Shear-Based Droplet Production for Biomaterial Printing

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Abstract

The highly uniform shear forces at millimeter and smaller dimensions can produce monodisperse particles spontaneously during continuous flow of immiscible fluids. Microfluidic techniques have also recently been used to generate and organize arrays of solid monodisperse microparticles with various material properties. Combining these developments with a well-engineered "chip-to-world" interface will provide a new high-resolution 3-D printing technology for rapid prototyping and biotechnology.

We produced hydrogel droplets at rates of 10-200 Hz in PDMS microreactors. Droplets were monodisperse, with 50-200 micron diameter dependent on flow rate, channel shape and the presence of surfactants. Droplets may be dispensed from the chip in their liquid state, or solidified by one of three mechanisms. Ultraviolet (UV) light was used to solidify photosensitized hydrogels. We also discuss other hydrogel systems that are solidified by a chemical reaction (sodium alginate and $CaCl_2$) or by thermal gelation (Pluronic F-127), methods to vary the droplets' chemical composition and mechanical properties, and methods for closed-loop electrical feedback on particle position for precision fabrication. Biocompatible hydrogels are of great interest for biomaterial applications such as drug delivery and tissue engineering.

Introduction

Three-dimensional printing methods that deposit a stream of viscous material are capable of rapid prototyping at a relatively low cost. For example, fused deposition modeling (FDM) and robocasting both operate by extruding material from a nozzle, with either the nozzle or the part moving under computer control. Because the deposited layers are relatively thick, parts can be built faster than by inkjet printing methods. However, because it takes some time for pressure or heat to start and stop flow at the nozzle, there is a serious tradeoff between build speed and a well-controlled cutoff of the deposited material.

This paper will review the work of our group and others with surface-tension based microfluidic techniques that use steady flow to produce a train of monodisperse droplets, rather than a continuous stream of material. These droplets are a potential source of individual voxels for three-dimensional printing. To apply the droplet generators as a printing technology, however, two important engineering developments are needed: (1) creation of droplets from materials that can quickly be solidified using various methods, and (2) precise control over droplet location as solid or liquid droplets exit the microfluidic device. Toward goal (1), materials development, we discuss three droplet solidification mechanisms: photopolymerization, chemical solidification, and thermal gelation, and provide examples of materials in each category. Toward goal (2), control over ultimate droplet location on the finished part, we discuss an electrode-based particle detection method that can sense the presence of droplets in a microfluidic channel. This information will help coordinate the translation stage with droplets' arrival at the chip outlet. With these developments, we envision microreactor print heads that generate, organize, and deposit arrays of solid microparticles with various material properties, having highly controllable shapes. This technology will enable rapid freeform fabrication of complex, high-quality parts directly from computer files by depositing small volumes of solid materials from chemicals that are mixed to the user's specification. Applications of the materials discussed here are especially promising in the medical and biotechnology fields.

Droplet formation

In the microfluidic regime (typical channel widths 1mm and smaller), when two immiscible fluids meet at a junction, the balance between surface tension and pressure can spontaneously produce monodisperse droplets (~2-5% coefficient of variation in diameter) [1,2]. Similar droplet trains form by flow focusing at bottlenecks during injection of oil and water streams at constant flow rates [3]. From around the year 2000 onwards, the problem of formation and manipulation of discrete droplets in two-phase flow inside microdevices has drawn significant attention [4]. While these spontaneously-formed droplet trains already have regular spacing that will be useful for predicting droplet arrival time in a printing application, droplet formation can also be electrically triggered at precise times by charging the water/oil or polymer interface in a microfluidic device [5]. Electrodes can switch fluids or droplets from one channel or another with no moving parts [6,7]. These techniques will enable electrically-controlled droplet release, addition of dyes, crosslinking agents, thermally depolymerizable substances or conductive liquids to selected droplets, and switching droplets into a stream in order to build an arbitrary sequence of colored/ differentially soluble/ conductive materials for deposition.

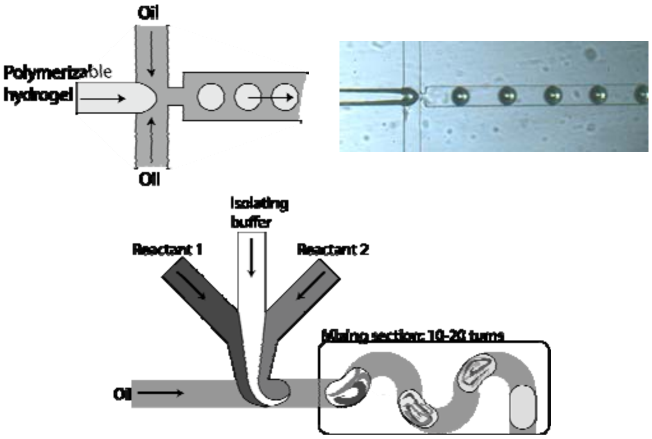

***Figure 1** Top left: schematic of a flow-focusing droplet reactor; top right: 100-micron diameter water droplets forming under steady inputs of water and oil in our reactor. Bottom: schematic of T-junction droplet reactor and mixer [8].*

Examples of microfluidic droplet-producing devices are shown in Fig. 1. Two immiscible fluids containing the hydrophobic and hydrophilic phases are injected into the microfluidic device using syringe pumps. Because a hydrophobic material was used for the channel walls in our experiments, the system produces aqueous or other polar droplets surrounded by a hydrophobic continuous phase (typically hexadecane or silicone oil). These microreactors create 50-200 micron diameter hydrogel droplets at a rate of 200-300 droplets per second.

Microreactor fabrication method

Droplet microreactors shown in Fig. 1 were produced by casting material onto a microfabricated mold master. The mold masters were made from SU-8 50 (MicroChem) negative photoresist on silicon. The devices were cast in poly(dimethyl)siloxane (PDMS) elastomer (Sylgard 184, Dow Corning Corporation). PDMS is widely used in the set of microfabrication techniques known as soft lithography [9]. It is a popular material for several reasons: it offers easy replication of microscopic features from reusable molds, transparency, low cost, biocompatibility, ability to bond to a smooth surface, and good control over wettability. A PDMS mold is prepared by mixing of PDMS prepolymer and curing agent in a 10:1 ratio and then degassing in vacuum to remove bubbles. The degassed PDMS mixture is then distributed onto the silicon master and cured at 60°C in an oven. After curing and demolding, holes are punched in the PDMS replica to add the fluid inlet lines. The replica and a PDMS coated glass substrate are both treated with oxygen plasma for 20 seconds and brought into contact to irreversibly bond to each other, forming a microchannel with all four walls made from hydrophobic PDMS. These fabrication steps are illustrated in Fig. 2. The cross-sectional dimensions of the final assembled microfluidic channels are 150 μm ×150 μm. Some devices have a large outlet reservoir (Fig. 3a) for collection and observation of the particles, and others have multiple inlets and a mixing section (Fig. 3b) for inducing solidification by a chemical reaction between two components in a droplet, followed by a long channel to allow the reaction to proceed.

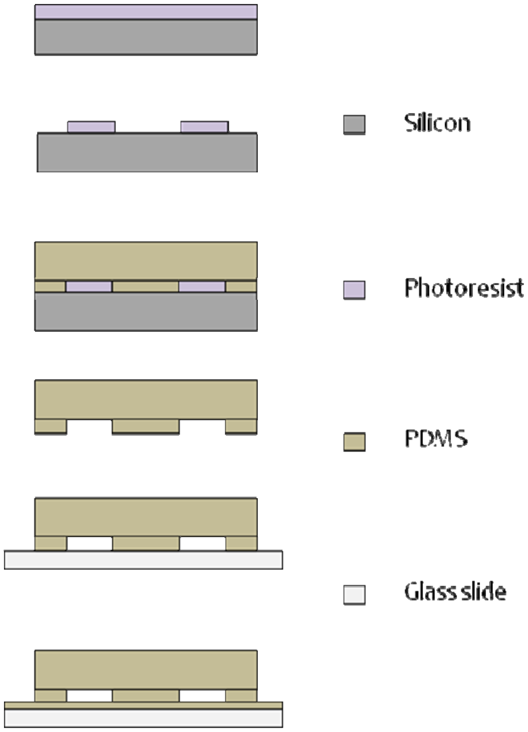

Figure 2 *Fabrication process steps for microfluidic device from poly(dimethyl siloxane) (PDMS). Devices are sealed to a glass slide with a thin layer of PDMS for an all-PDMS hydrophobic*

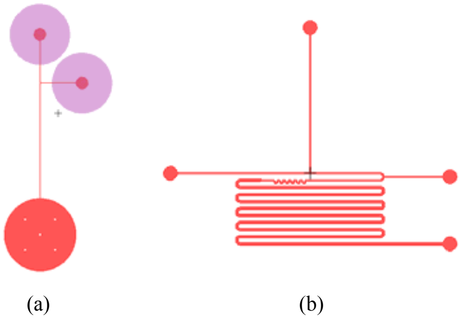

Figure 3 *Layouts of T-junction device ports: (a) Two inlets and one large outlet port for collection, (b) Three reagent injection device with mixing section and observation channel.*

Droplet materials and methods

Liquid droplets can be solidified by at least three different mechanisms: thermal solidification (by a phase change or polymerization), chemical reaction, and photopolymerization.

Thermal solidification Aqueous solutions of polymers such as Pluronic F-127 will undergo an inverse sol-gel transition, becoming solid when heated. Depositing these liquids from a cooled microfluidic device onto a room-temperature or physiological temperature substrate will solidify them into a biocompatible hydrogel with applications in tissue printing and bioengineering [10]. Typical concentrations are from 15 to 30 wt% in water. Without a dedicated cooled stage, however, we found 25% solutions of F-127 too viscous to readily undergo droplet formation, instead forming a parallel co-flowing stream with the continuous phase (hexadecane). Another possibility is to use more conventional phase-change mechanisms (freezing) to produce solids upon cooling of liquid precursors.

Chemical reaction Because sodium alginate and calcium chloride solutions gel rapidly when mixed, and are biocompatible, this system has been studied for biotechnological applications including cell encapsulation [12]. In this technique, aqueous sodium alginate and calcium chloride solutions are employed as a monomer and cross-linker, respectively. These solutions are slowly injected into two reagent inlets, supplying the dispersed phase, and an immiscible fluid (n-hexadecane) is injected into the other inlet in a perpendicular direction, supplying the continuous phase. Once the alginate solution comes into contact with the calcium ions, it immediately transforms into a gel due to the crosslinking between the guluronic acid blocks in the alginate by the Ca^{2+} ions. The interaction of these two precursors forms a fused droplet. The aqueous phases we used were a 1% by weight solution of sodium alginate in deionized water, and 20mM calcium chloride ($CaCl_2$) in water. The hydrophobic phase (continuous phase) was hexadecane containing surfactant (Span 80, 1 % by weight). Flow rate for both aqueous streams were set up at 0.01ml/h, while the continuous phase was maintained at 0.9 ml/h.

Photopolymerization Advantages of photopolymerization as a droplet solidification method are spatial and temporal control over polymerization, and extremely fast curing rates at room or physiological temperature. Photopolymerizable droplets can be made from acrylate-photoinitiator resins familiar to users of ultraviolet (UV) stereolithography and other three-dimensional photoprinting techniques. The UV light activates photo-initiators in the droplet which cause monomers to link to each other and this solidifies the material. Solid particles have been formed with these mixtures in droplet microreactors, but because these resins tend to be hydrophobic, the immiscible continuous phase is usually polar (water or another polar solvent). Therefore, the droplet reactors cannot be made from hydrophobic PDMS, but can be glass [13], plasma-activated temporarily hydrophilic PDMS [14], or urethane [11]. In contrast to these acrylates, photopolymerizable hydrogel droplets are polar and immiscible with oils, so the hydrophobic PDMS reactors described in Fig. 2 can successfully be used. If photoinitiators are chosen with low toxicity, these hydrogels will be useful for encapsulation of cells and other biological materials. We used a mixture that polymerized rapidly upon UV exposure to the mercury lamp in our fluorescence microscope, from [15]: 5 ml of 4-hydroxybutyl acrylate, 0.65 ml of acrylic acid, 58 µl of EGDMA (ethylene glycol dimethacrylate), and 30 mg DMPA (dimethylpropionic acid) photoinitiator, with similar flow rates to those used in the alginate system.

Results

We used surface-tension based microfluidic methods to produce droplet trains from two types of solidifiable materials. Photopolymerized hydrogel spheres with an average diameter of 60 microns were produced within a 100 micron diameter channel as shown in Figure 4a, and alginate hydrogel beads were produced by reaction with calcium chloride, in diameters ranging from 100-130 micron diameter within a 200 micron channel, as shown in Figure 4b. Photopolymerizable droplets had the advantage of rapid polymerization (~0.1 s or faster) so droplets were solidified before they could touch and merge in the outlet channel, producing monodisperse droplets even if surfactants were not present. However, alginate droplets' diameters were sensitive not only to flow rate and channel shape but also the presence or absence of surfactants, resulting in some larger merged droplets before solidification could occur, as seen in the middle of Figure 4b. Nevertheless, alginate beads are attractive due to their nontoxicity, biodegradability, and because they are formed from naturally occurring polysaccharides. These microfluidic devices are expected to find use in various fields, including high-resolution 3-D printing technology for rapid prototyping and biotechnology, and cell encapsulation [12].

Conclusions and future work

Photopolymerization of hydrogels allows good control over the spatial and temporal solidification process, as compared to solidification based on chemical reactions. A microfluidic three-dimensional print head can be envisioned for biomaterials using these chemicals with the same microfluidic chips but with miniaturized pumps, a microcontroller-based electrical interface, and a LED (395 nm) light source.

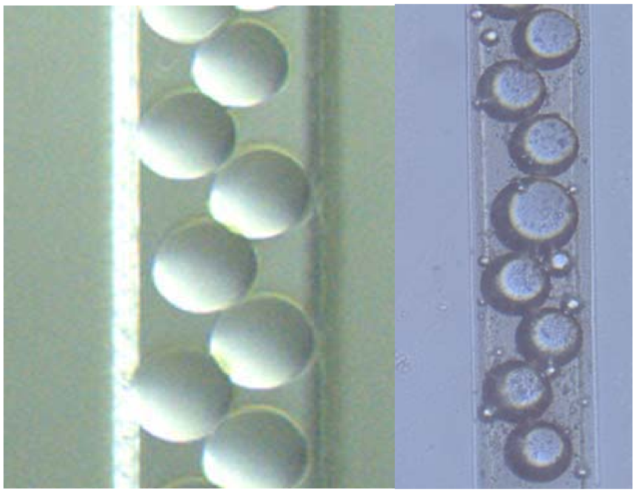

***Figure 4**: (a) Left: ultraviolet polymerized hydrogel spheres with average diameter 60 microns; (b) Right: alginate hydrogel droplets after reaction with calcium chloride.*

To deposit the particles in registry with parts under construction in a three-dimensional printing system, it will be important to sense the location and timing of particles as they emerge from the microreactor. The resulting information could be used to synchronize motion of the deposition system, or to shift particles into or out of the deposition stream. Here, microelectrode-based techniques should find several applications. Electrodes can apply fields for injecting, pumping and mixing fluids via electrophoresis, and for steering polarizable particles via dielectrophoresis. When a particle passes between a pair of electrodes in a medium having different electrical conductivity, the resulting impedance change signals the presence of the particle for closed-loop feedback during processing [16].

We described methods for particle formation in microfluidic reactors in relation to three-dimensional printing of biomaterials. Besides potential improvements in the quality and speed of three-dimensional printing, the technology promises to reduce the environmental impact of rapid prototyping by minimizing user exposure to hazardous reagents and providing opportunities to reuse materials. The process takes place in a closed channel, and hazardous reagents such as monomers are rendered harmless by polymerization before they exit the chip, while the immiscible nature of the droplet and continuous phase solutions allows continuous separation and reuse of unpolymerized material and carrier fluid. On-line detection of particle conductivity can prevent waste on a larger scale by sensing any defective particles and removing them before they are incorporated into the part.

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