

Manufacturing of microcirculation phantoms using rapid prototyping technologies

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Abstract— In this paper, we describe a method for the manufacturing of a microcirculation phantom that may be used to investigate hemodynamics using optics based methods. We made an Acrylonitrile Butadiene Styrene (ABS) negative mold, manufactured in a Fused Deposition Modelling (FDM) printer, embedded it in Polydimethylsiloxane (PDMS) and dissolved it from within using acetone. We successfully made an enlarged three-dimensional (3D) network of microcirculation, and tested it using red blood cell (RBC) analogues. This phantom may be used for testing medical imaging technology.

I. INTRODUCTION

Microcirculation networks consist of vessels $<100\mu\text{m}$ in diameter; arterioles, capillaries (where oxygen exchange takes place) and venules. Angiogenesis is a common feature of almost all diseases involving the proliferation of new blood vessels at the level of microcirculation [1]. There is considerable interest in understanding the role of microcirculation in terms of hemodynamics and in the development of techniques to measure perfusion [2-4]. 'Phantoms' mimic the geometry and physical properties of the tissues, allowing comparison of properties measured using imaging with known properties in the phantom. These are widely used in medical imaging [5]. Optically transparent phantoms may be used with optical measurement techniques to explore the local hemodynamics in detail. Optical measurement techniques include particle image velocimetry, laser Doppler anemometry and video imaging.

Manufacturing anatomically correct phantoms is challenging. Phantoms of arteries have been manufactured using a process in which a solid model is 3D printed in resin, followed by creation of a silicone mold from the resin model. Low-melting point alloy is poured into the mold to create an anatomical model, which is then incorporated into a phantom using a lost-core technique [6-8].

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For medical imaging phantoms, mimicking microcirculation has been extremely crude consisting of using the small cellulose vessels contained within a dialysis cartridge [9]. Microcirculation phantoms which are optically transparent have previously been described by Schirrmann [10] who were interested in creating 2D models of the pulmonary vasculature. To date there has been no microcirculation phantom which accounts for the 3D geometry.

The aim of the current paper is to develop a method for creating a 3D model of the microcirculation. In the first instance this is a magnified model to establish the manufacturing process. The methods used in this paper draw on the field of microfluidics, which is reviewed below.

The conventional method to manufacture microfluidic chips is via soft lithography. Commonly, the pattern is designed with CAD software, and manufactured into a photomask. A silicon or glass wafer is coated with light sensitive SU-8 and exposed with UV light through the mask to produce a mold. A soft pre-polymer can then be poured onto the mold wafer. Once cured it is peeled off the wafer and bonded to a plain layer of polymer or a glass plate to seal the channels. 3D structures can be achieved by making a pile of patterned layers, connected via vertical channels [11, 12]. Although soft lithography allows for complex designs with fine details, it remains a 2D process and requires access to specialized micro-fabrication facilities. Theriault et al. [13] developed a method to improve on soft lithography. They 3D printed a sacrificial material, creating a scaffold of circular channels overlapping each other. An epoxy resin was poured around the scaffold and cured before the sacrificial material was removed. Then the gaps were filled with a photocurable resin, before carefully designed masks were placed around the device, to selectively cure the resin. All the unexposed resin was washed out with water, leaving the desired network of channels behind. This technique avoids having to bond each layer one after the other and creates circular channels. Freitas and others [15] elaborated a methodology to make an optically transparent blood flow phantom. The fluidic network was modelled from CT scan data into a printable file. A negative mold was made out of ABS with a FDM printer and smoothed with sand paper. PDMS was then poured over the mold and cured. To finish the device, the mold was mechanically pulled out of the PDMS block, leaving a void. Geoghegan and others [16] proposed a different approach by using a sacrificial material. The adopted a different 3D printing technology with plaster

powder bound with small drops of glue to form the object. PDMS was injected around the mold and left to cure. Finally, the mold was washed away with water, leaving a fluidic network replicate. Unfortunately, those methods cannot be used to manufacture an intricate network of channels, like a realistic network of blood vessels.

PDMS is widely used in microfluidics because of its low cost, ease of use and biocompatibility [12, 17, 18]. PDMS will swell in solvents but the degree of swelling depends upon the solvent used [19, 20]. If the swelling is too large, it can damage the microfluidic device. Although it can be seen as problematic, these swelling properties can be used to bring a solvent inside the PDMS block, with the possibility to dissolve/destroy a solid material from within, like a mold. Polar solvents like acetone can have a dramatic effect on some common plastics, like ABS which is completely soluble in acetone. ABS is a common thermoplastic used in FDM printers by manufacturers such as Stratasys (Eden Prairie, MN 55344, USA). On the other hand, the swelling of PDMS in acetone is limited, 20% by mass on average [19, 21], so it should not have a structural effect on the microfluidic device. Furthermore, acetone evaporates quickly, under standard conditions, due to a high vapor pressure (24.5kPa at 20°C).

II. METHODOLOGY

A. A Fabrication of complex 3D micro-channels

The manufacturing of the microfluidic devices is achieved by following those steps:

1. We manufactured a sacrificial ABS fluidic network using a FDM 3D printer.
2. We sprayed the microfluidic structure with acetone and quickly dried it with compressed air in order to smooth the surface. To prevent damage to the microfluidic structure, we used successive, small exposures. This method was necessary because the FDM printing technology creates a rough surface, inadequate for an efficient flow.
3. We prepared the PDMS, degassed and poured it around the fluidic network. We took extra care to prevent the formation of bubbles during the pouring stage. We then placed the mold in a convection oven at 50°C. The amount of time it should stay in the oven depends upon the dimension of the device, the bigger the longer.
4. We removed the cured PDMS out of the mold and placed it in acetone to dissolve the ABS trapped inside. The microfluidic device remained in a closed container (at room temperature with no mixing) to prevent evaporation of the acetone.
5. After the ABS was liquefied we flushed it out of the channels.
6. Finally, we left the PDMS to rest in air until the acetone was fully evaporated.

Fig. 1 shows this process being used to manufacture a relatively simple 3D geometry.

B. Capillary network analogue design

We designed the vascular model to replicate the branching of blood vessels from a small arteriole to a bed of capillaries, with a scale of 150:1.

The inlet arteriole has a diameter of 3 mm and, following Murray's law, branches into several channels to form a capillary bed, with a diameter of 1.5 mm. The capillaries then merge back into a single channel to form the outlet of the vascular network. The dimensions of the vascular model are 154 x 54 x 33 mm. We produced the CAD design using SolidWorks 2012 (Dassault Systèmes SolidWorks Corporation, Waltham, MA 02451, USA).

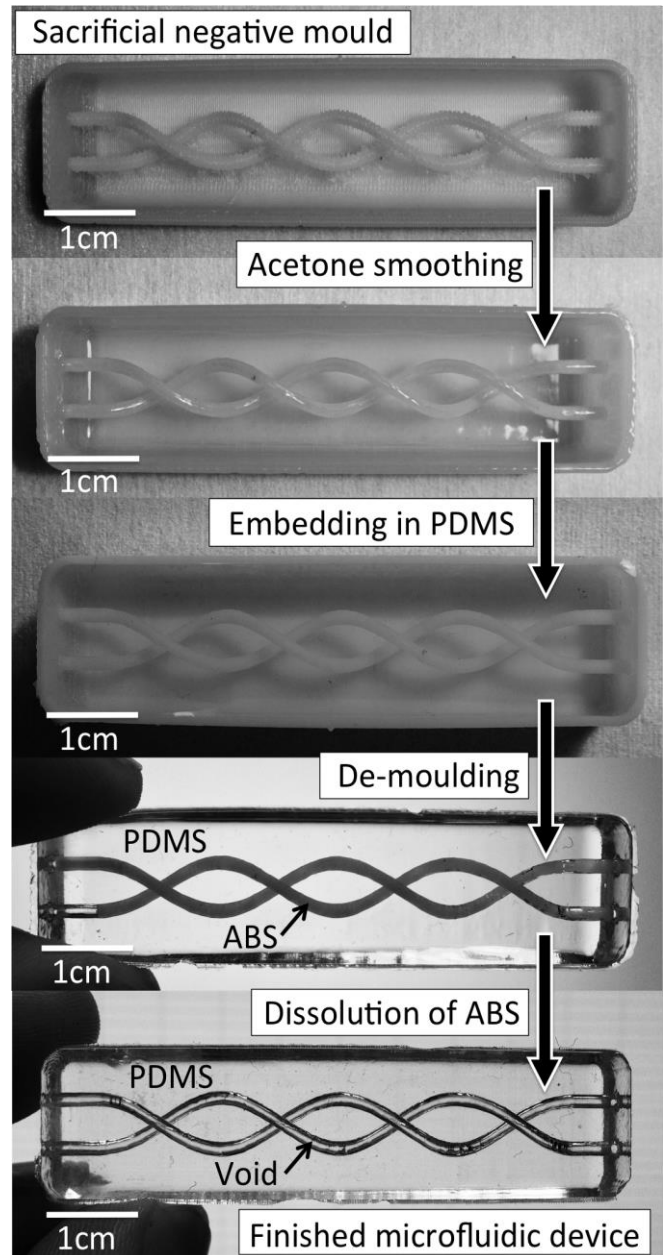


Figure 1. Process flow for the manufacturing of complex 3D networks in PDMS

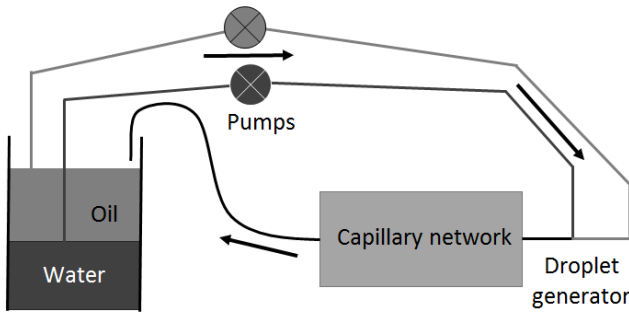


Figure 2. Experimental setup with water & oil reservoir, pumps, droplets generator and vascular network.

We left the vascular network replica in the oven overnight to cure the large volume of PDMS. It then took 2 days for the acetone to fully dissolve the ABS before we could flush it out.

Most of the liquefied ABS could be pushed out using a pipette but we had to clear some of the channels using compressed air, which took about 1 hour. Another 2 days was necessary for the acetone to fully evaporate out of the PDMS.

Fig. 2 shows a schematic diagram of the experimental setup. To represent the blood flow in the vascular network we used sunflower oil as a plasma analogue and water droplets as the RBC analogue. The water droplets are produced by a droplet generator before the blood analogue enters the vascular network. We set the flow rate of oil at 0.6 ml/s and the flow rate of water at 0.3 ml/s. We added blue dye to the water to help us to visualize movement of the droplets in the channels. The pictures and slow motion videos, at 240 FPS, were taken with an iPhone 6.

III. RESULTS

The general view of the phantom is presented in Fig. 3. We observe that when the bifurcation angle is reduced, the flow in the daughter channels is fast and contains a high density of droplets. This density reduces significantly when the channels located after a bifurcation are near vertical. Fig. 4 illustrates the flow distribution in the capillaries regarding their distance from a main arteriole, the flow is faster when the capillaries are closer to a bigger channel. We made those observations based on the slow motion video than can be downloaded from the link given in the appendix.

IV. DISCUSSION

During this experiment we were able to make a complex network representing arterioles branching in a capillary bed, with a flow analogue to blood flow. With this vascular network analogue we established proof-of-principle for the manufacturing technique. Future work will be concerned with reducing the scaling factor to make the dimensions more comparable with the microcirculation.

Future work will also be concerned with using the phantom to investigate hemodynamics, in the first instance

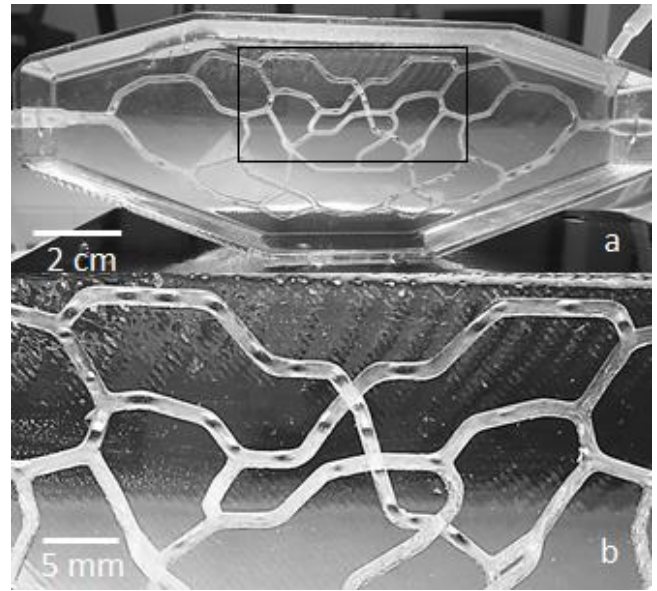


Figure 3. General view (a) and close-up view (b) of the vascular network showing the flow of water droplets (RBC analogue) in sunflower oil (plasma analogue).

by analysis of the video data, and also by automated measurement using embedded sensors. The current phantom would be of use in the development of macrovascular imaging methodologies such as Ultrasound Color Doppler or Spectral Doppler. However, a number of other tools are available, for example Diffusion MRI.

Large and complex vascular network could also be manufacture to study obstructions cause by blood clot or plaque rupture to observe the how the flow is modified in the whole network. Such networks could be used to study the effect on flow distribution when the vascular network is

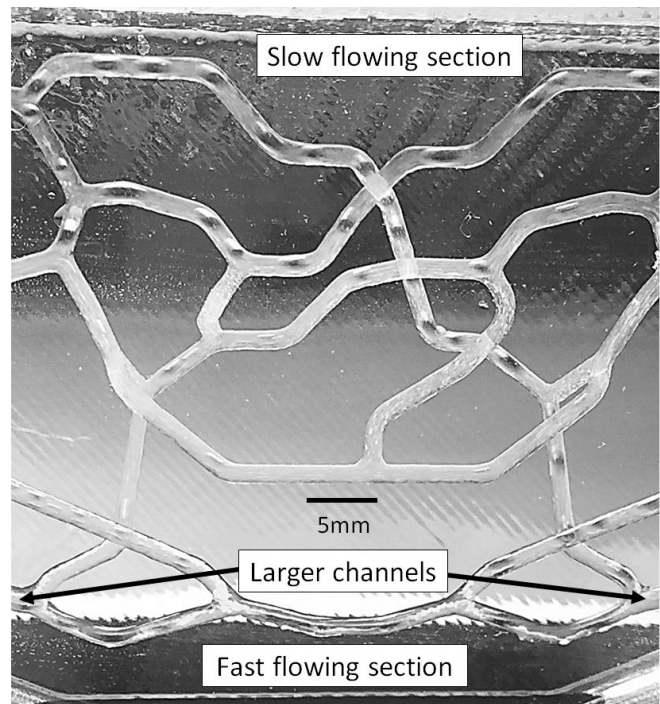


Figure 4. The flow velocity in capillary is high whenever the capillaries are close and lower when far from an arteriole.

affected by cardiovascular diseases.

This manufacturing method has the advantage of being easy to implement as it requires no expensive equipment or technical expertise and the materials are of low cost. By using a different 3D printing technology, it is also possible to reduce the dimensions of the channels and achieve true microvascular dimensions. UV cured resins can achieve much higher resolution, with layer thickness as low as 25 μm . Guo et al. [21] developed a technique in which a thermoplastic is dissolved in a solvent and used as an additive material for 3D printing. This ensures that the material used for the negative mold will be dissolvable once imbedded in PDMS.

V. CONCLUSION

We have demonstrated a manufacturing technique that is able to produce 3D models for future use in microcirculation phantoms. This methodology may be used to provide phantoms for the development of medical imaging modes.

APPENDIX

Videos of the flow in the vascular network can be downloaded at the following link:

<https://www.dropbox.com/sh/3zifx534c2tjm4n/AACkhjADXfuF-GVMaI8bCwn0a?dl=0>

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