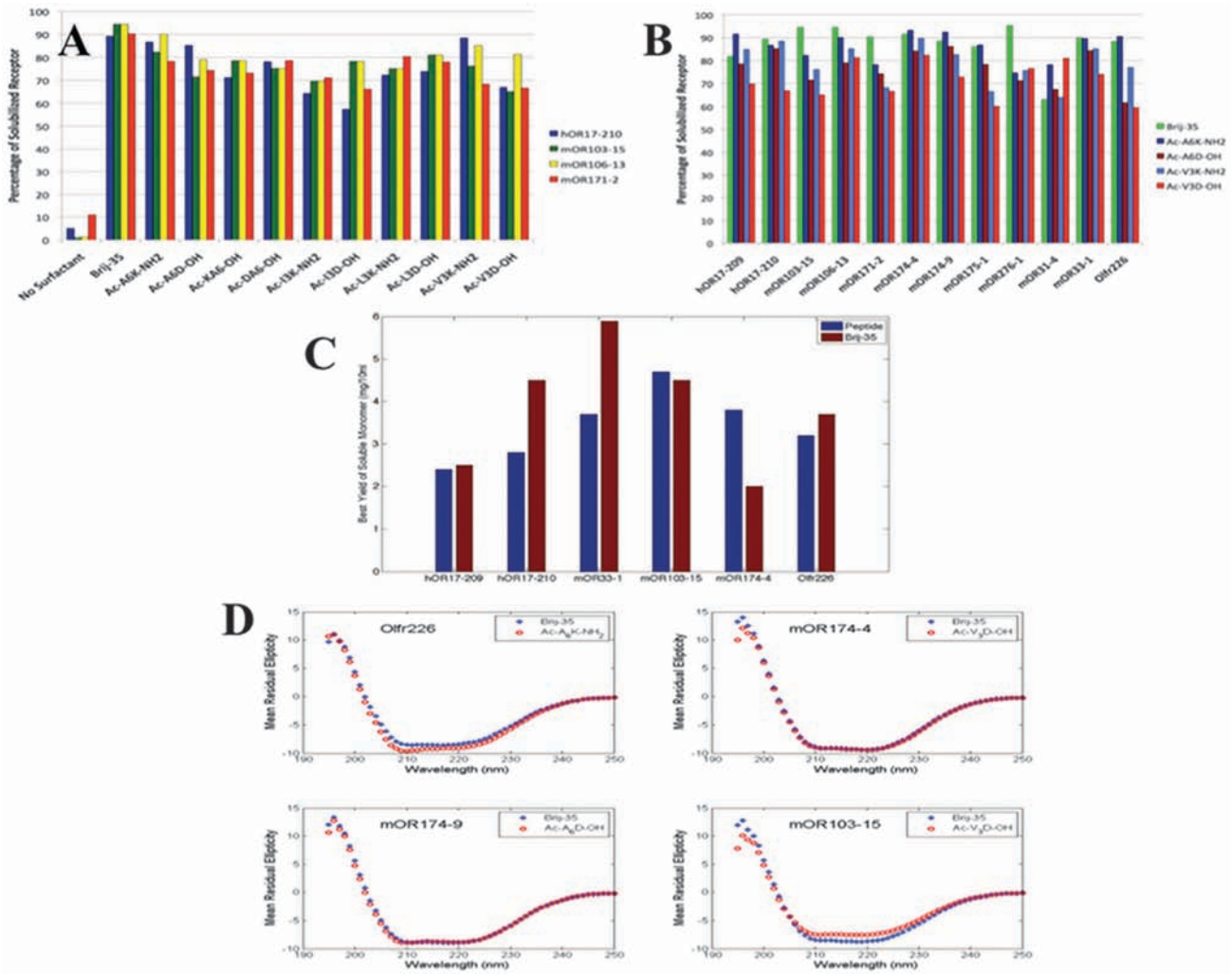

Fig. 12 Designer lipid-like peptides are used in the cell-free systems. Tens of functional olfactory receptors purification and secondary structure have been achieved. Olfactory receptors are soluble in Brij-35 and peptide detergents. Each receptor was expressed in the presence of Brij-35 or a peptide detergent using a commercial *E. coli* cell-free expression system (Qiagen, RiNA and Invitrogen). (A) The presence of a detergent was necessary to solubilize the olfactory receptors, and all of the peptide detergents were able to solubilize four unique receptors; (B) the detergent peptides and Brij-35 were able to solubilize similar fractions of protein. Peptides that were positively charged or had longer tails tended to solubilize higher fractions of receptors; (C) detergent peptides can yield milligram quantities of solubilized olfactory receptors, and the maximum yield of the monomeric form of all tested olfactory receptors expected in a 10 ml reaction. Only results from the most effective detergent peptide are shown; (D) CD spectra of Brij-35 and peptide detergent-produced olfactory receptors to Olfr226, mOR174-4, mOR174-9 and mOR103-15 (Images courtesy of Karolina Corin).[151]

been produced, solubilized and stabilized to maintain their structure and function (Fig. 12A–D).[54,151] These simple and inexpensive lipid-like peptide surfactants will likely make significant contributions to facilitate the rapid production of GPCRs, and perhaps other membrane proteins.[150,189,190,192]

We provide a schematic model for membrane protein stabilization using self-assembling peptides (Fig. 13).

5 Self-assembling peptides for human clinical studies

5.1. Self-assembling peptide scaffolds for accelerated-wound healing

Human wound healing has always been associated with surgical procedures and conflicts. At present the method of clinical processes still seem to be similar to procedures used many years ago, use of suture during operations, trying to prevent infection and other treatment measures. Accelerated-wound healing will benefit both patients and clinical workers. Since trauma was wide spread during human conflicts and wars,[193–198] accelerated-wound healing studies and practices have significantly improved, from using chondroitin sulfate,[199] zinc sulfate,[200,201] insulin,[202] collagen solutions[203]and amnion membrane grafts,[204] using external stimulations such as hyperbaric oxygen,[205] laser and infrared irradiation,[206,207] ultrasound[208–210] and pulsed electromagnetic fields,[211] using various growth factors,[212–220] growth hormone-releasing hormones,[221] even using p53,[222] gelatin hydrogels,[223,224] and medicinal moleucures,[225–228] using gene therapy,[229–232] or stem cell therapies.[233,234] However, accelerated surgical and trauma wound healing especially chronic diabetic ulcer wound healing,[234–237] is still problematic, the self-assembling medical

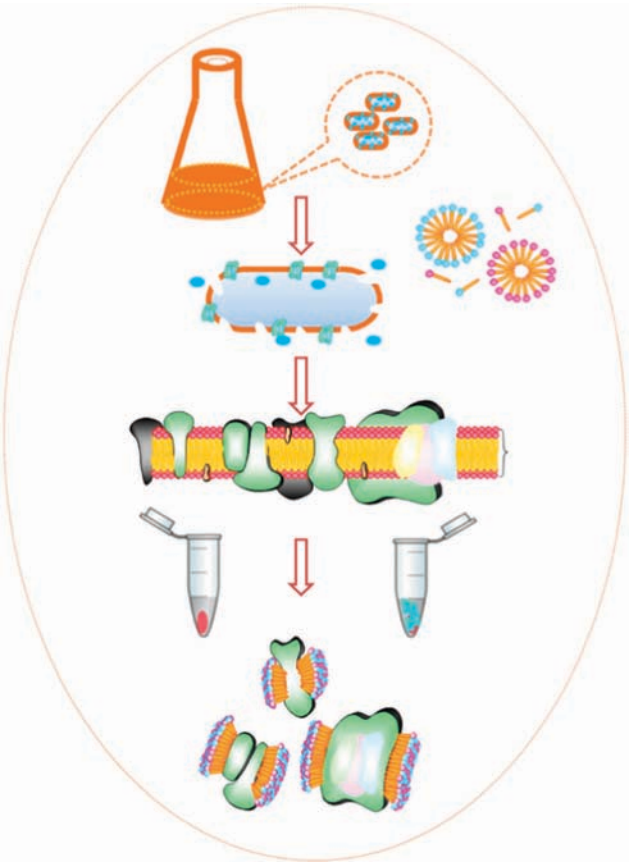

Fig. 13 Schematic illustration of a proposed model of membrane protein stabilization using self-assembling lipid-like peptides. From the starting to extract preparation to the cell lysis, added the self-assembling peptides, and proteins was extracted with other detergents from the membrane. After surfactant exchanges, the hydrophobic alanine tail of lipid-like peptide A_6D forms the protein–surfactant complex only on the hydrophobic belt area. Small peptide surround membrane protein and act to protect it from aggregation. This action may be similar to that of lipids and other surfactants. The proposed dimmeric GPCR are embedded in the cellular membrane. The lipids (shown with pink heads) of the membrane form bilayers. The surfactants (shown with gray heads) are other detergent, which is used for the initial purification, and peptide surfactant A_6D (shown with red heads). All hydrophobic tails are shown in yellow. The lipids have two tails, and both common detergent and A_6D.[150,189,190,192]

technology may alleviate the problem and be put to good practice.[228,232,237–246]

The self-assembling peptide scaffolds have been found to be permissible for all tissue cell cultures including human cells tested, and the degradation products of the scaffolds are natural amino acids that pose no harm to human body. Furthermore a variety of stringent animal tests with rigorous controls have showed that the peptides did not elicit noticeable immune responses, nor caused measurable inflammatory reactions through injections and surgical procedures at various tissue sites. It thus encouraged people to carry out human clinical trials for accelerated-wound healing indication.

Of all subjects treated for various surgical wounds including cardiovascular surgeries for coronary artery bypass and synthetic blood vessel replacement, gastrointestinal surgery for partial hepatectomy and gastrointestinal treatment for endoscopic excision of mucosa. Those clinical results are very encouraging and beneficial for patients. There were no observed adverse and undesirable side effects so far. This is not surprising since the designer self-assembling peptide scaffolds are totally pure synthetic amino acid based materials; there are no animal-derived impurities, no chemical and biological contaminations, no organic solvents, and no toxic compounds.

Since the success of human clinical trials, additional trials for several indications (Fig. 8) have been planned or launched for human tooth wound healing, skin wound healing from other diseases and injuries, in various parts of the world. Based on the previous successful clinical trials, it is anticipated that these clinical trials are likely to be successful. It is hoped that the self-assembling peptide scaffolds will become an enabling medical technology that will truly benefit society (Fig. 14).

6 Conclusion and perspective

Since the unexpected discovery of the self-assembling peptide EAK16-II in yeast Zuotin, we have come a long way, from initial surprises, puzzlement, no understanding at all to, in an outline, not only gradually understand the design principals at the molecular level, the molecular and fine material structures, interactions of the peptides, the dynamic self-assembly behaviours, but also how we can further improve their designs. From there, we not only subsequently expanded designer materials using 20 natural L-amino acids or some non-natural D-amino acids, but also we proceeded to optimize their sequence for delivering bioactive therapeutics such as drugs and growth factors. Recent advances in functionalization have also led to the development of better synthetic tissue culture bioactive scaffolds that promote cell proliferation, migration and differentiation for regenerative medicine.

As some of the non-functionalized self-assembling peptide scaffolds proceed through clinical trials, it is our hope that in the not too distant future, they will open the door for more clinical applications in biomedicine. Other studies which have expanded these fields, include the use as an immune adjuvant,[247] performs a light-harvesting function,[248] coatings designed for highly luminescent suspension of single-walled carbon nanotubes.[249] Consideration of chirality is the most interesting phenomenon in nature, we believe more surprising nanomaterials will be discovered in the area of chiral self-assembling peptide system.[250–255]

A few final words

In science there are numerous examples of curiosity-driven research and unintentional discoveries that led to technological breakthroughs and new economic development. They include DNA–RNA and RNA–RNA hybridizations, RNA splicing, RNA as enzymes, telomeres, programmed cell death, and the indispensible worldwide web – www. The discovery of the self-assembling peptide is another good example of an unexpected curiosity-driven discovery which led to the development of a biomedical technology that will benefit society. The recent example of successful clinical trials of the self-assembling peptide scaffolds for accelerating wound healing and regenerative medicine provide

Fig. 14 The timeline of chiral self-assembling peptides: from a serendipitous discovery to the benefit of society. It took over 20 years from the initial discovery in 1990 to successful human clinical trials in 2011. There have been a lot of ups and downs. After gaining full understanding and knowledge of various detailed aspects of amino acid chemistry, peptide structure properties, dynamic molecular self-assembly behaviors of the self-assembling peptides, we started to design new peptides with active biological functions that further enhance their usefulness for a wide range of applications. Seize the opportunity, one of us (S. Zhang) took the license and co-founded a startup biotech company 3DMatrix to translate the technology into new economy. To sum up the path from the discovery to a new medical technology: (1) to stimulate, encourage and support curiosity-driven scientific research in order to gain scientific knowledge, (2) to take risk and not be afraid of failure to translate scientific knowledge and research into enabling technologies, and (3) undeterred to develop the knowledge based economy for the benefit of mankind.

a glimpse of what is coming for wide spread uses of chiral self-assembling peptides (Fig. 14). Thus curiosity-driven research must be strongly encouraged and fully supported, despite the current emphasis of application-driven research.

Acknowledgements

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