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Advances in the design and higher-order assembly of collagen mimetic peptides for regenerative medicine

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Regenerative medicine makes use of cell-supporting biomaterials to replace lost or damaged tissue. Collagen holds great potential in this regard caused by its biocompatibility and structural versatility. While natural collagen has shown promise for regenerative medicine, collagen mimetic peptides (CMPs) have emerged that allow far higher degrees of customization and ease of preparation. A wide range of two and three-dimensional assemblies have been generated from CMPs, many of which accommodate cellular adhesion and encapsulation, through careful sequence design and the exploitation of electrostatic and hydrophobic forces. But the methodology that has generated the greatest plethora of viable biomaterials is metal-promoted assembly of CMP triple helices—a rapid process that occurs under physiological conditions. Architectures generated in this manner promote cell growth, enable directed attachment of bioactive cargo, and produce living tissue.

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Introduction

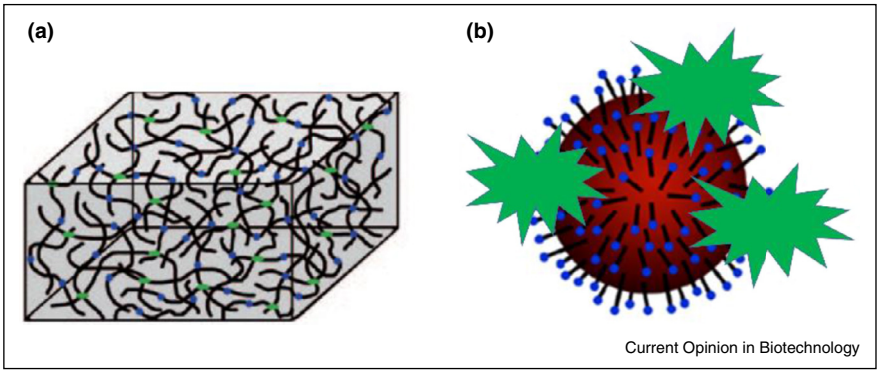
For a substance to be an effective biomaterial, it must be biocompatible, robust but malleable to facilitate implantation and customization, and mimic the endogenous extracellular matrix (ECM) well enough to support the growth and differentiation of stem cells. Microstructures fabricated from synthetic polymers [1,2], gelatin [3,4], and chitosan [5] have shown great promise as cell-supportive biomaterials for the replacement of lost or damaged tissue. Much attention, of late, has turned to collagen caused by its potential for structural diversity, allowing for tailor-made applications. Collagen is a protein that is abundant in the extracellular matrix (ECM), as well as in bone and ligaments. In nature, it exists as a left-handed polyproline type II (PPII) helix, characterized by proline-hydroxyproline-glycine (POG) repeats. A stable triple helical architecture results from hydrogen-bonding interactions between the amide hydrogens on glycine and other nearby main-chain carbonyl moieties [6], and steric repulsion between the bulky pyrrolidine rings on proline and hydroxyproline forces the main chains of the individual helices to form tighter, more cohesive bundles [7,8]. Collagen sequences also have a natural propensity for self-assembly. For instance, glycosylation of lysine residues in the collagenous domain of adiponectin causes the protein to adopt a highly-stable octameric conformation [9]. The stability and the wide-ranging functionality of collagen make it ideal for use as a scaffold for tissue engineering. In the last few years, crucial advancements have been made on the design and assembly of collagen-based and collagen peptide-based biomaterials. Some of these recent achievements will be reviewed in the work that follows.

Biological applications of natural collagen

Natural collagen is advantageous as a biomaterial caused by its ability to mimic the fibrous components of the ECM. Moreover, cells readily adhere to collagen microparticles, and, under the right conditions, collagen can support the growth and differentiation of stem cells into functional tissue. Recently, Van de Kamp et al. designed a hepatocyte growth factor (HGF)-loaded collagen scaffold that was capable of recruiting endogenous human mesenchymal stem cells (hMSCs) both *in vitro* and *in vivo* as a potential skin therapy for burn victims [10]. In another study, high-density cellularized tissues were fabricated through initial formation of a low-density, highly-interconnected fibrillar collagen-based cell encapsulating matrix, which was then subjected to confined compression [11•]. Additionally, Kopf et al. developed a 3D-printable Type I collagen/agarose hydrogel blend that could encapsulate human umbilical artery smooth muscle cells (HUASMCs) [12]. Furthermore, Wong et al. described an injectable collagen/alginate gel that encapsulated Glia-Derived Neurotrophic Factor (GDNF)-secreting HEK293 cells, continuously delivered GDNF in healthy rat eyes for 14 days, and, ultimately, promoted photoreceptor survival, a promising achievement in the treatment of eye diseases [13•].

A key advancement in the design of natural collagen biomaterials was the shift from bulky solid scaffolds to discrete, injectable microparticles (Figure 1). In fact,

Figure 1



Collagen cell-supportive biomaterial archetypes: **(a)** three-dimensional (3D) matrices, and **(b)** discrete microparticles (cells shown in green).

collagen can be formed into monodisperse spherical particles through the use of technologies such as microfluidics and emulsification [14]. This was exemplified by the use of collagen microspheres by Yamada et al. to support the growth and differentiation of oligodendrocyte progenitor cells (OPCs) as a potential therapy for spinal cord regeneration [15•]. More recently, Yao et al. [14] generated collagen microparticles with a rough surface morphology that enabled spheroid formation with primary rat hepatocytes. Spheroid formation is believed to be an important stage in the production of embryonic tissue [16–18]. Consequently, spheroids have been used as effective models of *in vivo* drug screening [19], as well as models for larger tissue functionality [20]. Additionally, Aravamudhan et al. demonstrated that Type I collagen nanofibers grown on a microporous polymeric substrate support *in vitro* osteogenesis of mesenchymal stem cells and have *in vivo* biocompatibility [21•].

However, it is challenging to introduce chemical diversity into the collagen triple helix. Consequently, researchers are limited in their ability to control the functionality and morphology of the scaffolds generated. Also, natural collagen is readily broken down by collagenases. Thermal and/or photochemical cross-linking has been shown to improve the stability of the triple helix, but this improved stability is coupled with a corresponding rise in toxicity [22]. An et al. have suggested recombinant bacterial collagen as an alternative to mammalian collagen biomaterials, since genetic engineering techniques result in easy customization of the peptide sequence [23]. In fact, one study uncovered a recombinant triple helical peptide, expressed in *Escherichia coli*, that self-assembles into periodically-banded, collagen-like mini-fibrils upon increasing pH and temperature [24]. Another team discovered a collagen-like peptide amphiphile through a phage display evolutionary screening and then proceeded to generate, through liquid crystal flow deposition, a precisely-templated, nanofibrous scaffold to which osteoblasts adhere and proliferate [25••]. However, the cost of large-scale production may remain a hindrance to this approach.

Collagen mimetic peptides (CMPs)

Recent research has focused on the design of a new set of scaffolds based on peptide mimetics, short sequences (15–45 residues) that closely imitate the structures of natural proteins [26]. Collagen-mimetic peptides (CMPs) are particularly noteworthy in that they may replicate the best features of natural collagen, while enabling greater control over higher-order assembly.

In order to emulate natural collagen, CMPs often contain a $(POG)_n$ backbone, allowing them to form a stable triple helical conformation. Remarkably, a recent CMP architecture was shown to be so robust as to aggregate gold nanoparticles through the formation of triple helices between oppositely-polarized peptides bound to adjacent particles [27•]. Modern structural studies have indicated that the triple helix can be further stabilized through substitution of a non-canonical amino acid in each triad. Raines et al. discovered that replacement of hydroxyproline with fluoroproline imparts additional stereoelectronic stability to the resulting helix and accelerates protein folding [28,29]. Likewise, substitution of one residue with its thioamide analog can result in enhanced structural stability caused by the modification of hydrogen-bonding interactions, as well as improved protease-resistance, enabling the collagen biomaterial to persist in biological environments [30]. Similarly, replacement of glycine residues with aza-glycine resulted in a significantly more stable helix caused by the availability of an additional hydrogen bond donor, although, in this case, a new type of helix may be forming caused by the unique CD signature observed with trimeric aza-glycine peptides [31•].