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# A novel concept for scaffold-free vessel tissue engineering: Self-assembly of microtissue building blocks

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ABSTRACT

Current scientific attempts to generate *in vitro* tissue-engineered living blood vessels (TEBVs) show substantial limitations, thereby preventing routine clinical use. In the present report, we describe a novel biotechnology concept to create living small diameter TEBV based exclusively on microtissue self-assembly (living cellular re-aggregates). A novel bioreactor was designed to assemble microtissues in a vascular shape and apply pulsatile flow and circumferential mechanical stimulation. Microtissues composed of human artery-derived fibroblasts (HAFs) and endothelial cells (HUVECs) were accumulated and cultured for 7 and 14 days under pulsatile flow/mechanical stimulation or static culture conditions with a diameter of 3 mm and a wall thickness of 1 mm. The resulting vessels were analyzed by immunohistochemistry for extracellular matrix (ECM) and cell phenotype (von Willebrand factor, α-SMA, Ki67, VEGF). Self-assembled microtissues composed of fibroblasts displayed significantly accelerated ECM formation compared to monolayer cell sheets. Accumulation of vessel-like tissue occurred within 14 days under both, static and flow/mechanical stimulation conditions. A layered tissue formation was observed only in the dynamic group, as indicated by luminal aligned α-SMA positive fibroblasts. We could demonstrate that self-assembled cell-based microtissues can be used to generate small diameter TEBV. The significant enhancement of ECM expression and maturation, together with the pre-vascularization capacity makes this approach highly attractive in terms of generating functional small diameter TEBV devoid of any foreign material.



## 1. Introduction

Due to an ever growing aging western population with cardiovascular disease and therefore increasing numbers of bypass and reconstructive vascular interventions, there is a substantial clinical need for appropriate vascular graft materials. In the context of coronary bypass surgery, small diameter vascular grafts, such as saphenous vein and mammary artery, are regularly in high demand thus resulting in the unwanted usage of native autologous materials. Development of arterial replacements to substitute native vein and artery segments for peripheral or coronary revascularization is more than 50 years old, but still native autologous replacements are considered to be the gold standard (L'Heureux et al., 2007). Synthetic grafts, especially for small diameter (<6 mm) applications, have been associated with various complications including acute thrombosis, restenosis and compliance mismatch, susceptibility to infections and lack of growth, (Hoerstrup et al., 2006; Zilla et al., 2007). As such, novel strategies to create allogeneic and autologous living tissue-engineered blood vessels (TEBVs) *in vitro* are considered to be acceptable alternatives and are therefore actively being pursued by numerous investigators. Since the first report of TEBVs by Weinberg and Bell (1986), a wide variety of biomaterials (synthetic and natural biopolymers) and cell sources have been investigated for their eligibility to produce TEBVs (Couet et al., 2007; Isenberg et al., 2006). Two scaffold-based conceptual strategies are prevalent: (1) implantation of instructive scaffold materials

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promoting *in vivo* cellular ingrowth and tissue maturation through recruitment of endogenous cells (Campbell et al., 1999) and (2) implantation of functional living tissue replacements generated *in vitro* produced from cells seeded on tubular scaffolds and matured in biomimetic bioreactors (Hoerstrup et al., 2001, 2006). However, when considering the importance of the immunological response to such scaffolds and the efficiency by which they can be produced, endogenous tissue derived from autologous cells may be deemed to be the most appropriate extracellular matrix. A successful scaffold-free concept has been introduced by L'Heureux et al. (1998), where single-cell sheets were wrapped in a multi-step process around a stiff support tube. The resulting tissue was reminiscent of native tissue and displayed good functionality *in vivo*, displaying the high potential of pure cell-based concepts. However, the whole production process required more than 4 months and necessitated multiple processing steps (L'Heureux et al., 2006).

Current approaches have shown high potential. But long production times and complex processing steps have hampered their routine clinical implementation. In the present report, we describe a scaffold-free concept to produce small diameter TEBV using microtissue composed of myofibroblasts and endothelial cells. Microtissues produce higher amounts of extracellular matrix required to create scaffold-free TEBV.

## 2. Material and methods

### 2.1. Isolation of primary cells

In order to obtain primary human artery-derived fibroblasts (HAFs), de-endothelialized vessel segments of human arteries were minced and cultivated in a 37 °C humidified 5% $CO_2$-containing atmosphere and Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal calf serum (FCS; cat. no. A-15-022, lot no. A01129-242; PAA Laboratories, Linz, Austria) and 1% penicillin/streptomycin solution

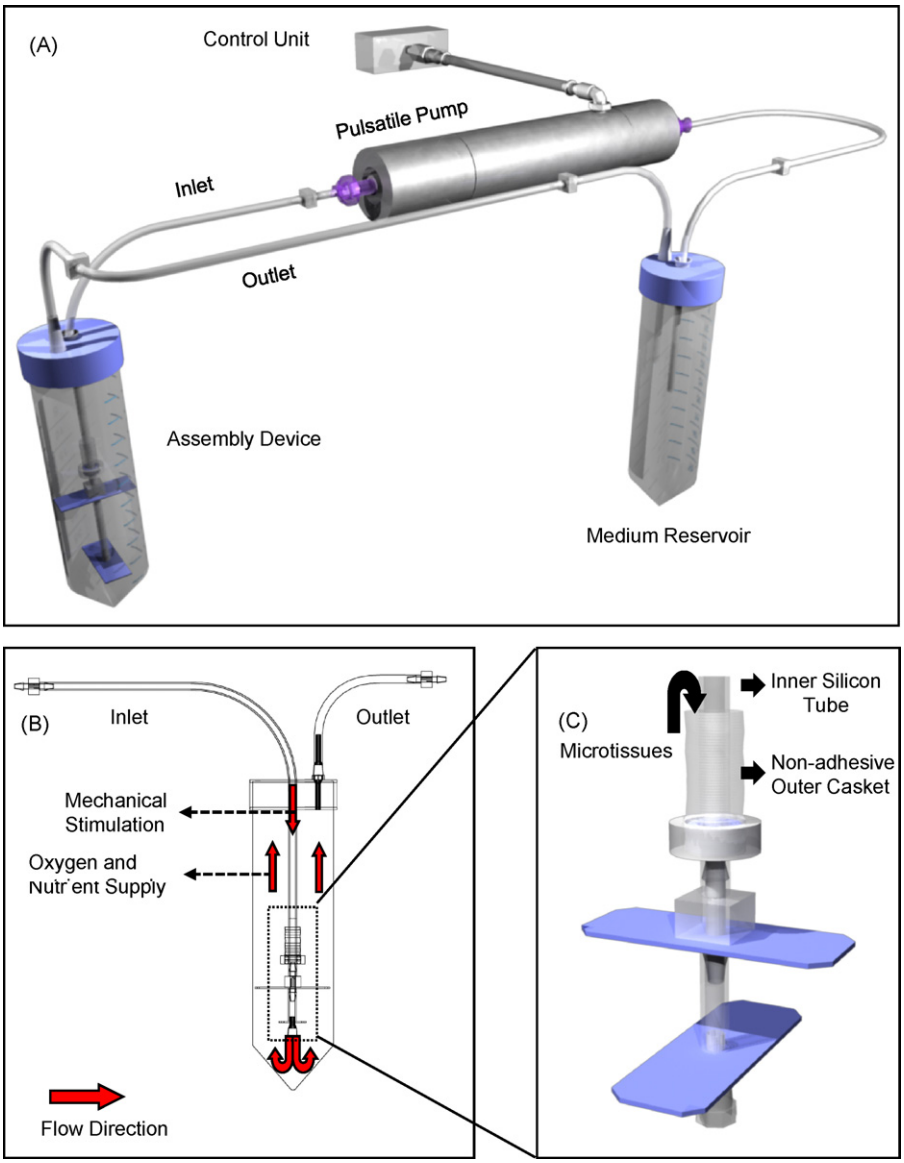


**Fig. 1.** A schematic image of the bioreactor setup, displaying the three components: (i) the pulsatile pump, (ii) the assembly device and (iii) the medium reservoir. (B) Cross-section through the microtissue assembly device with the inlet silicon tube enabling circumferential mechanical stimulation and a reverse medium outflow. (C) The casting mould to assemble the microtissues consist of an inner silicon tube (inflow, mechanical stimulation), two distance spacer of 1 mm thickness determining the vessel wall thickness and an outer non-adhesive, porous cask to retain the microtissues within the assembly device.