Designer Self-Assembling Peptide Scaffolds for 3-D Tissue Cell Cultures and Regenerative Medicine

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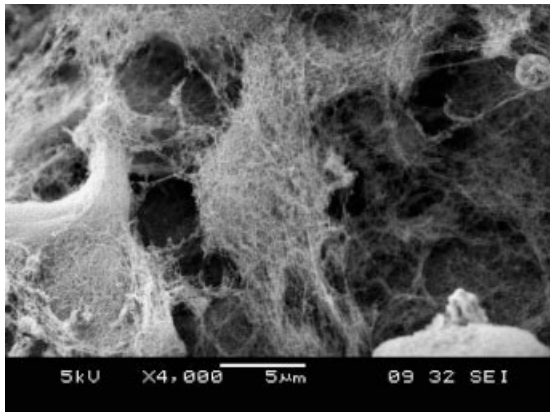
Biomaterial science has made enormous progress in the last few decades. Nonetheless, innovative biomaterials are still urgently needed to provide *in vitro* cell-culture models that more closely resemble three-dimensional (3-D) cell interactions and cyto-architectures in bodies and tissues. In this review, the recent advances toward this goal through molecular engineering of various designer self-assembling peptide scaffolds are discussed. These peptide scaffolds can be commercially and custom-tailor synthesized materials with high purity and may be not only useful for specific 3-D tissue cell cultures but also for tissue repair and regenerative therapies. Furthermore, these designer self-assembling peptide scaffolds have recently become powerful tools for regenerative medicine to repair nervous tissue, to stop bleeding in seconds, to repair infarctuated myocardia, as well as being useful medical devices for slow drug release.



Introduction

The advancement of biology often requires the development of new materials, methods, and tools that can in turn significantly accelerate scientific discoveries. The introduction of the Petri dish over 100 years ago provided an indispensable tool for culturing cells *in vitro*, thus permitted detailed dissection of seemingly intractable biological and physiological systems into manageable units and well-defined studies. This simple dish has had a profound impact on our understanding of complex biology, especially cell biology and neurobiology. However, recently developed technologies enable us to develop better *in vitro* models of various tissues and organs not only for gaining basic knowledge but also for applied medical science.

In a manner, regenerative medicine and tissue engineering require two complementary key ingredients. One of which is a biologically compatible scaffold that can be readily adopted by the body without harm, and the other are suitable cells, which include various stem cells or primary cells, that effectively replace the damaged tissues without adverse consequences. However, it would

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Fabrizio Gelain was born on November 18, 1976, in Milan, Italy. He received his degree in 2001 and his Ph.D. degree in Bioengineering from the Politechnic of Milan in 2005. During his Ph.D. research he worked under the supervision of Prof. Angelo Vescovi, co-director of the Stem Cell Research Institute–DIBIT in Milan where he studied the potential of hyaluronic acid based scaffolds and neural stem cells for regenerating spinal cord injuries and sciatic nerve transections. As a visiting Ph.D. student he joined the lab of Prof. Shuguang Zhang, associate director of the Center for Biomedical Engineering at MIT, Cambridge (MA). Under Zhang's supervision, Gelain developed new functionalized self-assembling peptides as new suitable bioinspired materials for 3D neural stem cell cultures and neural tissue engineering approaches. Since 2005 he has been a visiting scholar at Zhang's lab at MIT and a junior lecturer at the Biotechnology and Bioscience Department at the University of Milan-Bicocca. He is currently interested in developing and characterizing new functionalized self-assembling biopolymers and other nanotechnology-derived scaffolds for slow drug release and cell transplantation therapies in nervous system injuries.

Akihiro Horii received his Master of Science in Precision Machinery at the University of Tokyo in 1993. He joined Olympus Corporation in 1993 and dedicated to the development of minimally invasive diagnostic and therapy devices, especially diagnostical/surgical endoscopes using advanced optical technologies. He developed Endomicroscope (Endoscopic micro confocal scanning microscope) which enables clinical cellular observation during endoscopy in 2002. In 2004, he joined the research to study the self-assembling peptide scaffold for bone regeneration at Center for Biomedical Engineering, Massachusetts Institute of Technology as a visiting scientist. He holds 7 US granted patents, 2 US patent applications and 36 Japanese patent applications.

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be advantageous if one could apply suitable and active biological scaffolds to stimulate and promote cell differentiation, in addition to regenerating tissues without introducing foreign cells. The field is undergoing a rapid growth and it is impossible to cover it in a comprehensive manner in a few pages, thus in this review, we only focus on work that concerns synthetic designer self-assembling peptide scaffolds developed in our lab and by our colleagues since 1992. Readers interested in other aspects of advances in biomaterials can consult the other articles in this special issue and in the broader literature.

2-D or not 2-D

Although the Petri dish has had an enormous impact on modern biology, the Petri dish culture system, including multi-well plates, glass cover slips, etc, is less than ideal for the study of tissue cells for several reasons: i) It is a two-dimensional (2-D) system which is in sharp contrast to the three-dimensional (3-D) environment of natural tissues both in animals and plants. ii) The uncoated surface of a Petri dish is rigid and inert, again in sharp contrast to the *in vivo* environment where cells intimately interact with proteins located in the extracellular matrix or on other cell membranes, and can modify their surrounding adaptive microenvironment. iii) cell monolayers adhering and migrating on a 2-D coated surface, such as poly(L-lysine), collagen gels, fibronectin, laminin, and Matrigel[1] as well as other synthetic materials that contain segments of adhesion motifs, have only part of the cell surface attached to the materials and interact with neighboring cells. The remaining parts are often directly exposed to the culture media, unlike the tissue environment where every cell intimately interacts with its neighboring cells and extracellular matrix. Thus 3-D-matrix interactions display enhanced cell biological activities and narrowed integrin usage.[2] iv) The transport phenomena in 2-D and 3-D are drastically different. In 2-D culture systems, cytokines, chemokines, and growth factors quickly diffuse in the media across the culture dish. This is again in sharp contrast to the *in vivo* environment where chemical and biological gradient diffusion systems play a vital role in signal transduction, cell–cell communications and development.

In a landmark paper, Discher and colleagues[3] provide the first convincing evidence that a matrix can specify the lineage of mesenchimal stem cells (MSCs). Depending on the matrix physical stiffness the MSCs can differentiate into neurons, myoblasts, and osteoblasts. By demonstrating the crucial importance of the microenvironment's properties, their work suggests new intriguing possibilities offered by a 3-D approach.

The time has come to address the 3-D question because quantitative biology requires *in vitro* culture systems that more authentically represent the cellular

microenvironment in living organisms. In doing so, *in vitro* experimentation can become truly more predictive of *in vivo* systems than it has been so far.

The Ideal Biological Scaffolds

There are a number of strategies to fabricate biomaterials. However, the ideal biological scaffolds should meet several requirements. 1) The building blocks should be derived from biological sources. 2) Basic units should be amenable to design and modification to achieve specific needs. 3) They should exhibit a controlled rate of material biodegradation, 4) exhibit no cytotoxicity, 5) promote cell–substrate interactions, 6) elicit no or little immune responses and inflammation, 7) afford economically scaleable and reproducible material production, purification, and processing, 8) be readily transportable; 9) be chemically compatible with aqueous solutions and physiological conditions, and 10) integrate with other materials and in the body.

Current Widely Used Scaffolds

In the last three decades, several synthetic polymers, such as poly(D,L-lactide) (PLLA), poly[lactic-*co*-(glycolic acid)] (PLGA), and other biomaterials including alginate, collagen gels, and others, have been developed to culture cells in 3-D.[4–6] These culture systems have significantly advanced our understanding of cell–material interactions and fostered a new field of tissue engineering. However, high porosity scaffolds comprising these biomaterials are often made of microfibers with diameter of ≈5–50 μm or micro-pores of 10–50 μm. Since the size of most cells (≈5–10 μm) are similar to or smaller than these microstructures (≈10–100 μm), upon attachment the cells still exhibit a 2-D topography with a curvature depending respectively on the microfiber diameters or on the pore size. In order to culture cells in a truly 3-D microenvironment, these dimensions must be significantly smaller than cells so that the cells are fully surrounded by the scaffolds, much like the extracellular environment.

Biomaterials are often functionalized to promote desired biological activities through chemical reactions or coating. Because of their micrometer-scale sizes, their mechanical strength usually prevents further material structural adaptations from the forces exerted by the cytoskeleton of cells during their adhesion, migration, and maturation processes. Thus, although these microfibers provide an artificial extracellular environment, they are still far from the natural nanometer-scale extracellular matrix (ECM). In recent years a well-established technique, electrospinning, has been adopted to spin nanofibers of these same materials,[7,8] however, the harshness of the overall process and the presence of harmful chemical solvent prevent any possible addition of cells while the scaffold is forming. Attempts have been made to seed scaffolds under dynamic conditions or just on their surfaces, but even if a considerable cell migration occurred, a uniform and correct seeding of cells in real 3-D matrix is still an hurdle that has to be overcome.

For the encapsulation of labile bioactive substances and living cells, physically cross-linked nanofiber scaffolds are of great interest, especially if the scaffold formation occurs under mild physiological conditions without any organic solvent processes. There is a need for process scaffolds in an aqueous environment with a desired pH range, temperature, or specific catalyst that could control their microstructure properties.

Self-Assembling Peptide Scaffolds

The self-assembling peptide scaffold belongs to a class of biologically inspired materials. The first member of the family, EAK16-II (AEAEAKAKAEAEAKAK), was discovered from a segment in a yeast protein, Zuotin.[9] The scaffolds consist of alternating amino acids that contain 50% charged residues.[9,10] These peptides are characterized by their periodic repetition of alternating ionic hydrophilic and hydrophobic amino acids that spontaneously form β-sheet structures. These β-sheets have distinct polar and non-polar surfaces. They are isobuoyant in aqueous solution and readily transportable to different environments. Upon exposure to aqueous solutions with neutral pH, ions screen the charged peptide residues and alanines (which form the non-polar surfaces of the β-sheets) of different β-sheets pack together as a result of their hydrophobic interactions in water. This gives rise to double-layered β-sheet nanofibers, a structure that is found in silk fibroin from silkworm and spiders. Thus the final self-assembly step to create the peptide scaffold takes place under physiological conditions. Individual fibers are ≈10 nm in diameter. On the charged sides, both positive and negative charges are packed together through intermolecular ionic interactions in a checkerboard-like manner. In general, these self-assembling peptides form stable β-sheet structures in water, which are stable across a broad range of temperature, and pH, in high concentration of denaturing agent, urea, and guanidium hydrochloride. The nanofiber density correlates with the concentration of peptide solution and the nanofibers retain an extremely high hydration, >99% in water (5–10 $mg \cdot mL^{-1}$). A number of additional self-assembling peptides including RADA16-I (AcN- RADARADARADARADA-CNH_2) and RADA16-II (AcN-RARADADARARADADA-CNH_2), in which arginine and

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aspartate residues substitute lysine and glutamate, have been designed and characterized for salt-facilitated nanofiber scaffold formation.

The peptide synthesis method uses conventional F-moc mature solid-phase peptide synthesis chemistry. Depending on the length of the motifs, high purity peptides can be produced at a reasonable cost. Since the cost of the peptide synthesis has decreased steadily in the last few years, it has become affordable for most people.

Many scaffold-forming self-assembling peptides have been reported, and the numbers are still growing.[11–12] The formation of the scaffold and its mechanical properties are influenced by several factors, two of which are the level of hydrophobicity[13–18] and length of peptide sequence. As such, in addition to the ionic complementary interactions, the extent of the hydrophobic residues, i.e., Ala, Val, Ile, Leu, Tyr, Phe, Trp (or single letter code, A, V, I, L, Y, P, W), and consequently the number of repeats of the self-assembling motif, can significantly influence the mechanical properties of the scaffolds and the speed of their self-assembly. The higher the hydrophobic residue content and the longer the length of the peptide sequence, the easier it is for scaffold formation and the better the mechanical properties.[13,14,17,19]

In Vitro 3-D Cell Cultures

These new self-assembling peptide biological scaffolds have become increasingly important not only in the study of spatial behaviors of cells, but also in developing approaches for a wide range of innovative medical technologies, which include regenerative medicine. One example is in the use of the peptide scaffolds to support neurite growth and maturation,[20] neural stem cell differentiation, cardiac myocytes, and bone and cartilage cell cultures. The peptide scaffolds from RADA16-I and RADA16-II form nanofiber scaffolds in physiological solutions that stimulated extensive rat neurite outgrowth, and active synapse formation on the peptide surface was successfully achieved.[20]

A method to encapsulate chondrocytes within peptide scaffolds was developed using another self-assembling peptide KLD12 (AcN-KLDLKLDLKLDL-CNH_2) for cartilage repair purposes.[16] During four weeks of culture *in vitro*, chondrocytes seeded within the peptide scaffold developed a cartilage-like extracellular matrix (ECM) rich in proteoglycans and type II collagen indicative of a stable chondrocyte phenotype. Time dependent accumulation of this ECM was paralleled by increases in material stiffness indicative of deposition of mechanically functional tissue. The content of viable differentiated chondrocytes within the peptide scaffold increased at a rate that was four-fold higher than that in a parallel chondrocyte-seeded agarose culture, a well-defined reference chondrocyte culture system. These results demonstrate the potential of a self-assembling peptide scaffold as a scaffold for the synthesis and accumulation of a true cartilage-like ECM in a 3-D cell culture for cartilage tissue repair. Then the designer peptide scaffolds for additional explorations of other tissue types has become increasingly and widely accepted.

Designer Scaffolds for Regenerative Medicine

Ellis-Behnke and colleagues showed that self-assembling peptide material is a promising scaffold for neural regeneration medicine.[21] *In vivo* application to brain wounds was carried out using postnatal day-2 Syrian hamster pups. The optic tract within the superior colliculus (SC) was completely severed with a deep knife wound, extending at least 1 mm below the surface. At surgery, 10 animals were treated by injection into the wound of 10–30 μL of 1% RADA16/99% water. Control animals with the same brain lesion included three with isotonic saline injection (10 μL), and numerous additional cases, including 10 in which the dye Congo red was added into the peptide scaffold, and 27 earlier animals with knife cuts and no injection which survived 6–9 d. Animals were sacrificed at 1, 3, 6, 30, and 60 d for brain examinations. Histological specimen examinations revealed that only in the peptide scaffold-injected animals, but not in untreated 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, reinnervating the SC caudal to the lesion. Most importantly, functional tests proved a significant restoration of visual function in all peptide scaffold-treated animals.

In another work published by Lee's group, embryonic stem cells were suspended in RADA16-II peptide scaffold solutions and injected in the myocardium of 10-week old mice.[22] In that study it has been demonstrated that self-assembling peptides can be injected into the myocardium to create a 3-D microenvironment. After 7, 14, and 28 d these microenvironments recruit both endogenous endothelial and smooth muscle cells, and exogenously injected cells survive in the microenvironments: self-assembling peptides can thus create injectable microenvironments that promote vascularization.

In addition, Lee's group also developed an appealing drug-delivery strategy by using a biotinylated version of RADA-II to demonstrate a slow release of IGF-1 in infarctuated rat myocardia.[23] 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 trans-

plantation the strategy showed that cell therapy with IGF-1 delivery by biotinylated nanofibers significantly improved systolic function after experimental myocardial infarction.

Remarkably, since the building blocks of this class of designer peptide scaffolds are made of pure natural L-amino acids, RADA16, unlike most of the other synthetic microfibers, has been shown not to elicit detectable immune response, nor inflammatory reactions in animals,[21–23] and the degraded natural amino acid products can be reused by the body. Therefore, this class of scaffold may be useful as a bio-reabsorbable scaffold for tissue repair and neuro-engineering to alleviate and treat a number of trauma and neuro-degeneration diseases as well other tissue injures, damage, and ageing.

Alternative strategies are under evaluation to address the directionality and alignment of these scaffolds, for example, the nanofibers in the scaffolds do not have a predetermined porosity, pore orientation, and a predetermined 3-D oriented architecture, by using microfluidic or magnetic approaches to drive the self-assembling process.

Designer Self-Assembling Peptides

In a recent work,[24] we directly and systematically compared neural stem cell adhesion and differentiation on self-assembling RADA16-I scaffolds with other natural-based substrates including laminin, collagen I, fibronectin, and some of the most commonly used synthetic biomaterials in tissue engineering such as PLLA, PLGA, and poly(caprolactone). While natural derived substrates showed the best performances, the RADA16-I scaffold coaxed neural stem cell differentiation and survival to a similar degree as the other synthetic biomaterials.

Although self-assembling peptides are promising scaffolds, they show no specific cell interaction because their sequences are not naturally found in living systems. The next logical step is to search active and functional peptide motifs from a wealth of cell biology literature, thus the second generation of designer scaffolds will significantly enhance their interactions with cells and tissues.[25–27]

The simplest way to incorporate functional motifs is to directly synthesize them by extending the motifs on to the self-assembling peptides themselves (Figure 1).[28] The functional motifs are on the C-termini since peptide synthesis starts from the C-termini to avoid deletion of the functional motifs during synthesis. Usually a spacer comprising two glycine residues is added to guarantee a flexible and correct exposure of the motifs to cell surface receptors. Different functional motifs in various ratios can be incorporated in the same scaffold. Upon exposure to solution with neutral pH the functionalized sequences self-assemble to leave the added motifs flagging on both sides of each nanofiber. Nanofibers that take part in the overall scaffold thus give microenvironments functionalized with specific biological stimuli (Figure 1).

The self-assembling peptide scaffolds with functional motifs can be commercially produced with a reasonable cost. Thus, this method can be readily adopted for wide-spread uses, which include investigations into how cells interact with their local- and micro-environments, cell migrations in 3-D, tumor and cancer cell interactions with normal cells, cell processes and neurite extensions, cell-based drug test assays, and other diverse applications.

We have produced different designer peptides from a variety of functional motifs with different lengths.[28] We have shown that the addition of motifs (up to 12 additional residues) to the self-assembling peptide RADA16-I does not inhibit its self-assembling properties and nanofiber formation. Although the nanofiber structures appeared to be indistinguishable from the RADA16-I scaffold

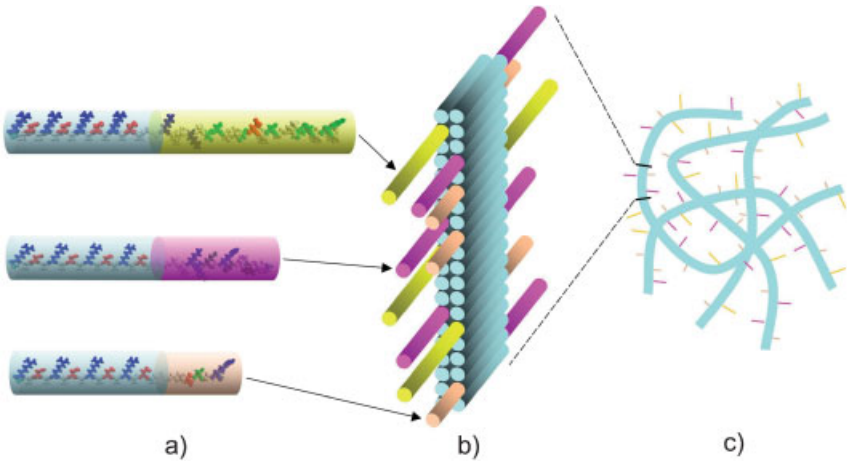

Figure 1. Schematic illustration of a designer self-assembling peptide scaffold. a) Direct extension of the self-assembling peptide sequence by adding different functional motifs. The alternating sequence of basic (blue), hydrophobic (white), and acid (red) residues is responsible for the self-assembling process. Additional motifs can be added by choosing various sequences including polar (green) residues. Light turquoise cylinders represent the self-assembling backbone and the yellow, pink, and tan lines represent various functional peptide motifs. b) Schematic representation of a self-assembling peptide nanofiber with functional motifs flagging from both sides of the double β-sheet nanofibers. Either mono or multiple functional (or labeled) peptide motifs can be mixed at the same time. The density of these motifs can be easily adjusted by simply mixing them in various ratios, 1:1 to 1:1 000 000 or more before the assembling step. c) They will then be part of the self-assembled scaffold.

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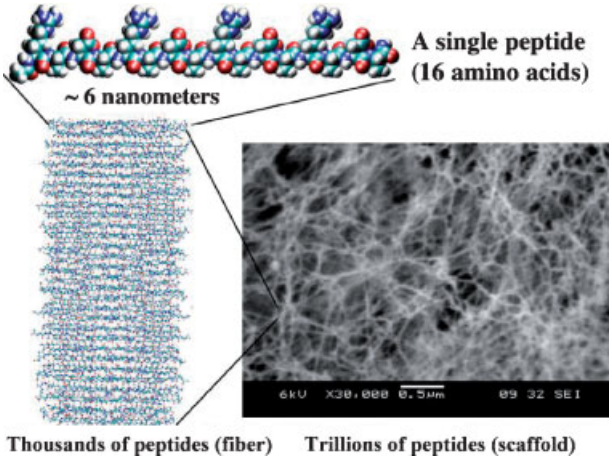

Figure 2. A designer self-assembling peptide nanofiber scaffold. A single peptide, ≈6 nanometers, is shown. Thousands of peptides self-assemble to form a single nanofiber, trillions of peptides or billions of nanofibers form the scaffold, which contains ≈99.5% water and 0.5% peptide materials. Positive and negative charges are labeled in blue and in red, respectively.

(Figure 2), the appended functional motifs significantly influenced cell behaviors.[28–31]

Using the designer self-assembling peptide nanofiber system, every ingredient of the scaffold can be defined and combined with various functionalities including the soluble factors. This is in sharp contrast to a 2-D Petri dish where cells attach and spread only on the surface. Instead cells reside in a 3-D environment where the extracellular matrix receptors on the cell membranes can bind to the functional ligands appended to the peptide scaffolds (Figure 3). It is possible that higher tissue architectures with multiple cell types, rather than monolayers, could be constructed using these designer 3-D self-assembling peptide nanofiber scaffolds.

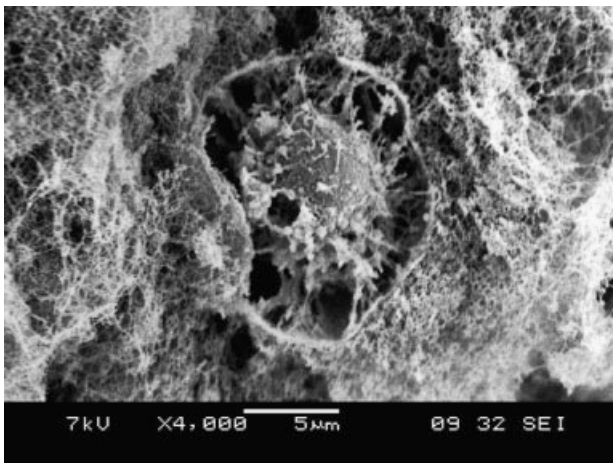

Figure 3. Scanning electron microscopy image (SEM) of a sectioned cell embedded in a 3-D self-assembled scaffold. The cell nucleus and cytoskeleton are visible. The scaffold completely wraps the cell body and membrane thus bringing the chosen functional motifs all over the cell membrane.

Even if only a fraction of functionalized motifs on the 3-D scaffold are available for cell receptor binding, the cells are likely to receive more external stimuli than when in contact with a coated 2-D Petri dish or arg-gly-asp (RGD)- or other motif-coated polymer fibers on a micrometer-scale, which are substantially larger than the cell surface receptors and in most cases, larger than the cells themselves. The cells are not in a real 3-D environment. Rather, they are in 2-D environment with wrapping around of the polymers with a curvature that depends on the diameter of the polymers. In a 2-D environment, where only one side of the cell body is in direct contact with the surface, receptor clustering at the attachment site may be induced. On the other hand, the receptors for growth factors, cytokines, nutrients, and signals are on the other sides that are directly exposed to the culture media. Thus cells may become partially polarized. In a 3-D environment, the functional motifs on the nanofiber scaffold surround the whole cell body in all dimensions and the factors may form a gradient in the 3-D nanoporous microenvironment.

Designer Peptide Scaffolds for Cell Differentiation and Migration

Designer self-assembling peptide nanofiber scaffolds have been shown to be excellent biological materials for 3-D cell cultures, and capable of stimulating cell migration into the scaffold as well repairing tissue defects in animals by adding specific cell binding and/or chemotactic sequences. We developed several peptide nanofiber scaffolds designed specifically for osteoblasts.[31] We designed one of the pure self-assembling peptide scaffolds RADA16-I through direct coupling to short biologically active motifs. The motifs included osteogenic growth peptide ALK (ALKRQGRTLYGF) bone-cell secreted-signal peptide, osteopontin cell adhesion motif DGR (DGRGDSVAYG) and 2-unit RGD binding sequence PGR (PRGDSGYRGDS). We made the new peptide scaffolds by mixing the pure RADA16-I and designer-peptide solutions, and we examined the molecular integration of the mixed nanofiber scaffolds using atomic force microscopy (AFM). Compared to the pure RADA16-I scaffold, we found that these designer peptide scaffolds significantly promoted mouse pre-osteoblast MC3T3-E1 cell proliferation. Moreover, alkaline phosphatase (ALP) activity and osteocalcin secretion, which are early and late markers for osteoblastic differentiation, were also significantly increased. We demonstrated that the designer self-assembling peptide scaffolds promoted the proliferation and osteogenic differentiation of MC3T3-E1. Under identical culture

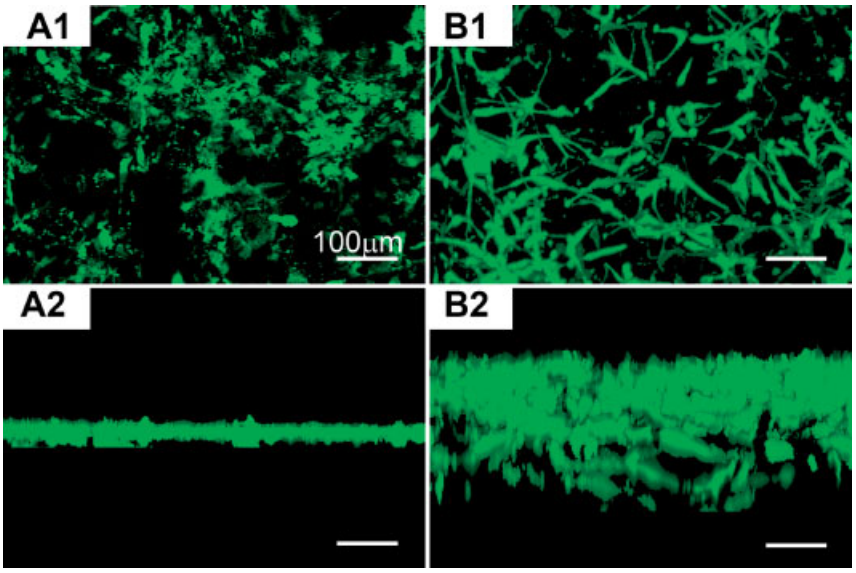

Figure 4. Reconstructed image of 3-D confocal microscope image of cells cultured on the different scaffolds consisting of a different mix ratio of RADA16-I 1% and PRG 1% using calcein-AM staining. The bar represents 100 μm. A1 and A2: PRG 10%. B1 and B2: PRG 70%. A1 and B1 are vertical views and A2 and B2 are horizontal views. In the case of the 10% PRG scaffold, the cells were attached on the surface of the scaffold whereas the cells migrated into the scaffold in the case of the 70% PRG scaffold. There is a drastic cell migration into the scaffold with a higher concentration of PRG motif. The scale bar in each panel is 100 μm.

medium conditions, confocal images unequivocally demonstrate that the designer PRG peptide scaffold stimulated cell migration into the 3-D scaffold (Figure 4).[31] Our results suggest that these designer peptide scaffolds may be very useful for promoting bone tissue regeneration.

In addition to laminin-derived self-assembling peptides previously studied by adding a long alkyl chain that promotes self-assembly,[29] another study evaluates common fibronectin and collagen-derived sequences as well.[30]

In our search for additional functional motifs, we found that a class of bone marrow homing peptides (BMHPs)[30] could be one of the most promising active motifs for stimulating neural stem cell adhesion and differentiation (Figure 5). This work opens the door to intriguing approaches that adopt functionalized self-assembling scaffolds and neural stem cells to regenerate brain ischemic focal injuries and spinal cord.

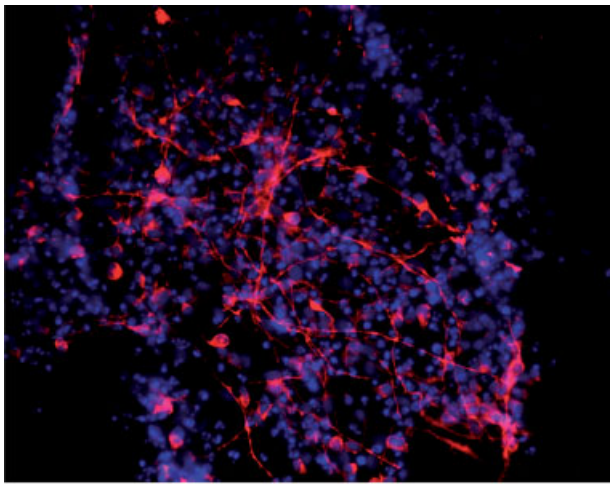

Figure 5. Human NSCs cultured for four weeks on RADA16+BMPH1 with staining for cell nuclei (DAPI in blue) and neurons (MAP2 in red). In long-term cell cultures, the self-assembling scaffold is particularly favorable to neuronal differentiation, maturation, and survival.

Why Designer Self-Assembling Peptide Scaffolds?

One may ask why one should choose designer self-assembling peptide scaffolds while there are a large number of biomaterials on the market, some of which have already been approved by FDA. There are several advantages of using the designer peptide nanofiber scaffolds. 1) Designer peptides can be readily modified at the single amino acid level at will, inexpensively and quickly. 2) Unlike Matrigel, which contains unknown ingredients and quality that varies from batch to batch, the designer self-assembling peptide scaffolds belong to a class of synthetic biological scaffolds that contain pure components and every ingredient is completely defined. 3) Because these designer peptide scaffolds are pure with known motifs, they can be used to study controlled gene expression or cell signaling processes. Thus these new designer nanofiber scaffolds have proven to be promising tools to study cell signal pathways in a selective way not possible with any substrates including Matrigel and collagen gels, which result in confusing cell signaling activation. 4) The initiation of the self-assembly process is through change of ionic strength under the physiological conditions without temperature influence. This is again unlike collagen gels, for which the gelation is through change of temperature that can sometimes induce unknown biological processes including cold or heat shocks. 5) These scaffolds provide the opportunity to incorporate a number of different functional motifs and their combinations to study cell behavior in a well-defined ECM-analogue microenvironment, not only without any chemical cross-link reactions but also with fully bio-reabsorbable scaffolds.

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The development of new biological materials, particularly of biologically inspired nanometer-scale scaffolds that mimic the *in vivo* environment and serve as permissive substrates for cell growth, differentiation, and biological function, is an active area of pursuit, which in turn could significantly advance regenerative medicine. These materials will be useful not only to further our understanding of cell biology in a 3-D environment, but also for advancing medical technology, tissue engineering, regenerative biology, and medicine.

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