3D NANOPRINTED MICROINJECTION NEEDLES *VIA EX SITU* DIRECT LASER WRITING

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ABSTRACT

Microinjection processes are integral to developmental biology, transgenics, and *in vitro* fertilization; however, the microneedles that underly such applications are limited by clogging susceptibility and undesired variations in tip shape and size. To address these issues, here we investigate the use of a submicron-scale additive manufacturing strategy termed "*ex situ* Direct Laser Writing (*es*DLW)" for 3D printing microinjection needles. Results for needles designed with 2.5 µm-thick walls and 10 µm inner diameters revealed microfluidic burst pressures above 70 kPa – the critical target for zebrafish embryo microinjection applications – including several cases in excess of 250 kPa.

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

INTRODUCTION

"Direct Laser Writing (DLW)" offers substantive 3D versatility at submicron scales; however, for microfluidic applications, such sizes are ill suited for printing macro-to-micro interfaces (*e.g.*, inlet/outlet ports). Although we previously reported "*in situ* DLW" approaches for printing microfluidic elements in enclosed microchannels [1, 2], applications like microinjection demand externally accessible microfluidics. Recently, researchers have demonstrated DLW-printing of architecturally complex optical [3, 4] and microfluidic components [5, 6] directly atop macroscale interfacing elements. Here we explore such "*ex situ*" strategies for 3D printing microinjection needles.

EXPERIMENTAL

We employ *es*DLW [6] to fabricate novel 3D microneedle designs directly atop (and fully integrated with) macroscale fluidic tubes *via* three steps: (*i*) a fused silica tube was mounted in a DLW printer (using a custom holder to support alignment) with the tip immersed in IP-Dip photoresist (**Fig. 1a**); (*ii*) the microneedle design was 3D printed onto the tube by scanning a tightly focused femtosecond IR laser to cure the photomaterial (**Fig. 1b**); and (*iii*) the print was developed to remove residual photoresist. For microfluidic burst pressure testing experiments, the fused silica tubes with printed 3D microneedles were coupled to silicone rubber tubing *via* an adapter (**Fig. 1c**) to facilitate fluidic infusion into and through the microneedle (**Fig. 1d**).

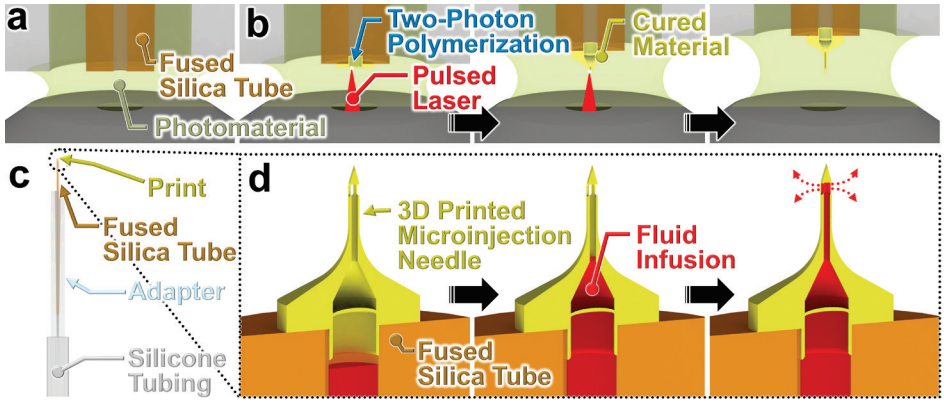

***Figure 1**: Illustrations of "ex situ Direct Laser Writing (esDLW)"-based microinjection needles. **(a)** A macroscale tube mounted in the 3D printer. **(b)** Point-by-point, layer-by-layer scanning of a tightly focused pulsed IR laser to initiate two-photon (or multi-photon) polymerization. **(c)** Assembly configuration for fluidic loading. **(d)** Fluid infusion process through an esDLW-printed architecturally complex 3D microneedle design.*

RESULTS AND DISCUSSION

We investigated the use of *es*DLW for two designs: (*i*) a complex 3D microneedle design (with four side outlets) that is infeasible to fabricate *via* conventional methods, and (*ii*) a control design that resembles industry standards. Simulations and corresponding micrographs of the *esDLW* printing process for the 3D design are presented in **Figure 2a** and **2b**, respectively. Printing results for both microneedles revealed effective fabrication (**Fig. 2c, d**). Microfluidic testing revealed unobstructed flow through the printed needles (**Fig. 3a, b**). Experimental results revealed burst pressure events consistently above the 70 kPa required for zebrafish embryo injections (**Fig. 3c, d**).

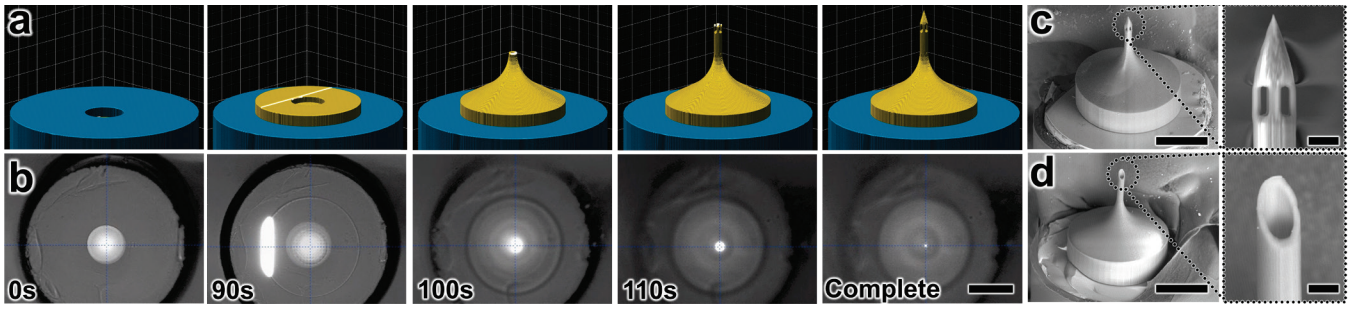

***Figure 2**: Fabrication results for esDLW-based printing of microinjection needles. **(a)** Computer-aided manufacturing (CAM) simulations and **(b)** corresponding sequential micrographs of the esDLW 3D printing process. **(c, d)** SEM micrographs of printing results for **(c)** architecturally complex and **(d)** control microneedle designs. Scale bars = 100 µm (insets = 10 µm)*

CONCLUSION

In this work, we investigated an *es*DLW strategy for printing architecturally complex 3D microinjection needle designs directly onto macroscale fused silica tubes. The *es*DLW fabrication and experimental results for proof-of-concept microneedle designs revealed effective structure-to-tube sealing, with fluidic integrity maintained during fluid transport from the tubing, into and through the printed 3D needles, and then out of the needle tips. The experimental results for burst pressure testing demonstrated that the macro-to-micro interfaces could withstand pressures in excess of 250 kPa in some cases, but with all trials above the 70 kPa associated with zebrafish embryo injection protocols. Future efforts should focus on investigating the use of such *es*DLW-based needles for embryo microinjections, and in particular, the potential for 3D needle tip designs to prevent undesired clogging events for serial injection protocols.

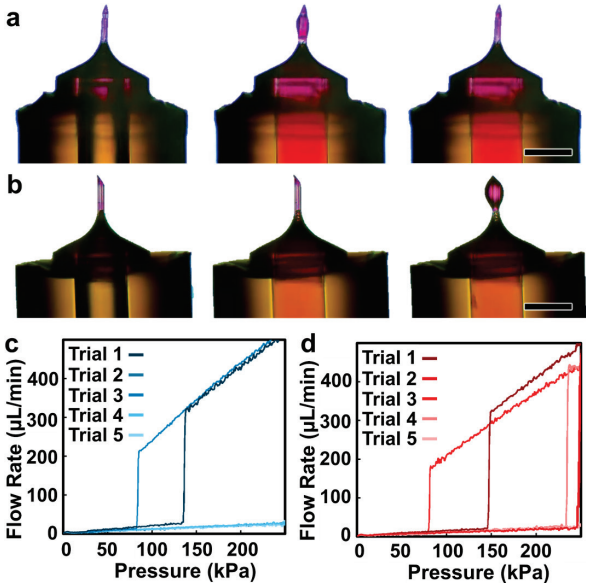

***Figure 3:** Experimental results for **(a, b)** infusion of Rhodamine B-dyed IPA through the microneedles and **(c, d)** microfluidic burst pressures corresponding to the **(a, c)** architecturally complex and **(b, d)** control designs. Scale bars = 100 µm*

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