Biomaterials 181 (2018) 126–139

Contents lists available at ScienceDirect

Biomaterials

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

Micropatterned cell sheets as structural building blocks for biomimetic vascular patches

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ARTICLE INFO

Article history:
Received 6 March 2018
Received in revised form
21 July 2018
Accepted 25 July 2018
Available online 30 July 2018

Keywords:
Cell sheet
Hydrogel
Vascular smooth muscle cell
Tensile testing
Patterning
Finite element modeling

ABSTRACT

To successfully develop a functional tissue-engineered vascular patch, recapitulating the hierarchical structure of vessel is critical to mimic mechanical properties. Here, we use a cell sheet engineering strategy with micropatterning technique to control structural organization of bovine aortic vascular smooth muscle cell (VSMC) sheets. Actin filament staining and image analysis showed clear cellular alignment of VSMC sheets cultured on patterned substrates. Viability of harvested VSMC sheets was confirmed by Live/Dead® cell viability assay after 24 and 48 h of transfer. VSMC sheets stacked to generate bilayer VSMC patches exhibited strong inter-layer bonding as shown by lap shear test. Uniaxial tensile testing of monolayer VSMC sheets and bilayer VSMC patches displayed nonlinear, anisotropic stress-stretch response similar to the biomechanical characteristic of a native arterial wall. Collagen content and structure were characterized to determine the effects of patterning and stacking on extracellular matrix of VSMC sheets. Using finite-element modeling to simulate uniaxial tensile testing of bilayer VSMC patches, we found the stress-stretch response of bilayer patterned VSMC patches under uniaxial tension to be predicted using an anisotropic hyperelastic constitutive model. Thus, our cell sheet harvesting system combined with biomechanical modeling is a promising approach to generate building blocks for tissue-engineered vascular patches with structure and mechanical behavior mimicking native tissue.

1. Introduction

A major goal in tissue engineering is to develop biomaterials that mimic the biomechanical characteristics of native tissue [1–3]. In vascular tissue engineering, a biomimetic approach is critical because mismatch of hierarchical structure and mechanical properties between native and tissue-engineered implants can cause complications negatively impacting regeneration and remodeling [4–6]. To avoid these complications, it is important to understand relationships between the complex structure and mechanical properties in the context of vessel function [7,8]. Vascular smooth muscle cells (VSMCs) are highly specialized cells with primary function of contraction and blood flow regulation [9]. In a native blood vessel, VSMCs align circumferentially to form the medial layer of arteries and provide structural support, contractility, and vessel elasticity [10]. In addition, the medial layer is composed of multilayers of highly ordered VSMCs and extracellular matrix (ECM) that are arranged in distinct helical configurations [11–13]. Several reports have shown correlations between structural organization of cells and ECM and function of vascular constructs, i.e. proteins such as fibronectin, elastin, and collagen play an important role in controlling structural integrity [14–16]. Aligned VSMCs in the medial layer play an important role to maintain vascular tone and actively regulate blood pressure through contraction/relaxation and change in arterial diameter, which together influence mechanical properties of the arterial wall [17].

The mechanics of native arterial wall are known to have nonlinear and anisotropic stress-strain behavior [18]. While the arterial wall is compliant in the low range of strain, collagen gradually begins to bear the load as strain increases, which results

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https://doi.org/10.1016/j.biomaterials.2018.07.047
0142-9612/

in the arterial wall stiffening (nonlinearity) [19,20]. The uniaxial tensile stress-stretch responses show that the mechanical response of the arterial medial layer in a circumferential direction tends to be stiffer than in a longitudinal direction (anisotropy) [21]. There is a strong relationship between the cells' complex helical arrangement and ECM around the circumference of the vessel; each elastic lamellae in the medial layer alternates with a layer of smooth muscle cells, collagen fibers and together, collectively organizing into a lamellar unit considered to be the functional unit of the vessel wall [22,23]. Therefore, it is clear that developing tissues with defined structure that mimics that of a native vessel is key for successful tissue-engineered vascular grafts [24]. Further, we have reported previously that characterization of the mechanical properties of VSMC layers combined with computational modeling could be a valuable strategy to provide insight and to predict the mechanical properties of engineered blood vessels, which would be an invaluable tool set to help prioritize the overwhelming number of design options [25].

Bottom-up tissue engineering approaches aim to create biomimetic engineered tissue by recreating the microstructural features of tissues, while traditional top-down approaches, in which cells are seeded on polymeric scaffold, often have difficulty to control the tissue microstructure [26,27]. As one of bottom-up approach, cell sheet engineering has progressed rapidly in the past decade and has emerged as a novel approach for scaffold-free, cell-based therapy [28]. Cell sheet technology, based on cells producing their own ECM [29], allows for viable, transplantable cell sheets for various tissue engineering applications [30,31]. It also involves building three dimensional layer-by-layer from monolayer cell sheets composed of cells and their ECM [32,33]. To recreate the microstructural features of tissues, micropatterning technology via photolithography have been combined with cell sheet engineering strategy using microgroove textured elastomeric substrates to provide topological cues [34–36], using elastomeric microcontact pattern printing to print the cell adhesive pattern on the substrate surface [37], or using cyclic mechanical stretching to guide cellular alignment [38]. Previous work from us and others has showed micropatterned substrate-guided cellular alignment and improved aspect ratio of VSMCs [22,39], as well as nonlinear and anisotropic mechanical behavior in aligned VSMC sheets [24,40]. However, in a bottom-up approach, bridging the gap between individual aligned cell sheets to tissue-like three dimensional multi-layered patches has yet to be characterized.

To achieve non-invasive release of intact cell sheets from underlying substrates, various stimuli systems have been reported, such as temperature, enzyme, electricity, and magnetic field [30,41,42]. Here, we use enzymatically degradable cellulose- or alginate-based hydrogels: these substrates are conjugated with tyramine in order to enable crosslinking between chains via phenol moieties using Horseradish peroxidase (HRP) [43–46]. Cellular adhesion and proliferation of fibroblasts first were confirmed on hydrogel substrates with varying phenol hydroxyl group content [47], peroxidase or H_2O_2 concentration [48], and a simple feasibility test of tyramine-conjugated cellulose for cell sheet applications has been reported [49]. Recently, we have further developed and optimized this system to generate multi-layer cell sheets [50,51]. Briefly, tyramine-conjugated alginate (Alty) or carboxymethylcellulose (CMCty) act as sacrificial substrates for cell sheet-based harvest and transfer system and do not compromise mammalian cells or ECM during the enzymatic degradation process of the underlying hydrogel substrate.

In this study, we fabricated aligned VSMC sheets to mimic the structure of cells in the native arterial medial layer. A surface-patterned degradable hydrogel substrate was used to produce patterned VSMC sheets that were subsequently stacked in alternating angles to make patches that mimic the multilayer structure of the medial layer in an artery. First, we analyzed the effect of surface patterning on the monolayer VSMC sheet cellular alignment. The morphology and cellular structure of VSMC sheets grown on patterned and nonpatterned hydrogel were observed, and their contractile marker expression was compared. After the VSMC sheets were harvested from the substrate, their cellular structure and viability were characterized. The mechanical properties of the harvested VSMC sheets were also characterized to determine the effect of patterning on mechanical non-linearity and anisotropy, which are known characteristics of native blood vessel walls. We also developed a strategy to stack VSMC sheets into bilayer VSMC patches without damaging their structure and confirmed strong bonding between the two layers of VSMC sheets. The structure of bilayer VSMC patches was characterized by pre-labeling each layer prior to stacking, and collagen content was characterized by staining and was quantified by standard assays. Moreover, patterned VSMC sheets were stacked in alternating angles mimicking the structure of native vessels. A finite-element model was developed to investigate the effect of geometric design parameters on the tensile mechanical response of the bilayer VSMC patches.

2. Materials and methods

2.1. Materials

2-(N-morpholino)ethanesulfonic acid (MES) was purchased from Alfa Aesar™ (Tewksbury, MA, USA); Krebs-Ringer Bicarbonate Buffer solution (Krebs) from Biotang Inc. (Lexington, MA, USA); human plasma fibronectin from MilliporeSigma (Bedford, MA, USA); and collagen type 1 rat tail from Corning Inc. (Corning, NY, USA). Low glucose Dulbecco's modified Eagle medium (DMEM), Fetal bovine serum (FBS), 0.05% trypsin-0.48 mM ethylenediaminetetraacetic acid (EDTA), 100x antibiotic-antimycotic (ABAM), 100x L-glutamine (200 mM), and phosphate-buffered saline (PBS) were purchased from Gibco BRL (Gaithersburg, MD, USA). Polydimethylsiloxane (PDMS) from Dow Corning (SYLGARD® 184 Silicone Elastomer Kit, Midland, MI). Unless otherwise specified, all other chemicals and solvents were obtained from Sigma (St. Louis, MO, USA).

2.2. Hydrogel substrate preparation

As shown in Table 1, tyramine-conjugated carboxymethyl cellulose (CMCty) and alginate (Alty) were synthesized based on the method previously reported with some modification [48,49]. Briefly, carboxymethylcellulose sodium salt (Medium viscosity) or alginate sodium salt were dissolved in MES buffer with stirring overnight, then tyramine hydrochloride was added. The next day, NHS (N-hydroxysuccinate), HOBt (1-Hydroxybenzotrizole hydrate) and EDC (N-(3-Dimethylaminopropyl)-N′-ethyl carbodiimide

Table 1
Components used to synthesize tyramine conjugated carboxymethyl cellulose (CMCty) and alginate (Alty).

	CMCty	Alty
MES (pH 6.0)	120 ml (0.05 M)	120 ml (0.1 M)
Sodium carboxymethyl cellulose	1.2 g	–
Alginate sodium salt	–	0.6 g
Tyramine hydrochloride	0.858 g	5.4 g
NHS	0.057 g	0.870 g
HOBt	0.134 g	–
EDC	0.473 g	2.899 g