TOWARD CONTROLLED-RELEASE DRUG DELIVERY MICROCARRIERS ENABLED BY DIRECT LASER WRITING 3D PRINTING

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ABSTRACT

Controlled-release, and especially long-acting, drug delivery systems hold promise for improving treatments for numerous medical conditions. Previously, we reported an additive manufacturing or "three-dimensional (3D) printing" approach for fabricating liquid-core-shell-cap microcarriers comprising standard photoresists. Here we explore the potential to extend this strategy to achieve microcarriers comprising biodegradable materials as a new pathway to controlled-release drug delivery options. Specifically, we investigate the use of "Two-Photon Direct Laser Writing (DLW)" as a means to 3D print microcarriers composed of: (*i*) a bottle-shaped "shell" with an orifice, (*ii*) an aqueous liquid "core", and (*iii*) a biodegradable "cap". The cap, which is DLW-printed directly onto the shell's orifice, is designed to degrade over time in the body—*e.g.*, with degradation time proportional to cap thickness—to ultimately facilitate release of the liquid core at desired time points. Fabrication results based on the use of a biodegradable poly(ethylene glycol) diacrylate (PEGDA) photomaterial for the cap revealed that shell designs incorporating microfluidic obstruction structures appeared to limit undesired entry of the liquid-phase PEGDA into the shell (*i.e.*, directly preceding cap printing), thereby resulting in improved retention of the liquid core after completion of the cap printing process. These results mark an important first step toward evaluating the utility of the presented DLW 3D printing strategy for possible drug delivery applications.

KEYWORDS

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

INTRODUCTION

Highly controlled and customizable drug delivery remains a long-term goal of medical fields as such technologies would allow for drug delivery architectures to be tailored directly to a patient's specific biological make-up and/or disease state [1]–[3]. There is growing interest in controlled-release approaches for drug delivery, particularly for treatments in which an overdose can be fatal [4], [5]. Although recent efforts have suggested that DLW is uniquely suited for emerging drug delivery applications [6]–[9], creating DLW-enabled systems that facilitate tightly controlled drug release remains a critical challenge. Previously, our group demonstrated the ability to fabricate liquid-core-shell-cap microcarriers using standard DLW-compatible photoresists—*i.e.*, photomaterials ill-suited for drug delivery-associated core-release functionalities [10]. As a preliminary study of the possibility for such DLW-based approaches to be extended for drug delivery applications, here we investigate the incorporation of a PEGDA photomaterial into the fabrication methodology as a route to achieve biodegradable "caps" for controlled-release applications.

MATERIALS AND METHODS

Concept

The overall fabrication strategy for the liquid-core-shell-cap microcarriers with biodegradable caps involves three main steps. First, DLW is used to 3D print the "shell" microstructures—each with a single, unenclosed orifice—onto a glass substrate (**Fig. 1a**). Second, following development, a poly(dimethylsiloxane) (PDMS) microchannel is aligned to the print and weakly bonded to the glass slide to facilitate microfluidic vacuum loading of the liquid core (**Fig. 1b**). Lastly, a liquid-phase biodegradable photomaterial is loaded into the PDMS microchannel and a "cap" is DLW-printed directly onto each shell's orifice,

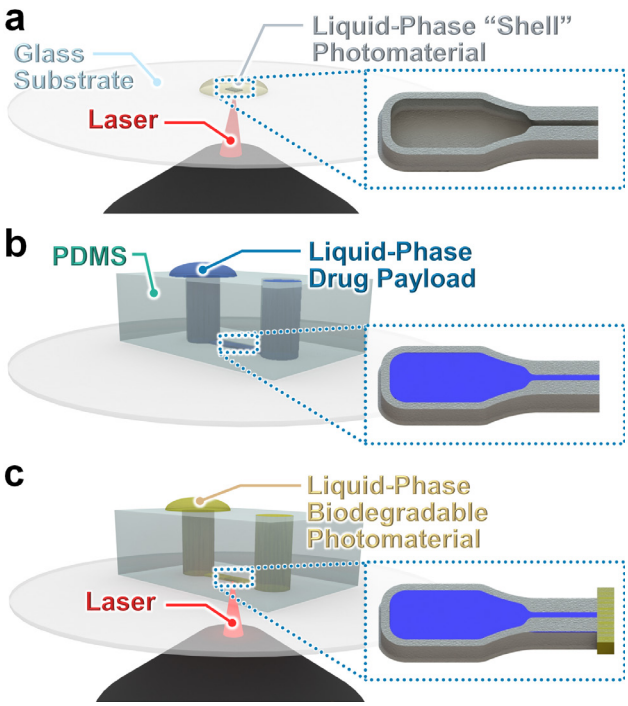

Figure 1: *Illustrations of the "Two-Photon Direct Laser Writing (DLW)"-based fabrication strategy for liquid-core-shell-cap drug delivery microcarriers (expanded views).* ***(a)*** *DLW 3D printing of bottle-shaped "shells" with an orifice.* ***(b)*** *Microfluidic vacuum loading of the liquid core via a weakly bonded poly(dimethylsiloxane) (PDMS) microchannel.* ***(c)*** *DLW 3D printing of the biodegradable "cap" directly onto the orifice.*

thereby fully enclosing the liquid core within the microcarrier (**Fig. 1c**). In principle, because the degradation time of the cap is proportional to its thickness (*e.g.*, thicker caps will require more time to fully degrade), the use of DLW would allow for each microcarrier to be designed with different cap thicknesses to yield distinct, yet controlled core release functionalities.

"Shell" Fabrication *via* "Direct Laser Writing (DLW)"

The shells were modeled using the computer-aided design (CAD) software, SolidWorks (Dassault Systemes, France), exported as STL files, and then imported into the computer-aided manufacturing (CAM) software, DeScribe (NanoScribe, Karlsruhe, Germany) for printing with the Nanoscribe Photonic Professional GT2 DLW 3D printer. The print parameters for hatching and layer height were set at 250 nm. Prior to printing, borosilicate glass substrates (Bioptechs Inc., Butler, PA) were silanized to enhance print-substrate adhesion. The shells were printed with the photoresist, IP-L 780 (Nanoscribe), using a 25× objective lens in the oil-immersion configuration. Following completion of the DLW printing process, the prints were developed in propylene glycol methyl ether acetate (PGMEA) for 2 hrs at 55 °C and then sonicated for 10 min in isopropyl alcohol (IPA). Lastly, the prints were dried on a hot plate set at 55 °C for 5 min.

Microfluidic Vacuum Loading of the Liquid "Core"

The aqueous core was achieved *via* a previously reported microfluidic vacuum loading technique [10], [11]. Briefly, DLW—in the Dip-in Laser Lithography (DiLL) configuration with a 10× objective lens—was used to print a negative master mold for microreplication of PDMS (Sylgard 184, Dow Corning, Corning, NY) to fabricate an unenclosed PDMS microchannel similar to methods described previously [12]–[14]. The unenclosed PDMS micro-channel was manually aligned to the previously printed shells and then, to ensure a firm attachment, the PDMS was weakly bonded to the glass substrate at 100 °C for 10 min. With the outlet port covered with tape (and the inlet port left open), the PDMS-glass assembly was placed inside a vacuum chamber for 20 min. Immediately thereafter, a droplet of methylene blue-dyed DI water—*i.e.*, the aqueous fluid for the liquid core—was deposited atop the inlet port, which was then passively drawn into the microchannel and the interiors of the shells. Lastly, the tape was removed from the outlet port.

"Cap" Fabrication *via* Microfluidic DLW

The cap fabrication protocol is leverages our "*in situ* DLW (*is*DLW)" and microfluidic DLW approaches [10], [14]–[16]. The caps were modeled and sliced for DLW printing akin to the shells. Two different formulations of PEGDA corresponding to distinct molecular weights, including PEGDA 250 and PEGDA 575, were used for cap printing in independent sets of experiments. PEGDA 250 was mixed with 3% (w/w) Irgacure 369 photoinitiator (Ciba) while PEGDA 575 was mixed with 50 mg/mL Parbenate (Ethyl-4-(Dimethylamin)benzoate) as a photoinitiator and 10 mg/mL 2-isopropyl-qH-thioxanthon-9-one as photosensitizer. To print the cap on the shell, first liquid-phase PEGDA was loaded into the microchannel (replacing the methylene blue-dyed DI water) and the PDMS-glass assembly was loaded into the DLW printer. After manual alignment, caps were printed directly onto the shell orifices—inside of the enclosed microchannel—using a 25× objective lens in the oil-immersion configuration (hatching, layer height = 350 nm). After completion of the cap printing process, the print was developed by loading IPA into the microchannel for 2 min.

Optical Characterization

All scanning electron microscope (SEM) images were captured using a TM4000 Tabletop SEM (Hitachi, Tokyo, Japan). Brightfield and fluorescence micrographs were captured using an Axio Observer Z1 inverted fluorescence microscope connected to an Axiocam 503 Mono charge-coupled device (CCD) (Zeiss, Jena, Germany).

RESULTS AND DISCUSSION

"Shell" Fabrication

CAM simulations and corresponding micrographs of fabrication results for DLW-based 3D printing of four demonstrative bottle-shaped shells with orifices are presented in **Figure 2a** and **2b**, respectively. SEM micrographs of example fabrication results for shell microstructures designed with wall thicknesses of 3 μm, 5 μm, 7.5 μm, and 10 μm are presented in **Figure 2c**. One deficit that may be associated with the development process used in this study was that the time required to remove residual photomaterial from the interiors of the shells appeared to result in diminished structural integrity of the shells. As a result, the majority of cases revealed undesired fractures and cracks. One note, however, is that such defects may have been caused by a current error in the z-drive feature for the 25× objective lens of the Nanoscribe 3D printer used in this study, which we observed led to defects in the intricate features of the bottleneck in particular. Thus, further studies are needed to determine whether the observed defects were caused by the printer's z-drive error or if they are caused by the development protocol itself, thereby requiring modifications to the presented protocols.

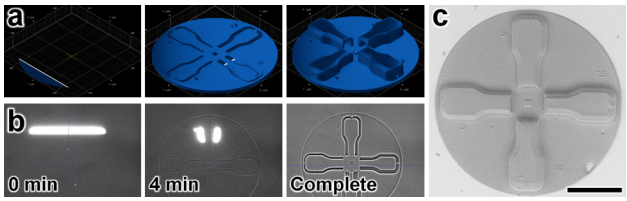

Figure 2: *Results for "shell" fabrication.* ***(a,b)*** *The DLW 3D printing process for four radially arrayed shells.* ***(a)*** *Computer-aided manufacturing (CAM) simulations.* ***(b)*** *Corresponding micrographs captured during the 3D printing process.* ***(c)*** *SEM micrograph of representative results. Scale bar = 100 μm.*

"Core" Loading and Intermediate Retention Efficacy

Both brightfield micrographs (**Fig. 3a,b**) and corresponding fluorescence micrographs (**Fig. 3c,d**) captured directly after vacuum loading of the liquid core (*i.e.*, methylene blue-dyed DI water) and infusion of liquid-phase PEGDA photomaterial (*i.e.*, prior to cap printing) are presented in **Figure 3a,c** and **3b,d**, respectively. Although we observed that the core vacuum loading

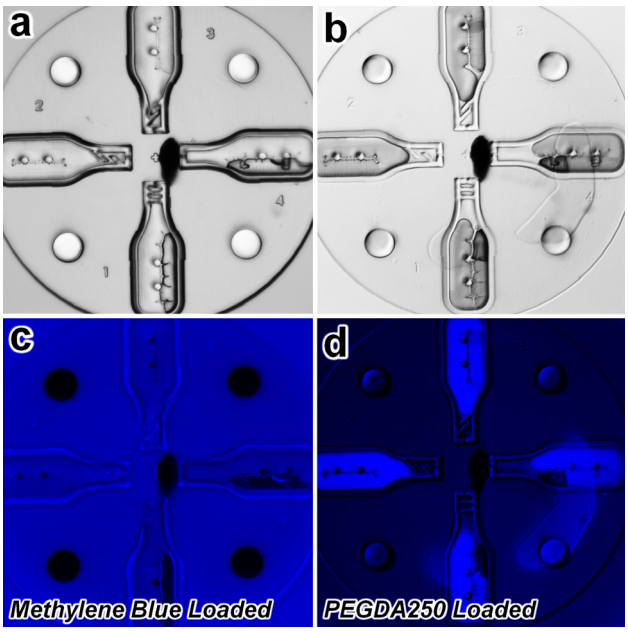

Figure 3: *Results for loading and retention of the liquid "core". **(a,b)** Brightfield and corresponding **(c,d)** fluorescence micrographs of results for **(a,c)** microfluidic vacuum loading of a liquid core (methylene blue-dyed DI water) and **(b,d)** retention of the core following loading of the liquid-phase photomaterial (PEGDA) for the cap.*

process was typically successful regardless of the interior design of the shell bottleneck adjacent to the orifice, the ability to retain the liquid core following loading of liquid-phase photomaterial (*i.e.*, for subsequent cap printing) appeared to vary in its efficacy based on the overall shell design and the design of the bottleneck's microchannel. By designing the bottleneck with microfluidic obstruction structures (*e.g.*, narrow internal channels to increase the hydraulic resistance); however, we found that both core loading and retention functionalities could be executed successfully (**Fig. 3**).

"Cap" Fabrication and Final Core Retention Efficacy

We investigated two variants of PEGDA with different molecular weights—specifically, PEGDA 250 and PEGDA 575—as materials for cap fabrication. Previously, researchers have found DLW-printed PEGDA 575 to yield effective biodegradation properties for drug and cell delivery applications [17]. In our studies, however, we observed that caps DLW-printed with PEGDA 575 resulted in porous, hydrophilic caps that appeared to absorb the liquid core directly into the caps. Consequently, experiments in which the caps were printed with PEGDA 575 failed to retain the methylene blue-dyed DI water in the core, rendering the material unsuitable for drug delivery within the context of the current study.

In contrast to PEGDA 575, PEGDA 250 exhibits a slower degradation rate and is insoluble in water [18]; however, liquid-phase PEGDA 250, which is denser and hydrophobic, presents challenges for the retention of the water-based liquid core. Preliminary results for DLW-printing the PEGDA 250 caps directly onto the orifices of the shells with liquid cores (*e.g.*, **Fig. 4**) revealed the ability to effectively maintain the core throughout the complete fabrication methodology (*e.g.*, **Fig. 5**). Despite these promising initial results, it is important to note that the fabrication variability and defect rates for the printed shells greatly diminished the ability to achieve such results with high repeatability (*e.g.*, that needed for clinical translation). Furthermore, critical microcarrier functionalities, such as cap biodegradation-mediated core release dynamics and storage stability, have not yet been investigated, but will be important to evaluate to assess the potential utility of the presented approach for drug delivery applications.

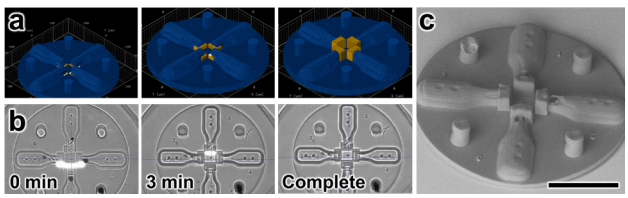

Figure 4: *Results for "cap" fabrication. **(a,b)** The DLW 3D printing process for the caps. **(a)** Computer-aided manufacturing (CAM) simulations. **(b)** Corresponding micrographs captured during the DLW 3D printing process. **(c)** SEM micrograph of representative results. Scale bar = 100 μm.*

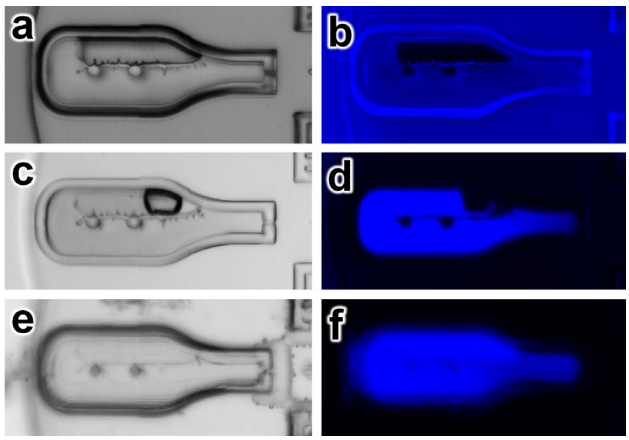

Figure 5: *Results for "cap" printing and retention of the liquid "core" for a single representative bottle-shaped microcarrier: **(a,b)** following microfluidic vacuum loading of the liquid core (methylene blue-dyed DI water), **(c,d)** following microfluidic loading of the liquid-phase cap photomaterial (PEGDA 250), and **(e,f)** after DLW-printing of the cap. Micrographs captured using **(a,c,e)** brightfield and **(b,d,f)** fluorescence microscopy.*

CONCLUSION

Additive manufacturing techniques offer powerful means for a variety of scientific fields [19]–[21], with DLW providing distinctive advantages for applications that demand high geometric control at micron-to-submicron length scales [22], [23]. In this work, we explored the utility of DLW as a route to achieve liquid-core-shell-cap microcarriers with biodegradable caps as a step toward potential controlled-release drug delivery applications. These preliminary results revealed that through geometric modifications of the bottle-shaped shell of the microcarriers (to enhance the separation between the liquid core and the liquid-phase cap photomaterial prior to cap printing), complete (*i.e.*, capped) microcarriers could be fabricated successfully. Importantly, investigations of cap mediated-core release functionalities—as well as the

potential to modify cap thickness or design to tune such functionalities [24]—have not yet been performed in the current work. Thus, future efforts should explore such potential capabilities, which would, in turn, hold unique promise for drug delivery applications.

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CONFLICT OF INTEREST

Sharon Flank is Founding CEO of InfraTrac, Inc., which has a potential interest in commercializing the microcarriers presented in this work.

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