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Higher-Order Assembly of Collagen Peptides into Nano- and Microscale Materials[†]

David E. Przybyla[‡] and Jean Chmielewski*[,‡,§]

[‡]*Department of Chemistry, Purdue University, 560 Oval Drive, West Lafayette, Indiana 47907, and*
[§]*Weldon School of Biomedical Engineering, Purdue University, 206 South Martin Jischke Drive, West Lafayette, Indiana 47907*

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ABSTRACT: The triple-helical structure of collagen peptides has recently been harnessed as a subunit in the higher-order assembly of unique biomaterials. Specific assembly signals have been designed within collagen peptides, including hydrophobic groups, electrostatic interactions, and metal−ligand binding, to name a few. In this way, a range of novel assemblies have been obtained, including nano- to microscale fibers, gels, spheres, and meshes, each with the potential for novel biological applications in drug delivery, tissue engineering, and regenerative medicine.

Collagen is a widely studied natural material because of its prevalence in mammals and excellent physical properties. The primary amino acid sequence of collagen contains repeating units of the sequence Xaa-Yaa-Gly. A variety of amino acids can be accommodated in the Xaa and Yaa positions, but the most abundant naturally occurring repeating sequence in mammals is Pro-Hyp-Gly, where Hyp is 4(*R*)-hydroxyproline (*1*). Collagen's unique structure is based on the association of three type II polyproline (PPII) chains into a right-handed triple helix (*2*−*4*). The high natural abundance of proline and hydroxyproline facilitates the formation of a PPII helix, with Hyp aiding in triple helix stability through hydrogen bonding and electronic effects (*3*, *5*). Also, the clustering of additional charged and hydrophobic residues along the triple helix can aid in directing the self-assembly into ordered structures (*6*, *7*).

To date, 28 different types of collagen have been identified, and these collagens can be categorized by their macromolecular structures (*8*−*11*). To be specific, collagen types I−III, V, XI, XXIV, and XXVIII form fibrils, types IV, VIII, and X form networklike structures, types IX, XII, XIV, XVI, XIX−XXII, and XXVII form fibril-associated collagens with interrupted triple helices (FACITs), type XXV forms membrane-associated collagens with interrupted triple helices (MACITs), and types XV and XVIII form multiple triple-helix domains and interruptions (MULTIPLEXINs). Each type of collagen described can exist as homotrimeric collagen sequences (i.e., types II, III, and VIII) or heterotrimeric collagen sequences, such as type I or IV. For instance, fibril-forming collagen will generate D-periodic collagen with gaps (D = 67 nm) between the fibrils resulting in collagen's unique banding pattern.

Collagen is a major component of the extracellular matrix and connective tissues, including ligaments, tendons, cartilage, and skin. Within the extracellular matrix, collagen exists as a network of fibers and provides the structural integrity and malleability to accommodate both cellular growth and tissue development (*12*). On the other hand, hyaline cartilage is comprised of type II collagen and utilizes collagen's diverse physical properties to generate a rugged yet elastic biological material (*13*). In contrast, the crystallization of type I collagen with hydroxyapatite gives bones their rigid stability and tensile strength (*14*). The drastically diverse physical properties of collagen observed among the extracellular matrix, cartilage, and bone highlight how controlling collagen's physical properties would provide an abundance of exceptional biological materials. Some of the current applications of naturally derived collagen matrices include scaffolds for drug delivery (*15*, *16*), bone repair (*17*, *18*), and tissue regeneration (*19*−*21*). Specifically, bovine collagen has been clinically used as a scaffold for generating tissue grafts by mimicking collagen's fibrous three-dimensional structure in the extracellular matrix (*22*). Other applications of natural collagen include bone substitutes (*23*) and cartilage implants (*24*).

Whereas natural collagen has proven to be useful in a variety of different applications, potential problems still exist. For instance, since collagen is typically derived from animal sources, there is an increased risk of the transfer of toxic agents, such as prions (*25*), as well as immunogenic responses (*26*, *27*) after implantation. Also, there is an inability to make sequence specific modifications to the collagen structure, and the collagen scaffolds used for transplants must be capable of withstanding degradation from collagenases in the extracellular matrix. To solve some of these problems, chemical or thermal/photo-cross-linking (*18*, *28*−*30*) techniques have been implemented; however, these techniques may result in increased toxicity (*31*, *32*).

In recent years, a variety of strategies have been developed that allow short, synthetic collagen triple helices to assemble into both nano- and macro-sized materials. The design of a successful self-assembling collagen system should include a number of essential features. First, the core of the collagen scaffold should consist of short and easily synthesizable monomers. Second, these monomers should be responsive to an external stimulus or trigger that results in the self-assembly of the collagen peptides under or near physiological conditions. Third, the collagen peptide's supramolecular structure should be fully reversible using mild reagents, resulting in limited stress on surrounding tissue. By utilizing these conditions, self-assembling collagen peptides will have the

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*To whom correspondence should be addressed: 560 Oval Dr., Department of Chemistry, Purdue University, West Lafayette, IN 47907. Phone: (765) 494-0135. Fax: (765) 494-0239. E-mail: chml@purdue.edu.

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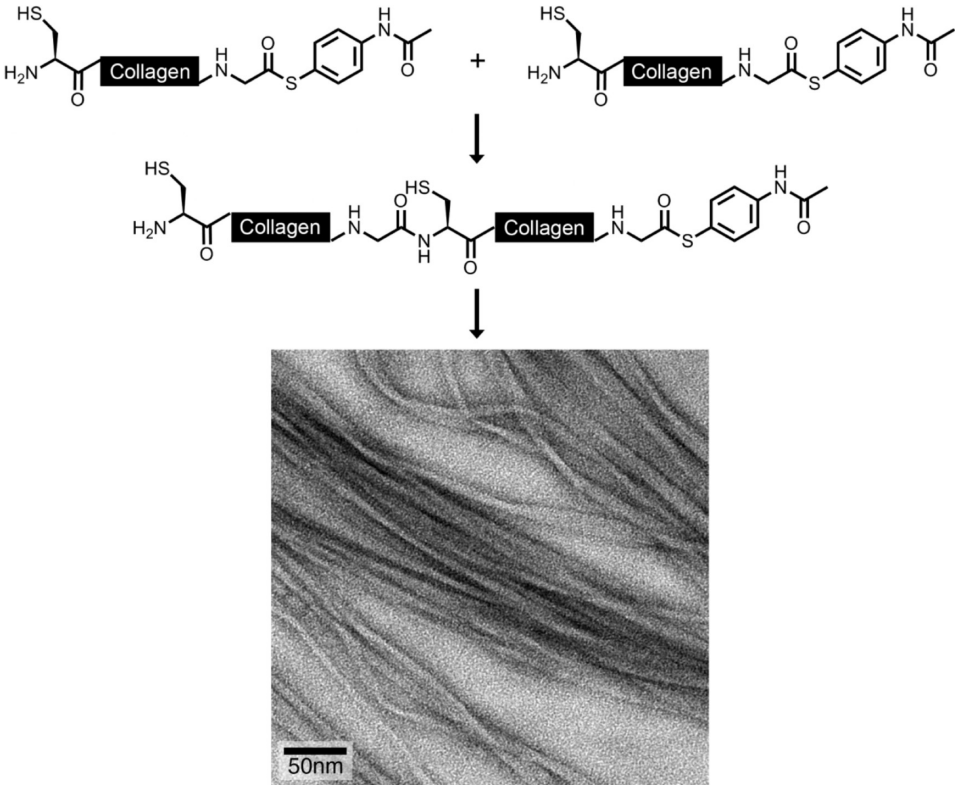

FIGURE 1: Native chemical ligation of short collagen sequences into collagen fibrils (*36*).

potential to play a major role in the development of new collagen-based materials.

HIGHER-ORDER ASSEMBLY OF UNMODIFIED COLLAGEN PEPTIDES

Collagen proteins are known to assemble into higher-order structures, such as fibrils and networks, as outlined above. Whereas extensive research with collagen peptides has delineated the forces involved in the formation of a triple helix, there has been limited evidence for higher-order assembly of collagen peptides. Brodsky and co-workers, however, were among the first to report that small, unmodified POG-based oligomers, such as $(POG)_{10}$, could undergo assembly beyond the triple helix (*33*). These researchers investigated a number of factors that resulted in the association of the collagen peptide into disordered aggregates with highly branched, filamentous structures, including peptide length, peptide concentration, and temperature. These studies concluded that assembly rates increased with peptide length and that high peptide concentrations (>1 mM) and temperatures just below the melting temperature for the triple helix both favored assembly. Interestingly, a homologous peptide, $(PPG)_{10}$, demonstrated no propensity to form higher-order structures. These data suggested that Hyp plays an important role in the supramolecular assembly of self-assembling collagen peptides. Indeed, a collagen protein lacking Hyp was found not to form the native fiber structure (*34*). Although the aggregates formed from $(POG)_{10}$ in no way mirror the highly ordered structure formed from collagen, these results are significant as they provided the ground rules for supramolecular assembly in unmodified POG sequences and provide a starting point for further studies that introduce additional assembly signals into collagen peptides.

Some of the earliest methods for generating synthetic collagen fibers from small collagen peptides involved chemical cross-linking the peptide termini into nonreversible collagen systems. For instance, $(Pro\text{-}Hyp\text{-}Gly)_{10}$ units were synthesized and cross-linked via amide bond-forming reactions (*35*). This process resulted in high-molecular weight products composed of more than 10 linked peptide chains with nanofiber-like structures that were longer than the aggregates of $(POG)_{10}$. Alternatively, Hartgerink and co-workers used native chemical ligation to polymerize small collagen fragments (Figure 1) (*36*). This was achieved by placing a cysteine at the N-terminus and a thioester at the C-terminus of a $(Pro\text{-}Hyp\text{-}Gly)_9$ sequence. The resulting native chemical ligation led to the generation of long nanofibers with diameters of 10−20 nm. These examples indicated that small collagen fragments could assemble into fibers that mimic the diameter of natural collagen fibrils. Cell adhesion sequences were also incorporated into these synthetic collagen assemblies, a key feature in generating collagen materials with cell binding activities.

CYSTEINE KNOTS AND SUPRAMOLECULAR ASSEMBLY OF HETEROTRIMERIC COLLAGEN PEPTIDES

A number of methods for improving the stability of collagen peptide triple helices have been developed. For instance, a range of functionality has been included at a single end of collagen peptides, including metal ligands (*37*−*39*), Kemp's triacid (*40*, *41*), cyclotriveratrylene (*42*), cyclopropane (*43*), tris(2-aminoethyl)-amine (*44*), lysine (*45*−*47*), glutamate knots (*48*, *49*), and cysteine knots (*50*−*54*), to successfully stabilize homotrimeric and heterotrimeric collagen triple helices. All of these strategies have been creatively designed to alter the thermal stability of the triple helix, but most have been limited in their biomaterials applicability as they have not been harnessed for higher-order assembly.

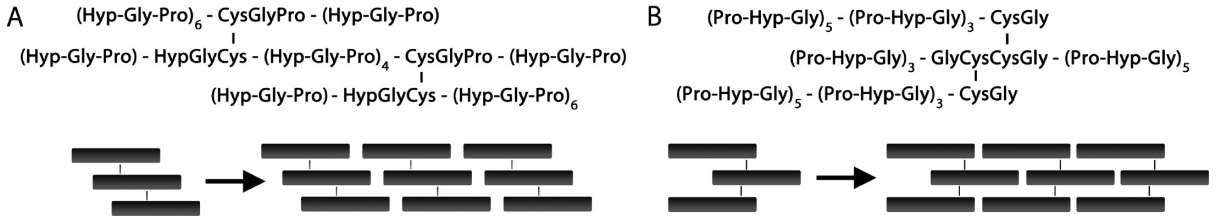

FIGURE 2: Staggered heterotrimeric collagen peptide sequences with disulfide knots. (A) Assembly strategy of Koide and co-workers. (B) Staggered collagen sequence of Raines and co-workers.

In the case of the cysteine knot, disulfide linkages have been used within the triple helix to covalently cross-link three unique collagen peptide sequences. The cysteine knot not only facilitated an increase in triple helix stability but also has been exploited for the controlled supramolecular assembly of heterotrimeric helices. Specifically, Koide and co-workers have used cysteine-knotted peptides to generate higher-order collagen peptide assemblies (*55*). In their design, three (POG)-based peptides were disulfide linked in such a way that they formed a heterotrimeric peptide structure with staggered overhangs to allow for self-complementary linear assembly of triple helices (Figure 2A). Supramolecular association was demonstrated for these heterotrimeric peptide, with particles sizes of approximately 0.6 and 14 μm, although the morphology of these particles was not explored by microscopy. This strategy was recently broadened by Koide and co-workers, whereby the size of the staggered overhang was sequentially lengthened (*56*, *57*), and was found to generate hydrogels. The authors describe the material obtained as a collagen-like gel; however, the thermal properties of this material were not compared to those of a collagen gel, nor was the gel imaged to determine if there are morphological similarities to collagen gels, such as Matrigel. These materials were further modified with an $\alpha 2\beta 1$ integrin-binding sequence, GFOGER, and found to function as a scaffold for cell binding and growth (*58*).

In another example of the utility of the cysteine knot, Raines and co-workers designed a collagen peptide assembly strategy in which three collagen peptides were also cross-linked with a cysteine knot (Figure 2B) (*59*). Dynamic light scattering confirmed that higher-order structures formed, with up to eight monomers associating in aqueous acetic acid-containing methanol, a solvent known to stabilize collagen triple helices. Microscopy confirmed the formation of fibrils that were anywhere from 20 to 400 nm in length and approximately 1 nm in width. In a more recent implementation of the cysteine knot, a heterotrimeric peptide lacking Hyp, but containing additional electrostatic interactions, was found to undergo supramolecular assembly into nanorods and micrometer-sized fibrils with diameters that are in the range of natural collagen fibers (*60*). In all of these ingenious studies, the cysteine knot has been successfully used to form heterotrimeric peptides with sticky ends that result in assemblies that in some cases mimic some of the features of the fibril morphology of natural collagen.

TERMINAL HYDROPHOBIC INTERACTIONS THAT MEDIATE HIGHER-ORDER ASSEMBLY

A sticky end approach has also been described that relies on hydrophobic, noncovalent interactions to promote collagen peptide assembly. Maryanoff and co-workers, for instance, designed a creative collagen peptide assembly strategy that relied on hydrophobic interactions from terminally placed aromatic residues (*61*). Specifically, a pentafluorophenylalanine residue

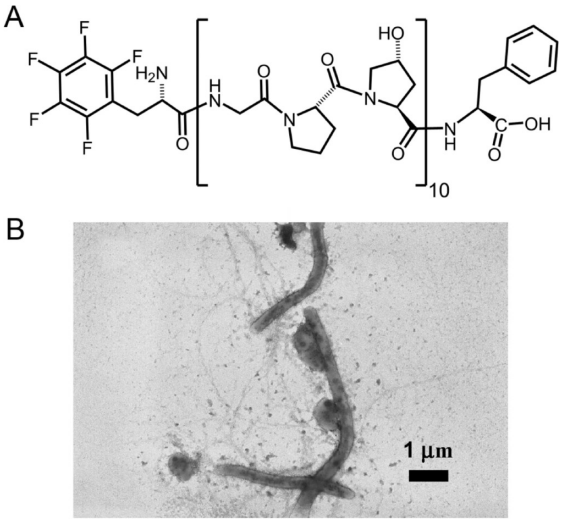

FIGURE 3: (A) Sequence of a self-assembling collagen peptide with hydrophobic termini. (B) TEM image of assembled collagen fibers (*61*).

was placed at the N-terminus and a Phe residue added to the C-terminus of a $(Pro\text{-}Hyp\text{-}Gly)_{10}$ sequence (Figure 3A). The spontaneous association of triple helices of this collagen peptide generated fibrils that were micrometers in length and resembled collagen fibers formed in murine aortic tissue, although the assembled fibrils are significantly thicker (~250 nm vs 50 nm) (Figure 3B). Biological activity was noted as the fiber-forming peptide induced platelet aggregation at a level that was similar to that of type I collagen from equine sources, demonstrating that synthetically derived collagen materials are capable of biological function. Subsequent work by this group demonstrated that similar fibril assemblies could be formed when both termini were functionalized with Phe, but not with a collagen peptide functionalized with Phe and Leu at each termini (*62*). This same assembly strategy, but with a lysine residue in the middle of the triple-helical peptide, was used as a template for the preparation of metallic nanowires, further demonstrating the potential applications of self-assembling collagen fibers (*63*).

The effect of having aromatic residues at the termini of collagen peptides was also analyzed by Brodsky and co-workers (*64*). In an effort to mimic the teleopeptide sequences found at the termini of collagen, the authors incorporated aromatic amino acids at the ends of a type IV collagen-based peptide. They then investigated the effect of these amino acids on the kinetics and structure of higher-order assemblies of the triple-helical form of the peptide. For instance, the peptide FT4Y, containing both Phe and Tyr at the N- and C-termini, respectively, aggregated at a concentration of ~1 mM within approximately 3 min, whereas peptides lacking either one or both of the aromatic amino acids showed no propensity to aggregate even after 48 h. These data

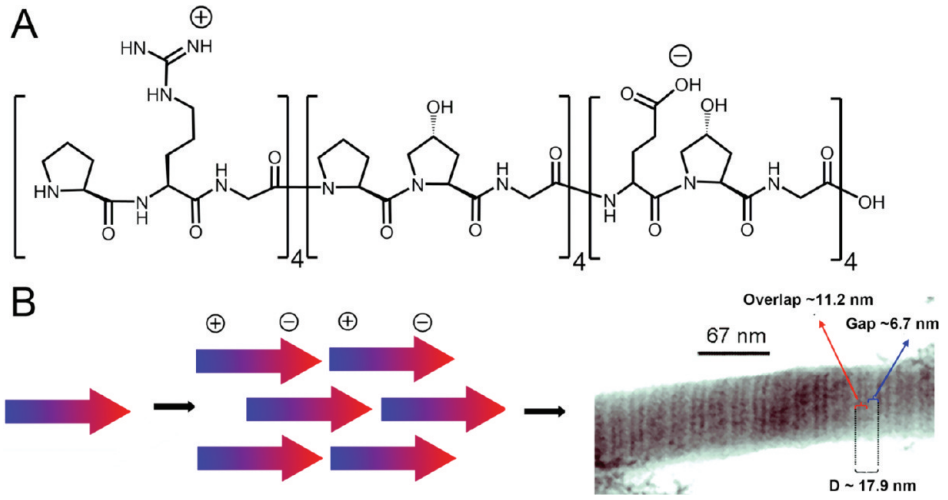

FIGURE 4: (A) Sequence of the D-periodic collagen peptide. (B) Assembly through electrostatic interactions by staggered charged Arg and Glu groups into a D-periodic collagen peptide fiber (*65*).

demonstrate accelerated assembly promoted by a single aromatic residue at each termini. Fibrillar suprastructures were observed for FT4Y that were similar to those obtained with peptides lacking one or both of the aromatic residues, with lengths exceeding 1 μm and widths of 20−40 nm, the latter value being similar to the diameters of fibrils found in natural collagen. These macroscopic assemblies were found to induce platelet aggregation, although, interestingly, the soluble form of FT4Y also allowed for platelet aggregation, perhaps because of smaller (~30 nm) assemblies of the peptide in solution.

Two different self-assembling strategies have both implemented hydrophobic interactions to promote collagen assembly. The additions of these different aromatic groups at both the N- and C-termini were shown to facilitate assembly presumably in a linear direction in both instances, followed by lateral association into fibrils. Interestingly, each assembly resulted in distinctly different fibril morphologies when imaged, yet both maintained the ability to promote platelet aggregation. This demonstrates the versatility of synthetic collagen scaffolds as materials with physiological applications.

ELECTROSTATIC ASSEMBLY

One goal of the design of synthetic collagen materials is to mimic natural collagen's physical and biological properties. In this regard, Chaikof, Conticello and co-workers took a major step forward by creating a self-assembling collagen peptide that forms a fibrous structure with a well-defined periodicity (*65*). The group designed the sequence $(Pro\text{-}Arg\text{-}Gly)_4$-$(Pro\text{-}Hyp\text{-}Gly)_4$-$(Glu\text{-}Hyp\text{-}Gly)_4$ in which terminal, charged residues facilitate linear assembly of the triple helix through electrostatic interactions at each end of the sequence (Figure 4A).

Micrometer-sized fibers were obtained with this peptide without thermal annealing of the sample, with diameters of 12−15 nm. Thermally annealed peptide solutions initially were found to form >100 nm long fibrils with tapered tips. These fibril ends are similar to those found in the tactoidal ends of native collagen fibers and are believed to be the point of further growth into fibers. Annealed peptide solutions after 9 days produced micrometer-sized fibers that were ~70 nm in diameter. Interestingly, however, these fibers exhibited periodic gaps in microscopy staining of approximately 18 nm, which shows some similarities to the D-periodicity (67 nm) of native collagen fibers. The authors propose a linear, staggered assembly of triple-helical units as the rationale for the periodicity (Figure 4B). The formation of an ordered material of this sort from the self-assembly of a single peptide chain is groundbreaking in the arena of collagen mimetics. Specifically, this self-assembling collagen peptide is the first example of a synthetic collagen peptide that generates D-periodicity, a major advance in creating a synthetic collagen fiber that mimics natural collagen. It will be interesting to see if the observed banding results in physical or biological properties not yet observed for the synthetic collagen scaffolds.

METAL-TRIGGERED ASSEMBLY

An alternate bottom-up approach to the directed assembly of collagen peptide-based materials has recently included the use of metal−ligand interactions as a means to promote directional assembly of triple helices. Chmielewski and co-workers, for instance, have incorporated metal binding sites into small collagen peptides and used metal−ligand interactions to drive self-assembly. Three designs have been reported: incorporating metal-binding ligands at the termini of a collagen peptide [linear design (Figure 5)], within the center of the collagen peptide [radial design (Figure 5)], and at both positions simultaneously [cross-linked design (Figure 5)]. In each case, the ligands were incorporated within a POG peptide core to produce modified triple-helical peptides with ample stability at room temperature. Furthermore, an important feature of the design strategy was that the assembled materials would be reversible under mild conditions by treatment with metal chelators.

For the terminally modified collagen peptide, the design consisted of two features: a central collagen-based core composed of nine repeating units of the Pro-Hyp-Gly tripeptide and distinct metal binding ligands at each terminus [**NCoH** (Figure 6A)] (*66*). Formation of triple helices of the individual **NCoH** strands would result in clustering of six histidines at one end and the triple helix and three NTA's at the alternate end. A range of metal ions were found to promote the higher-order assembly of **NCoH**, and interestingly, micrometer-sized florette-shaped particles were observed by SEM (Figure 6B). The surface of the former structures was found to be highly ruffled, and confocal microscopy with congo red staining confirmed that collagen peptide formed the core. Mechanistic studies confirmed the early formation of sheetlike structures, presumably due to linear and lateral

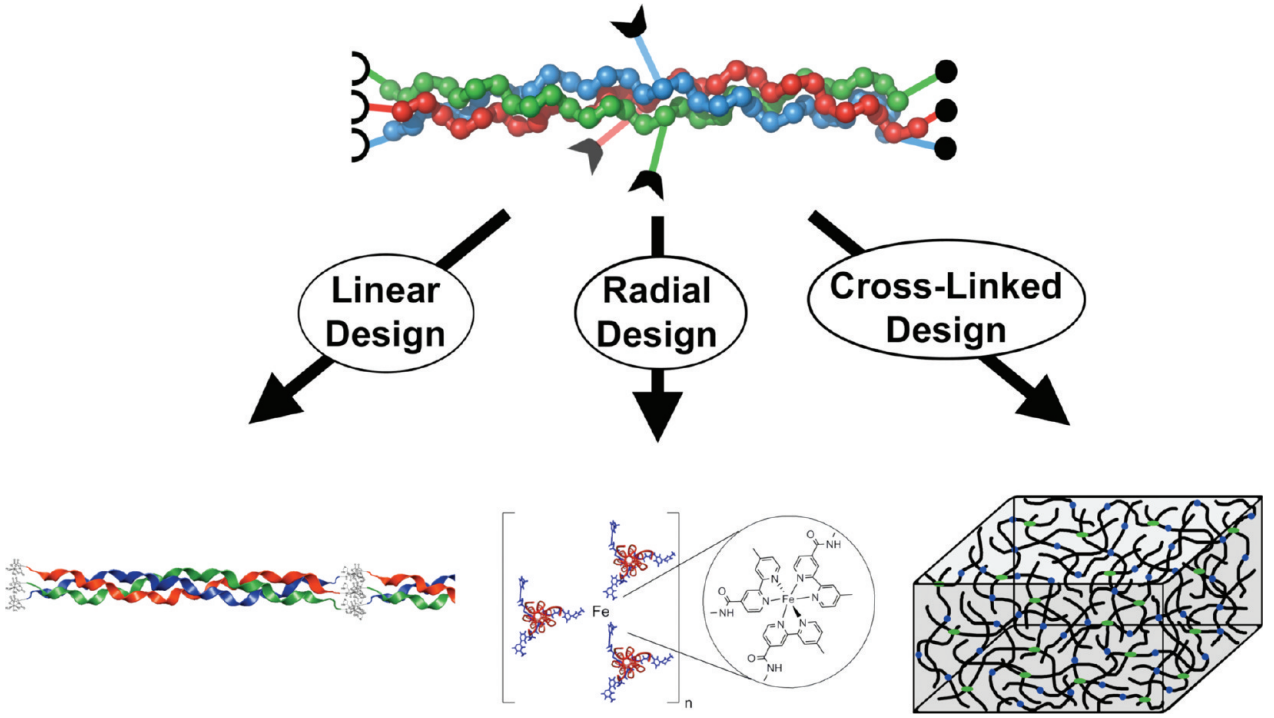

FIGURE 5: Design strategies for metal-promoted, directional assembly of collagen triple-helical peptides.

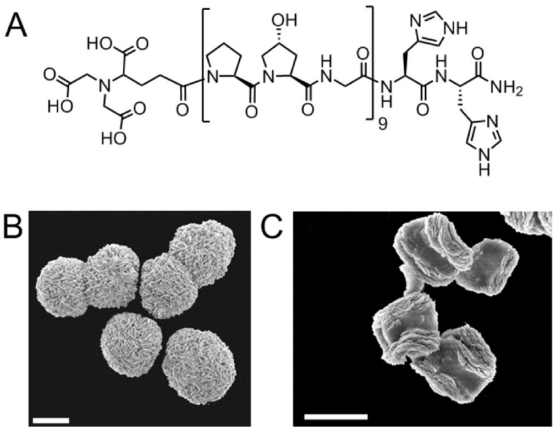

FIGURE 6: (A) Sequence of the **NCoH** peptide. (B) SEM image of the microflorette-shaped particles obtained with **NCoH** and Zn(II) at room temperature. (C) Stacked sheets obtained with **NCoH** and Zn(II) at 4 °C. The scale bars in panels B and C are 5 μm (*66*).

assembly of the peptide triple helices, followed by the curvature of stacked sheets to the spherical-shaped particles (Figure 6C). The unique morphology of these structures as opposed to collagen-based peptide fibrils and fibers may lend itself to equally new applications for collagen peptide materials.

In a departure from all of the collagen peptide assembly designs described above, Chmielewski and co-workers developed a radial design for higher-order assembly of triple-helical peptides that relies on the inclusion of a centrally positioned, bipyridine-modified amino acid within a $(POG)_9$ peptide (Figure 7A) (*67*). Formation of a triple helix with this peptide, **H-byp**, positions three bipyridyl ligands in the center of the triple helix, producing three potential directions for radial growth with the addition of metal ions (Figure 5, center). Imaging of the assemblies upon the addition of Fe(II) to the collagen peptide formed long (3−5 μm) branched fibers (Figure 7B). The unbranched regions of the fibers were composed of bundles of fibrillar structures with a width of approximately 10 nm, a value that corresponds to the radius of a trimer of triple helices and is on the low end for the diameter of a collagen fibril. Bipyridyl ligands displayed on the surface of the fibrils may be responsible for metal-mediated fibril association, a process with some analogies to the cross-linking found in collagen fibers.

In a final cross-linked design (Figures 5, right), Chmielewski and co-workers combined features of both their linear and radial growth designs into a single collagen peptide (*68*). The NTA/His2 terminal moieties of **NCoH** were combined with the central bipyridyl ligand in **H-byp** to yield the **NHbipy** peptide (Figure 8A). The multidirectionality of the metal ligands provides the opportunity for the formation of extensively cross-linked collagen peptide materials upon addition of metal ions, a feature that was confirmed with the addition of Ni(II), Cu(II), Co(II), and Zn(II) to **NHbipy**. The morphology of the assemblies was visualized by SEM, and highly cross-linked, fibrous meshes that were more than hundreds of micrometers in length were observed, with internal pores approximately 5−20 μm in diameter (Figure 8B), a network that is strikingly similar to the collagen matrix found in Matrigel (*69*) (Figure 8C).

The development of biocompatible three-dimensional scaffolds capable of cellular encapsulation and growth is of critical importance in the emerging fields of tissue engineering and regenerative medicine. Essential features of successful scaffolds for tissue engineering, for instance, would include the control of scaffold morphology on the nanometer to micrometer scale, the ability to include chemical diversity, such as bioactive agents, the ability to modulate physical properties, such as porosity, and the design of controlled scaffold degradation pathways. The three-dimensional (3D) network described above has potential in all of these areas and was further evaluated by Chmielewski and co-workers for inclusion of fluorescent and bioactive agents, and cell encapsulation and growth (*68*). Inclusion of low levels of peptide containing NBD or biotin (Figure 9A) within the **NHbipy**/Ni(II) mesh-forming reaction mixture with metal ions

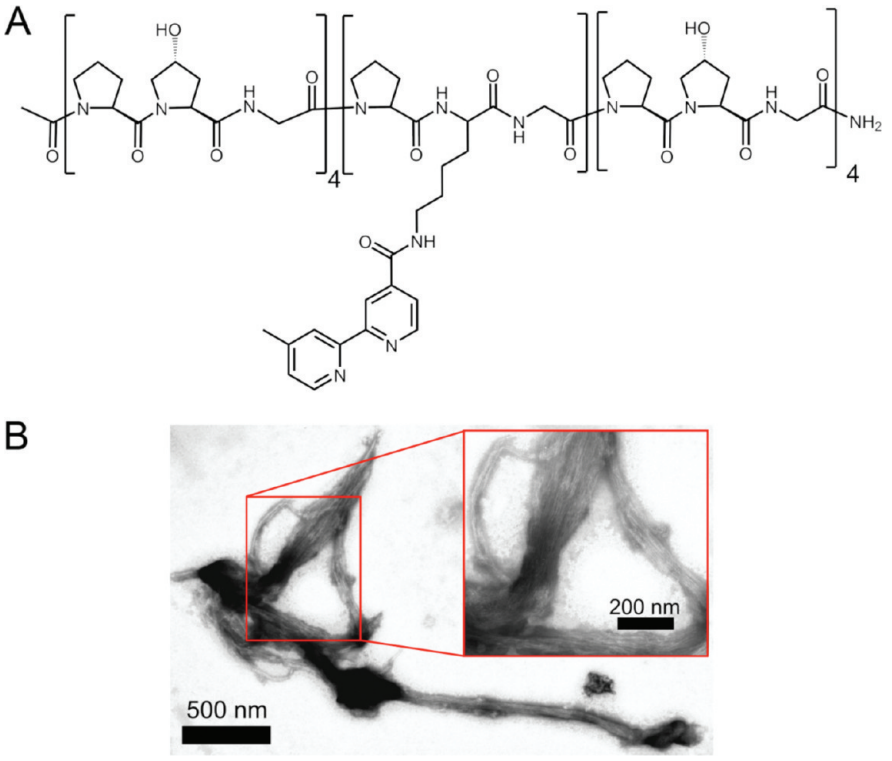

FIGURE 7: (A) Sequence of the **H-byp** peptide. (B) TEM image of the long, branched fibers obtained with **H-byp** and Fe(II) (*67*).

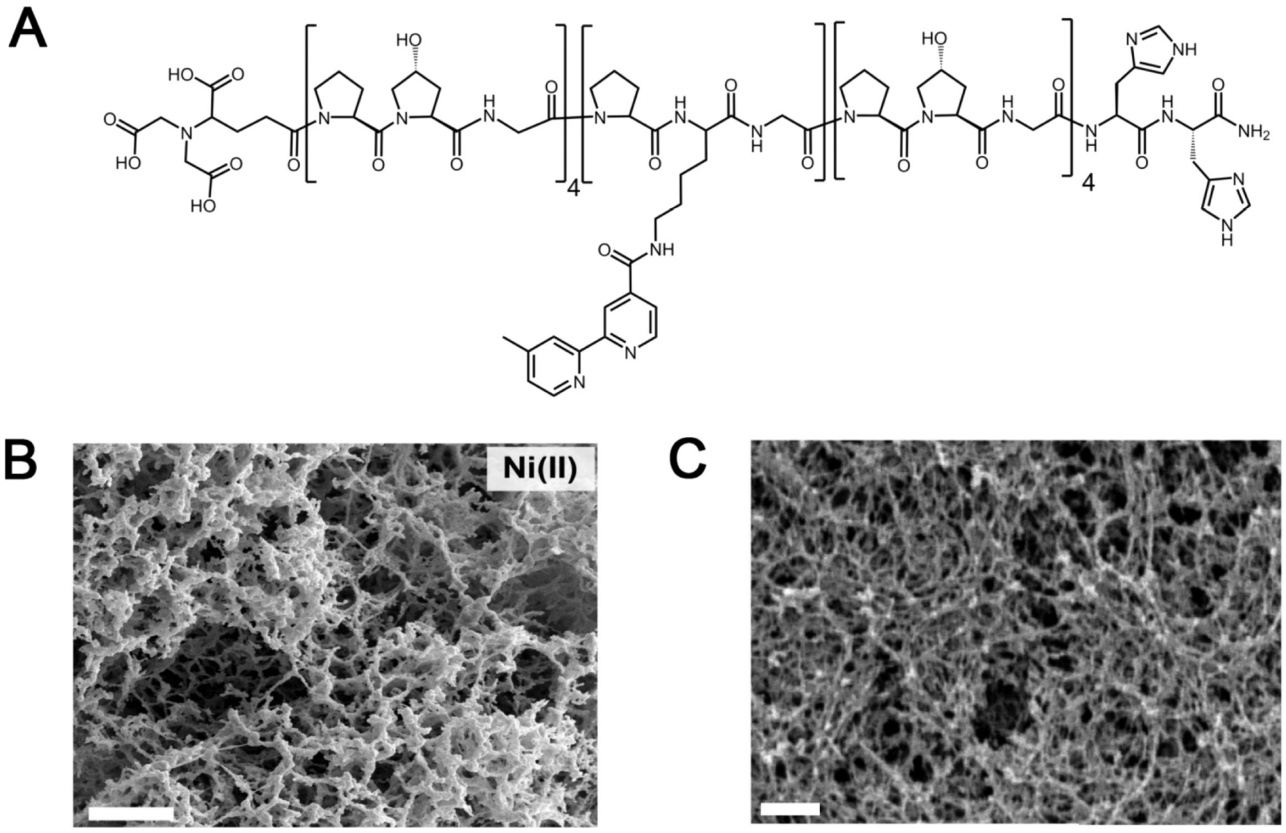

FIGURE 8: (A) Sequence of the **NHbipy** peptide. (B) SEM analysis of the material resulting from **NHbipy** and Ni(II), where the scale bar is 5 μm (*68*). (C) SEM analysis of the Matrigel, where the scale bar is 1 μm (*69*).

allowed for display of these small molecules in the 3D network. Encapsulation and growth of cells, such as HeLa, were accomplished simply via addition of the cells to the matrix-forming reaction mixture of **NHbipy** and Ni(II). Fluorescence microscopy (Figure 9B) and cryo-SEM (Figure 9C) imaging demonstrated that the cells were fully surrounded by the fibrous, collagen peptide network. Significantly, the cells maintained viability and continued to proliferate within the matrix, and a mild EDTA treatment of the scaffold was sufficient to release the cells, which were also viable and continued to grow.

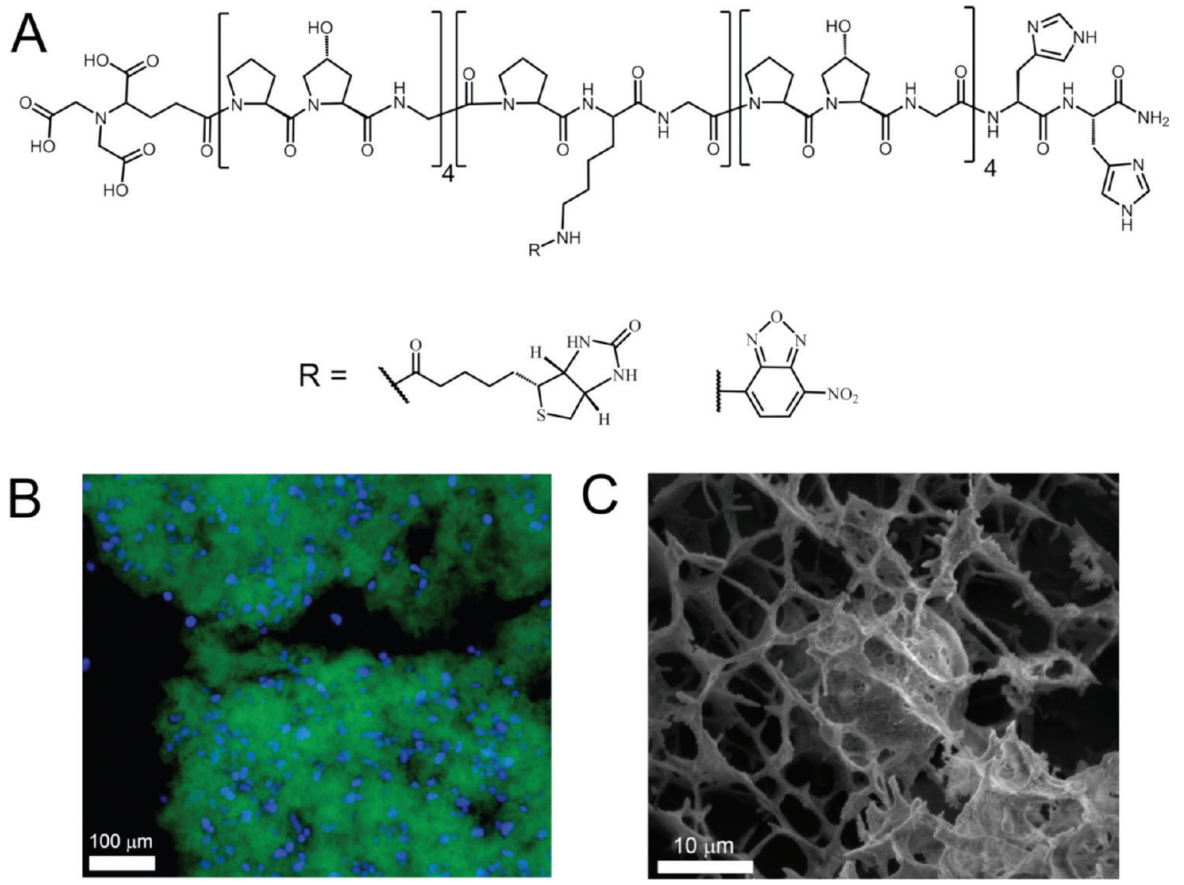

FIGURE 9: (A) Sequences of **NHnbd** and **NHbiot**. (B) Fluorescence image of the 3D network with added **NHnbd** encapsulating HeLa cells (blue nuclei). (C) Cryo-SEM image of encapsulated HeLa cells in the **NHbipy**/Ni(II) mesh.

These data confirm that the assembly of collagen-based peptides into triple helices, followed by their metal-promoted assembly, can lead to nanometer- to micrometer-scale florettes, fibers, and meshes. Significantly, the assembly processes proceed under mild conditions using a neutrally buffered aqueous solution at room temperature. The collagen peptide assemblies are fully reversible using a mild chelating agent, thereby providing the potential for precise temporal and spatial control of the construction of the scaffold. Collagen-based peptides that reversibly self-assemble under mild conditions in the presence of metal ions into large and well-ordered structures with tunable shapes and sizes may have a myriad of possible uses in tissue engineering and regenerative medicine.

HYBRID COLLAGEN PEPTIDE-BASED MATERIALS

The inclusion of other structural components that are covalently linked to collagen peptides has led to a number of other interesting collagen peptide-based assemblies. For instance, collagen peptides linked to dendrimers have produced stabilized collagen triple helices (*41*) and hydrogels that act as thermoresponsive drug carriers (*70*). Inclusion of collagen peptides covalently within PEG-based polymers also produced hydrogels with the ability to enhance tissue production with encapsulated chondrocytes (*71*). Hydrogels were also obtained when collagen peptides were included within triblock polymers (*72*), whereas triblock copolymers containing collagen peptide sequences were found to assemble into spherulites (*73*). The assembly of collagen−peptide amphiphiles has been shown to mimic protein architectures and form spheroidal and disklike micelles (*74*, *75*).

Collagen's biological diversity has made it an attractive synthetic material for a multitude of scientific fields. Specifically, harnessing collagen's unique physical properties in conjunction with other molecular architectures as described above opens up many possible applications for new hybrid materials for drug delivery and tissue engineering.

CONCLUDING REMARKS

Self-assembling peptides have proven to be a powerful technique and emerging field for generating novel biomaterials, especially with the development of coiled-coil (*76*), elastin mimetics (*77*), β-sheet (*78*, *79*), and peptide amphiphile assembly strategies (*80*). These materials have demonstrated potential in interdisciplinary applications in tissue engineering, regenerative medicine, and stem cell differentiation. Recent exciting advances with assembly of collagen triple-helical peptides demonstrate the potential of this peptide structure in the generation of novel biomaterials, including fibers, spheres, and meshes. These collagen self-assembling systems have been designed with the intent of utilizing the biological and physical properties of native collagen, while taking advantage of short, easily synthesizable sequences. These systems also allow for site specific modifications within the collagen sequence, resulting in tunable physical properties while accommodating biologically active sequences.

In terms of truly mimicking natural collagen's architecture, some outstanding advances have been made, such as D-periodic collagen peptide fibrils. There are still significant challenges, however. In the collagen peptide fibrils that have been obtained, the diameters of the materials are similar to those of collagen type I

fibrils, for instance, implying that lateral assembly is similar in natural and synthetic materials. Significantly shorter lengths have been obtained with the synthetic fibrils, however, as compared to those with natural collagen. Designs that improve upon linear growth mechanisms with collagen peptides are desirable. In terms of mimicking collagen's biological functions, early work with platelet aggregation and cell encapsulation and growth is promising.

While a variety of cleverly designed self-assembling collagen peptide systems have been explored, the development of these systems is still in its infancy. A better understanding of how to control the physical and chemical properties of the assembled peptides should lead to improved functions of these scaffolds in more complex biological environments. Introducing other key components, such as proteoglycans or elastin, may also lead to more complex biohybrid materials that more closely mimic the extracellular matrix. With the growing number of collagen peptide supramolecular structures, there should soon be a myriad of biological applications emerging. By incorporating cell adhesion signals, for instance, fibers and meshes may be used for cell binding and organization, and as scaffolds for cell and tissue growth. With similar cell binding signals, collagen peptide microflorettes could be used as cell delivery vehicles and with the appropriate growth factors could potentially direct stem cell differentiation. Incorporation of calcium binding units within hierarchical collagen peptide assemblies could result in hydroxyapatite binding and novel organic−inorganic materials that mimic bone. The addition of top-down assembly methods, such as the alignment of collagen-based materials using electrochemical strategies (*81*), to the hierarchical assembly of collagen peptides may also be an interesting avenue to unique structures. Given the fascinating range of biological activities of natural collagens, researchers will have many biomimetic options with collagen peptide-based biomatierals.

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