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Implantable tissue-engineered blood vessels from human induced pluripotent stem cells

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ABSTRACT

Derivation of functional vascular smooth muscle cells (VSMCs) from human induced pluripotent stem cells (hiPSCs) to generate tissue-engineered blood vessels (TEBVs) holds great potential in treating patients with vascular diseases. Herein, hiPSCs were differentiated into alpha-smooth muscle actin (α-SMA) and calponin-positive VSMCs, which were seeded onto polymer scaffolds in bioreactors for vascular tissue growth. A functional TEBV with abundant collagenous matrix and sound mechanics resulted, which contained cells largely positive for α-SMA and smooth muscle myosin heavy chain (SM-MHC). Moreover, when hiPSC-derived TEBV segments were implanted into nude rats as abdominal aorta interposition grafts, they remained unruptured and patent with active vascular remodeling, and showed no evidence of teratoma formation during a 2-week proof-of-principle study. Our studies represent the development of the first implantable TEBVs based on hiPSCs, and pave the way for developing autologous or allogeneic grafts for clinical use in patients with vascular disease.

1. Introduction

Vascular disease due to atherosclerosis, thrombosis or aneurysm is the largest cause of mortality in the developed world [1]. Autologous or synthetic vascular grafts are used in treating this disease to bypass and replace diseased vascular segments. However, some patients either lack suitable autologous tissue or cannot receive synthetic grafts due to the small size of the target vessels. Various approaches have been used to generate tissue-engineered blood vessels (TEBVs), some of which are currently in clinical trials with promising results [2]. However, the ability to produce functional TEBVs from human primary vascular smooth muscle cells (VSMCs) is limited not only by donor cells' difference in collagen matrix synthesis [3] but also by their variable and restricted proliferation potential. TEBVs have also been derived from human primary fibroblasts [4], but in contrast to VSMCs, fibroblasts may not respond effectively to vaso-regulatory signals. These fibroblast-based TEBVs may have limitations as arterial replacements due to observed dilatation after implantation. Other cell types such as mesenchymal [5], adipose [6] or hair follicle [7] stem cells have been used for TEBV generation. However, the inefficient SMC differentiation and limited accessibility of these cells may hinder their widespread application.

Human induced pluripotent stem cells (hiPSCs) can be derived

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from a person's own somatic cells by ectopic expression of stem cell factors. hiPSCs can self-renew and differentiate into virtually every cell type in the body, including functional VSMCs (hiPSC-VSMCs) [8,9]. Mesenchymal precursor cells derived from hiPSCs have previously been used to co-culture with endothelial cells in a collagen-fibronectin gel to form microvessels [10] or to create TEBVs with biodegradable polymer scaffolds [11]. However, the suture strength of TEBV based on these hiPSC-derived mesenchymal progenitors was relatively weak (30 g) and unsuitable for arterial implantation [11]. Additionally, TEBVs constructed with hiPSC-VSMCs and nanofibrous scaffolds have shown collagenous matrix deposition in a murine subcutaneous model [12]. In summary, mechanically strong TEBVs based on hiPSC-derived cells that are suitable for surgical handling and arterial implantation have yet to be developed.

We have previously generated VSMCs from iPSC lines derived from patients with supravalvular aortic stenosis (SVAS) for disease modeling and for mechanistic studies [13]. But for applications in tissue engineering, one of the challenges is to scale up the differentiation capacity to generate very large number of VSMCs. We have established a robust system to obtain over 40 million VSMCs from a single 6-well plate of feeder-free hiPSCs [14]. In this study, we hypothesize that hiPSC-VSMCs are able to generate strong and functional arterial grafts for implantation. Using an optimized culture medium, TEBVs were generated after 9 weeks' culture, and the grafts remained unruptured and patent while supporting active vascular remodeling in a rat model.

2. Methods

2.1. Animal use

The study was approved by the Yale University Institutional Animal Care and Use Committee. All animal care complied with the NIH Guide for the Care and Use of Laboratory Animals. Human tissue and cell populations were obtained using protocols approved by the Yale University Human Investigation Committee.

2.2. Differentiation of hiPSCs to VSMCs

The hiPSC line (Y6) was generated from human neonatal fibroblast cells isolated from a healthy female donor using SeV particles that encode OCT3/4, KLF4, SOX2, and c-MYC genes as previously described [14]. Y6 hiPSCs were differentiated to VSMCs via an EB approach as previously described [14]. Briefly, hiPSCs were cultured in mTeSR™1 self-renewal medium (STEMCELL Technologies) under feeder-free conditions until 80% confluency. hiPSCs were treated with dispase (1 mg/mL) for 15 min at 37 °C, and cell clusters of uniform sizes were seeded in a 6-well low attachment plate in mTeSR™1 medium. Culture medium was replaced with a 1:3 mixture of mTeSR™1 and EB differentiation medium [DMEM with 10% FBS (Hyclone), 1% (v/v) NEAA, 2 mM L-glutamine and 0.012 mM 2-mercaptoethanol] the next day. From day 3 to day 5, cells were cultured in EB differentiation medium. The EBs were then collected and seeded on a gelatin-coated 6-well plate for 5 days using EB differentiation medium. The cells were next harvested using 0.25% trypsin, seeded on Matrigel coated T75 flasks, and cultured until the VSMCs reached 80% confluence in SmGM-2 medium (Lonza, USA). hiPSC-VSMCs were also cultured in the maturation medium [DMEM with 5% FBS (Hyclone), 1% (v/v) NEAA, 2 mM L-glutamine and 0.012 mM 2-mercaptoethanol] for 7 days to further induce VSMC marker expression.

2.3. Culture of hiPSC-VSMCs on PGA scaffolds

PGA scaffold was cut into 5 mm × 5 mm squares and prepared for cell seeding as described previously [15]. PGA squares were coated with 0.1% gelatin for 2 h prior to cell seeding. Cells were seeded onto PGA squares at 5 million cells/mL and cultured in SmGM-2 at 37 °C, 5% CO_2 for 24 h. The medium was then replaced by one of the following bioreactor media modified those from previous studies that promote collagen synthesis [16]. These media included I: SmGM-2 with 5% FBS, EGF 0.5 ng/mL, bFGF 10 ng/mL, insulin 0.13 U/mL and Penicillin G with additional proline 50 μg/mL, glycine 50 μg/mL, alanine 20 μg/mL and $CuSO_4$ 3 ng/mL; II: DMEM with 20% human serum, EGF 0.5 ng/mL, bFGF 10 ng/mL and Penicillin G with additional proline 50 μg/mL, glycine 50 μg/mL, alanine 20 μg/mL and $CuSO_4$ 3 ng/mL; and III: DMEM with 20% FBS, PDGF-BB10 ng/mL, TGF-β1 1 ng/mL and Penicillin G with additional proline 50 μg/mL, glycine 50 μg/mL, alanine 20 μg/mL and $CuSO_4$ 3 ng/mL. The medium was refreshed weekly and ascorbic acid at 50 μg/mL was supplemented every 2 days. PGA squares were cultured for 3 weeks and harvested for analysis.

2.4. Engineered vessel culture in bioreactors

Engineered vessels were cultured from hiPSC-differentiated VSMCs on PGA scaffold as described previously [16,17]. Cells at 10 million cells/mL were seeded onto tubular PGA scaffolds that were mounted inside of sterilized glass bioreactors. Vessels were cultured in SmGM-2 medium at 37 °C, 5% CO_2 for 24 h. The medium was then replaced by the bioreactor medium (DMEM with 20% FBS, PDGF-BB 10 ng/mL, TGF-β1 1 ng/mL and Penicillin G with additional proline 50 μg/mL, glycine 50 μg/mL, alanine 20 μg/mL and CuSO4 3 ng/mL). The culture medium was refreshed weekly and ascorbic acid was supplemented every 2 days. The engineered vessels were cultured for 8–9 weeks without cyclic mechanical distension, and then harvested for implantation and further analysis.

2.5. Implantation of TEBVs into nude rats

To evaluate the mechanical strength of hiPSC-derived tissue engineered vessels and to determine the remodeling *in vivo*, TEBV segments of 2 mm inner diameter were implanted into $Foxn1^{rnu}$ nude rats, 3–4 month old, weighing 300 g (Charles River Laboratories, Boston, MA) as abdominal aorta interposition grafts. Briefly, rats were anesthetized with isoflurane. Animals were opened with a midline abdominal incision and the infrarenal abdominal aorta was exposed under standard sterile conditions. The abdominal aorta was cross-clamped and divided between the renal artery and the inferior mesenteric artery. A segment of the TEBV (6–8 mm in length) was inserted into the aorta "end-to-end" using a 10-0 monofilament nylon suture. After confirmation of graft blood flow and hemostasis post de-clamping, the wound was closed. The animals were recovered from surgery and maintained in the absence of anti-coagulants for up to 14 days post-operatively.

2.6. Assessment of grafts in vivo

At 3 days, 7 days and 14 days after implantation, animals were examined using a Vevo 770® Micro-ultrasound System (VisualSonics, Toronto, Canada) equipped with the RMV-704 scanhead (spatial resolution 40 μm) to determine graft patency and blood flow. The diameter of the graft at the midpoint was measured from both transverse and longitudinal axis ultrasound images. Grafts were explanted at 7 days (n = 2) or 14 days (n = 2) post-operatively and fixed in 10% formalin overnight.