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The Powerful Functions of Peptide-Based Bioactive Matrices for Regenerative Medicine

CHARLES M. RUBERT PÉREZ,[1] NICHOLAS STEPHANOPOULOS,[1] SHANTANU SUR,[1,3] SUNGSOO S. LEE,[2] CHRISTINA NEWCOMB,[2] and SAMUEL I. STUPP[1,2]

[1]Simpson Querrey Institute of BioNanotechnology, Northwestern University, 303 East Superior Street, 11th floor, Chicago, IL 60611, USA; [2]Department of Materials and Science & Engineering, Chemistry, Medicine, and Biomedical Engineering, Northwestern University, 2220 Campus Drive, Evanston, IL 60208, USA; and [3]Department of Biology, Clarkson University, 8 Clarkson Avenue, Potsdam, NY 13699, USA

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Abstract—In an effort to develop bioactive matrices for regenerative medicine, peptides have been used widely to promote interactions with cells and elicit desired behaviors *in vivo*. This paper describes strategies that utilize peptide-based molecules as building blocks to create supramolecular nanostructures that emulate not only the architecture but also the chemistry of the extracellular matrix in mammalian biology. After initiating a desired regenerative response *in vivo*, the innate biodegradability of these systems allow for the natural biological processes to take over in order to promote formation of a new tissue without leaving a trace of the nonnatural components. These bioactive matrices can either bind or mimic growth factors or other protein ligands to elicit a cellular response, promote specific mechano-biological responses, and also guide the migration of cells with programmed directionality. *In vivo* applications discussed in this review using peptide-based matrices include the regeneration of axons after spinal cord injury, regeneration of bone, and the formation of blood vessels in ischemic muscle as a therapy in peripheral arterial disease and cardiovascular diseases.

Keywords—Regenerative medicine, Tissue engineering, Biomaterials, Self-assembly, Bioactive peptides.

INTRODUCTION

Extending life expectancy with high quality of life through innovative therapies is the central goal of regenerative medicine. This field faces a great challenge in replacing or repairing damaged tissue or organs lost to disease, traumatic injury, genetic defects or aging. The existing clinical strategies that attempt to mimic and recreate structure or function of lost tissues include the use of permanent implants, prosthetics, and artificial devices. Clearly these strategies are unable to adequately mimic healthy organs or tissues, since they only provide partial function, and at times induce deleterious immune responses.[50] Furthermore, despite the promise of organ transplantation to treat patients, the lack of donors and potential for immune rejection creates a pressing need for *in vitro* organ growth or *in vivo* regeneration strategies.[1,70] A very active area of research to achieve regeneration has focused on the use of cell therapies drawing from rapidly advancing knowledge in stem cell biology.[14,18] However, over the past decade, the fields of biomaterials and nanotechnology have developed new strategies to regenerate tissue through the use of bioactive or biomimetic artificial matrices. These matrices are primarily composed of biological molecules or synthetic polymers that can be produced by scalable methods, then delivered to a tissue site as liquids or gels and promote cellular adhesion, growth and differentiation of stem or progenitor cells. These materials can be rationally designed at the molecular or supramolecular level to include biological signals or bioactive components, in order to appropriately communicate with cells to create a specialized *in vivo* niche and help guide regenerative processes.[22] In a general sense, one of the main functions of these artificial scaffolds is to serve as a physical support for cells and substitute for damaged extracellular matrix (ECM). Other features include the localization and delivery of drugs and biological therapeutics, such as growth factors, antibodies, DNA and siRNA in order to enhance their stability (e.g.,

Address correspondence to Samuel I. Stupp, Simpson Querrey Institute of BioNanotechnology, Northwestern University, 303 East Superior Street, 11th floor, Chicago, IL 60611, USA. Electronic mail: s-stupp@northwestern.edu

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protection from enzymatic degradation), improve local retention and prolong their release.[30] Most importantly, these scaffolds could be applied in cell therapies to effectively deliver and engraft both differentiated or stem cells to the site of injury to promote tissue formation.[12,37] Finally, the matrices can also be imbued with signals to recruit endogenous and reparative locally.

Much of the research in the past two decades has involved the use of short peptides engineered to self-assemble into matrices that act as ECM mimics. In many ways, peptides combine the best of both worlds: the synthetic tractability and scalability of polymeric systems with the biological function of native proteins. Furthermore, the supramolecular nature of these systems, held together by multiple weak noncovalent bonds, allows them to mimic the dynamic and reconfigurable nature of the ECM, which is impossible with covalently linked systems. The main goal of this review is to describe the chemistry and design of various self-assembling peptide scaffolds and their application to create biocompatible cell microenvironments. Although many elegant studies exist concerning fundamental peptide assembly, we will limit our discussion to systems that have been developed for the synthesis of functional fibrous hydrogels for regenerative medicine.

CURRENT PEPTIDE-BASED SCAFFOLDS

Why Use Peptides for Self-assembling Biomaterials?

Although a number of self-assembling platforms have been explored for applications in biomaterials, peptides represent particularly popular and functional building blocks for this aim for several reasons. First, peptides are synthetically accessible for the most part by automated solid-phase synthesis methods and purified, in most cases, with standard high-performance liquid chromatography (HPLC) methodology. Secondly, because of their small size, peptides can be designed to self-assemble by appending or engineering molecular components to create supramolecular nanostructures. Thirdly, a number of naturally occurring self-assembly motifs are well known in native proteins that can be applied to peptides by judicious choice of the amino acid sequence. For example, α-helices, β-sheets, coiled-coils, or electrostatic effects occurring in nature can be abstracted and used to drive peptide assembly.[41] Perhaps the main reason that peptides present such an attractive scaffold for biomaterials, is that they are, in effect, the main "signaling language" in the ECM. Thus, extracting the functional sequences that signal cells and incorporating them into a self-assembling peptide scaffold will imbue it with potent biological activity. In this section, we will outline some of the major approaches to peptide-based self-assembly for synthetic bio-scaffolds.

Naturally Occurring Protein Motifs

β-Sheets

The β-sheet motif has been used extensively for guiding self-assembly in peptide materials due to its well-understood design rules and relatively short sequences required. Sequences that possess alternating hydrophobic and hydrophilic amino acids endow the peptide backbone (which places the side chains of every other residue on the same side) with an amphiphilic character that is particularly conducive to the formation of β-sheets. For example, the P_{11}-II peptide sequence QQRFQWQFEQQ and derivatives thereof were found to form twisted β-sheet tapes, enforced by the naturally amphiphilic nature of the sequence, as well as the ionic interactions between the oppositely charged R and E residues (Fig. 1a).[16] These tapes bundled to form higher-order (and larger-diameter) structures, and could be triggered to form hydrogels by screening the charges between fibers (e.g., by adding counterions). The Collier group employed the use of β-sheet matrices for cell culture with the use of a similar peptide sequence (QQKFQFQFEQQ) termed "Q11".[27] In order to impart cell-signaling capabilities, peptide sequences such as RGDS and IKVAV were appended (derived from the ECM proteins fibronectin and laminin, respectively) to the Q11 backbone, resulting in constructs that allowed human umbilical vein endothelial cells (HUVECs) to attach, spread, and change their morphology.

Despite the relative simplicity of the β-sheet motif, judicious molecular design can be incorporated into supramolecular materials in a number of ways. Schneider and Pochan introduced a "β-hairpin" design based on a kinked peptide (with the sequence VKVKVKVK^{D}PPTKVKVKV, where ^{D}P superscript denotes *D* enantiomer of proline).[56] This peptide possesses the alternating hydrophilic-hydrophobic motif and a ^{D}PP "kink" that allowed intramolecular folding and β-sheet formation, yielding a "hairpin" structure that could subsequently associate into higher-order fibers and self-supporting hydrogels, when the pH was raised. Interestingly, this structural change was reversible, and lowering the pH dissolved the supramolecular structures. This stimulus-triggered change was particularly amenable to biological applications: simply adding biological media resulted in self-assembly and gelation (Fig. 1b).[31] These gels allowed fibroblast cell attachment and proliferation on their surface,

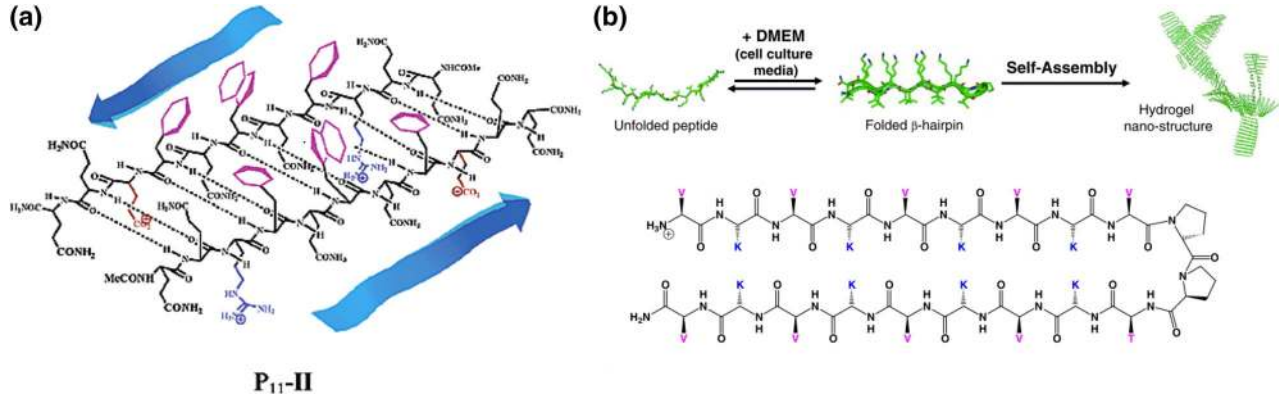

FIGURE 1. (a) Diagram showing the formation of H-bonding between two interacting QQRFQWQFEQQ (P_{11}-II) peptide molecules that promote the formation of fibril and ribbon structures. (b) Proposed self-assembly and chemical structure of the amphiphilic β-hairpin MAX 1. Panel (a) reprinted with permission from Fishwick *et al.*[16] Copyright 2003 American Chemical Society. Panel (b) reprinted with permission from Kretsinger *et al.*[31] Copyright 2005 Elsevier Ltd.

even in the absence of serum proteins or, a cell attachment signal like RGD. These hydrogels could be extended to three-dimensional cell culture by incorporating matrix metalloproteinase (MMP) enzyme cleavable sites, allowing cells to degrade them as they migrate and proliferate[21] as well as possess shear-thinning properties, allowing them to be injected as a viscous liquid and then re-gel *in vivo*.[23]

α-Helix/Coiled-Coil

Although the biophysical parameters governing α-helical structure have been known for decades, the key molecular and material design rules for incorporating this structure into artificial scaffolds for cell encapsulation has only recently been elucidated. The basic structure of the α-helix results from hydrogen bonding between backbone amides, yielding a right-handed helix with a periodicity of 3.6 residues per turn. The side chains of the amino acids involved protrude outwards from the helix, and are usually responsible for governing the interactions with other helices. Adapting α-helices to construct self-assembling materials generally involves tuning the structure in order to promote interactions between these side chains, thereby driving association into higher-order structures. In practice, this can be challenging to achieve, and in general longer lengths are usually required to yield stable α-helical interactions (20–30 residues in some cases) compared with β-sheets.

To assemble α-helices into higher order structures, the most common approach relies on using the "coiled-coil" motif: amphiphilic helices (with nonpolar residues on one face, and polar/charged residues on the other) such that they interact due to hydrophobic forces that can be further stabilized by charge interactions.[38] Despite the extensive literature on α-helix design and biophysical properties, not until 2009 was a hydrogel mainly composed of this motif reported by the Woolfson laboratory (Fig. 2a).[3] This design relied on two key innovations: (1) overhanging "sticky ends" (analogous to sticky ends in DNA) between coiled-coils in order to promote their assembly into long fibers; and (2) multiple weak interactions between the fibers to promote hydrogel formation. These α-helical hydrogels were explored as cell culture matrices, and were found to support adhesion, proliferation, and differentiation of PC12 cells into neurons, despite having no biological signals specifically engineered into their structure.[3] To further encourage bioactivity, the Woolfson group recently elaborated these α-helical hydrogels with the RGD epitope using click chemistry with an unnatural azide-containing amino acid. (Fig. 2b).[43]

Triple-helical Collagen Mimetic Peptides

Collagen is the most abundant ECM-derived protein in the human body, so it is not surprising that it has been used to a great degree as a scaffold for biomaterials. The vast majority of these investigations have focused on using naturally occurring collagen, which is ~1000 amino acids in length, and assembled into a complex hierarchical structure that spans several length scales. In parallel, however, several groups have investigated a more "bottom-up" approach towards collagen biomaterials, seeking to recreate the structural and functional properties of the protein with shorter peptide sequences. Although the amino acid sequence of native collagen is complex and varies greatly among the different types, one conserved motif is the GOX or GXO repeating triad (where X represents a different amino acid, in most cases proline, and O denotes hydroxyproline).[13] The signature structural component of a collagen fiber occurs when three peptide chains come together to form a triple helix.

Although collagen-mimetic peptides (CMPs) that possess the requisite triad repeat have been synthesized,

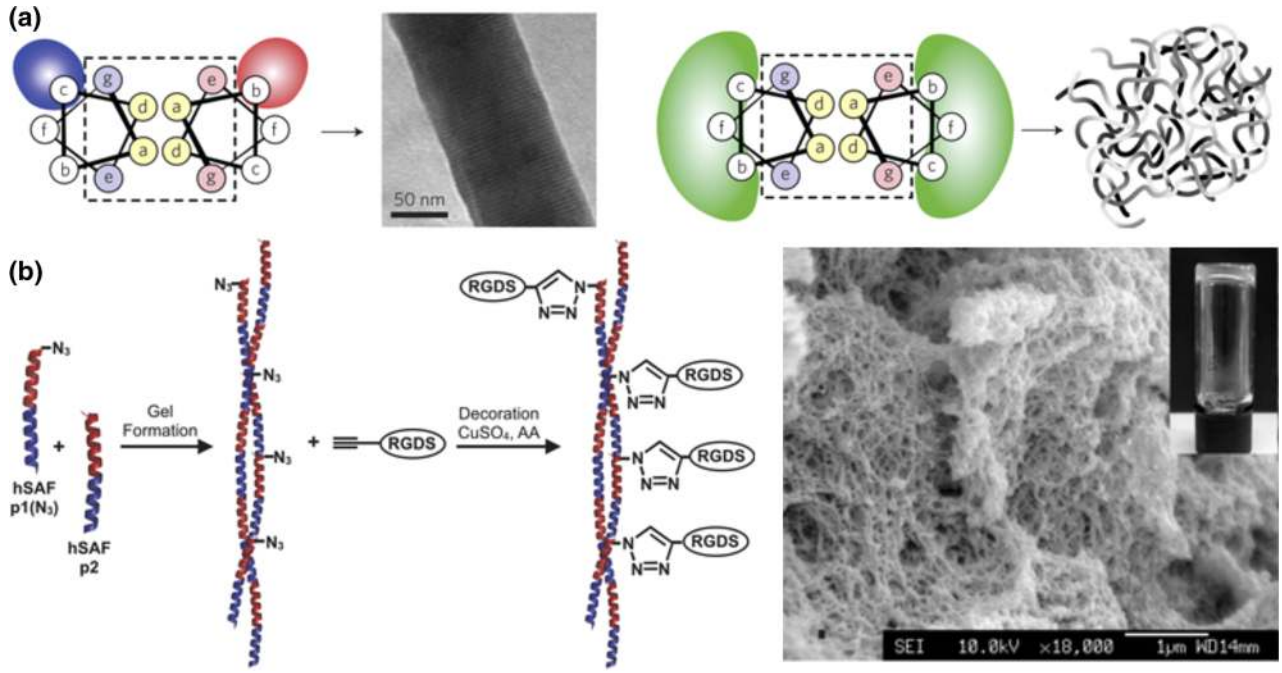

FIGURE 2. (a) Two different coiled-coil designs by the Woolfson group where changing ionic interactions between residues b and c yielded thick fibers (left), while changing residues b, c and f for weaker interaction such as hydrophobic and H-bonding resulted in fibrous hydrogels (right). (b) The same group was able to functionalized their coiled-coil peptides with RGDS via click chemistry without interrupting the formation of fibrous hydrogels as determined by SEM. Panel (a) reprinted with permission from Banwell *et al.*[3] Copyright 2009 Macmillan Publishers Ltd. Panels (b) reprinted with permission from Mehrban *et al.*[43] Copyright 2014 Wiley–VCH Verlag GmbH & Co.

building a biomaterials scaffold would require that multiple such subunits assemble into longer fibers that can then form inter-strand crosslinks to create three-dimensional structures. The first example of actually assembling CMP trimers into a three-dimensional scaffold came from the Chmielewski group in 2009.[52] Unlike other approaches mentioned thus far, which rely on supramolecular interactions typically found in proteins, the authors took a hybrid synthetic approach for the inter-strand self-assembly, installing metal-binding groups at the termini and center of the CMP peptides. Following assembly into triple helical components, the addition of metal ions resulted in inter-strand crosslinking, and the formation of a collagen mesh that could support and culture HeLa cells (Fig. 3a). The metal-based assembly had the additional advantage of being reversible: adding EDTA (a chelating agent) rapidly reversed the crosslinks and dissolved the material. Recently, the same group incorporated the RGDS adhesion sequences as well as His-tagged growth factors (His-EGF) to promote bioactivity into their scaffold.[25]

The Hartgerink group synthesized the first CMP hydrogels from purely peptide interactions in 2011, building on their previous work elucidating the pattern of amino acids that resulted in efficient and register-specific CMP trimers. Hartgerink and coworkers elucidated the design rules that yielded trimers with sticky-end overhangs that could direct them into longer fibers, usually through ionic and hydrogen bonding interactions.[47] CMPs with the sequence $(PKG)_4(POG)_4(DOG)_4$, assembled into long fibers that were able to form self-supporting hydrogels (Fig. 3b). These gels had similar mechanical properties to native collagen gels, and could even be degraded by collagenase enzymes. Recently, the authors extended their collagen hydrogel for use as a noninflammatory hemostat *in vitro*.[32]

Amphiphilic/Electrostatic Forces

Peptide Amphiphiles

One of the most extensively studied and successful platforms for self-assembling peptides in regenerative medicine is that of peptide amphiphiles (PAs). In 1995, one of the first reports emerged about PAs by Tirrell and co-workers with a design that consisted of a dialkyl ester tail conjugated onto a peptide sequence derived from collagen, which yielded the assembly of a monolayer at the air–water interface.[4] Later in 2001, The Stupp group introduced a PA molecule that could mimic the architecture of ECM fibers after assembly. A PA molecule of this variety typically consists of 4

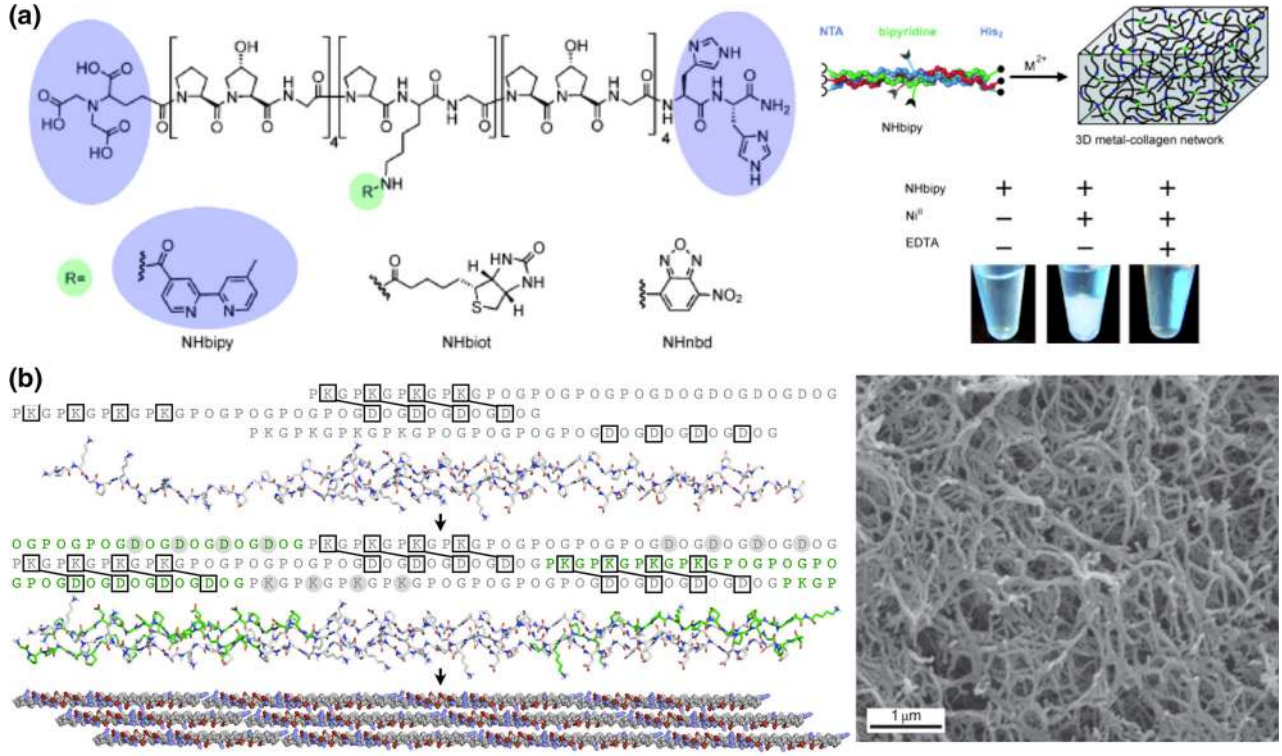

FIGURE 3. (a) Chemical structure of the metal ligand-containing collagen peptide designed by the Chmielewski group (left). In the presence a divalent metal (such as Ni(II)), the peptide is able to form a reversible (with the addition of the metal chelator EDTA) three-dimensional porous collagen scaffold for cell encapsulation. (b) The Hartgerink group was able to design an collagen peptide that self-assembles by electrostatic interaction initiated between Lys and Asp at the 'sticky end' of a collagen triple helix into fibrous hydrogels. Panel (a) reprinted with permission from Pires *et al.*[52] Copyright 2009 Wiley–VCH Verlag GmbH & Co. Panel (b) reprinted with permission from Kumar *et al.*[32] Copyright 2014 American Chemical Society and from O'Leary *et al.*[47] Copyright 2011 Macmillan Publishers Ltd.

regions (Fig. 4a): (1) a hydrophobic tail (usually palmitic acid); (2) a β-sheet sequence; (3) charged residues (to impart water solubility); and (4) an optional biological epitope. When dissolved in water, the hydrophobic tails drive the association of the PA molecules due to hydrophobic collapse and hydrogen bond formation particularly among amino acids in the β-domain.[68] Due to the stiff and planar nature of β-sheet supramolecular structures formed by many molecules, the assemblies of the PAs form one-dimensional objects that emulate the architecture of natural ECM fibers. The β-sheet sequence present in results in cylindrical nanofibers, 6–10 nm in diameter and often many micrometers in length (Fig. 4b). The extremely high aspect ratio of the fibers leads to the formation of self-supporting gels upon charge screening (e.g., in biological fluids), allowing for injection of the solution as a liquid and its gelation *in vivo*.[40] Self-assembly in water in these systems results in tight packing of the molecules within the fibers, and the hydrophobic tail orients the peptide segment toward the aqueous environment. This enables the precise display of signaling epitopes attached to the terminus of the peptide segment at a very high density on the surface of the fiber, allowing for the possibility of potent biological signaling.

Beniash, and Stupp reported the first fiber-forming PA molecule in 2001 and from the start the great biological potential of this platform was evident.[24] Because of the modular nature of the self-assembled fiber, multiple biological signals could be included, such as a phosphoserine residue and an RGDS signal. The nature of the PA one-dimensional nanostructures and the presence of a phosphoserine residue in the peptide segment allowed the mineralization of the nanofibers with hydroxyapatite in ways that mimics the nanoscale structure of bone ECM, becoming our first effort to integrate hydroxyapatite into polypeptides to create bone-interfacing biomaterials.[45]

In 2010, the Stupp group discovered a remarkable physical transformation that can occur in certain PA molecules that are treated by a thermal annealing process to form a liquid crystalline solution. When sheared gently (e.g., as by the force of pipetting), these PA solutions formed aligned monodomains; applying this shear in the presence of divalent cations like calcium resulted in string-like "noodle" gels with fiber alignment over macroscopic distances (Figs. 4c, 4d).[74]

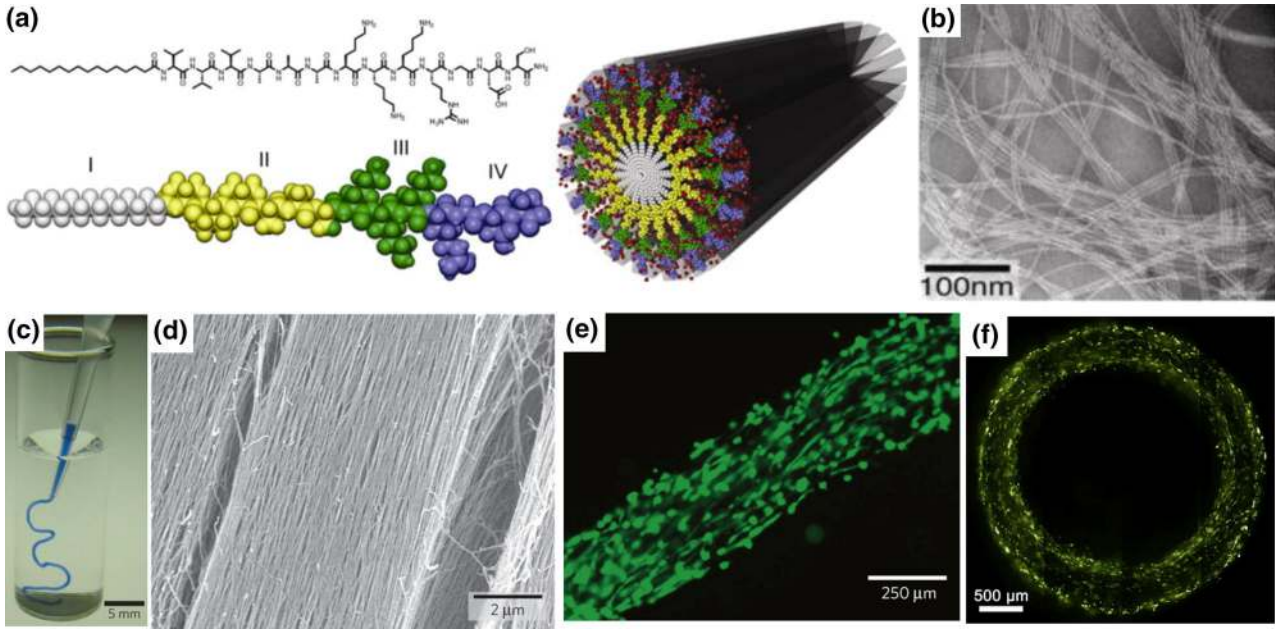

FIGURE 4. (a) Chemical structure of a canonical peptide amphiphile (PA) molecule and a pictorial representation of their assembly into nanofibers. The four regions depicted above represent the unbranched alkyl (usually palmitic acid) tail (region I), a β-sheet amino acid sequence to promote cohesion through H-bonding (region II), charged amino acids for solubility and fiber crosslinking (region III) and a peptide signaling epitope to induce a biological response (region IV). (b) TEM micrograph depicting the self-assembly of PAs in solution as high aspect ratio of nanofibers with micrometer length. (c) Formation of a PA, coloured with tryptan blue into a noodle-like string in a phosphate-buffered saline solution. (d) SEM micrograph of the PA gel strings showing highly aligned PA nanofibers bundles. (e) These PA gel strings can be used to efficiently encapsulate and align cells, such as hMSCs, along the axis of the gel. (f) Alignment of fluorescently stained SMCs along a PA tube gel after 7 days in culture for the potential application of artery reconstruction. Panel (a) and (b) reprinted with permission from Matson *et al.*[40] Copyright 2011 Elsevier Ltd. Panel (c), (d) and (e) reprinted with permission from Zhang *et al.*[74] Copyright 2010 Macmillan Publishers Ltd. Panel (f) reprinted with permission from McClendon and Stupp[42] Copyright 2012 Elsevier Ltd.

These gels could readily encapsulate cells prior to the alignment process (difficult with other methods such as electrospinning), and mesenchymal stem cells were shown to extend their processes along the aligned axis of the fibers (Fig. 4e). Another regenerative medicine target that would uniquely benefit from the natural alignment in these gels is blood vessel engineering, given the natural alignment of vascular smooth muscle cells (SMCs) circumferential to the blood flow. Applying a rotational shear to the heat-annealed PA, while simultaneously exposing it to gelation conditions resulted in a circular gel with the nanofibers aligned along the circumference.[42] SMCs could be incorporated in the material prior to shear and gelation, and were found to align with the nanofibers to form the native circumferential arrangement of cells present in native blood vessels (Fig. 4f).

Electrostatic Self-complementarity

Most of the examples mentioned above relied on facially amphiphilic peptides or helices to drive the assembly, with ionic interactions playing a secondary role for solubility and in enforcing the register or polymerization of the components into higher-order structures. Electrostatic effects, however, can be used as a primary driving force to assemble the individual molecules as well. This approach was first developed by the Zhang laboratory in 1993 with the sequence $(AEAEAKAK)_2$ derived from the yeast protein zuotin.[73] The alternating sequence of charged and hydrophobic residues mirrors the β-sheet peptides described above, but with the added ionic attraction between the positively charged K and negatively charged E residue side chains. The amphiphilic nature of the peptides resulted in their assembly into fibers, with the A residues packing together and the ionic faces assembling in a staggered conformation (Fig. 5a).

Shortly after the first report, ionic self-complementary peptides were used as scaffolds for cell encapsulation and attachment. The work utilized both the zuotin-derived sequence mentioned above, as well as a new sequence $(RARADADA)_2$ or RADA16, that replaces the E residues with D and the K residues with R (Fig. 5b).[72] These materials formed, upon charge screening with physiologically relevant salts, membranes comprised of the peptide fibrils with porous spaces between them. A number of cell lines were able to attach and grown on the membranes, interestingly, through a distinct mechanism relative to RGD-based

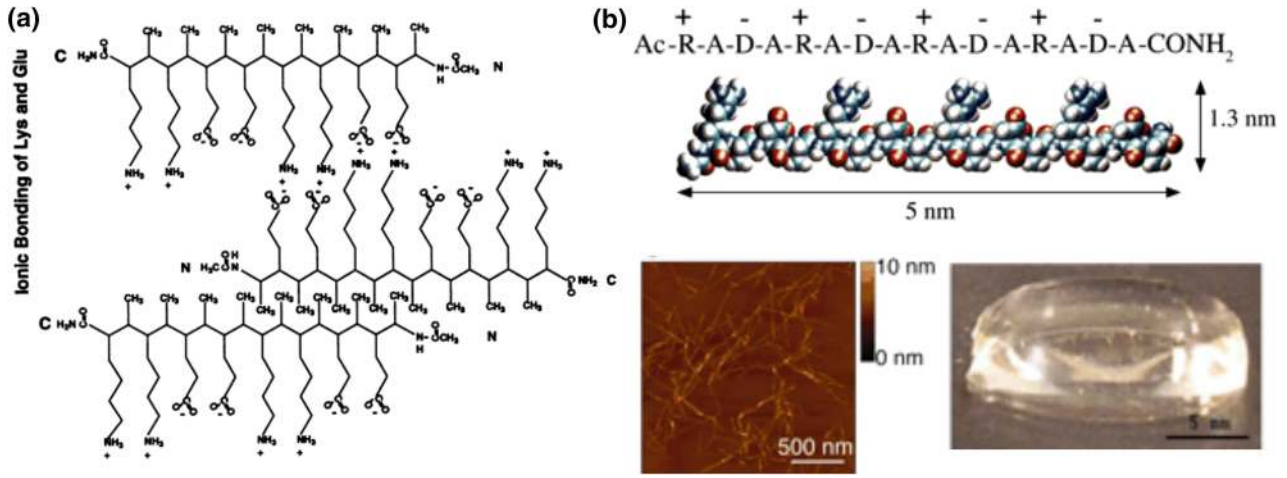

FIGURE 5. (a) Proposed mechanism of the assembly of EAK16 via antiparallel H-bonding in addition to the hydrophobic interactions between the alanine side chains and the ionic interactions between the charged Lys and Glu residues. (b) Diagram of the RADA16 molecule composed of both the positive (Arg) and negative residues (Asp) to promote the assembly of the peptide by electrostatic interactions into fibers and macroscopic hydrogels in buffer. Panels (a) and (b) reprinted with permission from Zhang *et al.*[73] and Yokoi *et al.*,[72] respectively Copyright 1993 and 2005 Proceedings of the National Academy of Science.

adhesion, as pre-incubating the cells with soluble RGD peptide did not inhibit their interaction with the membranes. The RADA-based peptide gels were also applied to generate hepatocyte spheroids from liver progenitor cells[57] or co-assembled with sequences from laminin or type IV collagen to enhance endothelial cell function.[20]

IN VIVO STUDIES

The peptide systems discussed above were designed to self-assemble into nanostructures and subsequent hydrogel scaffolds for biological applications. Thus far in our discussion we have highlighted proof-of-principle systems *in vitro*, but several of these platforms have already made the transition to more involved *in vivo* studies. In this section, we will discuss the efforts and challenges faced by our research group and others to translate these peptide-based biomaterials into functional devices or implants in regenerative medicine in the areas of neural, bone, and vascular regeneration.

Neural Regeneration

Damage to the central nervous system (CNS) due to injury or disease generally leads to devastating consequences with permanent disabilities or functional deficits. Re-establishing axonal connectivity between specific sets of neurons, which have been disrupted due to injury or disease, is essential for functional recovery. CNS injury that causes acute neuron degeneration often also leads to the formation of a glial scar at the site of injury due to proliferation of reactive astrocytes. This scar strongly impedes spontaneous regeneration of the growing nerve ends both by forming physical barrier as well as by secreting inhibitory molecules that block neurite growth.[59] Although the regenerative potential of the mammalian CNS is much less pronounced compared with fish and amphibians, evidence of axonal regrowth has been observed, especially when provided with an appropriate micro-environment.[26]

Fibrous peptide scaffolds can often be gelled by contact with tissue fluid for targeted delivery as well as evoke a lower inflammatory and immune response due to the low immunogenicity of the short self-assembling monomers as shown in an early study using self-assembled peptide materials, where the extent of infiltration of macrophages after injection of in the rat spinal cord was found to be similar to control saline injection.[19] Scaffold design pertaining to control over biological signal presentation,[62] nanostructure [44] and mechanical properties [51] have also been shown to influence neuronal adhesion, differentiation, neurite growth and directional guidance in a culture environment.[29,55] For example, a PA nanofiber scaffold presenting the laminin-derived epitope IKVAV was shown to selectively induce the differentiation of the neural stem cells into neurons (Fig. 6a).[58] Subsequent *in vitro* investigations confirmed the role of the IKVAV PA on improving neuronal survival and enhancing neurite outgrowth.[63,65] In addition, tuning the stiffness of the PA nanofibers has been demonstrated to significantly influence the developmental course hippocampal neurons (Fig. 6b).[64]

Unlike many other polymeric scaffolds, cells can be easily encapsulated in peptide scaffolds, such as the delivery of neural stem cells (NSCs) encapsulated in an

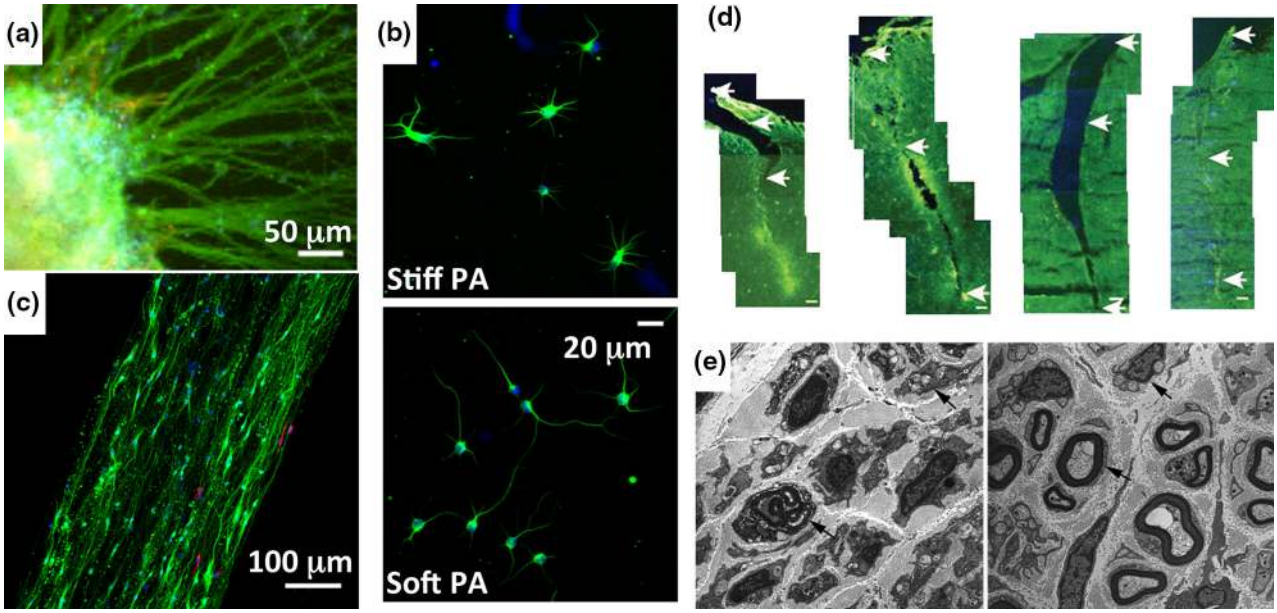

FIGURE 6. Self-assembled peptide materials for nervous tissue regeneration. (a) Neural stem cells (NSCs) differentiated when cultured with IKVAV epitope-presenting PA matrix (green, beta-tubulin III; red, GFAP). (b) Faster development of hippocampal neurons was observed on a softer PA substrate. (c) Aligned PA nanofibers guided the direction of neurite outgrowth. (d) Transmission electron micrographs of sections of cavernous nerve in adult rats after crush injury and treatment with BSA PA (left) or SHH PA (right) for 6 weeks. Myelinated and nonmyelinated fibers (indicated by arrows) show and an enhanced regeneration after SHH PA treatment. (e) Self-assembled peptide nanofiber scaffolds (SAPNS) were used to treat optic tract transection, where the lesion was made at superior colliculus (SC) in 2 days old hamster (left to right). Dark-field images of lesion in control and SAPNS injected animals after 1 day and after 30 days. Panel (a) reprinted with permission from Silva *et al.*[58] Copyright 2004 American Association for the Advancement of Science. Panels (b), (c) and (d) reprinted with permission from Sur *et al.*[64], Berns *et al.*[5] and Angeloni *et al.*[2] Copyright 2013 Elsevier B.V. Panel (e) reprinted with permission from Ellis-Behnke *et al.*[15] Copyright 2006 Proceedings of the National Academy of Science.

IKVAV epitope-presenting peptide scaffold to a rat brain wound defect.[8] The highly aligned nature of the axon tracts in spinal cord and certain other parts of brain has generated a substantial interest in developing scaffolds with fiber alignment to guide regenerating axons. Recent discovery of aligned monodomain gel afforded guidance of the neurite extension of encapsulated neurons (Fig. 6c) and also opened up the possibility of forming such scaffolds *in situ* within the spinal cord.[5] The effects of the IKVAV PA on neuronal differentiation, survival and neurite outgrowth *in vitro* inspired the Stupp and Kessler laboratories to test the regenerative potential of this material *in vivo*. Severe acute spinal cord injury (SCI) was performed at the T10 vertebral level in the adult mouse using a clip compression technique and IKVAV-bearing PA was then injected at the lesion site after 24 h of injury.[67] The IKVAV PA-treated animals demonstrated significantly higher BBB scores 9 weeks after injury compared with control animals injected with a nonbioactive PA or glucose. Histological analysis of the spinal cord demonstrated reduced astrogliosis and apoptotic cell death at the site of injury, associated with a significantly higher number of regenerating descending and ascending nerve fibers across the lesion site. PA scaffolds have also been investigated for auditory nerve regeneration in a rat model with transplantation of embryonic stem cells [49] as well as for peripheral nerve regeneration by delivering sonic hedgehog (SHH) in a rat cavernous nerve (CN) injury model (Fig. 6d).[2] Recently, PLGA scaffolds filled with aligned PA nanofibers were used as a simultaneous scaffold for axon regrowth and remyelination to regenerate the peripheral nervous system.[35]

In a separate study, the ability of a RADA16 peptide hydrogel to regain lost CNS function was examined by the Schneider group in a hamster model of an optic tract lesion.[15] In these experiments, the optic tracts of the animals were surgically transected to impair their vision, and then the fibers were injected at the lesion site. Substantial axon regeneration was observed across the lesion in scaffold-treated animals, accompanied with a functional return of vision (Fig. 6e).

Bone Regeneration

During osteogenesis, bone morphogenetic proteins (BMPs) regulate chemotaxis, mitosis, and differentiation of mesenchymal stem cells into osteoblasts.[54] The recombinant forms of BMP-2 and BMP-7, delivered

using an absorbable collagen sponge as a carrier, have been used clinically and have demonstrated efficacy in promoting improved bone regeneration and spinal fusion.[61] However, the supra-physiologic amounts of BMPs required for sufficient biological response can lead to complications including heterogeneous ossification, inflammation, and even bone resorption. One way to circumvent these side effects is by developing new BMP-2 carrier matrices that improve the localization of the osteogenic growth factor within the defect site, thereby reducing the amount necessary to promote the desired clinical outcome. In this context, supramolecular PA nanofibers have been investigated in depth as a synthetic ECM to both deliver growth factors while simultaneously serving as an organic template to promote bone formation.[48] When comparing different supramolecular assemblies, it has been found that the cylindrical nanostructures can direct the growth of HAP crystals along the long-axis of the fibrils, while flatter, ribbon-like nanostructures fail to promote the orientation found in biological systems.[46]

When PA nanofiber gels were implanted in a rat critical-size femoral defect model for four weeks, even in the absence of exogenous growth factors, the phosphorylated PA nanofiber gel promoted significantly higher bone formation relative to controls lacking the phosphorylated residues, even to the extent observed in animals treated with a clinically-used bone allograft.[39]

In the ECM, heparan sulfate-like glycosaminoglycans (HSGAGs) bind growth factors and enhance their signaling (Fig. 7a).[7] Accordingly, a PA was designed to bear a Cardin-Weintraub heparin-binding domain that would bind HSGAGs, creating a synthetic ECM with the potential to localize various growth factors.[53] Since BMP-2 possesses a heparin-binding domain, the HBPA-heparan sulfate system was evaluated *in vivo* to deliver BMP-2 and emulate native BMP-2 signaling (Fig. 7b).[33] When evaluated in a rat critical-size femur defect model (10 mm gap) using only 1 μg BMP-2, a dose less than one tenth the usual required dose for union in this model, the nanofiber system promoted enhanced bone regeneration with a high probability of

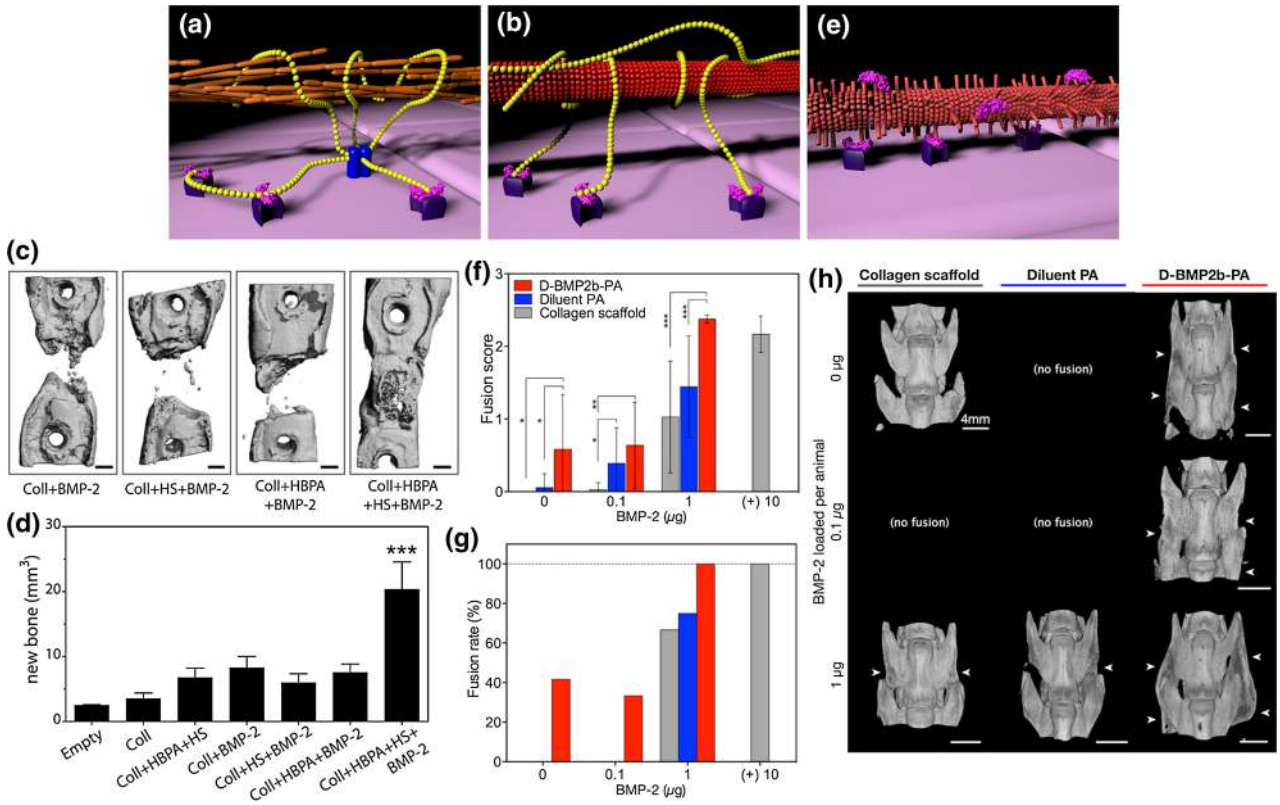

FIGURE 7. (a) In the ECM, the heparan sulfate (yellow)-syndecan (blue) complexes bind BMP-2 (pink) and regulate its interaction with the cell-surface receptor (purple). Fibronectin fibers (orange) also shown. (b) HBPA, which contains a consensus heparin-binding domain, can utilize heparin or heparan sulfate to deliver BMP-2. HBPA nanofibers-heparan sulfate nanofibers promoted enhanced bone regeneration in a rat critical-size defect model, as shown by (c) microcomputed tomography (μCT) reconstruction and (d) the amount of new bone. (e) PA nanofibers designed with an affinity to BMP-2 (BMP2b-PA) can bind the growth factor directly. The BMP-2-binding PA promoted greater spinal fusion than PA nanofibers without BMP-2-binding epitopes (Diluent PA) and absorbable collagen scaffold, as shown by (f) fusion scores from blind manual palpation analysis at 8 weeks, (g) fusion rate, and (h) representative μCT reconstruction. Panels (a–d) reprinted with permission from Lee *et al.*[33] Copyright 2014 Elsevier B.V. Panels (f–h) reprinted with permission from Lee *et al.*[34] Copyright 2014 Wiley–VCH Verlag GmbH & Co.

bridging (Fig. 7c). In order to create a completely synthetic ECM for bone regeneration, a new PA was also engineered to contain a peptide sequence TSPHVPYGGGS, discovered via phage display, which possesses a binding affinity to BMP-2 (Figs. 7d, 7e).[34] This BMP-2-binding PA augmented BMP-2-induced osteoblast differentiation of C2C12 pre-myoblasts *in vitro*. When evaluated in a rat posterolateral lumbar spinal fusion model, the PA nanofibers displaying the BMP-2-binding segments promoted superior spinal fusion rates, effectively lowering the required therapeutic dose of BMP-2 by a factor of ten. Interestingly, the bioactive nanofiber gel promoted a 42% fusion rate even in the absence of exogenous BMP-2, suggesting the ability to recruit endogenous growth factors and potentiate osteogenic signaling (Figs. 7f, 7g and 7h).

Angiogenesis

Angiogenesis is the process of sprouting and stabilizing of new blood vessels from existing ones. This process is important to cell survival and microvasculature perfusion for chronic wound healing, alleviation of ischemic tissue as in occurs in patients with type 1 diabetes,[66] and the regeneration of cardiac tissue after an episode of myocardial infarction (MI).[28] However, angiogenesis requires the spatially and temporally controlled expression and/or delivery of various angiogenic molecules such VEGF and FGF2 among others.[17]

Early work in this field in the Stupp laboratory used PA nanofibers with the ability to localize heparin, in order to deliver FGF2 and VEGF and promote the growth of new blood vessels. For this purpose, the heparin-binding PA (HBPA) gels (as mentioned in the bone regeneration section) containing both VEGF and FGF2 (HBPA-GF) were used and able to significantly increase neovascularization as compared with natural collagen gels in a rat cornea model.[53] In the following work, the HBPA-GF matrix was used for the treatment of type 1 diabetes and able to improve the engraftment of islets, induce vascularization and improve normoglycemia by 78% in a diabetic mouse model.[60]

Even though nanostructures protect growth factors from degradation and efficiently release them at the desired site, they are expensive and laborious to produce. As a result, there is a great effort in the field of protein and peptide chemistry to engineer short peptide sequences that can recapitulate the bioactivity of growth factors.[10] Towards this aim, a VEGF-mimicking PA nanofiber (termed VEGF-PA) was designed

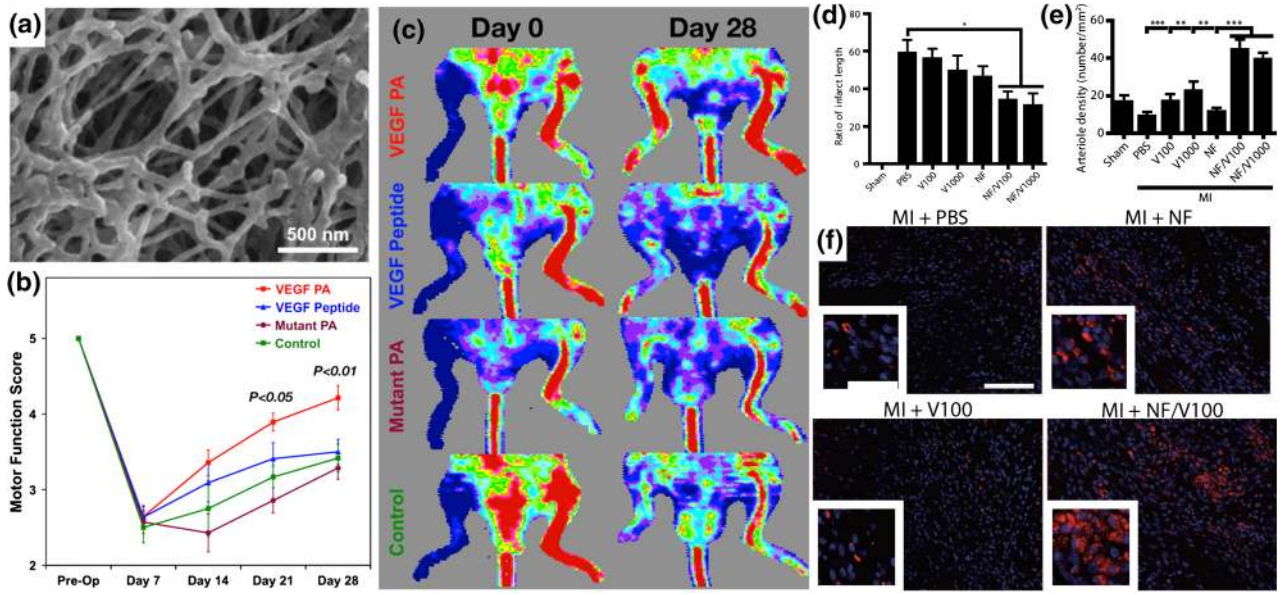

FIG. 8. (a) VEGF-PA, which contains the VEGF-mimetic sequence (QK) located at the C-terminus of the PA, can self-assemble into fibers to form a three-dimension gel as determined by (b) scanning electron microscopy (SEM). (b) Motor function scores were significantly higher when the PA was used in comparison to (QK), the mutant VEGF-PA and the saline control. (c) Laser Doppler Perfusion Imaging confirms the positive effect of VEGF-PA by portraying higher perfusion ratios after 28 days post-injection. (d) After 14 days post-injection of NFs with 100 and 1000 ng/mL (NF/V100 and NF/V1000), the infarct size has significantly decreased in size and (e) the arteriole density has increased indicating improved cardiac performance on the MI rat models through arteriogenesis (f) Dil fluorescence (red) corresponding to significant recruitment of circulating bone marrow cells (BMCs) into the infarct area with NF/V100. Panels (a–c) reprinted with permission from Webber *et al.*[69] Copyright 2012 Proceedings of the National Academy of Science. Panels (d–f) reprinted with permission from Lin *et al.*[36] Copyright 2012 American Association for the Advancement of Science.

containing the active peptide sequence KLTWQELYQLKYKGI (QK), derived from helix residues 17–25 of the native protein.[9] The VEGF-PA fibers was shown to effectively stimulate proliferation and migration of HUVECs *in vitro* through the phosphorylation of VEGFR1 and VEGFR2 (Fig. 8a).[69] To determine if the VEGF-PA could be used as a therapy for angiogenesis, the material was administered into a critical hind-ischemia model in the right femoral artery of mice. After 28 days, the leg treated with VEGF-PA showed a significant improvement in both motor function and tissue perfusion compared with the control leg (Figs. 8b, 8c).

Promoting angiogenesis can also be highly beneficial in cardiovascular therapies for myocardial infarction (MI), which is considered one of the leading causes of premature death.[71] After an episode of MI, there is a need for neovascularization to compensate for the drastic myocardial cell loss and pathological remodeling. In a recent study, RADA16-based nanofibers (NFs) were used for the regeneration of cardiac tissue in myocardial infarction rat and pig models, as inspired by previous work with this self-assembling peptide.[11] In their *in vivo* studies, Hsieh and coworkers encapsulated the angiogenic growth factor VEGF within the NFs at the infarcted site and detected a decreased infarct size as well as improved systolic function as compared with VEGF or the NFs alone after 28 days of injection (Fig. 8d).[36] Interestingly, they found that their procedure promoted arteriogenesis instead of angiogenesis, by analyzing vascular densities (Fig. 8e) and due to the presence of myofibroblast mural cells (Fig. 8f).

CONCLUSIONS

The examples we have covered in this review highlight the powerful functions and promise of self-assembled peptides for constructing bioactive matrices for regenerative medicine. In the past two decades, these systems have advanced from fundamental studies of novel self-assembly principles, to initial exploratory results using *in vitro* systems, to translationally-relevant *in vivo* models for novel therapies. Suites of self-assembly motifs and supramolecular interactions have been explored, and a diverse range of potential biomedical targets for clinical translation has been identified by recent research. However, the combination of biologically relevant signals and the similarity of noncovalent interactions utilized by biological systems and self-assembling peptides have allowed these materials to replicate certain features of the ECM in ways that are not possible with other approaches. Certain key challenges remain that must be addressed in order to realize the full potential of peptide-based materials. Only a handful of the platforms we discussed have been applied successfully to *in vivo* models. This suggests that many more potent platforms may exist with translational potential. However, a number of hurdles need to be overcome towards translation of these materials, not least, of which are the support of regulatory and federal agencies of costly translational research.

Another important horizon for biomaterials in regenerative medicine is to capture the highly dynamic nature of the ECM, with extensive remodeling in both time and space by cells. Although transplanted or endogenous cells will, to some extent, remodel a synthetic matrix, specifically engineering dynamic triggers that allow its composition and structure to be changed would be highly beneficial. Already some examples exist of dynamically triggered biomaterials and this field will continue to expand in order to imbue artificial materials with advanced function.[6] It should be noted that self-assembly is a natural candidate for creating such adaptable materials, as the components are not covalently fixed, and the supramolecular forces that hold them together can be reconfigured to change their properties.

In closing, we believe that self-assembled peptide matrices are necessary for the development of next-generation biomaterials, and will also be critical for cell therapies. They have shown great promise thus far, and with increased engineering and a greater push to *in vivo* investigations, we are confident that these materials will play a central role in regenerative medicine in the future.

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