Acta Biomaterialia xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Human adipose-derived stromal cells for the production of completely autologous self-assembled tissue-engineered vascular substitutes

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ARTICLE INFO

Article history:
Received 19 December 2014
Received in revised form 16 April 2015
Accepted 9 June 2015
Available online xxxx

Keywords:
Mechanical properties
Endothelialization
Vascular substitutes
Tissue engineering

ABSTRACT

There is a clinical need for small-diameter vascular substitutes, notably for coronary and peripheral artery bypass procedures since these surgeries are limited by the availability of grafting material. This study reports the characterization of a novel autologous tissue-engineered vascular substitute (TEVS) produced in 10 weeks exclusively from human adipose-derived stromal cells (ASC) self-assembly, and its comparison to an established model made from dermal fibroblasts (DF). Briefly, ASC and DF were cultured with ascorbate to form cell sheets subsequently rolled around a mandrel. These TEVS were further cultured as a maturation period before undergoing mechanical testing, histological analyses and endothelialization. No significant differences were measured in burst pressure, suture strength, failure load, elastic modulus and failure strain according to the cell type used to produce the TEVS. Indeed, ASC- and DF-TEVS both displayed burst pressures well above maximal physiological blood pressure. However, ASC-TEVS were 1.40-fold more compliant than DF-TEVS. The structural matrix, comprising collagens type I and III, fibronectin and elastin, was very similar in all TEVS although histological analysis showed a wavier and less dense collagen matrix in ASC-TEVS. This difference in collagen organization could explain their higher compliance. Finally, human umbilical vein endothelial cells (HUVEC) successfully formed a confluent endothelium on ASC and DF cell sheets, as well as inside ASC-TEVS. Our results demonstrated that ASC are an alternative cell source for the production of TEVS displaying good mechanical properties and appropriate endothelialization.

1. Introduction

The gold standard for coronary and peripheral bypass surgeries consists of using the patient's own saphenous veins or internal mammary arteries as grafting material. However, since a large number of patients lack adequate autologous vessels due to inherent disease or previous harvest, there is an important clinical need for small-diameter (<6 mm) vascular substitutes [1,2]. Unfortunately, current small-diameter synthetic grafts do not perform well and despite recent progress in engineering of synthetic and biological vascular substitutes, there is still no ideal alternative for patients.

Many tissue-engineered vascular substitutes (TEVS) have been developed in recent years but excessively long production time, poor mechanical resistance, thrombogenicity and immunogenicity are some of the drawbacks preventing their clinical use [3–6]. Among strategies for vascular reconstruction reviewed elsewhere [3–6], the engineering of entirely autologous vessel by the self-assembly approach has been first achieved by our group [7]. These tissue-engineered blood vessels made from dermal fibroblasts (DF), vascular smooth muscle cells (SMC) and endothelial cells (EC) comprised the three layers naturally present in native arteries, namely the adventitia, media and intima [7]. It was a significant innovation although it had limited clinical relevance because of its production time (about 28 weeks) and its SMC and EC source. Efforts were then devoted to improve the clinical applicability of the model by reducing its production time without compromising its mechanical properties and finding new cell sources [8,9].

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http://dx.doi.org/10.1016/j.actbio.2015.06.011
1742-7061/

As research continues to unveil adult stem cell potency and differentiation capabilities, mesenchymal stem cells are gaining a widespread attention in the field of regenerative medicine, including vascular tissue engineering. Adipose tissue was found to be an excellent source of adult mesenchymal stem cells for tissue engineering [10–13]. This tissue is easily obtained and the extracted stromal vascular fraction contains a large proportion of stem/stromal cells, even in elderly patients with vascular diseases [14]. Many studies have confirmed the adipose-derived stromal cells (ASC) potential for multilineage differentiation as well as their low immunogenicity [15–19]. More recently, ASC have also been investigated in the context of vascular tissue engineering [16,20–22]. Indeed, given ASC's differentiation capabilities, they were evaluated both as an EC source to form an endothelium-like monolayer in grafts lumen; or as a SMC source to seed vascular scaffolds. Among the research groups using ASC to replace the endothelium, some have seeded grafts with undifferentiated ASC and used them as is [23]; while most have differentiated ASC toward endothelial cell lineage *in vitro* before or after grafts' seeding [21,24]. Although one advantage of the use of ASC over mature EC to endothelialize vascular grafts relies on their ease of harvest and availability, the time required for differentiation and the suboptimal functionality of the resulting endothelial-like cells may counteract this advantage. On the other hand, ASC have also been used as a source of SMC for vascular tissue engineering. Various scaffolds such as decellularized saphenous veins and polyglycolic acid (PGA) meshes have been seeded with ASC previously induced toward the SMC lineage *in vitro* [22,25]. Since mature vascular SMC have limited proliferation capability and their isolation requires a vein biopsy associated with comorbidities, the use of ASC is a promising alternative.

However, an important biological property of ASC that has not been fully acknowledged in the field of vascular tissue engineering is their propensity to produce extracellular matrix (ECM) components under appropriate culture conditions. In this study, a new application of ASC in vascular tissue engineering has been investigated. Based on their similarities with fibroblasts, particularly in terms of ECM production when cultured under static conditions [10,12], we hypothesized that ASC could be used to produce a new and enhanced adventitia-like TEVS. ASC are also recognized for their important secretion of bioactive molecules, including growth factors, anti-apoptotic and proangiogenic molecules [26,27], which could potentially improve grafts patency and integration. The main objectives of this study were to demonstrate the potential of ASC to produce a completely autologous TEVS (ASC-TEVS) without any exogenous scaffolding material; to optimize the production protocol regarding the strength, the maturation time and the thickness of the TEVS; and to compare, histologically and mechanically, the ASC-TEVS to their counterparts made from DF (DF-TEVS).

2. Materials and methods

2.1. Cell isolation and culture

Human tissues were obtained after informed consent and all protocols were approved by the institutional committee for the protection of human participants (Comité d'Éthique de la Recherche du Centre Hospitalier Universitaire de Québec-Université Laval). Human DF were obtained from the dermis of adult breast skin of three donors (age 38, 18 and 21) as described previously [7]. Banked cells at passage (P) 4–5 were thawed and used at P6–7 for the engineering of substitutes. Fibroblasts were cultured in DMEM-Ham's F12 (DMEM-Ham; ratio 3:1; Invitrogen, Oakville, ON, Canada) containing 10% fetal-calf serum (FCS, HyClone, Logan, UT) and antibiotics (100 U/ml penicillin and 25 μg/ml gentamicin [Sigma]). Human umbilical vein endothelial cells (HUVEC) were obtained from an umbilical cord by enzymatic digestion with collagenase H, as described previously [28]. Banked cells at P5 were thawed and used in P7 for endothelialization experiments. HUVEC were cultured in EGM-2 MV media (Lonza, Walkersville, MD). ASC were extracted from lipoaspirates of three female donors (age 38, 46 and 35) undergoing cosmetic surgery procedures, as previously described [11]. Banked cells at P3–4 were thawed and used at P5–6 for the engineering of substitutes. For this study, ASC were cultured in the same medium as DF (DMEM-Ham; ratio 3:1; Invitrogen) containing 10% FCS (HyClone) and antibiotics (100 U/ml penicillin and 25 μg/ml gentamicin [Sigma]). All cells were grown under 8% CO_2 at 37 °C and media were changed three times a week.

2.2. Tissue-engineered vascular substitutes

TEVS were produced by the self-assembly approach, as previously described [7], using either human DF or ASC, resulting in DF-TEVS or ASC-TEVS (Fig. 1). Cells were seeded (1×10^4 cells/cm^2) in 245 mm × 245 mm tissue culture plates (Corning, Lowell, MA) and cultured in DMEM-Ham supplemented with 10% FCS, 50 μg/ml sodium L-ascorbate (Sigma) and antibiotics (Sigma). Cells were cultured for 21 days until their neosynthesized ECM proteins were sufficiently assembled to form adherent living cell sheets. These cell sheets were then separated into either four distinct 120 mm × 120 mm sheets (to produce 7 revolution-thick vessels) or two 120 mm × 240 mm sheets (resulting in 14 revolution-thick vessels). Each individual tissue sheet was gently detached from the culture flask using fine forceps, rolled onto a 4.7 mm outer diameter (OD) mandrel, and maintained in culture in DMEM-Ham supplemented with 5% bovine Fetal Clone II serum (HyClone), antibiotics, and 50 μg/ml of ascorbate. The vascular substitutes were then maintained in culture for a 35-day maturation period on the mandrel, except for those used to study different maturation time, at 37 °C in a humidified incubator containing 8% carbon dioxide. Culture medium was changed three times a week. To assess the impact of thickness and maturation time on the strength of the substitutes, 12 DF-TEVS (6 DF-TEVS-7R; 6 DF-TEVS-14R) and 12 ASC-TEVS (6 ASC-TEVS-7R; 6 ASC-TEVS-14R) were produced from one population of each cell type (DF38 and ASC38; not from the same donor). Then, for the comparative study of DF- and ASC-TEVS, eight substitutes were produced from each cell population (3 DF populations × 8 vessels = 24 DF-TEVS and 3 ASC populations × 8 substitutes = 24 ASC-TEVS for a total of 48 substitutes).

2.3. Thickness measurements

The wall-thickness of the TEVS was measured as described previously [29] using a high accuracy CCD digital micrometer (Keyence, Pointe-Claire, Quebec, Canada). Thickness measurements were performed on seven substitutes for each cell population used ($n = 7$ per population; $n = 21$ per cell type and $N = 3$ populations per cell type).

2.4. Uniaxial tensile testing

The TEVS were subjected to ring tensile testing on an Instron ElectroPuls E1000 mechanical tester (Instron Corporation, Norwood, MA) along with a ±50 N load cell (Instron Corporation) as described previously [29]. Briefly, 5-mm-long ring samples were cut from the substitutes and mounted between two hooks adapted to the mechanical tester. In order to eliminate the heterogeneity inherent to the rolling process, a 5-mm ring sample was first

Please cite this article in press as: K. Vallières et al., Human adipose-derived stromal cells for the production of completely autologous self-assembled tissue-engineered vascular substitutes, Acta Biomater. (2015), http://dx.doi.org/10.1016/j.actbio.2015.06.011