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A cost-effective micromilling platform for rapid prototyping of microdevices

Daniel P. Yen¹, Yuta Ando¹, and Keyue Shen^{1,2}

¹Department of Biomedical Engineering, Viterbi School of Engineering, University of Southern California, Los Angeles, CA 90089, USA

²Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, CA 90033, USA

Abstract

Micromilling has great potential in producing microdevices for lab-on-a-chip and organ-on-a-chip applications, but has remained under-utilized due to the high machinery costs and limited accessibility. In this paper, we assessed the machining capabilities of a low-cost 3-D mill in polycarbonate material, which were showcased by the production of microfluidic devices. The study demonstrates that this particular mill is well suited for the fabrication of multi-scale microdevices with feature sizes from micrometers to centimeters.

Keywords

Micromilling; Rapid Prototyping; Organ-on-a-Chip; Lab-on-a-Chip; Microfluidics; Microdevice

INNOVATION

There is a rapidly-growing demand for 3-dimensional (3-D) technologies with flexible material choices in biomedical research as it advances into the organ-on-a-chip era. 3-D milling is a subtractive technique that allows for milling of designs out of any machinable stock materials, the accuracy and precision of which can be further enhanced by the integration of computer numerical control (CNC) to the micrometer scale. However, high-accuracy CNC mills are expensive, inaccessible, and technically-challenging to most biomedical researchers. In this study, we examined the milling characteristics of an inexpensive, intuitive CNC mill platform and demonstrated its use for a microfluidic device. We show that it is capable of fabricating devices in polycarbonate with a $<28\ \mu\text{m}$ precision, and that high microdevice optical transparency can be achieved by surface treatments to allow for microscopic applications. Our study demonstrates this low-cost milling platform as a viable option for the production of microdevices for biomedical research.

NARRATIVE

The development of highly-controlled lab-on-a-chip devices for the studies of chemical, physical, and biological systems has led to considerable research discoveries and medical applications^{1–3}. These platforms miniaturize macroscale systems at a micrometer precision, thereby reducing quantity of reagents needed in larger models and capturing unique phenomena at microscale. In the field of biology, tissue or even organ function can be modeled by organ-on-a-chip devices, allowing for *in vitro* studies of biological processes under conditions that closely mimic *in vivo* cell environments^{1,4}. However, the expansion of research into tissue microenvironments of increasing complexity is limited by the engineering technology used to fabricate these platforms. Such microenvironments may require the integration of multi-scale features such as microfluidic channels and topological finishes within the same device^{5,6}. Additionally, these systems may require materials with particular physical and chemical properties that are not available to (or compatible with) a given fabrication technique^{5,7}. Thus, the approaches used to create micro-platforms for research is in itself an important area for study. The most commonly used technique for microdevice fabrication has been soft lithography, usually consisting of photolithography and poly(dimethylsiloxane) (PDMS) molding. Photolithography is an indirect fabrication method that typically involves extended workflow from mask design, photoresist development, to cleanroom operations. On the other hand, while PDMS is inexpensive, biocompatible, and optically transparent⁸, its manufacturing process is largely manual and difficult to automate; it is also undesirable for studies involving long-term cell cultures or specialized requirements such as gas impermeability^{9,10}. The elasticity of PDMS further constrains the aspect ratio of microdevices to avoid sagging or collapsing¹¹. Other methods such as injection molding, hot embossing, laser micromachining, and stereolithography either involve high initial costs that limit the low-volume, laboratory production of microdevices, or has emerged recently and is not as well characterized as other techniques⁵. Thus, alternative, well-characterized, and more accessible fabrication approaches are needed to meet the growing demands of organ-on-a-chip research.

Recently, micromilling has garnered increased interest in microdevice manufacturing due to its wide selection of working material, versatile applications, and rapid prototyping capabilities⁵. Milling is compatible with metals, glass, wood, and polymers. In particular, it can be used with biocompatible polymers such as polystyrene (the most common plastic for cell cultureware), polycarbonate, and poly(methyl methacrylate) (PMMA or acrylic), which are available as inexpensive stock materials. Micromilling can also be uniquely applied as a single-step process by machining final design features directly into the stock materials, while other techniques such as hot embossing and injection molding require initial creation of a master mold. As a result, it can produce precise prototypes of test designs in as few as 20 minutes⁵. Milling can alternatively produce master molds for other fabrication techniques including soft lithography. Additionally, in micromilling, adjustments to designs can be made and fabricated quickly. In practice, this flexibility and speed allow for rapid adaptation to experimental needs. However, this method remains under-utilized because CNC mills themselves are expensive: these machines commonly cost between \$20,000 and \$200,000, which is not competitive when compared with other more cost-effective techniques⁵. The

steep learning curve in the operations of traditional CNC due to the lack of user-friendly interface has also limited its application in a small-laboratory setting. However, with recent development of rapid prototyping technologies, entry-level CNC mills can now be purchased much more cheaply, at the price of a few thousand dollars. Importantly, new software interface has emerged to allow for intuitive operation of CNC milling equipment. In this study, the milling capabilities of an inexpensive CNC mill with the use of the manufacturer's free graphical software (Othermill V2 and Otherplan 0.31, Other Machine Co, Berkeley, CA) was evaluated for microdevice fabrication (Fig. 1a).

To characterize the accuracy and precision, we conducted tests using three flat end mills of different diameters ($1/16''$, $1/32''$, and $1/64''$), in which design dimensions were compared with the measured dimensions of the milled samples in both lateral and vertical dimensions. First, a $5\text{ }\mu\text{m}$ (L) $\times$ 5 mm (W) $\times$ 1 mm (H) rectangular prism protruding centrally from the top face of a larger polycarbonate base was milled and measured in X, Y, and Z-axes (Fig. 1b inset, $N = 5$). With all the three mill sizes, we consistently achieved $<28\text{ }\mu\text{m}$ precision, which is slightly larger than the manufacturer's specifications ($0.001''$ or $25\text{ }\mu\text{m}$). Next, a series of rectangular prisms were milled with each mill size to assess whether the X and Y precision could be maintained at multiple scales on a single sample (2 , 1 , 0.5 , and $0.25\text{ }\mu\text{m}$ squares with 1 mm height, $N = 3$) (Fig. 1b). The $<28\text{ }\mu\text{m}$ precision was consistently observed except in the 0.25 mm prisms (# in Fig. 1b), which was likely caused by slight bending of the small prism during the milling due to the higher aspect ratio. Additionally, we observed no statistical difference in the deviations of milled prism sizes from their target dimensions using the same mills, while there was a consistent undercut/overcut tendency by each mill possibly due to the variation of the actual tool size from the calibrated values in the milling program (Fig. 1b). Next, we assessed whether the established Z-axis resolution could be maintained at the microscale, as this is the scale most pertinent to studies involving microfluidic channel production in the form of channel depth. A sample piece with 10 channels of decreasing depth was milled with a $1/64''$ end-mill and perpendicularly cut with a $1/8''$ mill to expose the cross-section (Fig. 1c,d). The depth of channels was measured at three different locations with scanning electron microscopy (SEM), and the average values from three independent milling sessions were plotted and linearly regressed against the target dimensions (Fig. 1e). The linear relationship ($R^2 = 0.9927$) had a slope of 0.925 which indicates a slight trend of undercutting by the machine. Future designs in polycarbonate may be compensated using this relationship.

Surface roughness is an important parameter in organ-on-a-chip applications, as large surface roughness could adversely affect the distribution of cell populations within milled devices⁵. Additionally, surface roughness can affect optical transparency, a critical feature for imaging-based applications. Polycarbonate samples were milled flat using the three sizes of flat end mills ($1/16''$, $1/32''$, and $1/64''$). Mechanical polishing (by sanding) was used with and without vapor-polishing to reduce roughness and improve optical quality of milled surfaces (Fig. 2a–c). SEM imaging identified visible tool paths on the milled surfaces, which were reduced to minor traces upon sanding, and further smoothed by vapor-polishing (Fig. 2d–f). Surface roughness values (R_a) measured by contact surface profilometry from three milling sessions showed that, in general, smaller mills yielded rougher surfaces ($1/16''$ vs. $1/64''$, $p < 0.05$, Fig. 2j), likely due to denser tool passes. Sanding or sanding + vapor-

polishing reduced surface roughness to a similar level, with no statistical difference between mill sizes or treatment conditions. We further measured their optical transparency by examining the average absorbance of visible light ($\lambda = 400\text{--}700\text{ nm}$ with 1 nm steps) by the milled/polished surfaces, with higher absorbance corresponding to lower optical transparency. The absorbance of the milled surfaces closely correlates to their roughness (Fig. 2k). Interestingly, while sanding significantly reduced the roughness (Fig. 2j), it only had an averaging effect on the optical transparency (reduced transparency for 1/16" but improved it for 1/64" mill). On the other hand, while vapor-polishing did not significantly improve the roughness of the sanded samples (Fig. 2h–j), it did significantly reduce the optical absorbance and made the surfaces almost completely transparent, which is possibly due to nanoscopic (but not microscopic) smoothing effects by the chemical molecules (Fig. 2c,k).

Lastly, to demonstrate the ability of the CNC mill in fabricating microdevices with feature sizes covering multiple scales, we milled a prototype of a chaotic herringbone mixer¹² and compared its functionality with a control device without herringbone structures (Fig. 3). Instead of directly milling the microfluidic channels, we milled a master mold with protruding features to achieve smaller channel sizes which are otherwise restricted by the smallest flat end mill (1/100") available to us. Negative PDMS replicas were molded, plasma treated, and covalently bonded to a glass slide to form a microfluidic channel (Fig. 3a–f). Both the herringbone and the control devices featured two inlets and two outlets. Inlets were injected with aqueous solutions containing blue or yellow dyes that converged into a long mixing (Fig. 3e,g) or flat (Fig. 3f,h) channel and exited through two outlet channels. Mixing of the inlet streams was observed in the herringbone device as indicated by the green color in both outlet streams, while laminar flow and minimal mixing was seen in the control device, as indicated by the unmixed yellow and blue outlet streams (Fig. 3e–h).

This study demonstrates the milling capacity of a low-cost CNC mill and the surface treatment options for polycarbonate microdevices, with an example for microfluidic applications. While the combination of sanding and vapor-polishing yields the smoothest surfaces with optical transparency, it should be noted that manual sanding is not always possible within the small confinement of channels or topological features, in which case, only vapor polishing may be utilized. We are alternatively exploring the use of sanding bits as well as vapor-polishing alone to achieve desired roughness and optical properties. Notably, a surface roughness of $1.8\text{ }\mu\text{m}$ has been reported to reduce the apparent fluidic viscosity in small microfluidic channels ($\sim 50\text{ }\mu\text{m}$ height), while the impact largely diminishes in larger channels (height $> 100\text{ }\mu\text{m}$)¹³. In agreement, in our control microfluidic device with the untreated surfaces ($Ra < 2\text{ }\mu\text{m}$, Fig. 2j), we did not observe any disruptive effects of the roughness on the laminar flow pattern in the channels ($100\text{ }\mu\text{m}$ depth), with good optical transparency probably due to fluidic wetting effect (Fig. 3f,h). Therefore, the micromilling platform may be suitable for the fabrication of $> 100\text{ }\mu\text{m}$ microfluidic channel designs without sophisticated surface treatment. We are also assessing the milling capabilities of this machine using different types of thermoplastics that have similar or distinct surface and optical properties. In microfluidic design, as noted earlier, direct milling restricts the smallest channel sizes to the diameter of the mill used to create them; additionally, due to the circular shape of the mill, sharp concaved corners are impossible to

create, which may impose further design constraints. These issues can be mitigated if milling is used to create a master mold. Conversely, it is also noteworthy that conventional microdevice design has long been constrained by the fabrication methods such as photolithography. Vertical walls and flat lateral surfaces are the default topological features of microdevices, which can be overcome by 3-D milling. For instance, ball end mills allow for versatile 3-D topological features, while engraving bits can create angled grooves with arbitrary width (depending on the milling depth) for microfluidic channels or topological features. Considering the $<28\text{ }\mu\text{m}$ precision, this mill would be better suited for milling larger high-throughput channels or systems at scales of several hundred micrometers where the resolution is not as important. However, with the current rate of technological progress, it is likely that CNC machines with greater resolution will become available at a similar or lower price in the near future. Thus, micromilling has more than ever presented an attractive and feasible option for manufacturing microdevices in a small-laboratory setting for biomedical research.

METHODS

Test sample fabrication

For this paper, all designs and toolpaths were created using Autodesk Fusion 360 (Autodesk, San Rafael, CA), converted into gcode in Fusion 360, and then run using Otherplan (Other Machine Co, Berkeley, CA). Test samples were milled out of polycarbonate stock materials (each measured as 4" W $\times$ 5" L $\times$ 0.236" H). The most important settings for 3-D milling are spindle speed, cutting feed rate, plunge feed rate, and maximum stepdown size⁵. Values for these parameters were taken from the recommended tool settings found on Other Machine Co's website. Toolpaths were defined in Fusion 360 using flat end mills of varying diameters, depending on the resolution required in our design. During run time, a bit fan milled out of high density polyethylene (gcode obtained from Other Machine Co website) was inserted onto the flat end mills in order to blow away chips from the mill tip and the stock material during milling. Finally, chips that remained attached to the sample were carefully shaved or plucked off using a knife and tweezers, respectively, before taking measurements.

Measurements of X, Y, Z-dimensions in milled samples

Data collected for measurements followed similar guidelines to those obtained by Guckenberger *et al.*'s group for their milling characterization tests⁵. Measurements for the X- and Y-axis resolution tests were made using a micrometer (Digimatic Micrometer Series 293 MDC-MX Lite, Mitutoyo Corp., City of Industry, CA). Z-axis measurements for the micro-scale resolution test were made using scanning electron microscopy (Phillips XL-30, Koninklijke Phillips N.V, Amsterdam, NLD) by measuring differences in height between the edges of the channel, and the channel bottom in the left, right, and middle of the channel. The final channel depth for each channel was the average of these three values. With a given tool size and milling settings, the precision was calculated as 3 times the standard deviation of measured sizes at the same target value.

Surface finishes

Mechanical polishing was achieved using a 1000 grit sand paper (3M, Maplewood, MN). Samples were sanded down in alternating up-down, side-to-side patterns until no toolpaths could be observed on the polished surface. All vapor polishing procedures were conducted inside a fume hood, as methylene chloride gas is toxic. Approximately 10 mL of methylene chloride was poured into a Buchner flask. The top of the flask was then stoppered, so that the only pathway for gas to escape was through the hose nozzle. The flask was then heated on a hotplate set to 80 degrees Celsius. Samples were picked up using tweezers and passed several times through the escaping vapor stream, approximately 3–4 μm from the Buchner hose nozzle. Surface roughness was determined using a contact surface profilometer (Dektak iia, Veeco Instruments Inc., Town of Oyster Bay, NY) using 2 mm scan lengths at the low scan speed setting. The surface roughness parameter Ra was then calculated by the machine across the length of the scan. For the native surface finish, scans were made diagonally across toolpaths, as scans in the same direction of toolpaths yielded lower Ra values than perpendicular scans.

Measurement of optical transparency

Three samples consisting of a long 72 mm (L) $\times$ 18 mm (W) rectangular prism were milled to obtain a side-by-side test for absorbance. Each sample featured four 18 mm (L) $\times$ 18 mm (W) sections that were left as stock material surface as control, or faced 0.1 mm by three flat end mill sizes (1/16", 1/32", and 1/64"), respectively. Each sample represented a different surface treatment condition in the milled regions: milled, sanded, and sanded + vapor-polished. The non-machined section served as a blank for spectrum scans. Samples were loaded into the plate holder of a microplate reader capable of performing spectrum scans (Varioskan Lux, Thermo Fisher Scientific, Waltham, MA). Each 18 mm $\times$ 18 mm square section covers four wells of the plate holder. As such, four spectrum scans ($\lambda = 400\sim 700$ nm with 1 nm steps) were obtained for each section. The blank section's average absorbance was subtracted from the average absorbance values of each condition to obtain final average absorbance values influenced by surface roughness and mill size.

Fabrication of herringbone mixer and control device

A microfluidic herringbone micromixer device was designed in AutoCAD Fusion 360 (Autodesk, San Rafael, CA) (Fig. 3a) and milled into a polycarbonate stock material (Fig. 3b). The design was scaled up from the device from the Whitesides' group's paper¹² to accommodate the smallest flat end mill size (1/100") in the lab. The mixing microchannel was 100 μm tall, 1 mm wide, and 30 mm long. Herringbone ridges were 100 μm tall, 100 μm wide, with a 500 μm lateral period along the channel. The ridges formed a 45-degree angle with respect to the channel wall and were staggered, such that one shorter leg of the herringbone spanned one-third of the microchannel width, and the longer leg spanned the other two-thirds. Five herringbone ridges constituted a period. Consecutive periods alternated ridge leg lengths. It was determined experimentally that the most effective tool path for removing stock material without leaving chips was to first mill the herringbone ridges to the appropriate shapes, and then milled ridges to the appropriate height. Any remaining chips were removed using tweezers. The mold was then vapor-silanized with

trichloro(1H,1H,2H,2H-perfluorooctyl)silane overnight in a vacuum desiccator. Sylgard 184 PDMS was prepared at a 10:1 ratio of base to curing agent, and was poured into the polycarbonate mold and cured overnight. The PDMS was then peeled off (Fig. 3b), plasma treated, and permanently bonded to a plasma-treated glass slide. The second device was made with the same manufacturing procedure using the same channel dimensions without the herringbone features.

Statistical analysis

All data presented in graphs are in mean $\pm$ standard deviation, as stated in the figure legends. Statistical significance was assessed using one-way analysis of variation (one-way ANOVA); $p < 0.05$ was considered significant.

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Figure 1.

Machine setup and milling characteristics. **(a)** An Othermill V2 and its control software Otherplan. **(b)** Accuracy and precision for X- and Y-axis at multiple scales, measured and plotted against target dimensions (left Y-axis), as well as the size deviation from target value (right Y-axis) ($N = 5$ for $5 \text{ mm} \times 5 \text{ mm}$, and $N = 3$ for all other sizes; #: with larger size variation than all other mills and target sizes). *: n.s., not statistically-different; **: $p < 0.05$, by ANOVA test. **(c)** Z-axis micro scale test piece design and fabrication steps: 1) stock material faced by mill to the level of the microchannel top height, then individual channels are milled; 2) milling in transverse direction to channels to ensure perpendicular channel cross-sections. **(d)** Schematics of the side section of the microchannels and SEM views of Z-axis dimension in a test piece (figure inset: enlarged view under higher magnification); scale bar: 2 mm. **(e)** Z-axis microscale accuracy and precision test: SEM-measured channel depth values against target values, $N = 3$. Error bars: standard deviation (SD).

Figure 2.

Characterization of surface properties and surface treatments. Side-by-side visual comparison (a–c), SEM images (d–f), and surface roughness plots (g–i) of milled, sanded, and sanded + vapor-polished test pieces. (j) The effects of surface treatment on surface roughness at three flat end mill diameters ($N = 3$). (k) The effect of surface treatments on average absorbance of visible light wavelengths ($\lambda = 400\text{--}700\text{ nm}$, with 1 nm step size); blank sample: stock polycarbonate. Blank absorbance has been subtracted from the average absorbance of different surface conditions. (d–f) Scale bars: 200 μm . $p < 0.05$: statistically

different between the two conditions, or any of the two conditions from both sides, respectively, and n.s.: not statistically-different, by ANOVA test. Error bars: standard deviation (SD).

Figure 3.

Fabrication of a herringbone microfluidic mixer. (a) CAD image of the final design of the herringbone microchannel mixer in Fusion 360. (b) Close-up view of herringbone structure at the top of the central microchannel with design sizes. (c) Herringbone and (d) control PDMS replica pictured alongside polycarbonate mold used for casting. (e) Mixing occurring in the herringbone microchannel indicated by the two green outlet channels. (f) Laminar flow occurring in control group device, as indicated by yellow and blue outlet channels. (g,h) Microscopic images of sections of the herringbone and control devices with two colored dyes.