Biomaterials 33 (2012) 5713–5722

Contents lists available at SciVerse ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Tubular hydrogels of circumferentially aligned nanofibers to encapsulate and orient vascular cells

Mark T. McClendon [a], Samuel I. Stupp [b,c,d,e,*]

[a] Department of Chemical and Biological Engineering, Northwestern University, Evanston, IL 60208, United States
[b] Department of Chemistry, Northwestern University, Evanston, IL 60208, United States
[c] Department of Materials Science and Engineering, Northwestern University, Evanston, IL 60208, United States
[d] Institute for BioNanotechnology in Medicine, Northwestern University, Chicago, IL 60611, United States
[e] Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, United States

ARTICLE INFO

Article history:
Received 27 March 2012
Accepted 13 April 2012
Available online 14 May 2012

Keywords:
Peptide amphiphile
Smooth muscle cell
Biomimetic material
Self-assembly
Arterial tissue engineering
Circumferential alignment

ABSTRACT

There is a great clinical need for tissue engineered blood vessels that could be used to replace or bypass damaged arteries. The success of such grafts will depend strongly on their ability to mimic the cellular and matrix organization found in native arteries, but currently available cell scaffolds such as electrospun fibers or hydrogels lack the ability to simultaneously encapsulate and align cells. Our laboratory has recently developed liquid crystalline solutions of peptide amphiphile nanofibers that form aligned domains at exceedingly low concentrations (<1wt%), and can be trapped as gels with macroscopic alignment using low shear rates and ionic crosslinking. We describe here the use of these systems to fabricate tubes with macroscopic circumferential alignment and demonstrate their potential as arterial cell scaffolds. The nanofibers in these tubes were circumferentially aligned by applying small amounts of shear in a custom built flow chamber prior to gelation. Small angle X-ray scattering confirmed that the direction of nanofiber alignment was the same as the direction of shear flow. We also show the encapsulation of smooth muscle cells during the fabrication process without compromising cell viability. After two days in culture the encapsulated cells oriented their long axis in the direction of nanofiber alignment thus mimicking the circumferential alignment seen in native arteries. Cell density roughly doubled after 12 days demonstrating the scaffold's ability to facilitate necessary graft maturation. Since these nanofiber gels are composed of >99% water by weight, the cells have abundant room for proliferation and remodeling. In contrast to previously reported arterial cell scaffolds, this new material can encapsulate cells and direct cellular organization without the requirement of external stimuli or gel compaction.

1. Introduction

Heart disease is an unsolved problem accounting for over 30% all US deaths in recent years, and it is most often caused by damaged or weakened coronary arteries [1]. In such cases the affected blood vessels can be bypassed to restore blood supply to cardiac tissue. Synthetic materials have poor patency when used to bypass small diameter blood vessels (>5 mm) and autologous grafts are in short supply [2,3]. Therefore, there is a critical need for tissue engineered blood vessels that can be used to replace damaged and blocked arteries. After the pioneering work of Weinberg and Bell [4], a significant focus of vascular engineering has been the development of methods that mimic the native microscopic organization found in arteries [5–10]. The functions of arteries are dependent upon their cellular organization, and are known to fail when this organization is not present [11,12]. The key feature of arterial microarchitecture is the alignment of smooth muscle cells (SMCs) with their long axis extending in the circumferential direction in the medial layer [13]. Vasoactivity, the constriction or dilation of blood vessels, is controlled by the contractile force produced by circumferentially aligned SMCs, and the durable mechanical properties of arteries can be attributed to the circumferential alignment of SMCs and their fibrous extracellular matrix (ECM). Therefore, it has been established that the circumferential alignment of contractile SMCs is necessary for the successful design of artificial blood vessels [10].

* Corresponding author. Department of Materials Science and Engineering, Northwestern University, 2220 Campus Dr., Evanston, IL 60208, United States. Tel.: +1 847 491 3002; fax: +1 847 491 3010.
E-mail address: s-stupp@northwestern.edu (S.I. Stupp).

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doi:10.1016/j.biomaterials.2012.04.040

One of the first and most widely researched techniques used to align SMCs within vascular grafts was first suggested by L'Heureux et al. [14] using a collagen gel (and later fibrin gel) compacted around a non-adhesive mandrel [5,15]. While this method induces significant cellular alignment, it has inherent drawbacks such as the use of natural biopolymers that are known to influence cell behavior. For example, encapsulation of SMCs within collagen gels is known to inhibit the cellular production of elastin, a vital ECM component in arteries [10,16]. Other strategies have yielded similar cellular alignment via electrospinning of biocompatible polymers [6,7,17]. Macroscopic tubes can be made with highly aligned fibers using a rotating rod as the electrospinning target. However, the extremely high shear forces and organic solvents used during electrospinning can significantly damage cells and therefore they cannot be encapsulated into materials during the fabrication procedure. Instead cells must be seeded onto the surface of these tubes post-fabrication and allowed to infiltrate as the construct degrades. The infiltration of cells lengthens the maturation time of the graft, and the polymer degradation products will often negatively affect cell behavior [18]. Regardless of material, the application of a pulsed pressure on tubular scaffolds has been shown to preferentially aligned cells in the circumferential direction [9,19]. However, problems can arise due to mechanical stimulation causing SMCs to differentiate thus reducing their ECM production and proliferation capacity. Therefore an ideal scaffold for arterial tissue engineering would template the circumferential alignment of encapsulated SMCs while also displaying a select set of bioactive cues to induce a specific cell behavior. In this context, synthetic self-assembling fibrous materials offer a promising alternative to both electrospinning and the common biopolymers used in tissue engineering.

Over the past decade Stupp and co-workers have developed a class of peptide amphiphile (PA) molecules that self-assemble into high aspect ratio supramolecular nanostructures resembling ECM fibrils [20,21]. The PA nanofibers typically have diameters of approximately 6–10 nm and can be microns in length. These PA molecules can be modified with cell signaling amino acid sequences that, after self-assembly, are displayed in high density on the surface of nanofibers [22]. Previous research has investigated these signal-displaying nanofibers and demonstrated their ability to promote processes such as cell proliferation [23], cell adhesion [24,25], angiogenesis [26], axon elongation [27,28], bone regeneration [29], and for rational delivery of growth factors for cartilage regeneration [30] and islet transplantation [31].

We have shown recently that heating and cooling of certain PA solutions leads to a process of fusion and then rupture of two-dimensional plaques into fiber bundles approximately 40 nm in diameter [32]. In that work, it was observed that solutions of these fiber bundles form lyotropic liquid crystals at exceedingly low concentrations, <1 wt %. Using the low strain forces produced by dragging PA solutions from a pipette tip by hand (1–4 s^{-1}) it was possible to align these fiber bundles over macroscopic lengths and trap them in the aligned state through crosslinking by divalent ions. The procedure of pipetting and gelling an orientationally monodomain gel was found to be gentle enough to accommodate living cells suspended in solution. Once the matrix was gelled the cells responded to the anisotropy of fiber alignment by elongating processes parallel to the fiber bundles via contact guidance.

We investigate here the fabrication of tubular gels with the liquid crystalline PA nanofiber bundles and their potential use as cell scaffolds for tissue engineered arteries. Using small angle x-ray scattering (SAXS) and birefringent microscopy we have characterized the alignment of the nanofibers in these tubes following application of a circumferential strain prior to gelling. We have also encapsulated SMCs during fabrication of the tubular structures and measure their survival and proliferation in culture. Fluorescent microscopy and Fast Fourier Transform image analysis was used to investigate the cells' orientational response to the aligned matrix. The goal of this research was to develop a cell scaffold with the ability to template the alignment of encapsulated vascular cells and thus mimic the organization of native arteries. Such a scaffold could ultimately lead to a vasoactive graft capable of replacing damaged or weakened arteries.

2. Materials and methods

2.1. Synthesis, purification, and solution preparation of peptide amphiphiles

The PA molecule used for this study, C_{16}-$V_3A_3E_3(NH_2)$, was synthesized by standard solid-phase Fmoc chemistry on a CS Bio automated peptide synthesizer. Fmoc-protected amino acids, MBHA rink amide resin and HBTU were purchased from NovaBiochem and all reagents were purchased from Mallinckrodt. The resulting product was purified using standard reversed-phase high performance liquid chromatography. The PA was dialyzed against deionized water using 500 MWCO dialysis tubing and isolated by lyophilization. The purity and accurate molar mass for each PA was verified using liquid chromatography/mass spectrometry on an electrospray ionization quadruple time-of-flight mass spectrometer.

PA solutions were prepared at 10 mg/mL or 12 mg/mL in Tris buffer and pH adjusted using 1 M NaOH to pH 7.2. These solutions were sonicated for 15 min, annealed at 80 °C for 30 min, and slowly cooled overnight to room temperature. As previously described, these processing conditions create a liquid crystalline solution of PA nanofiber bundles [28].

2.2. Shear flow chamber

Custom made glass tubes were fabricated with inside diameters of 4 mm and lengths of approximately 6 cm (see Fig. 1). The glass tubes were designed with three O-ring grooves for isolation of PA solution and stabilizing the inner rotating rod during fabrication. The glass tube was fixed to a modified 50 mL Falcon tube cap. When the stainless steel rod (3 mm OD) was inserted into the custom glass tube it formed a water-tight seal with the O-rings completely isolating the annular gap. The annular gap measured 0.5 mm with a volume of approximately 275 μL. These dimensions were chosen to mimic the approximate size of the adult coronary artery medial layer.

2.3. Tube fabrication procedure

The tubular constructs were made by sequentially shearing the PA solution then gelling through exposure to Ca^{2+} ions. The previously describe PA solution at 10 mg/mL with suspended cells when specified was injected via an 18 gauge needle into the annular gap of the shearing chamber. The sheaing chamber with the inserted steel rod was fitted onto a 50 mL falcon tube filled with 160 mM NaCl, 15 mM $CaCl_2$, and then the entire device was loaded onto a modified metal lathe (Central Machinery 7 inch × 12 inch Precision Mini Lathe). The rotating chuck gripped the inner steel rod while the glass tube was fixed to the translational stage. The modified metal lathe was set to rotate at 116 ± 5 RPMs for 10 s then the translational stage was engaged. This retracted the inner rod from the glass tube at a rate of 0.42 ± 0.02 cm/s with constant rotation. As the inner rod retracts from the glass chamber the salt solution (160 mM NaCl, 15 mM $CaCl_2$) flowed into the glass tube, illustrated in Fig. 1C, thus gelling the cell suspension in the shape of a tube. This setup ensured that the PA solution is gelled immediately after the cessation of the applied shear force. When the inner rod was completely removed from the glass tube the resulting tubular gel was carefully extracted under sterile conditions and place in culture media when encapsulating cells. This fabrication procedure can be completed in less than 1 min. Two types of tubes were prepared: one prepared with the previously described shearing process and the other with the same method but without rotational shear. The non-sheared sample was prepared by retracting the inner rod at an extremely slow rate of approximately 6 mm/min but without rotation resulting in a strain rate less than 1 s^{-1}. These control samples are referred to as "non-aligned", and samples prepared at the normal rotation of 116 RPM are referred to as "aligned."

2.4. X-ray diffraction

SAXS measurements were performed using beam line 5ID-D, in the DuPont-Northwestern-Dow Collaborative Access team (DND-CAT) Synchrotron Research Center at the Advanced Photon Source, Argonne National Laboratory. An energy of 15 keV corresponding to a wavelength 0.083 nm was selected using a double-crystal monochromator. The data was collected using a CCD detector (MAR) positioned 245 cm behind the sample. The scattering intensity was recorded in the interval $0.008 < q < 0.25$ A^{-1} with an exposure time of 4 s. Samples were placed in a water-filled customized sample holder made from aluminum and mica sheets. Five 2-D SAXS images were averaged to produce one 2-D image.