MEMS-assisted spatially homogeneous endothelialization of a high length-to-depth aspect ratio microvascular network

Nisarga Naik, Vivek Kumar, Elliot L. Chaikof, and Mark G. Allen

Abstract—The endothelialization of an engineered microvascular network is constrained by the mass transport of the endothelial cells through high length-to-depth (l/d) aspect ratio microchannels. This paper presents a deformable, reentrant microvascular scaffold as a microelectromechanical systems (MEMS)-assisted approach for spatially homogeneous endothelial cell seeding of high l/d (>200) aspect ratio microvasculature. Nickel electroplating and micromolding were employed for the fabrication of the polydimethylsiloxane (PDMS) reentrant microvascular scaffold. A 'stretch – seed – seal' ('3S') operation was implemented for uniform incorporation of endothelial cells on the luminal surface of the elastomeric constructs. Confocal microscopy was utilized to establish the uniformity of endothelialization and to demonstrate the feasibility of this strategy.

I. INTRODUCTION

A substantial effort has been directed towards the development of vascularized tissues to ensure the nourishment and viability of engineered 'living' tissues. Although various approaches have been investigated for the creation of individual vascular channels with diameters that exceed 1 mm, recent applications of MEMS-based techniques has facilitated the development of microvascular networks. Micromachining strategies have afforded microchannel fluidic devices with complex shapes necessary to recapitulate a cell-seeded 'living' microvasculature. Micromolding, the most commonly used fabrication approach for microvascular networks, has been applied for the construction of both two-dimensional and three-dimensional structures [1-7]. PDMS has been widely utilized for this application on account of its high permeability, biocompatibility, and elastomeric nature [1-3].

The lumen of a native blood vessel is lined with a monolayer of non-thrombogenic endothelial cells. In the absence of an endothelial cell layer, small-diameter vascular conduits exhibit a high incidence of thrombosis with poor long-term patency. Reports suggest that endothelialized vascular grafts display a reduced thrombotic response as compared to non-seeded grafts [8]. Hence, several static and dynamic endothelial cell seeding strategies have been explored for the construction of a confluent endothelium on the luminal surface of synthetic vascular grafts [9-11].

Cell seeding of a tubular construct is a function of conduit diameter and length and is influenced by mass transport of the cells through the lumen of the tube. For high length-to-depth (l/d) aspect ratio conduits, such as microvascular constructs, efficient and uniform delivery of the cells throughout the interiors of the channels presents a challenge [12]. Approaches explored to enhance the uniformity and efficiency of cell seeding of small-bore (<6 mm) vascular grafts have included the use of dynamic perfusion-based systems [13], vacuum [14], and radial magnetic fields applied to cells tagged with magnetic micro/nanoparticles [12, 15]. Although these methods have demonstrated success for small-diameter grafts (4-6 mm), they may not be suitable for the seeding of microvascular conduits. Magnetic particles can result in flow obstruction due to plugging of microchannels and residual magnetic particles can potentially introduce toxicity. It may also be challenging to obtain radially uniform fluidic or magnetic force fields throughout a two-dimensional microvascular network. Additionally, the reported approaches may potentially subject cells to substantial mechanical stress that could induce cell death.

This paper reports the design of an elastomeric reentrant microvascular network that supports efficient and spatially homogeneous endothelial cell seeding. The deformability of the fabricated reentrant construct was utilized for simultaneous introduction of endothelial cells throughout the entire length of the microvascular network. The dynamic seeding process was followed by cell culture to promote tubular cellularization with enhanced uniformity.

II. DEVICE OPERATION

The proposed microvascular construct was comprised of deformable, reentrant microfluidic channels (Fig 1a). This construct allowed for a 'stretch - seed - seal' ('3S') operation, (Fig 1b-d). The width of the cleft on the sealing wall was designed to be approximately twice the diameter of an endothelial cell. This strategy enabled the incorporation of cells into microchannels when the cleft was in the open state (under tension), while preventing cell loss when the cleft was in the relaxed or closed state (under compression).

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To enable endothelial cell seeding, the device was stretched or bent outwards to widen the clefts followed by relaxation or compression of the elastomeric device to close the clefts. The seeded cells were then allowed to proliferate and conformally line the microvascular channels. It was postulated that the reentrant geometry of the deformable microvascular network and a '3S' approach would allow uniform cell deposition without mass transport limitations typically associated with high aspect ratio microchannels.

a) *Relaxed state*

Cleft

Sealing wall

Fibronectin

b) Cell seeding in the *open state* (under tension)

c) Cell culture in the *relaxed state*

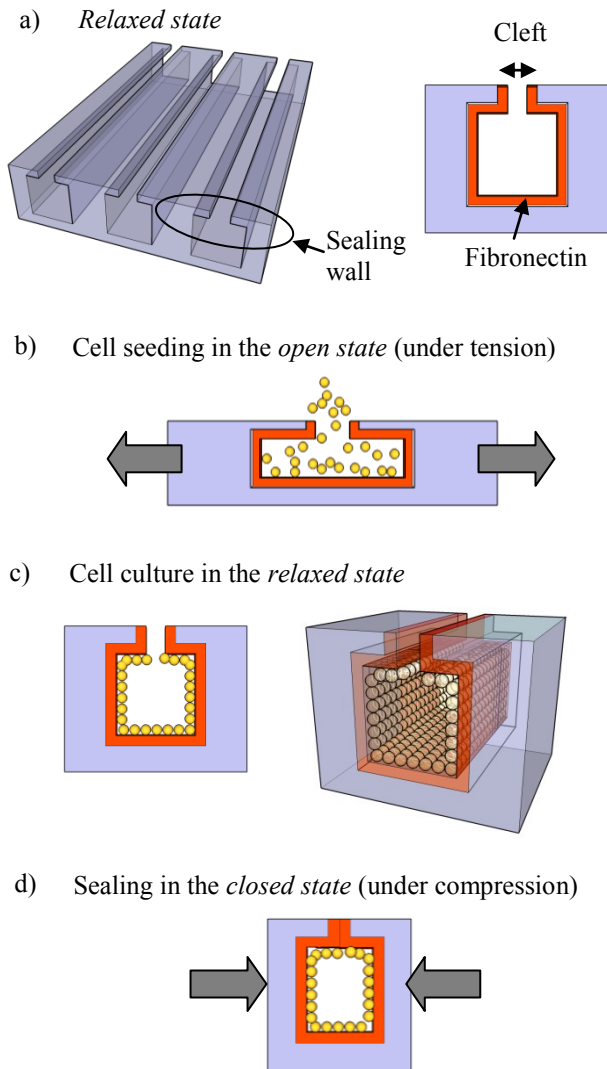d) Sealing in the *closed state* (under compression)

Fig. 1. A schematic illustration of the 'stretch - seed cells - seal' operation for endothelialization of a PDMS reentrant microvascular scaffold: a) reentrant microvascular scaffold in the relaxed state, b) stretch and seed cells in the open state, c) release and culture cells to form a spatially uniform endothelial cell layer, and d) seal in the closed state.

III. FABRICATION

The fabrication process for a PDMS reentrant microvascular scaffold is illustrated in Fig 2. A titanium-copper-titanium metal seed layer was sputter-coated on a (100) silicon wafer. A 10 μm thick positive photoresist AZ4620 (Hoechst Celanese Corp., Somerville, NJ) was spin-coated and patterned on the seed layer. This layer defined the thickness of the overhanging sealing wall. A second layer of negative photoresist NR21-20000P (Futurrex Inc., Franklin, NJ) was spin-coated and patterned on the existing layer of photoresist to form an 80-100 μm thick film defining the depth of the microchannels. The negative mold for the PDMS scaffold was constructed by electroplating nickel on the fabricated photoresist mold. The photoresist was etched away using acetone and oxygen/trifluoromethane plasma reactive ion etching (RIE) (250 mTorr, 300 W, 30 minutes), leaving behind the nickel negative mold (Fig 3a). PDMS (Sylgard 184, Dow Corning Co., Midland, MI) was cast on the nickel mold, degassed in vacuum, and cured at 60 °C for 10 hours. The PDMS conformed to the shape of the metal mold to form a reentrant microvascular construct. Finally, the PDMS construct with reentrant trenches was separated from the metal mold (Fig 3b-c).

The microchannel clefts (20-30 μm) were designed to be approximately 2 times the cell diameter (12-15 μm) and the microchannel width was designed to be 80 μm. An l/d aspect ratio of >200 (microchannel depth: 80-100 μm, microchannel length: 2 cm) was maintained. The cleft openings in the resultant deformable reentrant trenches could be widened and sealed by applying tensile and compressive loads, respectively (Fig 3d-e).

a) Seed layer deposition

b) Patterning of two photoresist layers

c) Nickel electroplating

d) Photoresist removal

e) PDMS molding

f) PDMS reentrant construct separation

Silicon

Metal seed layer

Photoresist layer-1

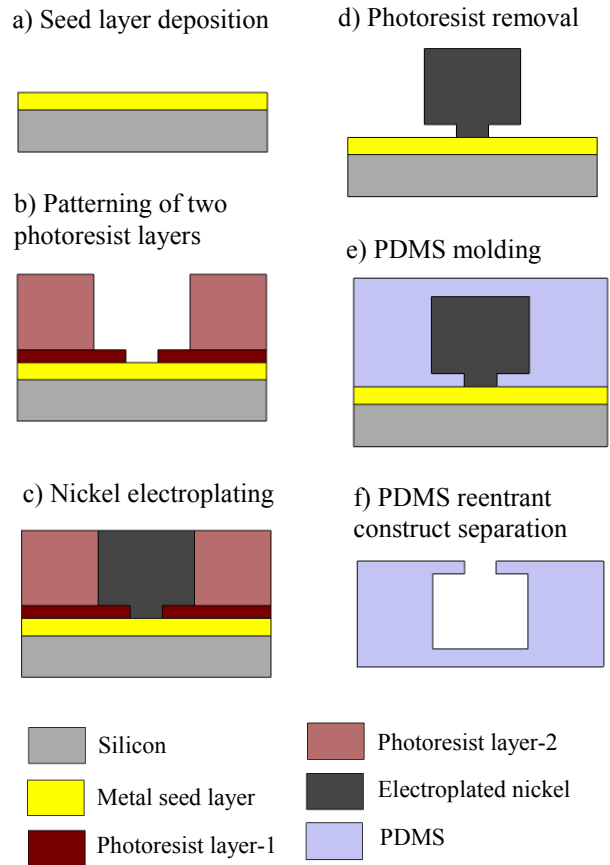Photoresist layer-2

Electroplated nickel

PDMS

Fig 2. Fabrication process flow for a PDMS reentrant microvascular scaffold.
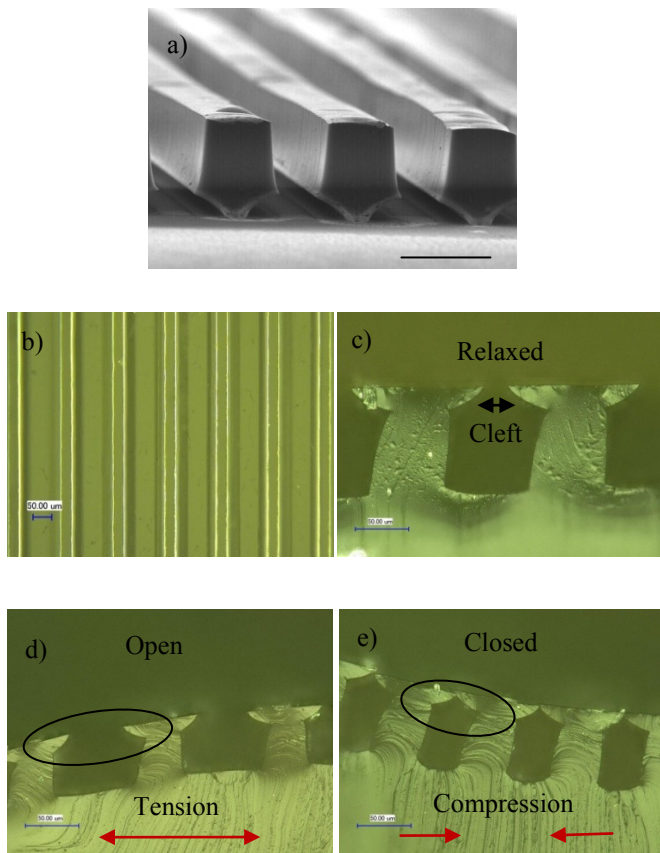
Fig 3. a) Electroplated nickel mold for a reentrant microvascular scaffold (scale bar 100 µm). b) Top view and c) cross section view of a PDMS reentrant microvascular scaffold in the relaxed state (scale bars 50 µm). A deformable reentrant microvascular scaffold in d) open and e) closed states (scale bars 50 µm).

IV. ENDOTHELIALIZATION OF THE REENTRANT MICROVASCULAR SCAFFOLDS

Cultured human umbilical vein endothelial cells (HUVECs) were utilized for endothelialization of the PDMS reentrant microvascular scaffolds. The HUVECs used in the current work were cultured using a standard protocol. Cytopreserved HUVECs (Lonza Inc.) were thawed and cultured in a cell culture flask with endothelial cell growth medium. Cultured HUVECs were spun down to a pellet and resuspended in serum free media at 10^6 cells/mL.

Endothelialization of the microvascular network was performed using the described '3S' approach. The cell seeding protocol was adopted from Rosano et al. [4]. In preparation for cell seeding, the reentrant microvascular scaffold was first treated with oxygen plasma (250 mTorr, 50 W, 30sec) to render it hydrophilic. It was then sterilized in ethanol for 2 hours. After vacuum drying, it was incubated in 8 mL of 50 µg/mL fibronectin (Sigma-Aldrich Inc.) for 24 hours at 4 °C to ascertain the adhesion of the cells to the microchannels. The reentrant microvascular scaffold was washed with 5 mL of PBS to remove excess fibronectin. It was placed in full serum media at 37 °C for 2 hours prior to cell seeding.

An acrylic tensile loading apparatus was used to load the PDMS reentrant microvascular scaffold in tension to widen the clefts (Fig 4). The apparatus consisted of two clasps to secure the scaffold and a screw joining the clasps for the application of a tensile strain (60-100 %) on the scaffold. HUVECs in the cell media (150 µL, 10^6 cells/mL) were released on the construct in the open state and the tensile loading apparatus was placed on a microplate shaker for 20 minutes. Seeding under dynamic conditions allowed the cells to assemble into the trenches and adhere to the interior walls of the scaffold. A total of 5 mL of cell media was introduced and the cells cultured in the relaxed scaffold for 5 days at 37 °C incubator.

After the prescribed time, the microchannel scaffold was washed in Dulbecco's phosphate buffer saline (DPBS) to remove the non-adherent cells. The microvascular scaffold was immersed in dilute (2 µM) calcein AM (Invitrogen Corp.) in DPBS to label the cells and incubated at 37 °C for 1 hour. The scaffold was then imaged using confocal microscopy (wavelength: 488 nm) to analyze the uniformity of cell seeding.
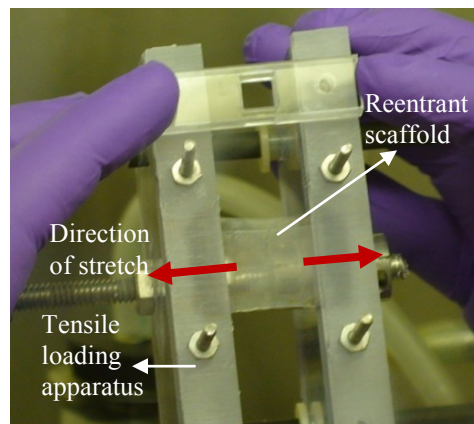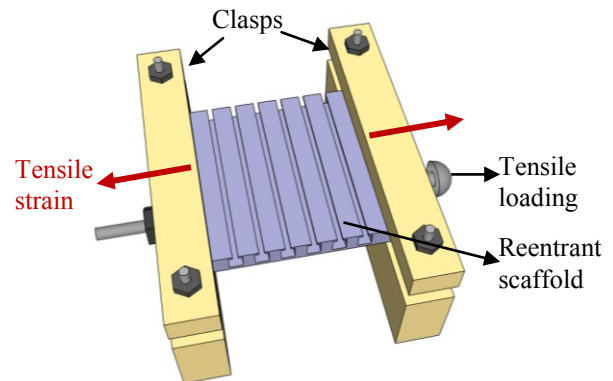

Fig 4. A tensile loading apparatus for widening the microchannel clefts prior to cell seeding.

Imaging demonstrated that HUVECs were successfully seeded throughout the entire 2 cm length of the microchannels (Fig. 5). Cells confluently lined the walls of the open channels forming tubular constructs. The cellular layer was observed to yield a cylindrical profile. The clefts could be sealed under compression resulting in a closed channel network (Fig. 5b), thereby, demonstrating the

feasibility of this approach for producing spatially uniform, endothelialized, high l/d aspect ratio microvascular networks. The rupture strength of the seal can potentially be enhanced by bonding a PDMS film to the construct or by reinforcing the cellular sheet bridging the seal with tissue matrix (such as collagen), if necessary.

a)

Top view illustrating HUVEC seeding on the vertical walls of a reentrant microvascular scaffold in the open state

Cross section view illustrating conformal tubular HUVEC seeding in a reentrant construct

b)

Top view illustrating a reentrant microvascular scaffold in the closed state with confluently seeded HUVECs
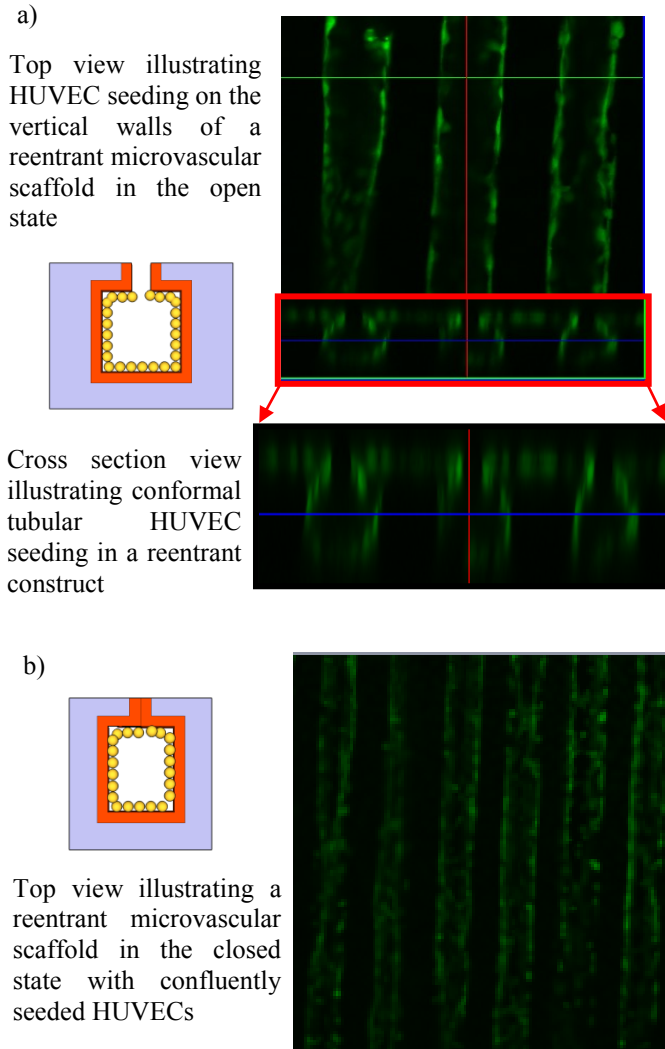
Fig 5. Confocal microscopy images of a 5-day endothelial cell culture in a reentrant microvascular scaffold in the a) open state, and b) closed state. The cells were conformally seeded inside the reentrant channels spanning the entire length to form tubular structures.

V. CONCLUSION

MEMS strategies were explored for the fabrication of a deformable PDMS microvascular scaffold with sealable reentrant microchannels. The deformability of the network could be exploited for a spatially homogeneous and efficient incorporation of endothelial cells onto the lumens of high l/d aspect ratio microvascular channels.

In principle, this approach can be extended to the fabrication of higher l/d aspect ratio 'microcapillary' constructs where microchannel dimensions may be on the order of 1-2 cell dimensions. The future work would focus on investigating the dimensional scalability of this strategy and also the biomechanical and fluid flow characteristics of the proposed microvascular construct.

REFERENCES

[1] Borenstein J. T., Terai H., King K. R., Weinberg E. J., Kaazempur-Mofrad M. R., Vacanti J. P, "Microfabrication technology for vascularized tissue engineering," Biomedical Microdevices, Jul 2002, 4(3), p 167-75

[2] Shin M., Matsuda K., Ishii O., Terai H., Kaazempur-Mofrad M., Borenstein J., Detmar M., Vacanti J. P., "Endothelialized networks with a vascular geometry in microfabricated poly(dimethyl siloxane)," Biomedical Microdevices, Dec 2004, 6(4), p 269-278,

[3] Shevkoplyas S. S., Yoshida T., Gifford S. C., Bitensky M. W., "Direct measurement of the impact of impaired erythrocyte deformability on microvascular network perfusion in a microfluidic device," Lab on a chip, 2006, 6(7), p 914-920

[4] Rosano J. M., Tousi N., Scott R. C., Krynska B., Rizzo V., Prabhakarpandian B., Pant K., Sundaram S., Kiani M. F., "A physiologically realistic in vitro model of microvascular networks," Biomedical Microdevices, Oct 2009, 11(5), p 1051-1057

[5] King K. R., Wang C. C. J., Kaazempur-Mofrad M. R., Vacanti J. P., Borenstein J. T., "Biodegradable microfluidics," Advanced Materials, Nov 2004, 16(22), p 2007+

[6] Armani D. K., Liu C., "Microfabrication technology for polycaprolactone, a biodegradable polymer," Journal of Micromechanics and Microengineering, , Mar 2000, 10(1), p 80-84

[7] Fidkowski C., Kaazempur-Mofrad M. R., Borenstein J., Vacanti J. P., Langer R., Wang Y., "Endothelialized microvasculature based on a biodegradable elastomer," Tissue Engineering, Jan 2005, 11(1-2), p 302-309

[8] Pasic M., MullerGlauser W., vonSegesser L., Odermatt B., Lachat M., Turina M., "Endothelial cell seeding improves patency of synthetic vascular grafts: Manual versus automatized method," European journal of cardio-thoracic surgery, May 1996, 10(5), p 372-379

[9] Burg K. J. L., Holder W. D., Culberson C. R., Beiler R. J., Greene K. G., Loebsack A. B., Roland W. D., Eiselt P., Mooney D. J., Halberstadt C. R., "Comparative study of seeding methods for three-dimensional polymeric scaffolds," Journal of Biomedical Materials Research, Sep 2000, 51(4), p 642-649

[10] Fernandez P., Deguette A., Pothuaud L., Belleannee G., Coste P., Bordenave L., "Quality control assessment of ePTFE precoating procedure for in vitro endothelial cell seeding," Biomaterials, Aug 2005, 26(24), p 5042-7

[11] Shan-hui H., I-jine T, Da-jun L, Chen, D. C., "The effect of dynamic culture conditions on endothelial cell seeding and retention on small diameter polyurethane vascular grafts," Medical Engineering & Physics, Apr 2005, 27(3), p 267-72

[12] Perea H, Aigner J, Hopfner U, Wintermantel E., "Direct magnetic tubular cell seeding: A novel approach for vascular tissue engineering," Cells Tissues Organs , 2006, 183(3), p 156-165

[13] He W., Ma Z. W., Teo W. E., Yi X. D., Robless P. A., Thiam, C. L., Ramakrishna S., "Tubular nanofiber scaffolds for tissue engineered small-diameter vascular grafts," Journal of Biomedical Materials Research Part A, Jul 2009, 90A(1), p 205-216

[14] Nieponice A., Soletti L., Guan J. J., Deasy B. M., Huard J., Wagner W.R., Vorp D. A., " Development of a tissue-engineered vascular graft combining a biodegradable scaffold, muscle-derived stem cells and a rotational vacuum seeding technique," Biomaterials, Mar 2008, 29(7), p 825-833

[15] Ito A, Ino K, Hayashida M, Kobayashi T, Matsunuma H, Kagami H, Ueda M, Honda H, "Novel methodology for fabrication of tissue-engineered tubular constructs using magnetite nanoparticles and magnetic force," Tissue Engineering, Sep 2005, 11(9-10), p 1553-1561