

MICROFABRICATION OF CIRCULAR CROSS-SECTION MICROCHANNELS FOR THROMBOSIS ASSAYS

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INTRODUCTION

The ability to accurately, inexpensively, quickly and repeatably fabricate sub-millimeter scale features has made soft-lithography based microfluidics a vital tool for the biomedical research and lab-on-a-chip assays. However, traditional methods used in this field, such as photolithography, deposition, and etching are generally limited to creating features with rectangular cross-sections, low aspect ratios, and feature heights below 200 μm [1]. These limitations cause several problems in the application of microfluidics to biological assays. Specifically, rectangular cross sections induce non-physiological gradients in fluid shear rate, velocity, and pressure in contrast with the rounded cross-sections found in most natural physiological systems, such as blood vessels and airways. Further, fabrication of features with multiple heights is complex and costly. Finally, casting and releasing around complex features can be problematic. Thus the development of methods for fabricating a wide range of heights and cross-sections within the same device would provide a valuable complement or replacement to standard microfabrication techniques.

Although previous groups have formulated methods for obtaining rounded channels such as reflow of photoresist during photolithography of the device mold, molding through the use of small wires, or by using additional coating hexanes within square channels, all of these methods have shown poor control over the resultant geometries and are unable to vary shapes and aspect ratios [1,2]. In contrast, micromilling channel molds is an option which can address a wide range of features and dimensions.

In this work we present a technique for the fabrication of an array of micro-scale channels of varying cross-sectional shape and areas with dimensions from 100 – 750 μm . In addition, we demonstrate the utility of these channels for investigating effects of varying local flow conditions on assessing

MATERIALS AND METHODS

Device Design and Modeling

Heart attacks occur when the coronary arteries fill with clots which prevent the flow of blood—a process known as thrombosis. Presence of local constrictions within these vessels can induce high shear rates and accelerate formation of occlusive clots. Assessment of heart attack susceptibility is often clinically characterized by the percentage of reduction in cross-sectional area as well as by the eccentricity of the vessel [3,4]. Recent intravascular angiographic clinical studies of human coronary arteries affected by atherosclerosis have shown that affected vessels exhibit localized constricted cross sections with predominantly eccentric elliptical or circular cross sections. Overall area reductions in cases of patients characterized as extremely high risk for thrombosis were reported in the range from 50-84% [3,4]. Thus there is great interest in designing instruments to better understand how and why these conditions of altered artery shapes impact patients' risk for thrombosis.

To study the effects of vessel shape, we designed an array of microchannels to study clot formation within four constricted regions with distinct cross-sectional areas: circular, square, semicircular, and eccentric (elliptical). Square and semicircular cross sections were chosen to

represent the dynamics of traditionally fabricated microchannels, while the circular and eccentric channels were chosen to represent the clinical conditions described previously. Square and circular cross sections had equal hydraulic diameters, while eccentric and circular cross sections had the same area constrictions were reduced in area by 84% relative to the rest of the channel to correspond to patients at very high risk for thrombotic stroke [3,4].

Device dimensions and added downstream resistance elements were chosen such that each channel would experience identical average shear rates of 7000 s^{-1} , an amount representative of conditions in patients at high risk for thrombosis and one which we have verified to cause clot formation in previous experiments [5,6]. In order to address such high shear rates for small volumes (less than 5 mL) of blood per trial, we required a wide range of feature heights and widths from 100 – 750 μm . These dimensions were modeled and verified using both an analytical Poiseuille flow analysis in conjunction with finite volume characterizations using Navier-Stokes equations (Ansys Fluent).

Fabrication

We first used micromilling to construct a mold from which we cast parts which were subsequently aligned, and bonded to create enclosed channels with the desired cross sectional areas.

The mold was cut from brass alloy 385 on a vertical milling machine (Haas OM-1) using square endmills from 1.57mm to 254 μm in diameter and a 508 μm ball endmill at spindle speeds from 5,000 - 30,000 rpm. For all tools, feed rates were 50.8 mm/minute with depth of cuts set to 1/3 the tool diameter. Surface milled toolpaths were generated using MasterCAM X3 software. After machining, the mold was treated for eight hours with a silanizing agent (UCS Chemicals) to facilitate polymer release during the casting step.

Next the mold was cast in the high fidelity, transparent polymer polydimethylsiloxane (PDMS) (Sylgard 184, Dow Chemical). After thermal curing, the polymer was peeled away from the mold and plasma bonded using high frequency corona bonding (Electrotech). Alignment of the layers before bonding was achieved manually under a stereomicroscope.

All channels except the semicircular channel were bonded to a mirror image. The complete process of milling and alignment is shown in *FIGURE 1(b)*.

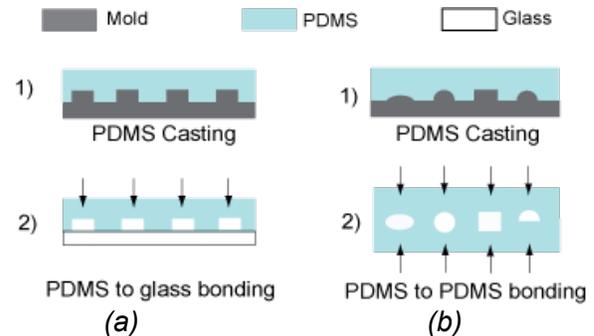


FIGURE 1. A comparison between (a) conventional microfluidic channel fabrication, and (b) our method for forming microfluidic round channels using micromilling and manual alignment.

Finally, blood was flowed through the final assembled device at a controlled pressure and shear rate while platelet based occlusion was monitored both by flow rate measurements and video microscopy.

RESULTS

Metrology

Verification of the mold's dimensions was performed using high-resolution digital microscopy (Keyence VHX-600). Special attention was given to the four constricted regions, where the modeled shear rate peaks at the desired 7000 s^{-1} . Results of metrology on these areas were characterized by figure error on curved surfaces as well as by absolute channel heights and widths. Figure error was calculated as the mean of the absolute value of the difference between the measured profile and the desired profile, results are shown in *TABLE 1*. Surface roughness along the flat face of the channel was sub-micrometer, and provided a sufficiently smooth surface for bonding castings of PDMS to PDMS along these regions.

Alignment and bonding of cast parts was performed manually under a stereomicroscope through which accuracy of 31 to 46 μm was achieved.

TABLE 1. Comparison between designed and fabricated mold dimensions within the constricted area of interest measured using digital microscopy.

	Eccentric	Circular	Square	Semi-circular
Designed Height(μm)	106	150	150	150
Measured Height(μm)	106.05	152.4	159.9	150.9
Designed Width(μm)	424	300	300	300
Measured Width(μm)	468	328	302.8	341.9
Figure Error (μm)	6.6	3.6	10.18	3.2

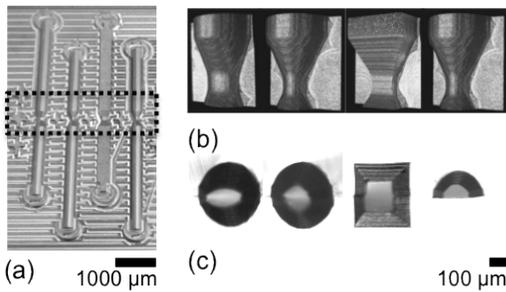


FIGURE 2. Images of the milled microchip mold (a) with enlarged images of the constricted regions of interest of varying cross sectional areas within the mold (b): eccentric, circular, square, and semicircular. Devices were cast from the mold in PDMS, bonded and aligned as shown in FIGURE 1. (c) Final stenotic cross sections.

Thrombosis Assays

The devices were used to assess the effects of variations in cross-sectional area on the formation of clots. Tests were run by flowing blood under specific shear rate conditions and monitoring the time it takes for clots to form and stop flow within each microchannel, simulating a heart attack. This quantity is defined as the time to occlusion [5,6]. Flow was monitored through the use of video microscopy and mass flow measurements with 10 μL resolution (TABLE 2). Clot formation for each channel was also monitored through the use of video microscopy (FIGURE 3).

TABLE 2. Experimental measurements of times for blood flow to occlude microchannels for varying cross-sectional shapes under conditions of identical shear rates ($n=3$ for all cross-sections).

	Eccentric	Circular	Square	Semi-circular
Time to Occlusion $\mu \pm \sigma$ (s)	78 +/- 47.5	313 +/- 55.2	170 +/- 41.7	66 +/- 48.1

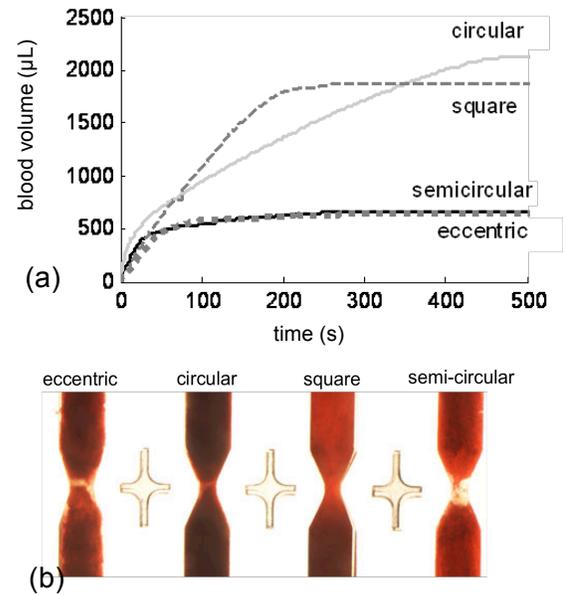


FIGURE 3. (a) Channel occlusion was measured by mass flow of blood through the microchannels of different cross-sectional areas at equal shear rates. (b) Images of blood clots in channels at 500 seconds.

DISCUSSION

We have developed a method for achieving a variety of three-dimensional features unachievable by traditional soft-lithography semiconductor fabrication processes. Employing this method, we demonstrated the creation of a single device mold containing four different cross sectional areas, with smoothly transitioning feature sizes ranging from 100 to 750 μm and aspect ratios ranging from 2:1 to 1:1. Variations in clotting time reveal the differential behavior of clot formation and nucleation over a range of cross-sectional areas.

The device fabricated in this work demonstrates the ability of micromilling to address heretofore-unattainable feature dimensions. Analysis of the

milled mold showed a wide range of heights and a surface shapes with figure error within 10 μm and absolute dimensions within 44 μm . Width dimensions showed the largest feature errors due to artefacts of the metrology method. The limiting factor for the entire fabrication method occurs in the manual alignment of the two molded parts, which could be improved through inclusion of specialized techniques such as temporary methanol bonding and the use of a mechanical stage. [7].

Our results showed that the time to occlusion for the circular channel was higher than within the square channel by almost three standard deviations. Since both channels had identical hydraulic diameters and shear rates, we conclude that this effect is largely due to differences in shear effects between square and circular cross-sectional shapes. Thus the estimation of hydraulic diameter for square cross-sections, a common practice in microfluidics, does not accurately predict fluid behavior for shear-dependent applications. Additional evidence for the importance of hydraulic diameter is provided by comparing the circular channel's time to that of the eccentric channel with the same percentage constriction and cross-sectional area. These variations in clotting time for channels of similar shear rates demonstrate the importance of both cross-sectional channel shape as well as hydraulic diameter and may provide substantial differences in thrombosis assays. These initial findings underscore the importance of developing a method for improved manufacturing techniques in microfluidic assays. Finally, it also suggests that the common clinical assessment of arterial constriction by area is insufficient to assess patient risk for thrombosis.

CONCLUSIONS

This work presents a simple method for creating three dimensional features, using a novel combination of micromilling and soft lithography. Future work on this method will include improvements in the alignment step of fabrication. Blood assay devices will continue to be developed towards pharmaceutical assays as well as biomedical research.

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