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Composition of intraperitoneally implanted electrospun conduits modulates cellular elastic matrix generation

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ABSTRACT

Improving elastic matrix generation is critical to developing functional tissue engineered vascular grafts. Therefore, this study pursued a strategy to grow autologous tissue in vivo by recruiting potentially more elastogenic cells to conduits implanted within the peritoneal cavity. The goal was to determine the impacts of electrospun conduit composition and hyaluronan oligomer (HA-o) modification on the recruitment of peritoneal cells, and their phenotype and ability to synthesize elastic matrix. These responses were assessed as a function of conduit intra-peritoneal implantation time. This study showed that the blending of collagen with poly(ε-caprolactone) (PCL) promotes a faster wound healing response, as assessed by trends in expression of macrophage and smooth muscle cell (SMC) contractile markers and in matrix deposition, compared to the more chronic response for PCL alone. This result, along with the increase in elastic matrix production, demonstrates the benefits of incorporating as little as 25% w/w collagen into the conduit. In addition, PCR analysis demonstrated the challenges in differentiating between a myofibroblast and an SMC using traditional phenotypic markers. Finally, the impact of the tethered HA-o is limited within the inflammatory environment, unlike the significant response found previously in vitro. In conclusion, these results demonstrate the importance of both careful control of implanted scaffold composition and the development of appropriate delivery methods for HA-o.

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1. Introduction

Surgical options for coronary heart disease are currently limited in more than 30% of patients due to the unavailability of suitable autologous vessels for bypass grafting [1]. While tissue engineered grafts have been investigated as potential alternatives, several challenges have yet to be overcome. One challenge is the poor generation of mature elastic matrix by most adult cells [2,3]. Elastic matrix in healthy arteries enables both vessel recoil after stretch and regulation of smooth muscle cell (SMC) phenotype [4]. Mature elastic matrix within engineered vascular grafts can serve a similar role, and critically determine graft mechanics after degradation of the initial scaffold material. Strategies have been developed to improve the inherently limited cellular production of elastic matrix (i.e. elastogenesis) [5], including stimulation by tetramers of hyaluronan (HA-o), transforming growth factor β [6,7] and insulin-like growth factor 1 [8]. While these factors improved elastogenesis, and can be incorporated within grafts, long-term small-diameter graft viability can still be compromised unless more mature elastic matrix is assembled within the three-dimensional (3-D) constructs. A second challenge is the inflammatory response that engineered constructs elicit post-grafting, which must be controlled. While inflammation is necessary for wound healing and tissue remodeling, a chronic response, or one that is too severe, can compromise these processes [9]. For vascular tissues, the consequences of a negative healing response can include graft occlusion via thrombosis [10] and tissue disruption from chronic cellular overexpression of proteolytic enzymes [11].

We have sought to address the above limitations of tissue engineered grafts by recruiting a potentially more elastogenic, autologous cell population within the peritoneal cavity. Most elastin in the body is generated during development, within microenvironments rich in stem and progenitor cell populations [12,13]. In addition, it has been recently shown that cell therapy with stem cells can improve elastic matrix production [14]. Thus, we hypothesize that stem or progenitor cell populations recruited from the peritoneal fluid to implanted conduits would represent a more elastogenic cell source than terminally differentiated cells isolated from adult vascular tissues [15]. Growing tissue within the peritoneal cavity can also provide additional benefits, including avoiding the high cost and extended culture times required for in vitro manipulation of seeded cells. Finally, since the initial wound healing response to the construct may subside while still in the peritoneal cavity, this strategy also has the potential to minimize inflammation, and potential thrombosis, after subsequent arterial grafting of the constructs.

Intraperitoneal implantation of silicone tubes has been previously performed to generate constructs that contain a mixture of

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cell types (e.g. macrophages, mesothelial cells and myofibroblasts) [15–19], and exhibit short-term viability after grafting into arteries. However, limited elastic matrix production was observed within these constructs, both in rat and in dog models. Despite these findings, the recruited peritoneal cells may still exhibit enhanced elastogenicity if the microenvironmental cues (e.g. conduit composition and architecture) are carefully controlled. In fact, elastic matrix production in vitro has been shown to be significantly modulated by scaffold composition (e.g. fibrin for SMCs [20]). Most of these in vitro studies utilized terminally differentiated cells, and single-cell types, unlike the mixed cell population recruited within the peritoneal cavity. Certain sub-populations of peritoneal cells have a greater potential for differentiation and inter-cell signaling [18], but it is not known how scaffold/conduit composition would modulate tissue generation in this complex microenvironment.

In this study, we specifically determined the impact of incorporating collagen with poly(ε-caprolactone) (PCL) into electrospun conduits, and the effects of tethering elastogenic HA-o to the conduit surface, on (a) the wound healing response within the peritoneal cavity and (b) the extracellular matrix (ECM) production. Collagen was specifically selected for incorporation since it is a dominant component of vascular ECM, and since it is an enzymatically degradable structural protein with specific cell binding sequences. While collagen-rich matrices have been previously shown to influence the general wound healing process [9,21], its impact may differ when blended with a synthetic polymer and implanted within the peritoneal cavity. In this study, PCL conduits with and without incorporated collagen, and with and without immobilized HA-o, were implanted in rat peritoneal cavities for 2, 4 or 6 weeks. We determined the impact of the conduit composition on matrix accumulation and distribution, particularly the elastic matrix, and on recruited peritoneal cell phenotype and differentiation.

2. Materials and methods

2.1. Materials

All disposables, chemicals and biological supplies were obtained from VWR International (West Chester, PA) unless specified otherwise. PCL (inherent viscosity 1.0–1.3 dl g^{-1} in chloroform) was from Lactel Absorbable Polymers (Pelham, AL), and acid-solubilized collagen (derived from calf skin) was from Elastin Products Co., Inc. (Owensville, MO). All antibodies were from Abcam (Cambridge, MA), unless specified otherwise. Hyaluronan oligomers were prepared by digestion of high-molecular-weight hyaluronan (1500 kDa, Sigma–Aldrich, St Louis, MO) with testicular hyaluronidase (Sigma–Aldrich), using a method we have published previously [22]. The digestate contained primarily HA-4 mers (83.9%, MW 657 Da) with the remainder as HA-6 mers, as determined with fluorophore-assisted carbohydrate electrophoresis.

2.2. Electrospinning and surface modification of conduits

2.2.1. Electrospinning

Electrospinning was performed from a 22% w/v solution of a collagen/PCL blend (25/75% w/w) in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) to generate conduits that present cell binding sequences, and exposed primary amines that can be used for biomolecule functionalization. Electrospinning was performed using a 15 kV voltage gradient, 0.8 ml h^{-1} flow rate, 22 gauge needle and 15 cm throw distance. Control conduits containing PCL alone were electrospun from a solution of 22% w/v poly(ε-caprolactone) (PCL) in 90% v/v chloroform and 10% v/v dimethylformamide. HFIP was avoided as a solvent for the conduits containing only PCL because of its toxicity, but it was required for the blend conduits since it is a good solvent for both components. For the PCL conduits, electrospinning was performed as described above, except for the use of an 11 kV voltage gradient, which was necessary to prevent charging, and a higher 3 ml h^{-1} flow rate. Electrospinning was performed for 7–30 min for the PCL solution, and 15 min–1 h for the collagen/PCL solution, onto an aluminum rod with a 1.6 mm diameter. A longer electrospinning time was necessary for the collagen/PCL blend to compensate for the lower flow rate and generate conduits with thick walls. However, the blend conduits still had thinner walls than the PCL ones (i.e. 157 ± 72.1 and 428 ± 139 μm, respectively). As described previously [7], the rod was both rotated slowly (i.e. <300 rpm) and moved laterally to maintain a consistent thickness throughout the circumference of the conduit and along the length of the drum. The conduits were removed from the rod after electrospinning, with ethanol if necessary, cut into 1 cm long sections and stored in a desiccator until further use.

2.2.2. Modification with HA-o

The electrospun conduits described in Section 2.2.1 were crosslinked using a procedure adapted from Croll et al. [23]. Briefly, the conduits were initially wetted with isopropanol, and then washed with a zero length crosslinking solution: 200 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC; Thermo Scientific, Rockford, IL), 100 mM *N*-hydroxysuccinimide (NHS; Thermo Scientific) and 10 mM trisodium citrate (pH 5.0, Sigma). The conduits were then reacted for 2 h with crosslinking solutions containing either 2.5 mg ml^{-1} of HA-o, or no HA-o (negative control). Afterward, the conduits were washed in phosphate buffered saline (PBS) then deionized water (two times, 5 min each) prior to lyophilization. The mass and length of the dry conduits were measured prior to implantation.

2.3. Characterization of electrospun conduits

2.3.1. Size and alignment of electrospun fibers

Conduit wall thickness was determined from bright-field images of cross-sections of the conduits obtained using an Olympus IX51 microscope (Olympus Corp., Center Valley, PA). The average diameters and degree of orientation of the electrospun fibers were determined from scanning electron microscopy (SEM) images. For SEM, the electrospun conduits were mounted onto aluminum stubs with their outer surface exposed, sputter-coated and then imaged in a JEOL JSM 5310 (Peabody, MA) with a working distance of 10 mm and operating at 15 kV. Analysis of the SEM and light microscopy images was performed with ImagePro Plus® software (Media Cybernetics, Bethesda, MD). The degree of orientation was characterized by angular standard deviation (ASD), where a reduced angular standard deviation is indicative of a conduit containing more aligned fibers [24].

2.3.2. Confirmation of collagen incorporation and HA-o immobilization

The presence of collagen within the blend conduits was confirmed with energy-dispersive X-ray spectroscopy (EDS). Samples were sputter-coated, mounted in a FEI Quanta 200 3D SEM (Hillsboro, OR) and elemental analysis was performed with an EDS detector. The accelerating voltage was maintained at 15 kV and the spot size and time constant were kept constant. The presence of collagen was determined by an N1s peak in EDS spectra and in elemental maps produced in NSS 3® software (Thermo Scientific). For the detection of HA (i.e. HA-6 mers or crosslinked HA-4 mers), the electrospun meshes were labeled first with biotinylated HA binding protein (HABP; bovine nasal cartilage, EMD Chemicals Inc., San Diego, CA), then reacted with a streptavidin conjugated Alexa 633 probe (Invitrogen, Grand Island, NY) and finally visualized with an Olympus IX51 microscope.

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