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Peptide Self-Assembly for Crafting Functional Biological Materials

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Abstract

Self-assembling, peptide-based scaffolds are frontrunners in the search for biomaterials with widespread impact in regenerative medicine. The inherent biocompatibility and cell signaling capabilities of peptides, in combination with control of secondary structure, has led to the development of a broad range of functional materials with potential for many novel therapies. More recently, membranes formed through complexation of peptide nanostructures with natural biopolymers have led to the development of hierarchically-structured constructs with potentially far-reaching applications in biology and medicine. In this review, we highlight recent advances in peptide-based gels and membranes, including work from our group and others. Specifically, we discuss the application of peptide-based materials in the regeneration of bone and enamel, cartilage, and the central nervous system, as well as the transplantation of islets, wound-healing, cardiovascular therapies, and treatment of erectile dysfunction after prostatectomy

Keywords

Peptide amphiphiles; Self-assembly; Bioactive materials; Regenerative medicine; Bone regeneration; Enamel regeneration; Cartilage regeneration; Angiogenesis; Islet transplantation; Bioactive membranes

1. Introduction

Demand for longer human lifespan and higher quality of life has long been the motivation behind the development of new medical technologies. A growing understanding of biology, chemistry, and medicine has led to the exploration of increasingly complex therapies, beginning with plant extracts, moving to small molecule synthetic drugs, and culminating in large protein therapeutics. The field of biomaterials has moved in the opposite direction, shifting from bulk materials towards nanomaterials that are increasingly well defined on the molecular and nanoscale. A highly promising class of biomaterials for medicine is composed of biocompatible small molecules capable of breaking down over time into

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benign metabolites. By rationally designing these small molecules to take advantage of secondary interactions, bottom-up construction of nanostructures such as spheres, cylinders, tubes, and other novel morphologies can occur via self-assembly in aqueous solution [1, 2]. Self-assembly can be further utilized to form functional hydrogels capable of supporting and signaling cells. In the design of self-assembling small molecules for medicine, units that are both biodegradable and bioactive should be preferred. The common building blocks of biology—sugars, amino acids, and nucleic acids—are inherently biodegradable and biocompatible and can provide an extensive platform to build new materials for cell signaling, proliferation, and differentiation. Peptide based materials represent an especially promising class of compounds due to their relative ease of synthesis and most importantly they have the same chemical structure of biological signals. With 20 canonical amino acids and many more that are synthesized in the body by post-translational protein modification, a great diversity of sequences can be synthesized. Incorporation of non-natural amino acids for bioconjugation, drug release, or *in vivo* molecular imaging is also generally facile [3]. The capabilities of rationally designed, biofunctional materials built from self-assembling, peptide-based building blocks are vast. Here we discuss some of the most important peptide-based functional gels and membranes investigated for therapeutic applications over the past few years.

2. Synthesis and self-assembly of peptide-based biomaterials

2.1 Self-assembling peptide amphiphiles

Peptide amphiphiles (PAs) are a broad class of molecules that have received significant attention over the past decade due to their potential as novel biomaterials for regenerative medicine [4, 5]. The Stupp laboratory has developed a class of PAs comprised of four domains, with the general structure shown in Figure 1 [1]. Domain I contains a hydrophobic moiety, usually an unbranched alkyl group with length and composition that can be changed to modify PA properties [6]. Palmitic acid, the most commonly used alkyl tail, is a natural 16-carbon saturated fatty acid found in many plant oils and proteins. Domain II is comprised of a β -sheet forming peptide sequence which can further be adjusted in order to tune PA mechanical properties and gelation kinetics [7, 8]. The formation of β -sheets is also important in directing nanostructure morphology, which can range from cylinders to twisted ribbons to larger nanobelts [9-11]. Domain III typically consists of one to three charged amino acids to impart aqueous solubility and further regulate gelation. Domain IV, which is not a structural necessity, typically incorporates a bioactive signaling epitope that is displayed on the nanofiber surface [12, 13].

The self-assembly of PAs in water is driven by hydrophobic collapse of alkyl tail domains and hydrogen bonding between β -sheet regions of neighboring PA molecules. The canonical PA assemblies are fibers that are approximately 6-12 nm in width and up to several microns in length [14]. Transmission electron microscopy (TEM), scanning electron microscopy (SEM) and small angle X-ray scattering (SAXS) are used to characterize PA assemblies (Figure 1C and 1D). Screening of charged residues by a change in solution pH or addition of salt or divalent counterions causes gelation of a PA solution even at concentrations of less than 1% by weight. The mechanical properties of the resulting gels can be tuned by adjusting any of the three structural domains of the PA as well as the method of gelation. Typical storage modulus values for PA gels are on the order of 10 kPa [15]. These highly hydrated PA nanofiber gels can display a high surface density of epitopes, making them ideal matrices for promoting cell viability and function as well as for regulating protein release *in vivo*. Additionally, PAs can be aligned using a heating and cooling process to achieve highly ordered, monodomain gels [16].

2.2 Self-assembling peptides

Self-assembling peptide systems based on molecules without a designated alkyl tail domain have also been designed. Inspired by the yeast protein zootin, Zhang has developed a series of self-complementary 16-residue peptides with alternating charged and uncharged residues [17, 18]. Upon β -sheet formation, these oligopeptides present one polar face with charged ionic side chains and one non-polar surface with hydrophobic side chains. Self-assembly of the folded peptides into one-dimensional nanostructures is driven by collapse of the hydrophobic face and is further stabilized by the polar face [19]. Gelation can be achieved by charge screening in a process akin to PA gelation. Similarly, a β -hairpin 20-residue peptide system has been developed by Pochan and Schneider, whereby assembly and interdigitation of the rod-like peptides under basic conditions leads to network formation (Figure 2) [20-22]. The shear-thinning behavior of β -hairpin gels allows for injection of the gel through a syringe followed by rapid stiffening at the injection site [23]. Self-assembling peptide-based materials that rely on intermolecular β -sheet structures (e.g. β -sheet tapes) have also been reported. The most notable examples include those made by Aggeli and Boden, which are inspired by the transmembrane domain of the channel-forming IsK protein [24, 25]. Hartgerink has also developed a series of peptide materials that form one-dimensional objects in solution based on ABA block motifs, where the B block is driven to assemble into β -sheets while the A block limits assembly [26]. Tuning the composition of the blocks and the method of gelation allows for variation in mechanical properties [27]. Similar to PAs, the Q11 peptide motif developed by Collier consists of a glutamine-rich sequence that can self-assemble into nanofibers with high β -sheet content and form nanofiber hydrogels at low concentrations [28, 29]. A common feature of these self-assembling peptides is the formation of 1D supramolecular aggregates spontaneously or by pH change and the formation of nanofiber networks by addition of salts or multivalent counterions.

3. Peptide-based hydrogels as synthetic matrices for tissue engineering

Interest in synthetic matrices for tissue repair and cell-based therapies has gained momentum due to the realization that cell viability and behavior are drastically affected by the chemical and mechanical properties of the surrounding environment. Synthetic scaffolds for cell culture and delivery have been developed in order to improve cellular function and retention at the treatment site. While these scaffolds have traditionally been polymeric, peptide-based hydrogels can more closely mimic the porosity and nanostructure of native extracellular matrix and can more readily incorporate signaling epitopes for cellular interaction. By taking advantage of bioconjugation techniques and orthogonal chemistry, peptide-based gels can be readily modified to display a wide variety of biologically relevant signals for enhanced therapeutic function. These signaling epitopes may be either peptidic or non-peptidic in nature. Common biomimetic peptides include the fibronectin-derived RGDS sequence [30-32], the laminin-derived IKVAV sequence [33], and the highly phosphorylated dentin-derived and bone sialoprotein-derived DS(P)S(P) and S(P)D sequences, where S(P) is phosphoserine [14, 34-37]. Several of the non-peptidic components that have been incorporated into PAs include fluorescent groups [38], non-peptidic signaling molecules [39], and nucleic acids [40], all of which have been attached to the canonical PA with little or no distortion to nanofiber morphology. The peptide-based molecules discussed here self-assemble into highly hydrated gels, relying on secondary interactions such as ionic bonding, β -sheet formation, and hydrophobic collapse to drive gel formation. In fact, in most cases gelation can be triggered using simple salt solutions [33, 41]. Due to the biocompatibility of this process, self-assembling peptide-based hydrogels are well suited for the facile encapsulation and 3D culture of both mature and proliferating cells.

3.1 Bone regrowth and repair

Traditional therapeutic strategies for injuries requiring bone regeneration have relied on the use of autografting. However, problems resulting from donor-site morbidity and patient discomfort have led to the exploration of a variety of peptide-based and other hybrid materials for use as synthetic biocompatible matrices for bone regeneration [42, 43]. A 2001 report from our group demonstrated that PAs displaying a phosphoserine residue and RGD cell-adhesion epitope can induce mineralization of hydroxyapatite (HAP), the crystalline inorganic component of bone, from solution onto a 2D substrate [14]. Most strikingly, the crystallographic c-axis of the mineralized HAP was shown to be aligned with the long axis of the PA nanofiber, indicating that PA materials can template the growth of the basic hierarchical organization in bone tissue. Research has further shown that 3D PA nanofiber gels can also nucleate HAP using alkaline phosphatase and an enzymatically-degradable organic phosphate source to regulate phosphate concentration [36].

PA gel matrices can provide advantageous biological cues for bone repair *in vivo* but are typically too soft to provide mechanical support in cases requiring load-bearing bone replacements. While traditionally used metal implants can provide the desired mechanical properties, they inherently lack the bioactivity needed to promote new bone formation. We addressed this issue by loading a 52% porosity titanium foam with pre-osteoblastic MT3T3-E1 cells and a PA mixture containing: 1) 5% of a bioactive PA that displayed an RGDS epitope and 2) 95% of a mineralization-promoting PA that contained a phosphoserine residue [44]. *In vitro* studies with such PA-loaded titanium foam plugs showed maturation of cells along the osteoblastic lineage and proliferation of cells throughout the hybrid material [45]. In a rat bone plug model, new bone growth was observed in and around the titanium plugs, with evidence of neovascularization around the implant also noted (Figure 3) [44]. These results indicate that this hybrid system is capable of promoting accelerated bone growth at the interface between implant and native bone.

PA gels assembled with 95% phosphoserine-displaying PA and 5% RGDS-displaying PA were further tested for their ability to regrow bone *in vivo* without the structural use of titanium foam [37]. Using a rat femoral defect model, PA gel was added into the critically sized defect while a titanium plate was used to secure the defect space. New bone growth was measured by micro-computed tomography and histology, showing enhanced bone regeneration in response to phosphoserine-displaying PA as compared to untreated controls and controls with only RGDS-displaying PA (Figure 4).

In other work, Jun and coworkers examined osteogenesis of human MSCs in PA gels that contained the RGDS and DGEA peptide sequences [46]. Differentiation was observed with and without the addition of osteogenic media, with delayed and less pronounced osteogenesis in the absence of osteogenic media. Similarly, Park and coworkers used a PA gel containing a peptide sequence derived from bone morphogenic protein (BMP)-2 as an osteoinductive scaffold for human bone marrow stromal cells [47]. Cells in PA gel matrices were cultured with osteogenic media, and cells cultured in gels assembled from BMP-2 derived PA showed greater proliferation and osteogenesis than those in matrices without the BMP-2 mimetic sequence. Other functionalized gels designed for enhancing bone regeneration include ionic self-complementary RADA peptide-based gels containing signaling peptide sequences derived from osteogenic growth peptide and the cell-adhesion domain of osteopontin, which contains an RGD group [48]. Osteogenesis of MT3T3-E1 cells in 2D culture on top of these gels was observed, with some cell migration into the scaffold when the osteopontin-derived peptide sequence was used. Mouse embryonic stem cells were similarly differentiated in two and three dimensions in RADA gels using osteogenic media [49].

Cell behavior is commonly known to be affected by substrate topology [50], but common microfabrication materials are typically not biocompatible. In an effort to add an additional aspect of surface topology to bone regeneration with PA gels, our group investigated micropatterned PA materials as substrates for osteogenesis [51]. Using an RGDS-displaying PA nanofiber gel with a UV-polymerizable hydrophobic tail, we produced micropatterned PA gels using soft lithographic techniques on silica substrates. These bifunctional gels (epitope and surface morphology) showed enhanced differentiation of pre-osteoblast cells in micropatterned gels with 40 μm wells, potentially due to mimicry of an enclosed, high signaling density environment *in vivo*.

3.2 Enamel growth

Though enamel is of ectodermal origin (as opposed to bone, which is derived from the mesoderm germ layer) and has several structural differences compared with bone (such as a lack of collagen), it is composed of the same inorganic building block: highly organized HAP crystallites. As enamel does not remodel, natural regeneration is severely limited, implicating the need for regenerative strategies for patients with severe enamel loss or genetic diseases that compromise tissue integrity. To address this need, we recently reported on a strategy for regenerating enamel using a bioactive RGDS-displaying PA in conjunction with ameloblast-like cells (LS8) or primary enamel organ epithelial (EOE) cells [52]. In an organ culture model, a solution of PA was injected into an embryonic incisor, with gelation occurring in response to salts present in the tissue. Cell proliferation, enamel gene expression, and accumulation of enamel-forming proteins were observed, with statistically significant increases for the RGDS-displaying PA compared with control PAs. In a follow-up study, embryonic incisors were treated with PA and transplanted under the kidney capsules in host mice for long-term culture [53]. After 8-10 weeks in culture, the teeth were recovered and analyzed for the presence of new enamel formation. The RGDS-displaying PA gel was found to induce proliferation and differentiation of mineral-forming ameloblasts, forming HAP with structures similar to those in authentic enamel.

In a similar enamel regeneration study from the Hartgerink laboratory, dental stem cells were cultured in PA gels containing an RGD cell-binding motif as well as an enzymatically cleavable linker in the β -sheet domain to allow for tissue remodeling in the presence of osteogenic media [54]. *In vitro* cell culture showed enhanced differentiation of two dental stem cell types into mineralizing osteoblasts and odontoblasts compared with control cells cultured in non-osteogenic media. *In vitro* models have also been used to show the mineralization of nanoscale β -sheet-tape-based materials when intra-oral conditions of pH cycling were simulated [55].

3.3 Central nervous system repair

Due to the lack of regenerative pathways in many adult neurons, permanent paralysis resulting from spinal cord injury has become a formidable biomedical challenge. Peptide-based gels that promote axonal regeneration across lesions are currently being intensively investigated for central nervous system repair. Derived from laminin, a protein which has been shown to direct neurite growth [56], the IKVAV peptide epitope has been shown to be a vital component in effective PA gel therapies for spinal cord repair. In 2004 our laboratory showed that a PA gel displaying the IKVAV epitope could induce rapid differentiation of encapsulated neural progenitor cells predominately into neurons while reducing astrocyte formation [33]. Differentiation appeared even more significant for cells exposed to IKVAV-displaying PA gels than for those exposed to laminin. In further studies, we applied IKVAV-presenting PA gels to spinal cord injury sites in a mouse model to inhibit glial scar formation, which is a significant factor preventing axonal regeneration [57]. Most strikingly, the IKVAV-displaying PA gel promoted regeneration of motor and sensory axons across the

injury site while axons in spinal cords treated with control PAs were unable to traverse the lesion (Figure 5). Because PA nanofiber solutions can gel in the presence of physiologically relevant salt concentrations, a free-flowing solution could be injected into the spinal cord, followed by gelation *in situ*. Compression and contusion models were investigated in a recent study on functional recovery from spinal cord injury in mice and rats [58]. Significant functional improvements were found in all cases when IKVAV-containing PA was injected but not when a control PA without the bioactive epitope was used. We attribute this result to axonal regeneration through the lesion and the increased presence of serotonin-containing nerve fibers promoted by the IKVAV PA.

Self-assembling and ionic self-complementary peptide gels have also been used in research on repair of the central nervous system. Zhang and co-workers demonstrated that such gels are capable of supporting neuronal cell attachment and facilitating neurite outgrowth, though the mechanism of attachment was unclear in the absence of a peptide sequence with known bioactivity [18]. Introduction of epitopes derived from laminin, fibronectin, collagen, and bone marrow homing peptides (BMHPs) led in some cases to increased adhesion and differentiation of neural stem cells [59]. Ionic self-complementary peptide gels have also been investigated for other central nervous system regeneration applications. Unfunctionalized RADA gels were shown to restore vision in a hamster model where the optic tract had been severed [60]. RADA-based peptide gels were also used as scaffolds for transplanted neural progenitor cells and Schwann cells in a rat spinal cord cavity model [61]. Cells were removed from donor rats and cultured for 7 days in peptide gels before transplantation into spinal cord cavities in experimental rats. Peptide gels effectively bridged the lesion, though gels without cells and not preconditioned in cell culture media did not integrate well with native tissue. Both cell types were shown to migrate throughout the gel and promote axonal regeneration in the defect site. Additionally, a traumatic brain injury model in rats was studied using RADA-based peptide gels to fill brain cavities [62]. Increased brain tissue restoration was observed in groups treated with the peptide gel due to migration of support cells into the scaffold, but no neurons were found to infiltrate the gel.

3.4 Cardiovascular treatment

The need for cell-based therapies to restore functional myocardium lost as a result of infarction arises from the limited regeneration of cardiomyocytes in mammals. Direct transplantation of embryonic stem cells or endothelial progenitor cells to ischemic tissue typically results in low cell viability, resulting in the need for a biocompatible and injectable delivery matrix to support cell proliferation and differentiation. We examined nanofiber gels self-assembled from PAs bearing RGDS epitopes as vehicles to encapsulate and deliver bone marrow-derived stem and progenitor cells *in vivo*. Compared with a scrambled RGDS sequence and non-epitope-bearing PAs, the RGDS-containing PA scaffold showed significantly greater cell survival and proliferation in an *in vivo* mouse model [32]. Such studies suggest the potential for peptide-based matrices bearing adhesion-promoting epitopes to overcome limitations facing cell-based therapies for ischemic injury.

Endothelial cells and myocardial cells have also been shown to survive well within gels assembled from ionic self-complementary peptides *in vitro* [63]. The *in vivo* injection of peptide gels containing neonatal cardiomyocytes and embryonic stem cells into the myocardium further showed the ability of these gels to recruit and promote survival of endothelial and smooth muscle cells for vascularization [64]. Bioactive peptide hydrogels formed from the self-assembly of aromatic short peptides derivatives Fmoc-FF and Fmoc-RGD have also been investigated for the encapsulation and 3D culture of dermal fibroblasts. These gels, which assemble via β -sheet formation and π - π stacking of Fmoc groups, have been shown to promote cell adhesion and proliferation *in vitro* [65].

4. Peptide-based hydrogels for delivery of soluble factors

Due to their versatility and ease of assembly, peptide-based hydrogels are well suited for therapeutic strategies that combine cell scaffold functions with controlled drug or protein release. While physical entrapment of drugs, enzymes, or growth factors within peptide-based hydrogels is most readily achieved [66-68], new methods for controlling delivery of therapeutics show promise as new biomaterials for drug release. Peptide sequences that specifically bind growth factors and proteins are under investigation, as are methods for controlling release of small molecule drugs and signals[69]. The biodegradable nature of peptide-based materials provides an ideal platform for sequestration and sustained release of soluble signals over periods of hours to weeks.

4.1 Pro-angiogenic peptide-based gels

Because successful tissue engineering and tissue repair in macroscopic dimensions require adequate blood supply for cell survival, promotion of neo-vascularization is frequently a necessary aspect of efficacious therapeutic strategy. Heparin and heparan sulfate are important glycosaminoglycan cofactors in angiogenesis, as they have been shown to bind, stabilize, and protect pro-angiogenic proteins such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (FGF-2). Our group reported in 2006 the design and synthesis of a PA bearing the Cardin-Weintraub heparin-binding domain to specifically bind and self-assemble into nanofiber hydrogels in the presence of heparin (Figure 6A and B) [70, 71]. This heparin-binding PA (HBPA) hydrogel exhibited prolonged protein release and promoted substantial neo-vascularization when delivered with nanogram quantities of VEGF and FGF-2 compared to controls in an *in vivo* rat corneal angiogenesis model (Figure 6C and D) [70]. Using a dorsal skinfold chamber model to dynamically monitor the interaction between nanofiber gel and microcirculation, we determined that HBPA hydrogels containing heparan sulfate persisted in tissue for up to 30 days and that such gels promoted *de novo* vascularization of connective tissue with minimal inflammation [72]. When combined with nitric oxide-releasing diazeniumdiolate molecules, HBPA gels exhibited sustained NO release and were able to inhibit neointimal hyperplasia as well as stimulate re-establishment of the endothelial barrier after arterial injury [73]. We further demonstrated the benefits of HBPA nanofiber gels in cardiovascular therapy by demonstrating their ability to bind paracrine factors from hypoxic conditioned stem cell media[74]. Injection of these conditioned HBPA gels following coronary artery ligation resulted in preservation of hemodynamic function in a mouse ischemia-reperfusion model of acute myocardial infarction and also promoted extensive revascularization in chronic rat ischemic hind limb models (Figure 7).

Efficacious transplantation of pancreatic islets for treatment of patients with type I diabetes is frequently compromised by loss of vascular networks during islet isolation from donor tissue. When VEGF, FGF-2, and heparin-containing HBPA gels in fibrous poly(L-lactic acid) matrices were implanted adjacent to donor islets in diabetic mouse omentum, recipients displayed increased blood vessel density at the transplant site and achieved higher rates of normoglycemia than control animals receiving growth factors alone or identical scaffolds without growth factors [75]. We further demonstrated that HBPA nanofibers co-delivered with heparin are able to infiltrate dense islets and retain growth factors in order to significantly improve cell viability and insulin secretion (Figure 8) [76].

4.2 Cartilage repair

With the steady extension in human lifespan, cartilage loss has become an increasingly problematic medical condition due to the lack of natural cartilage regeneration pathways. Cartilage growth requires chondrocytes, the cellular component of cartilage, but their

population is low in adults and their activity is reduced during aging. Injection of a material capable of delivering and stimulating cells to produce cartilage into defect sites *in vivo* is therefore a major goal in the field of regenerative medicine. In an early study on chondrocyte encapsulation, ionic self-complementary peptide gels were found to support cell survival and maintain chondrogenic phenotype expression. Increased production of glycosaminoglycans and type II collagen were observed, along with an increase in dynamic stiffness of the matrix material during the culture period [77]. More recently, these gels were tested with bone marrow stromal cells (BMSCs) against agarose controls using transforming growth factor β 1 (TGF- β 1) to induce chondrocyte differentiation [78]. Gene expression of chondrogenic markers was increased in peptide gels as compared to agarose gels, and expression of osteogenic and adipogenic markers were constant or decreased during 15 days in culture.

We recently reported differentiation of human MSCs in PA gels that released TGF- β 1 by employing a TGF- β 1 binding sequence [79]. Release of TGF- β 1 was three times slower when the TGF- β 1 binding sequence was employed versus non-bioactive control PA gel. Regrowth of cartilage was tested *in vivo* using the TGF- β 1-binding PA in rabbits in an articular cartilage full thickness defect model. Defect sites were treated with microfracture in the trochlea of rabbits to allow for bone marrow infiltration into the defect as PA solution was added and gelled. In comparison with controls of TGF- β 1 only and TGF- β 1 delivered with non-active filler PA, the groups that received TGF- β 1 with TGF- β 1-binding PA exhibited significantly higher cartilage regeneration as assessed by histological and visual examination (Figure 9). More importantly, rabbits treated with TGF- β 1-binding PA but without exogenous growth factor performed nearly as well as those treated with TGF- β 1-binding PA and growth factor, making the TGF- β 1-binding PA gel a promising candidate for cartilage regeneration.

4.3 Erectile Dysfunction

The capability of PA nanofiber gels to act as an injectable, noninvasive, and biocompatible vehicle for sustained protein release has been shown to be particularly advantageous for the delivery of apoptosis suppressants. Decreased expression of sonic hedgehog (SHH), a glycoprotein regulator of penile smooth muscle apoptosis, has been linked to the onset of erectile dysfunction, particularly after injury to the cavernous nerve (CN). SHH-containing PA nanofiber gels delivered into the sinuses of the corpora cavernosa showed SHH protein release for at least 6 days and caused a significant reduction in penile smooth muscle apoptosis after CN injury [80]. Furthermore, treatment of crushed CNs with SHH delivered via linearly aligned monodomain PA nanofiber gels suppressed penile apoptosis and promoted the function and speed of CN regeneration [81].

5 Peptide-Based Membranes for Biological Applications

While extensive research exists for peptide-based biomimetic hydrogels, alternative peptide-based constructs have remained relatively unexplored. Therapeutic structures in the form of planar sheets or membranes represent a particularly attractive architecture for tissue repair, as these constructs are well suited towards applications such as wound healing and large area tissue regeneration. The formation of therapeutic films by solution casting or electrospinning of naturally derived silk fibroin and by multilayer complexation of charged polypeptides has been studied for over two decades [82-85]. These membranes have demonstrated the potential ability to act as scaffolds for cell growth as well as for drug release and protein immobilization but will not be discussed here, as they do not incorporate synthetic peptides. Similarly, the review of peptide-based monolayers, coatings, and other surface modifications is beyond the scope of this paper.

Despite the potential utility of short, self-assembling peptide-based membrane constructs, the first such example was reported by our group in 2008 [86]. Using aqueous solutions of hyaluronic acid (HA), an anionic glycosaminoglycan, and a cationic PA displaying three terminal lysine residues, our group discovered the hierarchical self-assembly of a robust PA-HA hybrid membrane (Figure 10). The membrane formation phenomenon is characterized by a unique multi-step assembly, in which an instantaneous diffusion barrier is established via charge complexation and consequent PA nanofiber bundle assembly. Subsequently, osmotic pressure-driven diffusion of HA into PA solution leads to assembly and growth of aligned nanofiber bundles perpendicular to the interface over time. Due to the unique mechanism of formation, hierarchically assembled PA-HA membranes exhibit a novel ordered structure with regions of distinct morphology. In subsequent work, we reported the elastic modulus and permeability of these membranes as a function of HA solution concentration and incubation time in PA solution [87]. Furthermore, we determined that the pore size and mechanical robustness of PA-HA membranes lie advantageously between biomimetic hydrogels and liposomal membranes. Very recently we demonstrated the potential use of PA-HA membranes in wound healing applications by incorporating PA capable of binding heparin. Such membranes significantly and rapidly increased angiogenesis in *in vitro* models due to enhanced growth factor retention (Figure 11) [88].

6 Future Challenges and Prospects

Understanding of nanoscale phenomena in supramolecular chemistry, materials science, and cell biology has driven the recent rise in novel biologically-derived materials for regenerative medicine. As the field continues to mature, multifunctional scaffolds will be needed for clinical translation. Biomimetic, peptide-based gels are ideal systems since they can carry the same chemical signals that biology utilizes to mediate its pathways. They also have the capacity to recreate the architectures and physical properties of natural extracellular matrices, thus giving rise to an enormous future for artificial, biocompatible, and biodegradable cell scaffolds for regenerative medicine. The biology of tissue growth and regeneration is extraordinarily complex. Future efforts will need to build upon the successes of previous research and will need to further explore the mechanisms behind efficacious cell-material interactions. Specifically, the incorporation of cell signaling within peptide-based nanostructures and their gel networks will need to be optimized in terms of epitope combination and density in order to more fully mimic native developmental and regenerative environments. Another great challenge will be to learn how to design hierarchically structured scaffolds with order parameters, and also to find optimal designs for systems that can be delivered systemically and targeted to specific tissues. Optimal ways of delivering soluble small molecules and growth factors from bioactive gels is yet another dimension of the field and will most certainly develop in the future. In-depth, systematic studies of combined soluble and insoluble signals will greatly enhance the capabilities and biological understanding of biomaterials in regenerative medicine. The temporal profile of both surface-bound and released signals and the use of signal gradients should also be considered as potential avenues for additional functionality. Maximum control of cell growth and behavior *in vivo* will likely be enabled through the use of multiple signaling epitopes in combination with soluble factor release with an optimized temporal profile. Finally, the development of hybrid systems of self-assembling peptides with biologically relevant macromolecules into novel constructs represents a new direction in the field of bioactive materials. Achievement of the complex goals of regenerative medicine will require bottom-up self-assembly using new concepts and future discoveries in supramolecular chemistry. The continued rational design of peptide-based molecules capable of forming highly functional, structurally complex, and well-defined scaffolds will be required to achieve eventual clinical translation of peptidic biomaterials.

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Highlights

Biologically-active gels made from self-assembling peptide components are reviewed. Materials include peptide amphiphiles, β -hairpins and self-complementary peptides. Hierarchically structured peptide amphiphile-biopolymer membranes are also discussed. Regenerative medicine applications include bone, enamel and cartilage regeneration. Bioactive gels and membranes in angiogenesis and transplantation are also described.

Figure 1.

(A) Chemical structure and space filling model of an RGDS-bearing PA molecule, highlighting the four structural domains (I-IV). (B) Illustration of a self-assembled PA nanofiber, with red spheres representing water molecules. (C) TEM image of PA nanofibers cast from aqueous solution. (D) SEM image of a PA gel formed by mixing cell culture media with a PA solution. (E) Image of a PA gel after in vivo gelation and extraction from an enucleated rat eye. From reference [33] (Science), reprinted with permission from AAAS, and reference [6] (PNAS), Copyright 2002 National Academy of Sciences, USA.

Figure 2.

(A) Generalized chemical structure of β -hairpin gels and the proposed mechanism of thermally-triggered folding and gelation. (B) Illustration of folding and gelation of β -hairpin peptide gels. Adapted with permission from ref [21]. Copyright 2003 American Chemical Society.

Figure 3.

Images of histological sections of a PA-Ti hybrid with encapsulated MC3T3-E1 cells implanted in a rat femur after 4 weeks. Non-decalcified, plastic embedded samples were stained with methylene blue and basic fuchsin. (A) Mineralized bone formation (blue) occurs within an interior pore of the PA-Ti hybrid. (B) New bone formation is observed adjacent to and into the PA-Ti hybrid. The location and formation of these bone spicules are evidence of osteoconduction, with new mineralized bone (NMB, deep blue) on the exterior, new unmineralized bone (NUB, pink) in the middle, and collagenous fibers (C) against the PA-Ti hybrid. Reprinted from reference [44] with permission from Elsevier.

Figure 4.

MicroCT imaging of newly formed bone in rats with a critically-sized femoral defect after 4 weeks. Representative microCT images depict the difference in bone formation observed in animals implanted with a mixture of RGDS-containing PA and phosphoserine-containing PA in the defect site (A-C) versus those left untreated (D-F). Dashed white lines highlight the position of the slices from the PA treated (B, C) and from the untreated (E, F) animals. Higher levels of new calcified tissue (less dense, gray) were observed in animals treated with the PA gel. In these animals new bone formation was observed growing from both ends of the defect tending to bridge the gap (A-C) and expanding radially outwards (B). New bone at the edge of the defect coated both the outside (outer bone surface) and inside (medullary canal) of the original bone (more dense, white), which correspond to the locations where the PA matrix was observed to be present 48 h after implantation. Reprinted from reference [37] with permission from Elsevier.

Figure 5.

IKVAV PA promotes regeneration of motor axons after spinal cord injury. a, b, Representative Neurolucida tracings of BDA-labeled descending motor fibers within a distance of 500 μ m rostral of the lesion in vehicle-injected (a) and IKVAV PA-injected (b) animals. The dotted lines demarcate the borders of the lesion. c–f, Bright-field images of biotinylated dextran amine (BDA)-labeled tracts in lesion (c, e) and caudal to lesion (d, f) used for Neurolucida tracings in an IKVAV PA-injected spinal cord (a, b). g, h, Bar graphs show the extent to which labeled corticospinal axons penetrated the lesion. *The groups representing three control and three IKVAV PA mice and the tracing of 130 individual axons differ from each other at $p < 0.03$ by the Wilcoxon rank test. R, Rostral; C, caudal; D, dorsal; V, ventral. Scale bars: a–d, 100 μ m; e–f, 25 μ m. Reprinted with permission from reference [57].

Figure 6.

(A) Illustration of heparin binding to HBPA nanofibers. (B) TEM showing HBPA nanofibers covered with 10 nm heparin-gold nanoparticles (black dots). Scale bar = 40 nm. (C) In vivo rat corneal angiogenesis assay showing extensive neovascularization in a heparin-nucleated HBPA gel with growth factors versus (D) minimal neovascularization in a collagen gel with growth factors. Adapted with permission from ref [70]. Copyright 2006 American Chemical Society.

Figure 7.

In vivo bioluminescent imaging of transplanted bone marrow mononuclear cells (BMNCs) in mice 4 days after subcutaneous injection. Cells were injected in gels of (A) 10% RGDS-containing PA with 90% diluent PA, (B) 100% diluent PA, or (C) a saline solution. A 315% increase in signal was observed in group A from day 0 baseline, with signal increases of 127% (group B) and 147% (group C) for controls ($p < 0.001$), indicating that the RGDS-bearing PA matrix significantly retains and supports the transplanted cells. Reprinted from reference [32] with permission from Elsevier.

Figure 8.

Fluorescence microscopy images of murine islets at day 7 showing live (green) and dead (red) cells after culture in (a) media only (control) or media supplemented with (b) VEGF and FGF2 growth factors (GF), (c) HBPA and heparin (HBPA), and (d) HBPA, heparin, VEGF, and FGF2 (HBPA-GF). Islets in (c) and (d) appeared to have more live cells and maintained their normal rounded morphology. (e) Cell viability quantified using a fluorometric assay and normalized to DNA content. Viability is represented as percent of freshly isolated islets on day 0 (* $p < 0.05$). Reprinted from reference [76] with permission from Elsevier.

Figure 9.

Macroscopic views of articular cartilage defects in a full thickness articular cartilage rabbit model defects 12 weeks after treatment. Animals were treated with (A) TGF- β 1 growth factor (100 ng/mL), (B) diluent PA (without epitope) + TGF- β 1, (C) 10% TGFBPA + TGF- β 1, and (D) 10% TGFBPA alone. In groups treated with TGFBPA, both with and without growth factor (C and D), nearly complete tissue fill is observed. Reprinted from reference [79] (PNAS), Copyright 2010 National Academy of Sciences, USA.

Figure 10.

Mechanism of hierarchically-assembled HA-PA membrane. (A) SEM 30 min after initial contact of oppositely charged solutions of HA and PA. Numerals 1, 2 and 3 indicate the amorphous polymer region, the parallel fiber (diffusion barrier) region and the perpendicular fiber region, respectively. (B) SEM 4 days after initial contact. The yellow arrow designates the parallel fiber region. (C) SEM showing a cross sectional image of a mature membrane, highlighting the highly aligned nature of the perpendicular fibers. (D-F) Illustration of the mechanism of assembly of the membranes, whereby polymer (red) penetrates the diffusion barrier (D), which is made up of PA nanofibers (blue). Further assembly is initiated by polymer diffusion (E), which leads to increased growth and alignment of perpendicular fibers as the membrane matures (F). From reference [86], reprinted with permission from AAAS.

Figure 11.

Digital images of peptide membranes made from HA and HBPA in a chorioallantoic membrane in vivo angiogenesis assay. Membranes contain (a, b) 0 wt% heparin, (c, d) 0 wt% heparin + VEGF and FGF2 growth factors, (e, f) 0.5 wt% heparin, and (g, h) 0.5 wt% heparin + VEGF and FGF2 at days 0 and 1. Arrows in (h) indicate vessel morphology indicative of VEGF signaling. Graph shows quantification of vessel density relative to day 0 (***) p < 0.001). Reprinted from reference [88] with permission from Elsevier.