Minireview

Rational design of fiber forming supramolecular structures

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Abstract

Recent strides in the development of multifunctional synthetic biomimetic materials through the self-assembly of multi-domain peptides and proteins over the past decade have been realized. Such engineered systems have wide-ranging application in bioengineering and medicine. This review focuses on fundamental fiber forming α-helical coiled-coil peptides, peptide amphiphiles, and amyloid-based self-assembling peptides; followed by higher order collagen- and elastin-mimetic peptides with an emphasis on chemical / biological characterization and biomimicry.

Keywords: Tissue engineering, peptide chemistry, supramolecular chemistry, peptide amphiphiles, elastin-mimetic peptides

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Introduction

Numerous advances in biomimicry have transformed our understanding of the fundamental requirements for recapitulating the extracellular environment. Mimicry of supramolecular structures that provide an instructive scaffolding for cells, tissues, and organs is a mainstay for the fields of biomaterials, materials chemistry, and tissue engineering.[1–5] Through advances in nanotechnology and peptide chemistry it is now possible to recreate the fundamental building blocks of mammalian life—such as collagen and elastin in their native hierarchical structures.[6–8]

Peptide-based materials stand at the forefront of several tissue engineering strategies.[9–18] Owing to the modular nature peptide-based materials, a variety of different moieties can be introduced.[19] These moieties can guide self-assembly, bioactivity or both—as discussed in this review. Peptide-based materials originally focused on pharmaceutics. With a better understanding of secondary protein structure and how to tailor supramolecular self-assembly, short polypeptides have been used for a variety of applications from drug/ growth factor mimicry,[20] drug delivery,[21] inflammation modulation,[22] and orthogonal self-assembly with loaded liposomes.[23] Additionally, as detailed in this review, stable large scale hydrogels formed by non-covalent interactions of these peptide-based materials allow recapitulation of a nanofibrous extracellular matrix (ECM) mimetic scaffold.[19,24] Variants of these scaffolds have allowed for excellent cellular spreading *in vitro* and rapid infiltration without the formation of fibrous capsules *in vivo*.[20–23] Together, these biological and mechanical cues inform the design and application of a host of peptide-based materials, whose organization and core structure are analyzed in this review.

Two specific approaches can be used to fabricate novel biomaterials: top-down and bottom-up approaches.[24] The former utilizes understanding global structure with subsequent deconstruction and mimicry of macromolecular constituents. The top-down approach oftentimes fails at achieving specific structure and assembly. The consensus in the field is that construction of larger subunits of proteins and organized tissue requires a building-block bottom-up approach where assembly is dictated at the molecular level. De novo engineering with inspiration from nature allows programmed folding and self-assembly. Ultimately, secondary and tertiary protein structure dictate molecular arrangement of bioinspired materials.[17] Thermodynamic, entropic, and stereochemical factors guide non-covalent self-assembly of supramolecular structures that dictate mechanical and biological functionality.[3,25–27] Ranging from alternating hydrophobic and hydrophilic amino acids that create facial ampiphiles[28–30] to Xxx-Yyy-Gly sequences that predispose α-helix formation, nature has evolved a set of rules that govern arrangement at the molecular, nano, micro, meso, and macroscales.[31–33] The rational design of fiber forming biologically inspired materials, requires an understanding of these interactions for directed molecular self-assembly.[34]

α-Helical coiled-coils

α-Helical building blocks are fundamental supramolecular structures which comprise the majority of biological tissue.[4] By rationally designing sequence-to-structure relationships between α-helices, rules for engineered protein folding and

assembly can be achieved. At the amino acid level, Woolfson's group reports the predisposition of specific amino acids alanine, glutamine, glutamic acid, and lysine for a-helices over threonine and valine for β-structure.[17,35] Furthermore, given the small size and ability to pack efficiently, glycine and valine are commonly found at α-helical turns.[4] The canonical α-helical coiled-coil architecture involves a repeating *abcdefg* heptad that assembles to form an amphipathic α-helix secondary structure, and further into a left-handed dimer.[35] Noncanonical α-helical coiled-coils do not contain this characteristic heptad repeat, and as a result, do not necessarily form dimeric supercoils.[35] Similar to the majority of self-assembling peptide structures, non-covalent hydrophobic interactions between the side chains of different α-helices drive folding. Consequently, hydrophobic amino acids are usually spaced three to four amino acids apart to satisfy the hypothesized 3.6 amino acid α-helix geometry.[17,36] Most commonly, coiled-coil sequences take the form $(HPPHPPP)_{n\geq4}$, where hydrophobic (H) amino acids are at the *a* and *d* positions and polar (P) amino acids at all other positions.[37]

Pioneering this structure of self-assembling peptides, in 1998 Petka's group produced an early synthetic coiled-coiled α-helix that reversibly self-assembled into a hydrogel.[38] This peptide used a triblock architecture with two flanking leucine zipper motifs separated by a flexible alanylglycine-rich repeat. Each terminal leucine zipper comprised the characteristic α-helical *abcdefg* heptad repeat, with Leu frequently at positions *a* and *d.* Hydrophobic interactions between each of the amphipathic helices promoted the assembly of coiled-coil dimers between peptide strands, promoting aggregation, and subsequent gelation. Similarly, elevating pH or temperature disrupted coiled-coil aggregation, and thereby melted the hydrogel to a viscous liquid state.[38] Building upon this work, various α-helical coiled-coil systems have since been developed.[37,39–43] In 2000 Woolfson's group designed the first ''sticky-ended'' heterodimer that promoted the formation of long fibers.[37] Since then they have demonstrated the design of the MagicWand peptide, a single peptide that assembles to nanoscale fibers. This architecture directed the staggered assembly of α-helices to promote coiled-coil fibrillogenesis by incorporating an anionic core region with cationic flanking regions.[44] Another system they have developed promotes longitudinal fiber assembly of α-helical coiled-coils by substituting the *b* and *c* positions of the heptad repeat with oppositely charged residues to form complementary offset-register dimers with ''sticky-ended'' overhangs.[45] Ionic interactions between helices promoted strong fibril aggregation resulting in precipitation, limiting the utility of this system.

More recently, work on engineered α-helical architectures has shifted towards fiber-forming biocompatible hydrogels for application in mimicking the extracellular milieu.[42,46] For example, these hydrogel scaffolds are promising for the controlled delivery of drugs as well as supports for cell growth and tissue engineering.[17,47] Designer heptad repeats with altered *b, c,* and *f* positions substituted with either Ala or Glu residues were used to form weaker and more general hydrophobic interactions and hydrogen bonds between fibrils.[46] Instead of thick fiber aggregation and precipitation, a hydrogel with thinner, more flexible fiber bundles assembled. The incorporation of hydrophobic interactions was useful in controlling gelation, as an increase in temperature resulted in a stronger gel when the altered *b*, *c*, and *f* positions were Ala substituted. Cytocompatibility of hydrogels was tested by seeding rodent adrenal phechromocytoma cells and measuring neutrite outgrowth.[48] Hartgerink's group has demonstrated the design of blunt-ended coiled-coiled architectures that self-assemble into long nanofibers and form a hydrogel.[42] They have shown that the thickness of fibers can be controlled by varying the amino acids found at the *b*, *c*, and *f* positions of the heptad. Moreover, charged Lys residues at the periphery increased repulsion between coils resulting in thinner fibrils, while non-covalent interactions between hydrophilic amino acids promoted thicker fiber bundles.[42]

β-Hairpin peptides

β-Secondary structures constitute another underlying motif for self-assembly that incorporates both distinct material properties, biofunctionality and are commonly known for their preponderance in pathological amyloidosis.[49] These peptides usually integrate alternating hydrophobic (H) and polar (P) residues $(HPHPHP)_n$ and assemble to form facial amphiphiles with opposing hydrophobic and hydrophilic *faces.* As a result of non-covalent intermolecular van der Waals forces and hydrophobic packing, these secondary structures further assemble into higher order structures, including fibers, micelles, bilayers, and extended β-sheets. One important subset of β-based peptides is the β-hairpin motif. These sequences include two antiparallel β-strands that are joined by a tetrapeptide type-II β-turn.[48–50] The β-turn is one of the most common peptide secondary structures and is largely used for directional change in a polypeptide sequence.[51] In the β-hairpin architecture, this loop, along with the flanking β-strands, are critical components for the conformation and stability of the overall structure.[52] Alternating hydrophobic and hydrophilic amino acids populate the remaining peptide flanks, forming amphiphiles, which can then associate with other amphiphiles both facially and laterally to promote a nanoscale fibrillar structure and hydrogel network. In these strands, amino acids are organized to maximize interstrand hydrogen bonding and side-chain interactions.[51] In 1993 Blanco *et al.* reported Nuclear Overhouser Effect (NOE) NMR showing that the sequence YQ**NPDG**SQA had a significant population of isolated β-hairpins in aqueous solution attributed to the high turn probability of the central residues.[52] Schneider *et al.* in 2002 described the self-assembly of a 20-residue peptide composed of a (-V^DPPT-) β-turn with alternating Val and Lys residue flanking regions, MAX1.[53] MAX1 exhibited pH triggered self-assembly into a hydrogel under basic conditions. Val residues were used to promote hydrophobic collapse into a β-sheet architecture while Lys residues were incorporated to control gelation—at sufficiently low pH, electrostatic repulsion between positively charged Lys residues resulted in β-hairpin dissolution and

subsequent hydrogel dissolution.[53] Pochan *et al.* have since designed variations of MAX1 and have demonstrated precise control over hydrogelation of these peptides by changing conditions such as temperature,[54] pH,[55] ionic strength,[56] and light.[57] They have shown a rise in temperature triggered gelation by inducing a stronger degree of hydrophobic collapse between Val residues,[54] while an increase in ionic strength screened electrostatic repulsion between Lys residues.[56] As a result of built-in physical cross-linking, these gels also have a remarkable ability to shear-thin.[58] Demonstrating the tunability of the system for physiologic condition, more recently, Haines-Butterick *et al.* have designed MAX8, substituting a Lys residue in MAX1 for Glu, which decreases the net positive charge, allowing MAX8 to gel at physiological pH much more quickly.[59]

β-Sheet peptides

The principles underlying β-hairpin self-assembly are fundamentally similar to the structural organization motifs of self-assembling peptide amphiphiles. These designs generally involve the facial self-assembly of amphiphilic peptides to form long, high-aspect-ratio nanofibers.[60] Most commonly, these synthetic peptides comprise a region of β-sheet forming amino acids coupled with a solubilizing region that allows fiber and hydrogel formation in aqueous solution.[61,62] These assemblies provide a facile method for bioactive sequence incorporation to promote the adhesion / infiltration of cells.[62–64] There exist several examples of β-sheet-based peptide amphiphilies including Collier and Messersmith's Q11 peptide with alternating polar and aromatic residues,[65] and Goeden-Wood *et al.*'s $(AEAEAKAKAEAEAKAK)_9$ peptide which formed strong nanoscale fibrous hydrogels.[66] Jun *et al.* has demonstrated fiber-forming and hydrogelating systems with built-in bioactive functionality through the development of ''ionically complementary'' peptides, known as Lego peptides.[67] The formation of complementary ionic pairs between amino acids from different peptide chains can lead to self-assembling, electrostatically stable, higher-order aggregates, such as nanofibers or globular structures.[24] Zhang *et al.*'s first peptide of this type, EAK16-II was composed of complementary $(AEAEAKAK)_2$ sequences and associated into a β-sheet membrane structure through hydrophobic interactions and the formation of ionic bonds between oppositely charged Glu and Lys residues.[68,69] This stable self-complementary 12-amino-acid peptide gelled upon the addition of salt into a membranous structure, and demonstrated stability in heat, reducing conditions, and pH variations.[69] Derivations of this peptide—EAK16-I (nanofibers) and EAK16-IV (globular structures)—comprising identical amino acid compositions but with different sequence organizations were also demonstrated.[67] Of particular note—RADA16—has arguably gained much repute and is currently marketed under the name Puramatrix® by BD Biosciences.[70] This peptide substitutes Glu and Lys residues for Arg and Asp and undergoes molecular self-assembly into nanofibers to form a hydrogel scaffold.[71] The reader is directed to the following articles for more information on self-assembly, cytocompatibility and *in vivo* studies of Zhang and co-worker's work.[3,8,70,72,73] Aggeli *et al.* have reported the development of self-assembling amphiphilic β-sheet tapes, capable of forming hydrogels composed of nanometer-long fibers.[74–76] Fundamentally, these peptides aggregate to form antiparallel β-sheet tapes through cross-strand weak attractive forces.[74] To prevent the precipitation of aggregated β-sheet structures, lateral, one-dimensional assembly is ensured by incorporating molecular recognition mechanisms into side chain interactions: for example, aromatic residues can be used to provide intermolecular recognition by π–π stacking interactions. Moreover, strong solvent-to-surface interactions are ensured to control solubility in solvents of different polarity. The earliest of the group's designed peptides include the 24-residue peptide K24 and 11-residue peptide DN1, which self assemble in non-aqueous and aqueous solvents, respectively.[74] K24 was designed with an amphipathic primary sequence, with polar residues at the termini and nonpolar and aromatic residues in the core region, which was hypothesized to control one-dimensional β-sheet propagation. Similarly, the much shorter DN1 assembled through intermolecular π–π, hydrophobic, and ionic interactions. In both cases, association between aromatic amino acid residues significantly contributes to the peptides' self-assembly properties. More recent work by their group has demonstrated the self-assembly of stable β-sheet tapes without the use aromatic π–π interactions.[75] 7mer and 9mer peptides P_7-6 and P_9-6 contained predominantly aliphatic Leu residues on their hydrophobic faces, and still underwent a transition from monomeric random coils to β-sheet tapes at higher peptide concentration and neutral pH. At lower pH, protonated side chains decreased intermolecular attraction, and thus increased the critical concentration necessary for self-assembly. This study also demonstrated the increased propensity for self-assembly in polar organic solvents such as methanol, and the lower critical concentration needed for the self-assembly of longer peptides.

Recent advances in β-sheet forming systems have incorporated ancillary domains for greater control over fiber formation and hydrogelation. An important example of this has been demonstrated by Hartgerink's group with multidomain ABA triblock peptides that self-assemble to form highly ordered nanofibers in a hydrogel scaffold.[61,62,64,77–79] The self-complementary alternating hydrophobic–hydrophilic peptide cores pack to exclude hydrophobic residues from the aqueous environment, and additional peptides can then assemble laterally by intermolecular backbone hydrogen bonding, forming high aspect ratio nanofibers. Because such assembly can continue indefinitely until all monomeric peptides are depleted, flanking positively charged A blocks at neutral pH are incorporated to produce electrostatic repulsion between dimers. These flanks comprise 0-4 frustrated, positively charged lysine residues that counter the B block's strong affinity to associate, thus preventing complete aggregation and subsequent precipitation. Hydrogelation is optimized by the addition of multivalent ions that screen charge repulsion allowing a remarkable level of control over fiber formation and hydrogelation.[77] Demonstrably the sequence $K_n(QL)_mK_n$ showed that

varying the n/m ratio favored either self-assembly or disassembly; and only when the forces promoting self-assembly and opposing charge repulsion were balanced did the formation of controlled length nanofibers become possible.[77] Further, various derivatives of the $K_n(QL)_mK_n$ sequence, producing similar fiber-forming self-assembling hydrogels with added functionality have been developed. For example, a sequence in which Gln residues were substituted with Ser residues, $K_2(SL)_6K_2$, demonstrated a significantly greater storage modulus and greater degree of shear recovery than its Gln counterpart.[78] Such a system with highly tailorable viscoelastic and shear thinning properties has great implications with respect to injectable hydrogels for drug delivery and regenerative medicine.[20–23,61,64,78] In addition to conventional alternating hydrophilic–hydrophobic core regions, Hartgerink's group has demonstrated the self-assembly of triblock multi-domain peptide with an alternating hydrophilic-aromatic core region.[61] Recently they described a novel aromatic self-assembling peptide amphiphile. In contrast to the above-mentioned mechanism for self-assembly, the introduction of aromatic amino acids changes the packing characteristics between peptide chains and allows for the possibility of interstrand π–π stacking interactions. Interchanging Leu residues for aromatic Phe, Trp, and Tyr residues while keeping hydrophilic residues unchanged caused a significant change in fiber morphology when observed with electron microscopy. However, despite the large steric strain afforded by bulky aromatic residues, in all cases, these multi-domain peptides self-assembled and retained a basic nanofibrous structure.[61]

Amyloid-based self-assembly

Another class of β-based peptide biomaterials can be engineered from naturally occurring self-assembling amyloid fibers. In natural systems, amyloid fibers that arise as inappropriately folded polypeptides ultimately manifest as insoluble protein aggregates that have been linked to a variety of human diseases, including amyloidosis and a variety of neurodegenerative disorders.[80,81] Energetically, this occurs as conformational shifts from monomeric unfolded intermediates into β-sheet-rich structures is preferred. Most commonly, cross-β amyloid structures comprise β-sheet peptides organized into parallel or antiparallel β-strands. Two or more β-strands facially assemble to produce fibrils, which are stabilized by hydrogen bonds along the fibril axis and side chain interactions between fibrils.[82] These interactions are so prevalent that amyloid formation is not completely sequence specific: amyloid formation can be induced by seeding fibrils with the same or unrelated proteins.[83] It has been suggested that with sufficient time, all well-folded proteins undergo an irreversible structural transition to a ''correctly-folded'' aggregated β-sheet structure, which represents a global minimum in Gibbs energy for protein folding.[84] In engineered systems, however, these supramolecular assemblies, which range in states from liquid crystals to rigid nanofibers, can lead to synthetic biomaterials that introduce biological function and tailored mechanical properties.[85] Small oligomers of peptides serve as nucleation points for further aggregation and ultimately fibril formation, dictated by non-covalent bonds, notably hydrogen bonds,[86] hydrophobic interactions,[84] and π–π stacking.[87] One of the early examples of this architecture was designed by Hecht *et al.,* who demonstrated that simple alternating patterns of hydrophobic and polar amino acids resulted in aggregation between amphiphilic β-strands, forming β-amyloid fibers.[88] More sophisticated amyloid-based fiber-forming and hydrogelating biomaterials have since been designed. Notably, the Nilsson group has utilized π–π and hydrophobic side-chain interactions to demonstrate the formation of self-assembled materials inspired by amyloid materials.[89–91] To set the stage for his future work, Nilsson demonstrated that aromatic residues and π–π stacking interactions were not strictly necessary for amyloid formation with the amphipathic sequence $(FKFE)_2$.[89,91] Instead, residues with sufficient hydrophobicity or β-sheet propensity could drive amyloid fibril formation. When non-aromatic, highly hydrophobic cyclohexylalanine was substituted for phenylalanine residues, self-assembly still occurred and even exhibited enhanced hydrogelation properties. Using these principles, Nilsson has developed modified designs with wide-ranging applications as biomaterials. For example, a designed peptide sequence with the $(FKFE)_2$ motif and flanking cysteine residues takes on a cyclic structure when intermolecular disulfide bonding is present, but can undergo self-assembly into a linear β-sheet conformation and hydrogel with the addition of a reductive trigger.[92] Similarly, the Nilsson lab has also demonstrated the design of the coassembly of enantiomeric L-/D-peptides into rippled β-sheet fibrils with enhanced viscoelastic hydrogel properties.[93] Other recent examples of bioinspired self-assembling amyloid peptides includes work done by the Guler group. They have demonstrated the formation of a hydrogelating nanofiber scaffold with amyloid characteristics from oppositely charged peptides -E-FFAA-E- and -K-FFAA-K- at neutral pH.[94] In addition to the tailorability of this peptide architecture with respect to fiber formation and hydrogelation, engineered amyloid fibrils can also be chemically modified by incorporating functional peptide sequences.[85] The reader is directed to Bowerman and Nilsson for more details.[95]

Peptide amphiphiles

In addition to facially amphiphilic β-based self-assembling peptides, a class of peptide amphiphiles with appended alkyl chains have demonstrated various applications for tissue regeneration and bioengineering.[28,60,96] Pioneered by the Tirrell group, in 1995, a diblock system incorporating a long-chain dialkyl-ester lipid tail and collagen-model peptide head-group was used to form a biologically active cytocompatible evenly dispersed bilayer.[97] Since then, the Stupp lab has continued with the development of these straight-chain amphiphilic sequences. These peptide amphiphiles are composed of 4–5 regions: region 1 is composed of a long alkyl chain to promote hydrophobic collapse of individual molecules; region 2 largely drives the lateral association of peptide amphiphile molecules through either a

β-sheet forming segment stabilized by intramolecular backbone hydrogen bonding or covalent disulfide bonding; region 3 comprises a flexible glycine spacer or charged amino acids for solubility; and region 4 and 5 are functionalized to contain the specific peptide epitope for biological function or signaling. Hartgerink *et al.,* in 2001, produced a novel self-assembling peptide-amphiphile that formed cylindrical micelles and a nanoscale fibrous scaffold with properties similar to those of the ECM.[28] This peptide contained the characteristic peptide amphiphile motifs, with regions 4 and 5 containing a phosphorylated serine residue and arginyl-glycyl-aspartyl (RGD) epitope to promote hydroxyapatite mineralization and cell adhesion, respectively. Ultimately, this peptide amphiphile demonstrated the pH-triggered assembly of a chemically robust nanofiber scaffold that could direct ordered hydroxyapatite mineralization and support cell growth. Further studies on this peptide have shown conditions for triggering self-assembly by pH, ionic strength, and concentration, as well as varying amino acids and the alkyl chain for nanofibers of varying morphology and bioactivity.[98]

Other examples of designed peptide amphiphiles that self-assemble to form nanoscale fibers include the pH-induced assembly of hydrogel C(12)-GAGAGAGY based on silk fibroin,[99,100] aromatic Fmoc utilizing hydrogels,[101] and even binary mixtures of peptide amphiphiles containing oppositely charged residues.[102] Advances in this facile design allow tailoring the material properties of peptide amphiphile forming hydrogels.[103] A change in the position and increase in the number of Val residues were reported to form stronger, stiffer hydrogels, while an increase in Ala residues decreased the mechanical stiffness. Fourier transform infrared spectroscopy suggests that this effect is presumably due to the alteration in the alignment of hydrogen bonds along the long axis of the peptide amphiphile fibers: Val residues were hypothesized to form tightly packed, stiff β-sheet micelles/fibers, while the Ala-residue-populated β-sheet cores could not form hydrogen bonds as effectively, leading to more disordered peptide amphiphile micelles with twisted geometries.[103] Not only does this work demonstrate the significance of strong, ordered intermolecular hydrogen bonding in the mechanical properties of the resulting hydrogel, but also the potential to create softer hydrogels as potential injectable materials. More recently, work on nanofibrous peptide amphiphiles has focused on incorporating a variety of bioactive epitopes for biomaterials with tailored functionality. Peptide amphiphiles containing the neuron lineage driving IKVAV epitope have been developed,[104] as well as, an injectable vascular endothelial growth factor-mimetic peptide amphiphile with pro-angiogenic properties capable of restoring blood flow in a hind-limb ischemia model.[105] Moreover, peptide amphiphiles containing the heparin binding domain,[106,107] growth factor release domains,[108,109] hydroxyapatite nucleation domains,[110,111] RGD cell adhesion motif,[112–114] and cell-apoptosis-promoting regions for cancer treatment[115] have been reported. The reader is directed to reviews by Webber *et al.,* Cui *et al.,* and Matson and Stupp for details.[5,60,116]

Collagen-mimetic peptides

Building upon fundamental α- and β-secondary structures, a variety of other secondary structures have been explored as self-assembling biomaterials. The prime example of this has been recent work in the study of developing synthetic collagen mimetic peptides (CMPs).[117,118] These short peptide strands mimic natural collagen's Xxx-Yyy-Gly repeating triplet. Here, short-chain polypeptides, consisting of approximately 24–36 amino acids, self-assemble into triple helices and can further mimic all stages of natural collagen's multi-hierarchical self-assembly.[6,119] Most commonly, proline and 4-hydroxyproline occupy the Xxx and Yyy positions of natural collagen, while recent studies have begun to investigate self-assembling CMPs with a variety of amino acid mutations.[34] This repeating triplet plays a role in stabilizing triple helix formation. Glycine, the only necessary amino acid in the collagen-repeating motif, provides a compact methylene side chain that is oriented towards the interior of the triple helix and allows for the tight packing of individual polypeptide strands.[120] This close proximity facilitates inter-strand hydrogen bonding, in which the amine of glycine acts as a hydrogen donor to the carbonyl of proline on an adjacent polypeptide strand.[1,121] This further stabilizes triple helix assembly. In addition, the side-chain rings of proline and hydroxyproline have low degrees of freedom, contributing to the rigidity of the collagen backbone.[122,123] Inter-strand salt bridges between charged residues can also serve to stabilize peptide strands and triple helix formation.[124] Individual tropocollagen molecules then pack both linearly and laterally to form collagen nanofibrils.[125] Assembly can further continue to produce triple helices that pack into nanofibers, and finally compose a hydrogel scaffold.[96]

Self-assembly of the first generation CMPs largely stop after triple helix formation, limiting their widespread use. Initial studies have shown the substantial stabilizing role of electrostatic interactions, laying the foundation for higher-order structures similar to natural collagen. Early work by Brodsky's group studied homotrimeric CMP systems. Venugopal *et al.* prepared a collagen-like sequence $(POG)_4(EKG)(POG)_5$ in 1994.[126] This homotrimeric peptide self-assembled into a triple helix, and showed an increase in thermal stability when all ionizable side chain residues were charged, suggesting the stabilizing role of electrostatic interactions between ion pairs. To corroborate these results, a $(POG)_{10}$ peptide with uncharged amino acids displayed the least stability at neutral pH, due to charge repulsion between ionized C- and N-termini.[126] Similarly, at higher pH, in which N-termini were uncharged, the peptide gained about 2°C of stability in melting experiments.[127] Further work by Chan *et al.* has demonstrated the dependence of triple-helix stability on the identity, position, and environment of charged residues.[128] They embedded a collagen G-Y-X triplet in a host $Ac(GPO)_3$-GXY-$(GPO)_4$-GG-NH_2 sequence and substituted the Xxx and Yyy positions with ionizable residues. When the Yyy position was substituted with Glu, Asp, Arg, and Lys residues and the Xxx position with Pro, a significant range of triple helix thermal stability was observed. In contrast, when the

same substitutions were done in the opposite manner, only a narrow range of thermal stability was noticed. This was attributed to the Xxx position exhibiting a more favorable side chain orientation, in which they point outward in optimal directions for minimizing charge repulsion. Conversely, ionizable residues in the Yyy position appear to be slightly destabilizing due to potential charge repulsion between residues.[128] Kotch and Raines, in 2006, further demonstrated the self-assembly of a cysteine-containing synthetic collagen triple helix.[129] Cysteine disulfide linkages between peptide chains served as a means for linking the individual strands into a natural collagen mimetic triple helix. These covalent linkages between strands offset the strands and controlled their register. These ''sticky ends'', where the peptide is offset by a number of amino acids, allow additional peptide strands to add end-to-end, elongating the triple helix and driving fiber formation as previously demonstrated by Woolfson and others.[37,130] These charged, unpaired flanking sequences drive self-assembly by satisfying more and more charge pairs with each additional triple helix added. Examination by atomic force and electron microscopy revealed the self-assembly of one-dimensional nanofibrils of 20–120 nm in length, which may have potential as synthetic biocompatible materials.[129] Advanced collagen mimetic systems have revealed the self-assembly of CMPs into triple helices without the necessity of covalent linkages. In 2007 Chaikof's group developed the zwitterionic fiber-forming 36 amino acid sequence $(PRG)_4(POG)_4(EOG)_4$. This peptide self-assembled into a triple helix and subsequently nanofiber, without further assemble into a hydrogel.[33] More recent work on hierarchical constructs that are capable of mimicking both natural collagen's higher order fibrillation as well as the biological effects of specific natural tissue scaffolds, and potentially a variety of other novel functions, can be architecturally designed by incorporating specific short-peptide features into a single multi-domain peptide.[6] For example, Cejas *et al.* demonstrated this by adding C-terminal phenylalanine and N-terminal pentafluorophenyl flanks to a $(Pro\text{-}Hyp\text{-}Gly)_{10}$ CMP. Their system produced micrometer-length fibers with natural collagen-like properties, noting that without incorporation of aromatic flanking regions, fibrillation could not have been achieved.[131] Gottlieb *et al.* produced conductive synthetic nanowires tens of microns in length by conjugating the collagen mimetic sequence pentafluoro-F-$(GPO)_4$-GPK-$(GPO)_5$-F with gold nanoparticles.[127]

In 2007 the Hartgerink group demonstrated the development of a group of electrostatically stabilized ABC collagen mimetic heterotrimers.[132] These heterotrimeric systems were unique allowing tailored substitution in one, two, or all three peptide chains. This study demonstrated strategies for designing heterotrimers with separate net positive, neutral, and negative strands that assemble into triple helices with net neutral charge. Further, this study suggested that the formation of an Asp-Lys charge pair within a triple helix provides equivalent stability to helices containing collagen's characteristic Pro-Hyp-Gly triplet, which provides a foundation for designing more sophisticated collagen mimetic systems. They then demonstrated the first ABC heterotrimeric collagen mimetic system that self-assembled utilizing only supramolecular interactions,[133] a single-register ABC heterotrimer stabilized by electrostatic interactions,[134,135] and design strategies for designing collagen mimetic homotrimers,[2] AAB heterotrimers,[2] single-composition ABC heterotrimers.[136] This work has allowed them to determine design principles for register- and composition-controlled collagen mimetic homotrimers and heterotrimers. The work on CMPs from triple helix, to nanofiber, to hydrogel led to the design of the peptide $(PKG)_4(POG)_4(DOG)_4$.[6] This tri-block peptide (+,n,−) is unique since it is a homotrimeric system comprised of peptides with a net neutral charge that incorporates collagen's proline-hydroxyproline-glycine repeating unit in the central domain. Moreover, assembly is driven by the presence of stabilizing inter-strand side chain interactions between lysine and aspartate residues. These salt-bridge hydrogen bonds between oppositely charged ionized residues serve to stabilize a sticky ended triple helix formation, which nucleates fiber formation by allowing additional peptide strands to add end-to-end elongating the triple helix. As a result, self-assembly of KOD nanofibers is observed, which displays the characteristic triple helical packing of natural collagen fibrils. Current work with this synthetic collagen is focused on cytocompatibility and utility as a hemostat.[6,137]

Elastin-mimetic peptides

Similar to CMPs that exhibit tissue-like mechanical properties and architectures, elastin-mimetic peptides have been developed to mimic native tissue resilience for example in blood vessels, the lungs, and skin.[138,139] Natural elastin is found as a cross-linked protein comprising alternating hydrophilic polar domains rich in Ala and charged Lys necessary for chemical cross-linking, while the hydrophobic nonpolar domain, rich in Pro, Gly, and Val, is largely responsible for the material's elasticity and flexibility.[140,141] The hydrophobic nature elastin serves as a significant driving force in its self-assembly and aggregation.[142] Here, the well-characterized elastin repeats in the hexapeptide Val-Gly-Val-Ala-Pro-Gly and pentapeptide Val-Pro-Gly-Val-Gly are chemically cross-linked by the hydrophilic domain.[143] The cross-linked peptides then supramolecularly organize into a nanoscale fibrillar structures that orient in the direction of the tissue applied load.[142] Work on elastin-like synthetic sequences has been geared towards producing recombinant polypeptides that mimic both natural elastin's supramolecular assembly and its unique material properties.[142] While mature cross-linked elastin is insoluble, synthetic peptides mimicking elastin's characteristic repeat have been designed to gain a greater understanding of the structure–function relationship of the protein. Such work is consistent with the formation of a β-turn structure around the Pro-Gly doublet, and an overall β-spiral secondary structure.[144] In 2002 Conticello developed a recombinant elastin-mimetic polypeptide $[(VPGVG)_4(VPGKG)]_{39}$ that exhibited mechanical properties similar to that of natural elastin, as well as its characteristic filamentous morphology.[145–147] When crosslinking reagent *N*-hydroxysuccimide was added, a reversible,

temperature dependent hydrogel formed, presumably by an intermolecular condensation reaction between Lys residues and *N*-hydroxysuccimide substrates.[138] Work between Sallach *et al.* and Wu *et al.* described the design of a number of BAB recombinant co-block systems containing elastin-mimetic sequences.[26,148] In these systems, hydrophobic, elastin-mimetic polypeptide flanking sequences are separated by a central hydrophilic block. The elastin-mimetic domains are characterized by a repeating Val-Pro-Xxx-Yyy-Gly pentapeptide, while the central elastomeric domain is hydrophilic due to the presence of charged residues.[148] This co-block peptide can further aggregate, mimicking natural elastin's supramolecular structure while retaining its unique material functionality. Specifically, hydrophobic packing of the flanking domains in aqueous solvent drives self-assembly, while the central domain retains conformational flexibility and elasticity due to the presence of polar amino acid residues.[148] In contrast to chemical crosslinking between fibers as described by Wright and Conticello's 2002 study, this design utilized physical crosslinking to self-assemble, and demonstrated a number of advantages, including reversible self-assembly and the ability to control mechanical properties.[26,138] Further work reported similar elastin-mimetic polymers that combined both physical and chemical cross-linking into a single design.[26] In doing so, they were able to exert precise control over a variety of material properties unique to physical and chemical cross-linked systems. Le *et al.* demonstrated the design of a series of beaded fiber-forming elastin-mimetic double-hydrophobic block peptides establishing examples of next generation systems.[25] The reader is directed to the following reviews for more details.[29,149–151] These synthetic elastin matrices, oftentimes combined with synthetic collagen matrices, have been used to recapitulate features of the ECM in applications for tissue repair,[152–156] drug delivery,[29,157,158] and materials with tunable material properties.[150,154,159–161]

Conclusion

Bottom-up engineering of peptide-based supramolecular structures has allowed biomimicry at multiple length scales, including nanofibrous morphologies that mimic native ECM. Learning from natural self-assembly cues, bioinspired scientists can now generate a series of materials that can closely recapitulate higher order protein structure by tailoring primary and secondary composition to replace dependence on synthetic or animal derived matrices that may cause adverse host reactions.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: VAK has stock options in NangioTx, Inc which aims to translate some of the technologies presented in this manuscript towards clinical trials. No other authors report that they have no competing interests with the current work.

REFERENCES

1. Shoulders MD, Raines RT. Collagen structure and stability. *Annu Rev Biochem* 2009;**78**:929–58
2. Fallas JA, O'Leary LE, Hartgerink JD. Synthetic collagen mimics: self-assembly of homotrimers, heterotrimers and higher order structures. *Chem Soc Rev* 2010;**39**:3510–27
3. Zhao X, Pan F, Xu H, Yaseen M, Shan H, Hauser CA, Zhang S, Lu JR. Molecular self-assembly and applications of designer peptide amphiphiles. *Chem Soc Rev* 2010;**39**:3480–98
4. Woolfson DN. Core-directed protein design. *Curr Opin Struct Biol* 2001;**11**:464–71
5. Webber MJ, Kessler JA, Stupp SI. Emerging peptide nanomedicine to regenerate tissues and organs. *J Intern Med* 2010;**267**:71–88
6. O'Leary LE, Fallas JA, Bakota EL, Kang MK, Hartgerink JD. Multi-hierarchical self-assembly of a collagen mimetic peptide from triple helix to nanofibre and hydrogel. *Nat Chem* 2011;**3**:821–8
7. O'Leary LE, Fallas JA, Hartgerink JD. Positive and negative design leads to compositional control in AAB collagen heterotrimers. *J Am Chem Soc* 2011;**133**:5432–43
8. Gelain F, Bottai D, Vescovi A, Zhang S. Designer self-assembling peptide nanofiber scaffolds for adult mouse neural stem cell 3-dimensional cultures. *PLoS One* 2006;**1**:e119
9. Ramakers BE, van Hest JC, Lowik DW. Molecular tools for the construction of peptide-based materials. *Chem Soc Rev* 2014;**43**:2743–56
10. Cameron N, Deming T. Peptide-based materials for nanomedicine. *Macromol Biosci* 2015;**15**:7–8
11. Khadka DB, Haynie DT. Protein- and peptide-based electrospun nanofibers in medical biomaterials. *Nanomedicine* 2012;**8**:1242–62
12. Cavalli S, Albericio F, Kros A. Amphiphilic peptides and their cross-disciplinary role as building blocks for nanoscience. *Chem Soc Rev* 2010;**39**:241–63
13. Ikeda M, Tanida T, Yoshii T, Hamachi I. Rational molecular design of stimulus-responsive supramolecular hydrogels based on dipeptides. *Adv Mater* 2011;**23**:2819–22
14. de la Rica R, Matsui H. Applications of peptide and protein-based materials in bionanotechnology. *Chem Soc Rev* 2010;**39**:3499–509
15. Lowik DW, Leunissen EH, van den Heuvel M, Hansen MB, van Hest JC. Stimulus responsive peptide based materials. *Chem Soc Rev* 2010;**39**:3394–412
16. Ulijn RV, Woolfson DN. Peptide and protein based materials in 2010: from design and structure to function and application. *Chem Soc Rev* 2010;**39**:3349–50
17. Woolfson DN. Building fibrous biomaterials from alpha-helical and collagen-like coiled-coil peptides. *Biopolymers* 2010;**94**:118–27
18. Yu Z, Cai Z, Chen Q, Liu M, Ye L, Ren J, Liao W, Liu S. Engineering beta-sheet peptide assemblies for biomedical applications. *Biomater Sci* 2016;**4**:365–374
19. Webber MJ, Appel EA, Meijer EW, Langer R. Supramolecular biomaterials. *Nat Mater* 2015;**15**:13–26
20. Kumar VA, Taylor NL, Shi S, Wang BK, Jalan AA, Kang MK, Wickremasinghe NC, Hartgerink JD. Highly angiogenic peptide nanofibers. *ACS Nano* 2015;**9**:860–8
21. Kumar VA, Shi S, Wang BK, Li IC, Jalan AA, Sarkar B, Wickremasinghe NC, Hartgerink JD. Drug-triggered and cross-linked self-assembling nanofibrous hydrogels. *J Am Chem Soc* 2015;**137**:4823–30
22. Kumar VA, Taylor NL, Shi S, Wickremasinghe NC, D'Souza RN, Hartgerink JD. Self-assembling multidomain peptides tailor biological responses through biphasic release. *Biomaterials* 2015;**52**:71–8

23. Wickremasinghe NC, Kumar VA, Hartgerink JD. Two-step self-assembly of liposome-multidomain peptide nanofiber hydrogel for time-controlled release. *Biomacromolecules* 2014;**15**:3587–95
24. Zhang S. Fabrication of novel biomaterials through molecular self-assembly. *Nat Biotechnol* 2003;**21**:1171–8
25. Le DH, Hanamura R, Pham DH, Kato M, Tirrell DA, Okubo T, Sugawara-Narutaki A. Self-assembly of elastin-mimetic double hydrophobic polypeptides. *Biomacromolecules* 2013;**14**:1028–34
26. Sallach RE, Cui W, Wen J, Martinez A, Conticello VP, Chaikof EL. Elastin-mimetic protein polymers capable of physical and chemical crosslinking. *Biomaterials* 2009;**30**:409–22
27. McDaniel JR, Radford DC, Chilkoti A. A unified model for de novo design of elastin-like polypeptides with tunable inverse transition temperatures. *Biomacromolecules* 2013;**14**:2866–72
28. Hartgerink JD, Beniash E, Stupp SI. Self-assembly and mineralization of peptide-amphiphile nanofibers. *Science* 2001;**294**:1684–8
29. Kim W, Chaikof EL. Recombinant elastin-mimetic biomaterials: emerging applications in medicine. *Adv Drug Deliv Rev* 2010;**62**:1468–78
30. Zhang QH, Ma XQ, Ward A, Hong WX, Jaakola VP, Stevens RC, Finn MG, Chang G. Designing facial amphiphiles for the stabilization of integral membrane proteins. *Angew Chem Int Ed* 2007;**46**:7023–5
31. Fallas JA, Lee MA, Jalan AA, Hartgerink JD. Rational design of single-composition ABC collagen heterotrimers. *J Am Chem Soc* 2012;**134**:1430–3
32. Fallas JA, Dong JH, Tao YZJ, Hartgerink JD. Structural insights into charge pair interactions in triple helical collagen-like proteins. *J Biol Chem* 2012;**287**:8039–47
33. Rele S, Song YH, Apkarian RP, Qu Z, Conticello VP, Chaikof EL. D-periodic collagen-mimetic microfibers. *J Am Chem Soc* 2007;**129**:14780–7
34. Jalan AA, Demeler B, Hartgerink JD. Hydroxyproline-free single composition ABC collagen heterotrimer. *J Am Chem Soc* 2013;**135**:6014–7
35. Woolfson DN. The design of coiled-coil structures and assemblies. *Fibrous Proteins Coiled-Coils Collagen Elastomers* 2005;**70**:79
36. Pauling L, Corey RB, Branson HR. The structure of proteins; two hydrogen-bonded helical configurations of the polypeptide chain. *Proc Natl Acad Sci U S A* 1951;**37**:205–11
37. Pandya MJ, Spooner GM, Sunde M, Thorpe JR, Rodger A, Woolfson DN. Sticky-end assembly of a designed peptide fiber provides insight into protein fibrillogenesis. *Biochemistry* 2000;**39**:8728–34
38. Petka WA, Harden JL, McGrath KP, Wirtz D, Tirrell DA. Reversible hydrogels from self-assembling artificial proteins. *Science* 1998;**281**:389–92
39. Kojima S, Kuriki Y, Yoshida T, Yazaki K, Miura K. Fibril formation by an amphipathic alpha-helix-forming polypeptide produced by gene engineering. *Proc Jpn Acad Series B Phys Biol Sci* 1997;**73**:7–11
40. Potekhin SA, Melnik TN, Popov V, Lanina NF, Vazina AA, Rigler P, Verdini AS, Corradin G, Kajava AV. De novo design of fibrils made of short alpha-helical coiled coil peptides. *Chem Biol* 2001;**8**:1025–32
41. Ogihara NL, Ghirlanda G, Bryson JW, Gingery M, DeGrado WF, Eisenberg D. Design of three-dimensional domain-swapped dimers and fibrous oligomers. *Proc Natl Acad Sci U S A* 2001;**98**:1404–9
42. Dong H, Paramonov SE, Hartgerink JD. Self-assembly of alpha-helical coiled coil nanofibers. *J Am Chem Soc* 2008;**130**:13691–5
43. Zimenkov Y, Conticello VP, Guo L, Thiyagarajan P. Rational design of a nanoscale helical scaffold derived from self-assembly of a dimeric coiled coil motif. *Tetrahedron* 2004;**60**:7237–46
44. Gribbon C, Channon KJ, Zhang W, Banwell EF, Bromley EH, Chaudhuri JB, Oreffo RO, Woolfson DN. MagicWand: a single, designed peptide that assembles to stable, ordered alpha-helical fibers. *Biochemistry* 2008;**47**:10365–71
45. Papapostolou D, Smith AM, Atkins EDT, Oliver SJ, Ryadnov MG, Serpell LC, Woolfson DN. Engineering nanoscale order into a designed protein fiber. *Proc Natl Acad Sci U S A* 2007;**104**:10853–8
46. Banwell EF, Abelardo ES, Adams DJ, Birchall MA, Corrigan A, Donald AM, Kirkland M, Serpell LC, Butler MF, Woolfson DN. Rational design and application of responsive alpha-helical peptide hydrogels. *Nat Mater* 2009;**8**:596–600
47. MacPhee CE, Woolfson DN. Engineered and designed peptide-based fibrous biomaterials. *Curr Opin Solid State Mater Sci* 2004;**8**:141–9
48. Banwell EF, Abelardo ES, Adams DJ, Birchall MA, Corrigan A, Donald AM, Kirkland M, Serpell LC, Butler MF, Woolfson DN. Rational design and application of responsive alpha-helical peptide hydrogels. *Nat Mater* 2009;**8**:596–600
49. Rughani RV, Schneider JP. Molecular design of beta-hairpin peptides for material construction. *MRS Bull* 2008;**33**:530–5
50. Papapostolou D, Smith AM, Atkins ED, Oliver SJ, Ryadnov MG, Serpell LC, Woolfson DN. Engineering nanoscale order into a designed protein fiber. *Proc Natl Acad Sci U S A* 2007;**104**:10853–8
51. Stotz CE, Topp EM. Applications of model beta-hairpin peptides. *J Pharm Sci* 2004;**93**:2881–94
52. Blanco FJ, Jimenez MA, Herranz J, Rico M, Santoro J, Nieto JL. NMR evidence of a short linear peptide that folds into a beta-hairpin in aqueous-solution. *J Am Chem Soc* 1993;**115**:5887–8
53. Schneider JP, Pochan DJ, Ozbas B, Rajagopal K, Pakstis L, Kretsinger J. Responsive hydrogels from the intramolecular folding and self-assembly of a designed peptide. *J Am Chem Soc* 2002;**124**:15030–7
54. Pochan DJ, Schneider JP, Kretsinger J, Ozbas B, Rajagopal K, Haines L. Thermally reversible hydrogels via intramolecular folding and consequent self-assembly of a de novo designed peptide. *J Am Chem Soc* 2003;**125**:11802–3
55. Rajagopal K, Lamm MS, Haines-Butterick LA, Pochan DJ, Schneider JP. Tuning the pH responsiveness of beta-hairpin peptide folding, self-assembly, and hydrogel material formation. *Biomacromolecules* 2009;**10**:2619–25
56. Ozbas B, Kretsinger J, Rajagopal K, Schneider JP, Pochan DJ. Salt-triggered peptide folding and consequent self-assembly into hydrogels with tunable modulus. *Macromolecules* 2004;**37**:7331–7
57. Haines LA, Rajagopal K, Ozbas B, Salick DA, Pochan DJ, Schneider JP. Light-activated hydrogel formation via the triggered folding and self-assembly of a designed peptide. *J Am Chem Soc* 2005;**127**:17025–9
58. Yan C, Altunbas A, Yucel T, Nagarkar RP, Schneider JP, Pochan DJ. Injectable solid hydrogel: mechanism of shear-thinning and immediate recovery of injectable beta-hairpin peptide hydrogels. *Soft Matter* 2010;**6**:5143–56
59. Haines-Butterick L, Rajagopal K, Branco M, Salick D, Rughani R, Pilarz M, Lamm MS, Pochan DJ, Schneider JP. Controlling hydrogelation kinetics by peptide design for three-dimensional encapsulation and injectable delivery of cells. *Proc Natl Acad Sci U S A* 2007;**104**:7791–6
60. Cui H, Webber MJ, Stupp SI. Self-assembly of peptide amphiphiles: from molecules to nanostructures to biomaterials. *Biopolymers* 2010;**94**:1–18
61. Bakota EL, Sensoy O, Ozgur B, Sayar M, Hartgerink JD. Self-assembling multidomain peptide fibers with aromatic cores. *Biomacromolecules* 2013;**14**:1370–8
62. Galler KM, Aulisa L, Regan KR, D'Souza RN, Hartgerink JD. Self-assembling multidomain peptide hydrogels: designed susceptibility to enzymatic cleavage allows enhanced cell migration and spreading. *J Am Chem Soc* 2010;**132**:3217–23
63. Galler KM, D'Souza RN, Hartgerink JD, Schmalz G. Scaffolds for dental pulp tissue engineering. *Adv Dent Res* 2011;**23**:333–9
64. Bakota EL, Wang Y, Danesh FR, Hartgerink JD. Injectable multidomain peptide nanofiber hydrogel as a delivery agent for stem cell secretome. *Biomacromolecules* 2011;**12**:1651–7
65. Collier JH, Messersmith PB. Enzymatic modification of self-assembled peptide structures with tissue transglutaminase. *Bioconjug Chem* 2003;**14**:748–55
66. Goeden-Wood NL, Keasling JD, Muller SJ. Self-assembly of a designed protein polymer into β-sheet fibrils and responsive gels. *Macromolecules* 2003;**36**:2932–8
67. Jun S, Hong Y, Imamura H, Ha BY, Bechhoefer J, Chen P. Self-assembly of the ionic peptide EAK16: the effect of charge distributions on self-assembly. *Biophys J* 2004;**87**:1249–59
68. Zhang S, Lockshin C, Cook R, Rich A. Unusually stable beta-sheet formation in an ionic self-complementary oligopeptide. *Biopolymers* 1994;**34**:663–72

69. Zhang S, Holmes T, Lockshin C, Rich A. Spontaneous assembly of a self-complementary oligopeptide to form a stable macroscopic membrane. *Proc Natl Acad Sci U S A* 1993;**90**:3334–8
70. Holmes TC, de Lacalle S, Su X, Liu G, Rich A, Zhang S. Extensive neurite outgrowth and active synapse formation on self-assembling peptide scaffolds. *Proc Natl Acad Sci U S A* 2000;**97**:6728–33
71. Zhang S, Holmes TC, DiPersio CM, Hynes RO, Su X, Rich A. Self-complementary oligopeptide matrices support mammalian cell attachment. *Biomaterials* 1995;**16**:1385–93
72. Yokoi H, Kinoshita T, Zhang S. Dynamic reassembly of peptide RADA16 nanofiber scaffold. *Proc Natl Acad Sci U S A* 2005;**102**:8414–9
73. Schachner M. Neurobiology. Nervous engineering. *Nature* 2000;**405**:747–8
74. Aggeli A, Bell M, Boden NN, Keen JCB, McLeish T, Nyrkova I, Radford SE, Semenov A. Engineering of peptide [small beta]-sheet nanotapes. *J Mater Chem* 1997;**7**:1135–45
75. Davies RP, Aggeli A. Self-assembly of amphiphilic beta-sheet peptide tapes based on aliphatic side chains. *J Pept Sci* 2011;**17**:107–14
76. Aggeli A, Nyrkova IA, Bell M, Harding R, Carrick L, McLeish TC, Semenov AN, Boden N. Hierarchical self-assembly of chiral rod-like molecules as a model for peptide beta-sheet tapes, ribbons, fibrils, and fibers. *Proc Natl Acad Sci U S A* 2001;**98**:11857–62
77. Dong H, Paramonov SE, Aulisa L, Bakota EL, Hartgerink JD. Self-assembly of multidomain peptides: balancing molecular frustration controls conformation and nanostructure. *J Am Chem Soc* 2007;**129**:12468–72
78. Aulisa L, Dong H, Hartgerink JD. Self-assembly of multidomain peptides: sequence variation allows control over cross-linking and viscoelasticity. *Biomacromolecules* 2009;**10**:2694–8
79. Galler KM, Hartgerink JD, Cavender AC, Schmalz G, D'Souza RN. A customized self-assembling peptide hydrogel for dental pulp tissue engineering. *Tissue Eng Part A* 2012;**18**:176–84
80. Ramirez-Alvarado M, Merkel JS, Regan L. A systematic exploration of the influence of the protein stability on amyloid fibril formation *in vitro*. *Proc Natl Acad Sci U S A* 2000;**97**:8979–84
81. Eisenberg D, Jucker M. The amyloid state of proteins in human diseases. *Cell* 2012;**148**:1188–203
82. Rambaran RN, Serpell LC. Amyloid fibrils: abnormal protein assembly. *Prion* 2008;**2**:112–7
83. Hamley IW. Peptide fibrillization. *Angew Chem Int Ed Engl* 2007;**46**:8128–47
84. Gazit E. The ''Correctly Folded'' state of proteins: is it a metastable state? *Angew Chem Int Ed Engl* 2002;**41**:257–59
85. Cherny I, Gazit E. Amyloids: not only pathological agents but also ordered nanomaterials. *Angew Chem Int Ed Engl* 2008;**47**:4062–9
86. Perutz MF, Johnson T, Suzuki M, Finch JT. Glutamine repeats as polar zippers—their possible role in inherited neurodegenerative diseases. *Proc Natl Acad Sci U S A* 1994;**91**:5355–8
87. Gazit E. A possible role for pi-stacking in the self-assembly of amyloid fibrils. *FASEB J* 2002;**16**:77–83
88. Hecht MH, Das A, Go A, Bradley LH, Wei Y. De novo proteins from designed combinatorial libraries. *Protein Sci* 2004;**13**:1711–23
89. Bowerman CJ, Liyanage W, Federation AJ, Nilsson BL. Tuning beta-sheet peptide self-assembly and hydrogelation behavior by modification of sequence hydrophobicity and aromaticity. *Biomacromolecules* 2011;**12**:2735–45
90. Ryan DM, Anderson SB, Senguen FT, Youngman RE, Nilsson BL. Self-assembly and hydrogelation promoted by F-5-phenylalanine. *Soft Matter* 2010;**6**:475–9
91. Bowerman CJ, Ryan DM, Nissan DA, Nilsson BL. The effect of increasing hydrophobicity on the self-assembly of amphipathic beta-sheet peptides. *Mol Biosyst* 2009;**5**:1058–69
92. Bowerman CJ, Nilsson BL. A reductive trigger for peptide self-assembly and hydrogelation. *J Am Chem Soc* 2010;**132**:9526–7
93. Swanekamp RJ, DiMaio JT, Bowerman CJ, Nilsson BL. Coassembly of enantiomeric amphipathic peptides into amyloid-inspired rippled beta-sheet fibrils. *J Am Chem Soc* 2012;**134**:5556–9
94. Cinar G, Ceylan H, Urel M, Erkal TS, Deniz Tekin E, Tekinay AB, Dâna A, Guler MO. Amyloid inspired self-assembled peptide nanofibers. *Biomacromolecules* 2012;**13**:3377–87
95. Bowerman CJ, Nilsson BL. Self-assembly of amphipathic beta-sheet peptides: insights and applications. *Biopolymers* 2012;**98**:169–84
96. Hartgerink JD, Beniash E, Stupp SI. Peptide-amphiphile nanofibers: a versatile scaffold for the preparation of self-assembling materials. *Proc Natl Acad Sci U S A* 2002;**99**:5133–8
97. Yu YC, Pakalns T, Dori Y, McCarthy JB, Tirrell M, Fields GB. Construction of biologically active protein molecular architecture using self-assembling peptide-amphiphiles. *Methods Enzymol* 1997;**289**:571–87
98. Hartgerink JD, Beniash E, Stupp SI. Peptide-amphiphile nanofibers: a versatile scaffold for the preparation of self-assembling materials. *Proc Natl Acad Sci U S A* 2002;**99**:5133–8
99. Guo H, Zhang J, Xu T, Zhang Z, Yao J, Shao Z. The robust hydrogel hierarchically assembled from a pH sensitive peptide amphiphile based on silk fibroin. *Biomacromolecules* 2013;**14**:2733–8
100. Zhang J, Hao R, Huang L, Yao J, Chen X, Shao Z. Self-assembly of a peptide amphiphile based on hydrolysed Bombyx mori silk fibroin. *Chem Commun (Camb)* 2011;**47**:10296–8
101. Fleming S, Debnath S, Frederix PW, Tuttle T, Ulijn RV. Aromatic peptide amphiphiles: significance of the Fmoc moiety. *Chem Commun (Camb)* 2013;**49**:10587–9
102. Hamley IW, Dehsorkhi A, Castelletto V. Coassembly in binary mixtures of peptide amphiphiles containing oppositely charged residues. *Langmuir* 2013;**29**:5050–9
103. Pashuck ET, Cui H, Stupp SI. Tuning supramolecular rigidity of peptide fibers through molecular structure. *J Am Chem Soc* 2010;**132**:6041–6
104. Silva GA, Czeisler C, Niece KL, Beniash E, Harrington DA, Kessler JA, Stupp SI. Selective differentiation of neural progenitor cells by high-epitope density nanofibers. *Science* 2004;**303**:1352–5
105. Webber MJ, Tongers J, Newcomb CJ, Marquardt KT, Bauersachs J, Losordo DW, Stupp SI. Supramolecular nanostructures that mimic VEGF as a strategy for ischemic tissue repair. *Proc Natl Acad Sci U S A* 2011;**108**:13438–43
106. Rajangam K, Behanna HA, Hui MJ, Han XQ, Hulvat JF, Lomasney JW, Stupp SI. Heparin binding nanostructures to promote growth of blood vessels. *Nano Letters* 2006;**6**:2086–90
107. Ghanaati S, Webber MJ, Unger RE, Orth C, Hulvat JF, Kiehna SE, Barbeck M, Rasic A, Stupp SI, Kirkpatrick CJ. Dynamic *in vivo* biocompatibility of angiogenic peptide amphiphile nanofibers. *Biomaterials* 2009;**30**:6202–12
108. Stendahl JC, Wang LJ, Chow LW, Kaufman DB, Stupp SI. Growth factor delivery from self-assembling nanofibers to facilitate islet transplantation. *Transplantation* 2008;**86**:478–81
109. Shah RN, Shah NA, Del Rosario Lim MM, Hsieh C, Nuber G, Stupp SI. Supramolecular design of self-assembling nanofibers for cartilage regeneration. *Proc Natl Acad Sci U S A* 2010;**107**:3293–8
110. Mata A, Geng YB, Henrikson KJ, Aparicio C, Stock SR, Satcher RL, Stupp SI. Bone regeneration mediated by biomimetic mineralization of a nanofiber matrix. *Biomaterials* 2010;**31**:6004–12
111. Huang Z, Newcomb CJ, Bringas P, Stupp SI, Snead ML. Biological synthesis of tooth enamel instructed by an artificial matrix. *Biomaterials* 2010;**31**:9202–11
112. Sargeant TD, Oppenheimer SM, Dunand DC, Stupp SI. Titanium foam-bioactive nanofiber hybrids for bone regeneration. *J Tissue Eng Regen Med* 2008;**2**:455–62
113. Huang Z, Sargeant TD, Hulvat JF, Mata A, Bringas P, Koh CY, Stupp SI, Snead ML. Bioactive nanofibers instruct cells to proliferate and differentiate during enamel regeneration. *J Bone Miner Res* 2008;**23**:1995–2006
114. Rexeisen EL, Fan W, Pangburn TO, Taribagil RR, Bates FS, Lodge TP, Tsapatsis M, Kokkoli E. Self-assembly of fibronectin mimetic peptide-amphiphile nanofibers. *Langmuir* 2010;**26**:1953–9
115. Standley SM, Toft DJ, Cheng H, Soukasene S, Chen J, Raja SM, Band V, Band H, Cryns VL, Stupp SI. Induction of cancer cell death by self-assembling nanostructures incorporating a cytotoxic peptide. *Cancer Res* 2010;**70**:3020–6

116. Matson JB, Stupp SI. Self-assembling peptide scaffolds for regenerative medicine. *Chem Commun (Camb)* 2012;**48**:26–33
117. Li MH, Fan P, Brodsky B, Baum J. Two-dimensional NMR assignments and conformation of (Pro-Hyp-Gly)10 and a designed collagen triple-helical peptide. *Biochemistry* 1993;**32**:7377–87
118. Yu SM, Li Y, Kim D. Collagen mimetic peptides: progress towards functional applications. *Soft Matter* 2011;**7**:7927–38
119. Brodsky B, Ramshaw JA. The collagen triple-helix structure. *Matrix Biol* 1997;**15**:545–54
120. Ramachandran GN. Molecular structure of collagen. *Int Rev Connect Tissue Res* 1963;**1**:127–82
121. Zanaboni G, Rossi A, Onana AM, Tenni R. Stability and networks of hydrogen bonds of the collagen triple helical structure: influence of pH and chaotropic nature of three anions. *Matrix Biol* 2000;**19**:511–20
122. Persikov AV, Ramshaw JA, Kirkpatrick A, Brodsky B. Amino acid propensities for the collagen triple-helix. *Biochemistry* 2000;**39**:14960–7
123. Bansal M, Ramakrishnan C, Ramachandran GN. Stabilization of collagen structure by hydroxyproline residues. *Proc Indian Acad Sci A* 1975;**82**:152–64
124. Uitto J, Murray LW, Blumberg B, Shamban A. UCLA conference. Biochemistry of collagen in diseases. *Ann Intern Med* 1986;**105**:740–56
125. Krishna OD, Kiick KL. Supramolecular assembly of electrostatically stabilized, hydroxyproline-lacking collagen-mimetic peptides. *Biomacromolecules* 2009;**10**:2626–31
126. Venugopal MG, Ramshaw JA, Braswell E, Zhu D, Brodsky B. Electrostatic interactions in collagen-like triple-helical peptides. *Biochemistry* 1994;**33**:7948–56
127. Gottlieb D, Morin SA, Jin S, Raines RT. Self-assembled collagen-like peptide fibers as templates for metallic nanowires. *J Mater Chem* 2008;**18**:3865–70
128. Chan VC, Ramshaw JA, Kirkpatrick A, Beck K, Brodsky B. Positional preferences of ionizable residues in Gly-X-Y triplets of the collagen triple-helix. *J Biol Chem* 1997;**272**:31441–6
129. Kotch FW, Raines RT. Self-assembly of synthetic collagen triple helices. *Proc Natl Acad Sci U S A* 2006;**103**:3028–33
130. Jalan AA, Hartgerink JD. Pairwise interactions in collagen and the design of heterotrimeric helices. *Curr Opin Chem Biol* 2013;**17**:960–7
131. Cejas MA, Kinney WA, Chen C, Vinter JG, Almond HR Jr., Balss KM, Maryanoff CA, Schmidt U, Breslav M, Mahan A, Lacy E, Maryanoff BE. Thrombogenic collagen-mimetic peptides: Self-assembly of triple helix-based fibrils driven by hydrophobic interactions. *Proc Natl Acad Sci U S A* 2008;**105**:8513–8518
132. Gauba V, Hartgerink JD. Surprisingly high stability of collagen ABC heterotrimer: evaluation of side chain charge pairs. *J Am Chem Soc* 2007;**129**:15034–41
133. Gauba V, Hartgerink JD. Self-assembled heterotrimeric collagen triple helices directed through electrostatic interactions. *J Am Chem Soc* 2007;**129**:2683–90
134. Fallas JA, Gauba V, Hartgerink JD. Solution structure of an ABC collagen heterotrimer reveals a single-register helix stabilized by electrostatic interactions. *J Biol Chem* 2009;**284**:26851–9
135. Fallas JA, Hartgerink JD. Computational design of self-assembling register-specific collagen heterotrimers. *Nat Commun* 2012;**3**:1087
136. Fallas JA, Lee MA, Jalan AA, Hartgerink JD. Rational design of single-composition ABC collagen heterotrimers. *J Am Chem Soc* 2012;**134**:1430–3
137. Kumar VA, Taylor NL, Jalan AA, Hwang LK, Wang BK, Hartgerink JD. A nanostructured synthetic collagen mimic for hemostasis. *Biomacromolecules* 2014;**15**:1484–90
138. Wright ER, Conticello VP. Self-assembly of block copolymers derived from elastin-mimetic polypeptide sequences. *Adv Drug Deliv Rev* 2002;**54**:1057–73
139. Anwar RA. Elastin—a brief review. *Biochem Educ* 1990;**18**:162–6
140. Li B, Daggett V. Molecular basis for the extensibility of elastin. *J Muscle Res Cell Motil* 2002;**23**:561–73
141. Gray WR, Sandberg LB, Foster JA. Molecular model for elastin structure and function. *Nature* 1973;**246**:461–6
142. Pepe A, Bochiechio B, Tamburro AM. Supramolecular organization of elastin and elastin-related nanostructured biopolymers. *Nanomedicine* 2007;**2**:203–18
143. Patel D, Menon R, Taite LJ. Self-assembly of elastin-based peptides into the ECM: the importance of integrins and the elastin binding protein in elastic fiber assembly. *Biomacromolecules* 2011;**12**:432–40
144. Venkatachalam CM, Abukhaled M, Sugano H, Urry DW. Nuclear magnetic-resonance and conformational energy calculations of repeat peptides of elastin—conformational characterization of cyclopentadecapeptide cyclo-(L-Val-L-Pro-Gly-L-Val-Gly)3. *J Am Chem Soc* 1981;**103**:2372–9
145. McMillan RA, Conticello VP. Synthesis and characterization of elastin-mimetic protein gels for use in biomedical applications. *Abstr Papers Am Chem Soc* 2000;**219**:U453
146. Hong M, Isailovic D, McMillan RA, Conticello VP. Structure of an elastin-mimetic polypeptide by solid-state NMR chemical shift analysis. *Biopolymers* 2003;**70**:158–68
147. McMillan RA, Conticello VP. Synthesis and characterization of elastin-mimetic protein gels derived from a well-defined polypeptide precursor. *Macromolecules* 2000;**33**:4809–21
148. Wu X, Sallach RE, Caves JM, Conticello VP, Chaikof EL. Deformation responses of a physically cross-linked high molecular weight elastin-like protein polymer. *Biomacromolecules* 2008;**9**:1787–94
149. Jeon WB. Application of elastin-mimetic recombinant proteins in chemotherapeutics delivery, cellular engineering, and regenerative medicine. *Bioengineered* 2013;**4**:368–73
150. Gagner JE, Kim W, Chaikof EL. Designing protein-based biomaterials for medical applications. *Acta Biomater*. Epub ahead of print 2013
151. Bidwell GL 3rd, Raucher D. Cell penetrating elastin-like polypeptides for therapeutic peptide delivery. *Adv Drug Deliv Rev* 2010;**62**:1486–96
152. Lim DW, Nettles DL, Setton LA, Chilkoti A. *In situ* cross-linking of elastin-like polypeptide block copolymers for tissue repair. *Biomacromolecules* 2008;**9**:222–30
153. Nettles DL, Chilkoti A, Setton LA. Applications of elastin-like polypeptides in tissue engineering. *Adv Drug Deliv Rev* 2010;**62**:1479–85
154. Kumar VA, Martinez AW, Caves JM, Naik N, Haller CA, Chaikof EL. Microablation of collagen-based substrates for soft tissue engineering. *Biomed Mater* 2014;**9**:011002
155. Kumar VA, Caves JM, Haller CA, Dai E, Li L, Grainger S, Chaikof EL. Collagen-based substrates with tunable strength for soft tissue engineering. *Biomater Sci* 2013;**1**:1193–1202
156. Caves JM, Cui W, Wen J, Kumar VA, Haller CA, Chaikof EL. Elastin-like protein matrix reinforced with collagen microfibers for soft tissue repair. *Biomaterials* 2011;**32**:5371–9
157. McDaniel JR, Callahan DJ, Chilkoti A. Drug delivery to solid tumors by elastin-like polypeptides. *Adv Drug Deliv Rev* 2010;**62**:1456–67
158. Kim W, Xiao J, Chaikof EL. Recombinant amphiphilic protein micelles for drug delivery. *Langmuir* 2011;**27**:14329–34
159. Xu D, Asai D, Chilkoti A, Craig SL. Rheological properties of cysteine-containing elastin-like polypeptide solutions and hydrogels. *Biomacromolecules* 2012;**13**:2315–21
160. Kumar VA, Caves JM, Haller CA, Dai E, Liu L, Grainger S, Chaikof EL. Acellular vascular grafts generated from collagen and elastin analogs. *Acta Biomater* 2013;**9**:8067–74
161. Caves JM, Kumar VA, Martinez AW, Kim J, Ripberger CM, Haller CA, Chaikof EL. The use of microfiber composites of elastin-like protein matrix reinforced with synthetic collagen in the design of vascular grafts. *Biomaterials* 2010;**31**:7175–82