

# MICROFLUIDIC TISSUE: A BIODEGRADABLE SCAFFOLD WITH BUILT-IN VASCULATURE FOR CARDIAC TISSUE VASCULARIZATION AND SURGICAL VASCULAR ANASTOMOSIS

Boyang Zhang<sup>1,2</sup>, Miles Montgomery<sup>1,2</sup>, Aric Pahnke<sup>1,2</sup>, Lewis Reis<sup>2</sup>, Sara S. Nunes<sup>2,3</sup>, Milica Radisic<sup>1,2\*</sup>

<sup>1</sup>Department of Chemical Engineering and Applied Chemistry, University of Toronto, Canada

<sup>2</sup>Institute of Biomaterials and Biomedical Engineering, University of Toronto, Canada

<sup>3</sup>Toronto General Research Institute, University Health Network, Toronto, Ontario, Canada.

## ABSTRACT

To tackle tissue vascularization, a robust 3-D micro-patterning technique was developed to create complex bio-scaffolds from a synthetic biodegradable elastomer (Poly(octamethylene maleate (anhydride) citrate) by pre-patterning, solidifying, and stamping thin polymer sheets layer-by-layer to form intricate micro-structures. The bio-scaffold contains an 3-D perfusable branched network which supports the assembly of vascularized cardiac tissues with co-culture of human endothelial cells and cardiomyocytes (rat or human). Engineered microfluidic tissues were also surgically connected *in vivo* to the femoral artery of a murine model for the first time. Microfluidic tissues described here could act as a cornerstone for future functional tissue replacement strategies.

**KEYWORDS:** Microfluidics, Tissue Engineering, Vascularization, Biodegradable Polymer, Microfabrication

## INTRODUCTION

Leveraging tissue engineering in combination with stem cell technology has the potential to address the shortage of donor organs for transplantation and provide patient specific tissue replacement [1]. However, tissue vascularization and rapid vascular integration *in vivo* remain as two pervasive and long-standing obstacles. Modern fabrication techniques, such as 3-D printing, have prompted tremendous excitement in tissue engineering. 3-D sacrificial molding was recently introduced, where a rigid 3-D lattice of sugar filaments can be printed and embedded within an aqueous-based extracellular matrix in the presence of living cells and subsequently dissolved to leave behind a micro-channel network for endothelialization [2]. Although this method provides a simple approach to create hollow channel networks, the hydrogel provides no real structural support for the delicate lumen network. To establish a functional tissue at physiological cell density ( $10^8$  cells/cm<sup>3</sup>), extensive cell remodeling of the ECMs must take place, which inevitably alters the structure of the hydrogel and collapses any embedded micro-channels. For instance, macroscopic tissue contraction of heart muscle would be virtually impossible to establish without tight cell-cell connections at physiological cell density. Although a scaffold-free based approach potentially provide more rapid integration *in vivo*, the structural support provided through a synthetic biodegradable scaffold is also essential in establishing proper tissue assembly and function [1]. Therefore, in this work a hybrid approach was taken to create a microfluidic tissue containing a (1) a 3-D branched micro-channel network with thin channel walls to provide mechanical support to the built-in vasculatures composed of the biodegradable elastomer, (poly(octamethylene maleate (anhydride) citrate) (POMaC)) [3] and (2) a collagen based hydrogel embedded with cardiac cells cast around the network such that the cardiac cells remodel the aqueous matrix and compact around the built-in vasculatures to form macroscopically contracting vascularized cardiac muscle with physiological cell density.

## PRINCIPLE

The scaffold structure of the microfluidic tissue was realized through our 3-D micro-patterning technique. Thin biomaterial sheets were pre-patterned, solidified and stamped onto each other layer-by-layer to form complex suspended structures with precise alignment; hence, internal cavities were easily created without sacrificial materials (Figure 1A). We found POMaC to be an ideal material for this 3-D patterning technique for several reasons. POMaC can be photo-polymerized allowing fast processing under mild conditions, degrades through hydrolysis reaction in aqueous solution, and is synthesized from non-toxic monomers [3]. Importantly, POMaC exhibits non-permanent and differential adhesion to glass substrate (strong adhesion) and PDMS substrate (weak adhesion). This behaviour allows the transferring of thin patterned POMaC sheets from one substrate (PDMS) to another substrate (glass) easily and robustly without ever having to completely release the POMaC sheet during the process. Therefore, patterned POMaC sheets can be aligned and stacked to form 3-D structures with unprecedented accuracy and robustness (Figure 1B). Conventional layer-by-layer fabrication methods with biodegradable elastomer require complete release of patterned polymer sheet from the base substrate, restraining handling and making precise alignment either impossible or non-reproducible [4, 5].

## RESULTS AND DISCUSSION

To build a perfusable 3-D vascular network within a dense tissue, we first micro-fabricated a 3-D scaffold with a built-in branched network, mimicking a capillary bed, within a lattice matrix from POMaC. The built-in branched network includes a single inlet and outlet for perfusion with multiple bifurcations in between (Figure 1B) and branched networks on different levels were interconnected with vertical channels hence forming a 3-D interconnected network. In the current design the smallest micro-channel in the network was 100  $\mu$ m by 50  $\mu$ m with wall thickness of 50  $\mu$ m.

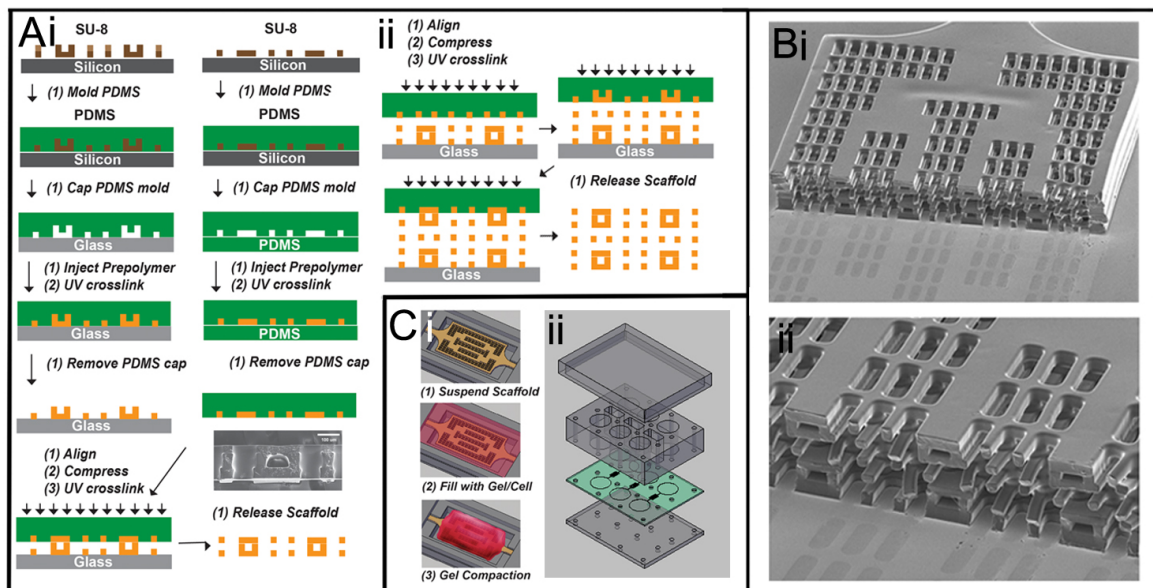


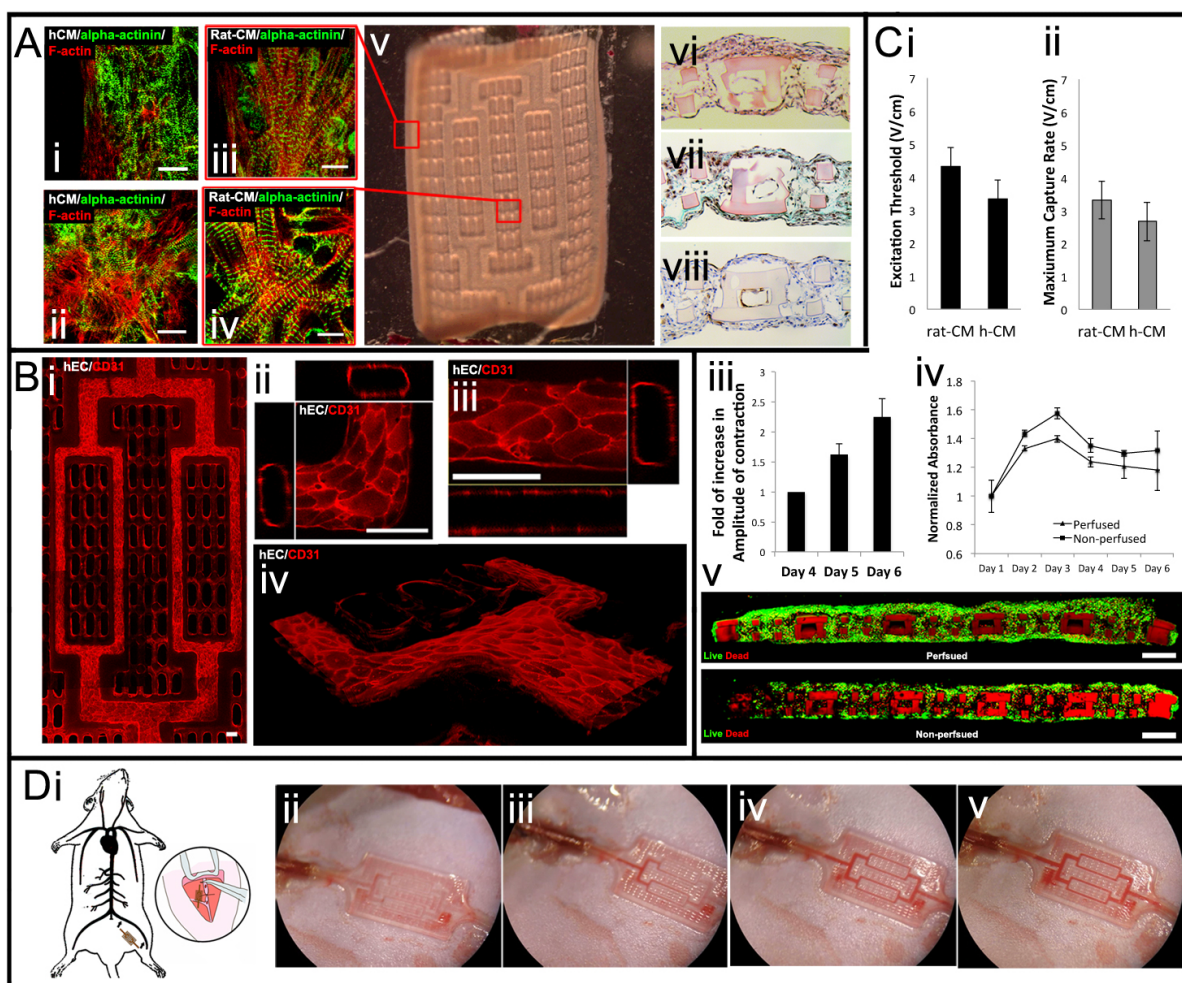
Figure 1: Fabrication of multi-layer microfluidic scaffold. (A) Schematic of microfluidic scaffold layer-by-layer molding and bonding procedures for (i) single layer and (ii) multi-layer network. (B) SEM images of the cross-section of a multi-layer microfluidic scaffold showing the channel lumens surrounded by the lattice matrix. (C) Schematic of (i) cardiac cell seeding/tissue formation and (ii) perfusion bioreactor assembly.

To further enhance oxygen and nutrient exchange through the micro-channel wall, nanopores were included within the bulk polymer structure by embedding and subsequently leaching of porogen (poly(ethylene glycol) dimethyl ether (PEGDM)) leaving behind wrinkled nanopores with a gap size of approximately 10 nm as described previously [6]. The permeability of the channel wall to small (Propidium Iodide, 668Da) and large molecules (TRITC-Dextran, ~70 kDa) were determined to be  $9.2 \times 10^{-6} \pm 2.5 \times 10^{-7} \text{ cm s}^{-1}$  and  $8.2 \times 10^{-6} \pm 4.4 \times 10^{-7} \text{ cm s}^{-1}$ , respectively.

The scaffold lattice structure was composed of multiple layers of rectangular shaped meshes supported by micro-posts in between. This design provided 100% interconnectivity within the structure allowing cardiomyocytes to form interconnected tissue fibres wrapping around the embedded micro-channel network (Figure 1B). The strut of the lattice matrix was designed to be 50  $\mu\text{m}$  thick to provide sufficient structural support while minimizing spatial interference to tissue connection and maximizing porosity. To mimic the anisotropic mechanical properties of an adult rat myocardium, we designed the scaffold so that its mechanical stiffness ( $E_{\text{CD}} 44 \pm 10$ ,  $E_{\text{LD}} 18 \pm 5$ ,  $E_{\text{CD}}/E_{\text{LD}} 2.4 \pm 0.2$ ) matches approximately to that of adult rat myocardium ( $E_{\text{CD}} 45 \pm 8$ ,  $E_{\text{LD}} 12 \pm 4$ ,  $E_{\text{CD}}/E_{\text{LD}} 3.7 \pm 0.2$ ). Through hydrolysis reaction, the scaffold degraded completely within 80 hr in 0.1M NaOH solutions and partially within 125 days in PBS. The degradation rate of the scaffold can be further modulated for specific application by altering post-polymerization condition or the molar ratio of citric acids.

To perfuse the built-in network, the scaffold was placed within a customized bioreactor (Figure 1C). Within the reactor, the microfluidic tissue was then assembled. First, human umbilical vein endothelial cells were seeded within the network through the network inlet and outlet and a confluent endothelialized network can be established overnight (Figure 2B). Then, either neonatal rat or human embryonic stem cell (hESC) derived-cardiomyocytes were seeded with collagen matrix outside of the endothelialized network and throughout the lattice matrix. Cardiomyocytes were able to quickly remodel the collagen matrix and compact around the vascular network forming a condensed tissue near physiological cell density ( $10^8 \text{ cells/cm}^3$ ) within 5 days (Figure 2A). Synchronous macroscopic tissue contraction can be seen as early as 4 days and the electrophysiological properties of the tissues fall within the standard range (Figure 2Ci-ii). Contracting tissues were able to compress the scaffold structure and the degree of compression was shown to increase over time indicating increasing forces exerted onto the scaffold as the tissue structure matures overtime (Figure 2Cii). Sarcomeric- $\alpha$ -actinin and actin structures of the cardiac cells were also visible and showed cell elongation on both the edge and center of the tissue patch (Figure Ai-iv). Co-culture of human endothelial cells and human cardiomyocytes showed cardiomyocytes distributed throughout the lattice matrix and densely packed around the micro-channel while endothelial cells coat the inner lumen of the micro-channel (Figure 2A vi-viii).

By examining the cross-section of the tissue with live and dead staining, we found more viable cells within the perfused tissue compared to non-perfused tissue, which clearly developed a necrotic core (Figure 2Cv). A lactate dehydrogenase activity (LDH) assay, used to quantify cell death over a 6 day period, revealed that perfusion helped to mitigate cell death (Figure 2Civ). To demonstrate the feasibility of directly connecting the microfluidic cardiac tissue to the host circulation system *in vivo*, we surgically connected the microfluidic scaffold to the femoral artery of an adult rat. Blood perfusion through the built-in network was established immediately after the surgical anastomosis (Figure 2D). Blood pulsation was observed throughout the network.



**Figure 2: Formation of functional microfluidic tissue and surgical anastomosis.** (A) Confocal fluorescent images showing sarcomeric- $\alpha$ -actinin (green) and F-actin (red) structure of hESC-derived cardiomyocytes and neonatal rat cardiomyocytes at (i, iii) the edge and (ii, iv) the center of (v) the microfluidic tissue shown in bright-field. (vi-viii) Histology section of the cross section of the microfluidic tissue with co-culture of cardiomyocytes and endothelial cells stained with (vi) H&E, (vii) Trichrome, and (viii) CD31. (B) Fluorescent images of endothelialized branched network stained with CD31. (C) Functional assessment of (i, ii) tissue excitation threshold and maximum capture rate  $n=3$ , (iii) amplitude of contraction, and (iv-v) viability w/o perfusion over time. (D) Surgical vascular anastomosis on the rat femoral artery showing the initiation of blood perfusion through the microfluidic scaffold.

## CONCLUSIONS

For the first time, a fully synthetic human cardiac tissue with a built-in 3-D vascular network was created and implanted with surgical vascular anastomosis. Blood perfusion through the engineered vascular network was established immediately upon implantation. The engineered tissues were functional and contracted macroscopically. Microfluidic tissue replacement could potentially be generated completely from stem cells and synthetic material without any need for prior surgical procedure from the patient.

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## CONTACT

\*Milica Radisic tel: +416-436-8382; m.radisic@utoronto.ca