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Sequential adaptation of perfusion and transport conditions significantly improves vascular construct recellularization and biomechanics

Aurore B. Van de Walle^{1,2}, Marc C. Moore^{2,3}, Peter S. McFetridge²

¹Laboratoire Matière et Systèmes, Complexes MSC, UMR 7057, CNRS & University Paris Diderot, Paris, France

²J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, Gainesville, FL

³Stephenson School of Biomedical Engineering, Carson Engineering Center, University of Oklahoma, Norman, OK

Abstract

Recellularization of ex vivo-derived scaffolds remains a significant hurdle primarily due to the scaffolds subcellular pore size that restricts initial cell seeding to the scaffolds periphery and inhibits migration over time. With the aim to improve cell migration, repopulation, and graft mechanics, the effects of a four-step culture approach were assessed. Using an ex vivo-derived vein as a model scaffold, human smooth muscle cells were first seeded onto its ablumen (Step 1: 3 hr) and an aggressive 0–100% nutrient gradient (luminal flow under hypotensive pressure) was created to initiate cell migration across the scaffold (Step 2: Day 0 to 19). The effects of a prolonged aggressive nutrient gradient created by this single luminal flow was then compared with a dual flow (luminal and abluminal) in Step 3 (Day 20 to 30). Analyses showed that a single luminal flow maintained for 30 days resulted in a higher proportion of cells migrating across the scaffold toward the vessel lumen (nutrient source), with improved distribution. In Step 4 (Day 31 to 45), the transition from hypotensive pressure (12/8 mmHg) to normotensive (arterial-like) pressure (120/80 mmHg) was assessed. It demonstrated that recellularized scaffolds exposed to arterial pressures have increased glycosaminoglycan deposition, physiological modulus, and Young's modulus. By using this stepwise conditioning, the challenging recellularization of a vein-based scaffold and its positive remodeling toward arterial biomechanics were obtained.

Correspondence: Aurore Van de Walle, Laboratoire Matière et Systèmes, Complexes MSC, UMR 7057, CNRS & University Paris Diderot, 75205 Paris, France. aure.vandewalle@univ-paris-diderot.fr, Peter McFetridge, J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, JG-56 Biomedical Sciences Building, P.O. Box 116131, Gainesville, FL 32611-6131. pmcfetridge@bme.ufl.edu.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

Keywords

recellularization; ex vivo-derived scaffold; vascular tissue engineering; biomaterial; vascular wall; biomechanics; small diameter vascular graft

1 | INTRODUCTION

Occlusive vascular disease remains highly prevalent, and autologous transplants continue to stand as the gold standard for small diameter bypass surgery (<6 mm). With these vessels in limited supply, new or alternative strategies are a priority. Early attempts creating biological passive conduits met with limited success and efforts became focused on improving biological functionality (Pashneh-Tala, MacNeil, & Claeysens, 2016). To do so, tissue engineering approaches are based on the repopulation of a scaffold with cells and their further conditioning. When considering the cell source used, two alternatives are most commonly preferred: either using primary cells matching the tissue type or using stem cells, which can be specifically differentiated, among which lie the induced pluripotent stem cells. In the case of vascular tissue engineering, a main drawback concerning the use of primary vascular cells is their limited availability if extracted from the patient own vessels directly, and patients with advanced arterial disease are likely to have cells with reduced growth or regenerative potential (Song, Rumma, Ozaki, Edelman, & Chen, 2018). For this reason, stem cells have gained interest as being highly proliferative and more readily available. However, a chemical or mechanical process needs to be engaged in order to drive their differentiation toward the appropriate cell type, process which, despite being widely described, still remains under investigation. Another approach, adopted herein, consists in the use of primary vascular cells derived from the umbilical cord. These cells are at the interface between the mother and the child and, as such, have increased immunocompatibility, can be easily harvested, matched to the patient and, more interestingly, are already differentiated in the cell type needed (M. C. Moore, Van De Walle, Chang, Juran, & McFetridge, 2017). For the scaffold choice, among the various possible approaches, ex vivo-derived materials such as decellularized vessels have the structural and mechanical advantage of being composed of a native extracellular matrix (ECM) and possess bioactive molecules that aid tissue homeostasis and regeneration (Bejleri & Davis, 2019; Chen & Liu, 2016; M. C. Moore et al., 2017). Indeed, positive cell interactions with a structurally appropriate ECM aid and guide the regenerative response (Dahan et al., 2017; Uzarski, Van De Walle, & McFetridge, 2013; Xu et al., 2017). Clinical success of decellularized tissues has been reported in a range of applications, including trachea (Gonfiotti et al., 2014; Hamilton et al., 2015), heart valve (Dohmen, Lembcke, Holinski, Pruss, & Konertz, 2011; Tudorache et al., 2016), right ventricular outflow tract (Bibevski et al., 2017; Konertz et al., 2011) as well as vascular grafts (Olausson et al., 2014, 2012). However, in the case of vascular grafts, the development of a vascular medial layer replete with physiologically dense smooth muscle cells (SMCs), which is critical in maintaining long-term mechanical graft integrity as well as conferring hemodynamic adaptability, has proven problematic with ex vivo scaffolds (Pashneh-Tala et al., 2016). Limitations in nutrient transport across the scaffolds thick vascular wall along with the subcellular ECM pore geometry requires cells to digest and remodel the ECM in

order to migrate. This often leads to poor cell integration requiring extended culture time frames (Scarritt, Pashos, & Bunnell, 2015).

In vivo, even with the presence of immune cells that help to coordinate the remodeling process and promote cellular penetration, recellularization is also limited, particularly at the grafts' anastomoses (Lin, Hsia, Ma, Lee, & Lu, 2018). Initial studies have shown these limitations can be overcome in vitro by optimizing key culture parameters. Cell migration across the vascular media has been improved by controlling transmural chemotactic nutrient gradients (M. Moore, Moore, & McFetridge, 2013; Tosun & McFetridge, 2013). More specifically, Tosun *et al.* showed that SMC seeded on the albumen of scaffolds and then cultured with a luminal nutrient supply dispensed under hypotensive conditions (in regard to arterial pressure) significantly improved cell migration from the albumen to the lumen (Tosun & McFetridge, 2013). This aggressive nutrient gradient was favorable for early cell migration as shown by a significant scaffold recellularization; however, these low pressure conditions also proved deleterious for the bulk graft mechanics as shown by decreasing Young's modulus and burst pressure. Evidence has shown that exposure to stimuli mimicking the arterial microenvironment is critical to drive positive construct development (Lu & Kassab, 2011; Niklason et al., 1999; Zhu et al., 2011). The transition from a hypotensive to a normotensive environment thus appears as an important culture parameter in order to create constructs capable of withstanding long-term hemodynamic stresses.

We here report that via a sequential adaptation of the culture conditions, we are able to engineer a cell-dense and robust vascular wall in a reduced time frame. With an initial hypotensive conditioning and directed nutrient gradients, a rapid scaffold recellularization is driven. A transition to normotensive conditions then induces a switch from cell migration to structural remodeling of the ECM, see Figure 1 for schematic of each perfusion conditions.

2 | MATERIALS AND METHODS

2.1 | Human umbilical vein isolation

Human umbilical veins (HUVs) were collected from Shands Hospital (Gainesville, FL). Veins were isolated from surrounding umbilical tissues using an automated dissection procedure as previously described (Daniel, Abe, & McFetridge, 2005). Briefly, cords were rinsed with water to remove residual blood and cut into 100-mm long segments. Sections were then mounted onto stainless steel mandrels (6-mm OD) through the vein lumen and progressively frozen down to -80°C . After at least 24 hr, frozen cords were positioned onto a CNC lathe and machined down to a uniform wall thickness of 0.75 mm. Immediately after dissection, veins were gradually thawed to room temperature.

2.2 | HUV treatment

Dissected veins were mounted into three bioreactors connected in series and a peristaltic pump (Cole Parmer L/S Digital Standard Drive, model EW-07551-10) was used to perfuse decellularization and sterilization solutions under defined flow conditions through the luminal or abluminal void (Figure 1a). Decellularization was performed by perfusing an

ethanol, acetone, and deionized (DI) water solution (Etac; 60:20:20 ratio) through the lumen at a flow rate of 30 ml/min and pressure of 50/30 mmHg for 24 hr. Sections were then rinsed with aliquots of DI water changed at the following time intervals: 5 min, 15 min, 40 min, 1 hr, 3 hr, 12 hr, and 24 hr. Veins were perfused with deoxyribonuclease I (Sigma-Aldrich, St Louis, MO; 70 U/ml, diluted in phosphate-buffered saline [PBS]) at 37°C for 2 hr to remove nucleic acids. Sections were then rinsed in DI water and terminally sterilized by perfusion of 0.2% peracetic acid and 4% ethanol solution in DI water (Montoya & McFetridge, 2009; Uzarski et al., 2013; Van de Walle, Uzarski, & McFetridge, 2015). Scaffolds were rinsed in DI water and pH balanced in PBS (pH 7.4) for 24 hr, followed by freeze-dried for 3 hr (Millrock Technology, Kingston, NY).

2.3 | Cell culture

Primary human smooth muscle cells were isolated as previously described (Siow & Pearson, 2001) from the umbilical artery and maintained in low glucose (2 g/L) Dulbecco's modified Eagle's medium (Hyclone, Carlsbad, CA), supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin (Hyclone) with 5% CO₂ at 37°C.

2.4 | Cell seeding and experimental design

The initial setup for all conditions followed the same initial seeding and perfusion profile until Day 19 when divergent conditions were applied. Initial conditions: primary human vascular SMC (Passage 3 to 4) suspended in media were pipetted into the abluminal void of the bioreactors (5.5×10^6 cells per scaffold in average) then scaffolds were placed under orbital rotation at 1.7 RPM for 2 hr to allow for uniform repartition of the cells on the abluminal surface. After rotation, luminal media flow was initiated using a peristaltic pump (Cole Parmer L/S Digital Standard Drive, model EW-07551-10). Initial flow rate was set at 2 ml/min and progressively increased up to a maximum flow of 50 ml/min at Day 7, with a pulse wave frequency of 1 Hz. Pressure was maintained at 12/8 mmHg due to improved cell migration, as previously evaluated (Tosun & McFetridge, 2013). Between Days 20 and 30 constructs were either exposed to flow only through the luminal flow circuit (single flow [SF30]) or dual perfusion with both luminal and abluminal flow (dual flow [DF30]; Figure 1b,c). Bioreactors were harvested at Day 30 and analyzed for cell migration, proliferation, and tensile properties.

Based on experimental data showing increased physiological and Young's moduli as well as improved cell migration, only SF conditions were carried forward to assess the effect of regulating pressure for extended periods on the recellularized constructs. The SF30 culture conditions (Day 0 seeding through Day 30) were replicated with another batch of similarly processed HUVs and similarly extracted SMCs, after which, on Day 31, the flow rate was increased to 120 ml/min, and the pulse wave frequency was fixed at 1 Hz and maintained until Day 45. The pressure was then adjusted using a one-way check valve with adaptable cracking pressure (SS-4C; Swagelok, Norman, OK) and monitored downstream using a pressure transducer (Sonometrics TRX-8, Canada). Control bioreactors were maintained at low pressure (8–12 mmHg) with single luminal flow, termed single flow hypotensive (SF-Hypo45), and compared with physiological values (120/80 mmHg), termed single flow normotensive (SF-Normo45; Figure 1b,c). At Day 45, constructs were

harvested and analyzed for cell proliferation, glycosaminoglycan (GAG) content, and tensile mechanical properties.

Three constructs were harvested per condition, and each construct was sectioned in several parts for analysis as follows: two ringlets of 1 cm each for metabolic measurements, two ringlets of 1 cm each for cell number and GAG content quantification, three ringlets of 0.5 cm each for mechanical testing, and three ringlets of 0.5 cm each for histology. Upon sectioning, metabolic activity was directly assessed using the alamarBlue assay, ringlets dedicated to cell number and GAG quantification were digested in papain, histology ringlets were rinsed and embedded in OCT medium, and samples for mechanical testing were kept in media in the incubator at 37°C and 5% CO₂ until analyses were performed (for up to 3 hr at the maximum).

2.5 | Tensile testing

Samples were cut into 5-mm-wide ringlets and subjected to uniaxial tensile testing (Instron 5542, Norwood, Mass, using a 10 N load cell). Tissue specimens were preloaded to a stress of 0.01 N then elongated at a rate of 2 mm/min until physiological loads followed by 5 mm/min until failure. Mechanical properties of the samples were analyzed over two regions: a low-strain region that reflects physiologic behavior (physiologic range between 80 and 120 mmHg meaning 11–16 kPa) and the failure region. Young's modulus was calculated to quantify material stiffness based on stress/strain data from the linear region prior to material failure. Tensile modulus within the physiological range was similarly calculated. Burst pressure was extrapolated from ring tensile testing, it was calculated via the Laplace's law as previously described (Laterreur et al., 2014).

2.6 | Metabolic activity, cell number, and GAG content

Metabolic activity of each construct ringlet was determined using the alamarBlue assay (Invitrogen). Briefly, the ringlets were rinsed in PBS before being transferred to a 24-well plate. In each well, 1 ml of culture media (Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin) homogenized with 100 µl of Alamar Blue was added, and the plate was placed in the incubator for 5 hr. The absorbance of the media of each well was then measured at the wavelengths 570 and 600 nm, a well without tissue ringlet served as control, and calculations were performed as per the manufacturer's instructions.

For assessment of cell number and GAG content, samples were first digested in papain solution (125-µg/ml papain, 5-mM L-cystein, 5-mM EDTA in PBS) at 60°C for 24 hr. Quantification of double stranded DNA was then performed using PicoGreen (Invitrogen, Grand Island, NY). Briefly, solutions for the PicoGreen assay were prepared as per the manufacturer's instructions. The digested samples were centrifuged to settle any remaining particles to the bottom of the tube, and the supernatant was diluted at least 10 times in 1X TE buffer (supplied in the kit), such as the measured fluorescence in function of the concentration of each sample becomes a straight line. In a black opaque well plate, 107 µl of the buffer solution, 43 µl of sample or standard, and 150 µl of the dye solution were added per well. The samples were incubated for 5 min in the dark, and fluorescence was measured

at wavelengths of ~480 nm (excitation) and ~520 nm (emission). DNA concentrations were determined by plotting a standard curve, made of known amounts of dsDNA. As the amount of dsDNA per cell remains constant, the cell number could be deducted.

GAG content was assessed using dimethylmethylene blue (Sigma-Aldrich). The color reagent was prepared by diluting 3.04 g of glycine, 2.37 g of NaCl, and 95 ml of 0.1 M HCl in 1 L of water, adjusting the pH to 3 (the absorbance at 525 nm of this solution should be of 0.31) and by dissolving 16 mg of dimethylmethylene blue in this solution. Dilutions of chondroitin sulfates at concentrations between 0 and 25 µg/ml were used as standards (Farndale, Buttle, & Barrett, 1986; Hoemann, Sun, Chrzanowski, & Buschmann, 2002). Papain-digested samples were diluted such as obtaining values within the standard curve and measurements were performed by adding 50 µl of papain-digested sample and 200 µl of color reagent (DMMB) per well of a 96-well plate with transparent bottom. Absorbance was immediately read at 525 nm.

2.7 | Histological analysis

Right after harvest, samples were embedded in OCT media and frozen down to -80°C. Samples were then sectioned using a cryostat to 10-µm thickness with the cutting edge perpendicular to the length of the ringlets. Evaluation of cell distribution and indirect assessment of cell viability was performed by staining the sections for RNA, using the SYTO RNASelect dye (Life Technologies, Carlsbad, CA), a nucleic acid stain exhibiting a very weak signal when bound to DNA whereas a strong one when bound to RNA (wavelengths: absorption ~490 nm, emission ~530 nm). Sections were fixed for 10 min using prechilled methanol (-20°C), washed twice for 5 min in PBS, and incubated in 500 nM of RNASelect green fluorescent stain (Invitrogen, USA) diluted in PBS for 20 min. Slides were then washed twice for 5 min in PBS, and images were taken with a Zeiss AxioImager M2 upright microscope coupled with a Zeiss Axiocam HRm digital camera (Zeiss, Thornwood, NY). For comparison of cell density within scaffold, each image was divided into sections (1–3) from ablumen to lumen (ablumen-mid-lumen), and fluorescence pixel intensity (% pixels white) was measured for each region. Hematoxylin and eosin staining was also performed to provide an overview of tissue structure, with hematoxylin coloring the cells' nuclei in dark purple and eosin staining the cytoplasm and ECM in pink.

2.8 | Scanning electron microscopy imaging

Samples were fixed in 2.5% glutaraldehyde, washed in PBS, fixed in 1% osmium tetroxide solution, and progressively dehydrated in 25%, 50%, 75%, 85%, 95%, and 3 × 100% ethanol solutions. Samples were then critical point dried, sputter coated with gold/palladium, and imaged using a Hitachi S-4000 FE-SEM at 10 kV.

2.9 | Statistics

All values are presented as mean ± standard deviation. Significance between two groups was determined using independent *t* test, and significance between three or more groups was determined using one-way analysis of variance test. If analysis of variance test indicated significance at $p < .05$, a Tukey post-hoc test was performed to compare group means ($n = 3$ for each sample group).

3 | RESULTS

Human umbilical veins were extracted mechanically from the umbilical cords, decellularized such as removing the cellular and nuclear contents while preserving the tridimensional structure and composition of the extracellular matrix, sterilized, and finally freeze-dried. The freeze-drying consists in the dehydration of the scaffold via sublimation, a step that can then allow long-term preservation of the decellularized veins at room temperature. Scanning electron microscopy and Young's modulus analyses show that the freeze-drying process only lightly alters the structure of the ECM, with the fibers being slightly more compacted on the abluminal side and with no significant difference in Young's modulus, after rehydration (Figure S1). Along cell seeding on the abluminal side, the dried scaffold then acts as a sponge; the cells resuspended in media can thus be integrated into the outer layer of the construct via adsorption. Upon seeding under orbital rotation, cells were cultured under a single luminal flow for 19 days. Histological images taken after 10 and 19 days of culture show that, over this initial culture period, the cells start migrating toward the lumen reaching however a limited penetration depth (Figure S2).

3.1 | Advanced recellularization via directed nutrient supply—Step 3 (Day 20 to 30)

Following seeding and the 19 days initial cell migration period, scaffolds were cultured for a further 11 days either under a single luminal flow (SF30) or a dual luminal + abluminal flow (DF30) to delineate the effects of single verses multiple (directional) nutrient sources on advanced recellularization. Scaffolds were harvested and examined on Day 30 using fluorescent RNA staining as an indirect method to evaluate cell viability and distribution. Histological images show that smooth muscle cells penetrated the vascular wall under both culture conditions, with the SF30 condition displaying improved (overall) migration toward the vascular intima (Figure 2a). Indeed, histological observation indicated DF30 conditions resulted in a higher proportion of cells remaining on the outer (seeded) surface, which was in agreement with the quantitative distribution analysis showing 71% of cells in this region ($p = .014$). Cells cultured under SF30 conditions displayed increased migration toward the constructs lumen with 45% of cells located in the outer abluminal region and 55% of the cells in the lumen and midlayers (Figure 2b). By contrast, only 29% ($p = .014$) cells in DF30 conditions were located in the lumen and midlayers. Additional histological images, stained with hematoxylin and eosin or SYTO RNA, are also displayed in Figures S3 and S4, for the SF30 and DF30 conditions, respectively. Overall, both culture conditions reached similar cell densities (SF30: 12.7×10^6 cells/g dry tissue, DF30: 14.4×10^6 cells/g dry tissue; $p = .435$), the different nutrient gradient thus only impacted cell distribution and not cell proliferation (Figure 2c). More detailed analysis showed an overall reduction in vessel wall thickness from the 550- μm scaffold thickness obtained after decellularization, which is significantly higher under SF30 (373 μm) compared with DF30 (527 μm ; $p = .010$; Figure 2d). An increased GAG synthesis was also noted under DF30 (5.06 $\mu\text{g}/\text{mg}$ dry tissue) compared with SF30 (2.86 $\mu\text{g}/\text{mg}$ dry tissue; $p = .023$; Figure 2e). Uniaxial tensile testing performed within the physiological and failure range (Figure 3a,b) shows that SF30 conditions display a higher physiological tensile modulus ($p = .003$), whereas no difference was observed for the Young's modulus ($p = .522$; Figure 3c,d). Burst pressure values for both conditions also remained similar (Figure 3e).

3.2 | Mechanical development via pressure conditioning—Step 4 (Day 31 to 45)

After 30 days of scaffold recellularization driven by SF30 culture conditions (previously analyzed), the effect of a switch to physiological pressure was assessed. Two parallel experiments were run. As the final goal is to develop a graft for arterial bypass, pressure conditions are here determined in regard to an artery. Under one condition, the pressure was thus kept low when compared with arterial pressure (8–12 mmHg; SF-Hypo45), whereas in the other set, the pressure was increased to physiological values (120/80 mmHg; SF-Normo45). Representative fluorescent RNA images at Day 45 show both grafts have cells present throughout the entire vascular wall (Figure 4a). Analysis of lumenal diameters shows SF-Normo45 scaffolds increased to 7.02 mm compared with 5.46 mm for the SF-Hypo45 ($p < .001$; Figure 4b). Structural analysis evidences increased GAG content for the SF-Normo45 scaffolds (8.72 $\mu\text{g}/\text{mg}$ dry tissue compared with 4.18 for SF-Hypo45; $p = .002$; Figure 4c) and increased cell count (SF-Normo45: 1.99×10^6 cells/g dry tissue, SF-Hypo45: 0.82×10^6 cells/g dry tissue; $p = .032$; Figure 4d) even though cell metabolic activity at Day 45 was found similar for both pressure conditions (SF-Normo45: 0.19%, SF-Hypo45: 0.21%; $p = .668$; Figure 4e). Arterial pressure was also shown to increase construct stiffness within the physiological range as shown by a physiological modulus of 0.55 MPa under SF-Normo45 while 0.26 MPa under SF-Hypo45 ($p = .001$; Figure 5a). Similar observations were evidenced in the failure range with a Young's modulus of 3.26 MPa under SF-Normo45 and 2.57 MPa under SF-Hypo45 ($p = .049$; Figure 5b). The burst pressure remained similar for both conditions (Figure 5c). The strain at failure and ultimate failure decreased under SF-Normo45 (32.6% and 36.7%, respectively) compared with SF-Hypo45 (46.9% and 91%, respectively; $p = .001$ and $p = .007$, respectively; Figure 5d,e), whereas the strength at failure and ultimate failure remained similar for both conditions (Figure 5f,g). In Figure 6, histological images stained with hematoxylin and eosin show the progressive development of the construct, from the native HUV (Figure 6a), to the decellularized scaffold (Figure 6b) and the recellularization and biomechanical conditioning either under SF45-Hypo (Figure 6c) or SF45-Normo (Figure 6d). A denser ECM structure is observed for the normotensive condition when compared with the hypotensive one, with cells throughout the vascular wall for both. Photograph of constructs conditioned with both approaches are also shown, right after harvest, at Day 45 (Figure 6e,f). They show that the normotensive grafts better retain their round-like shape when compared with the hypotensive ones.

4 | DISCUSSION

Ex vivo-based scaffolds hold the biological ECM composition of native tissues: a complex site-specific combination of biochemical and biomechanical cues challenging to synthetically reproduce. Its complexity is key for tissue regeneration as it provides a specific nanotopography as well as signaling molecules known to guide cell behavior and further tissue development (Garreta et al., 2017). Safe and effective decellularization techniques have been developed that allow for removal of immunogenic cellular materials while preserving the non-immunogenic ECM architecture and bioactive factors (Damodaran & Vermette, 2018; Gilpin & Yang, 2017; Porzionato et al., 2018). Scaffolds extracted from xenogeneic organisms and also from human origin have thus emerged and have become a

therapeutic actuality opening up the opportunity for full allogeneic graft development (M. C. Moore et al., 2017). Among the human sources, the placenta as well as the human umbilical vein and arteries have, for example, been assessed as scaffolds for vascular regeneration. They present the advantage of being widely available (placenta and umbilical cord are considered medical waste and as such are typically discarded upon delivery) with minimal ethical concern. Moreover, perinatal tissues have angiogenic, antimicrobial, and anti-inflammatory properties of main interest for tissue regeneration (M. C. Moore et al., 2017).

A drawback of these ex vivo-based tissues however has been their demanding recellularization (VeDepo, Detamore, Hopkins, & Converse, 2017). The low pore size geometry of the ECM structure limits cell penetration and results in the formation of a fibrous capsule at the construct periphery (Tosun, Villegas-Montoya, & McFetridge, 2011). Direct injection of cells into thick scaffolds, such as the ventricular wall, has been attempted by multiple groups; however, although dense cellularity was noted at the injection site, cells were not distributed throughout the scaffold even after extensive bioreactor culture (Scarritt et al., 2015). Another approach has been to use an artificial chemotactic nutrient-based gradient to drive cell migration from one side to the other of the thick scaffold (M. Moore et al., 2013; Tosun & McFetridge, 2013). For vascular grafts, cells were seeded on the ablumen of the vessel, and this nutrient gradient was created by applying media on the lumen and limiting its penetration through the scaffold for a full nutrient supply on the luminal side only. This nutrient gradient was created using a low luminal flow rate and a hypotensive pressure. Even though it provides recellularization, this model also induces the thinning of the construct, this degradation of the ECM can be due to enzymes such as matrix metalloproteinases (Jabło ska-Trypu , Matejczyk, & Rosochacki, 2016), and it results in the deterioration of the graft mechanics due to a lack of appropriate stimuli. Indeed, cyclic stretching induced by pulse waves at arterial pressures has been shown to play an important role in vascular cell phenotype and ECM synthesis (Lu & Kassab, 2011; Mao et al., 2012; Zhu et al., 2011). This dynamic stimulation is essential to improve construct development and translation to the in vivo microenvironment as it minimizes the negative effects of mechanical mismatching upon implantation (Post et al., 2019).

Here, using a double chamber bioreactor and associated flow circuits that allow for a precise control of the flow and pressure conditions, we were able to both fully recellularize and positively remodel scaffolds that were based on the human umbilical vein. A stepwise culture process was employed. Briefly, veins extracted from the human umbilical cord were first decellularized under perfusion and the acellular scaffolds obtained were seeded with human smooth muscle cells onto their ablumen. Recellularization of the vascular wall was then driven using a hypotensive luminal flow from Day 1 to 30 that drove cell migration from the ablumen to the lumen, leading to the development of a vascular wall replete with smooth muscle cells. The biomechanics of the cellular construct were then stimulated by increasing the luminal flow rate and pressure such as applying arterial-like cyclic stretch and shear stress (normotensive conditioning). Under these biomechanical stimuli, function of the smooth muscle cells was influenced and motivated an arterial-like remodeling as demonstrated by increased GAG content as well as increased physiological tensile and Young's moduli relative to the hypotensive conditioning.

Easing the vascular wall repopulation and biomechanical adaptation was needed for further development of ex vivo scaffolds as vascular grafts. Additional development of an endothelial layer to avoid thrombosis of the vessel upon implantation is also crucial (Sánchez, Brey, & Briceño, 2018). It has previously been demonstrated that the re-endothelialization of the acellular human umbilical vein can similarly be performed using sequential conditioning steps (Uzarski, Cores, & McFetridge, 2015). In this case, an initial step consisted in 5 days of static cell adhesion to the luminal surface and was followed by 24 hr of progressive flow rate increase up to reaching arterial shear stress and pressure. It led to the development of a fully confluent endothelium with limited inflammatory phenotype. In the future, combining both approaches will be of interest, to reduce pro-inflammatory and pro-thrombotic phenomenon upon implantation. A similar neo-endothelialization has proven difficult for numerous synthetic grafts and alternatives are being developed to either promote the endothelialization by adding anchoring molecules or to produce an inert luminal surface (Radke et al., 2018). The successful development of a recellularized vascular wall as well as the neo-endothelialization of the human umbilical vein thus brings promise regarding to its use and more generally the use of ex vivo-based tissues for the engineering of vascular tissues.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

Sequential culture conditions for the development of an improved vascular wall. (a) Design of the perfusion flow circuit used, made of three bioreactors connected in series and independent luminal and abluminal flow supplies. (b) Schematic of the culture conditions along the graft development. Uniform seeding was first performed by injecting smooth muscle cells resuspended in media on the abluminal side of the scaffold followed by orbital rotation of the entire bioreactor at 1.7 RPM for 2 hr. Initial recellularization of the constructs (Day 0 to 19) was then performed by applying a nutrient gradient (media on the luminal side of the scaffold only) that drove cell migration toward the lumen. The effect of the controlled nutrient supply on scaffold recellularization was then assessed from Day 20 to 30; a prolonged aggressive nutrient gradient generated by the luminal flow only (SF30) was compared with a luminal flow combined with an abluminal flow improving nutrient supply on the abluminal side of the construct (DF30). From Day 31 to 45, the effect of mechanical conditioning on vascular remodeling was then assessed; hypotensive conditions (SF-Hypo45) created by a luminal pulse pressure of 8–12 mmHg were compared with normotensive conditions (SF-Normo45) with pulse pressure cycling between 120 and 80 mmHg. (c) Detailed culture conditions. ECM, extracellular matrix; SMC, smooth muscle cell

FIGURE 2.

Analyses of Step 3 performed at Day 30—Recellularization can be modulated using defined nutrient gradients. (a) Histological sections were stained with SYTO RNA as an indirect method to assess cell viability and to localize cells (top row). Images were thresholded for more accurate quantitative analysis of cell distribution (bottom row), and three sections were defined: the abluminal (ab), middle (mid), and luminal (lum) regions. l represents the lumen, and a represents the ablumen. (b) White pixel intensity was measured in the abluminal (ab), middle (mid), and luminal (lum) regions and reported to the total white intensity as an analysis of cell distribution. Cells were more homogeneously distributed within the adventitial/medial layer with SF30, whereas for DF30, a high percentage of cells were located on the abluminal surface. † indicates significant differences in cell distribution ($p < .05$). (c) Quantification of cell number was comparable for both culture conditions. Asterisks indicate significant differences between SF30 and DF30 ($p < .05$). (d) Analysis of constructs thickness showed thinning of the scaffold when cultured with SF30, whereas DF30 preserved more of the scaffold thickness. Similarly, glycosaminoglycan (GAG) concentration was increased with DF30. † indicates significant differences in cell distribution ($p < .05$). * indicates significant differences between SF30 and DF30 ($p < .05$)

FIGURE 3.

Step 3—Nutrient gradient modulates construct mechanics during recellularization (Day 30).

(a) Uniaxial tensile testing is performed on 5-mm-wide sample ringlets. (b) Schematic of a stress/strain curve. Tensile moduli were calculated in the physiological and failure (Young's modulus) range. (c) Young's modulus is similar for both SF30 and DF30. (d) Physiological tensile modulus is higher for SF30 compared with DF30. (e) Burst pressure is similar for both SF30 and DF30. * indicates significant differences between SF30 and DF30 ($p < .05$)

FIGURE 4.

Step 4—Recellularized vessels structural characteristics are influenced by mechanical conditioning (Day 45). (a) Fluorescent images (stained for RNA) of vessels cultured under low (SF-Hypo45) and physiological (SF-Normo45) luminal pressure (scale bars: 100 μm). l represents the lumen, and a represents the ablumen. Analysis of lumenal diameter (b), glycosaminoglycan (GAG) concentration (c), and cell density (d) shows an increase with physiological pressure conditions (SF-Normo45). (e) The metabolic activity, however, was similar for both pressure conditions. * indicates significant differences between SF-Hypo45 and SF-Normo45 ($p < .05$)

FIGURE 5.

Step 4—Arterial pressure conditioning improves graft mechanics (Day 45). (a) Physiological tensile modulus and (b) tensile modulus at failure (Young's Modulus) increased when grafts were cultured under arterial pressure (SF-Normo45). (c) Burst pressure remains similar for both conditions. Strain values at failure (d) and ultimate failure (e) decreased with physiological pressure conditioning, whereas strength values at failure (f) and ultimate failure (g) remained similar for both culture conditions. * indicates significant differences between SF-Hypo45 and SF-Normo45 ($p < .05$)

FIGURE 6.

(a–d) Representative histological images stained with hematoxylin and eosin showing the processing steps of the HUV toward an arterial vessel (scale bars = 100 μ m). (a) The native HUV with the cells apparent. (b) The decellularized vein striped out of the cells but with the extracellular matrix remaining. The recellularized constructs conditioned for 45 days either under the (c) SF45-Hypo or (d) SF45-Normo conditions show a looser vascular wall structure for the Hypo condition. Photographs also show the (e) SF45-Hypo and (f) SF45-Normo constructs right after harvest. A zoom on a cross section of a SF45-Normo graft in the top right corner of (f) shows it keeps its round-like shape, to the opposite of the SF45-Hypo grafts