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Hybrid soft-lithography/laser machined microchips for the parallel generation of droplets†

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Abstract

Microfluidic chips have been developed to generate droplets and microparticles with control over size, shape, and composition not possible using conventional methods. However, it has remained a challenge to scale-up production for practical applications due to the inherently limited throughput of micro-scale devices. To address this problem, we have developed a self-contained microchip that integrates many ($N = 512$) micro-scale droplet makers. This $3 \times 3 \text{ cm}^2$ PDMS microchip consists of a two-dimensional array of 32×16 flow-focusing droplet makers, a network of flow channels that connect them, and only two inputs and one output. The key innovation of this technology is the hybrid use of both soft-lithography and direct laser-micromachining. The microscale resolution of soft lithography is used to fabricate flow-focusing droplet makers that can produce small and precisely defined droplets. Deeply engraved ($h \approx 500 \text{ }\mu\text{m}$) laser-machined channels are utilized to supply each of the droplet makers with its oil phase, aqueous phase, and access to an output channel. The engraved channels' low hydrodynamic resistance ensures that each droplet maker is driven with the same flow rates for highly uniform droplet formation. To demonstrate the utility of this approach, water droplets ($d \approx 80 \text{ }\mu\text{m}$) were generated in hexadecane on both 8×1 and 32×16 geometries.

Introduction

Microfluidic chips that generate droplets and microparticles have been utilized for a wide variety of applications, including the generation of droplets for digital biological assays,^{1–5} the fabrication of functional microparticles,^{6–11} and the synthesis of nanoparticles.^{12–17} The small feature-size of microfluidics enables fine control over size, shape, and composition of droplets and microparticles, with extremely high levels of uniformity.^{18,19} However, the limited throughput of micro-scale devices, make these approaches unsuitable for many practical applications.^{19,20}

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One promising approach to increase the throughput of microfluidic-based approaches is to incorporate multiple droplet makers onto a single chip for parallel operation. For conventional single-layer microfluidics the number of inputs and outputs n scales with $n \propto N$, the number of generators,¹⁹ (Fig. 1a) resulting in an impractically large number of connections to the outside world. By using a second layer of microfluidic channels to supply each generator, analogous to the architecture of an integrated circuit,²¹ large arrays can be packed onto a single chip with only a single set of inputs and outputs ($n = 3$ for single emulsions) (Fig. 1b). Previous work to integrate arrays of microfluidic droplet makers include the use of a machined metal fitting to feed a circular array of 256 PDMS molded droplet makers.²⁰ Additionally, devices have been developed that use multiple layers of microfabricated channels coupled together with either drilled²² or punched holes.^{23,24} Because these previous approaches rely on macroscopically defined through-holes, there is a practical limit to the number of generators that can be integrated onto a single chip ($N \sim 100$).^{20,23,24}

To address this issue, we have developed a fully microfabricated, self-contained $3 \times 3 \text{ cm}^2$ PDMS microchip that consists of a two-dimensional array of 32×16 (512) flow-focusing droplet makers, an integrated network of channels to drive each droplet maker, and only two inputs and one output. To build this chip, we used both soft-lithography and laser micromachining. The microscale resolution of soft lithography was used to fabricate flow-focusing droplet makers with an aperture of $30 \mu\text{m}$ that can produce small and precisely defined droplets. Laser micromachining enabled larger feature sizes and higher aspect ratios to create deeply engraved channels ($h \approx 500 \mu\text{m}$), which supply each of the droplet makers with its oil phase, aqueous phase, and access to an output channel. The engraved channels' low hydrodynamic resistance ensured that each droplet maker was uniformly driven to create homogenous droplets. To demonstrate the utility of this platform, $d \approx 80 \mu\text{m}$ water droplets were generated in hexadecane containing Span 80 (1.5% v/v) on both a single row 8×1 and a two dimensional array 32×16 (512) geometry.

Experimental design

To deliver fluid to each of the droplet makers, we used a ladder geometry with each maker connected to an oil, aqueous, and output line (Fig. 1b). The spine of the ladder is an engraved supply line and each rung of the ladder is a molded flow-focusing droplet maker. To ensure that each drop maker behaves identically, we designed the microchip such that the pressure drop along the supply channel P_r remains small compared to the pressure drop across the individual drop makers $P_r \ll P_d$. To enforce this design condition, we adopted the following design rule,²³

$$2N(R_r/R_d) < 0.01 \quad (1)$$

where R_r is the fluidic resistance along the delivery channel between each drop maker, R_d is the fluidic resistance of the individual droplet maker, and N is the number of droplet makers in the row (Fig. 1d). The row delivery lines and droplet makers consisted of rectangular microchannels, whose fluidic resistance R can be approximated²⁵

$$R = \frac{12\mu l}{wh^3} \quad (2)$$

where μ is the dynamic viscosity of the fluid and w , h , and l are the width, height, and length of the microchannel. The row delivery channel has a width $w_r = 0.6$ mm, height $h_r = 0.55$ mm, and length $l_r = 40$ mm. The flow-focusing droplet maker was modeled as rectangular channels in series with $w_d = 50$ μ m, 30 μ m, 50 μ m, and $l_d = 1600$ μ m, 380 μ m, 500 μ m, $h_d = 30$ μ m for oil, water, and their mixture respectively. For this geometry, we calculated that we can satisfy the design condition eqn (1) for $N_d < 360$. This number could be increased further by either increasing the size of the row delivery channel or decreasing the size of the droplet makers. For our two dimensional array, we set the number of droplet makers per row $N_d = 32$. A micrograph of a row of droplet makers is shown in Fig. 1f.

To deliver fluid to each of the rows, we used a ladder geometry with each row connected to a single arterial supply line (Fig. 1c). The supply line is a rectangular channel with $w_d = 1.4$ mm, $h_d = 0.55$ mm, and $l_d = 40$ mm. Because the resistance of the drop makers are much larger than that of the row delivery lines, we modeled the resistance from the arterial line through each row as $N_d = 32$ droplet makers in parallel $R_{\text{row}} \sim R_d/N_d$ (Fig. 1e). We calculated that the design condition eqn (1) can be satisfied for $N_r < 19$ (for both the water and oil channels). This number could be increased further by either increasing the size of the arterial line or decreasing the size of the droplet makers. We set the number of rows per device $N_r = 16$. A micrograph of the ladder geometry that supplies each row is shown in Fig. 1g. A photograph of the 3×3 cm² two dimensional array chip is shown in Fig. 1h next to a quarter dollar for scale.

Device fabrication

The fabrication strategy consisted of integrating three separate pieces: 1. a laser-engraved layer of PDMS that contains channels to deliver fluid to the droplet makers below (Fig. 2a). 2. An intermediate layer of spin-coated PDMS that is laser cut with through-holes to connect the engraved layer to the droplet makers (Fig. 2b). 3. A soft-lithography molded layer of PDMS that contains a two dimensional array of droplet makers (Fig. 2c). The three separate pieces were bonded together by stamping the pieces into spin-coated uncured PDMS,²⁶ aligning the pieces together under a stereoscope with a custom alignment rig, and baking (Fig. 2d). All pieces were fabricated using poly(dimethylsiloxane) (PDMS) (KR Anderson Company, Sylgard/elastomer kit). The dimensions of the droplet makers and connecting channels are given in the Experimental Design section above. The laser cut through-holes are 270×270 μ m² for the oil, 180×180 μ m² for the aqueous phase, and 270×270 μ m² for the output. The complete mask design is included in ESI.[†]

The laser engraved layer and the intermediate layer were machined using an infrared laser micromachining system (VLS3, VersaLaser). The molded layer was fabricated using standard single-layer SU-8 lithography (SU-8 2025, MicroChem) with 30 μ m thick features.²³ For the engraved layer, a 4 mm thick layer of PDMS was poured into a plastic

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petri dish and baked. This piece was then laser engraved using raster-based laser patterning, for a channel thickness $h \approx 500 \mu\text{m}$. For the intermediate layer, a $200 \mu\text{m}$ thick layer of PDMS was spin coated (ws-650mz-23npp, Laurell) and then baked. This spin coated layer was then laser cut using vector-based laser patterning to make through holes. Both the engraved and the intermediate layer were cleaned in a solution of tergazyme detergent (Sigma-Aldrich) in deionized water for 10 minutes in a sonicator, followed by a 10 minute soak in deionized water, followed by nitrogen blow drying. Washing using a detergent instead of isopropanol avoided swelling of the PDMS, which allowed for better alignment of the layers. Prior to use, the microfluidic channels were coated with Aquapel (PPG Industries) to ensure that they were preferentially wet by the hexadecane oil.

Results

Single row device

The first prototype that we built consisted of a single row of 8 droplet makers (Fig. 3a). Extending from each droplet maker is a 3 mm long channel, such that the droplets could be consistently imaged and droplet size and uniformity characterized. The aqueous phase input was set to $\Phi_{\text{aq}} = 0.8 \text{ mL h}^{-1}$ and the oil phase was set to $\Phi_{\text{oil}} = 3 \text{ mL h}^{-1}$, using two syringe pumps (NE 500, New Era Pumping Systems). Droplet formation was observed on all eight drop makers using an upright microscope (Leica, DM4000B) (Fig. 3b). The rate of droplet formation for the array was calculated to be 4×10^5 droplets per minute. The flow rates were chosen to facilitate characterization, by producing spatially separated droplets from each droplet maker. A video of droplet formation can be found the ESI.[†]

The droplet diameters were measured using image analysis software (ImageJ). The average diameter for the droplet makers was $d = 78.6 \mu\text{m}$ and the coefficient of variation CV of the individual droplet makers was 2.1%. The CV from the total output of all eight droplet makers was 3.6% (Fig. 3e). The variations in drop size did not significantly increase or decrease as a function of position along the ladder geometry, demonstrating that the pressure drop on the supply line was not significant. A photograph of the droplet maker device used in these experiments is shown in Fig. 3d.

Two dimensional droplet array

Based on the design constraints outlined in the sections above, we designed and fabricated a two dimensional droplet array consisting of 32 rows, with each row consisting of 16 drop makers (Fig. 4a). Each of the 512, $30 \mu\text{m}$ aperture flow focusing drop makers (Fig. 4b) connect to a source of oil and aqueous phase as well as to an output path. The fluid delivery lines run across each row in a ladder geometry. On the edge of the array, each row is supplied by an arterial supply channel (Fig. 4d). An underpass geometry connects each of the rows to the arterial output line, allowing it to pass under the arterial lines that supply the oil and aqueous phase. The underpass brings the fluid from the engraved level down to the molded level, where it passes under the oil or aqueous supply line, and back to the engraved level to meet with the arterial output line, which runs along both edges of the chip (Fig. 4c).

We tested the two dimensional array of droplet makers using two syringe pumps, one for the oil and one for the aqueous input. The aqueous phase input was set to $\Phi_{\text{aq}} = 8 \text{ mL h}^{-1}$ and the oil phase was set to $\Phi_{\text{oil}} = 20 \text{ mL h}^{-1}$. We observed droplet formation on all five hundred and twelve drop makers using an upright microscope (Leica, DM4000B). We calculated the rate of droplet formation, 4×10^6 droplets per minute. This rate was not limited by the device but by the syringe pumps and could be improved by switching to peristaltic pumps. A histogram was generated for the total output of the 512 droplet makers (Fig. 4e). The droplets had an average diameter of $d = 86.1 \text{ }\mu\text{m}$ and $\text{CV} = 9.0\%$. We hypothesize that the variations in droplet size from droplet-maker to droplet-maker increased relative to the 8 droplet device, due to the increased area of the chip which exacerbated small misalignment. Alignment could be improved by using a material more rigid than PDMS (e.g. fused silica²⁷).

Discussion

We have developed a novel technology for integrating many droplet makers onto a single PDMS chip. Our hybrid laser micromachined/soft lithography defined device uses the following elements to maximize the density of droplet makers, while maintaining droplet homogeneity: 1. deep, laser micromachined channels to deliver flow to each of the droplet makers with minimum variation in pressure, 2. fine-featured soft-lithography defined droplet makers to create small and uniform droplets. 3. Only two inputs and one output to enable simple use and integration. Furthermore, the all-PDMS construction of the device enables inexpensive manufacturing.

The device architecture developed in this paper can be extended to chips with more droplet makers than the 512 demonstrated in this paper. The main limitation to increasing the number of droplet makers is the design condition outlined in eqn (1). Due to this constraint, increases in the number of droplet makers N must be accompanied by a decrease in the flow resistance of the supply lines or an increase in the flow resistance of the droplet makers. By isotropically scaling the droplet makers from having an aperture size of $30 \text{ }\mu\text{m}$ to $2 \text{ }\mu\text{m}$, the flow resistance of the drop maker $R_d \propto l/(wh^3)$ would increase by a factor of $\sim 10^3$. This scaling would allow for approximately $1000\times$ more droplet makers N to be included for a given supply channel. However, to pack droplet makers this densely, the infrared laser micromachining used in this study, which has a resolution of $\sim 100 \text{ }\mu\text{m}$, would not be sufficient. We hypothesize that UV laser micromachining could be utilized, which can achieve $\sim 1 \text{ }\mu\text{m}$ feature size.²⁸ With such a device we estimate that on a $3 \times 3 \text{ cm}^2$ chip 2×10^6 droplet makers can be fabricated (1000×2000), with a throughput of $>10 \times 10^9$ droplets per minute. Additional opportunities for future development includes replacing the flow-focusing droplet makers with T-junctions²⁹ which require less space per drop maker and can be packed more densely or the addition of droplet splitters to further increase droplet generation.³¹ Additionally, more complex droplets can be generated that require $N > 2$ inputs per device, such as Janus microparticles³⁰ or multiple emulsions.²³

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

A hybrid soft-lithography/laser micromachined PDMS microchip for highly parallel droplet formation. a. For single-layer microfluidics, the topology of a droplet maker requires that the number of inputs/outputs n is proportional to the number of droplet makers $n \propto N$. b. By adding a second layer of microfluidics, the number of inputs for an array of droplet makers is $n = 3$ regardless of N . The droplet makers are molded into PDMS using soft lithography (green) and the delivery lines (blue) and through-holes (purple) are fabricated using laser micromachining. c. The droplet makers are organized into a ladder geometry, where each row of droplets is supplied by a single oil Φ_{oil} , aqueous $\Phi_{aq.}$, and output line Φ_{out} . Each row is supplied by an arterial row-delivery line, in a ladder geometry. d. The flow resistance of the ladder geometry is modeled as a series of flow resistors, with the resistance of the droplet makers R_d and that of the rows R_r . e. For the supply of fluid to each row, we model a similar circuit with the resistance of the arterial channel R_c and the total resistance of each row R_{row} . f. A micrograph of a row of droplet makers. The scale bar is 600 μm . g. A micrograph of the arterial supply line and underpass. The scale bar is 1.5 mm. h. A photograph of the chip. The scale bar is 20 mm.

Fig. 2.

Fabrication strategy. The hybrid chip consists of three layers, a. A laser engraved layer, which includes the delivery microchannels. b. A laser cut intermediate layer that contains the through-holes that connect the laser engraved layer and the droplet makers. c. A soft-lithography molded layer that includes the droplet makers. Uncured and cured PDMS are shown in red and orange, respectively. d. The layers are combined together using a stamping and baking technique, in which 1. a thin layer of PDMS is spun coat onto a silicon wafer, 2. one of the pieces is stamped into the uncured PDMS, 3. two layers are aligned and bonded using a custom built rig under a stereoscope, and 4. the pieces are baked to cure the PDMS.

Fig. 3. Characterization of a single row device. a. Eight flow focusing droplet makers are organized into a single row, with oil, aqueous, and output lines connected in a ladder geometry. b. Optical micrographs show uniform droplets are created by each droplet maker. The scale bar is 150 μm. c. Histograms of the diameter of the droplets from each individual droplet maker show an average diameter of $d = 78.6$ μm and an average CV of 2.1%. d. A photograph of the single row device. Scale bar: 1 mm. e. The histogram of the total output of the device shows a CV of 3.6%.

Fig. 4.

Two dimensional array. a. The array of droplet generators is organized into 16 rows, each row consisting of 32 droplet makers. The array is fed by a single aqueous (blue), oil (yellow), and output (purple) line. b. A schematic and stereoscope micrograph of the flow focusing droplet makers. The scale bar is 100 μm . c. A schematic and stereoscope micrograph of the underpass structure, which allows the output lines to pass under the oil line on the left edge and the aqueous line on the right edge of the array. The underpass sends the output from the engraved layer down to the molded layer (green), and then back to the engraved layer where it connects to an arterial line. Scale bar: 500 μm . d. A stereoscope micrograph of the droplet array. Scale bar: 3 mm. e. A histogram of the diameter of the droplets from the array of droplet makers show an average diameter of $d = 86.1 \mu\text{m}$ and a CV of 9.0%.