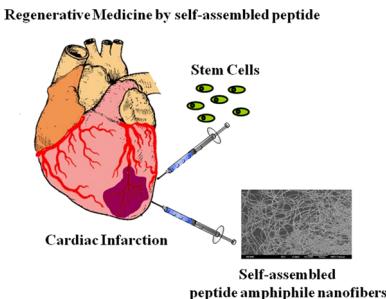


Self-Assembled Proteins and Peptides for Regenerative Medicine

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1. INTRODUCTION

Many diseases including cardiovascular disease, diabetes, osteoporosis, and cancers cannot be treated effectively with current clinical therapy because of a significant reduction in the level of tissue regeneration. Every day thousands of people of all ages are admitted to hospitals because of the malfunction of some vital organ. Because of the dearth of transplantable organs, many of these people will die. The evergrowing demand for donor organs to meet the needs of individuals on waiting lists will likely never be met. In short, the need for organs cannot be met by traditional methods of transplantation. Regenerative medicine may change that. Regenerative medicine is a new field of science that uses stem cells to regenerate biological tissues and improve tissue functions. Replacing these lost cells using stem cell-based therapies offers the possibility of exciting alternative treatments of current clinical medicine. Regenerative medicine will only be a topic of the future rather than the present and is based on combinational technology of materials science, stem cells technology, and reconstructive surgery that aim to regenerate natural tissues as well as create biological substitutes for defective or lost organs and tissues (Figure 1).

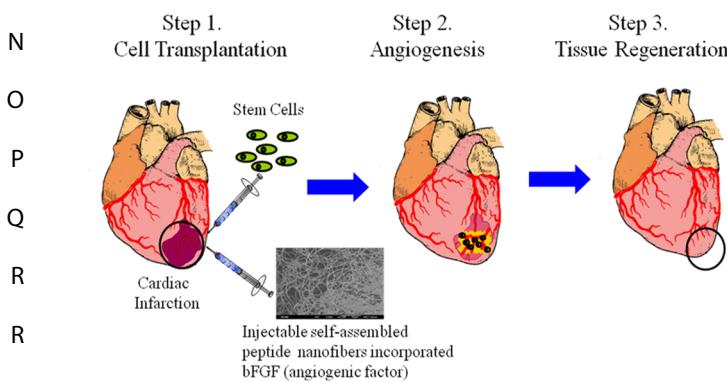


Figure 1. Strategy of regenerative medicine therapy based on combinational technology of materials science and engineering, stem cells technology, and reconstructive surgery.

Received: March 29, 2012

Design of materials that can regulate cell behavior such as proliferation and differentiation is a key component in regenerative medicine therapy. From the viewpoint of immune system response of the body, implanted biomaterials should mimic the structure and biological function of native extracellular matrix (ECM) in terms of chemical composition and physical properties. Tissue-engineered scaffolds used in regenerative medicine have micrometer dimensions that fail to mimic the structure of natural ECM. Biomaterials have been widely used to fabricate tissue-engineered scaffolds because of their good biological activity that current research appears to indicate for these materials. Remarkably, recent identification of nanotechnology with enhanced ability to mimic natural ECM proteins such as collagen has led to the discovery of a new class of biomaterials with specific properties for tissue-engineering applications. One of the common approaches to produce fibers similar to ECM proteins is self-assembling of collagen. This technique is sufficient to cause its rapid transduction into a variety of different tissues *in vitro* as well as *in vivo*. Moreover, this novel technique for proteins and peptides appears to circumvent many problems associated with cells and drug-based methods.

Nanomaterials technology has been approached in two ways: top-down and bottom-up approaches.¹ The top-down approach is miniaturization techniques to construct micro- and nanoscale structures from a macroscopic material or a group of materials by utilizing machining and etching techniques. The best example of this approach is the photolithography technique that has been widely used in semiconductor industry to fabricate components of an integrated circuit by etching micro- and nanoscale patterns on a silicon wafer.² The bottom-up approach refers to construction of macromolecular structures from atoms or molecules that have the ability to self-organize or self-assemble to form a macroscopic structure.³ In other words, this approach has been referred to as molecular nanotechnology. These nanomaterials can be effectively applied in the field of regenerative medicine, pharmacotherapeutics, and medical health care to discover novel drugs, drug delivery systems, imaging, and gene therapy. Self-assembly, electrospinning, and phase separation are three different approaches toward formation of nanofibrous materials.⁴ Fabricated materials by each method are very different in terms of physical and chemical characteristics, which lends to its development and biological applications. Self-assembly is a very unique and attractive method that can fabricate a small diameter of nanofibers in the range of natural ECM. Electrospinning can only form large diameter nanofibers that are very far from the range of natural ECM. Phase separation, on the other hand, can generate nanofibers in the same range as natural ECM and allows for design of macroporous structures.

Self-assembly is the autonomous organization of individual components into patterns or structures without human intervention. Self-assembly has been defined as ‘the non-covalent interaction of two or more molecular subunits to form an aggregate whose novel structures and properties are determined by the nature and positioning of the individual components’.⁵ Self-assembly has also been defined as ‘the spontaneous assembly of molecules into structured, stable, noncovalently joined aggregates’.^{6,7} Self-assembly is the inherent ability of numerous multimeric biological structures to assemble from their component parts through random movements of molecules and formation of weak chemical bonds between surfaces with complementary shapes. A few

examples of self-assembled structures that can be found in biological systems are the phospholipid bilayer of human cell membranes⁸ and RNA and DNA complexes.^{9,10} Detergent surfactant molecules exhibit a self-assembly phenomenon due to their amphiphilic properties.¹¹ The molecular building mechanisms underlying formation of bacteriophage and viral particles are all based on self-assembly.¹² Molecular self-assembly is a powerful phenomenon borrowed from nature by scientists for fabricating novel supramolecular architectures and defined as spontaneous organization of molecules under near thermodynamic equilibrium conditions into structurally well-defined and stable arrangements through noncovalent interactions.¹¹ This is mainly governed by weak noncovalent bonds like electrostatic interactions (ionic bonds), hydrogen bonds, hydrophobic and hydrophilic interactions, water-mediated hydrogen bonds, and van der Waals interactions. Although these forces are weak, their collective interactions can produce molecules that are structurally and chemically stable. Frequently, molecular self-assembly relies on chemical complementarity and structural compatibility.¹³ Molecular components need complementary properties such as specific surface characteristics, surface charge, and polarizability, mass and surface functionalities to self-assemble into different physiological forms.¹³ Biological molecules like proteins, peptides, nucleic acids, lipids, and other cellular components with complementary properties self-assemble to form the fundamental biological unit, the cell. Cellular events like amyloid fibril formation, antigen–antibody recognition, chromatin assembly, and phospholipid membrane self-assembly are excellent examples of molecular self-assembly.

This review will consider the self-assembled systems and recent developments for their potential applications in regenerative medicine. Self-assembling proteins and peptides for designing novel biomaterials and their potential applications in regenerative medicine and biomedical applications will be also discussed in detail from the viewpoint of their biological applications.

2. CLASSIFICATION OF SELF-ASSEMBLED SYSTEMS

Self-assembly is a native process. It can be classified into two types: static and dynamic.¹⁴ Most research studies on self-assembly have been focused on the static type, while studies on dynamic self-assembly are still poorly understood. Static self-assembly contributes to systems that are at global or local equilibrium and do not dissipate energy.¹⁴ In static self-assembly, formation of the ordered structure may require energy but is stable once it is formed. A few examples of the static self-assembly phenomenon tailored by nature are lipid molecules forming oil droplets in water, four hemoglobin polypeptides forming a functional hemoglobin protein, and combination of RNA and ribosomal proteins to form a functional ribosome. Dynamic self-assembly occurs when formation of an ordered state of equilibrium requires dissipation of energy. In other words, interactions responsible for formation of structures or patterns between components occur only if the system dissipates energy.¹⁴ Self-assembly takes place at molecular, mesoscopic, and macroscopic scales. On the basis of this criteria, self-assembly has been classified as molecular and nanoscale self-assemblies (classical form of self-assembly in chemistry involving atoms, molecules, and crystal formation) and meso- and macroscopic self-assemblies (e.g., engineered microparts). Molecular and nanoscale self-assembly can be further classified as intramolecular and intermolecular

self-assembly.¹¹ In intramolecular self-assembly, molecules are often complex polymers which can assemble from the random coil into a well-defined stable structure (secondary and tertiary structure). Protein folding to form secondary and tertiary amine structures is a good example of intramolecular self-assembly. Intermolecular self-assembly is the ability of molecules to form supramolecular assemblies (quaternary structure). Supramolecular micelle formation by surfactant molecules in solution and self-assembled monolayers (SAM) on a substrate are classic examples of intermolecular self-assembly. Magnetic, capillary, electrostatic, and gravitational forces play a vital role in self-assembly of structures on the meso- and macroscale levels.¹¹

2.1. Proteins and Peptides

Proteins are fundamental components of all living cells. They can be considered as a group of complex organic macromolecules containing carbon, hydrogen, nitrogen, oxygen, and sulfur. They are composed of one or more chains of amino acids. Two or more amino acids linked by a peptide bond form a peptide molecule. Large numbers of peptide molecules arrange themselves in different fashions to make up different kinds of proteins. Enzymes, hormones, and antibodies are a few examples of biological substances that are made up of proteins and required for proper function in living organisms. Structural analysis of protein molecules has revealed that they take up various shapes to form a stable macroscopic structure. Classification of proteins based on the structure and composition is shown in Table 1. Nature has used proteins

Table 1. Classification of Proteins

based on composition	<ul style="list-style-type: none"> (i) simple proteins: containing only amino acids (examples, albumins, globulins, glutelins, histones, scleroproteins, prolamines, and protamines) (ii) conjugated proteins: containing a nonproteinaceous prosthetic group and amino acids (examples: phosphoproteins, lipoproteins, flavoproteins, nucleoproteins, mucoproteins, glycoproteins, chromoproteins, and metalloproteins)
based on structure	<ul style="list-style-type: none"> (i) fibrous: long parallel polypeptide chains forming fibers and sheets (examples, collagen, elastin, keratin) (ii) globular: tightly folded polypeptide chains forming a spherical structure (examples, insulin, serum globulin) (iii) intermediate: fiber-shaped polypeptide chains (example, fibrinogen)

to build a vast array of structures like keratin, collagen, coral, pearl, shell, etc. In the past few decades extensive research has been done to understand the structural characteristics that influence self-assembly of protein and peptide molecules. Potential applications of self-assembling proteins and peptides have been effectively utilized to design novel biomaterials (Figures 2 and 3) which have tremendous applications in the fields of biotechnology and biomedicine.^{11–20} Application of self-assembled proteins and peptides has been outlined in Table 2.

2.2. Self-Assembly of Proteins and Peptides

Several peptides and proteins that self-assemble to form various nanostructures like nanotubes, vesicles, helical ribbons, and fibrous scaffolds have been discussed in detail. These structures are analyzed to design and fabricate new materials that will have potential applications in biomedical nanotechnology. The findings in the research paper have been discussed and grouped under different headings like self-assembling peptide systems, amphiphilic and surfactant peptides, peptides self-assembly into 3D matrix scaffolds, and designed peptide hydrogels for 3D cell

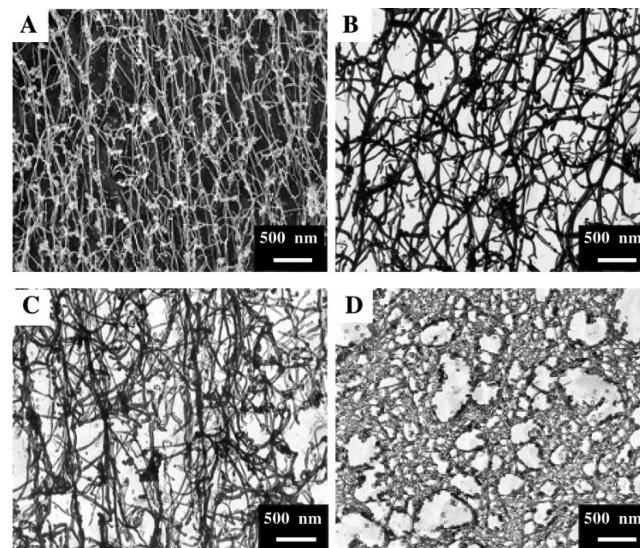


Figure 2. Quick-freeze/deep-etch TEM image of the surfactant peptides A_6D , V_6D , V_6D_2 , and L_6D_2 in water (4.3 mM). These peptides self-assembled into a dense network extended to several micrometers in length. Because the droplet solution containing the peptide nanotubes is in three dimensions, the network of a two-dimensional image appears denser than the actual structure, similar to looking at a picture of the branches on a tree without leaves: (A) A_6D , (B) V_6D , (C) V_6D_2 , and (D) L_6D_2 . Reprinted with permission from ref 18. Copyright 2002 National Academy of Sciences.

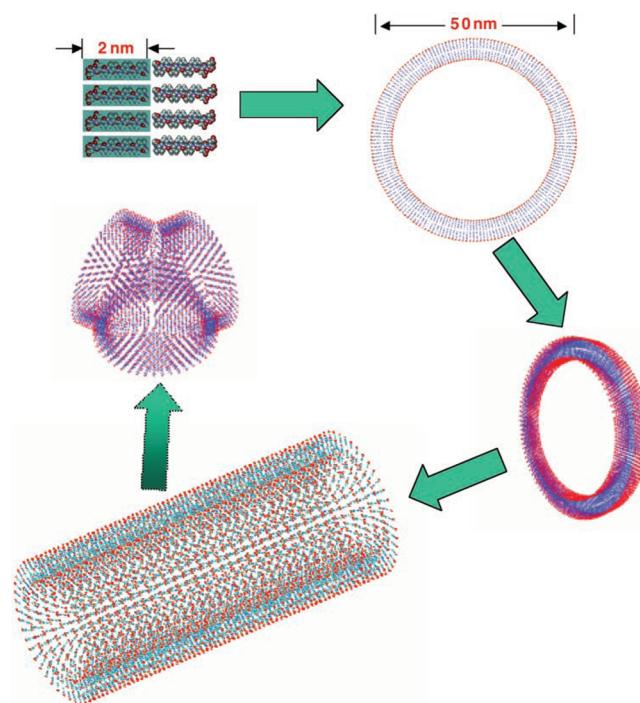


Figure 3. Potential pathway of V_6D peptide nanotube formation. Each peptide monomer is 2 nm, and the diameter of the modeled bilayer nanotube is 50 nm. Red, hydrophilic head; blue, hydrophobic tail. Each peptide may interact with one another to form closed rings, which in turn stack on top of one another, ultimately yielding a nanotube. Three nanotubes are connected to each other through a three-way junction. This phenomenon mirrors lipid microtubule structures. Reprinted with permission from ref 18. Copyright 2002 National Academy of Sciences.

Table 2. Application of Self-Assembled Proteins and Peptides

bionanotechnology applications and regenerative medicine	nanoengineering of molecular templates and supramolecular structures ¹² self-assembling amphiphilic peptide and protein systems that self-assemble to form various nanostructures like nanofibers, nanotubes, vesicles, helical ribbons, and fibrous scaffolds ¹³ self-assembly of amphiphilic peptides to nanoscale fibers forming nanostructured fibrous scaffold reminiscent of extracellular matrix ¹⁶ artificial proteins that self-assemble to form hydrogels in response to pH and environmental changes; protein hydrogels can be used for advanced wound closure and tissue repair in regenerative medicine and tissue engineering peptide nanofibers as target specific drug delivery systems with better biocompatibility used as scaffolds to fabricate nanowires, templates for metallization (for example, histidine-rich peptide nanotubes were metallized with gold nanocrystals, and the organic peptide scaffold was removed to make conducting gold nanowires) ¹⁷ supramolecular self-assembly of hydrophobic dipeptides into nanotubes which can serve as ion channels when incorporated in the phospholipid bilayer of the cell membrane self-assembled protein forming 3-dimensional scaffolds with bone regenerative peptide motifs for bone regeneration and tissue engineering enhancement of angiogenesis in self-assembled amphiphilic nanofibrous systems
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culture and regenerative biology.^{21,22} Wang et al. designed artificial proteins that self-assemble to form hydrogels.²³ The artificial proteins designed by the researchers were made up of ionic self-complementary peptide group that had an alternating polar and nonpolar fashion of arrangement of peptide molecules. These peptides formed stable β -strand and β -sheet structures which self-assembled to form nanofibers. These nanofibers form interwoven matrices that further give a scaffold hydrogel with high water content. This hydrogel structure has water as its dispersion medium and responds to changes in pH and other environmental factors. These protein hydrogels can be used for advanced wound closure and tissue repair in regenerative medicine and tissue engineering. Biodegradable protein hydrogels can be utilized in drug delivery systems, delivering pharmaceutical protein complexes in treatment of diseases like cancer. Adams et al. designed peptide nanotubes using surfactant-like peptides.²⁴ These peptide nanotubes can be used as templates for growing metal nanocrystals and to fabricate nanowires. Peptide nanotubes can also serve as ion channels when incorporated in phospholipid bilayer of the cell membrane. Recent studies also reported biomimetic protein structures and peptide systems that can form complexes with metals and semiconducting elements. Surface-binding peptides can bind covalently with metal surfaces like gold.²⁵ These peptides can also form complexes with DNA which in turn can be bound to a metal surface. This property can be exploited to design and fabricate nanobiosensors. Certain peptides with strong dipoles undergo drastic conformational changes between the α -helical and the β -sheet structures. These are called molecular switch peptides. Gold nanoparticles can be attached to these dipolar peptides to fabricate tiny molecular switches.²⁶

2.3. Amphiphilic and Surfactant-Like Peptides

Amphiphilic molecules have a hydrophilic (polar) and a hydrophobic (nonpolar) component. In the presence of water, they self-assemble into distinct structures whose shape is largely determined by the size and shape of the hydrophilic polar head. These molecules have one or two amino acids in the polar region and four or more consecutive hydrophobic amino acids in their nonpolar end. One such example discussed is the V₆D amino acid complex. The V₆D amino acid sequence (VVVVVVD) has six valine (V) residues that are hydrophobic and an aspartic acid (D) residue that is negatively charged.^{27,28} Valine (α -aminoisovaleric acid) is an essential amino acid, whereas aspartic acid is a nonessential amino acid. The V₆D peptide complex formed various nanostructures in aqueous solution like nanotubes and nanovesicles. Samples in aqueous

solution were frozen in liquid propane (-180°C) and surface coated with a thin layer of platinum and carbon to preserve the structures formed. Zhang et al. observed nanotubes and nanovesicles by transmission electron microscopy (TEM). Nanotubes measured about 30–50 nm.²⁹ It has been demonstrated that changing the sequence of the amino acids in the peptide chain and the environmental factors can modify the self-assembled structure. These peptide nanotubes can be incorporated into self-assembled membranes to use in bionanosensor devices (Figure 4).³⁰ It is also suggested that these surfactant peptides can be functionally engineered using techniques like biotinylation. Biotinylation is a process of incorporation of biotinyl groups into molecules to visualize specific substrates by incubating with avidin or streptavidin. It is a rapid method of detecting nucleic acids in Western blot technique. When these surfactant peptide nanostructures are made to undergo the process of biotinylation, they can bind to streptavidin-coated inorganic metal surface. Histidine-tagged peptides and proteins can be bound to nickel surfaces. Thus, these nanostructures can be attached to metallic surfaces.

2.4. Three-Dimensional Peptides Matrix

A wide variety of self-assembling proteins and peptides have inspired the search to fabricate nanoscale fibers and a fiber network.^{16,17} The main factor influencing the entire process was the chirality of the individual building components of the peptide complex. Two molecules are said to be chiral if their mirror images do not superimpose on each other. Zhang et al. studied the KFE₈ peptide complex which is an eight-residue peptide complex with a FKFEFKFE sequence.³¹ This structure represents a group of self-assembling peptides that spontaneously assemble under certain physiological conditions. This peptide self-assembles in aqueous solution into left-handed helical ribbons when the peptide backbone is twisted in the opposite direction.³¹ When certain amino acids (such as K) in the hydrophobic side chains were replaced with another amino acid sequence, little changes were observed. On the other hand, when a positively charged lysine (Lys) was replaced by a positively charged arginine (Arg) and likewise when a negatively charged glutamate (Glu) was replaced by a negatively charged aspartate (Asp), very little changes were observed in the nanofibers that were formed.³¹ However, when the positively charged residue was replaced by a negatively charged residue or vice versa, the peptides did not self-assemble. When the hydrophobic residues were replaced with alanine, there was a greater tendency to self-assemble and form peptide matrices with enhanced strength. This led researchers

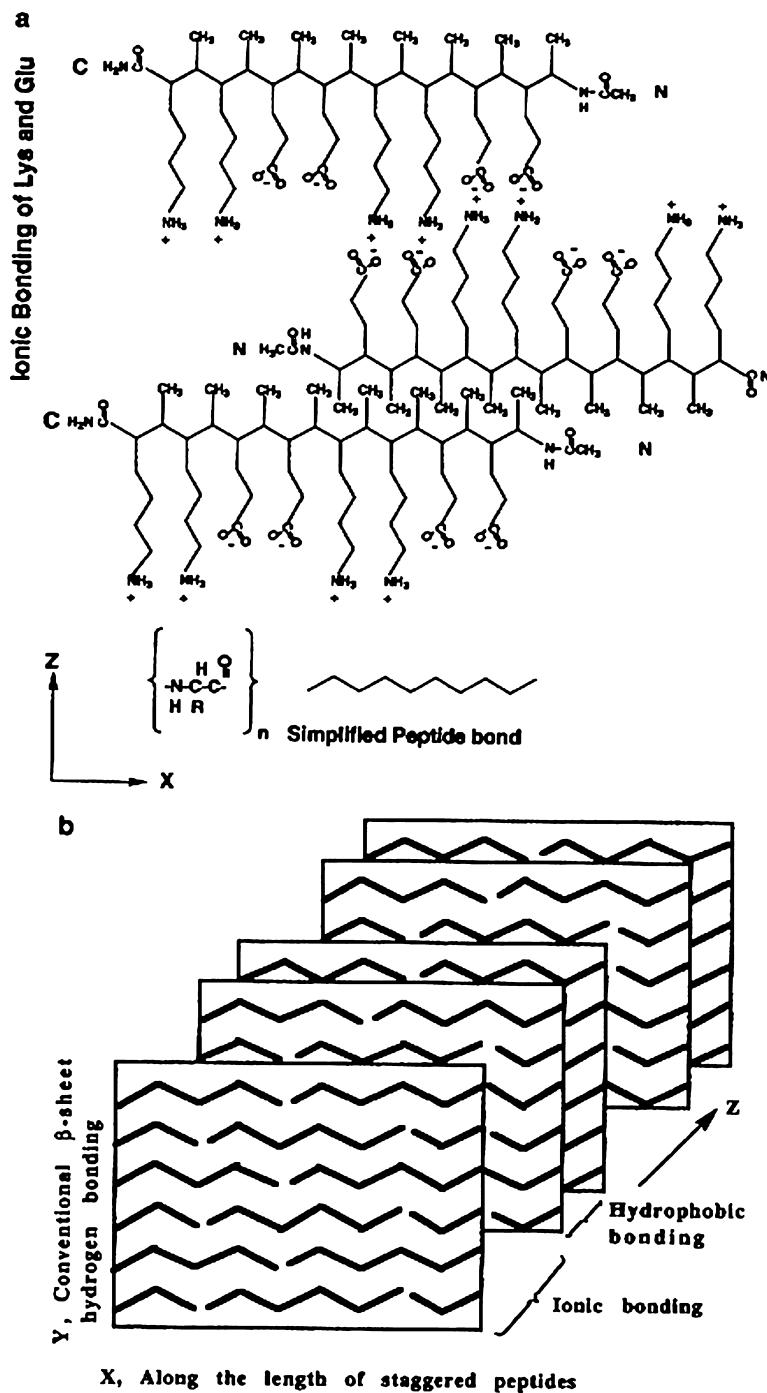


Figure 4. (a) Layers of an antiparallel 3-sheet, held together on one side by hydrophobic bonding between alanine side chains facing each other and the charged lysines and glutamic acid side chains facing each other to form ionic bonds. Structure can also be drawn as a parallel sheet. In either case, the peptide would be staggered along x , as shown in the diagram. (b) Stacking of β -sheets. Staggered peptides are oriented along the x axis. z axis has complementary ionic bonds between lysine and glutamic acid as well as hydrophobic bonds between alanines referred to in a. y axis contains conventional β -sheet hydrogen bonds. Similar interactions are found in sawfly silk fibroin containing 70–80% alanine and glutamine but without the ionic pairing. Reprinted with permission from ref 30. Copyright 1993 National Academy of Sciences.

to concentrate attention toward understanding the basis of protein conformational diseases. Protein conformational diseases are a group of disorders characterized by accumulation of malformed protein structures in cells. Proteins must fold into a proper three-dimensional structure to carry out their normal functions. However, when they do not fold properly they form malformed protein structures that accumulate in cells leading to pathological conditions. Alzheimer's disease, prion disease, and

Parkinson's disease are a few examples of protein conformational diseases. Thus, by understanding the mechanism of formation of peptide nanofibers and the factors controlling their self-assembly, researchers are aiming to formulate a remedy for protein conformational diseases.

Self-assembled peptide fibers can be classified into three theoretical models. The first model is the molecular model where the β -sheet peptides self-assemble into helical ribbons.

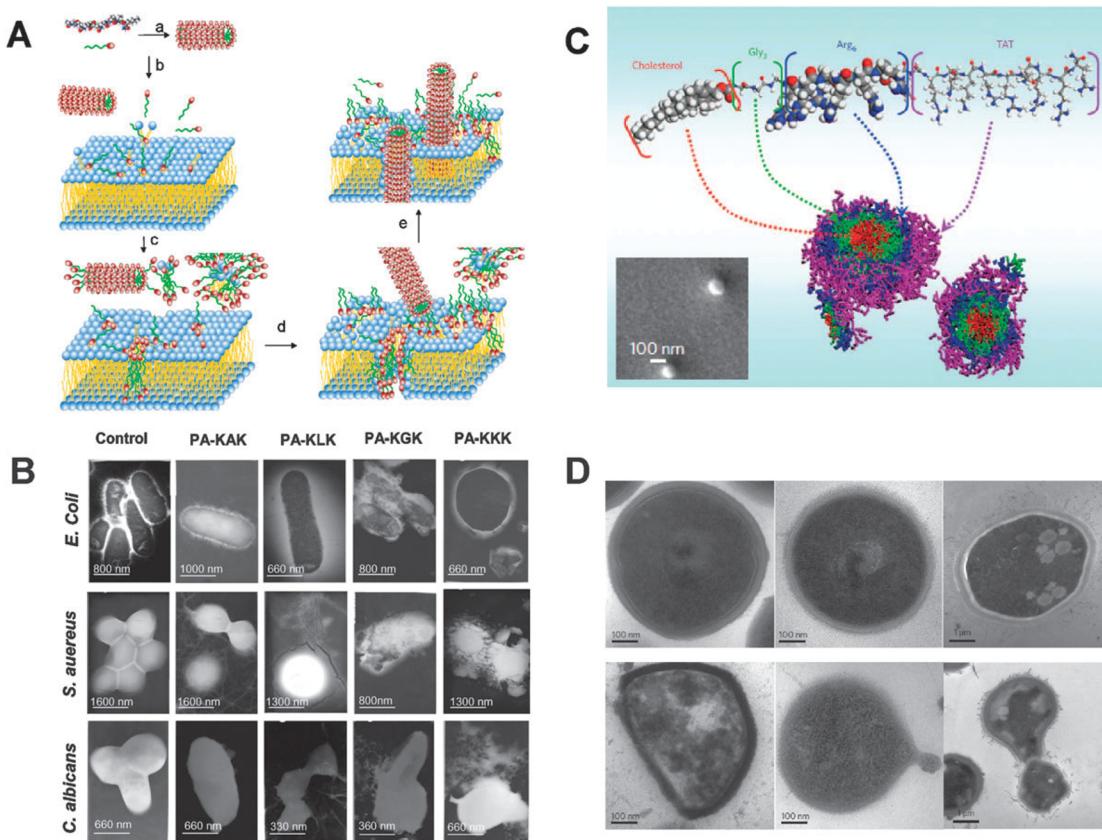


Figure 5. (A) Schematic illustration of mechanisms of action adopted by A₉K for bacterial membrane permeation and disruption. Red rods represent A₉K nanorods. A₉K molecules assemble into nanorods with the positive charges outside (step a). Monomers may also flap on and become inserted into the outer membrane surface (step b). They can then flip and become inserted into the inner leaf of the membrane, forming a “through barrel” or micelle to cause leakage or lysis (step c). Nanorods formed may also attack cell membrane through electrostatic attraction or local hydrophobic affinity, lifting some lipids out of the membrane and making the membrane bilayer (steps d and e). (B) Electron micrographs of negatively stained *E. coli* (top), *S. aureus* (middle), and *C. albicans* (bottom) untreated or treated with the C₁₆-KXX series of lipopeptides. Lipopeptides were used at their MICs. (C) Molecular cartoon of Chol-G₃R₆TAT and formation of micelles, simulated through molecular modeling using Materials Studio software. (SEM image of nanoparticles inserted). (D) TEM images of *S. aureus*, *E. faecalis*, and *C. neoformans* before (top row) and after (bottom row) incubation with 32 mM of nanoparticles for 2 h. Reprinted with permission from ref 32. Copyright 2010 Royal Society Chemistry.

The second model is the semicontinuum model where the peptides self-assemble to form elastic tape-like structures composed of brick-like building blocks. The third model is the full continuum model where the peptides self-assemble to form tubules. These approaches have helped researchers to know more about the mechanisms underlying formation of different structures when peptides and proteins self-assemble and will ultimately guide them to design efficient peptide-based and protein-based biomaterials.

3. IN VITRO BIOLOGICAL APPROACHES OF SELF-ASSEMBLED SYSTEMS

In the past several years, peptides and nucleotides have been considered to be useful building blocks for materials engineering; molecular self-assembly is known as an important route to produce novel materials which can cover a wide range of biological applications. Many amphiphilic peptides have been used in different applications thanks to their ability to form nanostructures.³²

3.1. Antimicrobial Activities

Cationic antimicrobial peptides can kill the microbes by interacting and disrupting bacterial cell membranes. After adopting stable secondary structures (α -helices, relaxed coils, β -

sheets), peptides sequences with 6–15 amino acids rich in hydrophilic moieties such as K, R, D, or E and in hydrophobic moieties such as A, V, I, L, F, W, or Y display characteristic amphiphaticity.^{33–37} Amphiphilic peptide A_mK ($m = 3, 6, 9$) displayed large antimicrobial activities with permeation and disruption to the bacterial membranes (Figure 5A).³² Mitra et al. studied lipopeptides-based molecules with P, F, or W as part of the head groups and C₁₄ as tails.³⁶ These lipopeptides showed high growth inhibition activity on both Gram-positive and Gram-negative bacteria and fungi. C₁₆-KXX series of lipopeptides (X is A, G, L, or K) have also been reported to be potent to both bacteria and fungi (Figure 5B). Among the lipopeptides studied, C₁₆-KKK is the best antimicrobial compound.

Recently, the membrane translocation sequence TAT (YGRKKRRQRRR) has been used to construct an antimicrobial agent:³⁸ Chol-G₃R₆TAT contains a hydrophobic tail of cholesterol, three glycine residues as spacer, and six arginine to enhance the performance of the membrane translocation sequence TAT (Figure 5C). Formation of micelles resulted in an increased cationic charge density outside of the nanoparticles, therefore enhancing antimicrobial activity. The presence of the TAT sequence could also help the nanoparticles to cross the blood–brain barrier (BBB) to the brain,

hereby making the molecule a good candidate for the brain infection treatment. Nanoparticles showed high antimicrobial effects against drug-resistant bacteria, yeast, and fungi. Electronic microscopic results revealed the disruption and lysis of cell walls of both bacteria and fungi (Figure 5D).

3.2. Three-Dimensional Cell Culture

The attractive features of nanostructures associated with gel network formation and antimicrobial effects from peptide amphiphiles make them good candidates as cell culture matrixes or scaffolds in tissue engineering and regenerative medicine. Extensive studies have already indicated their good biocompatibility.³⁹

Hydrogels constructed through self-assembly are receiving increasing attention for a variety of biomedical and biotechnological applications, including scaffolds for regenerative medicine, controlled release of therapeutics, and defined cell culture matrices.^{40–45}

β -Sheet fibrillizing peptides and peptide amphiphiles have received particular attention recently as matrices for cells owing to their stimulus-sensitive fibril formation, their ability to form hydrogels, their ease of synthesis, and the availability of many amino acid sequences known to influence cell behavior through binding of integrins and other receptors.^{41–53} These studies have been performed using the RAD₁₆ peptide self-assembling sequences from laminin or self-assembling sequences from transthyretin, demonstrating that several different β -sheet fibrill-forming peptides are able of presenting bioavailable ligands on their surfaces.^{45–50} Incorporation of ligands within β -sheet fibrillar hydrogels can significantly alter their mechanical properties, complicating the interpretation of cell behavior on these materials.^{48,54}

Some reports have described nonimmunogenic unfunctionalized fibrillar peptide assemblies and noninflammatory ligand-bearing assemblies.^{41,55–57} Jung et al.⁵⁸ investigated the coassembly, gelation, ligand presentation, technical properties, and immunogenicity of peptide hydrogels based on the sequence QQKFAQFQQ (Q₁₁), which has previously been utilized for producing substrates for endothelial cells with adjustable stiffness.⁵⁹ They reported a coassembling set of peptides based on the sequence of Q₁₁ that form hydrogels, where the display of multiple ligands may be adjusted simply by mixing different peptides in solution and inducing gelation. RGDS-Q₁₁ and IKVAVQ₁₁ were quantitatively incorporated into background gels of Q₁₁ in a wide range of peptide ratios, and ligand incorporation had no significant impact on fibril morphology or secondary structure. Gel viscoelasticity was minimally changed upon ligand inclusion, allowing ligand incorporation to be adjusted independently of gel mechanics. Both RGDS-Q₁₁ and IKVAV-Q₁₁ were presented on the surface of coassemblies with Q₁₁, and these ligands modulated HUVEC behavior in vitro. RGDS-Q₁₁ significantly affected HUVEC attachment, spreading, and proliferation, while IKVAV-Q₁₁ had a small effect on cell attachment and a subtle influence on cell morphology (Figure 6).

The ECM contains a lot of signals that activate various intracellular signaling pathways to control and guide cell behavior. Recapitulating the ECM regulatory mechanisms is of central importance in fundamental cell studies and cell-based applications such as tissue engineering. Methods have been established to capture the nanotopographical and biochemical characteristics in the natural ECM.^{60,61} Most of these methods, however, entail chemical syntheses that lack an adequate

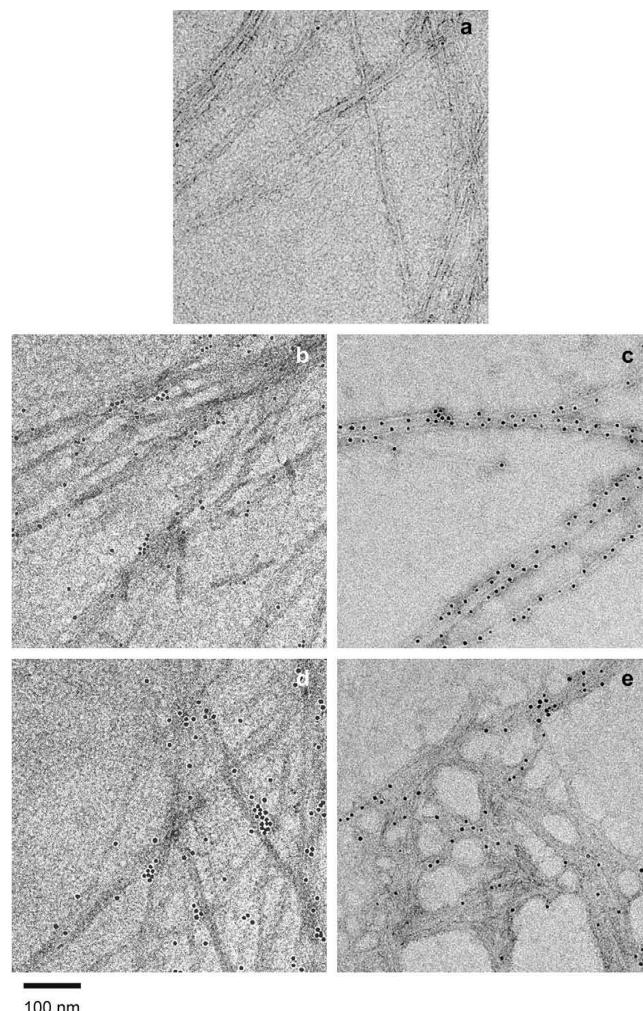


Figure 6. Ligands were displayed on the surface of Q₁₁ fibrils, as evidenced by the labeling of biotinylated ligand-bearing peptides with streptavidin–colloidal gold. Q₁₁ fibrils showed minimal background gold staining (a), whereas 10% biotin-RGDS-Q₁₁/90% Q₁₁ (b), 100% biotin-RGDS-Q₁₁ (c), 10% biotin-IKVAV-Q₁₁/90% Q₁₁ (d), and 100% biotin-IKVAV-Q₁₁/90% Q₁₁ (e) bound significant levels of avidin–gold. Reprinted with permission from ref 58. Copyright 2009 Elsevier.

flexibility to generate tunable biochemical patterns for rational design of extracellular environment. A comprehensive understanding of ECM cell interactions and screening of biomaterials may involve high-throughput studies that require systematically varying material and biochemical properties in a facile way.^{62,63} Chau et al.⁶⁴ explored a peptide-based platform for enriching biological functions in self-assembling three-dimensional scaffolds. A new family of biofunctional materials can potentially be obtained from automated peptide synthesis. Self-assembling peptides (SAPs) have recently emerged as an attractive class of 3D scaffolding materials, mainly due to their nanoscale fibrous and porous topographies that mimic the natural ECM features.^{65–67} Among them, (RADA)₄ peptides have been used to form scaffolds *in situ* for tissue-engineering applications.^{68,69} This material has been shown to support the growth and differentiation of a variety of cells, including those originated from human, mammals, mouse, and chicken, and covering stem cells, progenitor cells, and established cell lines.⁷⁰ Zhao et al. investigated a new SAP template with two modules:

one for generating ordered secondary structures to enable hydrogel formation, and the other for supplying biological cues to elicit specific cellular responses.

Fmoc-RGD mimicking the ECM has also been reported.⁷¹ These molecules self-assembled into nanofibers and bioactive hydrogels through $\pi-\pi$ stacking of the Fmoc groups, leaving the RGD groups outside the nanofiber surfaces. Self-assembled hydrogels displayed excellent performance in 3D cell culture using human adult dermal fibroblast cells.

Peptide amphiphiles have also been used to create three-dimensional (3D) microscale topographical patterns to study the behavior of human mesenchymal stem cells.⁷² The hydrophilic part of the amphiphile was a peptide sequence RGDSKKLLA(K) containing the cell adhesive epitope RGD, while the hydrophobic tail was an alkyl chain ($-C_8H_{16}-$ diacetylene- $C_{12}H_{25}$) bearing a photosensitive group diacetylene which could covalently link the self-assembled molecules upon UV irradiation. Cell growth on the patterned peptide amphiphile surfaces demonstrated that cells recognized not only the biomolecular signaling provided by RGDS epitopes but also the physical guidance provided by the topographical patterns. Mata et al. found that cell differentiation was significantly affected by the type of substrate created. Hole microtextures were better for the osteoblast differentiation than all other surfaces. The negatively charged $C_{16}-V_3A_3E_3$ peptide-coated surface was demonstrated to be suitable for growth of bone-marrow mononuclear cells. A binary peptide system containing 10 wt % $C_{16}-V_3A_3K_3RGDS$ and 90 wt % $C_{16}-V_3A_3E_3$ lipopeptide molecules was found to promote optimal cell adhesion.⁷³ In vivo delivery of luciferase-expressing cells using the binary lipopeptide nanofiber system into the mouse model revealed the enhanced viability and proliferation of associated bone marrow derived stem and progenitor cells. Lipopeptides with branched head groups containing RGDS also showed excellent performance as scaffolds for growth of human bladder smooth muscle cells.⁷⁴

3.3. Self-Assembled Systems in Drug Delivery

The ways in which drugs are administered have gained increasing attention in the past two decades. Normally, drug is administered in a high dose at a given time only to have to repeat that dose several hours or days later. As a consequence, increasing attention has been focused on methods of giving drugs continually for prolonged time periods and in a controlled fashion. In the pharmaceutical field, in addition to the importance of polymers, an understanding of the physiological barriers in the human body is also critical to developing appropriate controlled release systems.

Self-assembled proteins and peptide may serve as a new class of biomaterials for applications in drug delivery system. Given that the degradation products consist of the drug and amino acid, this drug delivery system has an advantage over the polymer-based drug delivery system that generates polymer fragments with heterogeneous chain lengths upon degradation that may present complex toxicity profiles.

Branco et al. and co-worker described in detail the application of self-assembling materials for therapeutic delivery.⁷⁵ As they described in their article, the first self-assembled materials used as drug carriers were predominantly prepared from lipids. Vesicles (liposomes) and lipid-based micelles have been extensively studied and investigated for drug delivery applications as early as the 1970s. As a result, a significant number of liposomal drug formulations are available

commercially, and many others are undergoing clinical trials, making these systems one of the leading drug vehicles today. Recently, novel polymeric and peptide self-assembling systems have been developed for drug delivery. These carriers mimic the capabilities of conventional lipid systems and in some cases demonstrate improved drug delivery qualities. Diblock copolymers are commonly used to construct micellar assemblies. In these systems, the hydrophobic blocks assemble, yielding a hydrophobic core shielded by a hydrophilic shell. Self-assembly of block copolymers is often spontaneous and can be performed in the presence of small molecules, affording micelles encapsulated with drugs that have diameters up to hundreds of nanometers. The majority of diblock copolymers have hydrophilic blocks of polyethylene glycol (PEG). Since the therapeutic is present during the self-assembly process, drugs are loaded easily. In addition to micelles, amphiphilic block copolymers can self-assemble into vesicles in water and are referred to as polymersome.^{75–77} Polymersomes have been used for delivery of small hydrophobic drugs and DNA/RNA molecules and possess similar drug delivery characteristics to micelles.

S-Fluorouracil (5-FU) is widely used in clinical practice to inhibit the fibroblasts to proliferate and improve the success rate of glaucoma-filtering surgery, but 5-FU has many toxic effects to normal ocular tissues. Liang et al. designed a novel targeting drug delivery system based on self-assembled peptide hydrogel which contains the RGD (arginine-glycine-aspartic acid) peptide sequence that acts as a specific receptor for proliferated fibroblasts adhesion.⁷⁸ They hypothesized that a novel peptide containing a bioactive RGD sequence was designed and prepared. When dissolving the peptide in distilled water, a supramolecular hydrogel with nanofibers was formed through self-assembly of the peptide. In addition, this self-assembled peptide hydrogel could integrate 5-FU into its nanofibers during the process of self-assembly of the peptide. The proliferated fibroblasts can be attached in the peptide hydrogel through recognition of the RGD sequence, and 5-FU is delivered to the fibroblasts by this model of targeting of drugs.

Zhao and co-workers synthesized an amphiphilic peptide, RATEA16, $[CH_3CO]-RATARAEARATARAEA-[NH_2]$ by a solid-phase method using the standard Fmoc strategy.⁷⁹ Peptide RATEA16 and insulin-FITC were mixed in Milli-Q water and Tris HCl buffer (pH 7.5), and the final concentrations of RATEA16 and protein in the mixture were 0.3–1.0 wt % and 30–200 μ M, respectively. Subsequently, drug release was investigated in vitro, and the release profiles of the insulin-FITC from the peptide hydrogels were examined after addition of Milli-Q water to the hydrogels containing insulin-FITC. The hydrogels stably retained these protein molecules, and its release ratio was less than 10 wt % over 48 h. It is concluded that the protein was very slowly released from the hydrogel which contained water more than 99 wt %. Koutsopoulos and co-workers reported that the acetyl-(Arg-Ala-Asp-Ala)₄-CONH₂ [Ac-(RADA)₄-CONH₂] peptide hydrogel is an efficient slow-delivery carrier of small molecules.⁸⁰ In this work, they used a variety of proteins, including lysozyme, trypsin inhibitor, BSA, and IgG, with differing physicochemical properties (pI 4.6, 11.4; molecular mass, 14.3–150 kDa) and morphologies and encapsulated them within the Ac-(RADA)₄-CONH₂ peptide hydrogel. Release kinetics and diffusion coefficients for all systems were determined using a single-molecule fluorescence correlation spectroscopy (FCS) method.

Their results show that large proteins are retained inside the hydrogel scaffold for longer times compared with smaller proteins. Also, they showed that protein release from the Ac-(RADA)₄-CONH₂ peptide hydrogel was more sensitive to physical size than protein charge. For example, lysozyme, which is the smallest among the proteins studied, was released fastest. Soybean trypsin inhibitor, which is slightly larger and oppositely charged compared with lysozyme, was released slower than lysozyme but faster than BSA, which is larger. IgG, the largest of the proteins examined, was released more slowly than all other proteins.

Curcumin, an antioxidant with anti-inflammatory and antitumorigenic properties, has poor water solubility and a relatively low bioavailability that limits its therapeutic use. Altunbas et al. showed a self-assembling peptide hydrogel to be an effective vehicle for localized delivery of curcumin over sustained periods of time.⁸¹ The curcumin–hydrogel is prepared *in situ* where curcumin encapsulation within the hydrogel network is accomplished concurrently with peptide self-assembly. Physical and *in vitro* biological studies demonstrated the effectiveness of curcumin-loaded β -hairpin hydrogels as injectable agents for localized curcumin delivery. Notably, rheological characterization of the curcumin-loaded hydrogel before and after shear flow indicated solid-like properties even at high curcumin payloads. *In vitro* experiments confirmed that encapsulation of the curcumin within the hydrogel does not have an adverse effect on its bioactivity. Most importantly, the rate of curcumin release and its consequent therapeutic efficacy can be conveniently modulated as a function of the peptide concentration.

Antisense oligonucleotides provide a promising therapeutic approach for several disorders including cancer. Gels composed of a nanofibrous peptide network have been used as carriers for controlled delivery of oligonucleotides. A self-assembled peptide nanofibrous system was formed by mixing a cationic peptide amphiphile (PA) with Bcl-2 antisense oligodeoxynucleotide (ODN) through electrostatic interactions.⁸¹ Self-assembly of PA-ODN gel was characterized by circular dichroism, rheology, atomic force microscopy, and scanning electron microscopy. These microscopy images revealed establishment of the nanofibrous PA-ODN network. Due to the electrostatic interactions between PA and ODN, ODN release can be controlled by changing PA and ODN concentrations in the PA-ODN gel. Cellular delivery of the ODN by PA-ODN nanofiber complex was observed using fluorescently labeled ODN molecule. Cells incubated with PA-ODN complex had enhanced cellular uptake compared to cells incubated with naked ODN. Furthermore, Bcl-2 mRNA amounts were lower in MCF-7 human breast cancer cells in the presence of PA-ODN complex compared to naked ODN and mismatch ODN as evidenced by quantitative RT-PCR studies. These results suggest that PA molecules can control ODN release, enhance cellular uptake, and present a novel efficient approach for gene therapy studies and oligonucleotide-based drug delivery.

Recently, a review on molecular self-assembly of peptides, peptide amphiphiles, and peptidomimetics into molecules through nanoarchitectures to hydrogels has been published.⁸² Their applications in the field of tissue engineering have been highlighted. The design rules of this rapidly growing field are centered mainly on construction of peptides in the form of peptide amphiphiles, aromatic short peptide derivatives, all-amino acid peptide amphiphiles, lipidated peptides with single

and multiple alkyl chains, and peptide-based block copolymers and polymer peptide conjugates. An overview of the diversity of the patent applications is also provided for self-assembling systems based on the nano- and/or microscale such as fibers, fibrils, gels, hydrogels, vesicles, particles, micelles, bilayers, and scaffolds.

Major efforts have been undertaken to develop responsive nanostructures that respond to applied stimuli and dynamically undergo defined changes, thereby producing switchable properties.⁸³ This introduction of stimuli-responsive functions into aqueous self-assembly provides an attractive approach for creation of novel nanomaterials that are capable of responding to environmental changes. Kim et al. described the general self-assembly of rod amphiphiles based on a rigid–flexible molecular architecture in aqueous solution. They then highlight the structural changes and optical/macrosopic switching that occurs in aqueous assemblies in response to external signals. For example, aqueous nanofibers formed through self-assembly of the rod amphiphiles respond to external triggers by changing their shape into nanostructures such as hollow capsules, planar sheets, helical coils, and 3D networks. When an external trigger is applied, supramolecular rings laterally associate and merge to form 2D networks and porous capsules with gated lateral pores.

Peptide amphiphiles are excellent candidates for drug delivery due to their trans-membrane capability. The amphiphilic nature of peptide molecules and self-assembled nanostructures can facilitate internalization of the drugs encapsulated by them. A number of cationic peptide amphiphiles have been reported as drug and gene delivery carriers. Self-assembled cholesterol-conjugated H₅R₁₀ and H₁₀R₁₀ oligopeptides outperformed PEI in plasmid DNA delivery into both HepG2 and HEK293 cell lines. Increasing the number of histidine residues was found to further enhance gene expression efficiency.⁸⁴ It was thought that weak cationically charged histidine served as a “proton sponge” and could enhance gene delivery. Peptide amphiphiles with different tails (NH₂-I₅-NH₂, NH₂-W₅-NH₂, NH₂-F₅-NH₂) but the same headgroup (-H₄R₈-CONH₂) have been compared for their gene delivery capability. Different gene expression efficiencies were observed, indicating the impact of tail hydrophobicity.⁸⁵

Peptide amphiphiles A₁₂H₅K₁₀ and homologues have shown comparable gene delivery efficiencies to PEI but with better biocompatibility. These peptides, when dissolved in aqueous solution, would form core–shell structured nanoassemblies with diameters around 800 nm. Seow et al. proposed that charge-mediated interaction occurred via the cationically charged micelles and DNA. The increased cationic charge density at the outside of the micellar shell offered better DNA binding capability and protected the DNA from enzymatic degradation. However, because free peptide amphiphile molecules were also present in solution, complexation with DNA might also proceed through direct molecular interaction. Molecular complexation might produce smaller complexes in greater numbers and might affect the entire transfection efficiency more significantly. Results have shown that addition of A₁₂ tail to H₅K₁₀ improved gene expression without causing a significant increase in cytotoxicity.⁸⁶ However, due to the weak hydrophobicity of the A₁₂ tails, these peptides were not effective at delivering hydrophobic drugs such as doxorubicin and paclitaxel.⁸⁷ To increase the hydrophobicity of the tails, six alanine residues inside the tail have been replaced by phenylalanine residues. The new cationic peptide amphiphile Ac-(AF)₆-H₅-K₁₅-NH₂ has been evaluated as a carrier for

codelivery of drug (doxorubicin) and genes (luciferase reporter gene and p53 gene). The nanostructures could then efficiently encapsulate doxorubicin into the micelles and achieve sustained release without an obvious initial burst. Compared with the free doxorubicin, micelles loaded with doxorubicin had better internalization capability into the HepG2 cells. Simultaneous delivery of a model drug (hydrophobic FITC) and gene (rhodamine-labeled DNA) have also been achieved. Codelivery of doxorubicin and p53-encoding plasmid using the self-assembled nanomicelles synergistically suppressed proliferation of HepG2 cells. Therefore, designed peptide amphiphiles have great potential as effective carriers for both drugs and genes for therapeutic applications.

3.4. Stabilization of Membrane Proteins

Membrane proteins are important natural molecular devices in living cells and involved in many life functionalities such as energy conversions, cell–cell communication, and ion transport. They also work as natural biosensors for sight, hearing, smell, taste, touch, and temperature.^{88–90} Therefore, they are particularly useful in current nanobiotechnology endeavor. However, there is a significant lack of understanding of their structures and functions due to the difficulties in extracting, purifying, and stabilizing them.⁹¹ Simple but improved methods that can obtain membrane proteins without disturbing their structures and biological functions have great potential in nanobiodevice fabrication.

Peptide amphiphiles have been reported as excellent materials to solubilize and stabilize membrane proteins.^{91,92} Peptides V₆D, A₆D, A₆K, and V₆K can significantly increase the activity and stabilize diverse membrane proteins including *E. coli* glycerol-3-phosphate dehydrogenase,⁹² G-protein-coupled receptor-bovine rhodopsin,⁹³ or multidomain protein complex photosystem-I (PS-I) on a dry surface⁹⁴ and in aqueous solution.⁹⁵ Peptide A₆K can stabilize the PS-I complex in a dried form at room temperature for at least 3 weeks. The polarity, number of charges on the headgroup, and size and hydrophobicity of the tails have important effects on different membrane proteins.⁴¹

Apart from peptide amphiphiles, other peptides or lipopeptides have also been considered as excellent stabilizers for membrane proteins (Figure 7). Natural lipid bilayers provide not only the surrounding interactions but also suitable pressure to membrane proteins to retain their structures. Many membrane proteins lose their native structures and biological functions during purification in the surfactant-solubilized state.

Lipopeptides have very similar properties to membrane lipids and can provide a suitable environment for membrane proteins. The lipopeptide used by McGregor et al.⁹⁶ has an α -helix structure. Polar residues are restricted to one side of the helix, while nonpolar alanine residues are on the opposite site. Two acyl chains are attached at the two ends of the α -helix and interact with the nonpolar alanine residues, forming a surfactant feature (Figure 7B). The length of the helical structure is similar to the width of a biological membrane. The lipopeptide has the capability to disrupt phospholipid bilayers and has proven to be effective at solubilizing helical membrane proteins including bacteriorhodopsin and lactose (lac) permease as well as the *E. coli* PagP protein. Experimental studies aiming at elucidating the location and extent of peptide binding in the future will certainly expedite this area of research, leading to design of more efficient peptides for protein stabilization.

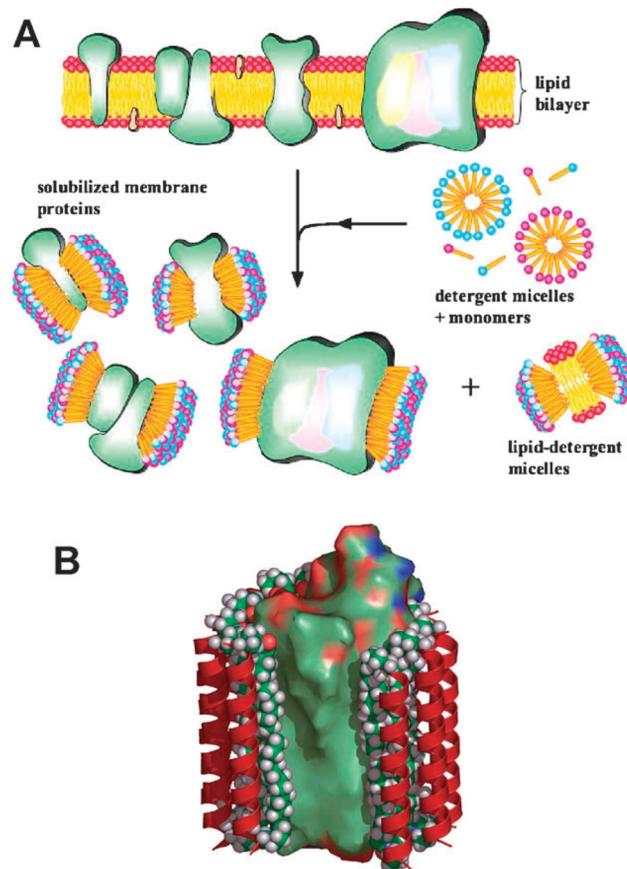


Figure 7. (A) Proposed scheme to illustrate how the designer peptide amphiphiles stabilize membrane proteins. Peptide amphiphiles have been used to solubilize, stabilize, and crystallize membrane proteins. They use their tails to sequester the hydrophobic part of membrane proteins, with their hydrophilic heads exposed to water. Thus, their binding and association make membrane proteins soluble and stable outside the native cellular lipid milieu. These peptides are very important for overcoming the barrier of high-resolution molecular structures for challenging membrane proteins. (B) Representation of a proposed protein–lipopeptide complex. Solubilized membrane protein is represented by the solid surface. Peptide backbone of LPD-14 is represented by red ribbons, and ornithines and alkyl chains are shown as space-filling spheres. Front-most LPD monomers are omitted for clarity. Reprinted with permission from ref 32. Copyright 2010 Royal Society Chemistry.

4. REGENERATIVE MEDICINE THERAPY

Regenerative medicine is an interdisciplinary field that combines engineering and live sciences in order to develop techniques that enable restoration, maintenance, or enhancement of living tissues and organs. Its fundamental aim is creation of natural tissue with the ability to restore missing organ or tissue function, which the organism has not been able to regenerate in physiological conditions. With that in mind, it aspires to improve the health and quality of life for millions of people worldwide and give solution to the present limitations: rejections, low quantity of donors, etc.⁹⁷ The term regenerative medicine is often used synonymously with tissue engineering, although those involved in regenerative medicine place more emphasis on the use of stem cells to produce tissues. Clinical trials on humans could begin in as little as 4 years said the company, which hopes to help supply an estimated \$6 billion market for organs and a similar market for cellular therapies to

treat diseases such as diabetes. Every day thousands of people of all ages are admitted to hospitals because of the malfunction of some vital organ. Because of a dearth of transplantable organs, many of these people will die. In perhaps the most dramatic example, the American Heart Association reports only 2300 of the 40 000 Americans who needed a new heart in 1997 got one. Lifesaving livers and kidneys likewise are scarce, as is skin for burn victims and others with wounds that fail to heal. The evergrowing demand for donor organs to meet the needs of individuals on waiting lists will likely never be met. While roughly 100 000 people have transplants in the United States, more than 10 million have implants. There are 20 000 transplants annually but 2 million implants. In short, the need for organs cannot be met by traditional methods of transplantation. Regenerative medicine and tissue engineering may change that. Table 3 shows potential applications of regenerative medicine therapy.

Table 3. Potential Applications of Regenerative Medicine therapy

people in the United States affected by diseases that may be helped by regenerative medicine and tissue engineering	
condition	number of persons affected
cardiovascular diseases	58 million
autoimmune diseases	30 million
diabetes	16 million
osteoporosis	10 million
cancer	8.2 million
Alzheimer's disease	4 million
Parkinson's disease	1.5 million
burns (severe)	0.3 million
spinal cord injuries	0.25 million
birth defects	150 000 (per year)
total	128.4 million

Tissue engineering is an interdisciplinary field that applies principles and methods of engineering toward development of biological substitutes to improve the function of damaged tissue and organs (Figure 8).⁹⁸ The motivation of using tissue engineering in regenerative medicine is due to the following: (i) since the 1970s, organ transplantation has become a common therapeutic approach for end-stage organ failure patients; (ii) demand \gg supply (UNOS National Patient Waiting List), for example: 19 095 patients (1989), 80 766 patients (December 2002); (iii) cost of organ replacement therapy \$305 billion (US, 2000). The interdisciplinary approach of tissue engineering is a combinational technology of using molecular biology, materials engineering, and reconstructive surgery.⁹⁹ Tissue engineering needs scaffolds to serve as a substrate for seeding cells and as a physical support in order to guide formation of the new tissue and is designed to regenerate natural tissues or create biological substitutes for defective or lost organs using cells.^{100–104} There is no doubt that a sufficient supply of nutrients and oxygen to the transplanted cells is vital for their survival and functional maintenance.¹⁰⁵ Mesenchymal stem cells (MSCs) are known as one of the most used cells in tissue engineering and have been rapidly improved by several researches to evaluate their therapeutic applications.⁹⁷

4.1. Scaffolding Materials in Regenerative Medicine

Material design of a scaffold for cell proliferation and differentiation is one of the key technologies for tissue engineering. In conventional cell culture, such as a static tissue culture dish (2D), the initial rate of cell growth is higher but proliferation stops once the cells reached confluence. Porous materials with three-dimensional (3D) structures have been investigated for the cell scaffold because they have a larger surface for cell attachment and proliferation than 2D materials and are preferable to assist formation of 3D cell constructs which may resemble the structure and function of body tissues. In addition, the 3D scaffold also plays an important role in the

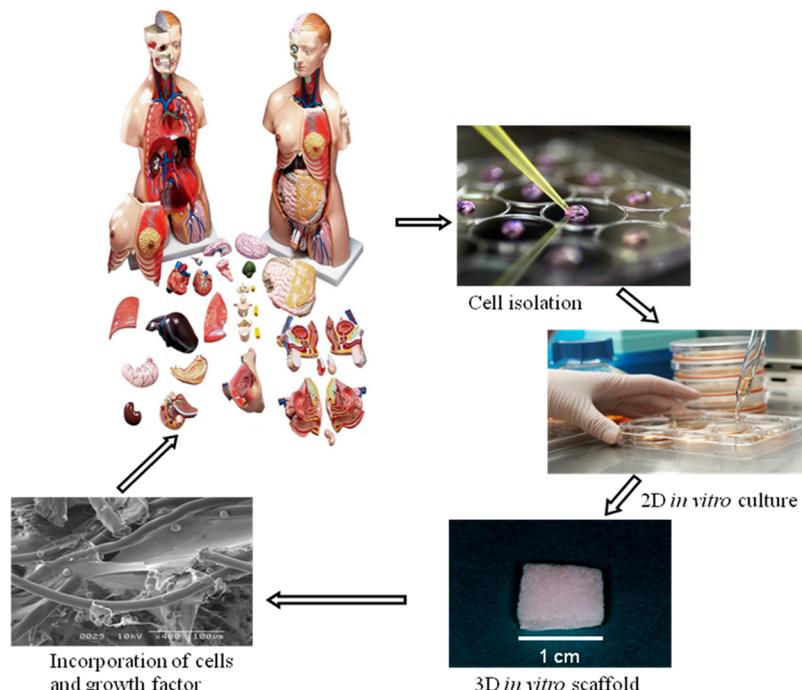


Figure 8. Schematic illustration of tissue regeneration based on the principle of tissue engineering.

Table 4. Examples of Synthetic and Natural Polymers in Tissue Engineering

synthetic polymers

advantages: control of the molecular weight during the synthesis and of the physical properties

disadvantages: lack of intrinsic biological activity

PGA (polyglycolic acid)

relatively hydrophilic, degradable through hydrolysis at the ester bond in aqueous solution or *in vivo* (2–4 weeks)

PLA (polylactic acid)

more hydrophobic than PGA, chirality, degradable in months or years

PCL (poly *e*-caprolactone)

hydrophobic, degrade at a much slower rate than PGA and PLA

natural polymers

advantages: possess intrinsic biological activity, enzymatically degradable

disadvantages: limited control over parameters such as molecular weight, potential for adverse immunological responses, variation in degradation rates due to difference in host enzyme levels, inferior mechanical properties

collagen

triple-helix structure, each polypeptide chain has general sequence of Gly-X-Y, where X and Y are often Pro and hydroproline, adhesive functions

chitosan

deacetylated derivatives of chitin, structure similar to cellulose, stable, insoluble above pH 7, cationic

GAGs (chondroitin-6-sulfate)
(hyaluronan)

unbranched, highly negatively charged, strongly hydrophilic, covalently linked to proteins in the form of proteoglycans which form hydrated gels to resist compressive forces in mammalian ECM

substrate for *in vitro* cell culture to increase the number of cells as high as clinically applicable.¹⁰⁸ 3D scaffolds able of regenerating or restoring tissue and/or organs have begun to revolutionize medicine and biomedical science. Scaffolds have been used to support and promote regeneration of tissues. Different processing techniques have been developed to design and fabricate three-dimensional scaffolds for tissue-engineering implants. However, there is not a simple, nonexpensive method that fulfills the main characteristics that a scaffold should have for application in tissue engineering.

Proliferation of cells in the 3D scaffold needs oxygen and nutrition supply. In this circumstance, 3D scaffold materials should provide such an environment for cells living in distance. Diffusion of nutrients, bioactive factors, and oxygen through 3D scaffolds is sufficient for survival of large numbers of cells for extended periods of time. A major constraint of biodegradable polymer scaffolds for vascular tissue engineering is poor cell adhesion and lack of signals for new tissue generation. The presence of ECM within the scaffold is desirable for growth of stem cells and *in vitro* formation of remodeled vascular conduit.¹⁰⁶ Tissue regeneration can be achieved by the following three key steps: cell proliferation, cell seeding in a suitable scaffold, and maintenance of the differentiation phenotype of the engineered tissues.¹⁰⁷ The property of scaffold material for cell attachment is one of the major factors contributing to their morphology, proliferation, function, and subsequent tissue organization.^{108–115} Ideally, a polymeric scaffold for tissue engineering should have the following characteristics: (i) have appropriate surface properties promoting cell adhesion, proliferation, and differentiation; (ii) be biocompatible; (iii) be highly porous, with a high surface area/volume ratio, with an interconnected pore network for cell growth and flow transport of nutrients and metabolic waste; (iv) have mechanical properties sufficient to withstand any *in vivo* stresses.^{116–120} The last requisite is difficult to combine with the high porosity in volume of the material. Thus, it is necessary to use polymeric matrices with special or reinforced properties, especially if the polymer is a hydrogel. On the basis of the extensive range of polymeric materials, different processing techniques have been developed to design and fabricate 3D scaffolds for tissue-engineering implants (see Table 4).^{121–129} These include (a) phase separation, (b) gas foaming, (c) fiber bonding, (d) photolithography, (e) solid free form (SFF), (f) solvent casting in combination with particle leaching.^{130–144} However, none of the techniques has achieved

a suitable model of three-dimensional architecture so that the scaffolds can fulfilled with their aims in the wanted way using equipment with high cost even for the reasons that are going to be discussed. Thus, using phase separation, a porous structure can be easily obtained by adjusting thermodynamic and kinetic parameters. However, because of the complexity of the processing variables involved in the phase-separation technique the pore structure cannot be easily controlled. Moreover, it is difficult to obtain large pores due to a lack of interconnectivity. Gas foaming has the advantage of room-temperature processing but produces a largely nonporous outer skin layer and a mixture of open and closed pores within the center, leaving incomplete interconnectivity. The main disadvantage of the gas foaming method is that it often results in a nonconnected cellular structure within the scaffold.¹³⁰ Fiber bonding provides a large surface area for cell attachment and rapid diffusion of nutrients in favor of cell survival and growth. However, these scaffolds, as the ones used to construct a network of bonded polyglycolic acid (PGA), lacked the structural stability necessary for *in vivo* use. In addition, the technique does not lend itself to easy and independent control of porosity and pore size.^{128,129}

Photolithography has also been employed for patterning, obtaining structures with high resolution, although this resolution may be unnecessary for many applications of patterning in cell biology (Figure 9). In any case, the disadvantage of this technique is the high cost of the equipment needed limits their applicability.¹³² SFF scaffold manufacturing methods provide excellent control over scaffold external shape and internal pore interconnectivity and geometry but offer limited microscale resolution. Moreover, the minimum size of global pores is 100 μm. Additionally, SFF requires complex correction of scaffold design for anisotropic shrinkage during fabrication and needs high-cost equipment. Finally, solvent casting in combination with the particulate leaching method, which involves casting of a mixture of monomers and initiator solution and a porogen in a mold, polymerization, followed by leaching out of the porogen with the proper solvent to generate the pores, is inexpensive but still has to overcome some disadvantages in order to find engineering applications, namely, the problem of residual porogen remains, irregular shaped pores, and insufficient interconnectivity.¹³³ The proposed scaffolds may find applications as structures that facilitate either tissue regeneration or repair during reconstructive operations.^{134,135} The new structure could also find applications

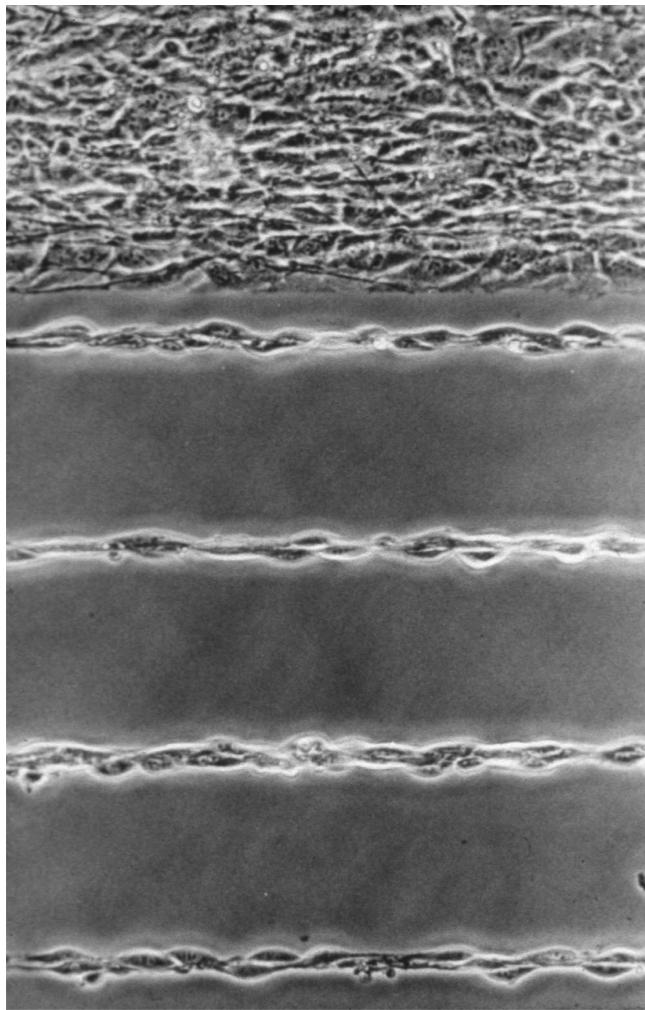


Figure 9. Capillary tube formation by bovine capillary endothelial cells cultured on 10 μm lines. Larger unpatterned region was included as an internal control. Reprinted with permission from ref 132. Copyright 1999 Elsevier.

in other areas in which the pore morphology plays an essential role, such as membranes and filters.^{136,137}

4.2. Specific Mediated Biomaterials

The design of materials that can regulate cell behavior such as proliferation and differentiation is a key component for fabrication of tissue-engineering scaffolds. From the viewpoint of immune system response of the body, the implanted biomaterials should mimic the structure and biological function of native ECM in terms of both chemical composition and physical structure as reported by Ma et al.¹⁴⁵ Therefore, in order to mimic the biological function of ECM proteins, the scaffold materials used in tissue engineering need to be chemically functionalized to promote tissue regeneration as ECM does. Collagen and elastin as ECM proteins are made from fibers in dimension smaller than micrometers.¹⁴⁵ It seems that artificial nanoscaled fibers have great potential application in the field of biomaterials and tissue engineering.

The initial report showed that nanoscaled features influenced cell behavior.¹⁴⁶ Nanoscaled surface topography has been found to promote osteoblast adhesion.¹⁴⁷ It has been demonstrated that osteoblast adhesion, proliferation, alkaline phosphatase activity, and ECM secretion on carbon nanofibers increased by decreasing fiber diameter in the range of 60–200 nm, whereas

adhesion of other kinds of cells such as chondrocytes, fibroblasts, and smooth muscle cells was not influenced.^{148,149} It has been supposed that the nanoscaled surface affects the conformation of adsorbed adhesion proteins such as vitronectin, thus affecting cell behavior.¹⁵⁰ In addition, the nanoscaled dimensions of cell membrane receptors such as integrins should also be considered. There are three different approaches toward formation of nanofibrous materials: phase separation, electrospinning, and self-assembly.¹⁵¹ Phase separation and self-assembling of biomolecules can generate smaller diameter nanofibers in the same range of natural ECM, while electrospinning generates large diameter nanofibers on the upper end of the range of natural ECM.¹⁵² Electrospinning is a common technique used to fabricate tissue-engineering scaffolds.¹⁵² It is an easy technique, is cheap, and can be applied for many different types of polymers. A recent study demonstrated that fabricated PGA/collagen nanofibers through electrospinning significantly enhanced cell adhesion compared with PGA/collagen microfibers.¹⁵³

One of the common approaches to produce fibers similar to ECM proteins such as collagen is self-assembly. It has been shown that peptide amphiphile that contains a carbon alkyl tail and several other functional peptides formed nanofibers through self-assembly by mixing cell suspensions in media with dilute aqueous solutions of the peptide.¹⁵⁴ These self-assembled nanofibers have been used recently to study selective differentiation of neural progenitor cells.¹⁵⁵ Another type of peptide containing 16 alternating hydrophobic and hydrophilic amino acids was fabricated to self-assemble into nanofibers under appropriate pH values.¹⁵⁶ Nanoscaled fibers produced by self-assembly of amphiphilic peptide may be a promising approach in designing the next generation of biomaterials for drug delivery and tissue engineering.

It would be beneficial for biomedical applications if scaffold materials could promote adhesion and growth of cells on their surfaces. The sequence of arginine-glycine-aspartic acid (RGD) has been discovered as a cell attachment sequence in various adhesive proteins present in the ECM and found in many proteins, such as fibronectin, collagen type 1, vitronectin, fibrin, and Von Willebrand Factor.¹⁵⁷ It has been recognized that the sequence of RGD interacts with various types of integrin receptors of mammalian cells. Ever since the RGD sequence was discovered as a cell attachment sequence in adhesive proteins of the ECM, there have been several efforts to synthesize bioactive peptides incorporating RGD for therapeutic purposes.¹⁵⁸ Micro- and nanopatterned scaffolds have been investigated less well in regard to stem cells, although two recent studies highlight their attractiveness.¹⁵⁹ In their study, Silva et al. included a five amino acid, laminin-specific cell-binding domain (which binds to specific integrins on cell surface) at the hydrophilic head of their amphiphilic structures and showed that neural stem cells could be induced to differentiate into neurons when cultured within peptide gel.¹⁵⁶ In contrast, cells grown in control scaffolds without the laminin-specific domain or on two-dimensional tissue culture plastic coated with laminin solution differentiated much less. This was hypothesized to be largely as a result of the density of the cells binding ligands to which the cells were exposed, indicating clearly the importance of extracellular matrix in influencing cell function. Recent studies indicated that when the laminin-specific domain in the amphiphilic molecule was replaced with the amino acid sequence, arginine-glycine-aspartate (RGD), a common cell-binding domain in many extracellular matrix

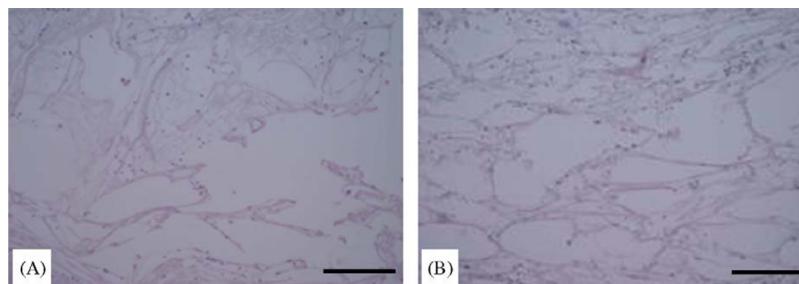


Figure 10. Distribution of cells attached to and proliferated in self-assembled PA nanofibers without RGD (A) and with RGD incorporation (B) 6 h after cell seeding. Scale bars: 500 μm . Reprinted with permission from ref 161. Copyright 2006 Elsevier.

proteins, especially collagen, differentiation of MSC to osteoblasts was significantly enhanced compared with amphiphilic nanofibers without this sequence or to two-dimensional controls (Figure 10).¹⁶¹ This is because the interaction of MSC integrins receptors with RGD of the peptide enhanced cell attachment on peptide nanofibers. Proliferation of cells in the 3D scaffold needs oxygen and nutrition supply. In this circumstance, 3D scaffold materials should provide such an environment for cells. The artificial scaffold formed by self-assembling molecules not only provides a suitable support for cell proliferation but also serves as a medium through which diffusion of soluble factors and migration of cells can occur. The result of the cell attachment and proliferation revealed that diffusion of nutrients, bioactive factors, and oxygen through these highly hydrated networks is sufficient for survival of large numbers of cells for extended periods of time.

4.3. Self-Assembled Systems in Regenerative Medicine therapy

For successful tissue regeneration, the cells constituting tissue to be regenerated, such as matured, progenitor, and precursor, are necessary. Considering the proliferation activity and differentiation potential of cells, stem cells are practically promising. Among them, MSC have been widely investigated to use by themselves or combining with scaffolds necessary for promotion of cell proliferation and differentiation. It was found that MSC have an inherent nature to differentiate into not only osteogenic lineage cells but also chondrogenic, myogenic, adipogenic, and neurogenic lineages.^{160–165} MSC have been experimentally used to demonstrate their *in vivo* potential to induce regeneration of mesenchymal tissues.^{166–170} Since it is reported that the cells are effective in inducing regeneration of tissues other than mesenchym, their feasibility in the cell source for regenerative medicine is highly expected. They are practically isolated from patient's themselves.^{171–174}

Another use of self-assembled peptides would be in tissue engineering. Considering the use of cells in the body, it is no doubt that a sufficient supply of nutrients and oxygen to the transplanted cells is vital for their survival and functional maintenance.¹⁷⁵ Without a sufficient supply, only a small number of cells preseeded in the scaffold or migrated into the scaffold from the surrounding tissue would survive. Rapid formation of a vascular network at the transplanted site of cells must be a promising way to provide cells with the vital supply. This process of generating new microvasculature, termed neovascularization, is a process observed physiologically in development and wound healing.¹⁷⁶ Basic fibroblast growth factor (bFGF) has been shown to promote such an angiogenesis process.^{176,177} Growth factors stimulate the appropriate cells (e.g., endothelial cells), already present in

the body, to migrate from the surrounding tissue, proliferate, and finally differentiate into blood vessels.¹⁷⁶ However, one cannot always expect the sustained angiogenesis activity when these proteins are only injected in the solution form, probably because of their rapid diffusional excretion from the injected site. One possible way for enhancing the *in vivo* efficacy is to achieve its controlled release over an extended time period by incorporating growth factor in a polymer carrier. If this carrier is biodegraded, harmonized with tissue growth, it will work as a scaffold for tissue regeneration in addition to a carrier matrix for growth factor release.

4.3.1. Cartilage Regeneration and Biomineralization in Self-Assembled Systems.

As understood from the findings, proteins and peptides can self-assemble into various structures like nanotubes, nanovesicles, and three-dimensional peptide matrices with interwoven nanofibers. Macroscopic three-dimensional peptide matrices can be engineered to form various shapes by changing the peptide sequence. Self-assembled peptide materials encouraged cell proliferation and differentiation. These peptide materials were also able to support various types of cell attachments. The ability of the peptides to support attachment of mouse neuronal cells has been fully studied.¹⁵⁵ The primary mouse neuron cells form active connections with the peptide scaffolds that form a valuable area of research for studying neuron regeneration. In regenerative medicine, these peptide matrices are used to cultivate chondrocyte extracellular matrix that can be used to repair cartilage tissue. Cartilage tissue engineering has been performed by placing the primary chondrocytes and mesenchymal stem cells into these self-assembled peptide hydrogels to produce collagen and glycosaminoglycans. These peptide matrices can also be used in regeneration of bone by incorporating a phosphorylated serine which can attract and organize calcium ions to form hydroxyapatite crystals and functionalize them with a cell adhesion motif like arginine-glycine-aspartic acid complex. Research studies have not been limited only to natural amphiphilic peptides. There are many research trials that have indicated synthesizing complex amphiphilic peptides by joining hydrophilic peptides into long alkyl chains. The peptide end of the molecule was designed to function and regulate biomineralization. For example, bone is produced as a result of deposition of calcium and phosphate ions to form hydroxyapatite crystals. This process is known as mineralization. Serine (nonessential amino acid) is incorporated with the synthetic amphiphilic peptide complex; it serves to attract and organize calcium and phosphate ions to form hydroxyapatite crystals. Furthermore, adding a cell adhesion motif has functionalized the synthetic amphiphilic peptides. RGD has been shown to attach to the C

terminal of the peptide. This can be used to study the ability of bone cells to differentiate, proliferate, and adhere to a biomaterial surface like titanium. Titanium is the most widely used biomaterial surface to produce orthopedic implants, dental implants, and hip replacements. Despite its excellent biocompatibility, titanium implants still fail. Most orthopedic implants have a lifetime of 15 years as the maximum. In order to produce a newer version of titanium implants that can stay in the body for a longer period of time, its surface has to be modified with nanosized surface patterns so that bone cells (osteoblasts) differentiate and migrate into these patterns for better bone–implant adhesion. For such a purpose, these synthetic amphiphiles can be used to regulate and control the osteoblasts.

Self-assembling peptide amphiphiles have great potential as templates for nanofabrication such as biomineratization, nucleation, nanowires, and nanocircuits.⁸⁹ A lipopeptide was designed and synthesized for biomineratization by the Stupp group.⁹⁰ The C₁₆ tail was connected to the N terminal of a peptide sequence which contained four cysteines, three glycines, a single phosphorylated serine, and a cell adhesion ligand RGD (C₁₆–C₄G₃S(p)RGD–OH). Connection of the C₁₆ tail made the molecule amphiphilic and facilitated self-assembly in aqueous phase into cylindrical micellar structure. C₁₆ acyl tails packed themselves in the center of the micelle as the hydrophobic core with the hydrophilic peptide sequences forming β -sheets at the outside.⁹¹ The intermolecular disulfide bonds formed by cross-linking of the 4-cysteine residues in the middle of the molecules made the self-assembled nanofibers robust and impervious to pH variation. Nanofibers were then used to direct mineralization of hydroxyapatite.⁹⁰ Hydroxyapatite nucleated on the surfaces of the lipopeptide nanofibers, and its crystals grew with their C axes oriented along the long axes of the nanofibers. This alignment was the same as that observed between collagen fibers and hydroxyapatite crystals in bone. Reported by the same group, self-assembled lipopeptide nanofibers have been used as bioactive materials to coat the bone implant materials consisting of a Ti–6Al–4 V foam.⁹² Results demonstrated that the nanofiber matrices occupied the pores of the metallic foam and that cells were encapsulated within the bioactive matrices. Meanwhile, nanofibers could also facilitate mineralization of hydroxyapatite. The inert titanium covered by the nanofiber matrix was transplanted into a rat model. Bone formation was observed around and inside the implant, and vascularization was also observed around the implant with the absence of a cytotoxic response. 3D bone-matrix mineralization using similar lipopeptides as templates has also been reported recently.⁹³

4.3.2. Angiogenesis in Self-Assembled Systems. Tissue engineering is designed to regenerate natural tissues or create biological substitutes for defective or lost organs by making use of cells. Considering the use of cells in the body, it is no doubt that a sufficient supply of nutrients and oxygen to the transplanted cells is vital for their survival and functional maintenance. Without a sufficient supply, only a small number of cells preseeded in the scaffold or migrated into the scaffold from the surrounding tissue would survive. Rapid formation of a vascular network at the transplanted site of cells must be a promising way to provide cells with the vital supply. This process of generating new microvasculature, termed neovascularization or angiogenesis, is a process observed physiologically in development and wound healing. It is recognized that basic fibroblast growth factor (bFGF) function

promotes such an angiogenesis process.¹⁸⁵ The growth factors stimulate the appropriate cells (e.g., endothelial cells), already present in the body, to migrate from the surrounding tissue, proliferate, and finally differentiate into blood vessels. However, one cannot always expect the sustained angiogenesis activity when these proteins are only injected in solution form probably because of their rapid diffusional excretion from the injected site. One possible way for enhancing the *in vivo* efficacy is to achieve its controlled release over an extended time period by incorporating the growth factor in a polymer carrier. If this carrier is biodegraded, harmonized with tissue growth, it will work as a scaffold for tissue regeneration in addition to a carrier matrix for growth factor release.

Some studies have demonstrated that bFGF promoted angiogenesis when used in combination with delivery matrices and scaffold.^{178–184} A recent study has indicated that a 3D network of self-assembled nanofibers was formed by mixing a bFGF suspension with an aqueous solution of peptide amphiphile as an injectable carrier for controlled release of growth factors. The feasibility of prevascularization by the bFGF release from the 3D networks of nanofibers has been tested.¹⁸⁵ Previous works have encapsulated bFGF within alginate, gelatin, agarose/heparin, collagen, and poly(ethylene-co-vinyl acetate) carriers.^{183,185,186} According to the results of these studies, it is conceivable to incorporate the angiogenic factor to a sustained releasing system prior to implantation. The bFGF-incorporated releasing system requires surgery for implantation, which is not welcomed. On the contrary, the bFGF incorporated in self-assembled peptide could be delivered to living tissues by simply injection of peptide amphiphile and bFGF solutions. The injected solutions would form a hydrogel at the injected site of tissue, and the release bFGF induced significant angiogenesis around the injected site, in marked contrast to bFGF injection or peptide amphiphile injection alone.¹⁸⁵ The *in vitro* release profile indicates that prolonged release of bFGF is continued for 750 h (Figure 11).

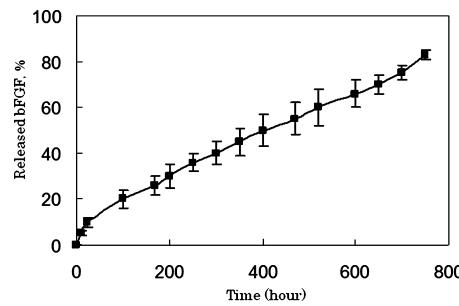


Figure 11. In vitro bFGF release kinetics from self-assembled PA nanofibers. Concentration of bFGF used is 0.2 $\mu\text{g}/\mu\text{L}$. $n = 6$, number of experiments for each time point. Reprinted with permission from ref 185. Copyright 2006 Elsevier.

When the release was halted, approximately 90% of total loaded protein had been released. However, it is possible to increase the cumulative amount of released molecules by increasing the concentration of the loading solution because the amount of loaded protein may be increased as the concentration of loading solution increases. This result is attributed to an increasing driving force, i.e., concentration difference for protein diffusion. However, the type of interaction forces acting between bFGF and peptide amphiphile molecules is not clear at present. The *in vivo* degradation rate of self-assembled

peptide amphiphile nanofibers and in vivo release profiles of bFGF were estimated in terms of the radioactivity loss of ^{125}I -labeled peptide amphiphile and ^{125}I -labeled bFGF. Therefore, injection of radioiodinated peptide amphiphile and bFGF into the mouse subcutis was carried out to evaluate the time profile of radioactivity remaining in vivo. The peptide amphiphile was degraded with time in the body, although the degradation rate was slow. The radioactivity of self-assembled peptide amphiphile nanofibers incorporating ^{125}I -labeled bFGF decreased with time, although the bFGF radioactivity was retained for longer time periods than that of free ^{125}I -labeled bFGF injection. The decrement order and pattern of bFGF and peptide amphiphile radioactivity reveals that the in vivo release rate of bFGF was faster than the in vivo degradation rate of peptide amphiphile. Taken together, the results of the in vitro and in vivo release profile indicate that bFGF was released from self-assembled peptide amphiphile nanofibers in the body as a result of combination of diffusion and degradation mechanism. The bFGF used here was originally characterized in vitro as a growth factor for fibroblasts and capillary endothelial cells and in vivo as a potent mitogen and chemoattractant for a wide range of cells. The most important of concern regarding delivery of proteins is whether or not the protein released in the body actually retains its biological activity. To evaluate protein activity, in vitro culture techniques are normally employed because of their simplicity and convenience, compared with in vivo animal experiments. However, any in vitro nondegradation system cannot be applied to evaluate the biological activity of released bFGF. Thus, to obtain information on the retention of bFGF activity, vascularization is directly assessed after subcutaneous injection of peptide amphiphile with bFGF in animals. Histological examination demonstrated that vascularization is remarkable around the injection site of self-assembled peptide amphiphile nanofibers incorporated bFGF, in contrast to sites injected with an aqueous solution of bFGF. Injection of bFGF in the form of a solution is not effective in inducing vascularization at all, and injection of bFGF-free peptide amphiphile alone does not induce any vascularization effect. This is in good accordance with the results of immunofluorescence analysis. The result also demonstrated that the number of smooth muscle cells of the capillary wall as well as endothelial cells of the vessel wall was greatly enhanced when release of bFGF is controlled by peptide amphiphile. The prolonged period of angiogenesis by the bFGF-incorporated self-assembled peptide amphiphile nanofiber is ascribed to the prolonged time period of bFGF release. The amount of tissue hemoglobin, which is measured by bFGF-induced neovascularization, notably increased within 1 day of injection of peptide amphiphile incorporated with bFGF, and the increased level is retained for several days, followed by a slow return over the time ranged studied. On the other hand, injection of an aqueous solution containing the same dose of bFGF, as a bFGF incorporated in self-assembled peptide amphiphile nanofibers, did not increase the amount of hemoglobin at the injection site over the time range studied; the level of tissue hemoglobin remained at approximately the same level as that found on injection of bFGF-free peptide amphiphile or in untreated mice. No increase in the amount of hemoglobin is observed even when the amount of bFGF in solution that was injected is increased to $10\ \mu\text{g}/\mu\text{L}$. This must be due to a rapid elimination of bFGF from the injection site. In contrast, the bFGF incorporated in self-assembled peptide amphiphile nanofibers enabled us to reduce the dose that was effective in inducing

significant vascularization to $0.2\ \mu\text{g}/\mu\text{L}$. This finding strongly suggests that the bFGF-incorporated self-assembled peptide amphiphile nanofibers still maintain its biological activity even though exposed to an in vivo environment. It is highly possible that the slow degradation of the bFGF-incorporated self-assembled peptide amphiphilic nanofibers achieves a longer period of bFGF release, resulting a prolonged angiogenesis effect. The in vivo degradation profile of self-assembled peptide amphiphile nanofibers indicates that the retention period of the self-assembled peptide amphiphile nanofibers-induced vascularization effect is shorter than the degree of degradation of peptide amphiphile. As described earlier, bFGF seems to be released from self-assembled peptide amphiphile as a result of a diffusion mechanism. The enhanced vascularization is due to sustained release of bFGF. Hosseinkhani's group recently demonstrated that stimulation of in vivo angiogenesis via controlled release of vascular endothelial growth factor (VEGF) was enhanced by incorporation of VEGF in self-assembled peptide amphiphile (unpublished work). They hypothesized that self-assembly hydrogel comprised of peptide amphiphile and VEGF can be used to fabricate tissue-engineering scaffolds for angiogenesis. To test this hypothesis, 3D hydrogels of self-assembled peptide amphiphile were fabricated by mixing VEGF suspension with aqueous solution of peptide amphiphile as an injectable carrier for controlled release of growth factors. We demonstrate the feasibility of this approach to induce angiogenesis by investigating the release kinetics of VEGF release from the 3D hydrogel improves angiogenesis. An injectable hydrogel can be formed by mixing a peptide aqueous solution with VEGF suspension. The in vitro release profile of VEGF from peptide gel and in vivo degradation of peptide were investigated through the radioactivity remaining of radiolabeled VEGF and peptide injected subcutaneously to the back of the rat. In addition, angiogenesis induced by released VEGF was assessed in a rat model using histological and immunological examinations. It was demonstrated that injection of an aqueous solution of peptide together with VEGF into the back of rats resulted in formation of a transparent 3D hydrogel at the injected site and induced significant angiogenesis around the injected site, in marked contrast to VEGF injection alone or peptide injection alone. Comparison of the in vitro degradation of peptide with the in vivo release profile of VEGF indicated that the governing mechanism of controlled release of VEGF was through degradation of peptide in the body.

4.3.3. Bone Regeneration in Self-Assembled Systems.

Bone defects and fracture nonunion are common problems, affecting as many as 1000 patients in the world every year, and difficult to heal using current therapies. Previously, these cases have been treated by surgery using techniques such as autologous bone grafting or artificial bone grafting. It has been reported that bone morphogenetic proteins (BMPs), transforming growth factor- β (TGF- β), and basic fibroblast growth factor (bFGF) can induce bone formation in both ectopic and orthotopic sites in vivo.^{188,189} However, use of these proteins alone require large amounts of protein because of its short half-life, and we cannot always expect the sustained biological activity when these proteins are only injected in solution form probably because of their rapid diffusional excretion from the injected site. One possible way for enhancing the in vivo efficacy is to achieve its controlled release over an extended time period by incorporating the growth factor in a polymer carrier. It is known that many growth factors in the body have short half-lives. To overcome

Table 5. Characteristics of Growth Factors Used in Tissue Engineering

growth factor	isoelectric point (IEP)	molecular weight (kDa)	biological substances for growth factor binding	functions of growth factor
basic fibroblast growth factor (bFGF)	9.6	16	heparin or heparan sulfate	stimulating the cells involved in the healing process (bone, cartilage, nerve, etc.); angiogenesis
transforming growth factor- β 1 (TGF- β 1)	9.5	25	heparin or heparan sulfate; collagen type IV; latency associated protein; latent TGF- β 1 binding protein	enhancing wound healing; stimulating the osteoblast proliferation to enhance bone formation
bone morphogenetic protein-2 (BMP-2)	8.5	32	collagen type IV	stimulating the mesenchymal stem cells to osteoblast lineage and inducing bone formation both at bone and at ectopic sites
vascular endothelial growth factor (VEGF)	8.5	38	heparin or heparan sulfate	stimulating endothelial cell growth, angiogenesis, and capillary permeability
hepatocyte growth factor (HGF)	5.5	100	heparin or heparan sulfate	stimulating matrix remodeling and epithelial regeneration (liver, spleen, kidney, etc.)

this limitation, growth factors have been encapsulated within different types of polymeric carriers. A potential limitation of the previously developed systems is that they require surgery for implantation. Injectable self-assembled peptides incorporating osteoinductive growth factors is superior compared with implantable scaffolds. BMP-2-incorporated self-assembled peptide amphiphile was found to be useful for growth factor release.¹⁸⁸ Hosseinkhani et al. and colleagues hypothesized that self-assembly hydrogels comprised of peptide amphiphile and BMP-2 can be used to fabricate tissue-engineering scaffolds to induce ectopic bone formation.¹⁸⁸ To test this hypothesis, 3D networks of self-assembled peptide amphiphile nanofibers were fabricated by mixing BMP-2 suspension with aqueous solution of peptide amphiphile as an injectable carrier for controlled release of growth factors. They demonstrated the feasibility of this approach to induce ectopic bone formation by showing that BMP-2 released from the 3D networks of nanofibers enhanced ectopic bone formation. Also, in another similar study Hosseinkhani et al. and colleagues examined the release system mentioned above that enabled inducing significant bone formation when peptide amphiphile and TGF- β solutions were subcutaneously injected to the back of the rat (unpublished work). The injected solutions of peptide and TGF- β formed a transplant gel, and sustained release of TGF- β induced significant ectopic bone compared with TGF- β injection. As a flexible delivery system, these scaffolds can be adapted for sustained release of many different growth factors and biomolecules. The ALP (alkaline phosphatase) activity increased rapidly and saturated at 3 weeks, while the temporal changes in the OCN (osteocalcin) content increased steadily with time, which was in good accordance with the course of bone formation in subcutaneous tissue. TGF- β -incorporated self-assembled PA nanofibers significantly increased both the ALP and the OCN levels compared with free TGF- β injection. Alkaline phosphatase activity is an ectoenzyme, produced by osteoblasts, that is likely to be involved in degradation of inorganic pyrophosphate to provide a sufficient local concentration of phosphate or inorganic pyrophosphate for mineralization. Therefore, ALP is a useful marker for osteoblast activity. OCN, also known as bone Gla protein, is a highly conserved noncollagenous protein that contains three γ -carboxyglutamic acid residues that allow it to bind calcium. Although the function of OCN is not quite clear, it is well recognized that only osteoblasts or cells with osteoblastic nature produce OCN. OCN is already known to play an important role in the process of ossification for bone formation. Like alkaline phosphatase, osteocalcin is also selected as a marker of osteogenic differentiation.^{189–194}

One important approach for bone healing and bone ingrowth is use of growth factors. Some of these factors, e.g., bone morphogenic proteins (BMPs),^{195,196} transforming growth factor- β (TGF- β),^{197–199} insulin-like growth factor (IGF),^{197,199} and basic fibroblast growth factor (bFGF), act as local regulators of cellular activity and seem to have osteoinductive and angiogenic potential.^{200–205} The growth factor of bFGF has been previously used as angiogenesis factor.¹⁸⁴ Interestingly, the same group has used bFGF as osteoinductive growth factor to induce bone at the skull defect of rabbits.²⁰⁶ bFGF is a growth factor to trigger proliferation of capillary endothelial cells in addition to osteoblast.²⁰⁶ It has been reported that bFGF stimulated cells involved in osteogenesis and angiogenesis in a vascularized bone graft.²⁰⁷ Although bFGF is not a strong osteoinductive growth factor compared with other growth factors such as BMP-2, the present study indicates that incorporation of bFGF in self-assembled peptides nanofibers as an injectable tissue-engineered scaffold is a promising technique to induce significant bone at the injected site. Table 5 summarizes common growth factors that are currently use in regenerative medicine.

4.3.4. Nerve Regeneration in Self-Assembled Systems. Ellis-Behnke et al. used self-assembled peptide as a 3D scaffold for neural regeneration medicine.²⁰⁸ In their animal model, they first made a brain wound defect that was carried out using postnatal day-2 Syrian hamster pups. The experimental groups of animals were then treated by injection into the wound of 10–30 mL of 1% RADA16/99% water. Histological observation showed that only in the peptide scaffold-injected animals the brain tissue appears to have reconnected itself together in all survival times. In addition, axons labeled from their retinal origin with a tracer molecule were found to have grown beyond the tissue bridge. Also, functional tests proved a significant restoration of visual function in all peptide scaffold-treated animals. In 2011, Liang's group succeeded to speed up the process of central nervous system recovery after injury that is needed for real-time measurement of axon regeneration to assess the extent of injury as well as the optimal timing and delivery of therapeutics and rehabilitation.²⁰⁹ In their study, they developed a chronic animal model with an *in vivo* measurement technique to provide a real-time monitoring and feedback system. They showed a successful chronic injury model to measure central nervous system regeneration, combined with an *in vivo* measurement system to provide real-time feedback during every stage of the regeneration process. Their results indicated that a chronic optic tract lesion was able to heal and axons were

able to regenerate when treated with a self-assembling nanofiber peptide scaffold. In a recent study developed by researchers from Sichuan University in China, Meng et al.²¹⁰ showed that earlier studies demonstrated that peptide RADA16-I (AcN-RADARADARADARA-CONH₂) could repair spinal cord injury and optical pathway and restore visual function. The objective of their research was to investigate the role of RADA16-I in regeneration of peripheral nerve injury in rats. Sciatic nerve injury was performed at female Sprague–Dawley rats. Two groups were conducted: crush group and sever group. There were RADA16-I treatment and blank control in each group. Sense nerve conduction parameters were evaluated on day 30 in the injured sciatic nerve by means of electrophysiological recording. Their results indicated that Sense nerve conduction in test groups showed a faster healing rate than blank control. In another interesting work developed by the Department of Neurology, Northwestern University, Tysseling et al.²¹¹ showed that injection into the injured spinal cord of peptide amphiphile (PA) conjugated isolucine-lysine-valine-alanine-valine (IKVAV) peptide mimicking laminin structure supports of the neural extracellular matrix, improved functional recovery after spinal cord injury in two different species, rat and mouse, and in two different injury models, contusion and compression. The results clearly showed that the improvement required the IKVAV epitope and was not observed with injection of an amphiphile displaying a nonbioactive sequence. They also showed that The IKVAV PA-injected groups also trended higher both in the total number neurons adjacent to the lesion and in the number of long propriospinal tract connections from the thoracic to the lumbar cord. IKVAV PA injection did not alter myelin thickness, total axon number caudal to the lesion, axon size distribution, or total axon area.

4.3.5. Cardiac Regeneration in Self-Assembled Systems. Stem cells have shown great interest in cardiac tissue engineering. In a work developed by Lee et al.²¹² embryonic stem cells were suspended in RADA16-II peptide scaffold solutions and injected in the myocardium of 10 week old mice. They injected self-assembling peptides into the myocardium to create a 3D microenvironment. After 7, 14, and 28 days these microenvironments recruited both endogenous endothelial and smooth muscle cells and exogenously injected cells survived in the microenvironments. They concluded self-assembling peptides created injectable microenvironments that promoted vascularization. In another work, they also developed a drug-delivery system using a biotinylated version of RADA-II to demonstrate slow release of IGF-1 in infarctuated rat myocardia.²¹³ The biotin sandwich strategy allowed binding of IGF-1 and did not prevent self-assembly of the peptides into nanofibers within the myocardium. In conjunction with cardiomyocytes transplantation, they showed that cell therapy with IGF-1 delivery by biotinylated nanofibers significantly improved systolic function after experimental myocardial infarction. In an interesting study, Davis's group hypothesized that a novel approach to promote vascularization would be to create injectable microenvironments within the myocardium that recruit endothelial cells and promote their survival and organization.²¹⁴ They demonstrated that self-assembling peptides could be injected and that the resulting nanofiber microenvironments were readily detectable within the myocardium. Furthermore, the self-assembling peptide nanofiber microenvironments recruited progenitor cells that express endothelial markers, as determined by staining with isolectin

and for the endothelial-specific protein platelet–endothelial cell adhesion molecule-1. Vascular smooth muscle cells were recruited to the microenvironment and appear to form functional vascular structures. In their animal model (adult, male C57BL/6 mice aged 8–10 weeks), after tracheal intubation, hearts were exposed by separation of the ribs. The peptide gel (10 μ L, RAD16-II peptide (AcN-RARADA-DARARADADA-CN₂)) was injected into the free wall of the left ventricle (LV) through a 30-gauge needle while the heart was beating. After injection, the chests were closed and animals were allowed to recover under a heating lamp. After the endothelial cell population, cells that express α -sarcomeric actin and the transcription factor Nkx2.5 infiltrate the peptide microenvironment. When exogenous donor green fluorescent protein-positive neonatal cardiomyocytes were injected with the self-assembling peptides, transplanted cardiomyocytes in the peptide microenvironment survived and also augmented endogenous cell recruitment. They demonstrated that self-assembling peptides created nanofiber microenvironments in the myocardium and that these microenvironments promoted vascular cell recruitment. Because these peptide nanofibers may be modified in a variety of ways, this approach may enable injectable tissue regeneration strategies.

4.3.6. Tooth Regeneration in Self-Assembled Systems. Recent advances in tissue engineering have drawn scientists to test the possibility of tooth engineering and regeneration. Tooth regeneration is normally referred to as regeneration of the entire tooth or root that can be integrated into the jaw bone. This technology is still at its infancy, and when it matures, it may be used to restore missing teeth and replace artificial dental implants when the tooth is damaged but still in a reparable condition; regeneration of parts of the tooth structure can prevent or delay loss of the whole tooth. To engineer and regenerate a whole tooth, the cell source, tissue-engineering strategies, and specific scaffolds need to be correct. Dental pulps as a source of adult stem cells have potential for skeletal tissue engineering. The dental pulp is a highly specialized mesenchymal tissue characterized by the presence of odontoblasts and by the fact that it is surrounded by a rigid mineralized tissue. Dental caries are still one of the most prevalent infectious diseases in the world. Currently, replacement of decayed soft and mineralized tissue together with some materials implantations is most applicable in dental technology. Galler et al.²¹⁵ applied a novel approach to develop engineered dental tissues. In their work, two dental stem cell lines were combined with peptide–amphiphile. These self-assembled peptides formed three-dimensional networks of nanofibers and living cells encapsulated inside them. Cell–matrix interactions were tailored by incorporation of the cell adhesion sequence RGD and an enzyme-cleavable site. Stem cells from human-exfoliated deciduous teeth and dental pulp stem cells were cultured in self-assembled peptide scaffolds for 4 weeks using osteogenic medium culture. Stem cells from human-exfoliated deciduous teeth showed a spindle-shaped morphology, high proliferation rates, and collagen production, resulting in soft tissue formation. In contrast, dental pulp stem cells reduced proliferation but exhibit an osteoblast-like phenotype, express osteoblast marker genes, and deposit mineral. They concluded that their technology could be suitable for engineering both soft and mineralized matrices for dental tissue regeneration. In the future, the success of regenerative endodontic therapy will depend on the ability to yield a functional pulp tissue within cleaned and shaped root canal

Table 6. Some Examples of Amino Acid Sequence of Self-Assembling Peptides

peptide name	peptide sequence	refs
K24	NH2-Lys-Leu-Glu-Ala-Leu-Tyr-Val-Leu-Gly-Phe-Phe-Gly-Phe-Phe-Thr-Leu-Gly-Ile-Met-Leu-Ser-Tyr-Ile-Arg-COOH	258
Lysb-21	AcNH-Gln-Ala-Thr-Asn-Arg-Asn-Thr-Asp-Gly-Ser-Thr-Asp-Tyr-Gly-Ile-Leu-Gln-Ile-Asn-Ser-Arg-NH2	259
EAK16	AcNH-Arg-Glu-Arg-Glu-Arg-Lys-Arg-Lys-Arg-Glu-Arg-Glu-Arg-Lys-Arg-Lys-COONH2	260
DN1	AcNH-Gln-Gln-Arg-Phe-Gln-Trp-Gln-Phe-Glu-Gln-Gln-NH2	259
P11-4	AcNH-Gln-Gln-Arg-Phe-Glu-Trp-Glu-Phe-Glu-Gln-Gln-NH2	261
RAD16-1	AcNH-Arg-Ala-Asp-Ala-Arg-Ala-Asp-Ala-Arg-Ala-Asp-Ala-COONH2	262
KLD12	AcNH-Lys-Leu-Asp-Leu-Lys-Leu-Asp-Leu-Lys-Leu-Asp-Leu-COONH2	263, 264

systems to revitalize teeth. This may be achieved by an *in vivo* approach, where pulp tissue is regenerated *in situ* into root canals, or by an *ex vivo* approach, which implies a *de novo* engineered pulp relying on the tissue-engineering triad (dental pulp stem cells, scaffold, growth factors).

4.3.7. Soft Tissue Regeneration in Self-Assembled Systems.

Skin functions to provide a physical and chemical interface that protects the host against invasion by toxins and microorganisms and prevents dehydration that can result from loss of barrier function. Loss of skin integrity and function due to wound injury has led to efforts designed to better comprehend the molecular and cellular mechanisms that can optimize wound repair. Schneider et al.²¹⁶ used an integrated approach using nanobiotechnology to augment the rate of wound reepithelialization by combining self-assembling peptide (RADA16-I, Ac-RADARADARADARA-CONH2) nanofiber scaffold and epidermal growth factor (EGF). This bioscaffold was tested in a bioengineered human skin equivalent (HSE) tissue model that enabled wound reepithelialization to be monitored in a tissue that recapitulates molecular and cellular mechanisms of repair known to occur in human skin. They found that self-assembled peptide underwent molecular self-assembly to form unique 3D structures that stably covered the surface of the wound, suggesting that this scaffold may serve as a viable wound dressing. They measured the rates of release of EGF from the SAP scaffold and determined that EGF was only released when the scaffold was in direct contact with the HSE. By measuring the length of the epithelial tongue during wound reepithelialization, they found that self-assembled peptide scaffolds containing EGF accelerated the rate of wound coverage 5-fold when compared to controls without scaffolds and 3.5-fold when compared to the scaffold without EGF. In another work published by Kao et al.,²¹⁷ a biocompatible peptide hydrogel scaffold as a three-dimensional synthetic skin that does not contain animal-derived materials or pathogens was prepared. They investigated preparation methods, proliferation, and functional expression of fibroblasts in the synthetic dermis and differentiation of keratinocytes in the epidermis. Synthetic dermis was prepared by mixing fibroblasts with peptide hydrogel, and synthetic skin was prepared by forming an epidermal layer using keratinocytes on the synthetic dermis. A fibroblast-rich foamy layer consisting of homogeneous peptide hydrogel subsequently formed in the synthetic dermis with fibroblasts aggregating in clusters within the septum. The epidermis consisted of 3–5 keratinocyte layers. Immunohistochemical staining showed human type I collagen, indicating functional expression around fibroblasts in the synthetic dermis, keratinocyte differentiation in the epidermis, and expression of basement membrane proteins. The number of fibroblasts tended to increase until the second week and was maintained until the fourth week but rapidly decreased in the fifth week. In the synthetic dermis medium, the human type I

collagen concentration increased after the second week to the fifth week. They suggested that peptide hydrogel acts as a synthetic skin scaffold that offers a platform for proliferation and functional expression of fibroblasts and keratinocytes.

Traumatic injury or surgery may trigger extensive bleeding. However, conventional hemostatic methods have limited efficacy and may cause surrounding tissue damage. Cheng et al.²¹⁸ used self-assembling peptides and specifically extend fragments of functional motifs derived from fibronectin and laminin to evaluate the capability of these functionalized peptides in the effect of hemostasis and liver tissue regeneration. From the results, these peptides could self-assemble into a nanofibrous network structure and became gelation into hydrogel with pH adjustment. In an animal study, the efficacy of hemostasis was achieved immediately within seconds in the rat liver model. Histological analyses by histological staining and immunohistochemistry revealed that self-assembled peptide with these functionalized motifs significantly enhanced liver tissue regeneration. They concluded that self-assembled peptides may have potential as pharmacological tools to extensively advance clinical therapeutic applications in hemostasis and tissue regeneration in the field of regenerative medicine.

4.3.8. Other Forms of Self-Assembled Systems in Regenerative Medicine Therapy.

A self-assembling system is an emerging field of science which utilizes biological molecules for biological applications.^{219–228} Molecular self-assembly technology takes advantage of the unique properties of biological molecules like amphiphilic peptides by utilizing their self-assembling property for bioengineering of molecular templates and supramolecular structures (see Table 6). Amphiphiles are molecules containing a nonpolar hydrophobic region, and a polar hydrophilic region will self-assemble in aqueous solution to form distinct structures such as micelles, vesicles, and tubules. When suspended in aqueous solution, the nonpolar hydrophobic regions of amphiphilic molecules are attracted toward each other and away from water (hydrophobic effect). The shape and dimensions of supramolecular structures formed from such assemblies will then depend on different factors, such as the structure of the polar headgroup and the shape of each amphiphile. Several self-assembling amphiphilic peptide and protein systems that self-assemble to form various nanostructures like nanofibers, nanotubes, vesicles, helical ribbons, and fibrous scaffolds have been described extensively for their potential applications in the field of biotechnology. The artificial scaffold formed by self-assembling molecules not only provides a suitable support for cell proliferation but also serves as a medium through which diffusion of soluble factors and migration of cells can occur. The results of cell attachment and proliferation revealed that diffusion of nutrients, bioactive factors, and oxygen through these highly hydrated networks is sufficient for survival of a large numbers of cells for extended

periods of time. As understood from the findings, proteins and peptides can self-assemble into various structures like nanotubes, nanovesicles, and three-dimensional peptide matrices with interwoven nanofibers. Macroscopic three-dimensional peptide matrices can be engineered to form various shapes by changing the peptide sequence.

Self-assembled peptide materials encouraged cell proliferation and differentiation. These peptide materials are also able to support various types of cell attachments. Self-assembling amphiphilic peptides are molecules engineered with hydrophilic heads and hydrophobic tails that, under correct conditions, can self-assemble into a network of nanofibers with the head sticking out into solution and the tails hidden in the core of the thread.¹⁶ These networks have the advantage of porosity, and living cells can be combined with such materials before the scaffold is fabricated. Silva's group included a five amino acid, laminin-specific cell-binding domain (which binds to specific integrins on cell surface) at the hydrophilic head of the peptide amphiphile and indicated that neural stem cells could be induced to differentiate into neurons when cultured within the laminin-specific domain or on two-dimensional tissue culture plate coated with laminin solution that differentiated much less.¹⁵⁵ Self-assembling nanofibers have been used to repair the spinal cord injury in a mouse model. Injection of peptide amphiphile increased the number of oligodendroglia at the site of injury. Furthermore, nanofibers promoted regeneration of both descending motor fibers and ascending sensory fibers through the lesion site.²²⁷ The Ellis-Behnke group showed that peptide nanofibers enabled the regenerated axons to reconnect to target tissues with sufficient density to promote functional return of vision, as evidenced by visually elicited orienting behavior.²¹⁷ In their study, they used the V₆D amino acid complex.^{27,28}

5. FUTURE PROSPECTS

As stated above, there is a large need for novel therapies for repair and regeneration of a multitude of tissues. Specifically, the primary significance is development and application of novel technology for identification of cell/biomaterial/signaling factor systems that promote tissue regeneration with optimal functional properties. There is currently great interest in harnessing these unique properties because by using stem cells it may be possible to generate cells in the laboratory that can be used to either replace damaged tissues inside the body or identify safer and more effective medicines.

Self-assembly of biological molecules forms the basic principle in formation of complex biological structures. The topic discussed above on nanostructured biological materials through self-assembly of peptides and proteins gives us a large amount of knowledge about the basic principle underlying molecular self-assembly of proteins and peptides. It gives a general overview of different kinds of self-assembling protein and peptide systems. Throughout the sections discussed above, it clearly guides the reader about potential applications of these self-assembled structures that can be applied for fabricating a wider range of novel biomaterials for use in medical applications. With appropriate references and examples, it opens up the reader's mind incorporating a wider range of knowledge about self-assembling proteins and peptide systems. These macroscopic structures have inspired researchers to use them in various areas of science like electronics, biotechnology, nanotechnology, and medicine.

Discussed in this review are a few applications and the advantages of self-assembled proteins and peptides in regenerative medicine therapy. Even though there are certain technological hurdles, these can be overcome by understanding the drawbacks of the individual systems and finding an alternative to overcome this drawback.^{227–230} However, it is necessary to create new and other alternative methods if we face any problems using the current technology in delivering biomolecules, such as proteins, growth factors, and DNA. Such systems have previously been created as one alternative method to enhance the *in vitro* and *in vivo* localization of biomolecules.^{231–257} Thus, self-assembling proteins and peptides and its versatile applications have kindled the interest of numerous scientists and researchers to find newer ways to apply it in the field of regenerative medicine. Tissue engineering is evolving rapidly with much more potential impacts in treatment of diseases like heart failure and diabetes. Numerous studies are being conducted worldwide to discover newer solutions that can be effectively applied to treat these diseases using self-assembled proteins and peptides. Results obtained in regenerative medicine are inspiring the scientific community to discover new innovative noninvasive tools at the micro- and nanoscale level for such purposes.

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Notes

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ACKNOWLEDGMENTS

This study was performed through National Science Council (NSC) of Taiwan, and supported by the Research Grant of NSC 99-2314-B-011-001-MY3.

REFERENCES

- (1) Wang, Y.; Brown, P.; Xia, Y. *Nat. Mater.* **2011**, *19*, 482.
- (2) Dvir, T.; Timko, B. P.; Kohane, D. S.; Langer, R. *Nat Nanotechnol.* **2011**, *6*, 13.
- (3) Farokhzad, O. C.; Langer, R. *Adv. Drug Delivery Rev.* **2006**, *58*, 1456.
- (4) Smith, L. A.; Ma, P. X. *Biointerfaces* **2004**, *10*, 125.
- (5) Peer, D.; Karp, J. M.; Hong, S.; Farokhzad, O. C.; Margalit, R.; Langer, R. *Nat. Nanotechnol.* **2007**, *2*, 751.
- (6) Mrsny, R. J. *Adv. Drug Delivery Rev.* **2009**, *61*, 172.
- (7) Whitesides, G. M.; Boncheva, M. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4769.
- (8) Ng, C. C.; Cheng, Y. L.; Pennefather, P. S. *Biophys. J.* **2004**, *87*, 323.
- (9) Nasalean, L.; Baudrey, S.; Leontis, N. B.; Jaeger, L. *Nucleic Acids Res.* **2006**, *34*, 1381.
- (10) Won, J.; Chae, S. K.; Kim, J. H.; Park, H. H.; Kang, Y. S.; Kim, H. S. *J. Membr. Sci.* **2005**, *249*, 113.
- (11) Duval-Terrié, C.; Huguet, J.; Muller, G. *Colloids Surf., A: Physicochem. Eng. Asp.* **2003**, *220*, 105.
- (12) Chung, W. J.; Oh, J. W.; Kwak, K.; Lee, B. Y.; Meyer, J.; Wang, E.; Hexemer, A.; Lee, S. W. *Nature* **2011**, *478*, 364.
- (13) Whitesides, G. M.; Grzybowski, B. *Science* **2002**, *295*, 2418.
- (14) Lee, J.; Macosko, C. W.; Urry, D. W. *Biomacromolecules* **2001**, *2*, 170.
- (15) Zhang, S.; Marini, D. M.; Hwang, W.; Santoso, S. *Curr. Opin. Chem. Biol.* **2002**, *6*, 865.
- (16) Hartgerink, J. D.; Beniash, E.; Stupp, S. I. *Science* **2001**, *294*, 1684.
- (17) Niece, K. L.; Hartgerink, J. D.; Donners, J.; Stupp, S. I. *Chem.* **2003**, *125*, 7146.
- (18) Vauthey, S.; Santoso, S.; Gong, H.; Watson, N.; Zhang, S. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5355.
- (19) Kisiday, J.; Jin, M.; Kurz, B.; Hung, H.; Semino, C.; Zhang, S.; Grodzinsky, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 9996.
- (20) Zhang, S.; Gelain, F.; Zhao, X. *Semin. Cancer Biol.* **2005**, *15*, 413.
- (21) Hosseinkhani, H.; Hosseinkhani, M. *Chem. Today* **2008**, *26*, 30.
- (22) Hosseinkhani, H.; Hosseinkhani, M. *Chem. Today* **2008**, *26*, 35.
- (23) Wang, K.; Keasling, J. D.; Muller, S. J. *Int. J. Biol. Macromol.* **2005**, *36*, 232.
- (24) Adams, D. J.; Holtzmann, K.; Schneider, C.; Butler, M. F. *Langmuir* **2007**, *25*, 12729.
- (25) McMillan, R.; Meeks, B.; Bensebaa, F.; Deslandes, Y.; Sheardown, H. *J. Biomed. Mater. Res.* **2001**, *54*, 272.
- (26) Sun, X.; Sheardown, H.; Tengvall, P.; Brash, J. L. *J. Biomed. Mater. Res.* **2000**, *49*, 66.
- (27) Pilkington, S. M.; Roberts, S. J.; Meade, S. J.; Gerrard, J. A. *Biotechnol. Prog.* **2010**, *26*, 93.
- (28) Zhang, S.; Yan, L.; Altman, M.; Lässle, M.; Nugent, H.; Frankel, F.; Lauffenburger, D. A.; Whitesides, G. M. *Biomaterials* **1999**, *20*, 1213.
- (29) Sharma, J.; Chhabra, R.; Andersen, C. S.; Gothelf, K. V.; Yan, H.; Liu, Y. *J. Am. Chem. Soc.* **2008**, *130*, 7820.
- (30) Zhang, S.; Holmes, T. C.; Lockshin, C.; Rich, A. S. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 3334.
- (31) Zhang, S. *Mater. Today* **2003**, *20*.
- (32) Zhao, X.; Pan, F.; Xu, H.; Yaseen, M.; Shan, H.; Hauser, C. A. E.; Zhang, S.; Lua, J. R. *Chem. Soc. Rev.* **2010**, *39*, 3480.
- (33) Brogden, K. A. *Nat. Rev. Microbiol.* **2005**, *3*, 238.
- (34) Zasloff, M. *Nature* **2002**, *415*, 389.
- (35) Chen, C. X.; Pan, F.; Zhang, S. Z.; Hu, J.; Cao, M. W.; Wang, J.; Xu, H.; Zhao, X. B.; Lu, J. R. *Biomacromolecules* **2010**, *11*, 402.
- (36) Mitra, R. N.; Shome, A.; Paul, P.; Das, P. K. *Org. Biomol. Chem.* **2009**, *7*, 94.
- (37) Makovitzki, A.; Baram, J.; Shai, Y. *Biochemistry* **2008**, *47*, 10630.
- (38) Liu, L.; Xu, K.; HuayingWang, J. T. P. K.; Fan, W.; Venkatraman, S.; Li, S. L.; Yang, Y. Y. *Nat. Nanotechnol.* **2009**, *4*, 457.

- (39) Ghanaati, S.; Webber, M. J.; Unger, R. E.; Orth, C.; Hulvat, J. F.; Kiehna, S. E.; Barbeck, M.; Rasic, A.; Stupp, S. I.; Kirkpatrick, C. J. *Biomater.* **2009**, *30*, 6202.
- (40) Kisiday, J.; Jin, M.; Kurz, B.; Hung, H.; Semino, C.; Zhang, S. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 9996.
- (41) Silva, G. A.; Czeisler, C.; Niece, K. L.; Beniash, E.; Harrington, D. A.; Kessler, J. A. *Science* **2004**, *303*, 1352.
- (42) Ramachandran, S.; Yu, Y. B. *BioDrugs* **2006**, *20*, 263.
- (43) Law, B.; Weissleder, R.; Tung, C. H. *Biomacromolecules* **2006**, *7*, 1261.
- (44) Zhang, S.; Holmes, T. C.; DiPersio, C. M.; Hynes, R. O.; Su, X.; Rich, A. *Biomaterials* **1995**, *16*, 1385.
- (45) Horii, A.; Wang, X.; Gelain, F.; Zhang, S. *PLoS ONE* **2007**, *2*, 190.
- (46) Haines-Butterick, L.; Rajagopal, K.; Branco, M.; Salick, D.; Rughani, R.; Pilarz, M. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 7791.
- (47) Gelain, F.; Bottai, D.; Vescovi, A.; Zhang, S. *PLoS ONE* **2006**, *1*, 119.
- (48) Genove, E.; Shen, C.; Zhang, S.; Semino, C. E. *Biomaterials* **2005**, *26*, 3341.
- (49) Kasai, S.; Ohga, Y.; Mochizuki, M.; Nishi, N.; Kadoya, Y.; Nomizu, M. *Peptide Sci.* **2004**, *76*, 27.
- (50) Gras, S. L.; Tickler, A. K.; Squires, A. M.; Devlin, G. L.; Horton, M. A.; Dobson, C. M. *Biomaterials* **2008**, *29*, 1553.
- (51) Hartgerink, J. D.; Beniash, E.; Stupp, S. I. *Science* **2001**, *294*, 1684.
- (52) Storrie, H.; Guler, M. O.; Abu-Amara, S. N.; Volberg, T.; Rao, M.; Geiger, B. *Biomaterials* **2007**, *28*, 4608.
- (53) Guler, M. O.; Hsu, L.; Soukasene, S.; Harrington, D. A.; Hulvat, J. F.; Stupp, S. I. *Biomacromolecules* **2006**, *7*, 1855.
- (54) Chau, Y.; Luo, Y.; Cheung, A. C.; Nagai, Y.; Zhang, S.; Kobler, J. B. *Biomaterials* **2008**, *29*, 1713.
- (55) Holmes, T. C.; de Lacalle, S.; Su, X.; Liu, G.; Rich, A.; Zhang, S. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6728.
- (56) Davis, M. E.; Hsieh, P. C. H.; Takahashi, T.; Song, Q.; Zhang, S. G.; Kamm, R. D. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 8155.
- (57) Kapadia, M. R.; Chow, L. W.; Tsihlis, N. D.; Ahanchi, S. S.; Eng, J. W.; Murar, J. J. *Vasc. Surg.* **2008**, *47*, 173.
- (58) Jung, J. P.; Nagaraj, A. K.; Fox, E. K.; Rudra, J. S.; Devgun, J. M.; Collier, J. H. *Biomaterials* **2009**, *30*, 2400.
- (59) Jung, J. P.; Jones, J. L.; Cronier, S. A.; Collier, J. H. *Biomaterials* **2008**, *29*, 2143.
- (60) Lutolf, M. P.; Hubbell, J. A. *Nat. Biotechnol.* **2005**, *23*, 47.
- (61) Stevens, M. M.; George, J. H. *Science* **2005**, *310*, 1135.
- (62) Anderson, D. G.; Levenberg, S.; Langer, R. *Nat. Biotechnol.* **2004**, *22*, 863.
- (63) Flaim, C. J.; Chien, S.; Bhatia, S. N. *Nat. Methods* **2005**, *2*, 119.
- (64) Chau, Y.; Luo, Y.; Cheung, A. C. Y.; Nagai, Y.; Zhang, S. G.; Kobler, J. B.; Zeitels, S. M.; Langer, R. *Biomaterials* **2008**, *29*, 1713.
- (65) Zhang, S. G. *Nat. Biotechnol.* **2003**, *21*, 1171.
- (66) MacPhee, C. E.; Woolfson, D. N. *Curr. Opin. Solid State Mater. Sci.* **2004**, *8*, 141.
- (67) Holmes, T. C. *Trends Biotechnol.* **2002**, *20*, 16.
- (68) Holmes, T. C.; de Lacalle, S.; Su, X.; Liu, G. S.; Rich, A.; Zhang, S. G. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6728.
- (69) Kisiday, J.; Jin, M.; Kurz, B.; Hung, H.; Semino, C.; Zhang, S. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 9996.
- (70) Zhao, X. J.; Zhang, S. G. *Macromol. Biosci.* **2007**, *7*, 13.
- (71) Zhou, M.; Smith, A. M.; Das, A. K.; Hodson, N. W.; Collins, R. F.; Ulijn, R. V.; Gough, J. E. *Biomaterials* **2009**, *30*, 2523.
- (72) Mata, A.; Hsu, L.; Capito, R.; Aparicio, C.; Henrikson, K.; Stupp, S. I. *Soft Matter* **2009**, *5*, 1228.
- (73) Webber, M. J.; Tongers, J.; Renault, M. A.; Roncalli, J. G.; Losordo, D. W.; Stupp, S. I. *Acta Biomater.* **2010**, *6*, 3.
- (74) Harrington, D. A.; Cheng, E. Y.; Guler, M. O.; Lee, L. K.; Donovan, J. L.; Claussen, R. C.; Stupp, S. I. *J. Biomed. Mater. Res., Part A* **2006**, *78*, 157.
- (75) Branco, M. C.; Schneider, J. P. *Acta Biomater.* **2009**, *5*, 817.
- (76) Rajagopal, K.; Schneider, J. P. *Curr. Opin. Struct. Biol.* **2004**, *14*, 480.
- (77) Lee, K. Y.; Yuk, S. H. *Prog. Polym. Sci.* **2007**, *32*, 669.
- (78) Liang, L.; Yang, J.; Li, Q.; Huo, M.; Jiang, F.; Xu, X.; Zhang, X. J. *Biomater. Nanobiotechnol.* **2011**, *2*, 622.
- (79) Zhao, Y.; Tan, T.; Yokoi, H.; Tanaka, M.; Kinoshita, T. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 4927.
- (80) Koutsopoulos, S.; Unsworth, L. D.; Nagai, Y.; Zhang, S. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 4623.
- (81) Altunbas, A.; Lee, S. J.; Rajasekaran, S. A.; Schneider, J. P.; Pochan, D. J. *Biomaterials* **2011**, *32*, 5906.
- (82) Bulut, S.; Erkal, T. S.; Toksoz, S.; Tekinay, A. B.; Tekinay, T.; Guler, M. O. *Biomacromolecules* **2011**, *12*, 3007.
- (83) Kumar, P.; Pillay, V.; Modi, G.; Choonara, Y. E.; du Toit, L. C.; Naidoo, D. *Recent Pat. Drug Delivery Formulation* **2011**, *5*, 24.
- (84) Kim, H. J.; Kim, T.; Lee, M. *Acc. Chem. Res.* **2011**, *44*, 72.
- (85) Guo, X. D.; Tandiono, F.; Wiradharma, N.; Khor, D.; Tan, C. G.; Khan, M.; Qian, Y.; Yang, Y. Y. *Biomaterials* **2008**, *29*, 4838.
- (86) Seow, W. Y.; Yang, Y. Y. *Adv. Mater.* **2009**, *21*, 86.
- (87) Wiradharma, N.; Khan, M.; Tong, Y. W.; Wang, S.; Yang, Y. Y. *Adv. Funct. Mater.* **2008**, *18*, 943.
- (88) Wiradharma, N.; Tong, Y. W.; Yang, Y. Y. *Biomaterials* **2009**, *30*, 3100.
- (89) Dickerson, M. B.; Sandhage, K. H.; Naik, R. R. *Chem. Rev.* **2008**, *108*, 4935.
- (90) Hartgerink, J. D.; Beniash, E.; Stupp, S. I. *Science* **2001**, *294*, 1684.
- (91) Jiang, H.; Guler, M. O.; Stupp, S. I. *Soft Matter* **2007**, *3*, 454.
- (92) Sargeant, T. D.; Guler, M. O.; Oppenheimer, S. M.; Mata, A.; Satcher, R. L.; Dunand, D. C.; Stupp, S. I. *Biomaterials* **2008**, *29*, 161.
- (93) Spoerke, E. D.; Anthony, S. G.; Stupp, S. I. *Adv. Mater.* **2009**, *21*, 425.
- (94) Wallin, E.; Von-Heijne, G. *Protein Sci.* **1998**, *7*, 1029.
- (95) Loll, P. J. *J. Struct. Biol.* **2003**, *142*, 144.
- (96) Nilsson, J.; Persson, B.; von-Heijne, G. *Proteins: Struct., Funct., Bioinf.* **2005**, *60*, 606.
- (97) Privé, G. G. *Curr. Opin. Struct. Biol.* **2009**, *19*, 379.
- (98) Yeh, J. I.; Du, S.; Tortajada, A.; Paulo, J.; Zhang, S. *Biochemistry* **2005**, *44*, 16912.
- (99) Zhao, X.; Nagai, Y.; Reeves, P. J.; Kiley, P.; Khorana, H. G.; Zhang, S. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 17707.
- (100) Kiley, P.; Zhao, X.; Vaughn, M.; Baldo, M. A.; Bruce, B. D.; Zhang, S. *PLoS Biol.* **2005**, *3*, 1180.
- (101) Matsumoto, K.; Vaughn, M.; Bruce, B. D.; Koutsopoulos, S.; Zhang, S. *J. Phys. Chem. B* **2009**, *113*, 75.
- (102) McGregor, C. L.; Chen, L.; Pomroy, N. C.; Hwang, P.; Go, S.; Chakrabarty, A.; Privé, G. G. *Nat. Biotechnol.* **2003**, *21*, 171.
- (103) Dazzi, F.; Horwood, N. J. *Curr. Opin. Oncol.* **2007**, *19*, 650.
- (104) Hosseinkhani, H. *Curr. Drug Saf.* **2012**, *7*, 37.
- (105) Langer, R. S.; Vacanti, J. P. *Sci. Am.* **1999**, *280*, 86.
- (106) Langer, R. J.; Vacanti, P. *Science* **1993**, *260*, 920.
- (107) Hosseinkhani, M.; Hosseinkhani, H.; Khademhosseini, A.; Bolland, F.; Kobayashi, H.; Prat, S. *Stem Cells* **2007**, *25*, 571.
- (108) Hosseinkhani, H.; Hosseinkhani, M.; Tian, F.; Kobayashi, H.; Tabata, Y. *Biomaterials* **2006**, *27*, 5089.
- (109) Wintermantel, E.; Mayer, J.; Blum, J.; Eckert, K. L.; Lüscher, P.; Mathey, M. *Biomaterials* **1996**, *17*, 83.
- (110) Hosseinkhani, H.; Inatsugu, Y.; Hiraoka, Y.; Inoue, S.; Shimokawa, H.; Tabata, Y. *Tissue Eng.* **2005**, *11*, 1459.
- (111) Hosseinkhani, H.; Inatsugu, Y.; Hiraoka, Y.; Inoue, S.; Tabata, Y. *Tissue Eng.* **2005**, *11*, 1476.
- (112) Colton, C. K. *Cell Transplant.* **1995**, *4*, 415.
- (113) Pankajakshan, D.; Krishnan, V. K.; Krishnan, L. K. *J. Tissue Eng. Regener. Med.* **2007**, *1*, 389.
- (114) Hosseinkhani, H.; Hosseinkhani, M.; Kobayashi, H. *J. Bioact. Compat. Polym.* **2006**, *21*, 277.
- (115) Hosseinkhani, H.; Hosseinkhani, M.; Kobayashi, H. *Biomed. Mater.* **2006**, *1*, 8.
- (116) Xie, Y.; Yang, S. T.; Kniss, D. A. *Tissue Eng.* **2001**, *7*, 585.

- (117) Hosseinkhani, H.; Azzam, T.; Kobayashi, H.; Hiraoka, Y.; Shimokawa, H.; Domb, A. J.; Tabata, Y. *Biomaterials* **2006**, *27*, 4269.
- (118) Noshi, T.; Yoshikawa, T.; Ikeuchi, M.; Dohi, Y.; Ohgushi, H.; Horiuchi, K.; Sugimura, M.; Ichijima, K.; Yonemasu, K. *J. Biomed. Mater. Res. Part A* **2000**, *52*, 621.
- (119) Hosseinkhani, H.; Yamamoto, M.; Inatsugu, Y.; Hiraoka, Y.; Inoue, S.; Shimokawa, H.; Tabata, Y. *Biomaterials* **2006**, *27*, 1387.
- (120) Ma, T.; Li, Y.; Yang, S. T.; Kniss, D. A. *Biotechnol. Bioeng.* **2000**, *70*, 606.
- (121) Hosseinkhani, H.; Hosseinkhani, M.; Khademhosseini, A. *Yakhteh Med. J.* **2006**, *8*, 204.
- (122) Thomson, R. C.; Wake, M. C.; Yaszemski, M. J.; Mikos, A. G. *Adv. Polym. Sci.* **1995**, *122*, 245.
- (123) Hutmacher, D. W. *Biomaterials* **2000**, *21*, 2529.
- (124) Whang, K.; Thomas, C. H.; Healy, K. E.; Nuber, G. *Polymer* **1995**, *36*, 837.
- (125) Taboas, J. M.; Maddox, R. D.; Krebsbach, P. H.; Hollister, S. J. *Biomaterials* **2003**, *24*, 181.
- (126) Gomes, M. E.; Ribeiro, A. S.; Malafaya, P. B.; Reis, R. L.; Cunha, A. M. *Biomaterials* **2001**, *22*, 883.
- (127) Hutmacher, D. W.; Schantz, T.; Zein, I.; Ng, K. W.; Teoh, S. H.; Tan, K. C. *J. Biomed. Mater. Res.* **2001**, *5*, 203.
- (128) Cai, Z.; Cheng, G. *J. Mater. Sci. Lett.* **2003**, *22*, 153.
- (129) Cai, Q.; Yang, J.; Bei, J.; Wang, S. *Biomaterials* **2002**, *23*, 4483.
- (130) Zein, I.; Hutmacher, D. W.; Tan, K. C.; Teoh, S. H. *Biomaterials* **2002**, *23*, 1169.
- (131) Lin, A. S. P.; Borrows, T. H.; Cartmell, S. H.; Guldberg, R. E. *M. Biomaterials* **2003**, *24*, 481.
- (132) Oxley, H. R.; Corkhill, P. H.; Fitton, J. H.; Tighe, B. J. *Biomaterials* **1993**, *14*, 1064.
- (133) Chirila, T. V.; Constable, I. J.; Crawford, G. J.; Vijayasekaran, S.; Thompson, D. E.; Chen, Y. C.; Fletcher, W. A.; Griffin, B. J. *Biomaterials* **1993**, *14*, 26.
- (134) Zhang, X.; Jiang, X. N.; Sun, C. *Sens. Actuators A* **1997**, *77*, 149.
- (135) Chen, G.; Ushida, T.; Tateishi, T. *Mater. Sci. Eng. C* **2001**, *17*, 63.
- (136) Chen, G.; Ushida, T.; Tateishi, T. *Biomaterials* **2001**, *22*, 2563.
- (137) Lin, A. S. P.; Borrows, T. H.; Cartmell, S. H.; Guldberg, R. E. *Biomaterials* **2003**, *24*, 481.
- (138) Kane, R. S.; Takayama, S.; Ostuni, E.; Ingber, D. E.; Whitesides, G. M. *Biomaterials* **1999**, *20*, 2363.
- (139) Taboas, J. M.; Maddox, R. D.; Krebsbach, P. H.; Hollister, S. J. *Biomaterials* **2003**, *24*, 181.
- (140) Hosseinkhani, H.; Hong, P. D.; Yu, D. S.; Chen, Y. R.; Farber, I. V.; Domb, A. J. *Int. J. Nanomed.* **2012**, *27*, 3035.
- (141) Hutmacher, D. W.; Ng, K. W.; Kaps, C.; Sittiger, M.; Kläring, S. *Biomaterials* **2003**, *24*, 4445.
- (142) Blacher, S.; Maquet, V.; Schils, F.; Martin, D.; Schoenen, J.; Moonen, G.; Jérôme, R.; Pirard, J. P. *Biomaterials* **2003**, *24*, 1033.
- (143) Nikpour, M.; Chaouk, H.; Mau, A.; Chung, D. J.; Wallace, G. *Synth. Met.* **1999**, *99*, 121.
- (144) Cavallini, A.; Notarnicola, M.; Berloco, P.; Lippolis, A.; Leo, A. D. *J. Microbiol. Methods* **2000**, *39*, 265.
- (145) Pastist, C. M.; Mulder, M. B.; Gautier, S. E.; Maquet, V.; Jérôme, R.; Oudega, M. *Biomaterials* **2004**, *25*, 1569.
- (146) Schmidt, C. E.; Leach, J. B. *Biomed. Eng.* **2003**, *5*, 293.
- (147) Kang, H. W.; Tabata, Y.; Ikada, Y. *Biomaterials* **1999**, *20*, 1339.
- (148) Blacher, S.; Maquet, V.; Schils, F.; Martin, D.; Schoenen, J.; Moonen, G.; Jérôme, R.; Pirard, J. P. *Biomaterials* **2003**, *24*, 1033.
- (149) Plant, G. W.; Harvey, A. R.; Chirila, T. V. *Brain Res.* **1995**, *671*, 119.
- (150) Shugens, C. H.; Maquet, V.; Grandfils, C.; Jérôme, R.; Teyssie, P. H. *Polymer* **1996**, *37*, 1027.
- (151) Maquet, V.; Martin, D.; Scholtes, F.; Franzen, R.; Schoenen, J.; Moonen, G.; Jérôme, R. *Biomaterials* **2001**, *22*, 1137.
- (152) Ma, Z.; Kotaki, M.; Inai, R.; Ramakrishna, S. *Tissue Eng.* **2005**, *11*, 101.
- (153) Rosenberg, M. D. *Science* **1963**, *139*, 411.
- (154) Webster, T. J.; Siegel, R. W.; Bizios, R. *Biomaterials* **1999**, *20*, 1221.
- (155) Pricea, R. L.; Waidb, M. C.; Haberstroha, K. M.; Webster, T. J. *Biomaterials* **2003**, *24*, 1877.
- (156) Elias, K. L.; Price, R. L.; Webster, T. J. *Biomaterials* **2002**, *23*, 3279.
- (157) Webster, T. J.; Schalder, L. S.; Siegel, R. W.; Bizios, R. *Tissue Eng.* **2001**, *7*, 291.
- (158) Smith, L. A.; Ma, P. X. *Biointerfaces* **2004**, *10*, 125.
- (159) Kameoka, J.; Verbridge, S. S.; Liu, H.; Czaplewski, D. A.; Craighead, H. G. *Nano Lett.* **2004**, *4*, 2105.
- (160) Tian, F.; Hosseinkhani, H.; Hosseinkhani, M.; Khademhosseini, A.; Yokoyama, Y.; Esterada, G. G.; Kobayashi, H. *J. Biomed. Mat. Res., Part A* **2008**, *84*, 291.
- (161) Hartgerink, J. D.; Beniash, E.; Stupp, S. I. *Science* **2001**, *294*, 1684.
- (162) Silva, G. A.; Czeisler, C.; Niece, K. L.; Beniash, E.; Harrington, D. A.; Kessler, J. A.; Stupp, S. I. *Science* **2004**, *303*, 1352.
- (163) Hong, Y.; Legge, R. L.; Zhang, S.; Chen, P. *Biomacromolecules* **2003**, *4*, 1433.
- (164) Ruoslahti, E.; Pierschbacher, M. D. *Science* **1987**, *238*, 491.
- (165) Williams, J. A. *Pathol. Biol.* **1992**, *40*, 813.
- (166) Evans, N. D.; Gentleman, E.; Polak, J. M. *Mater. Today* **2006**, *12*, 26.
- (167) Hosseinkhani, H.; Hosseinkhani, M.; Tian, F.; Kobayashi, H.; Tabata, Y. *Biomaterials* **2006**, *27*, 4079.
- (168) Vandenberghu, H.; Del Tatto, M.; Shansky, J.; Lemaire, J.; Chang, A.; Payumo, F.; Lee, P. *Hum. Gene Ther.* **1996**, *7*, 2195.
- (169) Petite, H.; Viateau, V.; Bensaid, W. *Biotechnology* **2000**, *18*, 959.
- (170) Peretti, G. M.; Randolph, M. A.; Villa, M. T.; Buragas, M. S.; Yaremchuk, M. J. *Tissue Eng.* **2000**, *6*, 567.
- (171) Woerly, S.; Plant, G. W.; Harvey, A. R. *Biomaterials* **1996**, *17*, 301.
- (172) Ishaug, S. L.; Crane, G. M.; Miller, M. J.; Yasko, A. W.; Yaszmski, M. J.; Mikos, A. G. *J. Biomed. Mater. Res.* **1997**, *36*, 17.
- (173) Freed, L. E.; Vunjak-Novakovic, G. *Adv. Drug Delivery Rev.* **1998**, *33*, 15.
- (174) Freed, L. E.; Vunjak-Novakovic, G.; Langer, R. *J. Cell Biochem.* **1993**, *51*, 257.
- (175) Ishaug, S. L.; Crane, G. M.; Miller, M. J.; Yasko, A. W.; Yaszmski, M. J.; Mikos, A. G. *J. Biomed. Mater. Res.* **1997**, *36*, 17.
- (176) Niklason, L. E.; Gao, J.; Abbott, W. M.; Hirschi, K. K.; Houser, S.; Marini, R.; Langer, R. *Science* **1999**, *284*, 489.
- (177) Ma, P. X.; Langer, R. *J. Biomed. Mater. Res.* **1999**, *44*, 217.
- (178) Schneider, A. I.; Maier-Reif, K.; Graeve, T. *In Vitro Cell Dev. Biol.: Anim.* **1999**, *35*, 515.
- (179) Papas, K. K.; Long, R. C.; Sambanis, A.; Constantinidis, I. *Biotechnol. Bioeng.* **1999**, *66*, 219.
- (180) Griffith, M.; Osborne, R.; Munger, R.; Xiong, X. J.; Doillon, C. J.; Laycock, N. L. C.; Hakim, M.; Song, Y.; Watsky, M. A. *Science* **1999**, *286*, 2169.
- (181) Kloth, S.; Ebenbeck, C.; Kubitzka, M. S. *FASEB J.* **1995**, *9*, 963.
- (182) Colton, C. K. *Cell Transplant.* **1995**, *4*, 415.
- (183) Polverini, P. J. *Crit. Rev. Oral Biol. Med.* **1995**, *6*, 230.
- (184) Ware, J. A.; Simons, M. *Nat. Med.* **1997**, *3*, 158.
- (185) Thompson, J. A.; Anderson, K. D.; Dipietro, J. M.; Zwiebel, J. A.; Zametta, M.; Anderson, W. F.; Macing, T. *Science* **1988**, *241*, 1349.
- (186) Tabata, Y.; Nagano, A.; Ikada, Y. *Tissue Eng.* **1999**, *5*, 127.
- (187) Tabata, Y.; Ikada, Y. *Biomaterials* **1999**, *20*, 2169.
- (188) Cai, S.; Liu, Y.; Shu, X. Z.; Prestwich, G. D. *Biomaterials* **2005**, *26*, 6054.
- (189) Dogan, A. K.; Gumusderelioglu, M.; Aksoz, E. *J. Biomed. Mater. Res. B: Appl. Biomater.* **2005**, *74*, 504.
- (190) Edelman, E. R.; Mathiowitz, E.; Langer, R.; Klagsburn, M. *Biomaterials* **1991**, *12*, 619.
- (191) Hosseinkhani, H.; Hosseinkhani, M.; Tian, F.; Kobayashi, H.; Tabata, Y. *Biomaterials* **2006**, *27*, 5836.

- (192) Downs, E. C.; Robertson, N. E.; Riss, T. L.; Plunkett, M. L. *J. Cell Physiol.* **1992**, *152*, 422.
- (193) Iwakura, A.; Tabata, Y.; Tamura, N.; Doi, K.; Nishimura, K.; Nakamura, T.; Shimizu, Y.; Fujita, M.; Komeda, M. *Circulation* **2001**, *104*, 1325.
- (194) Hosseinkhani, H.; Hosseinkhani, M.; Khademhosseini, A.; Kobayashi, H. *J. Controlled Release* **2007**, *117*, 380.
- (195) Hosseinkhani, H.; Hosseinkhani, M.; Tian, F.; Kobayashi, H.; Tabata, Y. *Tissue Eng.* **2007**, *13*, 1.
- (196) Shin, H.; Zygourakis, K.; Farach-Carson, M. C.; Yaszemski, M. J.; Mikos, A. G. *J. Biomed. Mater. Res.* **2004**, *69*, 535.
- (197) Yoshikawa, T.; Ohgushi, H.; Tamai, S. *J. Biomed. Mater. Res.* **1996**, *32*, 481.
- (198) Lee, Y. M.; Seol, Y. J.; Lim, Y. T.; Kim, S.; Han, S. B.; Rhyu, I. C.; Baek, S. H.; Heo, S. J.; Choi, J. Y.; Klokkevold, P. R.; Chung, C. P. *T. J. Biomed. Mater. Res.* **2001**, *54*, 216.
- (199) Wang, Y.; Uemura, T.; Dong, J.; Kojima, H.; Tanaka, J.; Takeishi, T. *Tissue Eng.* **2003**, *9*, 1205.
- (200) Dohi, Y.; Ohgushi, H.; Tabata, S. *J. Bone Miner. Res.* **1992**, *7*, 1173.
- (201) Subramani, K.; Pathak, S.; Hosseinkhani, H. *Dig. J. Nanomater. Bios.* **2012**, *7*, 85.
- (202) Urist, M. R. *Science* **1965**, *150*, 853.
- (203) Urist, M. R.; Lietze, A.; Dawsen, E. *Clin. Orthop.* **1984**, *187*, 277.
- (204) Schmidmaier, G.; Wildemann, B.; Heeger, J.; Gabelein, T.; Flyvbjerg, A.; Bail, H. J.; Raschke, M. *Bone* **2002**, *31*, 165.
- (205) Yu, Y.; Yang, J. L.; Chapman-Sheath, P. J.; Walsh, W. R. *J. Biomed. Mater. Res.* **2002**, *60*, 392.
- (206) Blumenfeld, I.; Srouji, S.; Lanir, Y.; Laufer, D.; Livne, E. *Exp. Gerontol.* **2002**, *37*, 553.
- (207) Rifkin, D. B.; Moscatelli, D. *J. Cell Biol.* **1989**, *109*, 1.
- (208) Abraham, J. A.; Wlang, J. L.; Tunolo, A.; Mergin, A.; Friedmann, J. *EMBO J.* **1986**, *5*, 2523.
- (209) Wans, J. S.; Aspenberg, P. *J. Orthop. Res.* **1996**, *14*, 316.
- (210) Globus, R. K.; Petterson-Bückendahl, P.; Gospodarowicz, D. *Endocrinology* **1988**, *123*, 98.
- (211) Gospodarowicz, D.; Ferrara, N.; Schweigerer, G.; Neufeld, G. *Endocrinol. Rev.* **1987**, *8*, 95.
- (212) Jingnski, S.; Hedemann, A.; Kana, S. K.; Mecey, L. R.; Bolander, M. E. *J. Orthop. Res.* **1990**, *8*, 364.
- (213) Tabata, Y.; Yamada, K.; Miyamoto, S.; Nagata, I.; Kikuchi, H.; Aoyama, I.; Tamura, M.; Ikada, Y. *Biomaterials* **1998**, *19*, 807.
- (214) Ellis-Behnke, R. G.; Liang, Y. X.; You, S. W.; Tay, D. K.; Zhang, S.; So, K. F.; Schneider, G. E. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 5054.
- (215) Liang, Y. X.; Cheung, S. W.; Chan, K. C.; Wu, E. X.; Tay, D. K.; Ellis-Behnke, R. G. *Nanomedicine* **2011**, *7*, 351.
- (216) Meng, H.; Chen, R.; Xu, L.; Li, W.; Chen, L.; Zhao, Z. *Life Sci. J.* **2012**, *9*, 42.
- (217) Tysseling, V. M.; Sahni, V.; Pashuck, E. T.; Birch, D.; Hebert, A.; Czeisler, C.; Stupp, S. I.; Kessler, J. A. *J. Neurosci. Res.* **2010**, *88*, 3161.
- (218) Davis, M. E.; Motion, J. P.; Narmoneva, D. A.; Takahashi, T.; Hakuno, D.; Kamm, R. D.; Zhang, S.; Lee, R. T. *Circulation* **2005**, *111*, 442.
- (219) Davis, M. E.; Hsieh, P. C.; Takahashi, T.; Song, Q.; Zhang, S.; Kamm, R. D.; Grodzinsky, A. J.; Anversa, P.; Lee, R. T. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 8155.
- (220) Davis, M. E.; Motion, J. P. M.; Narmoneva, D. A.; Takahashi, T.; Hakuno, D.; Kamm, R. D.; Zhang, S.; Lee, R. T. *Circulation* **2005**, *111*, 442.
- (221) Galler, K. M.; Cavender, A.; Yuwono, V.; Dong, H.; Shi, S.; Schmalz, G.; Hartgerink, J. D.; D'Souza, R. N. *Tissue Eng., Part A* **2008**, *14*, 2051.
- (222) Schneider, A.; Garlick, J. A.; Egles, C. *PLoS ONE* **2008**, *3*, e1410.
- (223) Kao, B.; Kadomatsu, K.; Hosaka, Y. *Tissue Eng.: Part A* **2009**, *15*, 2385.
- (224) Cheng, T. Y.; Wu, H. C.; Chang, W. H.; Huang, M. Y.; Lee, C. H.; Wang, T. W. Functionalized Self-Assembling Peptides for Immediate Hemostasis and Accelerative Liver Tissue Regeneration. *Technical Proceedings of the 2012 NSTI Nanotechnology Conference and Expo*, Taipei, Taiwan, 2012, Vol. 3.
- (225) Weiss, A. P.; Olmedo, M. L.; Lin, J. C.; Ballock, R. T. *J. Hand Surg.* **1999**, *20*, 94.
- (226) Hosseinkhani, H.; Hosseinkhani, M.; Khademhosseini, A.; Gabrielson, N. P.; Pack, D. W.; Kobayashi, H. *J. Biomed. Mater. Res., Part A* **2008**, *85*, 47.
- (227) Peppas, N. A.; Langer, R. *Science* **1994**, *263*, 1715.
- (228) Langer, R.; Peppas, N. A. *Biomaterials* **1981**, *2*, 201.
- (229) Hosseinkhani, H. *Int. J. Nanotechnol.* **2006**, *3*, 416.
- (230) Mohammad-Taheri, M.; Vasheghani-Farahani, E.; Hosseinkhani, H.; Shojaosadati, S. A.; Soleimani, M. *Iran Polym. J.* **2012**, *21*, 239.
- (231) Hosseinkhani, H.; Tabata, Y. S. *J. Nanosci. Nanotechnol.* **2006**, *6*, 2320.
- (232) Langer, R.; Tirrell, D. A. *Nature* **2004**, *428*, 487.
- (233) Tysseling-Mattiace, V. M.; Sahni, V.; Niece, K. L.; Birch, D.; Czeisler, C.; Fehlings, M. G.; Stupp, S. I.; Kessler, J. A. *J. Neurosci.* **2008**, *28*, 3814.
- (234) Horii, A.; Wang, X.; Gelain, F.; Zhang, S. *PLoS ONE* **2007**, *2*, e190.
- (235) Kayser, O.; Lemker, A.; Trejo, N. H. *Curr. Pharm. Biotechnol.* **2005**, *6*, 3.
- (236) Neuberger, T.; Schöpf, B.; Hofmann, H.; Hofmann, M.; Rechenberg, B. V. *J. Magn. Magn. Mater.* **2005**, *293*, 483.
- (237) Hosseinkhani, H.; Hosseinkhani, M. *Curr. Drug Saf.* **2009**, *4*, 79.
- (238) Hosseinkhani, H.; Hosseinkhani, M.; Vasheghani, E.; Nekoomanesh, M. *Adv. Sci. Lett.* **2009**, *2*, 70.
- (239) Tian, F.; Hosseinkhani, H.; Estrada, G.; Kobayashi, H. *J. Phys. (Paris)* **2007**, *61*, 587.
- (240) Hosseinkhani, H.; Kushibiki, T.; Matsumoto, K.; Nakamura, T.; Tabata, Y. *Cancer Gene Ther.* **2006**, *13*, 479.
- (241) Hosseinkhani, H.; Hosseinkhani, M.; Khademhosseini, A. *Drug Discovery* **2006**, *1*, 32.
- (242) Konishi, M.; Tabata, Y.; Kariya, M.; Hosseinkhani, H.; Suzuki, A.; Fukuhara, K.; Mandai, M.; Takakura, K.; Fujii, S. *J. Controlled Release* **2005**, *103*, 7.
- (243) Hosseinkhani, H.; Tabata, Y. *J. Controlled Release* **2005**, *108*, 540.
- (244) Hosseinkhani, H.; Azzam, T.; Tabata, Y.; Domb, A. *J. Gene Ther.* **2004**, *11*, 194.
- (245) Hosseinkhani, H.; Tabata, Y. *J. Controlled Release* **2004**, *97*, 157.
- (246) Hosseinkhani, H.; Tabata, Y. *J. Controlled Release* **2003**, *86*, 169.
- (247) Hosseinkhani, H.; Aoyama, T.; Ogawa, O.; Tabata, Y. *Curr. Pharm. Biotechnol.* **2003**, *4*, 109.
- (248) Hosseinkhani, H.; Aoyama, T.; Ogawa, O.; Tabata, Y. *J. Controlled Release* **2003**, *88*, 297.
- (249) Aoyama, T.; Hosseinkhani, H.; Yamamoto, S.; Ogawa, O.; Tabata, Y. *J. Controlled Release* **2002**, *80*, 345.
- (250) Hosseinkhani, H.; Aoyama, T.; Ogawa, O.; Tabata, Y. *J. Drug Targeting* **2002**, *10*, 193.
- (251) Hosseinkhani, H.; Aoyama, T.; Ogawa, O.; Tabata, Y. *Pharm. Res.* **2002**, *19*, 1469.
- (252) Hosseinkhani, H.; Aoyama, T.; Ogawa, O.; Tabata, Y. *J. Controlled Release* **2002**, *83*, 286.
- (253) Hosseinkhani, H.; Aoyama, T.; Ogawa, O.; Tabata, Y. *Proc. Jpn. Acad., Ser. B* **2001**, *77*, 161.
- (254) Hosseinkhani, H.; Kobayashi, H.; Tabata, Y. *Pept. Sci.* **2006**, *2005*, 341.
- (255) Hosseinkhani, H.; Kobayashi, H.; Tabata, Y. *Pept. Sci.* **2006**, *2005*, 63.
- (256) Hosseinkhani, H.; Hosseinkhani, M.; Hattori, S.; Matsuoka, R.; Kawaguchi, N. *J. Biomed. Mater. Res., Part A* **2010**, *94*, 1.

- (257) Lindstrom, S.; Iles, A.; Persson, J.; Hosseinkhani, H.; Hosseinkhani, M.; Khademhosseini, A.; Lindstrom, H.; Andersson, H. *JBSE* **2010**, *5*, 272.
- (258) Abdullah, S.; Yeo, W. Y.; Hosseinkhani, H.; Hosseinkhani, M.; Masrawa, E.; Ramasamy, R.; Rosli, R.; Rahman, S. A.; Domb, A. J. *J. Biomed. Biotechnol.* **2010**, *2010*, 284840.
- (259) Subramani, K.; Hosseinkhani, H.; Khraisat, A.; Hosseinkhani, M.; Pathak, Y. *Curr. Nanosci.* **2009**, *5*, 134.
- (260) Mahmoudi, M.; Hosseinkhani, H.; Hosseinkhani, M.; Boutry, S.; Simchi, A.; Journeay, W. S.; Subramani, K.; Laurent, S. *Chem. Rev.* **2011**, *111*, 253.
- (261) Mohageri, S.; Hosseinkhani, H.; Ebrahimi, N. G.; Solimani, M.; Kajbafzadeh, A. M. *Tissue Eng., Part A* **2010**, *16*, 3821.
- (262) Abedini, F.; Ismail, M.; Hosseinkhani, H.; Azmi, T.; Omar, A. R.; Pei Pei, C.; Ismail, N.; Farber, I. Y.; Domb, A. J. *J. Cell Anim. Biol.* **2010**, *4*, 170.
- (263) Abedini, F.; Hosseinkhani, H.; Ismail, M.; Domb, A. J.; Omar, A. R.; Pei Pei, C.; Hong, P. D.; Yu, D. S.; Farber, I. Y. *Int. J. Nanomed.* **2012**, *7*, 4159.
- (264) Semino, C. E. *J. Dent. Res.* **2008**, *87*, 606.
- (265) Aggeli, A.; Boden, N.; Cheng, Y. L.; Findlay, J. B.; Knowles, P. F.; Kovatchev, P. *Biochemistry* **1996**, *35*, 16213.
- (266) Aggeli, A.; Bell, M.; Boden, N.; Keen, J. N.; Knowles, P. F.; McLeish, T. C. B. *Nature* **1997**, *386*, 259.
- (267) Zhang, S.; Holmes, T.; Lockshin, C.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 3334.
- (268) Kirkham, J.; Firth, A.; Vernalis, D.; Boden, N.; Robinson, C.; Shore, R. C. *J. Dent. Res.* **2007**, *86*, 426.
- (269) Zhang, S.; Lockshin, C.; Cook, R.; Rich, A. *Biopolymers* **1994**, *34*, 663.
- (270) Kisiday, J.; Jin, M.; Kurz, B.; Hung, H.; Semino, C.; Zhang, S. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 9996.