7.1 Introduction

The most promising paradigm for regenerative medicine is to engineer a nanostructured environment that mimics the complex hierarchical order and self-assembled formation of native tissue, as opposed to trying to adopt traditional materials to a biomedical need. This approach is emphasized by the ongoing research of biomimetic peptide scaffolds that employ a bottom-up tissue engineering strategy. To capture the self-assembling complexity required, bioactive scaffolds need to emulate the intrinsic nanoscale properties of the desired tissue and surrounding extracellular matrix (ECM). The ECM is a viscoelastic three-dimensional (3D) network consisting of nanofibrillar proteins and polysaccharides that self-assembles into complex supramolecular structures and serves as a critical component in the development and maturation of tissues (Patrick et al. 1998; Hubbell 2003; Daley et al. 2008). In particular, cell–ECM interactions can be recapitulated to directly regulate cell behaviors, such as cell proliferation, growth, survival, polarity, morphology, migration, and differentiation (Kleinman et al. 2003). With this approach, self-assembled nanomaterial constructs can be tailored to precise tissue regenerative needs and other biomedical applications by incorporating specific functional peptide moieties, such as cellular adhesive ligands and enzyme-mediated degradable sites. Furthermore, versatile biomimetic microenvironments can be created by introducing hybrid functionality into the self-assembling peptide (SAP) scaffolds. To this end, peptide-based biomaterials can potentially be combined with other polymers, metals, nanotubes, or growth factors to improve mechanical stability, direct cellular responses, guide degradation, or deliver therapeutic drugs in controlled manners. Overall, this chapter offers only
a glimpse into the vast amount of knowledge available. Thus, only a few peptide-based biomaterials have been selected for a more in-depth examination. The focus is on self-assembling nanofibrous structures that have the potential to mediate biological activity and other biomedical applications by closely following the principles of naturally derived phospholipids, molecules critical for the structural stability of membranes in biological systems (Shimizu et al. 2005). The proceeding discussion first starts by describing the self-assembly process of phospholipids, followed by synthetic peptide-based biomaterials developed to mimic this natural self-assembly mechanism.

### 7.2 Self-Assembly of Phospholipids

Phospholipids occur in nature as an important class of biomolecules. Structurally, they consist of three components—a polar head, one or more hydrophobic tails, and a backbone linking the two parts. Given the versatility of the head and tail regions, lipids are classified based on their backbone. The amphiphilicity of lipids drives their self-assembly in solution. They can assume several shapes based on their structure, concentration, and temperature. Common lamellar and non-lamellar self-assembled structures are shown in Figure 7.1 (Collier and Messersmith 2001). Lamellar bilayers are formed when the hydrophobic alkyl chains are too bulky to fit within a circular micelle, otherwise a non-lamellar conformation is assumed.

![Common lamellar and non-lamellar self-assembled structures of lipids](image-url)

Due to their inherent biocompatibility and capacity to form self-assembled compartment or layered structures, phospholipids have emerged as attractive candidates for biomedical applications, such as vesicles for drug delivery, tubule and ribbon structures to make scaffolds for tissue engineering, and monolayer or bilayer membrane-like materials for biocompatible coatings of medical devices and implants (Schnur 1993; von Segesser et al. 1993; Gregoriadis 1995). Among the potential functions, considerable efforts have been devoted to developing stimulus-responsive lipid vesicles for site-specific controlled drug delivery. Various stimuli methods for the lipid vesicles or liposomes have been investigated, including temperature, light, and pH change (Thompson et al. 1996; Gerasimov et al. 1999). Messersmith and coworkers have investigated the temperature-responsive approach to engineer therapeutic delivery systems, as chemical reactions between the encapsulated species and the extravascular species were driven by ambient change in temperature (Messersmith and Starke 1998; Sanborn et al. 2002; Pederson et al. 2003; Burke et al. 2007). The entrapped substances were released from the liposomes at the melting transition temperature ($T_m$) of the lipid chains. Exploring this temperature sensitive mechanism, the permeability of the bilayers was found to be significantly enhanced at the melting transition temperature, $T_m$. The bilayers maintained a liquid state below their $T_m$ value but shifted to a gelatinous phase as the temperature increased above the $T_m$. Both of these states expressed low permeability. However, the permeability of the lipid bilayers was several order of magnitudes higher during the transition between phases, owing to the existence of defect-rich interfacial regions between coexisting gel and fluid domains (Figure 7.2) (Honger et al. 1997).

Applying this methodology to clinical treatments, saturated phosphatidylcholines have been used to make vesicles with a $T_m$ near 37°C to facilitate release of calcium upon injection into the body cavity. The exact $T_m$ value can be determined by the chain length of the phosphatidylcholines. Hence, by selecting miscible phospholipids of appropriate chain length, the $T_m$ of the bilayer has been tailored to fall within 23°C–41°C. This strategy has been used to elicit calcium phosphate mineralization, as the thermally responsive liposomes can be activated to release calcium that reacts with extravascular phosphate when subjected to physiologic temperatures (Messersmith and Starke 1998).

The liposome strategy has also been used to trigger rapid in situ formation of polymer hydrogels in response to thermal or photochemical stimuli. In this approach, liposomes reacted with CaCl$_2$ were combined with 16 amino acid SAPs called FEK16. (SAPs are discussed in more detail in the following section.) This composite system was created by dispersing liposomes in a low viscosity solution of FEK16, allowing for the long-term maintenance of a fluid state at ambient temperatures. Upon heat activation after exposure to body temperature (37°C) or infrared light (NIR excitation, 800 nm), CaCl$_2$ is released from the liposomes within the composite system (Thompson et al. 1996). The released CaCl$_2$ triggers salt-dependent self-assembly of FEK16, thereby giving rise to a polymer hydrogel. Thus, these suspensions can be stored as stable fluid precursors at room temperature, but they rapidly form polymer hydrogels when induced at physiological conditions (Messersmith and Starke 1998; Collier and Messersmith 2001; Collier et al. 2001; Westhaus and Messersmith 2001).

7.3 Self-Assembling Peptides

Over the past decade Zhang and coworkers have significantly contributed to the field of biologically inspired SAPs that follow some of the same principles as natural phospholipid self-assembly. EAK16-II was the first member of this family and was discovered in the yeast protein, Zuoitin (Zhang et al. 1992). Since this initial discovery, a number of peptides have been added to the group (Table 7.1) (Zhang 2002). These SAPs are characterized by an alternating sequence of hydrophobic and hydrophilic residues, as the hydrophilic residues alternate in turn between a positive and a negative charge. Self-assembly is spontaneous, and the peptides are held together by various ionic and nonionic, hydrophobic, and van der Waals interactions (Whitesides et al. 1991; Zhang 2002). Four different types of SAPs have been investigated, differing in their charge distribution and resulting in secondary and tertiary self-assembled structures.

7.3.1 Type I Self-Assembling Peptides

Type I SAPs are characterized by the presence of both a hydrophobic and hydrophilic composite face, which leads to β-sheet formations in aqueous solution. They are also termed as “molecular lego” structures due to their striking similarity to Lego bricks, as they have “pegs and holes” and can only assemble into particular structures at the molecular level. Variations can be made within the peptide sequence to increase the size of the “pegs” and the “holes,” producing such sequences as RARADADA and RARARADADADA. Due to their hydrophilic surfaces, SAPs are known to form complementary ionic bonds consisting of regular repeating peptide blocks. The ionic bond arrangements can follow various patterns and serve as the basis for classifying the SAPs into different electrically charged groups (Table 7.1). For example, the molecules within the Type I class have positively (+) and negatively (−) charged amino acids repeating as + − + − + −. Similarly, Type II class SAPs will have amino acids arranged as + + − − + + − and so on (Zhang et al. 1993).

The alternating charge within SAPs drives the nanofibrous self-assembly when subjected to the right stimuli. Self-assembly of these SAPs into nanofibers can be triggered by exposing the peptides to physiological media or monovalent alkaline cations. This creates a bulk mesh of individually assembled fibers that are typically about 10–20 nm in diameter and up to a few microns in length, as determined by scanning electron microscopy (SEM) and atomic force microscopy (AFM). Pores are prevalent throughout the interwoven fibrillar meshwork and are usually on the order of 50–200 nm, which is the same scale as many vital biomolecules. This size scale is conducive for diffusing biological molecules, along with the subsequent creation of a concentration gradient for programmable drug delivery applications. The density of the nanofibers assembled can be easily controlled, depending on the employed concentration of the peptide solution. Overall, the nanofiber meshwork is very strong as peptide self-assembly is stable over a wide range of pH values, temperatures, and denaturing agents (e.g., urea guanidium hydrochloride) (Zhang et al. 1995; Leon et al. 1998; Caplan et al. 2000; Holmes et al. 2000).

In order to understand the self-assembly of these SAPs, a proposed model for complementary molecular pairing between positively charged lysines and negatively charged glutamates has been described by Zhang et al. (1995). It was found that replacing the charged residues with other amino acids of similar charges does not significantly affect the self-assembly process, as neither the replacement of a positively charged lysine with a positively charged arginine nor a negatively charged glutamate with a negatively charged aspartate had any bearing on the assembled structures. However, replacing the amino acids with the residues of the opposite charge prevented self-assembly. For example, self-assembly cannot occur after substituting a positively charged lysine with a negatively charged glutamate, even though β-sheet structures can still form when exposed to cations (Caplan et al. 2000). Furthermore, enhancing the hydrophobicity of the peptides by replacing an alanine with more hydrophobic residues (e.g., leucine, isoleucine, phenylalanine) helps to promote faster self-assembly and results in improved mechanical strength (Leon et al. 1998). These self-assembled nanofibers become fragmented when subjected to sonication but are able to reassemble after removing the disruptive forces. The kinetics of reassembly
### TABLE 7.1  List of Self-Assembling Peptides Studied

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (N→C)</th>
<th>Ionic Modulus</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>RADA16-I</td>
<td>+−−−−−−−−</td>
<td>I</td>
<td>Beta</td>
</tr>
<tr>
<td>RGDA16-I</td>
<td>+−−−−−−−−</td>
<td>I</td>
<td>r.c.</td>
</tr>
<tr>
<td>RADA8-I</td>
<td>+−−−</td>
<td>I</td>
<td>r.c.</td>
</tr>
<tr>
<td>RAD16-II</td>
<td>+−−−−−−−−</td>
<td>II</td>
<td>Beta</td>
</tr>
<tr>
<td>RAD8-II</td>
<td>+−−−−</td>
<td>II</td>
<td>r.c.</td>
</tr>
<tr>
<td>EAKA16-I</td>
<td>−−−−−−−−</td>
<td>I</td>
<td>Beta</td>
</tr>
<tr>
<td>EAKA8-I</td>
<td>−−−−</td>
<td>I</td>
<td>r.c.</td>
</tr>
<tr>
<td>RAE16-I</td>
<td>+−−−−−−</td>
<td>I</td>
<td>Beta</td>
</tr>
<tr>
<td>RAE8-I</td>
<td>+−−−</td>
<td>I</td>
<td>r.c.</td>
</tr>
<tr>
<td>KADA16-I</td>
<td>+−−−−−−−−−−−−−−</td>
<td>I</td>
<td>Beta</td>
</tr>
<tr>
<td>KADA8-I</td>
<td>+−−−</td>
<td>I</td>
<td>r.c.</td>
</tr>
<tr>
<td>EAH16-II</td>
<td>−−−−−−−</td>
<td>II</td>
<td>Beta</td>
</tr>
<tr>
<td>EAH8-II</td>
<td>−−−−</td>
<td>II</td>
<td>r.c.</td>
</tr>
<tr>
<td>EFK16-II</td>
<td>−−−−−−−−</td>
<td>II</td>
<td>Beta</td>
</tr>
<tr>
<td>EFK12-I</td>
<td>−−−−−−</td>
<td>I</td>
<td>Beta</td>
</tr>
<tr>
<td>EFK8-II</td>
<td>−−−−−−</td>
<td>I</td>
<td>Beta</td>
</tr>
<tr>
<td>ELK16-II</td>
<td>−−−−−−−−</td>
<td>II</td>
<td>Beta</td>
</tr>
<tr>
<td>ELK8-II</td>
<td>−−−−−−</td>
<td>II</td>
<td>Beta</td>
</tr>
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<td>EAK16-II</td>
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<td>II</td>
<td>Beta</td>
</tr>
<tr>
<td>EAK12</td>
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<td>IV/II</td>
<td>Beta/alpha</td>
</tr>
<tr>
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<td>II</td>
<td>r.c.</td>
</tr>
<tr>
<td>KAE16-IV</td>
<td>+−−−−−−−</td>
<td>IV</td>
<td>Beta</td>
</tr>
<tr>
<td>EAK16-IV</td>
<td>−−−−−−−−−−−−−−</td>
<td>IV</td>
<td>Beta</td>
</tr>
</tbody>
</table>

(continued)
heavily depend on time and are best explained by the sliding diffusion model (Yokoi et al. 2005). On the charged face of the peptide, both positive and negative charges are packed together through intermolecular ionic interactions in a checkerboard pattern. When fragments of the nanofibers first meet, the hydrophobic sides may not fit perfectly together, creating gaps in the formations. However, nonspecific hydrophobic interactions permit each nanofiber to slide along the cylindrical axis in either direction, which minimizes the exposure of hydrophobic residues and eventually seals any gaps.

These SAPs have been extensively tested with different types of mammalian cells to evaluate cellular attachment and growth behaviors. Zhang et al. have systematically studied the adhesion and differentiation behavior of neural stem cells (NSCs) on RAD16-I and compared the results to several naturally derived materials, including collagen, fibronectin, and synthetic polymers (e.g., poly(lactic acid), poly(lactic-co-glycolic acid)). The RADA16-I scaffold was found to support NSC survival and elicit differentiation to a similar degree as other synthetic biomaterials (Gelain et al. 2007). The follow-up evaluation investigated the ability of SAPs to encapsulate cells and ensure viability. Specifically, Zhang et al. explored the utility of these nanofibers to provide a suitable growth environment for endothelial cells (Davis et al. 2005). In this study, 1% RAD16-II peptides were injected into the left heart ventricle of adult mice and established as 3D microenvironments. The recipient hearts were excised at different time points, and hematoxylin and eosin staining of the fixed sections distinguished the synthetic microenvironment from the surrounding tissues. The sectioned peptide scaffolds were found to be populated with both endothelial and smooth muscle cells within 2 weeks. The implanted peptide matrix also recruited α-sarcomeric actin-positive cells that are responsible for developing monocytes, as indicated by positive

### Table 7.1 (continued) List of Self-Assembling Peptides Studied

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (N→C)</th>
<th>Ionic Modulus</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>KLD12-I</td>
<td>+ + + + + + + +</td>
<td>I</td>
<td>Beta</td>
</tr>
<tr>
<td>KLE12-I</td>
<td>+ + + + + + + +</td>
<td>I</td>
<td>Beta</td>
</tr>
<tr>
<td>RAD16-IV</td>
<td>+ + + + + + + +</td>
<td>IV</td>
<td>Beta</td>
</tr>
<tr>
<td>DAR16-IV</td>
<td>− + + + + + + + +</td>
<td>IV</td>
<td>Beta/alpha</td>
</tr>
<tr>
<td>DAR16-IVα</td>
<td>− + + + + + + + +</td>
<td>IV</td>
<td>Beta/alpha</td>
</tr>
<tr>
<td>DAR32-IV</td>
<td>− + + + + + + + +</td>
<td>IV</td>
<td>Beta/alpha</td>
</tr>
<tr>
<td>EHK16</td>
<td>+ + + + + + + + + + + +</td>
<td>N/A</td>
<td>r.c.</td>
</tr>
<tr>
<td>EHK8-I</td>
<td>+ + + + + + + + + + + +</td>
<td>N/A</td>
<td>r.c.</td>
</tr>
<tr>
<td>VE20*(NaCl)</td>
<td>− + + + + + + + + + + +</td>
<td>N/A</td>
<td>Beta</td>
</tr>
<tr>
<td>RF20*(NaCl)</td>
<td>+ + + + + + + + + + + +</td>
<td>N/A</td>
<td>Beta</td>
</tr>
</tbody>
</table>


Beta, β sheet; alpha-helix; r.c., random coil; N/A, not applicable. The numbers that follow the name denote the length of the peptides.

* Both VE20 and RF20 are in β-sheet form when they are incubated in solution containing NaCl.
staining for the NKX2.5 transcription factor. Conversely, Matrigel was injected as the control, and minimal penetration of endothelial cells was observed, along with no evidence of putative monocyte precursors. In addition, several therapeutic studies with Type I SAPs have been conducted with other in vivo animal models. Primary rat hippocampal neurons were shown to form fully functional synapses on the peptide scaffolds, indicating the support of neurite outgrowth and active synaptic transmission (Holmes et al. 2000). The peptide scaffolds have been injected into the optical nerve area of severed mice brains and found to promote healing, as the incision was sealed after 2 days (Ellis-Behnke et al. 2006). Bovine chondrocytes seeded within the peptide hydrogel have been shown to deposit cartilage-like ECM within 4 weeks, signifying potential application as a treatment for cartilage tissue repair (Kisiday et al. 2002).

The resourcefulness of SAPs has also been expanded to incorporate cell-specific sequences that promote cell–cell and cell–tissue interactions. Many different designer SAPs inscribed with biologically inspired peptide sequences have been synthesized by Zhang et al. Two examples of biologically functionalized SAPs created by Zhang et al. include RADA-16-Bone Marrow Homing Peptide-1 (BMHP1) and RADA16-BMHP2. Both the BMHP1 and BMHP2 SAPs contain cell-specific signals for osteogenic tissue. The addition of these functional motifs did not interfere with the self-assembly of RAD-16, indicating that all SAPs have the potential to be functionalized without any adverse effects. Also, the density of the presented peptide sequences in RADA-16 can be easily controlled as different ratios can be incorporated into the self-assembled nanofiber scaffolds. For cell encapsulation with these biologically functionalized SAPs, the peptide scaffolds were found to significantly enhance adult mouse neuronal stem cell survival after 7 days without external growth factors added to the cell culture media (Gelain et al. 2006). The level of induced neuronal differentiation promoted by these designer scaffolds was very similar to the Matrigel positive control.

### 7.3.2 Type II Self-Assembling Peptides

Type II SAPs depart from the long-held assumption that assembled secondary peptide structures maintain long-term stability. These peptides have also been described as “molecular switches” because of their predisposition to abruptly transform their secondary molecular structure from \( \alpha \)-helix to \( \beta \)-sheet form in response to temperature or pH changes. Similar to Type I SAPs, they have distinct hydrophobic and hydrophilic faces that promote \( \beta \)-sheet assembly (Zhang et al. 1993). A distinguishing feature of SAPs in this class is the clustering of negatively charged residues (e.g., aspartic acid, glutamic acid) toward the N terminus, while positively charged amino acids cluster (e.g., arginine, lysine) toward the C terminus. This distribution of charge balances the \( C \rightarrow N \) dipole moment and facilitates formation of \( \alpha \)-helices (Aurora and Rose 1998).

The transition from \( \beta \)-sheets to \( \alpha \)-helices and vice versa is usually abrupt and depends on ambient temperature or pH. For example, a 16 residue self-complementary oligopeptide developed by Zhang et al. called DAR16-IV has a \( \beta \)-sheet structure at room temperature that is 5 nm long, but its self-assembled formation undergoes an abrupt structural transition when heated to 60°C to form a stable \( \alpha \)-helix with a 2.3 nm length (Zhang and Rich 1997). The temperature at which this transition takes place depends on the peptide sequence. Peptides with more stable \( \beta \)-sheet structures will have to be heated to higher temperatures to transform into \( \alpha \)-helices. Once formed, it takes weeks for the \( \alpha \)-helices to revert back to the initial \( \beta \)-sheet form. Similarly, adjusting the pH can induce structural transformations, depending on the charge expressed by the constituent amino acids (Altman et al. 2000).

Notably, the slow conversion of \( \alpha \)-helix to \( \beta \)-sheet form is similar to the conformational changes implicated in neurological disorders like Alzheimer’s disease (Zhang and Rich 1997). These findings offer many potential applications for Type II SAPs, including the study of protein–protein interactions and protein foldings in normal physiology or diseased states, such as Parkinson’s or Alzheimer’s disorders. These peptides could also be used for other biomedical applications, such as designing peptide biosensors that rapidly respond to in vivo or in vitro ambient pH or temperature changes. Biosensors with such features could potentially be developed as personalized diagnostic devices.
7.3.3 Type III Self-Assembling Peptides

The Type III peptides are designed to self-assemble onto surfaces rather than among themselves, functioning as “molecular paint” or “molecular Velcro” (Zhang 2008). They can be used to form monolayers onto different surfaces, providing recognition or interactive sites that promote the attachment of specific cell types or other molecules. In general, this SAP class has three components, namely, a ligand, an anchor group to facilitate binding to different surfaces, and a linker to connect the ligand and the anchor (Zhang et al. 1999). The ligand can be varied according to the target cell or molecule desired. The peptide anchor displays a specific chemical group that is designed to react with the desired surface and create a self-assembled coating. Besides serving as a connector, the linker also provides versatile mechanical control, as it can be modified to provide flexibility or stiffness depending on the choice of amino acids. For example, incorporating a glycine backbone sequence into the linker segment provides a more flexible structure, as opposed to a chain of valine that imparts a stiff connection (Mrksich et al. 1996).

While investigating Type III SAPs, Zhang et al. designed the following peptide assemblies: RADS RADS and RADR ADS RADS, which were linked to cysteine anchors at the C-terminus by connection sequences of 3–5 alanines (Prieto et al. 1993). Both of these SAPs were self-assembled as monolayers onto micropatterned gold-coated surfaces via the thiol groups in the cysteine anchors. Mammalian cells were seeded on these patterned monolayer surfaces and they aligned in a well-defined manner, reflecting the presence or absence of cell adhesion motifs (Zhang et al. 1999). This simple system can be used to address many questions regarding specific cell–cell and cell–tissue biological interactions. Furthermore, using the cell-responsive ligand as a molecular hook, “intelligent” diagnostic devices can be developed for surface molecular detection.

7.3.4 Type IV Self-Assembling Peptides

This last class of SAPs is designed to mimic the properties of polymeric and lipid surfactant molecules. For this class, the peptide structure is amphiphilic, as the leading head group is composed of at least one charged amino acid followed by a string of six identical hydrophobic amino acids (e.g., alanine, valine) to form the hydrophobic tail. Both the cationic and anionic amphiphiles self-assemble into tubular morphologies at neutral pH. Specifically, it is proposed that the peptides first assemble into bilayers to sequester the hydrophobic tails from an aqueous solution, followed by formation of higher order structures, such as tubular or vesicle formations, that are facilitated by hydrogen bonding between adjacent units (Santoso et al. 2002). Interestingly, when the anionic SAPs are folded into the aggregates, they do not seem to resemble the typical β-sheet or α-helix assemblies. Instead, the folding displays an unusual confirmation of an unknown nature. For the cationic systems, the pH of the surrounding environment is critical because if the pH exceeds the pI value of the head group segment, the assembled tubes collapse into membranous sheets, no longer producing well-defined nanostructures. The anionic systems have only been studied at neutral pH and possess a charged head group in all cases (Maltzahn et al. 2003). By incorporating molecular recognition sites, these vesicles and tubes can be utilized for drug delivery to specific cell types. Additionally, the peptide backbone can be modified to include reactive amino acids that facilitate coupling onto other nanosurfaces for fabrication of devices at the nanoscale.

To conclude, taking cues from the ubiquitous self-assembly found in natural systems, scientists have developed designer peptide-based biomimetic systems. The bottom-up approach in these nanofibrillar peptide assemblies offers numerous potential functionalities, such as molecular switches, Velcro, etc. Thus, the development of these SAPs paves the way for building supramolecular structures with a highly controllable hierarchy that are very attractive for tissue regeneration applications.
7.4 Peptide Amphiphiles

Several synthetic peptide-based fibrillar hydrogels have been investigated as potential ECM mimics, which vary in structure to include diblock co-polypeptide amphiphiles, oligopeptides, or peptide amphiphiles (PAs). Among them, the PAs made from hydrocarbon alkyl chains attached to hydrophilic peptide segments have been known to self-assemble into nanofiber networks similar to the fibrillar mesh-like structure of the ECM. Several distinguishing characteristics of these nanofibrous PA scaffolds peaked interest in studying them as a true ECM microenvironment for tissue engineering. In general, these PAs are very versatile molecules, as their composition can be self-assembled to allow for the concurrent control of nanostructure and biological functionality. Broad utility exists for these peptide-based biomaterials; they can be easily adapted due to amino acid interchangeability and have the potential to inscribe various biologically active sequences. Stupp’s laboratory was one of the first to provide valuable research into these PAs, investigating the ability of the molecules to form higher order nanostructures coined as “one-dimensional assemblies” (Hartgerink et al. 2002). They have been designated as 1D because the nanostructure possesses a single dimension that is much longer than the other two, typically showing a 100- to 1000-fold increase (Palmer et al. 2007). Overall, these PAs serve as a synthetic biomaterial designed to interact with cells and proteins in a specific, controllable manner to provide regenerative medicine alternatives. This holds great promise because of the inherent ability of these self-assembling PA systems to direct nanoscopic architecture and alignment, while separately being able to integrate biological functionality.

In 2001, Hartgerink et al. originally investigated these PAs for bone regeneration scaffolding, creating a composite scaffold that combined the organic and inorganic bone phases at the lowest hierarchical level (Hartgerink et al. 2001). This was an innovative system that was designed for these organic PAs to promote nucleation of inorganic hydroxyapatite (HA) on the surfaces of the fibers. The PA structure designed for this study consisted of three functionally distinct peptide regions as shown in Figure 7.3. Four consecutive cysteine amino acids were inscribed next to the hydrophobic core and their inclusion resulted in disulfide bonding between adjacent peptides to stabilize the supramolecular structure. Phosphoserine was also incorporated to provide the proper environment for biomineralization because the phosphorylated amino acid is abundantly common in non-collagenous bone matrix proteins and is believed to interact with HA (Mai et al. 2008). Finally, an Arg-Gly-Asp (RGD) peptide sequence was included as the last region. The RGD motif is a general cell recognition site commonly found in ECM molecules, such as fibronectin and laminin (Hersel et al. 2003). By incorporating this bioactive sequence into the exposed outer domain, these PAs presented a general cell adhesion ligand to promote cell adhesion and growth. This designed PA was induced to self-assemble by lowering the pH, creating a cross-linked fiber network. Successful biomineralization was observed on this PA template, as HA crystals were preferentially aligned down the long fiber axis. Early follow-up studies further expanded the utility of these PAs beyond applicability as a bone tissue regeneration scaffold. In particular, the versatility of these PAs was demonstrated by modifying the molecular structure with different alkyl tail lengths and amino acid compositions, and comprehensively investigating different self-assembly methods (Hartgerink et al. 2002). These variant PAs were all able to self-assemble into 1D nanostructures using several induction methods, such as lowering pH, addition of divalent ions, and drying onto surfaces. These more expansive material characterizations of PAs demonstrated that the biomaterial is tolerant to most chemical modifications, as a vast array of peptide ligands can be incorporated into the molecule. Therefore, this nanofibrous peptide-based system has vast potential for both biological and nonbiological applications, which are subsequently described later on in this chapter.

After the introduction of these PAs as a self-assembling biomaterial, numerous investigations have been conducted and documented in the literature. In general, the structural composition of these PAs consisted of a hydrophilic peptide segment, containing a varying amount of amino acids (6–15 residues), coupled via an amide bond to a hydrophobic alkyl chain that usually fluctuated in length from 10 to 22 carbon atoms (Beniash et al. 2005). The self-assembled configuration of these molecules
FIGURE 7.3 (a) Chemical structure of the PA, highlighting five key structural features. Region 1 is a long alkyl tail that conveys hydrophobic character to the molecule and, when combined with the peptide region, makes the molecule amphiphilic. Region 2 is composed of four consecutive cysteine residues that when oxidized may form disulfide bonds to polymerize the self-assembled structure. Region 3 is a flexible linker region of three glycine residues to provide the hydrophilic head group flexibility from the more rigid cross-linked region. Region 4 is a single phosphorylated serine residue that is designed to interact strongly with calcium ions and help direct mineralization of HA. Region 5 displays the cell adhesion ligand RGD. (b) Molecular model of the PA showing the overall conical shape of the molecule going from the narrow hydrophobic tail to the bulkier peptide region. (c) Schematic showing the self-assembly of PA molecules into a cylindrical micelle. (From [Hartgerink, J.D., Beniash, E., and Stupp, S.I., Self-assembly and mineralization of peptide-amphiphile nanofibers, Science, 294(5547), 1684–1688, 2001]. Reprinted with permission of AAAS.)
mimics native phospholipids and other biological membrane-forming structures (Tovar et al. 2005). In the self-assembled arrangement, the hydrophobic alkyl tails comprise the core, while the hydrophilic peptide segments form a shielding outer surface. Standard solid phase chemistry is typically used for synthesizing the peptide sequences. Single-tailed PAs with only one ionic peptide segment are most commonly studied (Hartgerink et al. 2001). However, PAs with multiple or branched peptide architecture have also been used in past research (Guler et al. 2006; Harrington et al. 2006; Storrie et al. 2007). The branched PAs provide another means for diversifying the nanostructure. In particular, different densities of epitopes can be maintained to control the receptor clustering and signal accessibility (Storrie et al. 2007). This is potentially relevant for studying cell–matrix interactions with the PA because it has been shown that the ligand density affects cellular attachment, spreading, and migration (Massia and Hubbell 1991; Hubbell et al. 1992).

### 7.4.1 Controlling the Self-Assembly Process of Single-Tailed Peptide Amphiphiles

Overall, these PAs are advantageous because of their ability to self-assemble into sheets, spheres, rod-like fibers, disks, or channels, depending on the shape, charge, and environment (Israelachvili et al. 1977). PA self-assembly creates an intricate nanomatrix environment that is driven by the hydrophobic nature of the covalently attached alkyl tail and primarily stabilized by hydrogen bonding between the adjacent peptides, along with further support provided by electrostatic attraction, ionic bridging, van der Waals forces, and molecular geometry in relation to amphiphilic packing (Hartgerink et al. 2002; Claussen et al. 2003; Stendahl et al. 2006; Jiang et al. 2007). The self-assembly mechanism is initialized by screening the charged groups within these PAs by adjusting the pH or adding soluble metal ions, which results in high-aspect-ratio nanofibers via hydrophobic collapse (Tovar et al. 2005; Palmer et al. 2008). The amphiphilic character of the molecule provides thermodynamic incentive for the assembled formations to maintain peptide shielding and reduce entropically unfavorable interactions between the alkyl tails, especially in an aqueous environment (Stendahl et al. 2006). The formed “one-dimensional assemblies” are typically presented as cylindrical micelle nanostructures because the conical shape of the hydrophilic peptide segment is relatively bulkier than its narrow hydrophobic tail and the employed peptide sequences have a strong β-sheet disposition (Hartgerink et al. 2001, 2002). The β-sheets form parallel to the long axes and are packed radially within the nanofibers, as the hydrophilic peptide segments extend outward toward the surface (Figure 7.4) (Jiang et al. 2007).

The self-assembled PA nanostructures are able to form robust non-covalent cross-links between the fibers, resulting in an interwoven network that gives rise to a macroscopic, self-supporting gel. Rheological characterization has been performed on these PAs to confirm self-supporting gelation, indicating that most of the deformation energy was recovered during elastic stretching rather than being lost as heat during viscous sliding (Stendahl et al. 2006). The gelation process can be induced over a wide PA concentration range, as stable gels can be assembled at concentrations as low as 0.25% by weight (Beniaish et al. 2005). Additionally, the gelation kinetics can be controlled without altering any inscribed bioactive epitopes, as described by Niece et al. (2008). Without modifying the outer bioactive peptide domain, they demonstrated that increasing the hydrophobic character of PAs by incorporating specific residues into the peptide core accelerated self-assembly, but the self-assembling formation was suppressed by including more hydrophilic or bulky peptides. Within the self-assembled gels, the morphology of the high-aspect-ratio nanofibers has been well documented as shown in Figure 7.5 under several different high magnification modalities, including transmission electron microscopy (TEM), SEM, and AFM (Palmer et al. 2008). The general nanomatrix observed was a network of cylindrical nanofibers, ranging from 6 to 10 nm in diameter, depending on the length of the self-assembling molecules that form them (Beniaish et al. 2005). In principle, there is no limitation on how far each nanofiber can extend along the long axis because of the high potential for orthogonal β-sheet linking between the PA molecules (Jiang et al. 2007). Typically, however, the nanofibers only achieve a length up to several microns.
FIGURE 7.4 (See color insert.) Schematic representation of β-sheets within PA nanofibers. As depicted in the inset, β-sheets are oriented parallel to the long axis of the nanofibers (inter-β-strand hydrogen bonds are represented as yellow lines; carbon, oxygen, hydrogen, and nitrogen atoms are colored grey, red, light blue, and blue, respectively). (From Jiang, H., Guler, M.O., and Stupp, S.I., The internal structure of self-assembled peptide amphiphiles nanofibers, Soft Matter, 3, 454–462, 2007. Reproduced by permission of The Royal Society of Chemistry.)

FIGURE 7.5 Schematic illustration of the RGD-PA and the self-assembly into a nanofiber. The low magnification (a) and high magnification (b) scanning electron micrographs and the transmission electron micrograph (c) show fibrous bundles, made up of PA nanofibers approximately 5–7 nm in diameter. The scanning electron micrographs were taken of a critical point dried PA gel, while the transmission electron micrograph was taken of nanofibers dried on a TEM grid and stained with phosphotungstic acid. (Reprinted with permission from Palmer, L.C., Newcomb, C.J., Kaltz, S.R., Spoerke, E.D., and Stupp, S.I., Biomimetic systems for hydroxyapatite mineralization inspired by bone and enamel, Chem. Rev., 108(11), 4754–4783. Copyright [2008] American Chemical Society.)
Furthermore, because of the ionic nature of the molecule, self-assembly can be reversibly induced by increasing the pH of the PA nanomatrix (Hartgerink et al. 2001, 2002; Guler et al. 2005). This reversibility provides another beneficial mechanism for these peptide-based biomaterial to respond to the local environment by assembling, disassembling, or changing shape, especially as a self-assembled 3D gel (Hartgerink 2004).

From earlier studies, it was revealed that these single-tailed PAs became nanofibers as a result of the hydrophobic interactions between aliphatic carbon chains (Hartgerink et al. 2001; Paramonov et al. 2006a, b). It was also reported that the β-sheet formations between the peptide segments stabilize the nanofibers and electrostatic interactions between the peptide secondary structures influence their stability (Behanna et al. 2005; Paramonov et al. 2006a; Stendahl et al. 2006). Recently, after a more in-depth analysis of the internal peptide region, Paramonov et al. showed that the amino acids closest to the core of the nanofibers form the most critical β-sheet hydrogen bonds needed to achieve higher order assemblies (Paramonov et al. 2006b). Any disruption occurring at these core hydrogen bonds eliminates the ability of these PAs to form elongated, cylindrical nanostructures. The basic structure for all PAs used in this study by Paramonov et al. is depicted in Figure 7.6.

To determine the exact role of hydrogen bonding in the self-assembly process, a series of PAs were prepared, consisting of 19 N-methylated variants listed in Table 7.2. After preparing these N-methylated PAs, the ability of each PA to self-assemble into fibers (indicated “F”) and produce a self-supporting gel (indicated “Gel” or “wGel”) was observed. Within the N-methylated PA variants, two groups were synthesized to elucidate the relative importance of specific hydrogen bonding locations for nanofiber formation. The first series (PAs 2–8) N-methylated a single glycine at position 7 in PA 2 and then progressively added more N-methyl groups, moving toward position 1 until all seven linker glycines were methylated. The second series (PAs 9–19) reversed the order of methylation, and a few select variants (PAs 9–14) only contained one N-methylated glycine at each position in the glycine linker region. Overall, the introduction of methylated glycine residues lowered the storage modulus values, resulting in weaker gels. Interestingly, the elimination of one hydrogen bond in the core region (methylating a glycine between glycine positions 1–4) disrupted the gel formation, while its absence could be tolerated in the periphery (methylating a glycine between glycine positions 5–7). These findings rationalize that the amino acids further away from the core of the PA nanofiber are less restricted in their conformation and only play a minor role in stabilizing the nanostructure and corresponding macroscopic gel. Thus, there is greater freedom for incorporating bioactive moieties, such as cell adhesive ligands and degradable sites, onto the end of the PA to control cellular behaviors. However, consideration must be given to the resulting assemblies in the hydrophilic peptide region, as unfavorable β-sheet conformations due to random protein folding could reduce the availability of bioactive moieties in the peptide backbone (Paramonov et al. 2006a).

Molecular simulation of PA nanofiber formation has also been investigated to further understand the molecular interactions taking place during self-assembly because a detailed explanation had not been fully realized from experimental characterizations. Velichko et al. used a course-grained model to simulate PA self-assembly (Velichko et al. 2008). This allowed for a simplified simulation that did
not account for any specific chemical structures of PAs; instead, the amphiphilic molecule was divided into three general regions—hydrophobic, peptide, and epitope head group. The theoretical simulations determined that PA self-assembly into cylindrical nanofibers followed an open association model, indicating that initial hydrogen bonding into β-sheet formations first occurs before reorganization into extended cylindrical nanofibers. This shows that the molecular structure and the balance of dominant intermolecular forces (i.e., hydrogen bonding and hydrophobicity) are the most important factors for achieving an equilibrium state during the PA self-assembly process.

Finally, the environmental conditions must be considered in the PA self-assembly process, especially for biological applications that require a physiological environment to ensure viability. Based heavily on initial factors, such as pH and salinity, PAs can assemble into cylindrical or spherical micelles, an intermediate structure between the two, or not form at all. Thus, Tsonchev et al. built a semiquantitative pH/salinity phase diagram shown in Figure 7.7 to better illustrate how all of these parameters and their corresponding interactions direct PA self-assembly (Tsonchev et al. 2008). The model constructed was based on the competition between electrostatic and hydrophobic forces using both theoretical modeling and experimental data. In general, a disposition toward higher hydrogen bonding favors cylindrical PA formations, but this can be negated with increased salinity. Several more self-assembly generalizations were also formulated based on the pH/salinity interactions investigated by Tsonchev et al. PA molecules tend to stay bundled as cylindrical nanofibers over the pH range of 2–4 at low salt concentrations. The nanostructures become disassembled as the pH is lowered to 0 because of increased protonation breaking apart the hydrogen bonding. However, spherical assemblies are still possible at these highly acidic conditions if the salinity

### Table 7.2 Summary of N-Methylated Peptide Amphiphiles Prepared

<table>
<thead>
<tr>
<th>Glycine Position</th>
<th>Nanostructure</th>
<th>Rheology</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA 1</td>
<td>G G G G G G</td>
<td>F Gel</td>
</tr>
<tr>
<td>PA 2</td>
<td>G G G G G G</td>
<td>F Gel</td>
</tr>
<tr>
<td>PA 3</td>
<td>G G G G G G</td>
<td>NMeG NMeG</td>
</tr>
<tr>
<td>PA 4</td>
<td>G G G G G G</td>
<td>NMeG NMeG</td>
</tr>
<tr>
<td>PA 5</td>
<td>G G G NMeG</td>
<td>NMeG NMeG</td>
</tr>
<tr>
<td>PA 6</td>
<td>G NMeG NMeG</td>
<td>NMeG NMeG</td>
</tr>
<tr>
<td>PA 7</td>
<td>G NMeG NMeG</td>
<td>NMeG NMeG</td>
</tr>
<tr>
<td>PA 8</td>
<td>NMeG NMeG</td>
<td>NMeG NMeG</td>
</tr>
<tr>
<td>PA 9</td>
<td>NMeG G G G G</td>
<td>NMeG NMeG</td>
</tr>
<tr>
<td>PA 10</td>
<td>G NMeG G G G</td>
<td>F − −</td>
</tr>
<tr>
<td>PA 11</td>
<td>G NMeG G G G</td>
<td>F − −</td>
</tr>
<tr>
<td>PA 12</td>
<td>G G NMeG G G</td>
<td>F − −</td>
</tr>
<tr>
<td>PA 13</td>
<td>G G G G NMeG</td>
<td>G G F Gel</td>
</tr>
<tr>
<td>PA 14</td>
<td>G G G G NMeG</td>
<td>F Gel</td>
</tr>
<tr>
<td>PA 15</td>
<td>NMeG NMeG</td>
<td>G G G G G</td>
</tr>
<tr>
<td>PA 16</td>
<td>NMeG NMeG</td>
<td>NMeG NMeG</td>
</tr>
<tr>
<td>PA 17</td>
<td>NMeG NMeG</td>
<td>NMeG NMeG</td>
</tr>
<tr>
<td>PA 18</td>
<td>NMeG NMeG</td>
<td>NMeG NMeG</td>
</tr>
<tr>
<td>PA 19</td>
<td>NMeG NMeG</td>
<td>NMeG NMeG</td>
</tr>
</tbody>
</table>

Source: Adapted with permission from [Paramonov, S.E., Jun, H.W., and Hartgerink, J.D., Modulation of peptide-amphiphile nanofibers via phospholipid inclusions, Biomacromolecules, 7(1), 24–26, Copyright [2006] American Chemical Society.

“F” indicates that the nanofibers were the dominant nanostructure present as observed by vitreous ice cryo-TEM; “−” means no fibers were present, and the sample was principally composed of spherical micelles and amorphous aggregates. For the column indicating rheology, options are Gel or wGel (weak gel), or “−” meaning no gel was formed.
is increased enough to sufficiently screen the charges. Conversely, increasing the pH with a negligible salt concentration, increases the electrostatic repulsion between the PA molecules, but the hydrogen bonding, albeit weaker, remains strong enough to still produce cylindrical nanofibers capable of forming gels. For a pH above 9, though, the electrostatic repulsion becomes too high to tolerate PA self-assembly, unless the salinity is greatly increased to screen the repelling forces and allow for cylindrical formations. Altogether, self-assembly of these PA molecules is capable of transitioning across several phases based on the pH and salinity, but for tissue regenerative applications to succeed, a physiologically relevant microenvironment is a required necessity. Hence, this highly controllable PA self-assembly system within the targeted neutral pH range offers numerous opportunities for regenerative treatments based on cell encapsulation within the nanomatrix to direct biological responses.

### 7.4.2 Incorporation of Enzyme-Mediated Degradation Sites into Single-Tailed Peptide Amphiphiles

These initial molecular characterization studies provided insight into the self-assembly of single-tailed PAs and laid the necessary groundwork for future investigations oriented toward tissue engineering applications. Progressing with this approach, the ultimate goal is to develop an ECM-mimicking
biomaterial, capturing both the chemical and biological complexity needed. By including degradation sites and cell adhesive ligands isolated from the ECM, one can potentially control cellular behaviors with this PA nanomatrix. Moreover, degradation by cell-mediated enzymes allows the cells to create pathways for migration. Therefore, an ideal ECM-mimicking biomaterial should have all of these vital characteristics. In this regard, an approach demonstrated by Jun et al. is of particular interest (Jun et al. 2005). In this study, single-tailed PAs were utilized to create cell-responsive PA nanofiber networks that simulate several essential properties of the ECM, including self-assembling of nanofibers, presence of cell-adhesive ligands, and cell-mediated degradation.

As shown in Figure 7.8, this degradable PA molecule consisted of three regions: cell-mediated enzyme sensitive peptide sequence (GTAGLIGQ), calcium binding sites via a glutamic acid residue and C-terminal carboxylic acid, and cell adhesive ligand (RGDS). The featured matrix metalloproteinase-2 (MMP-2) specific cleavage site allows for cell-mediated degradation of the PA nanofiber network, thereby enabling a pathway for cellular migration and remodeling. To test the efficiency of the incorporated degradable sequence, PAs were prepared as self-assembled disk-shaped gels and incubated in Type IV collagenase. After 1 week, these PA gels lost 50% of their original weight (Figure 7.9a), and by week 3, egg-shaped fibrillar aggregates that associated into multistranded twisted ribbons were observed (Figure 7.9c). This indicated that an accumulation of defects within these PA nanofibers eventually broke the assemblies into small fragments that diffused out of the gel and decreased the stability.

Finally, rat maxillary incision pulp cells, which play an important role in dentin mineralization and dental tissue development, were encapsulated in these self-assembled nanofibers to assess the ability of these degradable PAs to support cell adhesion and proliferation. The RGDS ligand inscribed within these PAs used for cell encapsulation was varied at different densities to investigate bioactive signal availability. Although the encapsulated cells exhibited a round morphology within the nanofibrous gel after 1 day for all conditions, the cells grown in gels presenting at least 50% of the RGDS ligand eventually formed dense cell colonies throughout the gel, which became fully spread after 4 days (Figure 7.10a and c). However, cells exposed to less than 50% of the RGDS ligands remained spherical throughout (Figure 7.10b and d). This signifies the ability of the encapsulated cells to enzymatically make migratory pathway and track along the adhesive ligands through the network, remodeling the PA nanomatrix. By fully integrating an enzyme-sensitive degradable site, these cell-responsive PA nanofibers offer a more sophisticated biomaterial for mimicking native ECM. The use of single-tailed PAs in this manner provides a large step forward in the development of next-generation biomaterials capable of manipulating cell adhesion, migration, proliferation, and differentiation.
7.4.3 Modifications of Single-Tailed Peptide Amphiphiles

The hydrophobic core of these PAs provides a potential region that can be adapted to create synthetic ECM biomaterials directed toward a specific biomedical need. Exploring this versatility, phospholipids have been utilized to modulate the mechanical properties and secondary peptide structures of the self-assembling hydrogels (Paramonov et al. 2006b). Specifically, 1-palmitoyl-2-hydroxy-sn-glycerol-3-phosphocholine was chosen as the phospholipid and was conjugated with PAs as depicted in Figure 7.11. Rheologically, it was demonstrated that these modulated PAs were capable of forming self-supporting gels with lipid inclusions up to 20% molarity. Within this range, normal nanofiber formation was observed with an average diameter of 10.8 ± 0.8 nm; however, increasing the lipid inclusion up to 20% molarity destabilized the hydrogels. Overall, the maximum storage modulus occurred at 5% molarity, indicating the optimal hydrogen bonding and molecular packing of these PA molecules constituting the nanofibers. The ability to introduce small hydrophobic molecules, such as phospholipids, into PAs further expands the resourcefulness of this molecule, especially as a drug delivery system.
In a follow-up study with the enzyme-sensitive PAs described earlier, Jun et al. demonstrated that the selection and combination of specific peptide sequences could be tailored to create a diverse range of ECM-like gels to better control bioactivity, degradability, and mechanical properties (Jun et al. 2008). Investigating this tunable system, three different peptide combinations were used to synthesize three individual PA molecules: a MMP-2 only PA (GTAGLIGQES; PA1), degradable PA containing the cell adhesive RGDS (GTAGLIGQERGDS; PA2), and a scrambled RDGS control (GTAGLIGQERDGS; PA3). All three PAs included the cleavage site (GTAGLIGQ) and a hydrophobic tail composed of palmitic acid. Inducing nanofiber self-assembly with calcium ions ($M_r = 2, M_r = [\text{Ca}^{2+}]/[\text{PA}]$), PA1, which has three lesser amino acids, formed shorter nanofibers with an average length of 500 nm, while PA2, containing the RGDS motif, self-assembled into long nanofibers with a length of several microns. If PA1 and PA2 were mixed at a 1:1 molar ratio, nanofibers with an intermediate length were obtained. Interestingly, the length of the nanofibers affected the mechanical properties of the PA networks. Based on rheometry, the storage modulus ($G'$) of PA2 only amounted to a fraction of the value for PA1 (Figure 7.12a). However, the nanofiber network modulated to 25% of PA1 and 75% of PA2 showed a storage modulus six times
higher than that of PA2 alone, whereas the storage modulus of the 75% PA1 and 25% PA2 composite was 60-fold higher than pure PA2. When the different PA ratio mixtures were incubated with Type IV collagenase, the incubation time needed to achieve a 50% weight reduction for PA2, a 50:50 mixture of PA1/PA2, and PA1 were approximately 1, 2, and 4 weeks, respectively (Figure 7.12b). Therefore, altering the length of the nanofibers also changes the degradation kinetics of PA gels. This follow-up study vividly shows that the nanofiber length is another influential factor in the self-assembly process of PAs into viscoelastic networks. Clearly, these PAs have a promising future as a biomaterial for biomedical applications because of their versatility to form pseudo ECM-like nanostructures with controllable mechanical properties and degradability within the microenvironment.

7.4.4 Biomedical Applications for Peptide Amphiphiles

Potential tissue regenerative applications with these PA molecules are far reaching, as numerous examples are present in the literature and range from directed biological response via cell encapsulation to hybrid scaffolding dual functionality to growth factor delivery. Tissue-specific deviations of this PA nanomatrix have served as bioactive scaffolds for many cell types, including neural progenitor cells, mouse calvarial pre-osteoblastic (MC3T3-E1) cells, primary enamel organ epithelial cells, and pancreatic islets (Silva et al. 2004; Beniash et al. 2005; Huang et al. 2008; Sargeant et al. 2008; Stendahl et al. 2008). In all cases, this PA encapsulation approach has proven to be biocompatible. Specifically, Beniash et al. demonstrated that cell encapsulation within this PA nanomatrix does not deter cell proliferation or
motility, and surprisingly, that the entrapped cells were able to internalize the surrounding nanofibers by endocytosis (Beniaș et al. 2005).

Progressing into directed cellular signaling, the PA biomaterial has also been investigated as an instructive scaffold for proliferation and differentiation along specific tissue lineages. For example, neural progenitor cells have been encapsulated within a self-assembled PA nanomatrix expressing the IKVAV epitope in the outer peptide domain (Silva et al. 2004). The IKVAV peptide sequence was isolated from laminin and has shown the ability to promote neurite growth (Wheeler et al. 1999; Kam et al. 2001; Yeung et al. 2001). The presentation of this bioactive epitope within this PA fibrous network was found to selectively enhance differentiation of the progenitor cells into neurons with minimal astrocyte development, as confirmed by neurite outgrowth morphology and positive β-tubulin immunohistological staining. In a similar study, PAs have effectively been used as instructive cell encapsulating scaffolds for enamel formation and long-term tooth regeneration. Specifically, Huang et al. employed a branched PA molecule displaying the RGD
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Peptide signal and co-cultured ameloblast-like cells and primary enamel organ epithelial cells (Huang et al. 2008). This cell-encapsulated PA gel was implanted in embryonic mouse incisors and found to promote proliferation and enhanced expression of differentiation markers, while still maintaining long-term viability. Therefore, these peptide-based biomimetic scaffolds for cell entrapment provide the necessary bioactivity and physical properties desired for regenerative medicine treatments.

Expanding the scope and utility further, these easily tunable PAs can be effortlessly functionalized to create a wide range of hybrid scaffolds and therapeutic delivery vehicles. One such beneficial aspect of this dual functionality is the potential to add bioactive signals to relatively inert surfaces, while still maintaining the physical properties of the original material. Using this approach, PAs have been combined with metal orthopedic implants and carbon nanotubes (Arnold et al. 2005; Sargeant et al. 2008). Sargeant et al. was able to create a hybrid bone implant material consisting of Ti-6Al-4V foam that integrated self-assembled PAs throughout the interconnected pores (Sargeant et al. 2008). These incorporated PAs served as a mineralization template for HA and enhanced bone ingrowth from the surrounding tissue, thus allowing for improved implant fixation, osseointegration, and long-term stability. PAs have also been non-covalently functionalized with carbon nanotubes to provide lacking bioactivity. The carbon nanotube is a hydrophobic material that displays an extremely high length-to-diameter ratio and extraordinary strength (Zheng et al. 2004). Its potential functionality canvases a wide range of applications, such as mechanical, electronic, optical, sensing, and biological (O’Connell et al. 2002; Chen et al. 2003; Dalton et al. 2003; Javey et al. 2003; Kam et al. 2004). Arnold et al. was able to successfully encapsulate carbon nanotubes with several different PAs, as the hydrophobic alkyl tails were strongly attracted to the hydrophobic nanotube surfaces (Arnold et al. 2005). Both of these examples are just one of many endless possibilities for introducing a directed biological response onto other inert materials through the self-assembled formations of PAs.

Finally, the versatility of PAs provides opportunities for developing therapeutic delivery systems, as the peptide segment can serve as a binding construct for growth factors or imaging contrast agents (Bull et al. 2005; Rajangam et al. 2006; Stendahl et al. 2008). For example, heparin-binding sequences have been inscribed into PA molecules by Rajangam et al. (Rajangam et al. 2006). Heparin, itself, is a biological molecule with a strong binding affinity for angiogenic growth factors (Tanihara et al. 2001; Ishihara et al. 2003). Thus, heparin was attracted to specific binding regions in the designed PA, which served as an intermediate for delivering vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FGF-2). In vivo studies with these heparin-binding PAs and attached growth factors were found to stimulate significant new blood vessel formation in a rat cornea angiogenesis model, (Rajangam et al. 2006) and the validity of the binding sequence was confirmed by stable heparin interactions and the resulting biological response (Rajangam et al. 2008). These heparin-binding PAs have also been exploited to deliver encapsulated isologous islets and angiogenic growth factors (i.e., VEGF, FGF-2) into a diabetic mouse omentum (Stendahl et al. 2008). The transplantation resulted in increased neovascularization and improved islet engraftment, thereby enhancing the normoglycemia rate in the mice recipients. Besides drug delivery, PAs have been modified to uptake magnetic resonance imaging (MRI) contrast agents, such as Gd(III) (Bull et al. 2005). The magnetic resonance contrast agent was covalently linked to a specific binding sequence isolated from tetraacetic acid and inscribed within PA molecules. By conjugating Gd(III) to these PAs, the relaxivity of the agent was increased, which produced significantly better imaging contrast for longer in vivo observations. This allows for improved image sensitivity and potential cell tracking within these peptide-based scaffolds.

7.5 Conclusions

The potential applications for these peptide-based biomaterials translate across many different fields of biomedical research. This chapter has only highlighted the opportunities presented by two types of peptide molecules—SAPs and PAs. Both work in principle to synthetically capture the self-assembled formations of phospholipids naturally observed under physiological conditions. However, the differences
lie in the basic structure, as SAPs are purely peptide based and PAs contain added hydrocarbon tails. Each has its own merits as a self-assembling biomaterial due to the versatility within the internal amino acid composition and ease of conjugating with other biomaterials to diversify functionality. Many different self-assembled configurations are possible based on the endowed physical properties, but the focus here is on nanofibrous assemblies, which present a complex nanostructured environment in the mold of native tissue formations at the most basic level. For both biomaterials, the self-assembly into nanofibers is driven by the inherent amphiphilic nature that results in the outer hydrophilic peptides shielding the inner hydrophobic core in a thermodynamically efficient arrangement. As discussed, this is a highly controllable process that can be directed by molecular and environmental factors, such as charge, hydrophobicity, pH, and salinity. Altogether, these factors work in concert to create cell-responsive peptide-based nanofibers with great promise for biomedical applications in regenerative medicine. Many such examples have been presented, encompassing tissue-engineered scaffold to encapsulate cells and direct cellular responses, therapeutic drug delivery, bioactive implant coatings, and diagnostic biosensors. By having the capacity to concurrently control the nanostructure and biological complexity, the future of peptide-based self-assembling nanofibers as a biomaterial is full of endless possibilities.

References


