Bioconjugate Chemistry

Review

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Biomedical Applications of Self-Assembling Peptides

This paper was originally submitted for the "Biofunctional Biomaterials: The Third Generation of Medical Devices", published as the July 15, 2015, issue of Bioconjugate Chemistry (Vol. 26, No. 7).

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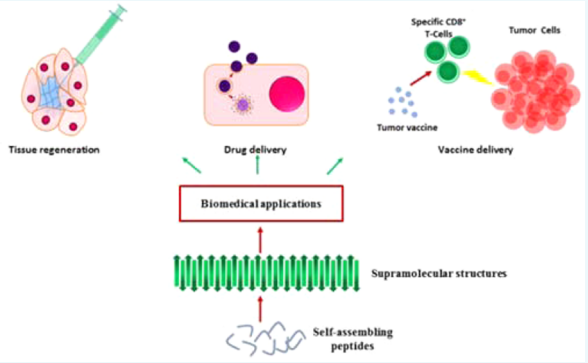
ABSTRACT: Self-assembling peptides have gained increasing attention as versatile molecules to generate diverse supramolecular structures with tunable functionality. Because of the possibility to integrate a wide range of functional domains into self-assembling peptides including cell attachment sequences, signaling domains, vaccine epitopes, and even therapeutic moieties, complex nanostructures can be obtained with a wide range of applications in the biomedical field. The first part of this Review provides a concise overview of how peptide primary and secondary structure dictate the way such self-assembling peptides organize into higher ordered, supramolecular structures. Next, an overview of the literature will be given on recent studies on peptide self-assembly for application in drug delivery, vaccination, and tissue engineering.



1. INTRODUCTION

With our increasing understanding of the mechanisms of protein folding we have now reached a stage that we can begin to build peptides and proteins to assemble into predefined but complex structures for diverse applications in materials and biomedical sciences. Through rational design and engineering of (poly)peptides many different kinds of assemblies can be formed, all driven by inter- and intramolecular forces such as hydrogen bond formation, hydrophobic and electrostatic interactions, van der Waals forces, and $\pi-\pi$ stacking. Moreover, peptides can be modified with lipids or synthetic and natural polymers to gain even more flexibility in the self-assembling properties. Because of these features, self-assembling peptides are interesting candidates for biomedical applications.

This Review will focus on the biomedical applications of self-assembling peptides. First, a concise overview will be given of the various secondary structures that peptides can adopt that facilitate the formation of well-defined supramolecular structures. Second, different types of self-assembling peptides will be discussed, focusing on their biomedical applications such as drug delivery, vaccination, and tissue engineering. Although the field of self-assembling peptides is rather broad, this Review will exclusively focus on unmodified peptides <30 amino acids or peptides with small chemical modifications at the N- or C-terminus that have an intrinsic capacity to self-assemble into ordered supramolecular architectures. We will not discuss the literature on peptides that are conjugated to large chemical structures such as polymers.

2. SECONDARY STRUCTURES

Since the supramolecular self-assembly of peptides is governed by the way these individual peptides fold in aqueous solution, it is important to understand which secondary structures exist and how these can be used for self-assembly.

2.1. α-Helix. α-Helices are a type of protein secondary structure of which the amino acids have a tendency to form hydrogen bonds between the oxygen of the carbonyl group and the hydrogen of every third amide group, providing some stabilization to the peptide backbone (Figure 1C). The side chains of the amino acids extend outward from the outer surface of the α-helix.[1] The α-helix is in itself not a thermodynamically stable conformation, but by assembling together with other α-helices, a more stable structure is created.[2,3] A typical assembly of α-helices is the coiled coil.

2.1.1. Coiled-Coil. The coiled-coil structure is a structure that is often found in nature. It consists of two or more α-helices intertwined with each other in a way that the hydrophobic parts are excluded from the aqueous environment (Figure 1E,F).[4] The molecular basis for this structure is the repeated heptad sequence: $(abcdefg)_n$. In this amino acid sequence, positions a and d are occupied by amino acids with hydrophobic side chains and e and g are often charged amino acid residues. Peptides conforming to these rules will form into a right-handed α-helical shape, assembling into helical bundles with left-handed supercoils. Adequately designed coiled coils can self-assemble into larger supramolecular assemblies such as nanofibrils or nanoparticles, structurally supported by the

Received: February 9, 2015
Published: October 16, 2015

DOI: 10.1021/acs.bioconjchem.5b00487
Bioconjugate Chem. 2016, 27, 3−18

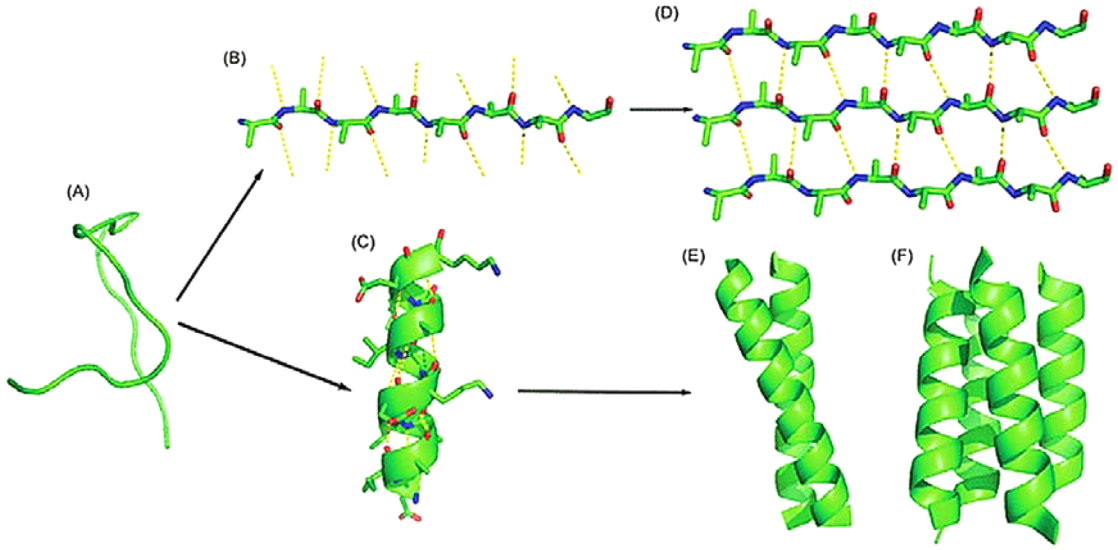


Figure 1. Schematic representation of a polypeptide folding. (A) polypeptide chain, depending on amino acid composition, can fold into a β-strand (B) or and an α-helix (C) (yellow dashes represent the hydrogen bindings). Further, α-helices can form coiled coils (E) and helical bundles (F) while β-strands develop β-sheets (D). Reprinted with permission from Boyle, A. L., and Woolfson, D. N. (2011). *Chem. Soc. Rev. 40* (8), 4295−4306. Copyright 2011 RSC publishing.

hydrophobic interactions between the amino acids at positions a and d and the electrostatic interactions between the amino acids at positions e and g.[5] A more thorough explanation of coiled-coils can be found in a review by Woolfson.[6]

2.2. β-Sheet. Another type of secondary protein structure apart from the α-helix is the β-strand. In a β-strand, the peptide backbone is stretched, and the hydrogen bonding groups point orthogonally to the direction of the peptide chain. When β-strands are connected laterally through hydrogen bonding, they form a β-sheet.[7] The sheet-like structure is created by the hydrogen bonds between the amino acids in different peptide strands (Figure 1B,D). The interpeptide and interchain bonds add considerably to the rigidity of the structure.[8,9] Peptides that are prone to form β-sheets and self-assemble into supramolecular structures are usually around 16−20 amino acids long and often feature alternating patterns of hydrophobic and polar amino acids. The alignment of strands in β-sheets is parallel or antiparallel, which results in different hydrogen binding patterns for these two forms. Computational analyses indicated that antiparallel β-sheets, because of the well aligned hydrogen bonds, are energetically more favored than parallel forms.[10,11]

Most β-sheet forming peptides currently described for self-assembly form indefinite assemblies, i.e., assemblies with no discrete dimensions. An example of such indefinite assemblies is peptide fibers with hundreds of nanometers to a few micrometers in length.[12,13] However, there are also examples that β-sheet forming peptides assemble into definite structures such as vesicles and micelles with discrete dimensions.[14,15]

The β-hairpin structure is an example of an antiparallel β-sheet structure. A 20-amino-acid-long peptide was designed by Schneider et al. consisting of two β-strands with alternating lysine and valine residues and contains a fold in the middle of the molecule.[16] The β-hairpin will self-assemble, facially or laterally, into a hydrogel, with possible applications such as promoting cell proliferation or as an antimicrobial agent.[17−19]

2.3. Poly(L-Proline) Type II (PPII) Helices. Apart from the α-helix and β-sheet there is a third, less common type of secondary structure, namely, the poly(L-proline) type II helix. The poly(L-proline) type II (PPII) helix is a left-handed helix with 3 amino acids per turn, having the shape of a triangular prism. While the name suggests it contains many proline residues, this is not necessarily the case. However, proline-rich peptides have a high propensity to assemble into PPII helices.[20] The PPII helix might be relevant in the self-assembly of peptides because the backbone of the structure is exposed and available for intermolecular hydrogen bonds, contributing to the stability of oligopeptide vesicles.[21] The PPII helix is also an important component of collagen, in which it is assembled into a triple helix, thereby contributing to the stability of collagen.[22] Peptides undergoing this assembly might be relevant for tissue regeneration purposes.

3. SUPRAMOLECULAR STRUCTURES FORMED BY THE INTERMOLECULAR SELF-ASSEMBLY

The supramolecular structures formed by the secondary protein structures can be divided into definite assemblies such as micelles and vesicles that have discrete dimensions and indefinite assemblies such as tubes, fibers, tapes, and ribbons that are several hundred nanometers long. These two types of structures will be further described below.

3.1. Definite Structures. *3.1.1. Micelles.* Spherical micelles can be formed when building blocks with a hydrophilic head and a hydrophobic tail self-assemble into a definite supramolecular core−shell structure (Figures 2B and 3B). They can differ in size and shape depending on factors such as the temperature, pH, concentration, and interactions between the peptides. Schuster et al. designed an all-amino-acid peptide that self-assembled into spherical micelles.[23] This peptide has a repetitive sequence of the hydrophobic amino acids leucine and tryptophan and three charged lysine residues at the headgroup ($H\text{-}K_3\text{-}[WL]_3\text{-}W\text{-}NH_2$). Electrostatic repulsion leads to the formation of micelles, possibly created from an intermediary α-helical structure.

To make the hydrophobic effect of the peptides stronger, an acyl chain can be conjugated to the tail of peptides, creating so-called peptide amphiphiles.[24] Shimada et al. designed a peptide, C_{16}-WAAAAKAAAAKAAAAKA, which has an α-helix propen-

DOI: 10.1021/acs.bioconjchem.5b00487
Bioconjugate Chem. 2016, 27, 3−18

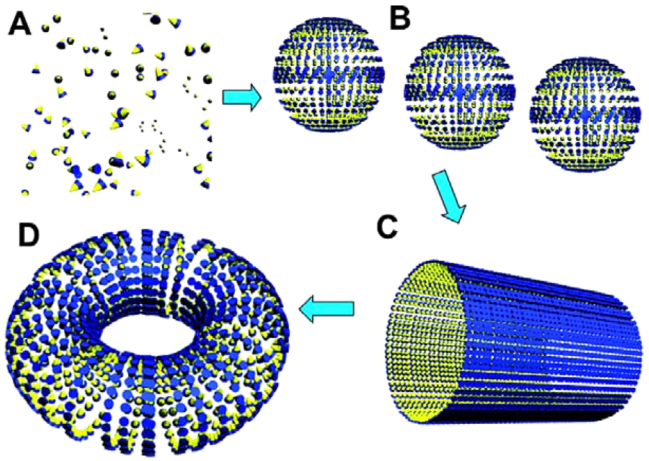


Figure 2. Proposed assembly mechanism of the nanodonut structure. (A) Nonassembled peptides at a low concentration. (B) Self-assembling into micelles above critical micelle concentration (CMC). (C) Elongation of micelles into nanotubes. (D) Ultimately, bending of the nanotube into a nanodonut shape. Reprinted from Khoe, U., Yang, Y., and Zhang, S. (2008). *Langmuir* 25 (7), 4111–4114. Copyright 2009 American Chemical Society.

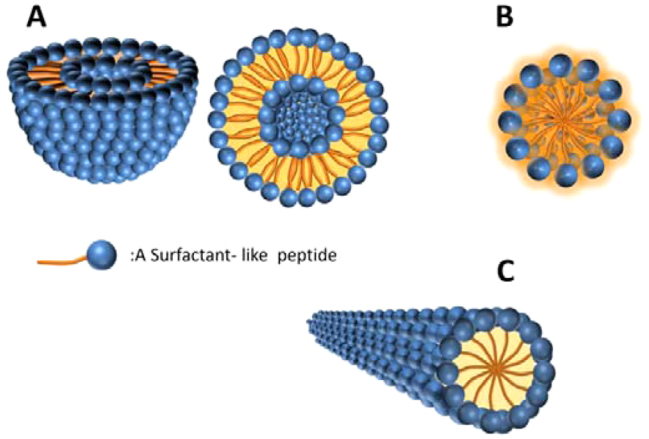


Figure 3. Schematic representation of a vesicle (A), a micelle (B), and a fiber formed by surfactant-like peptides in an aqueous solution.

sity and self-assembles into spherical micelles into an aqueous solution. After a few days of incubation, however, the conformation changes to a worm-like micelle.[25] It is likely that this transformation is caused by a change in secondary structure to a more stable β-sheet conformation. This change leads to an enhanced hydrophobicity of the tail part and the subsequent transformation to a worm-like micelle structure.[25] A particular kind of micelle formation is seen in the so-called "nanodonut" shape (Figure 2). The peptides that generate this assembly are relatively short and cone-shaped (Ac-GAVILRR-NH_2); the hydrophobicity and size of the first five amino acids increase toward the positively charged arginine residues. They first assemble into spherical micelles, which then fuse to form cylindrical micelles or nanopipes. Hydrophobic interactions between the tail parts cause the nanopipes to bend and ultimately form an enclosed nanodonut shape.[26]

Looking at all the studies showing micelle formation by self-assembling peptides indicates that the peptide building blocks can be very different from each other.[27] The common factor, however, is that there is always a hydrophilic (and most of the time charged) headgroup and a hydrophobic tail, often conjugated with an acyl group. This design favors the formation of micelles from self-assembling peptides.

3.1.2. Vesicles. Vesicles, contrary to micelles, have an aqueous interior compartment because the amphiphilic building blocks form a bilayer or sheet in which the hydrophilic parts point to both the outer and the inner surface (Figure 3A). How the vesicles are exactly being formed from the peptides is not entirely clear at present. It is, however, suggested that the peptide monomers first create a bilayer by connecting tail to tail and subsequently convert into a spherical or tube-like shaped structures due to hydrophobic interactions.[28] Some surfactant-like peptides designed by the group of Zhang et al. were found to self-assemble into vesicles of about 30–50 nm in diameter.[29] The hydrophilic head part consisted of 1 or 2 aspartic acid residues and either 6 alanine, valine, or leucine residues constitute the hydrophobic tail part (A_6D, $V_6D_{1/2}$, or L_6D_2, respectively). Hydrophobicity of the peptide's tail seemed to have the biggest influence on the type of supramolecular structures formed. Whereas L_6D_2 with the most hydrophobic tail formed mixed structures of nanotubes, entwined rod-like micelles, and vesicles as determined by transmission electron microscopy, A_6D_2 with lower hydrophobicity only formed nanotubes. Interestingly, TEM images of V_6D_2 peptide (with hydrophobicity in between that of L_6D_2 and A_6D_2) showed a transition state of nanotubes with budding nanovesicles. Also, work done by Van Hell et al. showed that acetylated oligopeptides of 10 or 15 amino acid residues self-assembled into vesicles.[30] The sequence of these peptides is Ac-AAVVLLLWEE-COOH (SA_2) and Ac-AAVVLLLWEEE-EEEE-COOH (SA_7) which, compared to the sequence of the self-assembling peptides from Zhang et al., has a more conical shape as the side groups of these amino acids increase in bulkiness nearing the charged glutamate residues at the C-terminus. Further structural characterization using ssNMR showed that SA_2 peptides, when assembled in nanovesicles, developed antiparallel β-sheets and consequently formed an intercalated bilayer instead of a typical tail to tail alignment.[14]

For surfactant-like peptides, it is suggested that the hydrophobic domain arrangement has more influence on the final self-assembly structure rather than the differences in ratios between the hydrodynamic volumes of the hydrophobic and hydrophilic domains as is the case for hydrocarbon surfactants.[30] For example in A_6D_2 and A_6D or SA_2 and SA_7, each set containing the same hydrophobic domain but with hydrophilic domains that differ in size, the obtained supramolecular structures are the same. These examples may indicate some deviations in behavior compared to hydrocarbon surfactants whose self-assembly can be well described by Israelachvil's packing parameter equation.[29−32] Shorter peptides, consisting of only two amino acid residues, α,β-dehydrophenylalanine (ΔPhe) coupled with either lysine or glutamic acid, are also able to self-assemble and form nanovesicles.[33] Furthermore, they were proteolytically and thermally stable and were able to trap molecules inside, making these nanovesicles potentially appealing for drug delivery applications.[33]

3.2. Indefinite Structures. *3.2.1. Fibers.* Fibers can be very similar to micelles in the way that the hydrophobic tails of the peptide monomers are attracted toward the center of the structure, and the surface is hydrophilic. Fibers, however, are not spherical, but cylindrical and can, therefore, be seen as elongated micelles (Figure 3C). It is, therefore, not surprising

DOI: 10.1021/acs.bioconjchem.5b00487
Bioconjugate Chem. 2016, 27, 3–18

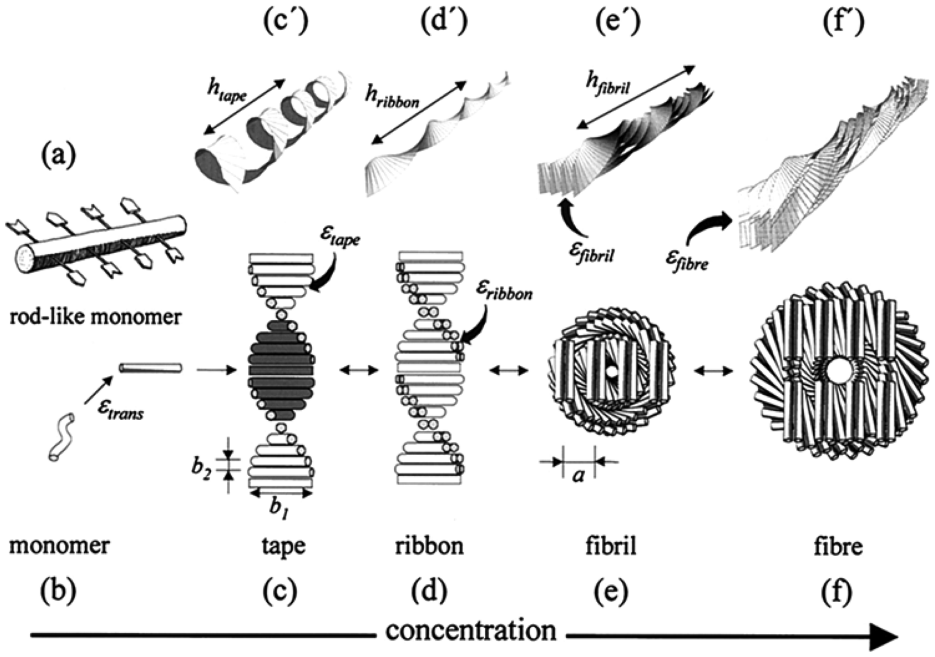


Figure 4. Schematic representation of β-sheet forming supramolecular structures. Peptides are depicted as chiral rod-like units (a, b). Arrows show the complementary donor and acceptor groups. The monomers interact to form a tape (c); these tapes can stack together with their hydrophobic side to form a ribbon (d). When the concentration increases, monomers can assemble into fibrils (e) or fibers (f); both show the front view of the edges. c′−f′ represent the global equilibrium conformations corresponding to c−f, respectively. Reprinted with permission from Aggeli, A., et al. (2001) *Proc. Natl. Acad. Sci. U.S.A. 98* (21) 11857−11862. Copyright 2001 National Academy of Sciences.

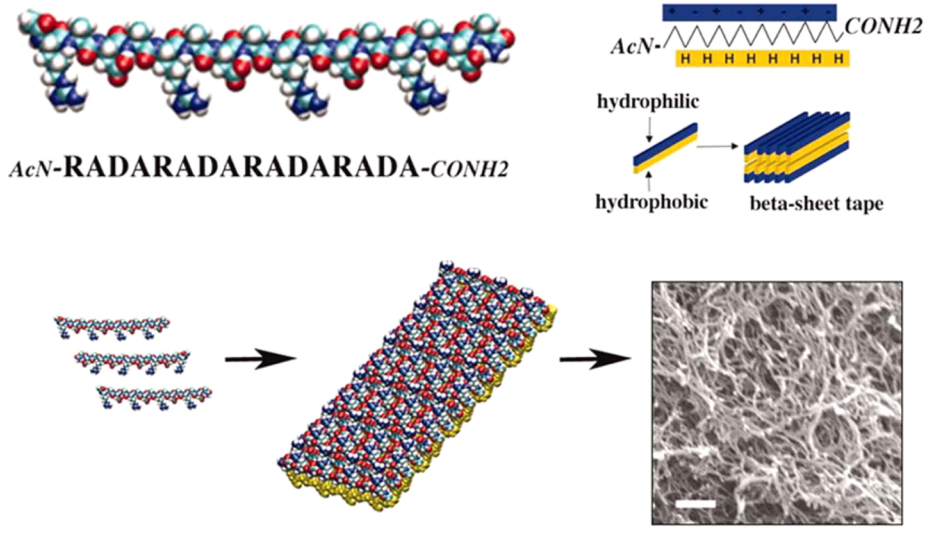


Figure 5. Illustration the RADA16-I hydrogel formation by parallel β-sheet alignment of peptides. Reprinted with permission from Semino, C. E. (2008) *J. Dental Res. 87* (7) 606−616. Copyright 2008 SAGE Publications.

that peptides that are able to form micelles are very similar to the peptides that form fibers.

An example of a well-designed peptide amphiphile is the molecule made by the group of Stupp et al.[34] This peptide has an alkyl tail, 4 cysteine residues for forming disulfide bonds, 3 glycine residues functioning as a flexible linker, a phosphorylated serine residue to interact with calcium ions, and finally arginine-glycine-aspartic acid (RGD), a cell adhesion ligand. These peptides self-assemble in a tightly packed nanofiber that can direct mineralization of hydroxyapatite, that eventually creates a bone-like composite material. A much shorter peptide (C_{12}-VVAGK) also self-assembles into fibers.[35]

However, amphiphilicity of a peptide is not a prerequisite to forming a fiber. Peptides that will form a β-sheet can also assemble into fibers, depending on the concentration. The peptides Ac-RADARADARADARADA-$CONH_2$ (RADA16-I) and P_{11}-I (Ac-QQRQQQQQEQQ-NH_2), for example, form fibers as well (Figures 4 and 5).[36,37] Thus, it is hard to define a single rule about the required structural properties for fiber forming peptides. Some examples of biomedical applications of peptide fibers will be given in the following sections of the Review article.

3.2.2. Tubes. In the same way that fibers are basically elongated micelles, tubes are elongated vesicles. The structure is open-ended, has a hollow inside, and the monomers are

DOI: 10.1021/acs.bioconjchem.5b00487
Bioconjugate Chem. 2016, 27, 3−18

linked together by their hydrophobic tail parts and their hydrophilic head parts are directed outward. Generally, the same peptides that are capable of forming vesicles can also form tubes. In fact, Yan et al. showed that dipeptide nanotubes can be rearranged into nanovesicles upon dilution and can then be used as a carrier of oligonucleotides.[38] The aforementioned peptides designed by Zhang et al. are also able to self-assemble into nanotubes.[28] The proposed model for these peptides to self-assemble into a nanotube is that the monomers first form a bilayer, which then forms a closed ring. Subsequently, these rings stack on top of each other, which causes tube formation.[27] For drug delivery purposes, a vesicle might be more preferable than a tube since it is able to encapsulate the drug. However, it can still work as a carrier system for bigger structures such as proteins and genes.[39]

3.2.3. Tapes. The most studied tape structures have been designed by Aggeli et al.[40] First, the peptide K24 (NH_2-KLEALYVLGFFGFFTLGIMLSYIR-COOH) was found to assemble into a tape and form a gel when dissolved in methanol. In another study, peptides P_{11}-I (CH_3CO-QQRQ-QQQQEQQ-NH_2) and P_{11}-II (CH_3CO-QQRFQWQFEQQ-NH_2) were designed. These peptides are shorter, but due to the charged residues were still able to interact electrostatically with each other, resulting in again a tape structure and a gel-like substance when dissolved.[41] These tapes were several hundred nanometers long consisting of β-sheets in a helical or twisted shape. The helical twist was caused by the intrinsic chirality of the amino acids (Figure 4). Since the tape was essentially a β-sheet, it had one side that was predominantly hydrophobic and one that was mainly hydrophilic.[37]

Another tape-like structure based on the β-sheet, but without a helix, is the "nanobelt" designed by Cui et al. This large one-dimensional structure consists of peptides of only four amino acids (VEVE) coupled to an aliphatic group ($C_{16}H_{31}O$).[42]

For tape(-like) structures, it is especially necessary to have an amino acid sequence that tends to form β-sheets, for example, by incorporating the negatively charged glutamate between hydrophobic amino acid residues. The length of the monomer does not seem to be a very crucial factor, as proven by Cui et al.; most tapes will form a gel-like substance and can be used for tissue engineering and cell culture applications.[43]

3.2.4. Ribbons. Ribbons are described as two stacked tapes. Because both sides of the tape have different surfaces, in an aqueous environment the hydrophobic parts will be shielded off by the conjoining of two tapes (Figure 4). The peptide structure does not change, only the supramolecular structure. This was demonstrated with the P_{11}-1 and P_{11}-II peptides from Aggeli et al.[37] Above a certain concentration of the peptides, ribbons started to form instead of tapes. When the concentration increased further, first fibrils (several ribbons stacked together) and subsequently fibers were formed. In another study, an interesting phenomenon was observed when ribbons were kept in solution at 25 °C for 4 weeks. A morphological transformation occurred where the peptide ($C_{16}O-F_3E_3$) assembly switched from a twisted ribbon to a helical ribbon.[44] In a twisted ribbon conformation, the β-sheets near the outside can be less twisted than those near the center; the helical ribbon conformation is, therefore, more energetically stable since all β-sheets make the same twist.[44] With a similar peptide but without phenylalanine ($C_{16}O$-A_3E_3), ribbon conformation and the aging phenomenon were not observed indicating that this process is sequence dependent.

In conclusion, driven by various molecular forces peptides can self-assemble in different supramolecular structures. The combinations of all these forces make it difficult to predict the behavior of self-assembling peptides. Peptides that have an electrostatically charged (or hydrophilic) head and a hydrophobic tail part will likely self-assemble in spherical micelles or vesicles. From these structures on, elongation into fibers or tubes respectively is possible. Peptides with a β-sheet propensity tend to assemble into flat structures such as tapes or ribbons. However, when the concentration of the peptides increases these tapes and ribbons will stack on each other and form more tightly packed fibers. More knowledge of how all the molecular forces interact is essential to design more efficient, chemically stable, and practical peptide self-assemblies.

4. SELF-ASSEMBLING PEPTIDES FOR DRUG DELIVERY APPLICATIONS

Drug delivery systems are often complex systems in which drugs are being combined with carrier molecules and targeting ligands. They often involve encapsulation, complexation, or conjugation of drug molecules to the nanoparticulare carrier, sizing of the nanoparticles, removal of the unincorporated drugs, and conjugation of targeting ligands to obtain cell specificity. This is a laborious procedure that, due to the use of multiple components, can lead to pharmaceutical incompatibilities. Self-assembling peptides can be designed in such a way that multiple functionalities are united in a single molecule. For example, a polypeptide can be generated that consists of a targeting sequence, a self-assembly domain, and a biologically active peptide. Such monopartite delivery systems are advantageous from a pharmaceutical point of view, as they obviate the need for complex formulation work (e.g., using surfactants, high shear homogenization) or chemical reactions (e.g., post modifications) to obtain multifunctional nanoparticles. Moreover, peptides are naturally biodegradable, which is not always the case for polymer-based drug delivery systems. If peptide-based drug delivery systems are constructed from de novo peptides, immunogenicity might be an issue. However, with the current knowledge of how antigenic epitopes generated from proteins and peptides are being presented to the immune system, it has become possible to engineer proteins and peptides in such a way that they are poor substrates for MHC class I and II presentation.[45−47] Such combinatorial deimmunization can also be applied to self-assembling peptides with the aim to reduce potential immune responses without losing the self-assembling capacity. Nevertheless, the repetitive nature of the surface of supramolecular structures formed by self-assembling peptides may trigger T-cell-independent antibody formation as has been shown for protein aggregates.[48] Therefore, the immunogenicity of the peptide supramolecular structures should be monitored at all times.

Different supramolecular architectures have been studied for drug delivery applications. Self-assembling peptides that form definite nanostructures, such as micelles, rods, or vesicles, can be used for the generation of nanocarriers for local or systemic drug delivery. Alternatively, self-assembling peptides forming fibrils or hydrogels may be used as macroscopic drug depots for sustained drug release.

Since the majority of self-assembling peptides that have been reported so far form indefinite nanofibers, the application of such peptides has been focused on the formation of hydrogels for sustained release applications or tissue engineering. There are only a few studies describing the use of self-assembling

DOI: 10.1021/acs.bioconjchem.5b00487
Bioconjugate Chem. 2016, 27, 3−18

Table 1. Selection of in Vitro Characterized Self-Assembling Peptides As Drug Delivery Vehicle

composition/sequence	supramolecular structure	secondary structure	cargo	chemically(C)/ physically(P)[a]	ref.
L-diphenylalanine: NH_2-FF-COOH	microtubes	----	Rhodamine B	P	63
Fmoc-FF-COOH	hydrogel nanoparticles	---	5-fluoro-uracil (5-Fu), Doxorubicin	P	64
RADA16-I: Ac-$(RADA)_4$-$CONH_2$	nanofiber hydrogel	β-sheet	Pindolol, Quinine, Timolol maleate	P	65
RADA16-I: Ac-$(RADA)_4$-$CONH_2$,	nanofiber hydrogel	-----	Human IgG	P	66
KLD12: Ac-$(KLDL)_3$-$CONH_2$					
$(Ac\text{-}FLIVI)_2KKKKK$-$CONH_2$	nanovesicles	β-sheet	5(6)-carboxyfluorescein, rhodamine 6G carboxytetramethylrhodamine (Covalently)	P/C	15
$(Ac\text{-}FLIVIGSII)_2KKKKK$-$CONH_2$					
MAX8: VKVKVKVK-$V^DPPTKVEVKVKV$-NH_2	fibrils	β-hairpin	curcumin	P	67
SA_{2-7}: Ac-$AAVVLLLW(E)_{2-7}$-COOH	nanovesicles	PPII	zinc-phthalocyanine (TT-ZnPcNH2)	P	56
SA_2 C_3: Ac-ACVCLCLWEE-COOH					
RAD16-I: Ac-$(RADA)_4$-$CONH_2$,	nanofiber hydrogel	β-sheet	Cytokines: βFGF	P	54
Ac-$(RADA)_4$-GGDGEA-$CONH_2$			BDNF		
Ac-$(RADA)_4$-GGPFSSTKT-$CONH_2$			VEGF		
RADA16-I: Ac-$(RADA)_4$-$CONH_2$	nanofiber hydrogel	β-sheet	lysozyme, trypsin inhibitor, BSA, IgG	P	53
NH_2-PSFCFKFEP-COOH	"'beads-on-a-thread"' type nanofibers	β-sheet	pyrene	P	68
MAX8: VKVKVKVK-$V^DPPTKVEVKVKV$-NH_2	fibrils	β-hairpin	lysozyme, α-lactalbumin, myoglobin, lactoferrin, bovine serum albumin (BSA), human immunoglobulin G (IgG),	P	69
EAK16-II: NH_2-AEAEAKA-KAEAEAKAK-COOH	EAK16-II: nanofibers	EAK16-II: β-sheet	ellipticine	P	70
EAK16-IV: AEAEAEAEA-KAKAKAK-COOH	EAK16-IV: globular nanostructures, short nanofibers:	EAK16-IV: β-turn			
EFK16-II: FEFEFKFKFE-FEFKFK-COOH	EFK16-II nanofibers	EFK16-II: Not reported			

[a]Cargo molecules be either chemically conjugated to the peptides or physically loaded into the peptide scaffolds.

peptides forming discrete nanoparticles for the delivery of drugs into cells. On overview of both indefinite and discrete supramolecular peptide assemblies used for drug delivery is given in Table 1.

In this section we will divide the self-assembling peptides that have been developed for drug delivery into 3 categories: (a) peptides consisting of natural amino acids with no or minor N- or C-terminal modifications; (b) self-assembling peptides containing unnatural amino acids; (c) lipidated self-assembling polymer−peptide conjugates.

4.1. Self-Assembly Based on Unmodified Peptide. The majority of peptides used for engineering structures driven by self-assembly consist of natural L-amino acids with or without standard N-(acetylation) or C-terminal (amidation) modifications to remove unwanted terminal charges. Self-assembly of such peptides is entirely driven by the properties of the amino acid sequence. The main advantage of this class of self-assembling peptides is that they can be easily synthesized using standard solid-phase synthesis techniques without the need for laborious chemical modifications. The majority of unmodified peptides used for self-assembly form indefinite structures such as fibers or hydrogel-based networks. The best example of a self-assembling peptide that form hydrogels is RADA16-I.

RADA16-I comprises 4 repetitive units of arginine (R), alanine (A), aspartic acid (D), and alanine (A). In aqueous solution, hydrophobic interactions of the alanines among 2 peptide molecules expose the negatively charged aspartate residues and positively charged arginine residues toward the exterior face. Consequently, complementary charge interactions between the formed pieces result in nanofiber formations with a width of 3−8 nm. At high concentrations, such large numbers of nanofibers generate nanoporous hydrogels (Figure 5).[49−51] Hydrogels, because of their tailorable properties like cross-link density, water content, and degradability, are frequently studied as matrices for the controlled release of proteins.[52]

RADA-16 has been developed for commercial use as cell matrix for tissue engineering and sustained drug delivery, and is called PuraMatrix.[53−55]

Koutsopoulos et al. studied the release profile of different proteins with different molecular weights which were loaded in RADA16-I hydrogels.[53] They found that the release was governed by diffusion, in which the diffusion coefficients are dependent on the molecular weight of proteins and the concentration of peptide used to form the geometrically uniform hydrogel. For example, IgG (MW: 150 kDa) was released more slowly than lysozyme (MW: 14.5 kDa) when using hydrogels with the same peptide densities. By a 1% (wt/vol) peptide hydrogel, 40% of embedded IgG was released over 60 h of incubation, compared to >60% of lysozyme after 10 h of incubation.

DOI: 10.1021/acs.bioconjchem.5b00487
Bioconjugate Chem. 2016, 27, 3−18

Table 2. Selection of Peptide Amphiphiles Used As a Drug Delivery Carrier

composition/sequence	supramolecular structure	secondary structure	cargo	chemically(C)/ physically(P)[a]	ref.
C16 V2A2E2K(Hyd)[b]	nanofiber hydrogel	β-sheet	6-propionyl-2-dimethylaminonaphthalene (Prodan)	C	71
C16 V2A2K(Hyd)E2[b]					
C16 V2K(Hyd)A2E2[b]					
C16K(Hyd)V2A2E2[b]					
C16 V3A3E3K(ßD)[c]	nanofiber gels	β-sheet	[Ru(CO)3Cl2]2 for CO delivery	C	74
C16 V2A2E2	nanofiber gels	β-sheet	Nabumetone	C	75
C16 V2A2E2	nanofiber gels	β-sheet	Dexamethasone	C	72
C16A4G3E3	nanofiber gels	β-sheet	Camptothecin	P	76
C16 V3A3E3	nanofiber gels	β-sheet	Sonic hedge- hog (SHH) protein	P	77

[a]Cargo molecules either chemically conjugated to the peptides or physically loaded. [b](Hyd) = hydrazide. [c]ßD indicates that the Asp residue was attached to its side chain carboxylic acid.

Charge interaction between proteins and peptidic scaffold did not play a major role in the release kinetics of entrapped proteins, since the isoelectric point of the peptidic scaffold was very close to physiological pH, with a slightly negative charge at pH 7.4. However, the electrostatic interaction of entrapped proteins with the peptide matrix and thereby the release kinetics of such proteins can be tailored by changing the pI of the scaffold.[53]

There are few designed peptides that are able to form discrete nanoparticles which could in principle be used for intravenous drug delivery. Surfactant-like peptides have been shown to form vesicular structures, depending on the primary sequence, peptide concentration pH, and ionic strength of the dispersing media.[15,30,56] Van Hell et al. reported a number of surfactant-like peptides (SLPs) which can form nanovesicles.[21,30,56] One of these self-assembling peptides is the SA2 peptide (Ac-AAVVLLLWEE), which forms discrete nanovesicles with a radius of 60 nm when dispersed in aqueous media at physiological pH. The formed peptide vesicles precipitated out of solution at pH values below the pK_a of the glutamic acid side groups, which could be fully reversed when pH was raised again to 7.4.[30]

SA2 peptide vesicles could be loaded with a photosensitizer with virtually no water solubility. Incubation of this photosensitizer loaded into SA2 peptide vesicles with different cells (HUVECS, COS-7, and C26) in culture resulted in accumulation inside the cells. Upon illumination to excite the delivered photosensitizer, concentration-dependent cytotoxicity was observed which was absent if the cells were not illuminated.[56]

Tomich and co-workers designed and synthesized two types of branched self-assembling peptides to mimic phospholipid structure.[15] The hydrophobic part (FLIVIGSII and FLIVIGS) is a segment of a transmembrane helix of the human dihydropyridine sensitive L-type calcium channel, and the hydrophilic part comprised 5 lysines. In order to branch out the molecule, hydrophobic segments were conjugated to the α and ε-amine of the N-terminal of the oligo-lysine. The amphipathic nature of this peptide resulted in the formation of nanovesicles with 50−200 nm in diameter and a positive surface charge in water. These nanovesicles were loaded by 5(6)-carboxyfluorescein and rhodamine 6G as the model drugs and showed internalization in N/N 1003A rabbit lens epithelial cells in culture media.[15] An overview of the literature describing the use of peptide nanostructures for drug delivery is given in Table 1. Most of these studies report in vitro data, and only a few studies were performed in vivo.

4.2. Self-Assembling Peptides Contain Unnatural Amino Acids. Incorporation of unnatural amino acids in self-assembling peptides (e.g., β- and γ-amino acids, α,β-dehydro amino acids, D-amino acids, and α-aminoisobutyric acid) can increase the proteolytic resistance, thereby extending their in vivo half-lives, which broadens the scope of their biomedical applications.[57−59] For example, Alam et al. synthesized a dipeptide, methionine-dehydrophenylalanin (MΔF), and assessed its capacity for drug delivery compared to methionin-phenylalanin dipeptide (MF), which contained L-phenylalanine rather than ΔF. The designed dipeptide (MΔF) self-assembled into spherical nanoparticles with a mean diameter of 40 nm while MF assembled into smaller nanostructures (4 nm in diameter). Delivery of curcumin encapsulated inside these nanoparticles increased the antitumor activity of curcumin on L-929 cells grown in culture and decreased the mortality rate of tumor-bearing Balb/c mice. Biocompatibility, single step dipeptide synthesis, and low risk for immunogenicity were mentioned to be the main advantageous for these dipeptide systems.[60]

In another study done by Parween et al. the capacity of nanotubes formed by two designed dipeptides (βF−F and βF−ΔF) for drug delivery was evaluated and compared with the standard F−F dipeptide.[61] Findings indicated that βF−ΔF was stable after 24 h incubation at room temperature with a highly nonspecific proteolytic enzyme (proteinase K) while βF−F was relatively stable (20% degradation) compared to F−F (70% degradation). Higher cytotoxicity of mitoxantrone entrapped in peptide nanotubes in comparison with the free drug was observed, whereas loaded βF−ΔF showed the highest cytotoxicity on B6F10 cell line.[61]

Besides resistance to proteolysis, the use of unnatural amino acids offers an efficient means to design novel supramolecular structures. For example, the integration of a D-proline in MAX peptides formed a β-hairpin which has been utilized for drug delivery (Table 1) and tissue engineering (section 6.1.2). In another example, alternating D- and L-amino acids in cyclic peptides is able to form a nanotube that showed potential use in drug delivery.[62]

4.3. Lipidated Self-Assembling Peptides. Peptide self-assembly can be greatly influenced by chemical modifications of the amino acid side groups or the N- and C-terminus. For example, the addition of alkyl tails to N- or C-terminus can drive the self-assembly of such lipopeptides.

Stupp and co-workers have developed a series of lipid−peptide molecules comprising a hydrocarbon chain (e.g., palmitoyl) covalently attached to an amphiphilic peptide (e.g.,

DOI: 10.1021/acs.bioconjchem.5b00487
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$V_nA_nE_n$) which is able to form β-sheet-rich supramolecular structures. These peptide amphiphiles (PAs) when dispersed in water form hydrogels at concentrations as low as 1% (w/v) in the presence of calcium ions that triggered gelation through charge screening. Interestingly, these PAs kept their hydrogel-forming capacity even after covalent conjugation of dexamethasone or prodan to the peptide moieties via acid-cleavable hydrazone bond.[71,72] This striking feature makes these PAs a versatile system for sustained release of a wide range of medicines (Table 2). In most cases, PAs were utilized for drug delivery in hydrogel forms. In a recent study, PA fibers conjugated to a collagen binding peptide showed promising results for targeted delivery to the injured artery after the vascular intervention via systemic administration. Importantly, findings indicated that applying the specific targeting ligands in combination with fibrous morphology were crucial to get the optimal binding at the site of interest in the vasculature.[73]

5. APPLICATION OF SELF-ASSEMBLING PEPTIDES FOR VACCINATION

The way antigens are presented to the immune system determines to a great extent the quality and longevity of the evoked immune response. As such, proper presentation of antigens is of utmost importance in the development of effective vaccines. Although the trend in vaccine formulation is toward "clean" and well-defined systems making use of well-characterized synthetic antigens, such as peptide epitopes, these synthetic systems suffer from poor immunogenicity since the antigen presentation far resembles the antigen as part of a living pathogen and the uptake by professional antigen-presenting cells (APC) is suboptimal. Many studies showed that administration of soluble peptides with adjuvants may not necessarily result in induction of efficient immune responses.[78−82] This is partly because of poor antigen uptake by APCs, e.g., dendritic cells (DCs) and inefficient activation of DCs. Self-assembling peptide systems have been investigated for vaccination purposes with the aim of reconstructing virus-like structures making use of β-sheet or α-helical coiled-coil forming peptides for assembly into well-defined nanostructures.[78,83] In general, self-assembling peptide particles have several advantages over other antigen carrier systems such as ease of production, biodegradability, high density of surface-exposed antigen epitopes, control on particle size, direct conjugation of ligands or imaging probes to the peptides,[80] and, most importantly, increase of antigen uptake by APCs.[79−82] Moreover, in contrast to virus-like particles (VLPs) and genetic vaccines based on viruses, self-assembling peptide particles have shown hardly any cytotoxicity.[84] Due to the simplicity and using databases to avoid immunogenic sequences, the self-assembly domain of the peptide vector itself would not be immunogenic so that any evoked immune reactions will be directed against the incorporated antigenic epitopes. This is in contrast to the use of VLPs where the viral proteins themselves are sometimes very immunogenic, causing problems with pre-existing antibodies against the vaccine carrier or leading unwanted immune responses.[85−87] In the following section, we highlight some examples of self-assembling peptides for vaccination, limiting the discussion to all-peptide systems or to bioconjugates in which the peptide moiety is critical for self-assembly.

Boato et al. constructed a synthetic virus-like particle (SVLP), which comprised a lipo-peptide having a coiled-coil sequence. Peptide monomers formed a trimeric coiled-coil helical structure, which in turn formed micelles with a mean diameter of about 20−30 nm (Figure 6).[83]

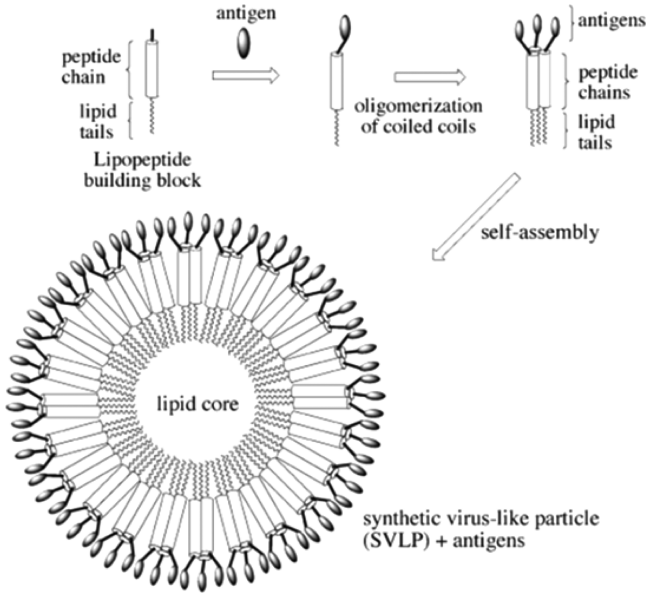

Figure 6. Synthetic virus-like particle (SVLP) formation through self-assembly of a trimeric coiled-coil helical structure and hydrophobic interaction of lipophilic tails. Reprinted with permission from Boato, F., et al. (2007) *Angew. Chem. 119* (47) 9173−9176. Copyright 2007 John Wiley & Sons Inc.

The immunogenicity of the synthetic VLPs fused to a sequence derived from the V3 region of gp120 from HIV-1 was tested in vivo. The synthetic VLP vaccines showed high antigen-specific antibody titers, in vaccinated New Zealand white rabbits without the need for additional adjuvants. The authors suggest that in these systems, the lipid tail can be either a phospholipid or a toll-like receptor (TLR) ligand (e.g., Pam2Cys or Pam3Cys), which can be coupled to the N-terminus of the self-assembling peptide.[83] In another study, the same authors showed that these synthetic VLP vaccines were efficiently taken up by DCs mainly through caveolin-independent lipid raft macropinocytosis and subsequent antigen processing and MHC-restricted presentation by DCs.[88]

Rudra et al. evaluated the ability of a self-assembling peptide (Q11: Ac-QQKFQFQFEQQ-am) for vaccination. This peptide is able to form β-sheet fibrillar structures of approximately 5−10 nm wide and 100−1000 nm in length in salt-containing aqueous solutions.[78] They showed that these peptide fibers are nonimmunogenic and did not elicit a detectable immune response even coadministered with complete Freund's adjuvant (CFA). However, nanofibers fused to $OVA_{323-339}$ peptide (containing T-helper and B-cell epitope) at the N-terminus of Q11 peptide elicited a high level of IgG1, IgG2a, and IgG3 antibodies against OVA epitope (Figure 7). The antibody levels were comparable to those induced by the peptide formulated in CFA adjuvant.[80,86] The effectiveness of this adjuvant-free nanofiber vaccine in the induction of humoral immune response against *Plasmodium falciparum* circumsporozoite was shown in animals immunized with $(NANP)_3$ malaria peptide coupled to Q11. The vaccinated mice showed strong antibody responses up to 40 weeks without the necessity for regular boosts. The authors also showed that two different epitope-

DOI: 10.1021/acs.bioconjchem.5b00487
Bioconjugate Chem. 2016, 27, 3−18

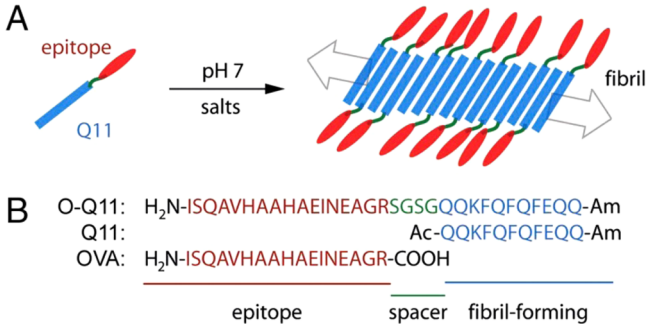

Figure 7. (A) Alignment of self-assembled Q11-epitope to form fibers. (B) Sequences of self-assembled Q11-OVA (O-Q11), Q11, and ovalbumin epitope ($OVA_{323-339}$). Adapted from Rudra, J. S., et al. (2010) *Proc. Natl. Acad. Sci. U. S. A. 107* (2) 622−627.

bearing Q11 peptides could coassemble without decreasing of the immune response to either epitope.[86]

In another study by Rudra et al. it was shown that the immunogenicity of the self-assembled peptide vaccines is T-cell dependent and strongly associated with the self-assembling structure. The fibril formation and thereby the immunogenicity of the vaccine was fully demolished by mutating key amino acid residues in the self-assembling domain.[80] The authors also tested another self-assembling peptide domain (KFE8: FKFEFKFE-Am) and proved that the adjuvant effect was not limited to a specific self-assembling peptide sequence.[80] In a followup study, Rudra et al. investigated the mechanism of immunological activity and adjuvanticity of the self-assembled vaccines in vivo.[86] They hypothesized that the fibril structures resemble bacterial flagellin and thus might activate innate immune system through interaction with TLRs or activate inflammasomes due to their particulate structures. They demonstrated that the antigen-specific immune response was T-cell and MyD88 dependent, but they could not find any specific interactions with TLR2 and TLR5 as well as NALP3, as an inflammasome activation marker.[80] Recently Chen et al. evaluated the cytotoxicity and reactogenicity of the above nanofiber vaccines.[81] They proved, contrary to the alum-adsorbed $OVA_{323-339}$ vaccine, that $OVA_{323-339}$-Q11 nanofibers did not induce any swelling and local inflammation at the site of injection and did not recruit inflammatory cells after intraperitoneal (i.p.) administration. In vitro studies showed that OVA-Q11 was safe and did not cause any cell death in a range of concentrations, whereas the alum formulation elicited a dose-dependent cell death. They also demonstrated that fluorescently labeled OVA-Q11 nanofibers were taken up by APCs 10-fold higher than labeled OVA protein and the isolated DCs from mice with positive fluorescent signal had significantly increased expression DCs activation markers (CD80 and CD86). Further, it was shown that OVA-Q11 nanofibers elicited the formation of B-cell germinal center and high-titer, high-affinity neutralizing IgG responses in an in vitro flu model.[81] Rudra and co-workers recently demonstrated that a self-adjuvanting nanofiber vaccine based on Q11 peptide was able to mount a robust specific $OVA_{257-264}$ CD8+ T cell response in OT-I transgenic mice. Taken together, the results indicate that these self-adjuvanting vaccines not only can elicit CD4+ T cell responses but also increase CD8+ T cells which play a pivotal role in immunotherapy of viral infections and cancers.[89]

6. SELF-ASSEMBLING PEPTIDES FOR TISSUE REGENERATION APPLICATIONS

Advances in the field of stem cell research and the technical possibilities to revert differentiated cells back to pluripotent stem cells make it in principle possible to regrow damaged tissue using the patient's own cells. However, this requires a good understanding of the organizational and signaling events that govern the growth and differentiation of (pluripotent) stem cells in a complex 3D microenvironment, with subsequent translation of these principles into man-made engineered scaffolds.

3D cell cultures require sophisticated scaffolds onto which the cells can adhere and, in addition, can provide the necessary signals for growth and differentiation. Scaffolds can be formed from natural components, such as decellularized extracellular matrix components. However, such biological scaffolds have the risk of potential disease transmission, limited range of mechanical properties, complex structures which make them difficult for manipulation and to obtain reproducible manufacturing results. As a consequence, scaffolds composed of synthetic polymeric networks or peptide scaffolds have gained increasing attention as a well-characterized scaffold with tailor-made properties such as mechanical properties, stimuli-responsiveness, and degradation rate.

Compared to polymeric scaffolds, peptide scaffolds have the added benefit that biological functionalities, such as cell adherence or growth stimulating factors, can be easily incorporated as an intrinsic component of the peptide scaffold. They show excellent biocompatibility and can be metabolized through a series of enzyme reactions in vivo. In order to construct a 3D scaffold, self-assembling peptides should form indefinite structures upon the self-assembly. Self-assembling peptides most frequently used for tissue engineering are β-structured peptides that form hydrogelating fibrous scaffolds, thereby mimicking the extracellular matrix (ECM).

6.1. Self-Assembling Peptides for Tissue Engineering.

Peptide self-assembly has been extensively used to form porous 3D scaffolds to which cells can adhere. The advantage of using peptides as building blocks is that these can be readily extended to include biologically active sequences to facilitate cell attachment or growth. Both natural peptide sequences as well as do novo designed peptide sequences have been used to construct such 3D scaffolds. A representative selection of literature on the application of self-assembling peptides for regenerative medicine is given below.

6.1.1. EAK-16 Family Peptides. A well-known example of self-assembling peptides copying natural protein sequences is the EAK-16 peptide.[90] Zhang et al. derived the sequence from the yeast protein zuotin, a putative nucleic acid binding protein, with a repetitive sequence AEAEAKAKAEAEAKAK which forms fibrous hydrogels upon addition of salts and is stable at high temperatures. A striking feature is that once the hydrogel has been formed the peptides show remarkable resistance to proteolytic degradation, despite the presence of protease recognition sequences.[90] The EAK16 peptide and several variants derived from it, among which is the RADA16 peptide, were used for cell attachment and were shown to be a suitable scaffold for a variety of cell types.[91] Competition experiments using soluble RGD indicated that cell attachment was not integrin-specific. These hydrogels successfully promote the culture of chondrocytes, bone, nerves, and other cell types in vitro and in vivo models.[92] To improve the weak mechanical

DOI: 10.1021/acs.bioconjchem.5b00487
Bioconjugate Chem. 2016, 27, 3−18

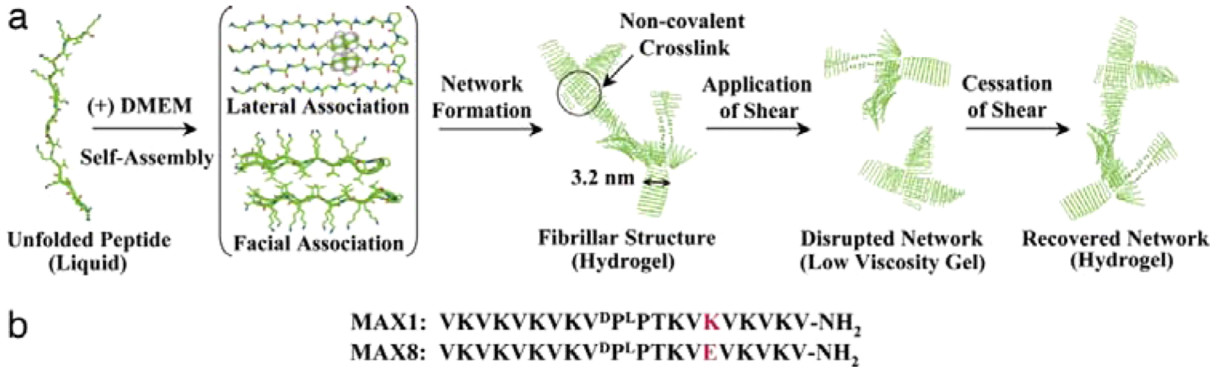

Figure 8. (a) Schematic illustration of triggered self-assembly of β-hairpin peptides by adding the culture medium (DMEM). Shear-thinning and self-healing property of the hydrogel make the system suitable for biomedical applications such as cell delivery. (b) Amino acid sequences of Max1 and Max8. Reprinted with permission from Haines-Butterick, L., et al. (2007) *Proc. Natl. Acad. Sci. U. S. A. 104* (19) 7791−7796. Copyright 2007 National Academy of Sciences.

Table 3. In Vivo Studies on Self-Assembled Peptide Hydrogels for Tissue Engineering Applications

peptide sequence	binding moieties	second structure	animal (spices)	organ	ref.
RADA16-I	N/A	β-sheets	Syrian hamster	nervous system (optic tract)	113
RADA16-I	N/A	β-sheets	rat	N ervous system(spinal cord)	114
RADA16-I	N/A	β-sheets	rat	brain (cortical tissue)	118
RADA16-I	N/A	β-sheets	Syrian hamster	brain, spinal cord, femoral artery, liver, or skin (hemostasis)	119
RADA16-I	N/A	β-sheets	rat	Kidney (hemostasis)	120
RAD16-II: H_2N-RARADADARARADADA-COOH	biotin (to bind to IGF-1 - streptavidin complex)	β-sheets	mouse	heart	121
RADA16-I, RAD16-II	PDGF-BB (platelet-derived growth factor) loaded into the hydrogel	β-sheets	rat	heart	110
RADA16-I	N/A	β-sheets	rat	Skin(wound healing)	122
RADA16-II mixed with R-G7-JAG1: [RADA16-II]-[Glycine]$_7$- [CDDYYYGFGCNKFCRPR-COOH]	Notch ligand Jagged-1: H2N-CDDYYYGFGCNKFCRPR-COOH	N/A	rat	heart	123
RADA16-II	PDGF-BB and FGF-2 (fibroblast growth factor) loaded into hydrogel	N/A	rat	heart	124
RAD16-I	N/A	β-sheets	pig	heart	125
KLD 12	TGF-β1, dexamethasone, and IGF-1 as chondrogenic factors (CF) mixed with the hydrogel	β-sheets	rabbit	joints (cartilage)	93
KLD12 mixed with KLD12-SP: AcKLDLKLDLKLDLGRPKPQQFFGLM-CONH$_2$	Substance P (SP): RPKPQQFFGLM	β-sheets	mouse	bone	116
C16−AAAAGGGLRKKLGKA-COOH	heparin-binding domain (LRKKLGKA)	β-sheets	at	bone	126
C16−V2A2K3GKLTWQELYQLKYKGI-CONH$_2$	VEGF-mimetic epitope(KLTWQELYQLKYKGI)	β-sheets	mouse	ischemic tissues (angiogenesis)	127
HSNGLPLGGGSEEEAAAVVV(K)-CO(CH$_2$)$_{10}$CH$_3$	TGF-binding domain(NH2-HSNGLPL-COOH)	β-sheets	rabbit	joints (cartilage)	128
C16-VVAAEEEEGIKVAV-COOH, C16-VVAAEERGDS-CCOH	a neurite-promoting laminin epitope(IKVAV), Integrin binding sequence(RGDS)	β-sheets	rat	spinal cord	115
16C-4×Cys-3×Gly-1×phosphorylated serine	phosphoserine (an inducer of mineralization)	β-sheets	rat	bone	129
SLanc: AcKSLSLSLRGSLSLSLKGKLTWQELYQLKYKGICONH$_2$	an angiogenic sequence: KLTWQELYQLKYKGI	β-sheets	rat	ischemic tissues (angiogenesis)	130
P11−4	N/A	β-sheets	human	dental caries	101,117

stability of EAK16 and RADA16 to apply as a scaffold for cartilage repair, self-assembling peptide KLD12 was designed by the same group.[93,94]

6.1.2. β-Hairpins. The MAX peptide series consists of de novo designed peptides that can form thermally reversible hydrogels (Figure 8).[16,95] Pochan and colleagues have shown that these peptides have a random coil conformation at room temperature and high pH but form β-hairpin structures upon heating, which in turn leads to self-assembly into a hydrogel network. Upon cooling, complete dissolution of the hydrogel occurs. By changing the hydrophobicity of the peptides, the temperature at which hydrogel formation occurs can be tuned between 25 and 60 °C.[95]

Since the MAX1 gelation rate was too slow, MAX8 was designed by the same group to improve the gelation kinetics. This amendment led to better spreading of cells within the MAX8 hydrogel network. They demonstrated that MAX8 and MAX1 were able to recover back to the measured elastic

DOI: 10.1021/acs.bioconjchem.5b00487
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modulus when shear was applied. Cell viability results in the majority of viable cells in the MAX8 gel−cell construct which was injected into a well plate by a syringe. These features of MAX peptides make them suitable for cell-delivery applications.[96]

6.1.3. Coiled Coils. Woolfson et al. have developed and studied α-helical coiled-coil peptides that self-assemble into hydrogelating self-assembling fibers (hSAFs). Hydrogel properties can be altered by modifying the peptide sequences.[6,97,98] For example, hSAFQQQ peptide formed a hydrogel at low temperature but melted by heating, whereas the most hydrophobic peptide hSAFAAA showed an opposite behavior. Such coiled-coil-forming peptide hydrogels have been used to support growth and differentiation of rat adrenal pheochromocytoma cells (PC12) for 2 weeks.[99]

6.1.4. P11 and Q11. The P_{11} series of peptides are an example of such de novo β-sheet forming peptides. They were designed to keep both the chemical complexity of the primary structure and the conformational complexity to a minimum.[100] They consist of 11-mer peptides with alternating polar and aromatic amino acids, typically incorporating several glutamine residues to drive the formation of β-sheets which, dependent on the peptide concentration, can form higher-order structures such as tapes, fibrils, and fibers. An interesting feature of these peptides is that they can undergo pH and ionic strength-triggered self-assembly. These properties enable the injection of the peptides in a liquid form, prior to peptide self-assembly. For tissue engineering, gels of P_{11} peptides can be conveniently prepared by adding the lyophilized peptide directly to the cells in culture, leading to cell incorporation into the peptide matrix. These peptides are currently being tested as a scaffold for enamel regeneration in patients with carious lesions.[101]

A peptide derived from the P_{11} series of peptides, which allows chemical end-modification or fibril elongation, is the Q11 peptide. This peptide has an N-terminal cysteine residue and a C-terminal thioester to join individual peptide fibrils or to functionalize peptide fibrils with cell adhesion ligands via native chemical ligation (NCL). Hydrogels in which the individual peptide fibrils were ligated with NCL showed increased stiffness compared to the nonligated hydrogel, which resulted in the better growth of HUVECs.[102]

6.1.5. Aromatic Peptide Derivatives. Short peptides with strategically positioned aromatic groups can self-assemble in aqueous solutions through $\pi-\pi$ stacking in addition to hydrogen bonding interactions. Gazit and co-workers reported one of the shortest self-assembling peptides. They demonstrated that diphenylalanine (FF) peptides and lately Fmoc-FF formed linear nanotubes, amyloid fibers, and cylindrical architectures in aqueous solution. They exploited these nanofibers for casting and fabrication of metallic nanowires and nanocables.[43]

Ulijn and colleagues showed the usefulness of such diphenylalanine peptides for forming hydrogel scaffolds; peptides Boc-FF-COOH, Nap-FF-COOH, and Fmoc-FF-COOH all generated hydrogels with a nanofibrous network with diameter 10−100 nm under physiological conditions.[103,104] In addition, the group has shown that Fmoc diphenylalanine peptides increased survival of the chondrocytes and human dermal fibroblasts in 2D and 3D cell culture media.[105,106]

6.1.6. Peptide Amphiphile. Stupp and co-workers designed a series of peptide amphiphiles consisting of an alkyl tail and a peptide headgroup with increasing hydrophilicity toward the C-terminus such as C16-VVAAEE-NH2.[107] Hydrophobic interaction between alkyl chains and hydrogen bonding through β-sheet formation between the peptide head groups assisted in peptide self-assembly forming elongated fibers with a diameter of approximately 5−7 nm and a length of several micrometers that assembled into hydrogels above a critical concentration. The hydrogels formed showed promising results for tissue engineering such as neuronal repair (see Table 3).

6.3. Functionalization for Proper Cell Differentiation. To improve the functionality of the designed scaffolds including cell adhesion, growth, and differentiation, several active motifs have been studied and applied mainly in 2 forms, either conjugated or mixed with the self-assembled peptides.[92,93,108−110] Gelain et al. conjugated several bioactive motifs, including cell adhesion, differentiation, and bone marrow homing motifs, to the RADA16-I peptide and evaluated their effects on the biofunctionality of the scaffold. They showed that these peptide scaffolds containing bone marrow homing motifs not only significantly increased the survival of neural stem cell, but also promoted cell differentiation in the absence of any added growth and neurotrophic factors to the cell culture media.[111] Wang et al. functionalized the RADA16-I with "Link N" peptide (AcN-(RADA)4-GGRLNSDNYTLDHDRAIH-$COHN_2$) and evaluated bone marrow stem cells in the obtained scaffold by mixing RADA16-I and RADA16-Link N (1:1). Though this modification could not stimulate cell proliferation, however, it increased cell adhesion and promoted the production and deposition of type II collagen and aggrecan.[112] Stupp et al. also designed several amphiphile peptides conjugated to the bioactive motifs such as VEGF-mimetic epitope and TGF-binding domain which elevated regenerative functionality of in vivo tested scaffolds (Table 3).

6.4. In Vivo Applications. Although most designed peptide scaffolds are still in the research and in vitro phase, a number of self-assembling peptide hydrogels have been tested in animal models and some systems are even undergoing clinical translation (Table 3).

Among all the peptide based scaffolds, RADA16 has been extensively studied in vivo due to its well characterized physicochemical properties, which are promising for in vivo applications. For instance, applied RADA16-I hydrogel on the deep transection of the optic tract in the Syrian hamster could regenerate the axons and rejoin the injured brain tissue together, eventually with functional return of vision.[113] In another study, the injured spinal cord was reconnected through migration of host cells and growth of blood vessels and axons into the RADA16-I scaffold, consequently with the recovery of locomotor functions in the subjected rats.[114]

Several in vivo studies also have been conducted on peptide amphiphile hydrogels. Designed constructs including active ligands such as IKVAV (a neurite-promoting laminin epitope) and RGDS (integrin binding sequence) were shown to promote the regrowth of seeded nerve cells in animal models.[115] Substance P (SP) (an 11-mer neuropeptide) has been indicated for its efficacy in wound healing. Kim et al. exploited this motif in conjugation with KLD12 peptide for bone tissue regeneration without cell transplantation. A coated PLA (poly(L-lactide)) scaffold with β-tricalcium phosphate (β-TCP) which was filled with a mixture of KLD12/KLD12-SP showed a chemoattraction for mesenchymal stem cells (MSCs) followed by bone tissue formation and regeneration.[116]

DOI: 10.1021/acs.bioconjchem.5b00487
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Hydrogels of the P11-4 peptide are now available on the market as Curodont and used to repair early dental caries. The results showed that mineral deposition in the dental lesions was significantly augmented due to increased remineralization and inhibition of demineralization.[101,117]

Although many peptide scaffolds showed safety in vitro and in vivo, there are some concerns about their short- and long-term toxicity and adverse effects. For instance, Westermark et al. showed that there is a possibility that synthetic fibrils may act as nucleating seeds for pathologic amyloid formation and deposition.[131] They showed some amyloid deposits in some of the subjected mice with designed peptides including RADA16-I and silver nitrate (that can induce AA-amyloidosis), while no amyloid deposits were seen in the control group that only received silver nitrate. However, further studies are needed to confirm these findings.[131]

7. CONCLUSIONS

A variety of studies on peptide self-assembly in the different fields of science including medicine and engineering indicate the capacity of such peptides to generate well-defined nano-architectures with unique functionalities. Since many different functionalities can be incorporated into the peptide sequence, including self-assembly domains, cell attachment, or signaling domains, versatile, multifunctional structures can be generated from a single molecular entity. This, combined with ease of production (recombinant or chemical synthesis), makes self-assembling peptides attractive for various biomedical applications. In this paper, we reviewed the structural features of peptide self-assembly and reported recent advances in their applications in drug delivery, vaccination, and tissue regeneration. Although very useful examples have appeared in the literature on how self-assembling peptides can be used for drug delivery, tissue engineering, or vaccination, there are still some hurdles that need to be overcome. Many publications on self-assembling peptides are descriptive, with the researchers describing how their self-assembling peptides behave under specific conditions, without being able to predict their self-assembling properties in advance or make changes to obtain a desired supramolecular structure. Moreover, since self-assembly is influenced by many factors, some of which seem trivial and are therefore not always well described, it is very difficult to reproduce results from the laboratory to laboratory.[132] Nevertheless, with an increased understanding of the intricate interplay of forces that drive the self-assembly, we can now start to predict the self-assembly behavior of simple peptides. This is further aided by the development of advanced molecular dynamics simulations that enable researchers to better predict the self-assembly behavior of self-assembling peptide candidates in advance.[14,133,134]

Moreover, an aspect, hardly addressed in the literature so far, is the potential toxicity[131] and immunogenicity[78] of these peptide-based self-assembling systems. As reviewed in this paper, most self-assembling peptides consist of novel sequences or are derived from naturally existing amino acid sequences with a repetitive nature. Such repetitive, self-assembling polypeptides are generally suitable substrates for innate immune activation and subsequent activation of adaptive immune responses. Not many studies have looked into the immune activating potential of biomaterials based on self-assembling peptides, and this is a point of concern for future development. Furthermore, exposed hydrophobic patches often present in SA peptides may cause toxicity to cells by directly interacting with cell membranes.[135] These issues should be considered and tested when developing self-assembling peptides for biomedical use.

In the coming decade many of these hurdles will without doubt be tackled, and more sophisticated self-assembly designs will be created, which will pave the way for widespread applications of peptide-based drug delivery systems for a variety of biomedical applications.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

M.R.M was sponsored by a fellowship from the Iranian Ministry of Health.

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DOI: 10.1021/acs.bioconjchem.5b00487
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DOI: 10.1021/acs.bioconjchem.5b00487
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