CHAPTER 14

Self-assembly of Nanomaterials for Engineering Cell Microenvironment

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14.1 Overview

This chapter reviews the applications of self-assembling proteins and peptides toward designing novel biomimetic nanomaterials and their potential applications in nanobiotechnology and biomedicine. Numerous proteins and peptides have been emerging as nanobiomaterials due to their ability to self-assemble into nanoscale structures like nanotubes, nanovesicles, helical ribbons, and fibrous scaffolds. Self-assembly can be defined as the ability of certain multimeric biological structures to assemble from their component parts through random movements of the molecules and formation of weak chemical bonds between surfaces with complementary shapes. In other words, self-assembly can be defined as the spontaneous organization of individual components into an ordered structure without human intervention [1, 2]. A few examples of self-assembled structures that can be found at the microscopic level are phospholipid bilayer of human cell membranes [3], RNA [4], and DNA complexes [5]. The detergent molecules exhibit self-assembly phenomena due to their amphiphilic properties. The molecular building mechanisms underlying the formation of bacteriophage and viral particles are based on self-assembly. The other common examples of self-assembly phenomena tailored by nature are lipid molecules forming oil droplets in water, four haemoglobin polypeptides forming a functional haemoglobin protein, and the combination of RNA and ribosomal proteins to form a functional ribosome.

Molecular self-assembly is a powerful phenomenon borrowed from nature by scientists for fabricating novel supramolecular architectures. Molecular self-assembly is mainly governed by weak noncovalent bonds like electrostatic interactions (ionic bonds), hydrogen bonds, hydrophobic interactions, water-mediated hydrogen bonds, and van der Waals interactions [2–4]. Although these forces are weak, their collective interactions can produce structurally and chemically stable structures. Self-assembly of biological molecules like proteins, peptides, nucleic acids, lipids, and other cellular components governs the biological structure and function of a living cell. Cellular events like amyloid fibril formation, antigen-antibody recognition, chromatin assembly, and phospholipid membrane self-assembly are excellent examples of molecular self-assembly.

14.2 Proteins and Peptides

Proteins are fundamental components of all living cells. They can be classified as a group of complex organic macromolecules containing carbon, hydrogen, nitrogen, oxygen, and sulphur. They are composed of one or more chains of amino acids. Amino acids contain an amino (-NH2) and a carboxyl (-COOH) group. 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 the proper functioning of a living organism. Structural analysis of protein molecules has revealed that they take up various shapes to form a stable macroscopic structure. Nature has used proteins to build a vast array of structures like keratin, collagen, coral, pearl, shell, and the like. Molecular self-assembly is exhibited by proteins and peptides. In the past few decades, numerous researches have been done to understand the structural characteristics that influence the self-assembly of protein and peptide molecules. The potential applications of self-assembling proteins and peptides have been effectively utilized to design novel biomimetic nanomaterials that have tremendous applications in the fields of nanobiotechnology and biomedicine.

14.3 Self-assembly of Proteins and Peptides

Various authors have discussed in detail several self-assembling peptide and protein systems that self-assemble to form various nanostructures like nanotubes, vesicles, helical ribbons, and fibrous scaffolds. These structures are analysed to design and fabricate new materials that will have potential applications in biomedical nanotechnology. The findings discussed in this chapter have been grouped under different headings: “Self-assembly of Proteins and Peptides,” “Findings about Amphiphilic and Surfactantlike Peptides,” “Findings about Three-dimensional Peptide Matrix Scaffolds,” and “Use of Peptide Hydogels in Regenerative Biology and Three-dimensional Cell Culture.” A group of scientists has designed artificial proteins that self-assemble to form hydrogels (Figure 14.1). The artificial proteins designed by the researchers were made up of an ionic self-complementary peptide group that had an alternating polar and nonpolar fashion of arrangement of peptide molecules. These peptides formed stable â-strand and â-sheet structures, which self-assembled to form nanofibres. These nanofibres formed interwoven matrices that further formed a scaffold hydrogel with high water content. Hydrogel 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 act as drug-delivery systems delivering pharmaceutical protein complexes in the treatment of diseases like cancer.

Another group of scientists has designed peptide nanotubes using surfactantlike peptides (Figure 14.2). These peptide nanotubes can be used as templates for growing metal nanocrystals; thus, nanowires can be fabricated. Peptide nanotubes can

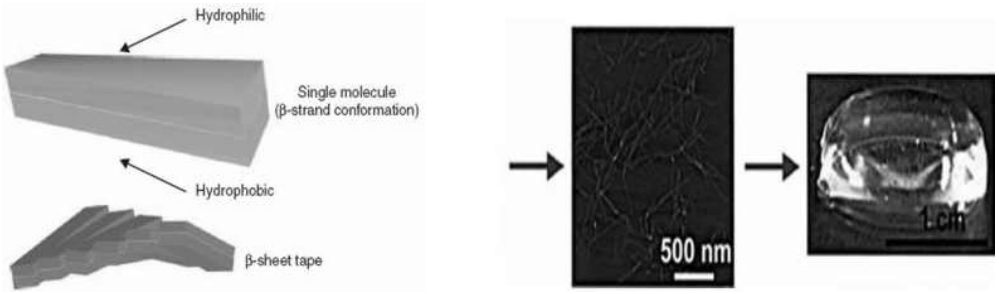

Figure 14.1 Amphiphilic peptides in â-strand and â-sheet conformation self-assemble into interwoven matrices that further form a scaffold hydrogel. (*Source:* www.nature.com/nbt/jour-

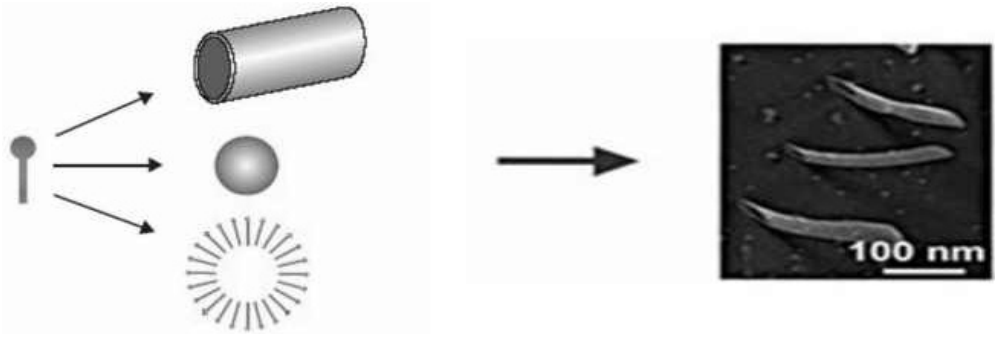

Figure 14.2 Surfactantlike peptides self-assemble to form nanotubes. (*Source:* www.nature.com/nbt/journal/v21/n10/fig_tab/nbt874_F1.html.)

also serve as ion channels when incorporated into the phopholipid bilayer of the cell membrane.

Recent results also refer to the researches done into 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 (Figure 14.3). 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 á-helical structure and the â-sheet. These are called molecular switch peptides. Gold nanoparticles can be attached to these dipolar peptides to fabricate tiny molecular switches (Figure 14.4).

14.4 Findings about Amphiphilic and Surfactantlike 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. The paper describes in detail the authors' research into amphiphilic and surfactantlike peptides conducted in their laboratory. These molecules have one or

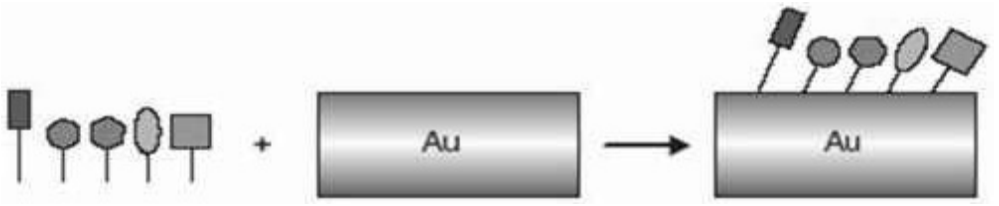

Figure 14.3 Surface-binding peptides binding with gold surface. (*Source:* http://cba.media.mit.edu/publications/articles/02.00.zhang.pdf.)

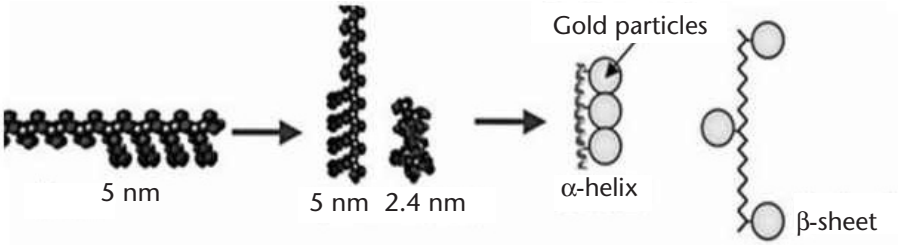

Figure 14.4 Helical dipolar peptides undergoing conformational changes can be used as tiny molecular switches with gold nanoparticles attached. (*Source:* www.puramatrix.com/NatureBiotechny_2003.pdf.)

two amino acids in the polar region and four or more consecutive hydrophobic amino acids at 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 (Figures 14.5 and 14.6).

Valine (α-aminoisovaleric acid) is an essential amino acid, and aspartic acid is a nonessential amino acid. This V6D peptide complex formed various nanostructures in aqueous solution like nanotubes and nanovesicles. The samples of the 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, and when they investigated them using transmission electron microscopy (TEM), the researchers observed

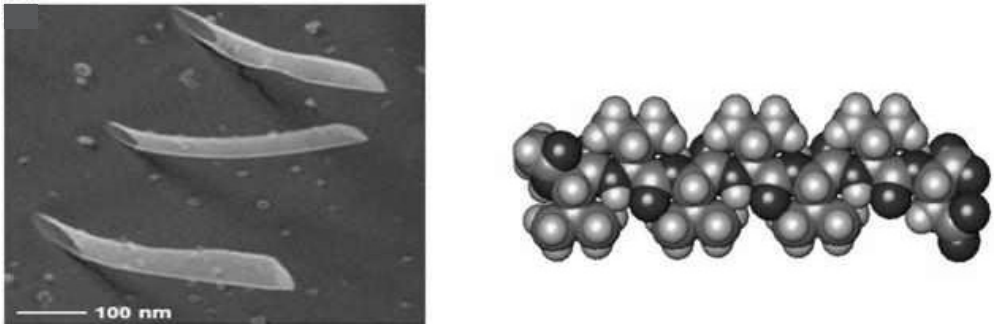

Figure 14.5 Molecular model of surfactantlike peptide V6D (VVVVVVD). (*Source:* www.templeton.org/biochem-finetuning/papers/zhang_paper.doc.)

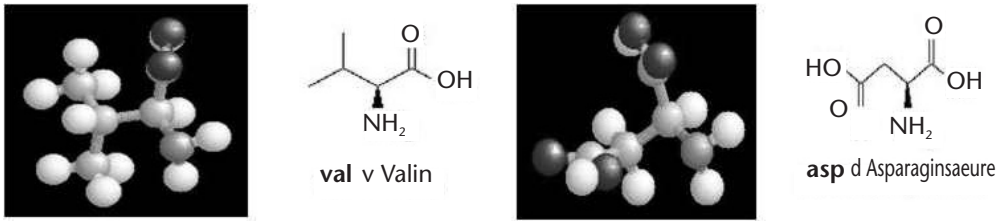

Figure 14.6 Three-dimensional structure and structural formula of valine and aspartic acid. (*Source:* http://ntri.tamuk.edu/cell/chapter3/val3d.html,http://ntri.tamuk.edu/cell/chapter3/asp3d.html.)

nanotubes and nanovesicles. The nanotubes measured about 30 to 50 nm (Figure 14.7). It has been indicated that the self-assembled structure can be modified by changing the sequence of the amino acids in the peptide chain and the environmental factors. These peptide nanotubes can be incorporated into self-assembled membranes for use in bionanosensor devices (Figure 14.8).

It is also suggested that these surfactant peptides can be engineered for improved functionality by using techniques like biotinylation. Biotinylation is a process of incorporation of biotinyl groups into molecules to visualise specific substrates by incubating them with biotin-labelled probes and avidin or streptavidin. It is a rapid method of detecting nucleic acids for use in the Western blot technique. When these surfactant peptide nanostructures are made to undergo the process of biotinylation, they can be bound to streptavidin-coated an inorganic metal surface. Histidine-tagged peptides and proteins can be bound to nickel surfaces. Thus, by utilizing the standard techniques in peptide chemistry, these nanostructures can be attached to metallic surfaces.

14.5 Findings about Three-dimensional Peptide Matrix Scaffolds

A wider variety of self-assembling proteins and peptides have inspired researchers to fabricate nanoscale fibres and fibre network scaffolds. The main factor influencing the entire process was the chirality of the individual building components of the

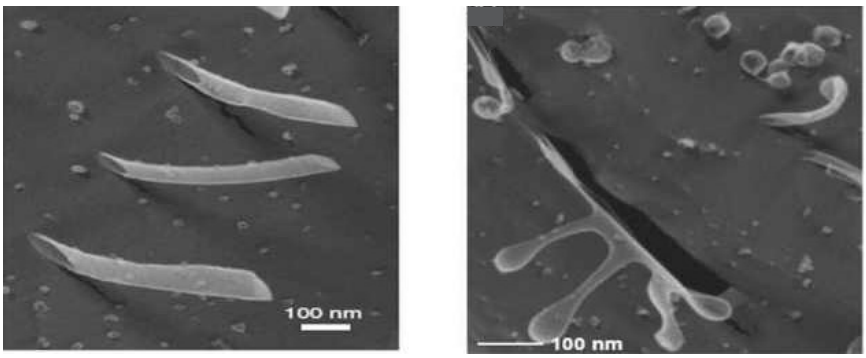

Figure 14.7 Quick-freeze/deep-etch transmission electron micrographs of V6D peptide complex: (a) Nanotubes with a diameter of 30 to 50 nm, and (b) nanovesicles and nanotubes. (*Source:* http://web.mit.edu/lms/www/PDFpapers/Zhang_MaterialsToday3BEBE.pdf.)

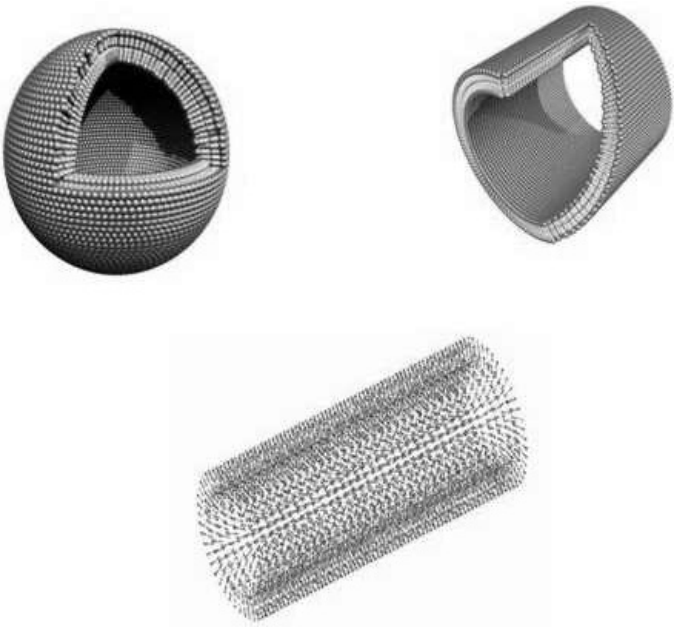

Figure 14.8 Molecular models of V6D peptide nanovesicle and nanotube. (*Source:* www.templeton.org/biochem-finetuning/papers/zhang_paper.doc.)

peptide complex. Two molecules are said to be chiral if their mirror images are not superimposed. Researchers have studied the KFE8 peptide complex, which is an eight-residue peptide complex. The KFE8 has the peptide chain sequence of FKFEFKFE (Figure 14.9).

This structure represents a group of self-assembling peptides that spontaneously self-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 (Figure 14.10). It has been reported that when certain amino acids in the hydrophobic side chains were replaced with another amino acid sequence, little change was observed. When a positively charged lysine (Lys) was replaced with a positively charged arginine (Arg), or when a negatively charged glutamate (Glu) was replaced with a negatively charged aspartate (Asp), very little

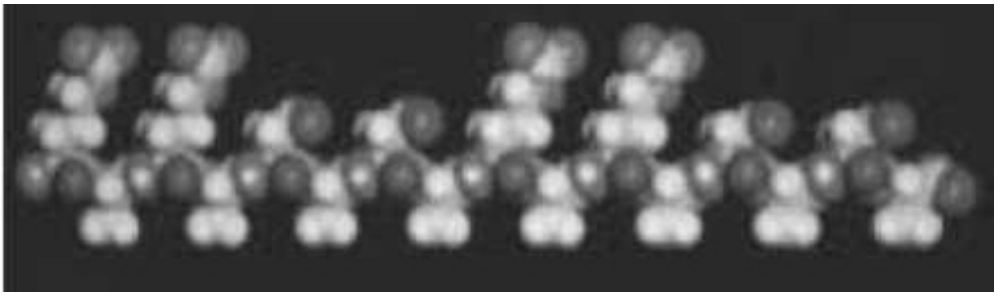

Figure 14.9 Molecular model of KFE8 peptide. (*Source:* http://web.mit.edu/lms/www/PDFpapers/Zhang_MaterialsToday3BEBE.pdf.)

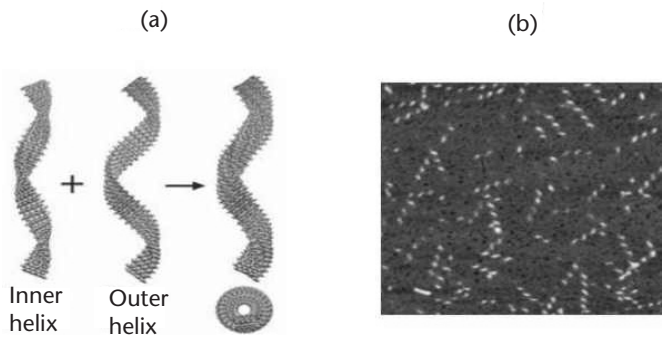

Figure 14.10 (a) The inner and outer â-helices of KFE8 peptide form a double-sheet helix with hydrophobic side chains sandwiched between the two layers. (b) atomic force microscopy image (500 × 500 nm) of peptide solution deposited over mica. (*Source:* http://cba.media.mit.edu/publications/articles/02.00.zhang.pdf.)

change was observed in the nanofibres that were formed. But when the positively charged residue was replaced with a negatively charged residue or vice versa, the peptides did not self-assemble. When the alanines were replaced with hydrophobic residues, there was a greater tendency to self-assemble and form peptide matrices with enhanced strength.

This has led researchers to concentrate their attention on understanding the basis of protein-conformational diseases. Protein-conformational diseases are a group of disorders characterized by the accumulation of malformed protein structures in cells. Proteins must fold into a proper three-dimensional structure to carry out their normal functions. When they do not fold properly, they form malfolded protein structures that accumulate in cells, leading to pathological conditions. Alzheimer's disease, prion diseases, and Parkinson's disease are a few examples of protein-conformational diseases. Thus, by understanding the mechanism of formation of peptide nanofibres and the factors controlling their self-assembly, researchers aim to formulate a remedy for protein-conformational diseases.

The authors have divided the self-assembled peptide fibres into three theoretical models. The first model is the molecular model, where the â-sheet peptides self-assemble into helical ribbons. The second model is the semicontinuum model, where the peptides self-assemble to form elastic tapelike structures composed of bricklike building blocks. The third model is the fully continuum model, where the peptides self-assemble to form tubules. These approaches have helped researchers to learn more about the mechanisms underlying the formation of different structures when peptides and proteins self-assemble and will ultimately guide them to design efficient peptide-based and protein-based biomaterials.

14.6 Use of Peptide Hydogels in Regenerative Biology and Three-dimensional Cell Culture

Material design of scaffolds for cell proliferation and differentiation is one of the key technologies for tissue engineering. The scaffold should mimic the structure and

biological function of native extracellular matrix (ECM) as much as possible, both in terms of chemical composition and physical structure [5]. Native ECM does far more than just provide a physical support for cells. It also provides a substrate with specific ligands for cell adhesion and migration and regulates cellular proliferation and function by providing various growth factors. A well-known feature of native ECM structures is the nanoscaled dimensions of their physical structure. In typical connective tissue, structural proteins fibers, such as collagen fibers and elastin fibers, have diameters ranging from several tens of nanometers to several hundred nanometers [5]. The nanoscaled protein fibers entangle with each other to form a nonwoven mesh that provides tensile strength and elasticity and laminin, which provide a specific binding site for cell adhesion and also exist as nanoscaled fibers in ECM.

The initial report showed that nanoscaled features influenced cell behaviors [6]. Nanoscaled surface topography has been found to promote osteoblast adhesions [7]. It has been demonstrated that osteoblast adhesion, proliferation, alkaline phosphatase activity, and ECM secretion on carbon nanofibers increased with decreasing fiber diameter in the range of 60 to 200 nm, whereas the adhesion of other kinds of cells, such as chondorocytes, fibroblasts, and smooth muscle cells, was not influenced [8, 9]. It has been supposed that the nanoscaled surface affects the conformation of adsorbed adhesion proteins such as vitronectin, thus affecting cell behavior [10]. In addition, the nanoscaled dimensions of cell membrane receptors such as integrins should also be considered.

Three different approaches toward the formation of nanofibrous materials have emerged: self-assembly, electrospinning, and phase separation [11]. Each of these approaches is very different and has a unique set of characteristics that lend to its development as a scaffolding system. For instance, self-assembly can generate small-diameter nanofibers in the lowest end of the range of natural ECM collagen, while electrospinning has only generated large-diameter nanofibers on the upper end of the range of natural ECM collagen. Phase separation, on the other hand, has generated nanofibers in the same range as natural ECM collagen and allows for the design of macropore structures. These attempts at an artificial ECM have the potential to accommodate cells and guide their growth and subsequent tissue regeneration. Self-assembly, that is, the autonomous organization of molecules into patterns or substrates without human intervention, is common throughout nature and technology. Self-assembly of natural or synthetic macromolecules produces nanoscaled supramolecular structures and nanofibers. Specifically designed amphiphilic peptides that contain a carbon alkyl tail and several other functional peptide regions have been synthesized and self-assembled into nanofibers with a diameter of 7.6 ± 1 nm [12]. These self-assembled nanofibers have been used recently to study the selective differentiation of neural progenitor cells [13]. Another kind of peptide containing sixteen alternating hydrophobic and hydrophilic amino acids was induced to self-assemble into nanofibers under appropriate pH values [14]. Nanoscaled fibers produced by self-assembly of peptide amphiphile seem to have great potential applications in the field of biomaterials and tissue engineering.

For successful tissue regeneration, the cells constituting the 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, mesenchymal stem cells (MSCs) have been widely investigated for use by themselves or in combination with the scaffolds necessary for the promotion of cell proliferation and differentiation. It was found that MSCs have an inherent nature to differentiate into not only osteogenic linage cells but also chondrogenic, myogenic, adipogenic, and neurogenic lineages [15–19]. It has been recognized that induction of tissue regeneration based on tissue engineering can be achieved by the following three key steps: the proliferation of cells, the seeding of cells and proliferation in a suitable scaffold, and the maintenance of the differentiation phenotype of the engineered tissues [20]. The property of scaffold material for cell attachment is one of the major factors contributing their morphology, proliferation, and functioning, as well as the subsequent tissue organization [21]. At first, cells attach to the material surface of scaffold, then spread and proliferate. A three-dimensional scaffold can provide larger surface area for cell attachment and spreading than a two-dimensional scaffold (i.e., tissue-culture plate). Xie, Yang, and Kniss [22] have reported that the initial rate of cells growth was higher for the two-dimensional culture, but once the cells reached confluence, their proliferation stopped. However, the cells' growth in the three-dimensional scaffold was continued for longer periods than in two-dimensional scaffolds. Other reports have demonstrated that cell proliferation was superior in the three-dimensional scaffold than the two-dimensional one [23–27]. It would be beneficial for biomedical applications if scaffold materials could promote the adhesion and growth of cells on their surfaces. The sequence of arginine-glycine-aspartic acid (RGD) has been discovered to be a cell-attachment sequence in various adhesive proteins present in the ECM and found in many proteins, such as fibronectin, collagen type I, vitronectin, fibrin, and the Von Willebrand factor [28]. It is well recognized that the sequence of RGD interacts with various types of integrin receptors of mammalian cells. Ever since the RGD sequence was discovered to be a cell-attachment sequence in adhesive proteins of the ECM, there have been several efforts to synthesize bioactive peptides incorporating RGD for therapeutic purposes [29].

Micro- and nanopatterned scaffolds have been investigated less well with regard to stem cells, although two recent studies highlight their attractiveness [30]. In one study, Silva et al. included a five amino acid, laminin-specific, cell-binding domain (which binds to specific integrins on the cell surface) at the hydrophilic head of their amphiphiles and showed that neural stem cells could be induced to differentiate into neurons when cultured within peptide gel [13]. 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 a result of the density of the cell-binding ligands to which the cells were exposed, indicating clearly the importance of ECM in influencing cell function. Our recent studies have indicated that when the laminin-specific domain in the amphiphilic molecule was replaced with the amino acid sequence, RGD, a common cell-binding domain in many ECM proteins, especially collagen, differentiation of MSCs to osteoblasts was significantly enhanced compared with amphililic nanofibers without this sequence or to two-dimensional controls (Figure 14.11) [31]. This is because the interaction of MSC integrin receptors with RGD of the peptide enhanced cell attachment on peptide nanofibers. The proliferation of cells in the three-dimensional scaffold needs an oxygen and nutrition supply. In this circum-

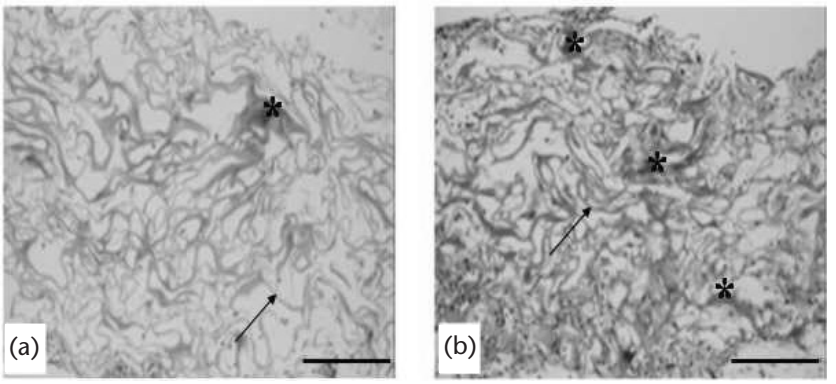

Figure 14.11 Histological cross sections of MSC attached to the self-assembled peptide nanofibers (a) without RGD and (b) with RGD incorporation four weeks after the culture in the osteogenic medium. The scale bar measures 100 ìm in higher magnification views of center in gel. Arrows indicate the residual gel of the self-assembled peptide nanofibers. Asterisks indicate the newly formed bone. (*Source:* www.sciencedirect.com/science/journal/01429612/2006.pdf.)

stance, the three-dimensional scaffold materials should provide such an environment for cells. The artificial scaffolds formed by self-assembling molecules not only provide suitable support for cell proliferation but also serve 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 the survival of 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 nanofibres. 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. The primary mouse neuron cells formed active connections with the peptide scaffolds that formed a valuable area of research for studying about neuron regeneration. In regenerative medicine, these peptide matrices were used to cultivate chondrocyte ECM that can be used to repair cartilage tissue. Thus, cartilage-tissue engineering has been done by placing the primary chondrocytes and MSCs into these self-assembled peptide hydrogels to produce collagen and glycosaminoglycans. These peptide matrices can also be used in the regeneration of bone by incorporating a phophorylated serine, which can attract and organize calcium ions to form hydroxyapatite crystals, and functionalizing them with a cell-adhesion motif like arginine-glycine-aspartic acid complex. Research studies have not been limited only to natural amphiphilic peptides. Many research trails have indicated on the synthesizing of complex amphiphilic peptides by joining hydrophilic peptides into long alkyl chains. The peptide end of the molecule was designed to function and regulate biomineralization. Bone is produced as a result of the deposition of calcium and phosphate ions to form hydroxyapatite crystals. This process is known as mineralization. Serine is a nonessential amino acid. When a

phophorylated serine was incorporated with the synthetic amphiphilic peptide complex, it served to attract and organize calcium and phosphate ions to form hydroxyapaptite crystals. Furthermore, the peptides the synthetic amphiphilic peptide have been functionalized by adding a cell-adhesion motif. It was the RGD that was attached to the C-terminus of the peptide. This can be used to study the ability of the bone cells to differentiate, proliferate, and adhere to a biomaterial surface like titanium. Titanium is the most widely used biomaterial surface to produce orthopaedic implants, dental implants, and hip replacements. Inspite of its excellent biocompatibility, titanium implants still fail. Most orthopaedic implants have a lifetime of fifteen years at 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 nano-size 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.

Another use of self-assembled peptides is in tissue engineering. Tissue engineering is designed to regenerate natural tissues or to create biological substitutes for defective or lost organs by making use of cells. Considering the use of cells in the body, there is no doubt that a sufficient supply of nutrients and oxygen to the transplanted cells is vital for their survival and functional maintenance [32]. 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 [33]. It is recognized that basic fibroblast growth factor (bFGF) functions to promote such an angiogenesis process [33, 34]. 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 [33]. However, one cannot always expect 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 to enhance the in vivo efficacy is to achieve its controlled release over an extended period by incorporating the growth factor into a polymer carrier. If this carrier is biodegraded and harmonized with tissue growth, it will work as a scaffold for tissue regeneration in addition to serving as a carrier matrix for growth factor release. Some studies have demonstrated that bFGF promoted angiogenesis when used in combination with delivery matrices and scaffold [35–40]. Our recent study has indicated that a three-dimensional network of self-assembled nanofibers was formed by mixing bFGF suspension with an aqueous solution of peptide amphiphile as an injectable carrier for controlled release of growth factors and used it for feasibility of prevascularization by the bFGF release from the three-dimensional networks of nanofibers in improving the efficiency of tissue regeneration [41]. Previous work has encapsulated bFGF within alginate, gelatin, agarose/heparin, collagen, and poly(etyhylene-co-vinyl acetate) carriers [40, 42, 43]. According to the results of these studies, it is conceivable to incorporate the angiogenic factor to a sustained releasing system and to use it prior to the implantation. The bFGF incorporated

these releasing system requires surgery for implantation, which is not welcome. On the contrary, the bFGF incorporated in self-assembled peptides could be delivered to living tissues simply by injecting a liquid (i.e., peptide amphiphile solutions) and bFGF solution. The injected solutions would form a solid scaffold at the injected site of the tissue, and the releases bFGF would induce significant angiogenesis around the injected site, in marked contrast to bFGF injection alone or PA injection alone [41].

14.7 Applications of Synthetic Amphiphilic Peptides in Other Fields of Nanotechnology

Various authors have referred to the potential use of self-assembling amphiphilic peptides in various other areas of nanotechnology. The amphiphilic peptide molecules can be used as scaffolds to fabricate nanowires. A nanowire is a solid, metallic, cylinderlike structure with a diameter ranging from ten to several hundred nanometers. Nanowires can potentially be applied in electronics, chemistry, and biomedical engineering and therefore have greater applications in nanotechnology. Amphiphilic peptide nanotubes can be used as templates for metallization. One such example of the peptide sequence used was the histidine-rich peptide nanotube. This structure was metallized with gold nanocrystals, and the organic peptide scaffold was removed to make a conducting gold nanowire (Figure 14.12). Specific peptide sequences recognize specific metal ions. Thus, by varying the peptide sequence, much more efficient metal coatings can be coated on peptide nanotubes. Examples of other efficient metals that can be coated on peptide nanotubes include silver, platinum, copper, and nickel.

Various other methods to attach the peptides and the gold nanocrystals have recently been developed. A group of researchers fabricated nanotubes from bolaamphiphile peptides. They used crystalline glycylglycine bolaamphiphile

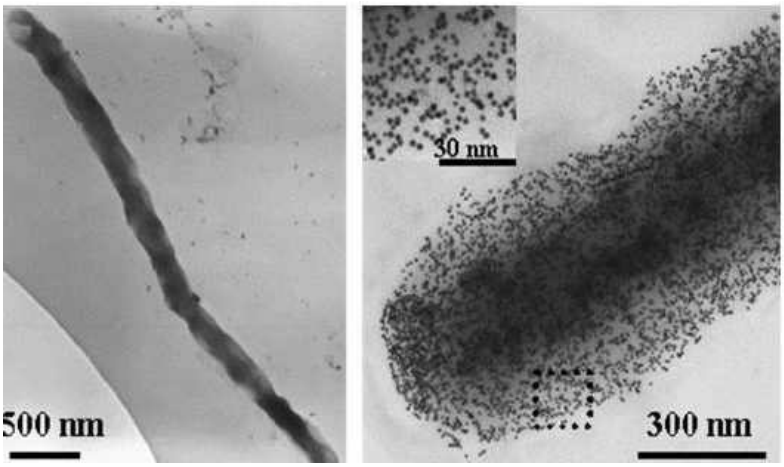

Figure 14.12 TEM image of gold nanowire (left) made from histidine-rich peptide sequence as template. Image on the right shows gold nanocrystal particles coated over peptide nanotube. (*Source:* http://oasys2.confex.com/acs/225nm/techprogram/P611826.HTM.)

tubules and studied the pH-sensitive structural transformation of the peptide tubules. These bolaamphiphiles undergo self-assembly in solution and form tubular structures with diameters ranging from 20 nm to 1 μm. The peptide nanotube was coated with a metalloporphyrin compound. Metalloporphyrins are a group of chemical compounds that have a metallic group attached to a porphyrin ring. Porphyrins are a group of closely related tetrapyrollic pigments occurring widely in nature and play a vital role in various biological processes. The heme component of the haemoglobin is a good example of this group of compounds. Researchers utilized the electron-transferring property of the metallophophyrins to bind to the peptide nanotubes. They were able to grow and immobilize the peptide nanotubes on a gold substrate. They also demonstrated that the peptide nanotubes can be coated with avidin, which enabled them to bind to gold surfaces treated with biotinylated self-assembled monolayers (SAMs) (Figures 14.13 and 14.14).

Thus, a wider range of applications and the design flexibility of self-assembling protein and peptide complexes, together with the researchers' knowledge of cell biology, biochemistry, and molecular biology, have enabled them to identify potential new applications in the field of material science and nanotechnology.

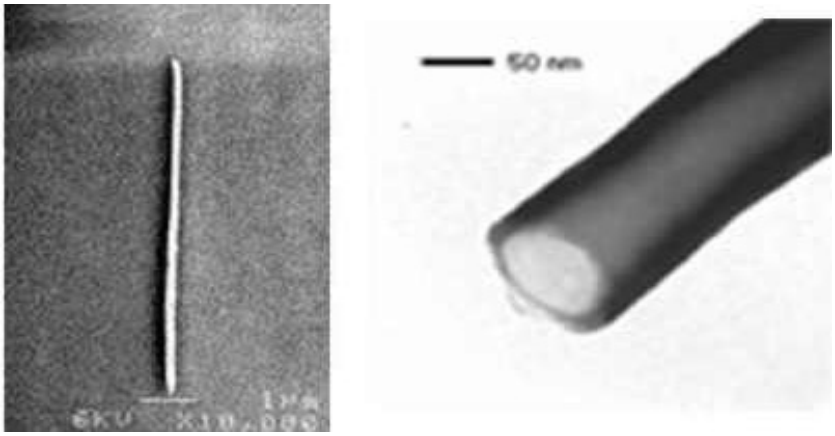

Figure 14.13 Scanning electron micrograph (SEM) and TEM image of a peptide nanotube. (*Source:* http://patsy.hunter.cuny.edu/matsui.html.)

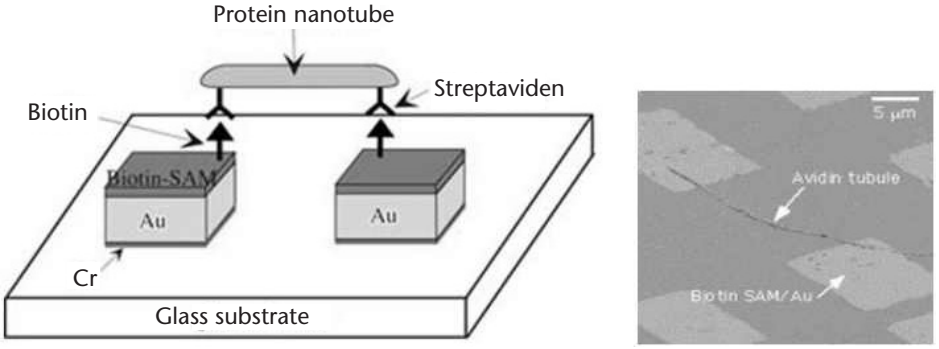

Figure 14.14 Schematic set-up of the process and SEM image of protein nanotube immobilized on biotin-SAM/Au surface. (*Source:* http://patsy.hunter.cuny.edu/matsui.html.)

14.8 Conclusion

The increasing interest in bionanotechnology has stimulated researchers to scrutinize biological elements and learn from nature. Self-assembly of biological molecules forms the basic principle in the formation of complex biological structures. The topic discussed above on nanostructured biological materials through self-assembly of peptides and proteins gives us wide knowledge of the basic principle underlying the molecular self-assembly of proteins and peptides. It gives a general overview of different kinds of self-assembling protein and peptide systems. The research discussed in this chapter clearly guides the reader as to the potential applications of these self-assembled structures for fabricating a wider range of novel biomaterials for use in bionanotechnology. With appropriate references and examples, it opens the reader's mind to 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.

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