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Šeila Selimović,[ab] Mark W. Tibbitt,[c] Mehmet R. Dokmeci[ab] and Ali Khademhosseini*[abde]

Capillary networks for *in vitro* permeability screening

With few exceptions, the tissues of the body are permeated by a complex vascular network that serves to transport nutrients, signaling molecules, and waste to and from the cells that reside within the tissue.[1] Often, this vascularization is exploited to deliver antibodies, growth factors, or small molecule drugs locally to tissues in order to mitigate disease or encourage regeneration.[2] However, to design effective therapeutics, delivery systems, and dosing strategies, an improved understanding of the barrier and transport properties of the microcapillaries that comprise the periphery of the vasculature tree is required. Furthermore, improved strategies to engineer and construct macroscale vessel analogs will improve the translation of tissue engineering and replacement. Thus, there remains a desire to design scalable methods to produce capillary analogs *in vitro* to study molecular transport and incorporate functional vessels into engineered tissue replacements.

To address this need, Yoshida *et al.* have recently reported a unique strategy to fabricate multilayered capillary analogs within 3D hydrogel constructs.[3] The capillaries were formed within uniaxial microchannels inside poly(γ-glutamic acid) (γ-PGA) hydrogels by coating the templated channels with umbilical artery smooth muscle cells (UASMCs) and human umbilical vein endothelial cells (HUVECs). By forming these multilayered vessels within γ-PGA hydrogels the authors were able to recapitulate the multicellular structure of native capillaries while preserving the ability to study molecular transport across the engineered vessel. In this manner, these engineered capillaries could be employed to study the transport properties of human vasculature and to perfuse neo-tissue with functional vasculature.

To form the templated microchannels, Yoshida and colleagues crosslinked γ-PGA hydrogels around silica microtubes that were then removed (Fig. 1). The diameter of the silica microtubes dictated the inner diameter of the channel (~100s of microns) and the arrangement of the silica microtubes enabled the formation of an interconnected network throughout the γ-PGA hydrogel. Upon microchannel formation, multilayered capillaries were formed by the hierarchical cell manipulation technique, which was previously employed by the authors to form multilayered cell sheets.[4] In short, a layer-by-layer technique was exploited to coat the channels with a thin film of fibronectin–gelatin (FN–G) to which most adherent cells can attach. Then, the hydrogel construct was immersed in a cell suspension of UASMCs to form a smooth muscle layer on the interior of the microchannels. This process was repeated to coat the UASMCs with a thin film of FN–G and then the construct was placed in a cell suspension of HUVECs to attach a layer of endothelial cells to the engineered capillary (Fig. 1).

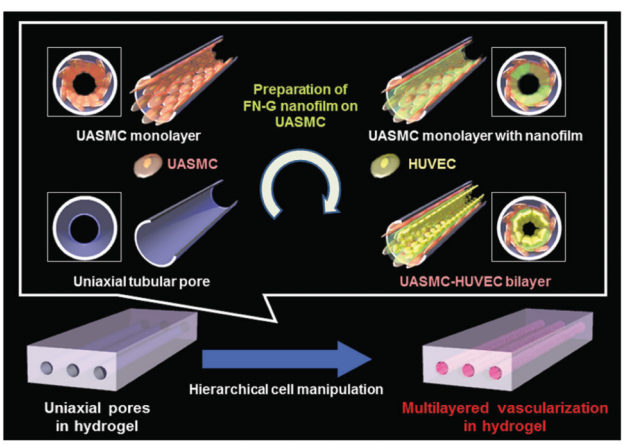

Fig. 1 Schematic of capillary analog fabrication within hydrogel microchannels, through a sequential deposition of fibronectin–gelatin thin films and smooth muscle cell or endothelial cell monolayers. Figure reprinted with permission from Yoshida *et al.*[3]

[a]*Center for Biomedical Engineering, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Cambridge, Massachusetts 02139, USA. E-mail: alik@rics.bwh.harvard.edu*
[b]*Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA*
[c]*Department of Chemical and Biological Engineering, University of Colorado, Boulder, Colorado 80303, USA*
[d]*Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, Massachusetts 02115, USA*
[e]*World Premier International – Advanced Institute for Materials Research (WPI-AIMR), Tohoku University, Sendai 980-8577, Japan*

After forming capillary analogs through this sequential process, the authors demonstrated that intact capillaries (over 1 cm in length) could be liberated from the γ-PGA hydrogels by dissolving the gels in a cysteine solution that reduces the disulfide bonds that form the gel. In subsequent experiments, the authors perfused the microchannels of the γ-PGA gels with fluorescently labeled macromolecules to investigate the barrier properties of the capillary analogs. Naked microchannels free of cells allowed for the diffusion of macromolecules into the bulk of the surrounding hydrogel whereas cell coated microchannels limited diffusion. For fluorescently labeled albumin the diffusion rate in naked channels was 7-fold higher than that for the cell coated channels, which indicated that the cell bilayers were able to recapitulate the barrier properties of native capillaries.

The multilayered capillary analogs formed within hydrogel microchannels present unique opportunities to create vasculature and to investigate transport and barrier properties of capillaries outside of the body. The scalable and facile synthesis method should enable the translation of this technique to study systematically how drugs and cells interact with capillary tissue. Further improvements may enable the fabrication of tree-like vasculature in co-culture with other cell types during *ex vivo* organ growth and development.

DNA detection on a printed circuit board with integrated microfluidics

A number of novel diagnostic microfluidic devices use nucleic acid extraction and sequence-specific detection to identify and quantify exogenous or endogenous nucleic acid sequences. While the sensitivity of the applied nucleic acid extraction methods has steadily been improving and allowing the use of whole blood samples, it has been difficult to incorporate a cell lysis technology into these systems that does not require external control, such as pressure-controlled flow.[5] A fully integrated device including cell lysing, on-chip mixing, and nucleic acid extraction all on the same platform can be a valuable asset to the diagnostics community allowing detection without any need for standard bench-top sample preparation techniques.

Santiago and co-workers have recently introduced a new approach to achieving this goal.[6] They reported an integrated microfluidics chip made on a printed circuit board with electronic components that allow on-chip cell lysis and nucleic acid extraction. The cells are broken open by heat to release the nucleic acids, and the nucleic acids are then extracted and purified *via* isotachophoresis (ITP). In this analytical chemistry technique, an electrical field is applied to a discontinuous buffer system to separate different constituents of the sample. More specifically, the sample is contained in a low-conductivity terminating electrolyte (TE) and allowed to migrate under an applied electrical field towards the high-conductivity leading electrolyte (LE). Because of the difference in conductivities, the electrical field is stronger in the TE than in the LE. Nucleic acids are highly charged so they outpace the co-ions in the TE, and focus quickly at the interface between the two electrolytes, while slower molecules fall behind. This separation allows selective collection of the nucleic acids in the sample. A schematic detailing this process is shown in Fig. 2.

The device fabricated by Marshall *et al.*[6] consisted of a printed circuit board (PCB) with custom surface-mount heating elements, a polyurethane microfluidic layer (molded from a poly(dimethylsiloxane) master), and a poly(methyl methacrylate) sealing layer that contained the fluidic access ports. The microfluidic structure itself was simple – it included a single 3.7 cm long straight channel connecting the two 15 μl large reservoirs for the TE and LE.

The authors tested the device using whole human blood samples spiked with malaria parasites that had been cultured inside human red blood cells. The LE was first injected into the microfluidic channel and the LE reservoir, and then the TE reservoir was filled with the corresponding electrolyte and the sample in the volumetric ratio of 14 : 1. Upon sealing both reservoirs with an adhesive film, the on-chip heaters were activated for 3 min. The applied current raised the temperature of the TE to a lysing temperature of approximately 70 °C. The efficiency of this lysis protocol was quantified by counting the lysed and whole cells before and after the procedure and the lysis efficiency was determined to be 90%. In addition, fluorescently labelling DNA-containing parasite cells made it possible to visualize them.

After lysing, the adhesive covers were removed and electrodes were inserted into the two reservoirs, with the TE electrode serving as the ground and the LE electrode set to +500 V. The effect of ITP was monitored, among others, by imaging the fluorescently labelled DNA that collected at the ITP interface. ITP was complete when the DNA band eluted into the LE reservoir (see schematic in Fig. 2). The concentrated sample was extracted and evaluated off-chip using quantitative polymerase chain reaction. The nucleic acid product was confirmed to be pure enough to perform PCR, and that the parasites were detectable to a limit of detection of 500 parasites per μL.

The presented device combined temperature heaters and sensors with microfluidics on a printed circuit board to achieve a clinically important evaluation of foreign DNA in whole human blood samples. It did so by integrating all

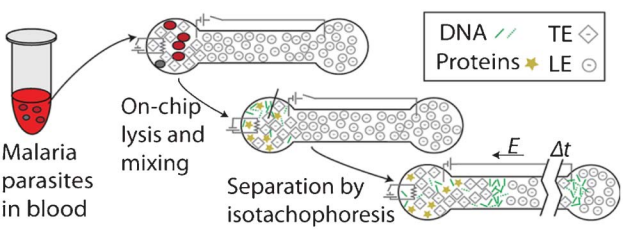

Fig. 2 Schematic of the isotachophoresis process, adapted and reprinted with permission from Marshall *et al.*[6] Copyright 2012 American Chemical Society.

processing steps onto the platform and making them independent from off-chip controls and bench-top scale techniques. Even the current source can be integrated into the device by using a battery. Aside from the high purity and concentration values achieved on-chip, this approach stands out due to the innovative combination of electronics and microfluidics to solve an engineering problem. This example shows how microfluidics can reach its full potential in biomedical research by integrating it with simple electronics platforms in an interdisciplinary approach.

Particle sorting with microfluidic logic circuits

Separation of droplets and (bio-)particles according to their physical properties, such as size or deformability, using microfluidic devices has notable applications in bioengineering and medicine.[7] For example, cells from different tissues in the body may differ in size, while cells found in healthy and cancerous tissues often have different elastic properties. Hence, it is important to develop a microscale approach to particle separation that is label-free, fast, and low-cost with good resolution.

A solution has recently been proposed by Karnik and colleagues[8] in the form of a microfluidic logic gate. By carefully designing the hydrodynamic resistance (the ratio of the pressure drop across a channel to the fluid flow rate along the channel) of individual branches of a simple microfluidic network, Cartas-Ayala *et al.*[8] were able to predict the flow direction of an incoming particle, regardless of its size and deformability. In their analytical approach, the authors assumed a network consisting of an inlet channel that divides into a sensing and a bypass channel, and ends in a sorting and a rejection channel (Fig. 3). According to their calculation, a particle whose size is similar to that of the channel or slightly smaller always travels towards the rejection channel. In contrast, a larger deformable particle is directed towards the sorting channel. This behavior was shown to be a consequence of the changes in the hydrodynamic resistance along the whole fluidic path in the presence of a particle. Namely, the channel dimensions were chosen such that the flow rate through the rejection channel is higher than that through the sorting channel even when a small particle is present in the sensing area. However, when a high-resistance particle enters the sensing channel, thereby partially obstructing it, the flow rate through the bypass channel increases and changes the flow rates at the junction of the sorting and rejection channels. At this point, the highest instantaneous flow rate is found at the entrance to the sorting channel, and the particle follows this path.

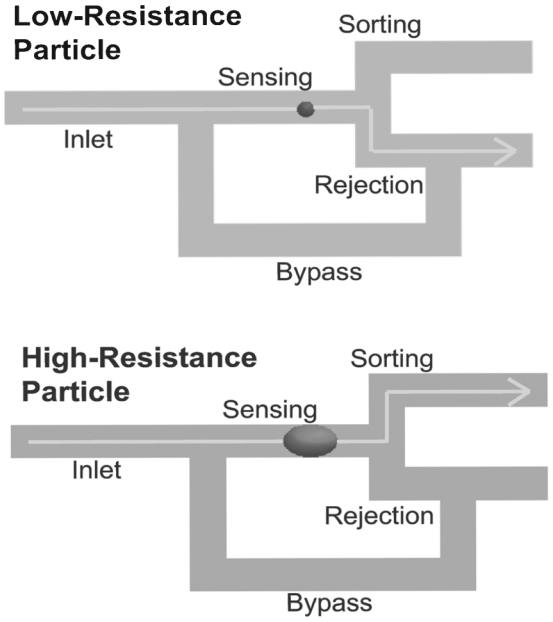

Fig. 3 Schematic of the flow path for a low-resistance and a high-resistance particle, adapted and reprinted with permission from Cartas-Ayala *et al.*[8]

The authors used gelatin particles immersed in mineral oil to validate their design. They chose gelatin as an example of a common biological material with a deformability value similar to that of cells. The particles were generated off-chip and were then introduced into the poly(dimethylsiloxane) device. The diameters of the particles ranged from $\sim$0.6 to 1.2 of the channel width which was 6 μm. The smallest particles ($<0.6D_H$, the hydrodynamic diameter of the channel) were too small to noticeably affect the hydrodynamic resistance of the channel, so they followed the streamlines into either the sorting or rejection channels and were not sorted. When the particle size was in the range of $0.6 < D_H < 1.2$, the particles ended up in the rejection channel (logic gate = 0). Moreover, all the larger particles were sorted into the selection channel (logic gate = 1) with a relatively fast rate of up to 100 particles per second.

In the second experiment, the authors showed that they could select the particles solely on the basis of their deformability. To achieve this goal, they used an up-stream T-junction to generate droplets of UV-curable glue in mineral oil, and then randomly crosslinked them by shining UV light. Thus, all the particles had the same size ($1.2D_H$), but different deformabilities. The crosslinked particles increased the hydrodynamic resistance of the channel more than the droplets did, which was reflected in the logic output of the microfluidic network: the droplets were rejected, while the solid particles entered the sorting channel.

The microfluidic device was highly selective, with a resolution of 13%, meaning that the selected and rejected particles differed by at least a change in diameter of 13% or 1 μm in size. This cut-off is appropriate for sorting biological samples, such as cells. The logic gates break down when two or more particles occupy the channel network simultaneously, however this problem is easily avoided by tuning the feeding rate of particles at the channel input. The current limitation of the device lies in its inability to control particles that are much smaller than the hydrodynamic diameter of the channel. In

practice, this means that impurities in a sample cannot be removed by using this device, making a secondary filtering step necessary. However, perhaps adding secondary bypass channels to the microfluidic network could sufficiently increase the sensitivity of the device allowing it to filter very small particles. Most importantly, this device shows that although the field of microfluidics has made great strides in multiple disciplines, there is still opportunity for traditional fluid mechanics research to achieve significant results for interesting biomedical applications.

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