HIGH-THROUGHPUT SYNTHESIS OF ENCODED HYDROGEL PARTICLES FOR BIOSENSING USING CONTACT FLOW LITHOGRAPHY

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ABSTRACT

We report an automated cost-efficient method for the high-throughput fabrication of graphically encoded hydrogel microparticles for use in multiplexed biosensing. By combining automated flow lithography in a multichannel microfluidic device with contact lithography and a high capacity 1-inch UV source, we improve our production rate of chemically homogeneous particles by at least two orders of magnitude (> 10^6 100-micron sized particles per hour).

KEYWORDS: hydrogel particles, graphical encoding, contact lithography, high-throughput patterning

INTRODUCTION

Suspension arrays of graphically encoded hydrogel particles are compelling alternatives to optically encoded beads for multiplexed biosensing due to their biocompatibility, high probe density, ease of production and versatility in encoding. The Doyle group previously developed a projection lithography technique for the continuous photopolymerization of PEG particles in PDMS microchannels, subsequently demonstrating numerous biosensing applications [1, 2]. However, in order to supply multiplex screens at ultra-high throughput, such as drug discovery campaigns, higher sustainable production rates still need to be achieved. To increase the synthesis rates by two orders of magnitude, we developed a method for particle photo-polymerization in microchannels based on contact photolithography, using a bench-top cost-efficient instrument.

EXPERIMENTAL

The contact lithography station is depicted in Figures 1a and 2b. A chrome photomask is secured on a holder on top of the 1" LED source (365nm). The LED position is adjusted to align the mask with the device placed on the stage, and to bring them in contact, using the top view imaging module for control.

The principle of particle synthesis is depicted in Figure 2b. A PDMS mold with 50µm deep channels

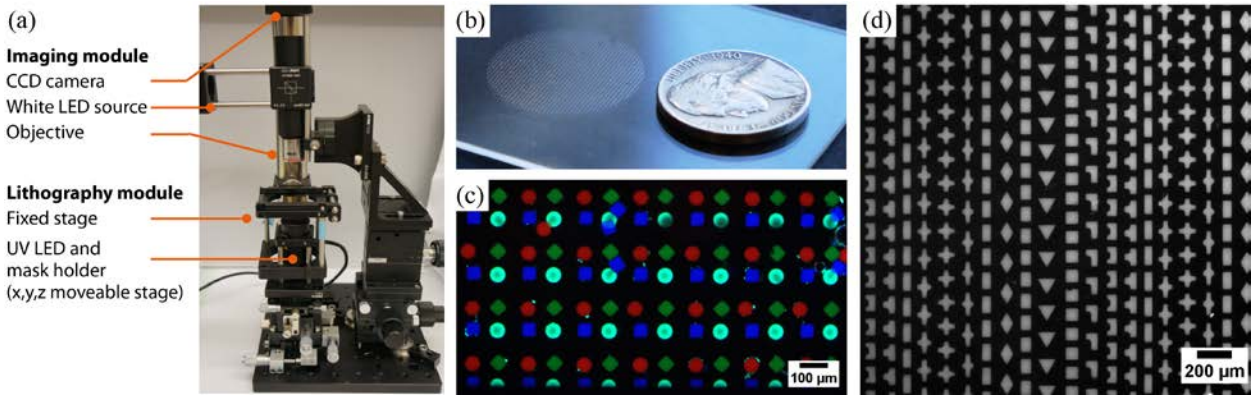

Figure 1: Contact lithography station. (a) View of the instrument. (b) Full polymerization area (2cm). (c) Composite fluorescence image of polyethylene diacrylate (PEGDA) posts patterned inside a 50µm tall glass channel using four different materials, each one being labeled with a different fluorophore. (d) Array of rhodamine-B labeled shape-encoded PEGDA structures photo-polymerized on a glass slide.

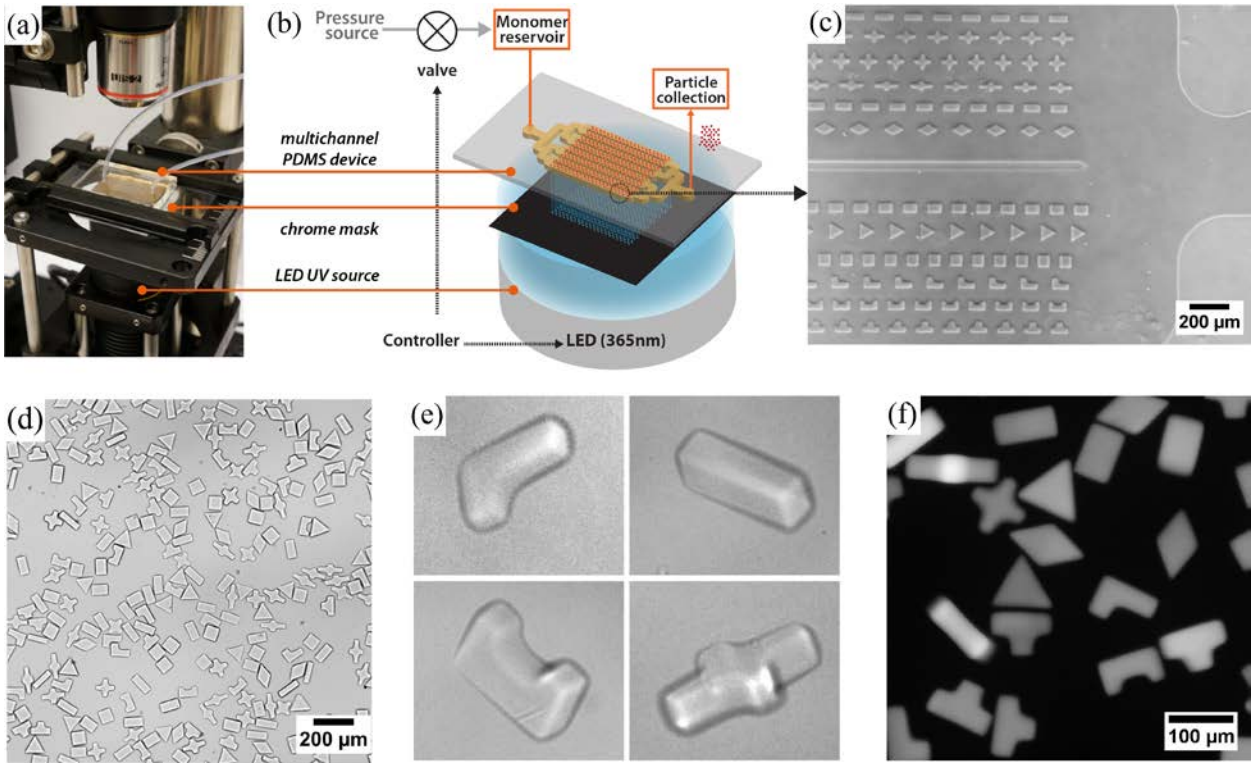

Figure 2: Synthesis of shape-encoded particles using stop-flow contact lithography. (a) Detailed view of the stage with the device and chrome photomask in place. (b) Experimental set-up. (c) Crosslinked PEGDA particles in the microchannels right after exposure. (d, e) Brightfield images of the collected particles. (f) Fluorescence image of rhodamine-B labeled PEGDA particles.

is assembled with a 150µm thick glass slide spin-coated with PDMS to form the multichannel device (8parallel channels per inlet, 1mm wide). A switch valve controls the flow of monomer solution in the device. Extensive details about stop-flow lithography can be found elsewhere [1, 2]. The automated synthesis cycle operates as follows: (i) the channels are filled with the monomer blend; (ii) UV exposure through the mask induces polymerization of graphically-encoded particles; (iii) particles are collected at the outlet, while the device is filled with fresh monomer and (iv) a new polymerization cycle starts.

PEGDA particles were UV-polymerized from various blends of PEGDA (MW700), photoinitiator (Darocur1173) and Tris-EDTA buffer. Acrylated biomolecules (fluorophore, oligonucleotide) mixed in the monomer blend were covalently captured in the particles during gelation. PEG (MW600) was added in the monomer blend as a porogen to enhance the gel porosity when necessary.

RESULTS AND DISCUSSION

The collimated LED UV source provides a circular homogenous illumination area over 2cm, a diameter 20 times larger than using the projection lithography technique (Figure 1b-c). The light density is sufficient to polymerize thin objects in tens of milliseconds, yielding sharp and well resolved objects.

To make the most of this increased polymerization area, flow lithography was parallelized by designing a multichannel device. However, the overall throughput of particle synthesis depends not only on the polymerization area but also on the device dimensions which impact the time required to flow the particles out of the device and for PDMS to relax and completely stop the flow in the device. A theoretical study showed that those times scale as the length of the channel, which was then kept at 1mm. Simulations were used to design a device with a homogeneous flow rate across the different channels. For each inlet, the flow is divided in 8 parallel channels, and two of these modules can be run in parallel, covering a 16mm*10mm polymerization zone and producing 10 000 particles per exposure (Table 1).

Table 1. Comparison of projection and contact stop-flow lithography parameters

Technique	Light source	Device dimensions	Typical cycle time	Particles (75μm) per exposure	Throughput (particles/h)
Projection SFL	mercury lamp + filter	straight channel h=40μm, w=300μm, l=1cm	1s	20	72 000
Contact SFL	LED lamp (365nm)	8 parallel channels per inlet h=50μm, w=950μm, l=1cm	6s	10000	6 000 000

With this technique, free-floating particles functionalized with a sensing bioprobe (oligonucleotide, protein) and/or a fluorophore were produced at high speed and reproducibility (CV<7% for fluorescence intensity). A library of simple shapes was successfully created for mid-plex bioassays (Figure 2d).

CONCLUSION

To our knowledge, none of the contact lithography [3, 4] and replica molding [5] techniques reported for the production of 100-μm shape-encoded particles dedicated to biosensing has been automated and particle collection is often laborious. As for hydrogel particle production using microfluidic droplet generation tools, although such techniques have reached high-throughput, they do not offer flexibility for graphical encoding [6]. With the present technique, millions of particles are produced in an hour and such cost-efficient and simple synthesis stations could be easily parallelized towards industrial production. Moreover, complex libraries can be generated by simultaneously encoding particles with varying levels of a fluorophore or using bitcoding.

Beyond particle synthesis, this apparatus provides an affordable solution for rapid and accurate microstructure patterning of any photopolymerizable material onto surfaces or in channels. Thanks to the fine alignment capability, complex patterning of multiple materials using multiple masks can be achieved (Figure 1d).

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