3D NANOPRINTED EXTERNAL MICROFLUIDIC STRUCTURES *VIA EX SITU* DIRECT LASER WRITING

Ruben Acevedo[1], Ziteng Wen[1], Ian B. Rosenthal[1], Emmett Z. Freeman[1], Michael Restaino[2] Noemi Gonzalez[2], and Ryan D. Sochol[1,2]

[1]Department of Mechanical Engineering, University of Maryland, College Park, Maryland USA
[2]Fischell Department of Bioengineering, University of Maryland, College Park, Maryland USA

ABSTRACT

Additive manufacturing (or "three-dimensional (3D) printing") technologies offer unique means to expand the architectural versatility with which microfluidic systems can be designed and constructed. In particular, "direct laser writing (DLW)" supports submicron-scale 3D printing *via* two-photon (or multi-photon) polymerization; however, such high resolutions are poorly suited for fabricating the macro-to-micro interfaces (*i.e.*, fluidic access ports) critical to microfluidic applications. To bypass this issue, here we present a novel strategy for using DLW to 3D print architecturally complex microfluidic structures directly onto—and notably, fully integrated with—macroscale fused silica tubes. Fabrication and experimental results for this "*ex situ* DLW (*es*DLW)" approach revealed effective structure-to-tube sealing, with fluidic integrity maintained during fluid transport from macroscale tubing, into and through demonstrative 3D printed microfluidic structures, and then out of designed outlets. These results suggest that the presented DLW-based printing approach for externally coupling microfluidic structures to macroscale fluidic systems holds promise for emerging applications spanning chemical, biomedical, and soft robotics fields.

KEYWORDS

Microfluidics, Additive Manufacturing, 3D Printing, Direct Laser Writing, Two-Photon Polymerization

INTRODUCTION

Additive manufacturing technologies have garnered increasing interest in the microfluidics community as a means to circumvent the geometric restrictions inherent to conventional microfabrication protocols (*e.g.*, soft lithography) [1]. For example, investigators have applied direct ink writing (DIW)—an extrusion-based 3D printing approach—to resolve fluidic channels in the millimeter-to-submillimeter regime [2,3]; however, the ineluctable trade-off between feature size and print time has led researchers to focus instead on alternative additive techniques [4]. To print microfluidic systems with resolutions on the order of 100–200 μm, several groups have employed inkjet-based additive manufacturing techniques, such as "Multijet Modeling" [5,6] and "PolyJet 3D Printing" [7]. At present, light-based additive manufacturing methods represent the predominant means by which researchers 3D print microfluidic systems [8,9]. In particular, several groups have used stereolithography (SLA) and digital light processing (DLP) printers to resolve microfluidic channels with dimensions on the order of tens of microns [10-12]. For microfluidic applications that require high geometric versatility at micron-to-submicron length scales, only one technology is suitable: "direct laser writing (DLW)" [13].

DLW is a 3D microfabrication approach by which a photocurable material is crosslinked in a point-by-point, layer-by-layer manner *via* two-photon (or multi-photon) polymerization phenomena [14,15]. One challenge associated with the use of DLW for microfluidics manufacturing is that the submicron-scale size of the printing volume element (voxel) renders the technology inefficient for constructing the macro-to-micro interfaces (*i.e.*, inlet and outlet ports) required for fluidic loading [16]. To address this issue, researchers previously introduced "*in situ* DLW"—an approach for printing microfluidic systems directly inside of enclosed microchannels [16,17]. Although this technique is beneficial for microchip-based use cases [18,19], a number of emerging applications demand DLW-printed microfluidic structures fabricated *ex situ* (*i.e.*, outside of enclosed microchips). In prior reports, Gissibl *et al.* and Dietrich *et al.* used DLW to print architecturally complex optical elements (*e.g.*, lenses and micromirrors) directly onto optical fibers and photonic components [20,21]. In this work, we seek to demonstrate microfluidic analogue of these works by investigating a novel "*ex situ* DLW (*es*DLW)" strategy for printing 3D microstructures with internal fluidic microchannels directly atop (and fully integrated with) macroscale tubes.

CONCEPT

The *es*DLW protocol investigated herein consists of three fundamental steps. First, a macroscale fused silica tube is mounted in a DLW 3D printer in the Dip-in Laser Lithography (DiLL) configuration, with both the surface of the tip and the object lens immersed in a photocurable material and aligned parallel to each other (**Fig. 1a**). Next, a 3D microfluidic structure is printed directly onto the tip of the fused silica tube by scanning a tightly focused femtosecond IR laser point-by-point, layer-by-layer, to polymerize the photomaterial in target locations (**Fig. 1b**). Lastly, the tube-print assembly is removed from the DLW printer for development to eliminate any residual uncured photomaterial. To support microfluidic loading (and ease of manual handling), the fused silica tube can be connected to flexible silicone tubing *via* a glass adapter (**Fig. 1c**). Thereafter, fluid can be infused into the 3D microfluidic channels of the printed structure and out the designed port (**Fig. 1d**). Alternatively, the outlet could instead be used as the point of infusion—*i.e.*, by applying a vacuum pressure to the tube to draw in fluid volume from an external source.

MATERIALS AND METHODS

*"es*DLW"-Printing of 3D Microfluidic Structures

All *es*DLW protocols in this work are based on the use of a Nanoscribe Photonic Professional GT2 DLW 3D printer (Nanoscribe GmbH, Karlsruhe, Germany). Prior to

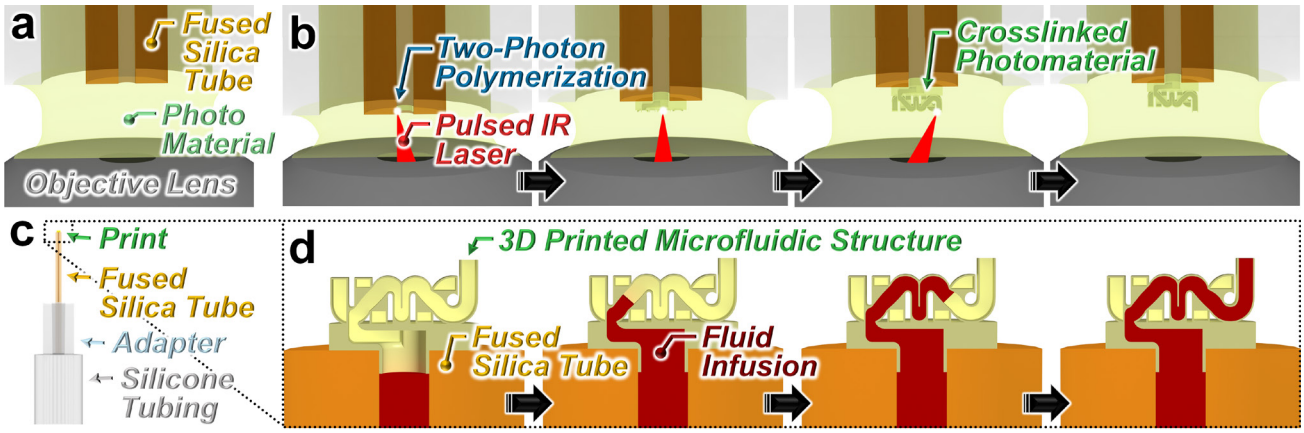

Figure 1: Conceptual illustrations of the "ex situ direct laser writing (esDLW)" strategy for printing three-dimensional (3D) microfluidic structures directly onto—and fully integrated with—macroscale fluidic components (e.g., tubes). *(**a**) A macroscale fused silica tube is mounted in the DLW 3D printer, immersed in a liquid-phase photocurable material. (**b**) Point-by-point, layer-by-layer scanning of a tightly focused femtosecond pulsed IR laser to initiate two-photon (or multi-photon) polymerization of a 3D microfluidic structure directly onto the tube. (**c**) Assembly configuration to support facile microfluidic loading. (**d**) Example microfluidic infusion process in which a fluid is delivered via the fused silica tube into and through a demonstrative esDLW-printed 3D microfluidic structure.*

beginning the printing process, fused amber silica polyimide-coated smooth solid tubes (Molex LLC, Lisle, IL) with an inner diameter (ID) of 75 μm and an outer diameter (OD) of 360 μm were scored and cut at a length of approximately 2 cm. The fused silica tubes were prepared for printing through successive infusions of acetone through both openings by coupling the tubes to silicone rubber tubing (#51135K11, McMaster-Carr, Elmhurst, IL) *via* inner-lok GC union adapters (Molex). After removing the fused silica tube from the adapter, the surface designated for print adhesion was then washed and rinsed with acetone, isopropyl alcohol (IPA), and DI water. Once dry, the fused silica tube was mounted into the Nanoscribe DLW printer using a custom-built holder, with a single droplet of the photoresist, IP-Dip (Nanoscribe), placed in contact with the designated print surface of the fused silica tube.

The computer-aided design (CAD) software, SolidWorks (Dassault Systèmes, France), was used to generate 3D models of demonstrative microfluidic structures to evaluate the efficacy of the *es*DLW approach. Each model was exported as an STL file and then imported into the computer-aided manufacturing (CAM) software, DeScribe (Nanoscribe). The UMD design included 500 nm hatching and 300 nm layer heights, while the other two designs included 1 μm hatching and 500 nm layer heights. All of the designs were printed using the Nanoscribe Photonic Professional GT2 DLW 3D printer with the 25× objective lens in DiLL mode with a laser power of 27.5 mW and a laser scanning speed of 100,000 μm/s. To promote fluidic sealing between the DLW-printed part and the fused silica tube, each design included an ID 10 μm smaller than the ID of the fused silica tube (*i.e.*, 65 μm). Also, to account for any unintended deviations associated with tilting of the tube, the surface interface of the fused silica tube was found manually. The printing process initiated with approximately 35 μm of overlap into the tube (with respect to the surface interface). Following the DLW process, the tube-print assembly was removed from the printer, developed using propylene glycol methyl ether acetate (PGMEA) for 30 min, and then allowed to dry under ambient conditions.

Optical Characterization

Scanning electron microscope (SEM) images of *es*DLW-printed were captured using a TM4000 Tabletop SEM (Hitachi, Tokyo, Japan). Brightfield microscopy during microfluidic testing was performed using an inverted microscope (Motic AE31, Motic, Canada) connected to a charge-coupled device (CCD) camera (Moticam Pro 285B, Motic).

Microfluidic Experimentation

Fused silica tubes with printed 3D microfluidic structures were coupled to inner-lok GC union (Molex) tube adapter, which was attached to silicone rubber tubing (McMaster-Carr). The opposite end of the silicone tubing was then connected to the Fluigent Microfluidic Control System (MFCS) to regulate the fluid flow rate *via* the MAESFLO software (Fluigent, France). For microfluidic testing, fluorescently labeled isopropyl alcohol (IPA) (Rhodamine B, MilliporeSigma, St. Louis, MO) was inputted under an applied pressure of approximately 2 kPa.

RESULTS AND DISCUSSION

*es*DLW-Based Fabrication

As a preliminary investigation of the efficacy of our *es*DLW strategy, we fabricated three proof-of-concept 3D microfluidic structures: (*i*) a University of Maryland-inspired "UMD" design, (*ii*) a statue of the University of Maryland mascot, "Testudo", and (*iii*) a design featuring various landmarks in Washington, DC. CAM simulations and corresponding printing results for the point-by-point, layer-by-layer, *es*DLW fabrication process for the "UMD" design interfaced with the fused silica tube are presented in **Figure 2a** and **2b**, respectively. The entire DLW process was completed in less than 10 min. Due to the larger hatching and layer height conditions, the total print time for the Testudo and landmark designs were approximately

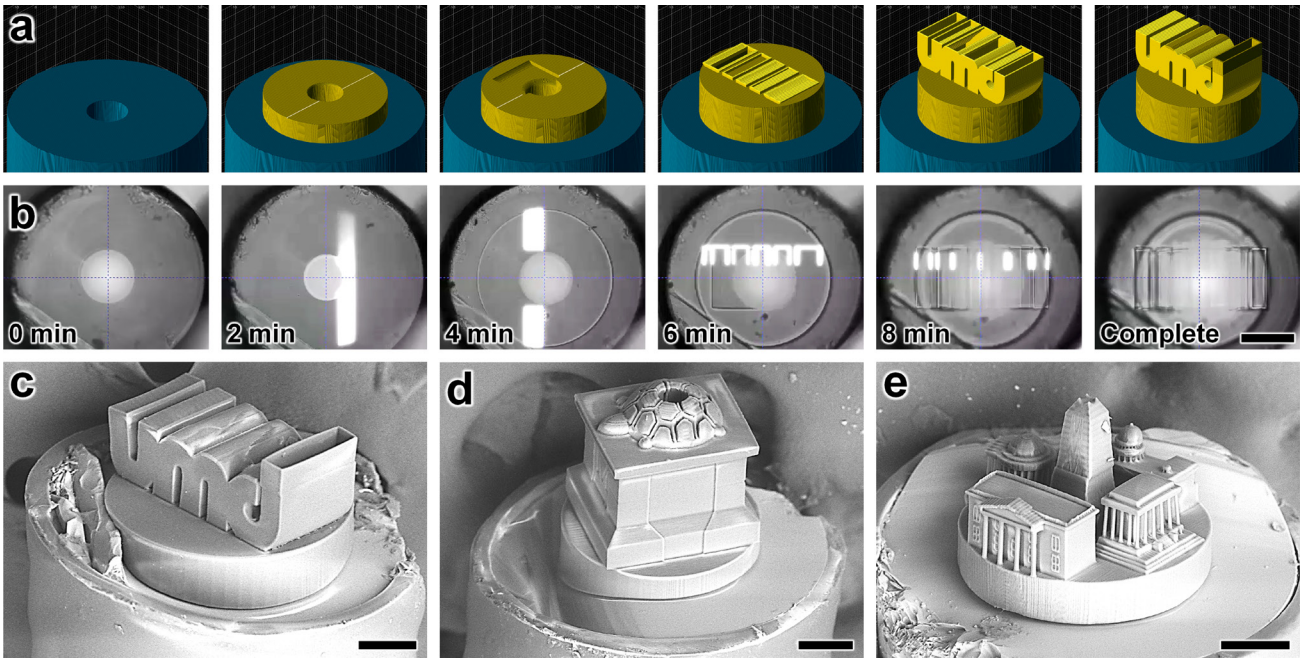

Figure 2: Fabrication results for esDLW-based printing of 3D microfluidic structures directly onto fused silica tubes. *(**a**) Computer-aided manufacturing (CAM) simulations and (**b**) corresponding micrographs of the esDLW printing process. Scale bar = 100 µm; Print time = 9 min. (**c–e**) SEM micrographs of the three demonstrative 3D microfluidic structures: (**c**) "UMD" design, (**d**) Testudo statue design, and (**e**) landmarks of Washington, DC, design. Scale bars = 50 µm.*

3 min and 2 min, respectively. Following the development process, all of the printed designs exhibited effective adhesion to the fused silica tube (**Fig. 2c–e**).

Microfluidic Results

To evaluate the integrity of the fluidic pathway from the fused silica tube into and through the *es*DLW-printed microfluidic structures, we connected the opposing end of the tube to silicone tubing *via* a glass adapter (**Fig. 3a**). Due to the scale at which the structures were printed, we did not find any of the designs to be observable to the naked eye. Using an applied pressure of approximately 2 kPA, we infused Rhodamine B-dyed IPA through each assembly while monitoring the flow through the microfluidic

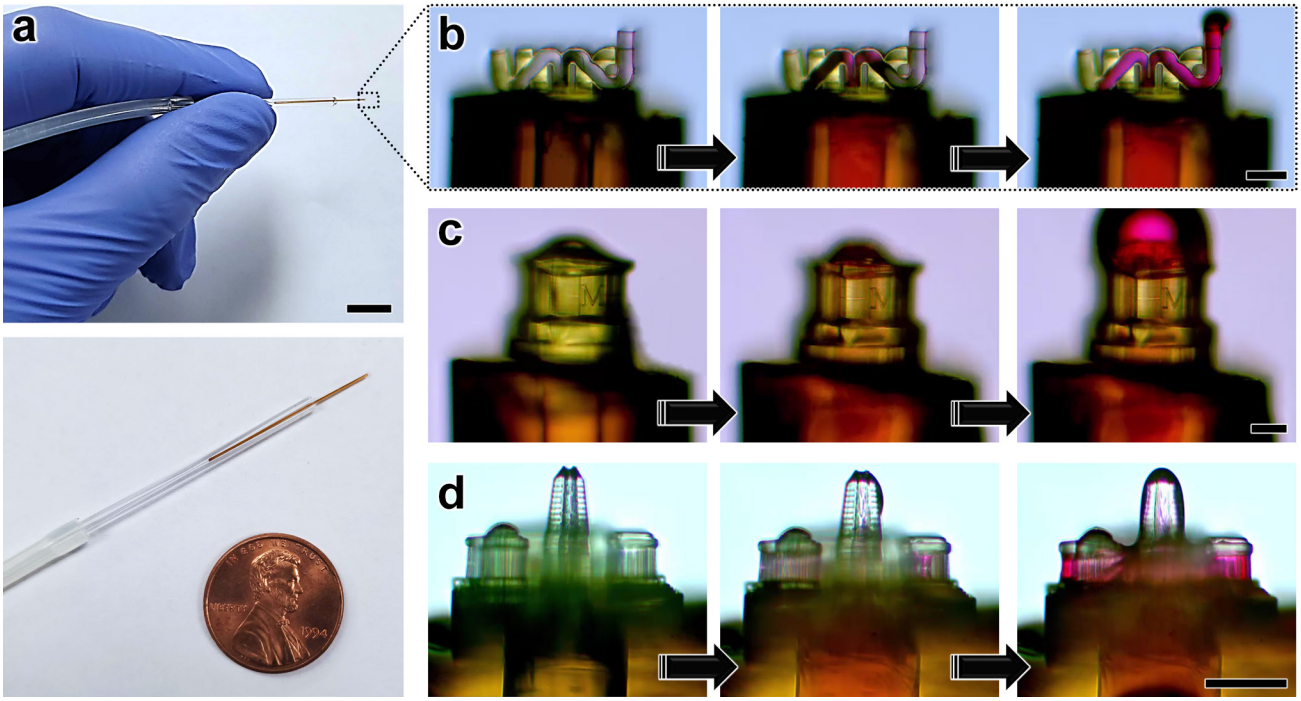

Figure 3: Experimental results for microfluidic infusion through esDLW-printed 3D microstructures. *(**a**) Photographs of the assembly configuration to support facile microfluidic loading, with esDLW-printed microfluidic structures atop the fused silica tube. Scale bar = 1 cm. (**b–d**) Sequential micrographs of infusion of Rhodamine B-dyed IPA through each of the demonstrative 3D microfluidic structures: (**b**) "UMD" design, (**c**) Testudo statue design, and (**d**) landmarks of Washington, DC, design. Scale bars = 50 µm.*

structures under brightfield microscopy. We did not observe any signs of undesired leakage during any of the microfluidic experiments (**Fig. 3b–d**). Rather, inputted fluids exited the structures only at the designed outlet locations. For example, in the "UMD" structure, the fluid flowed from letter to letter within the embedded "M"-shaped channel (**Fig. 3b**). We also observed that fluid exclusively exited the Testudo design through the 30-μm-in-diameter orifice of the turtle shell (**Fig. 3c**), and the landmark design through the 10-μm-in-diameter port atop the Washington Monument (**Fig. 3d**). In combination, these results suggest effective fluidic sealing between fused silica tubes and the *es*DLW-printed microfluidic structures.

CONCLUSION

In this work, we investigated a novel strategy for using DLW to print architecturally complex 3D microfluidic structures directly onto macroscale fused silica tubes with full fluidic integration. The *es*DLW fabrication and experimental results for proof-of-concept microfluidic components revealed effective structure-to-tube sealing, with fluidic integrity maintained during fluid transport from the tubing, into and through the printed 3D microstructures, and then out of designed outlets. These results suggest that the presented *es*DLW approach for externally coupling microfluidic structures to macroscale fluidic systems holds promise for emerging chemical, biomedical, and soft robotics fluidic applications.

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CONTACT

*R. Acevedo, tel: +1 (949) 683-7773; raceved3@umd.edu