Acta Biomaterialia xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Mechanical properties of endothelialized fibroblast-derived vascular scaffolds stimulated in a bioreactor

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ARTICLE INFO

Article history:
Received 23 September 2014
Received in revised form 22 December 2014
Accepted 28 February 2015
Available online xxxx

Keywords:
Vascular grafts
Tissue engineering
Bioreactor
Endothelialization
Mechanical properties

ABSTRACT

There is an ongoing clinical need for tissue-engineered small-diameter (<6 mm) vascular grafts since clinical applications are restricted by the limited availability of autologous living grafts or the lack of suitability of synthetic grafts. The present study uses our self-assembly approach to produce a fibroblast-derived decellularized vascular scaffold that can then be available off-the-shelf. Briefly, scaffolds were produced using human dermal fibroblasts sheets rolled around a mandrel, maintained in culture to allow for the formation of cohesive and three-dimensional tubular constructs, and then decellularized by immersion in deionized water. Constructs were then endothelialized and perfused for 1 week in an appropriate bioreactor. Mechanical testing results showed that the decellularization process did not influence the resistance of the tissue and an increase in ultimate tensile strength was observed following the perfusion of the construct in the bioreactor. These fibroblast-derived vascular scaffolds could be stored and later used to deliver readily implantable grafts within 4 weeks including an autologous endothelial cell isolation and seeding process. This technology could greatly accelerate the clinical availability of tissue-engineered blood vessels.

1. Introduction

Cardiovascular diseases are the leading cause of death in industrialized countries. In 2010, 397,000 coronary artery bypass graft (CABG) procedures were performed in the USA [1]. The autologous saphenous vein and the internal mammary artery are considered as the gold standard biologic materials for this procedure. However, these vessels can be restricted both in quality and quantity, therefore impeding accessibility. This limited access to adequate replacement vessels is especially problematic for patients requiring repeated bypass procedures [2]. Synthetic prostheses, while very appropriate for large-diameter applications (>6 mm), have intrinsic inadequate patency rates when used as CABG or for peripheral vascular repair below the knee related to their prothrombotic characteristics [3,4]. Since the pioneering but incomplete work of Weinberg and Bell [5], many tissue-engineering approaches have been developed to solve the clinical need for small-diameter (<6 mm) vascular grafts [6–10]. However, most of the proposed solutions have displayed subphysiological mechanical properties and low patency results both *in vitro* and *in vivo*.

Our laboratory has developed an entirely biological tissue-engineered blood vessel (TEBV), completely devoid of any synthetic or exogenous material, using our self-assembly approach [6]. This methodology relies on the capability of cells to produce extracellular matrix (ECM) components when cultured in the presence of ascorbic acid and other appropriate growth conditions [11,12]. The resulting cell sheets can be comprised of human cells such as fibroblasts or smooth muscle cells and their ECM, and can be rolled around a mandrel to generate cohesive cylindrical structures [6,13]. Endothelial cells can then be seeded in the lumen to create an endothelium [6]. However, the production of such constructs can take from 6 weeks [14] to as long as 28 weeks, depending upon the required thickness and the cell types used [13]. Recent advances from our group combining the use of fibroblast-derived ECM scaffolds and smooth muscle cells reduced the production time of this specific tissue-engineered substitute, following smooth muscle cell seeding, from 6 weeks to 4 weeks [14].

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http://dx.doi.org/10.1016/j.actbio.2015.02.026
1742-7061/

In order to produce TEBV using 3D scaffolds, many groups have investigated the combination of allogeneic vascular matrices derived from human cadaveric or animal tissue and vascular or mesenchymal cells, or induced pluripotent stem cells [9,15,16]. Another strategy without the use of cells involved heparin-coated decellularized porcine aortas [17]. Recently, a case study was published on the use of allogeneic fibroblast-derived grafts for hemodialysis access, revealing no evidence of an immune response by known reactive antibodies [18]. Indeed, the literature suggests that cultured dermal fibroblasts do not express major histocompatibility complex class II antigens [19] and there is clinical evidence that allogeneic fibroblast tissues are well tolerated by the immune system following implantation [20,21].

The current study presents the development of a tissue-engineered fibroblast-derived vascular scaffold (FDVS). The self-assembly methodology was used to develop a fibroblastic tubular vessel that was ultimately decellularized to obtain a scaffold. Following the decellularization process, TEBV were created by seeding endothelial cells in the lumen of the scaffolds and perfusing the construct for 1 week in a bioreactor. The mechanical and biological properties of these vessels at various stages of production were characterized.

2. Materials & methods

2.1. Dermal fibroblasts and endothelial cells isolation and culture

All protocols were approved by the institutional committee for the protection of human subjects (Comité d'Éthique de la Recherche du Centre Hospitalier Universitaire de Québec). Human dermal fibroblasts were obtained from an adult specimen after reductive breast surgery of a healthy subject as described previously [14]. Cells were grown in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen, Burlington, ON, Canada) containing 10% fetal calf serum (FCS) (Hyclone, Logan, UT, USA) and antibiotics (100 U/ml penicillin and 25 mg/ml gentamicin) under 8% CO_2 at 37 °C and media were changed 3 times a week. Endothelial cells were obtained from human umbilical veins as described elsewhere [22]. Cells were expanded using Endothelial Basal Medium-2 supplemented with the EGM-2 MV kit (Lonza, Mississauga, ON, Canada).

2.2. Production of tissue-engineered vessels

Fibroblasts (passage seven) were cultured in 500 cm^2 plates (Corning Life Sciences, Tewksbury, MA, USA) in DMEM supplemented with 10% FCS to which ascorbic acid was added at a concentration of 50 μg/ml to promote the assembly of the ECM, leading to the production of tissue sheets comprised of cells and ECM. After 3 weeks in culture, tissue sheets were carefully cut with a scalpel to 120 mm in length and 80 mm in width and rolled around 4.7 mm diameter mandrels for a total of 7 rolls. In order to allow the cohesion of the layers, the tissues were cultured for 4 weeks in DMEM supplemented with 1/4 Ham's F12 nutrient mixture (Invitrogen) and 10% FCS.

2.3. Tissue decellularization

Engineered vessels were decellularized through hypo-osmotic shock by immersion in deionized water. The scaffolds were rinsed 2 times on the first day of decellularization, then two more times throughout 1 week during which they were kept at 4 °C in deionized water. Scaffolds that were not used immediately were preserved at 4 °C in deionized water.

2.4. Endothelial cells seeding and bioreactor conditioning

The scaffolds were cannulated within a perfusion bioreactor chamber (Lumegen, Instron TGT, Norwood, MA, USA). Confluent endothelial cells were trypsinized, counted, and seeded in the scaffold lumen by injecting a 4 ml cell suspension (0.75 million cells per ml of EGM-2) through the lumen. Alternatively, controls were filled with EGM-2 without suspended cells. The bioreactor chambers were rotated at 1 rpm for 4 h at 37 °C to allow cell adhesion to the inner wall of the scaffolds.

The chambers were mounted on the bioreactor, which held up to 6 parallel chambers. The culture medium used inside the vessels under perfusion was 50% (1/2) EGM-2, 12.5% (1/8) Ham's F12 nutrients, and 37.5% (3/8) DMEM. The tissues were perfused at a rate of 40 ml/min using a peristaltic pump with a 6-roller head. The following Hagen–Poiseuille equation was used to calculate the shear stress: $\tau = 4\eta Q/\pi r^3$, where τ is the shear stress in dynes/cm^2, η is the viscosity in Poise ($P = 1\ g\ cm^{-1}\ s^{-1}$), Q is the flow in ml/s and r is the radius of the vessel in cm. Given a viscosity of 0.001 P for DMEM with 10% FBS [23], the calculated shear stress was 0.65 dynes/cm^2. The hydrostatic pressure, controlled by a restriction valve, was kept between 25 and 40 mmHg and monitor using a pressure gauge linked to a computer (Fig. 1B3). The pressure was adjusted frequently during the initial 4 h of operation and once the following morning after which no adjustment of the restriction valve was necessary for the remainder of the week. To avoid any disturbance during the bioreactor conditioning week, no medium change was performed and 1 L of medium was used to fill the tubing and the reservoir. After 1 week under perfusion, the samples were removed from the bioreactor for analysis.

2.5. Mechanical testing

2.5.1. Burst pressure, compliance and thickness

The burst strength was measured in accordance with the ANSI/AAMI/ISO 7198:1998/2001 standard which is used for the testing of synthetic vascular prostheses [24] and as described previously [25]. Briefly, 5-cm long vessels were cannulated in a custom-made chamber and pre-stressed with a longitudinal load of 30 g. Preconditioning was then performed by varying the pressure inside the vessel from 80 to 120 mmHg for 15 cycles at an average rate of 0.5 Hz. The hydrostatic pressure was then raised at an average rate of 150 mmHg/s until failure. The internal diameter of the vessel was calculated by continuously recording the external diameter using an optical micrometer (LS-7600, Keyence, Itasca, IL, USA) and using the equation $R_{ipx} = \sqrt{R_{epx}^2 - (R_{im} + t_m)^2 + R_{im}^2}$, where R_{epx} is the measured external radius at pressure x, R_{im} is the internal radius on the mandrel and t_m is the wall thickness measured on the mandrel. The compliance was calculated between 80 and 120 mmHg or 50 and 200 mmHg and was expressed as a percentage of internal diameter variation per 100 mmHg.

To measure the thickness, a high-precision optical micrometer (LS-7600, Keyence) was used to measure the external vessel and external mandrel diameters. Measurements were taken at six different locations for every sample. The average wall thickness of each vessel was then calculated by subtracting the average mandrel external diameter from the external vessel diameter and dividing the result by two.

2.5.2. Suture retention strength

Suture strength was measured in conformity with the ANSI/AAMI/ISO 7198 standard (article 8.8.4.1). A 15 mm-long segment was cannulated except for the last 2 mm. Three 5–0 prolene sutures, separated by 120 degrees, were inserted 2 mm from the end of the vessels through one wall to form a half loop. The sutures

Please cite this article in press as: Tondreau MY et al. Mechanical properties of endothelialized fibroblast-derived vascular scaffolds stimulated in a bioreactor. Acta Biomater (2015), http://dx.doi.org/10.1016/j.actbio.2015.02.026