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4342 *Current Pharmaceutical Design,* **2015,** *21,* **4342-4354**

Self-Assembling Peptide Nanofibrous Hydrogel as a Versatile Drug Delivery Platform

Zhiqiang Yu[1,#], Quan Xu[2,#], Chenbo Dong[3], Su Seong Lee[4], Liqian Gao[4,*], Yiwen Li[5], Mathew D'Ortenzio[6] and Jun Wu[7,*]

[1]*School of Pharmaceutical Sciences, Guangdong Provincial Key Laboratory of New Drug Screening, Southern Medical University, Guangzhou 510515, China;* [2]*Institute of New Energy, State Key Laboratory of Heavy Oil Processing, China University of Petroleum (Beijing), Beijing, 102249, China;* [3]*Department of Chemical Engineering, West Virginia University, Morgantown, WV, 26505, USA;* [4]*Institute of Bioengineering and Nanotechnology, 31 Biopolis Way, The Nanos, 138669, Singapore;* [5]*Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, CA, 92093, USA;* [6]*University of Waterloo, 200 University Avenue West, Waterloo, ON N2L 3G1, Canada;* [7]*Laboratory of Nanomedicine and Biomaterials, Department of Anesthesiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, 02115, USA*

Jun Wu

Abstract: Molecular hydrogels have been widely explored in various biomedical applications, such as cell culture, tissue engineering and drug delivery. Peptide-based hydrogel nanoparticles represent a promising alternative to current drug delivery approaches and cell carriers for tissue engineering, due to their encapsulation stability, water solubility and biocompatibility. This review focuses on recent advances in the use of self-assembling peptide nanogels for applications in drug delivery. We firstly introduce a self-assembly mechanism for small molecules used in the peptide hydrogel, and then describe recent methods for controlling the assembly of molecular hydrogelations. A particular emphasis is placed on recent advances in the use of different types of peptide hydrogels as drug delivery carriers. Lastly, the current challenges and future perspectives for self-assembling peptide hydrogels in drug delivery applications are discussed.

Keywords: Self-assembly, peptide hydrogel, drug delivery, hydrogelator, biomaterials.

1. INTRODUCTION

Hydrogels consist of hydrophilic polymers, which contain a significant amount of water, while maintaining a distinct three-dimensional self-assembled networked structure [1-3]. Many hydrogenating molecules can be assembled, under specific conditions, into nanogels through the self-assembly of small molecules. In the last two decades, molecular hydrogels have attracted considerable attention as promising biomaterials for various biomedicine and nanomedicine applications such as cell culture [4, 5], tissue engineering [6, 7], drug delivery platform [8, 9], cancer therapy [10, 11], and regenerative medicine [12, 13]. Until now, several systems of molecular hydrogelators have been reported, including sugar-based molecules [14, 15], peptides [16-20], and amino acid derivatives [21-23] *et al.* Among these mentioned gelation systems , peptide-based nanogels are particularly attractive as molecular building blocks due to their versatile synthesis, excellent gelation ability, good biocompatibility and bioactivity [24]. Self-assembling peptides can adopt a huge diversity of 2D or 3D architectures, and also provide necessary control over self-assembly through physicochemical factors such as pH, temperature, ionic strength, enzyme, solvent and light [25, 26]. This feature article summarizes the recent developments of a novel method of molecular hydrogelations, by the mechanism used for molecular hydrogelations and the application of peptide hydrogels as drug delivery vehicles.

2. MECHANISM FOR MOLECULAR HYDROGELATIONS

Considerable attention has focused on peptides that might be involved in the regulation of a multitude of physiological functions in vertebrates and mammals, including humans [27-30]. Peptide molecules can form specific secondary, tertiary and quaternary structures, which provide unique opportunities for the design of nano-materials that don't exist with traditional organic molecules and polymers. Additionally, various chemical functionalities found in naturally occurring and artifical occurring amino acids give the ability to self-assemble into different forms of nano-materials with desired structures and chemical functions [31]. With developments and a better understanding of the relationships between the sequences and structures, it is now possible to design new protein and peptide based materials [32, 33]. By virtue of rational design, various types of biomaterials can be formed via self-assembly, ranging from nanometer to microscale materials [34-39]. The properties of self-assembling peptides can be easily designed, synthesized and modulated, by controlling the secondary structural motifs of peptides (Fig. **1**) or their hierarchical self-assembly process (Fig. **2**).

Currently, a well-designed and synthetic peptide can form hydrogels, which allows the development of a new platform with the ability to fine-tune the properties of self-assembled structures with the assistance of external stimuli. The high successful rate for these kinds of self-assembled materials is due to the fast development and deep understanding of the molecular interactions and the requirements for such interactions.

Interestingly, there has been an increase in the reported number of peptide based self-assembling materials (fibres, tapes, sheets, wires ribbons and hydrogels), which have been applied in many areas [40-49]. There are four main factors that are important to self-assembly of small molecules: (a) hydrophobic interaction, (b) π-π stacking, (c) hydrogen bonding, and (d) electrostatic interactions [50-55].

Peptide can meet all the requirements for self-assembly via self-aggregation process by adjusting the peptide sequences. Hydrogelation via self-assembly is a hierarchical process, and is shown in Fig. (**2**). Usually, small peptide molecules in solution can form a specific secondary structure, and then self-assemble to form nanofibres when treated by appropriate stimuli or suitable physical conditions.

*Address correspondence to these authors at the Institute of Bioengineering and Nanotechnology, 31 Biopolis Way, The Nanos, 138669, Singapore; Tel: +65-68247140; E-mail: lqgao@ibn.a-star.edu.sg

Laboratory of Nanomedicine and Biomaterials, Department of Anesthesiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, 02115, US; Tel: 1-631-3274855; E-mail: junwu1@mit.edu

[#]These authors contributed equally to this work.

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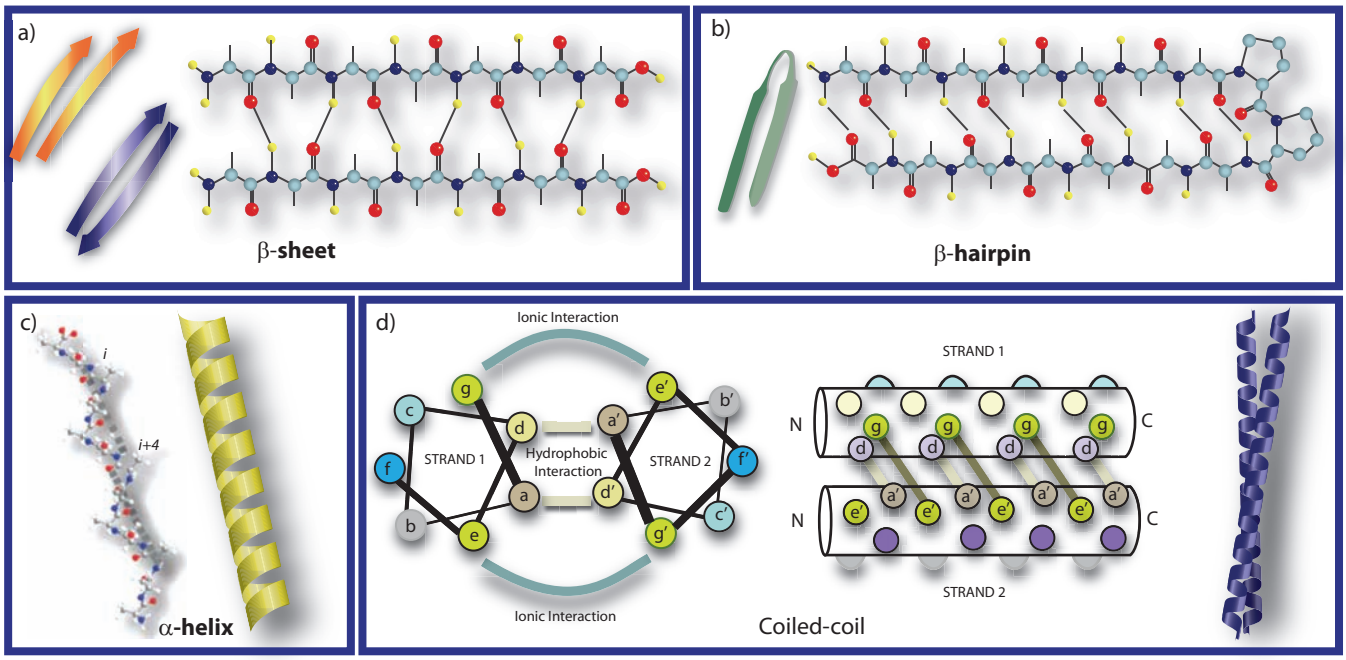

Fig. (1). Scheme for peptides self-assembly to form different secondary structures: (a) β-sheet, (b) β-hairpin, (c) α-helix and (d) coiled-coil. Adopted from RSC Advances.2013; 3: 9117 [39].

As a result, thicker and longer fibres in three-dimensional space can gradually form, thus leading to a fibrillary network (Fig. **2**). Water molecules can be entrapped in these 3D networks of peptides, which resulting in a self-supporting hydrogel. The properties of the hydrogels can be adjusted by changing the peptide sequence, thus making the final synthetic materials tunable.

To better design oligopeptides as to generate hydrogelators, it is necessary to understand the mechanism involved in gel formation. Although it is difficult to accurately predict the likelihood of the peptide's ability to form a hydrogel, numerous existing examples which have either failed or succeeded to form gels can provide useful information for the design of peptide based hydrogels.

2.1. Hydrophobic interactions

Peptides can form hydrogels, or many other well-ordered supramolecular structures, by multiple non-covalent interactions. Many cases have shown that peptide amino acids can easily form hydrogels when they are conjugated to a large aromatic group. It has been emphasized that aromatic interactions play a very important role in the hydrogelation (especially for LMW gelators), which can be indicated by comparing the different chemical conjugates of similar peptides or amino acids. For example, although it was reported that Fmoc-Tyr can easily undergo self-assembly and form hydrogel [56], change of the Fmoc group with non-aromatic tert-butyloxycarbonyl (Boc) protecting group or the smaller aromatic group Cbz will fail to form hydrogels [57]. The same problems also happen to Fmoc-Phe [58] and Fmoc-protected pentafluorophenylalanine (Fmoc-F5-Phe) [57] when Fmoc protection groups were changed into Cbz. Xu and co-workers [58] rationally designed hydrogelators based on aromatic-aromatic interactions. They reported that phenylalanine derivatives, composed of Phe conjugated to an aromatic group (fluorenyl, naphthyl, naphthalenoxyl, or cinnamoyl), were efficient hydrogelators. Interestingly, they identified the cinnamoyl group as the smallest aromatic group that provided sufficient aromatic-aromatic interactions for N-modified phenylalanine to become a hydrogelator.

Besides, Xu group also investigated whether the tetrapeptide (Gly-Phe-Phe-Tyr) can form hydrogels when they were capped by diverse aromatic capping reagents. They found that Cbz-Gly-Phe-Phe-Tyr is a less efficient hydrogelator than Fmoc-, Nap- or PTZ-protected Gly-Phe-Phe-Tyr tetrapeptide [59]. They also revealed that the minimum gelation concentration for Cbz-Gly-Phe-Phe-Tyr was 5 wt. %, while it was only 0.08 wt. % for Fmoc- or Nap-protected tetrapeptides. Furthermore, it was found that PTZ-Gly-Phe-Phe-Tyr can form gels at ultra-low concentrations of 0.01 wt.%. To further study the important role of aromatic groups in regulating the self-assembly process and influencing the structural and physical properties of the hydrogels formed, a library of Fmoc-peptides was designed and investigated to elucidate their detailed functions [60-63].

2.2. π-π interactions

It was previously reported that Gly-Phe-Ile-Leu was a more efficient hydrogelator than Gly-Ala-Ile-Leu. To elucidate the contribution of aromatic residues in the self-assembly process, investigating how Fmoc-Tyr form hydrogels may provide us an answer to this question. After investigation, Xu group revealed that four possible modes of π-π interactions may coexist to drive the hydrogel formation. Furthermore, one of these modes was resulting from the presence of the fluorenyl group overlapping with phenyl group of tyrosine [56]. Besides, other recent studies for the supramolecular structure of Fmoc-Phe-Phe [63] and the assembly of Fmoc-Ala-Ala [64] also indicated that π-π stacking as driving forces plays a very important role in the self-assembly to form the final hydrogel structure. Furthermore, π-π stacking as the main driven force for hydrogel formation is also proved by a molecular dynamics simulation experiment using Fmoc-DAla-DAla hydrogelator [65].

2.3. Hydrogen bonding

α-helix is one of most important secondary structure elements for protein structures, and it is usually formed by the winding of the polypeptide backbone into a right-handed helix with a periodicity of 3.6 amino acids per turn. For this kind of secondary structure, the internal backbone hydrogen bonding is mainly responsible for the stabilization, which is formed between the carbonyl oxygen atoms

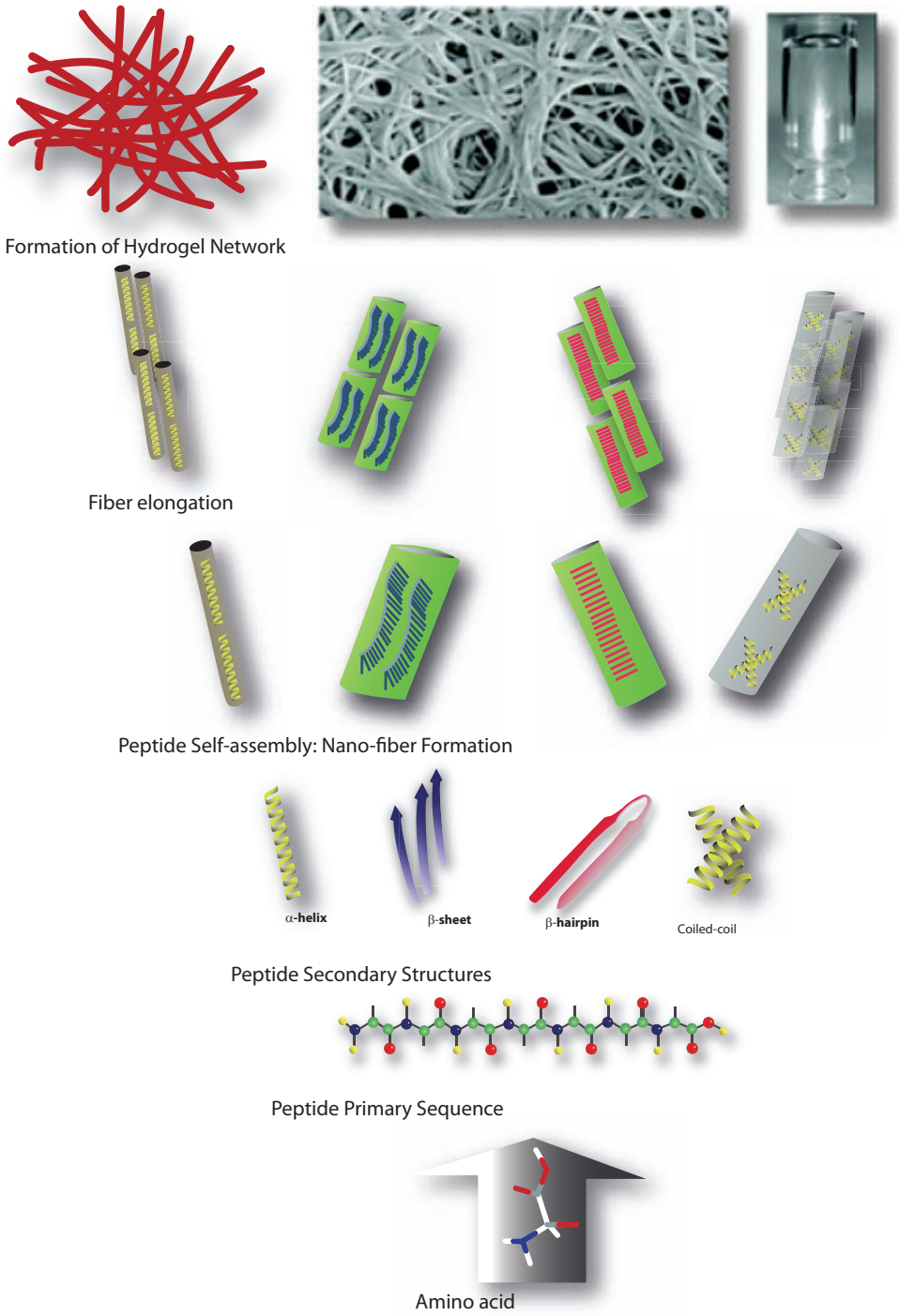

Fig. (2). Representative scheme for hydrogel formation via hierarchical self-assembly process from peptide molecules. Adopted from RSC Advances. 2013; 3: 9117 [39].

of residues i, with the amide hydrogen, of residues i + 4. To make proteins structure more stable, α-helices can also pack together through other forces, such as hydrophobic effect and van der Waals' forces. Different from β sheet structures, the hydrogen bonding in α-helices belongs to intramolecular interaction, which will make it discrete building blocks [66, 67].

2.4. Electrostatic interactions

Compared to the strategies mentioned above, another different and efficient method to facilitate hydrogel formation is based on the electrostatic attraction of oppositely charged peptides. One typical examples is that, Zhuo *et al*. in 2010 reported several Fmoc-oligopeptide hydrogelators: Fmoc-Val-Arg-Gly-Asp-Val, Fmoc-Gly-Arg-Gly-Asp-Gly and Fmoc-Lys-Lys-Arg-Gly-Asp-Lys [68].

When mixing 2 of these 3 oligopeptide hydrogelators in H_2O at a neutral pH, supramolecular hydrogels can easily form due to the electrostatic attraction assisted co-assembly. Besides, by virtue of electrostatic interactions as the main driving force, hydrogels can also easily form even for larger building blocks [69-72].

2.5. Amino Acids Order

It was also found that the order of amino acids was of great importance for hydrogelation. It was proved by several typical examples as below that peptides failed to form hydrogels once the order of amino acids in the peptide sequences was changed. The first example was Fmoc-Phe-Gly, which can form hydrogels at at <1 wt. %, while peptide Fmoc-Gly-Phe failed to form gels [73]. This change for the hydrogelation formation could be resulting from the change in the self-assembly kinetics due to the presence of two adjacent aromatic moieties in Fmoc-Phe-Gly.Similar phenomenon also happened to the dipeptides between Nap-Gly-Ala and Nap-Ala-Gly.Nap-Gly-Ala can easily form hydrogels when decreasing pH, while Nap-Ala-Gly cannot form hydrogels [74]. To find out the real reason for different self-assembly behavior of these two peptides, computational methods and X-ray diffraction studies suggested that it could be due to the variation in conformational and hydrogen bonding preferences between these two peptide sequences.

The importance of the amino acids order for the hydrogel formation was also confirmed by Hamley and his co-workers in several studies [75, 76].

3. METHODS FOR MOLECULAR HYDROGELATIONS

3.1. Ultrashort Peptide Hydrogels from Protected Single Amino Acid

So far, Xu group was the first one to report the fibrillation and subsequent hydrogelation by using Fmoc protected single amino acids [77]. In this study, it was reported that hydrogelation of a mixture of Fmoc-Lysine and Fmoc-Valine could successfully formed under systematic basification. Besides, the same group also developed new strategies to incorporate external stimuli for the hydrogel formation using Fmoc protected amino acids [77-82].

3.2. Short Peptide Hydrogels Based on Small Peptides

During the history of peptide hydrogel developed as biomaterials, Janmey and her coworkers firstly reported that Fmoc protected dipeptides derivatives could functionalize as hydrogelators. For example, Fmoc-Leu-Asp, and its analogues Fmoc-Ala-Asp and Fmoc-Ile-Asp, can successfully form hydrogels [83]. Besides, other dipeptides Phe-Phe and its derivative peptides (Boc-Phe-Phe-COOH, Z-Phe-Phe-COOH and Fmoc-Phe-Phe-COOH peptides) self-assemble into tubular and amyloid-like structures [84-86].

Different from dipeptides systems, tripeptide based hydrogel systems were not so that many [87-90]. One of typical example was that Banerjee and coworkers successfully identified three self-assembling, pH sensitive tripeptide based systems, which can successfully form nanofibrillar networks at basic pH values (between pH 11.0 and 13.0) with a common peptide structure of Boc-Phe-X-Phe-OH, where X = Val, Leu, Phe [87].

3.3. Long Peptide-Based Hydrogels

3.3.1. Hydrogels Based on β-Sheet Forming Peptides

The β-sheet or β-pleated sheet is another form of regular secondary structures in proteins.

In order to better design a peptide containing minimal complexity, three simple criteria was summarized by Boden and his coworkers as below: the most important one belongs to cross-strand attractive forces (hydrophobic, electrostatic and hydrogen-bonding) between their side chains; the second one is lateral recognition between adjacent β-strands to constrain their self-assembly to one dimension and avoid heterogeneous aggregated β-sheet structures; last one is strong adhesion of solvent to the surface of the tapes to control solubility [91]. Besides, a theoretical model of hierarchical self-assembling chiral rod-like units for β-sheet tapes, ribbons, fibrils and fibers was also proposed (for details see ref [92]).

Following these standards, Zhang *et al*. reported several interesting β-sheet forming peptides such as (H_2N-AEAEAKAK-COOH; Ac-NH-AEAEAKAKAEAE-$CONH_2$; Ac-NH-AEAEAKA KAEAEAKAK-$CONH_2$; can-KLDLKLDLKLDL-$CONH_2$; Ac-RADARADARADARADA-$CONH_2$) [93-97]. These special kinds of self-assembling peptides contain periodic repeats of hydrophilic and hydrophobic amino acids, which can result in discrete polar and nonpolar faces. These short peptides (about 2.5-5 nm in length) with 8 to 16-residue can easily form stable β-sheet structures in water. Furthermore, two peptides named Fmoc-Leu-Gly and peptide H_2N-VKVKVKVKV^D*P* PTKVTVKVKV-NH_2 not only self-assemble to form stable nanofibres, but also form higher order nanofibre scaffolds, which means hydrogels with extremely high water content [99.5 (wt/vol)% water] [95-97].

3.3.2. Hydrogels Based on β-Hairpin Forming Peptides

As one of another important secondary structure, β-hairpin (β-ribbon or β-β) involves two beta strands forming a hairpin shape [91, 92]. It was reported that Schneider and his co-workers identified a series of de novo designed peptides which can form hydrogel via stimuli driven folding to β-hairpin and self-assembly [98-100]. For these peptides, a tetra-peptide is in the center with high type II' β-turn propensity, which is flanked by two extended strands. For these 2 strands, they contain an alternating arrangement of hydrophobic and hydrophilic amino acids to help the formation of a β-sheet structure [98-100].

3.3.3. Hydrogels Based on the α-Helix Forming Peptides

Based on the structure, a supercoil (either right or left-handed) usually contains two or more strands of α-helices [101-108]. Native proteins usually contain coiled-coils in the structure, and coiled-coil motif is also useful and important for hybrid gel formation. A typical coiled-coil usually contains seven important residue repeats, which are designated as ''a, b, c, d, e, f, g'' (Fig. **1d**). An interhelical hydrophobic core is formed by the hydrophobic interactions between the hydrophobic residues ''a'' and ''d'' of two helices, which usually stabilizes the coiled-coil architecture. In positions ''e'' and ''g'', they stand for the charged residues that contribute to coiled-coil stability and mediate specific association among helices.

It was reported that Woolfson *et al*. developed a new approach to create a self-assembled hydrogel by using the coiled-coil aggregation [109]. Woolfson presented many fibrous biomaterials based on α-helical coiled-coils, where two 28-residue peptides were designed to co-assemble to form an α-helical dimer with complementary sticky ends [110,111]. Rational architectural changes in the peptide sequences lead to the formation of hydrogels with temperature sensitivity.

3.4. Hybrid Peptide Based Hydrogels

Hybrid hydrogels usually possess at least two distinct classes of components. For example, these 2 different classes of components may belong to synthetic polymers and biological macromolecules. To form a hybrid gel, these 2 different components are connected covalently or non-covalently [112].When combining two types of structures together, the new materials may possess unprecedented levels of structural organization and novel properties.

3.4.1. From Hybrid Block Copolymers

Hybrid block copolymers is very important in materials sciences because they can self-assemble to form many different kinds of nanostructured materials including vesicles, micelles, rod-like aggregates, and hydrogels [113]. Especially there are many literatures about the self-assembly into micelles of hybrid block copolymers composed of PEG and poly-amino acids for anticancer drug and/or gene delivery [114].

During these copolymers, diblock copolymers of PEG and β-sheet forming peptides are one of the typical systems which have been extensively studied (reviewed in Refs. [115,116]). A great

number of peptides, including β-amyloid mimics [117,118], elastin mimics [119,120] and silk mimics [121-123] have been used as the protein/peptide block.

To prepare hybrid multiblock copolymers of PEG and GAGA peptides, people usually used step-growth polymerization. Based on the design structure of Bombyx mori silk, when the amorphous regions of the protein structure were replaced by PEG, the GAGA peptides assembled intra- or inter-molecularly into parallel or anti-parallel β-sheets [124].

3.4.2. From Synthetic Polymers and Peptide Domains

Compared to the strategies mentioned above, a novel strategy to form hydrogel could be easily achieved by virtue of the self-assembly of synthetic polymer chains mediated by genetically engineered protein domains. It has been confirmed that it is feasible to impose properties of a well-defined coiled-coil protein motif onto a hybrid hydrogel containing synthetic polymer primary chains [125]. For example, hybrid hydrogels were successfully formed based on a N-(2-hydroxypropyl) methacrylamide (HPMA) copolymer with side chains terminating in iminodiacetic acid moieties charged with Ni^{2+} and His-tagged coiled-coil-forming peptides (CC1 and CC2) through metal complexation. CC1 stands for a segment of the stalk region of the Drosophila motor protein, kinesin, and CC2 was a *de novo* designed coiled-coil sequence ($[VSSLESK]_6$).

3.4.3. From Hybrid Graft Copolymers

Another type of polymer architecture, which could be used for peptide mediated self-assembly, is the graft copolymers containing a synthetic polymer backbone and associative peptide grafts. As a typical example of such graft copolymer, it is the HPMA copolymer backbone and coiled-coil-forming peptide grafts [126,127].

3.4.4. From Hybrid Star Copolymers

Another important hybrid copolymers are star-architecture-type macromolecules, which could self-assemble into 3D structures, and also can form hydrogels when synthesized with two 4-arm PEGS [128,129]. As for the first star-type macromolecule, it was reported and prepared by the attachment of the heparin-binding domain of the heparin interacting protein (HIP) to a 4-arm PEG. The HIP peptide containing a N-terminal cysteine (CRPKAKAKAKAKDQTK) could react via Michael's addition with vinyl sulfone-modified 4-arm star PEG.

3.4.5. Other Designs of Hybrid Hydrogels

Besides of the methods mentioned above, there are still other hybrid copolymers for forming hydrogels. As is well known, peptide/protein segments (within crosslinks) were usually used to induce degradability into hydrogels [130-132]. Due to the different degree of swelling and the size of the particular enzyme, the degradation usually proceeds through either in bulk or as surface erosion. As a result, protein grafted PEG hydrogels, which mimicked the natural extracellular matrix, were prepared via a three-step process. Firstly, an artificial protein was produced by genetic engineering. After that, it was grafted by the PEG diacrylate, and lastly the acrylate groups were photopolymerized, and finally formed hydrogels. The protein sequence contained the RGD bio-recognition peptide and two plasmin degradation sites. These hydrogels are versatile biomaterials that permit cell attachment and proteolytic penetration [133]. If we want to modify design of materials to permit 3D cell migration within the hydrogel matrix, it could be achieved via the reaction of cysteine thiols (inside the protein structure) with vinyl sulfone moieties of end-functionalized PEG [134,135].

4. MOLECULAR PEPTIDE HYDROGELS FOR DRUG DELIVERY

Molecular hydrogels, especially peptide-based hydrogels have been widely used in the biomedical field including scaffold for cell culture and therapeutic molecular carriers for drug delivery [136-138]. Therapeutic molecular carriers can be covalently bound to hydrogels and released from the gel via hydrolysis of chemical bonds causing degradation of gels. Besides using hydrogels as delivery system for drug transportation to a target, molecular hydrogel and/or its derivatives has been reported to act as carrier-free self-delivery therapeutic agents [139]. In this section, we will focus on peptide hydrogel application as a therapeutic drug delivery tool or self-delivery therapeutic agents for therapy.

4.1. Peptide Hydrogel as A drug Delivery Tool

Hydrogels with a capacity to absorb and hold water within a porous, swelled structure make it a great candidate as a material for many biomedical applications [140]. Peptides have emerged as promising molecular candidate for hydrogel-based biological oriented applications especially as drug delivery system due to their broad range of physical properties as well as chemical adaptability [141]. Unlike traditional polymer hydrogels, peptides may self-assemble to form highly ordered structures with their unique set of chiral amino acid building blocks. The self-assembly reaction is governed via the van der Waals' and electrostatic interactions, hydrogen bonding, and the hydrophobic effect in a controlled manner. Soukasene *et al.* generated self-assembling peptide amphiphilic (PA) nanofibers to encapsulate the hydrophobic chemotherapy agent camptothecin (CPT) [142]. The hybrid drug delivery system of incorporating CPT to PA had been found to improve the solubility of CPT to 50-fold and led to inhibited tumor growth in the mouse orthotopic model of human breast cancer. Accordingly, Mao *et al.* reported on the creation of a peptide drug delivery hydrogel tool loading with two complementary anticancer drugs used for chemotherapy for the first time (Fig. **3**) [143]. The stability of anticancer agents was greatly enhanced by loading the agents on hydrogels to which these drug molecules can have a controlled released via ester bond hydrolysis. This system shows potential to be used for the long-term controlled release of anti-cancer drugs.

Koutsopoulos *et al.* designed and synthesized a self-assembling peptide hydrogel fiber in nanoscale for controlled release of functional protein [144]. The biocompatibility of the self-assembling peptide hydrogel based delivery system did not alter the protein conformation structure and its functionality. It displayed great potential as a molecular carrier for functional therapeutic biomolecular delivery and controlled release. Additionally, the amphiphilic peptides could form stable nanowebs, which had ability to release incorporated hydrophobic drugs slowly, as well as accelerate animal hemostasis [145]. Ruan *et al.* prepared a 9-residue peptide (N-Pro-Ser-Phe-Cys-Phe-Lys-Phe-Glu-Pro-C) formed fishnet-like nanostructures, which could encapsulate pyrene drug and release pyrene to liposomes slowly from coated microcrystall, and thus has potential as a drug delivery tool. Curcumin could be encapsulated into hairpin hydrogels as an injectable agent for localized delivery [146]. *In vitro* experiments, performed on a medulloblastoma cell line, indicated that the encapsulation of curcumin within the hydrogel had little adverse effect on its bioactivity. Matson *et al.* investigated the potential of high aspect ratio peptide nanofibers for drug delivery [147]. They synthesized different self-assembling peptide amphiphiles (PAs) with a lysine 3-aminederivatized hydrazide, which was located at various positions along the peptide sequence backbone C16V2A2E2. These compounds were found to have the ability to release prodan with a near zero-order release profile, and their half-life was dependent on the location of the fluorophore at the PA sequence. Wang *et al.* evaluated the RGD peptide based hydrogel biocompatibility in posterior segment of the eye, and demonstrated that the biomaterial could potentially be used as a sustained drug delivery tool [148]. Clinical results indicated that the RGD peptide based hydrogel exhibit great ability to tolerate the vitreous cavity well. It could also disappear from the injection sites progressively, exhibiting excellent biocompatibility in the rabbit eyes's posterior segment, and thus can be considered a suitable biomaterial to deliver agents to the choroid and retina.

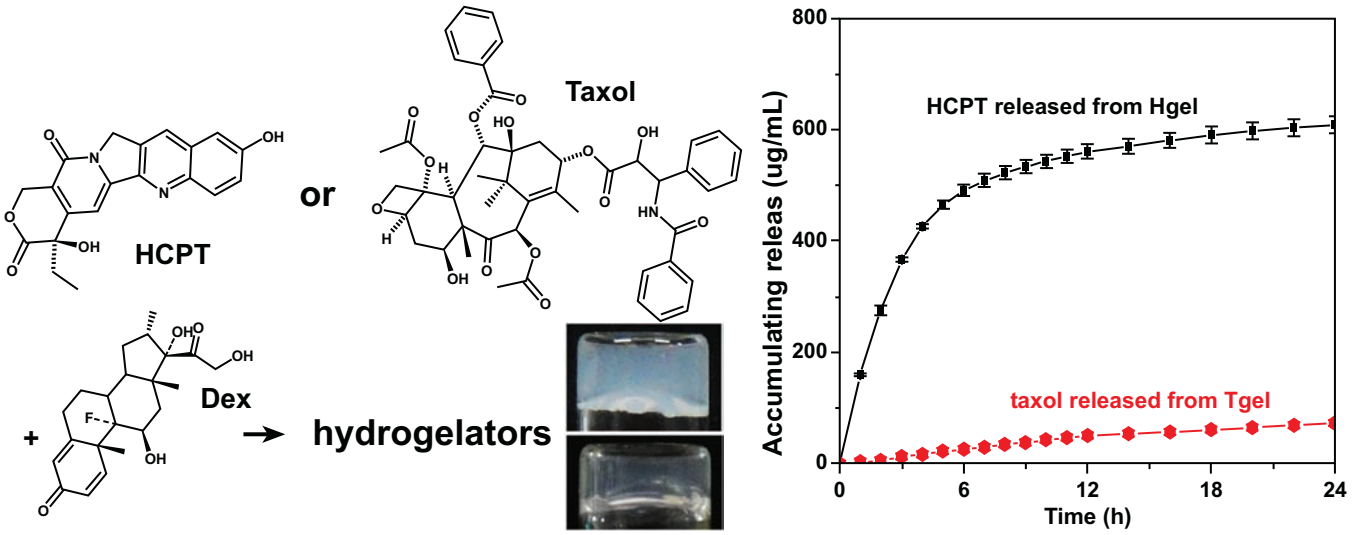

Fig. (3). Combination of two complementary anti-cancer drugs confers molecular hydrogels used as a drug delivery system, Adopted from Chem. Commun. 2012; 48: 395 [143].

4.2. Small Molecular Peptide Hydrogels as Drug Delivery Systems

Small peptide hydrogels could possess the similar advantages as normal peptide hydrogel systems and are highly attractive recently, because of their relative low cost and user controlled-manipulability [149]. The very first report about an amino acid based hydrogel occurred almost a century ago. Since then, people have developed new concepts involving small peptides, as well as their specific biomedical applications, especially as a drug delivery tool. Qin *et al.* synthesized L-glutamic acid dendron based on the shrinkable Metallo peptide hydrogel and investigated its ability for the stepwise release of drugs [150]. The novel hydrogel displayed selective shrinkage in the presence of positively charged species and maintained gel status in the presence of negatively charged species. This shrinkable peptide hydrogel could delivery drugs in a stepwise manner, where the negatively charged drug was released first and the second agent was released later upon a pH trigger. Baral synthesized a novel tripeptide-based hydrogel which was sustainable at native physiological pH and temperature (Fig. **4**) [151]. This product was thixotropic, injectable and had been employed for the incorporation and controlled release of antibiotic vancomycin molecular and vitamin B12. Ischakov *et al.* developed a scalable process for the formation of peptide-based hydrogel nanoparticles, which could be employed as effective drug delivery carriers, from aromatic dipeptide building blocks [152]. Encapsulation doxorubicin (Dox) and 5-flourouracil, within the hydrogel nanoparticle matrix, could result in the controlled release of therapeutic agents based on their chemical structure, molecular weight and surface property.

Yang developed a novel small peptide hydrogel system with a folic acid (FA)-Taxol conjugate [153]. These peptide hydrogels were generated via sulfide bonds reduction, using glutathione (GSH), and could achieve Taxol release via ester bond hydrolysis to inhibit tumor growth. Small peptides could also be conjugated to hydrogel structure to achieve excellent drug delivery ability. Qin *et al.* introduced a route to modify small peptide-decorated polymeric nanoparticles (NP), which can delivery anticancer drugs such as doxorubicin (Dox) for cancer therapy. The incorporation of the nucleolin-targeting F3 peptide could significantly enhance the internalization of co (CEA-AAm) Nano Particles (NPs) toward the drug-resistant NCI/ADR-RES cancer cell line. In addition, improved loading amount and controlled release of doxorubicin were gained [154]. Ashley *et al.* discovered a small peptide modified hydrogel drug delivery tool. Such system had tunable drug release properties [155]. They used β-eliminative linkers to co-combined cross-link PEG hydrogels and tether drugs, and, and illustrated controlled drug rate release as well as hydrogel erosion rates in long time. Such β-eliminative linkers could extend the half-life to regulate polymer degradation, and thus the system can be controlled to release the drug before gel undergoes complete degradation.

4.3. Sugar Functionalized Peptide Hydrogel as a Drug Delivery System

The majority of peptide hydrogels deliver drugs via passive targeting, and therefore it is desirable to develop a peptide hydrogel delivery system for actively targeting specific cells with enhanced performance of delivery and little side effects [156]. For this purpose, using "ligands" (which can be incorporated into various peptide systems) as moieties to facilitate drug targeting has gained intensive attention [157]. Sugar is one of the most widely used ligands that can be applied to drug delivery receptor targeting. Besides drug targeting, association of sugar to peptide hydrogel carriers could also offer different beneficial properties such as biostability, enhanced solubility, bioadhesive properties, as well as reduced toxicity for drug delivery. Xu *et al.* constructed a therapeutic glycopeptide hydrogel containing an N-fluorenyl-9-methoxycarbonyl phenylalaninephenylalanine-aspartic acid (FMOC-Phe-Phe-Asp) sequence and a glucosamine moiety used as a new substitute for proliferation inhibition drugs. Such new drug could be used to retard postoperative scar formation (Fig. **5**) [158]. Within 21 days after filtration surgery, the intraocular pressure (IOP) in a rabbit's eyes was low after the administration of such therapeutic sugar modified peptide hydrogels. Such glycopeptide hydrogels show similar therapeutic effects as traditional antiproliferative drug. Importantly, intraoperative administration of such novel therapeutic glycopeptide hydrogels could prevent the toxicity side effect of antiproliferative drugs against tissues, which shows that these hydrogels could make a promising potential utilization for glaucoma treatment. Tian *et al*. detailed the preparation and *in vitro* investigation of novel amphiphilic glycopeptide functional copolymers system as drug delivery tool for controlled drug release [159]. They aggregated into nanoparticles loaded with therapeutic agents in the presence of doxorubicin, exhibiting controlled release characteristic in water environments. These copolymers, specifically the lactobionolactone grafting ones, offer opportunity to be served as the tar-

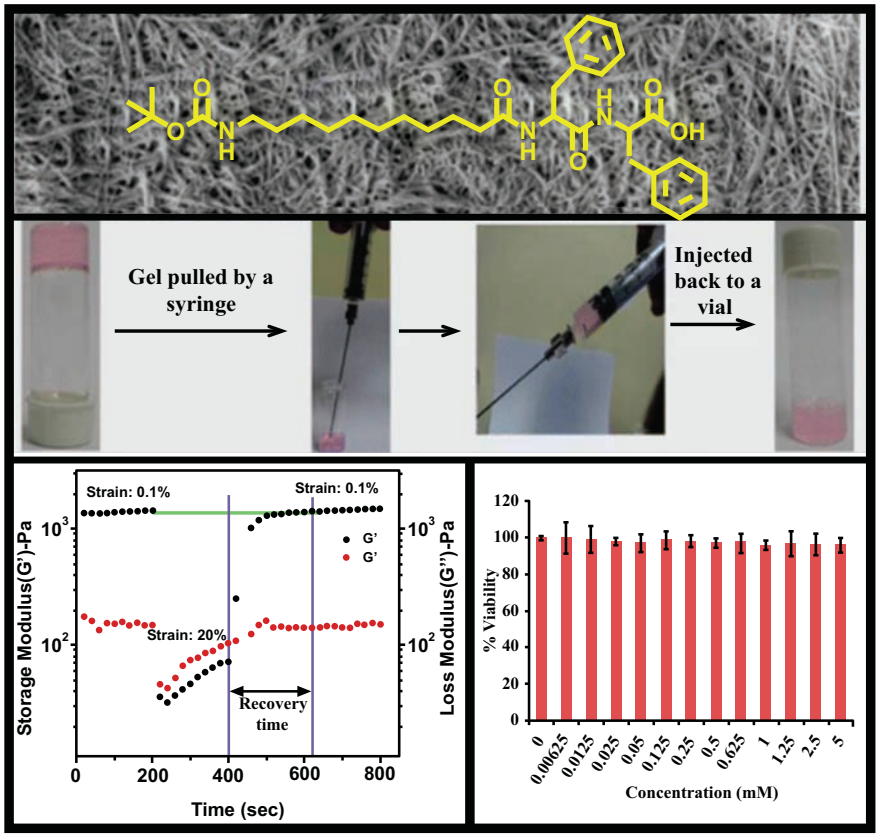

Fig. (4). Assembly of a noncytotoxic injectable peptide-based hydrogel for release of drugs. Adopted from Langmuir. 2014; 30 (3): 929 [151].

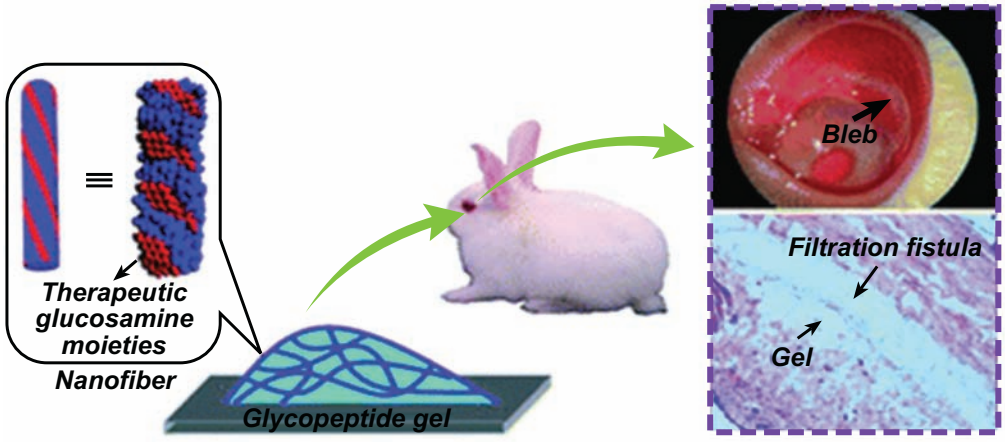

Fig. (5). Generation of therapeutic glycopeptide hydrogels as a novel substitute for antiproliferative molecular drugs to retard postoperative scar formation. Adopted from J. Mater. Chem.2012; 22:18164 [158].

get sites for the delivery system of drug release in controlled manner. Stohr *et al.* created glyco-NCAs by combining peracetylated sugars using a thiourea linker to the ε-amino group of *α*-Boc-lysine and *α*-Z-lysine [160]. The peptide hybrid hydrogels were shown to be specifically up taken by human T lymphocytes, demonstrating great potential as a drug delivery system. Xu *et al.* reported on the preparation of glycopolypeptide nanoparticles (GPNPs), with Mn doped ZnS quantum dots (QDs), which formed a hybrid shell/core nanostructure through the initiated N-carboxyanhydrides polymerization on surface and condensation polymerization with carboxymethyldextran on QDs [161]. They also investigated the immobilization and release characteristics of ibuprofen on the nanoscale drug delivery system. It was found that the GPNPs displayed high loading capabilities, as well as the controlled release characteristic in medium. By incubating HEK293T cell lines to the nanoparticles, the cellular toxicity of the GPNPs was also investigated, and low cytotoxicity was observed. Such hybrid structures appeared to be a great promising candidate system for targeted drug delivery.

4.4. Peptide Hydrogels as Self-Delivery Therapeutic Agents

Besides being used as a drug delivery carrier, peptide hydrogels can also serve as direct self-delivery therapeutic agents [162]. Compared with traditional antibiotics, the anti-inflammatory effects of peptides are extremely rapid, and can involve multiple cellular targets [163]. Xu *et al.* synthesized a peptide hydrogel with combi-

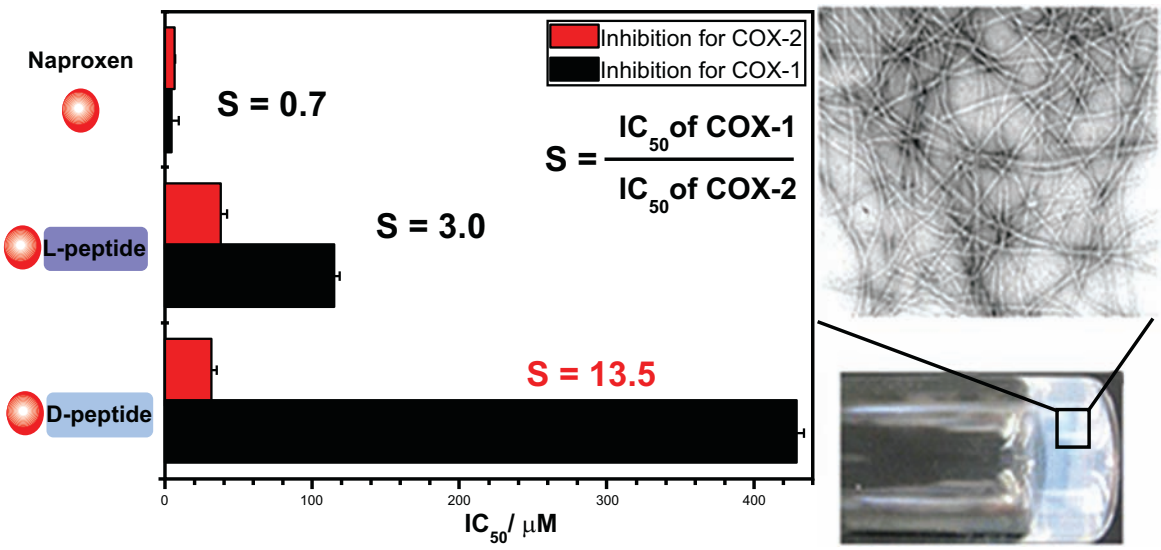

Fig. (6). D-amino acids boost the selectivity and confer supramolecular hydrogels of a nonsteroidal anti-inflammatory drug (NSAID). Adopted from J. Am. Chem. Soc. 2013; 135 (2): 542 [166].

nation of two N-(fluorenylmethoxycarbonyl) amino acids that displayed anti-inflammatory abilities [164]. Xu and co-workers also found that covalent bonding of D-amino acids and Naproxen could significantly enhance the drug selectivity toward COX-2, while maintaining high activity of Naproxen [165].

Self-assembling β-hairpin peptides, with high arginine content, exhibited extremely good performance in killing bacteria (both gram-positive and gram-negative bacteria), for example, Pseudomonas aeruginosa with multi-drug resistant property [166] (Fig. **6**). These hydrogel materials required no addition of antibacterial agents, displayed high efficiency against bacteria, and were biocompatible with human erythrocytes and mammalian mesenchymal stem cells. Studies with rheology demonstrated that the gel shows moderately stiff and exhibits shear recovery characteristics, allowing its delivery behavior through simple syringe. He *et al*. designed antimicrobial peptides that showed considerable selectivity for both *P. aeruginosa* and *Streptococcus mutants* [167]. This effect was achieved by combining a nonspecific widespread antimicrobial agent, as well as a specifically targeted antimicrobial agent. In addition, the peptide-based β-hairpin hydrogel surface showed excellent antibacterial ability. Studies indicated that such peptide hydrogel surface is very good at killing both gram-positive and gram-negative bacteria [168]. Derivatives of the peptide based hydrogel system also exhibited strong bacterial extermination abilities [169]. Hilpert *et al.* described a high throughput approach to screen peptides with enhanced antimicrobial abilities in large scale. Such approach relied on peptide synthesis from a cellulose support, and a pseudomonas aeruginosa strain which constitutively expressed bacterial luciferase [170]. They generated 12-mer peptides exhibiting a broad-spectrum ability. The minimal inhibitory concentrations (MIC) toward *Escherichia coli* of such peptides was as low as 0.5 μg/ml. They also designed an 8-mer substituted peptide with broad spectrum activity. The 80-mer substituted peptide show strong killing ability toward *E. coli* and *Staphylococcus aureus* at an MIC of 2 μg/ml. Mygind *et al.* isolated a plectasin-based peptide antibiotic from saprophytic fungus and investigated its therapeutic potential [171]. In the test, plectasin exhibited very low toxicity in animal models. Additionally, experimental peritonitis and pneumonia due to S. pneumonia were cured with such plectasin-based peptide antibiotic. These findings open the door to use fungi as a novel source of antimicrobial treatments, and also show the therapeutic potential of plectasin. Liu *et al* synthesized an innovatively designed peptide made up of two antibacterial peptide sequences as well as a central tetrapeptide linker. The peptide hydrogel displayed inherent antibacterial activity against *Escherichia coli* [172]. Yang *et al.* created a series of novel formyl hydroxyamino derivatives and evaluated their antibacterial activities. The *in vivo* studies confirmed that these compounds are mildly toxic, have a good pharmacokinetic profile, and protective effects. Therefore, it can be concluded that this class of compounds has the potential for use in future antibacterial drugs [173]. Besides the above-mentioned antibacterial applications, the peptide hydrogels also have been demonstrated to be used as wound healing agents and other therapeutic instruments under different conditions [174].

5. BIOCOMPATIBILITY

The formation of hydrogel peptides affect the structure and stability of hydrogel peptides which correlated with safety of such functional biomaterials. Thus, the safety of hydrogel peptides is controlled by physical physiological conditions such as pH, calcium ions, temperature, *et al.* Zarzhitsky *et al.* pointed that pH played important roles in regulating the second structure of charged hydrogel [175]. They found that the peptide dissolved in the pH range 4-9, specially, in the pH range from 7.4-9, the hydrogel peptides unfolded. Calcium ion could also control hydrogel peptides properties. The high calcium ion concentration could cause dissolve of hydrogel peptide dissolved and low concentration of calcium ion could maintain the stability of hydrogel. Chenite *et al.* demonstrated that gelation became temperature dependent in the pH [176]. Fu *et al.* discussed the role of temperature on morphological transitions of hydrogel nanostructures self-assembled by peptide amphiphiles using dynamic molecular simulation methods. It was shown that with the temperature increase, the β-strand percentage is not affected, the α-helix percentage decrease first then reach the plain, however the random coil percentage increase upon temperature then reach the plain [177].

6. PERSPECTIVES AND CHALLENGES

This review article mainly focuses on recent progress in the development of the synthesis of functional, biocompatible, self-assembling peptide nanostructure hydrogels and their specific applications for drug delivery. Although promising progress has been achieved, there are still many challenges and shortcomings of such systems. Firstly, the bioactivity of the drug needs to be maintained

at high levels in peptide hydrogel hybrid drug delivery systems and molecular peptide based hydrogel systems, without compromising the activities of the therapeutics. Additionally, the toxicity of peptide-based hydrogels should be evaluated comprehensively, including investigations on the cyto/genotoxicities of peptide molecular hydrogels therapeutics. Studies should be conducted on specific disease models such as the mouse breast cancer model and the rabbit ophthalmic disease model. Furthermore, bioresponsive physical and chemical triggers that could prompt drug release *in vivo* or *in vitro* need to be studied intensively. For reasons mentioned above, future research on the development and understanding of bioactive drug molecular transport through peptide hydrogels, under 'real' conditions, must be further explored.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by Grants from Natural Science Foundation of China (NSFC) Project (51403042, 31370781). We also acknowledge the funding support from the Institute of Bioengineering and Nanotechnology (Biomedical Research Council, Agency for Science, Technology and Research, Singapore).

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Received: May 14, 2015 **Accepted: August 17, 2015**