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Characterization of Leaf-Inspired Microfluidic Chips for Pumpless Fluid Transport

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Abstract

Microfluidic networks are extensively used in miniaturized lab-on-a-chip systems. However, most of the existing microchannels are simply designed and the corresponding microfluidic systems commonly require external pumps to achieve effective fluid transport. Here we employed microfabrication techniques to replicate naturally-optimized leaf venations into synthetic hydrogels for the fabrication of pumpless microfluidic chips. The unique properties of leaf-inspired microfluidic network in convectively transporting fluid were characterized at different inclination angles. Flow velocity inside these microfluidic networks was quantitatively measured with Particle Image Velocimetry (PIV). Mass diffusion from biomimetic microfluidic network to surrounding bulk hydrogels was investigated. The results demonstrate that the leaf-inspired microfluidic network can not only effectively transport fluid without the use of external pumps, but also facilitate rapid mass diffusion within bulk hydrogel chips. These leaf-inspired microfluidic networks could be potentially used to engineer complex pumpless organ-on-a-chip systems.

Keywords: leaf-inspired, microfluidic network, pumpless, lab-on-a-chip system, biomimetics

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1 Introduction

Lab-on-a-chip systems take advantage of microfluidics to develop miniaturized laboratory platforms for various academic researches and engineering applications[1–3]. In comparison with conventional laboratory systems, lab-on-a-chip systems could be flexibly controlled with low reagent consumption and high-throughput production[4]. Currently, most of the existing lab-on-a-chip systems are driven by external forces such as mechanical, centrifugal, electric or magnetic pumps. The weakness associated with these pump-actuated systems is the demand of expensive and complicated external equipment[5].

Pumpless microfluidic devices have thus been developed with the advantages of simplicity, cost effectiveness, portability and no bubbles[6–9]. Beebe's group conducted extensive investigations on passive-pump based microfluidic systems, which have been used for high-throughput cell culture, tissue engineering and cell-free protein expression[10–13]. Khademhosseini's group employed similar passive pump principles to generate concentration or material gradient in a simple microchannel for high-throughput drug testing and cell-biomaterial interaction studies[14,15]. Filter chip was also developed to isolate plasma and viruses from unprocessed whole blood based on size exclusion without the use of a pump[8]. However, the microfluidic networks in these pumpless systems were relatively simple, which were not enough to construct complex lab-on-a-chip systems such as vascularized organ-on-a-chip systems[16–18].

Natural leaves contain complex venation networks. These naturally-optimized microfluidic networks could effectively deliver water and nutrients to every location of leaf without dead zones. The unique property of leaf venations in transporting fluid upward inspires us to design novel pumpless lab-on-a-chip systems with biomimetic microfluidic networks. Although leaf-inspired microfluidic networks were previously embedded into an epoxy matrix using a direct-writing technique[19], it is technically difficult to fabricate capillary-scale channel networks of leaf venations (<50 μm), and external force was required to facilitate fluid flow in

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these microfluidic channels. Microfabrication techniques have been previously employed to accurately transfer multiscale leaf venations into biomaterial hydrogels for the fabrication of perfusable vascularized tissue constructs[20]. Here microfluidic chips with leaf-inspired microchannel networks were fabricated to systematically investigate their unique property in convectively transporting fluid at different inclination angles without the use of external pumps. Flow velocity inside these microfluidic networks was quantitatively measured with Particle Image Velocimetry (PIV). Mass diffusion from biomimetic microfluidic networks to surrounding hydrogels was investigated.

2 Materials and methods

2.1 Fabrication of leaf-inspired microfluidic hydrogel chips

Leaf-inspired microfluidic networks were fabricated using well-established microfabrication techniques[20]. Briefly, mulberry leaf venations were obtained by removing soft tissues of fresh leaves in alkaline solution. After coated with a thin layer of chrome, the leaf venation was used as photomask to transfer biomimetic microfluidic networks into silicon wafer via an inductively coupled plasma etching process. Polydimethylsiloxane (PDMS) mould with negative pattern of leaf venation (Figs.1a and 1b) was replicated from the silicon mould. To fabricate leaf-inspired microfluidic chips, 1 wt% agarose solution was maintained at about 80˚C, cast onto plasma-treated PDMS mould and cooled at 4˚C for 5 minutes. Microfluidic agarose hydrogel was obtained after removing the PDMS mould. A thin layer of agarose solution was uniformly coated and gelled on a transparent glass slide (75 mm × 25 mm). The microfluidic agarose hydrogel was carefully assembled with the gel-coated glass slide to form a closed microchannel network with no bubbles inside. A triangle inlet and a flat outlet were produced to expose the central stem channel. To facilitate the visualization of microchannels inside the microfluidic chip, a red dye solution was perfused into the microfluidic channels. Figs. 1c and 1d show the replicated microfluidic chips as well as internal leaf-inspired microchannels. It is clear to see that the leaf-inspired chips consist of multiscale microchannels with the width ranging from 30 μm to 1 mm and the depth of 150 μm.

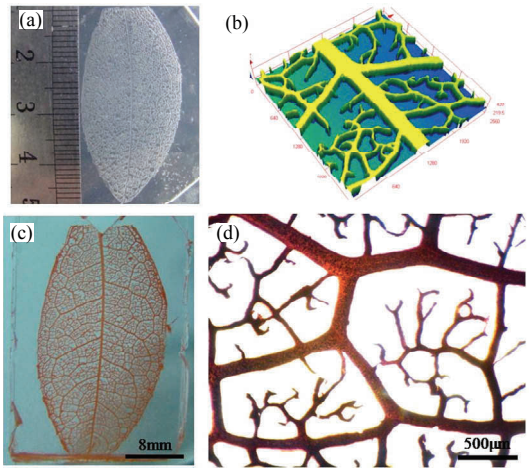

Fig. 1 Micromolding of leaf-inspired microfluidic chip. (a) PDMS mould with negative patterns of leaf-inspired microfluidic network; (b) 3D microstructure of PDMS mould; (c) microfluidic chip prefilled with red dye solution; (d) leaf-inspired internal microchannels.

2.2 Characterization of pumpless flow inside leaf-inspired microfluidic chips

To investigate the pumpless flow within the leaf-inspired microfluidic networks, the microfluidic chips were placed at different inclination angles of 90˚, 60˚, 30˚, 0˚, −30˚, −60˚ and −90˚. A red dye solution was prepared and gradually pipetted into the triangle inlet of the microfluidic chips. The continuous flow of the red dye solution inside the microfluidic network was recorded using a Sony camcorder. To quantitatively characterize the fluid flow, the perfused images were extracted from the flow video every 4 seconds and the perfusion area was measured in ImageJ software. The perfusion ratio was calculated as the ratio of perfusion area to the whole area of the microfluidic region.

2.3 Pumpless transport of microparticles inside leaf-inspired microfluidic chips

To investigate the pumpless transport of microparticles within the leaf-inspired microchannels, the microfluidic chip was placed horizontally. Fluorescent microbeads with 10 μm diameter (Thermo Scientific), similar to the size of mammalian cells, were diluted by 10 times and pipetted into the triangle inlet. The flow of fluorescent microbeads inside the microfluidic network was recorded with a fluorescent microscope (Nikon Ti-5, Japan). To quantitatively characterize the flow velocity inside the leaf-inspired microfluidic network, smaller