Design and Fabrication of Flow-Focusing Devices for Tissue Engineering Applications

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DESIGN AND FABRICATION OF FLOW-FOCUSING DEVICES FOR TISSUE ENGINEERING APPLICATIONS

SAMUEL HOTALING

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ABSTRACT

While the lifespan of humans has increased, the durability of cartilage has not, leading to increasing rates of arthritis in aging humans. As both natural and surgical methods for repairing osteochondral defects tend to fall short, UVM’s Engineered Biomaterials Research Laboratory (EBRL) is working towards a solution where biomimetic, polymeric, and porous engineered tissue scaffolds are seeded with drugs and human mesenchymal stem cells (hMSCs). The seeded scaffold is then implanted or injected into the patient’s osteochondral defect, where the hMSCs differentiate and grow a new cartilaginous extracellular matrix to heal the defect as the artificial scaffold breaks down.

Microspheres in three distinct size ranges are required to create pores and embed drugs and cells in the scaffold. In order to produce these microspheres, we turn to the field of microfluidics, which examines fluid interactions at micro-scale geometries and flow rates. A microfluidic flow-focusing device (MFFD) leverages the low Reynolds numbers and pronounced effects of surface tension in such flows to create highly monodisperse droplets of one fluid in a second.

This project investigates the design and fabrication of MFFDs for the production of homogeneous microspheres. A MFFD must be consistently reproducible, readily characterized, and easy to test and use. MFFDs show great potential to successfully play a role in the EBRL’s investigation of engineered tissue scaffolds.
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Motivation

UVM’s Engineered Biomaterials Research Laboratory (EBRL) has identified that surgical processes deployed to repair osteochondral defects are largely ineffective. Osteochondral defects (damage to articular cartilage and the underlying bone), caused either by trauma or chronic inflammation, must be treated to avoid the development of chronic osteoarthritis and the degeneration of surrounding joint tissues. Traditionally, surgical procedures take one of three forms: 1) total joint replacement, 2) removal of the damaged tissue and grafting of tissue-engineered cartilage into the osteochondral lesion, or 3) stimulation of the body’s natural repair mechanisms by introduction of microfractures in the subchondral bone.

None of these treatments effectively and permanently heal an osteoarthritic joint, and the number of patients experiencing osteoarthritis continues to increase. The EBRL’s proposed solution is a porous, implantable or injectable, three-dimensional, biocompatible tissue scaffold, seeded with drugs and/or human mesenchymal stem cells (hMSCs). To create such a scaffold, biomimetic polymers emulating natural, healthy osteochondral tissue (cartilage and subchondral bone) is poured over a pre-mold of microspheres. The microspheres will dissolve once in the body, releasing drugs and/or hMSCs at a controlled rate, and leaving behind a porous scaffold to support cartilage regrowth. It is crucial that monodisperse microspheres be produced within a set of relevant size ranges. The EBRL has identified microfluidic flow-focusing devices (MFFDs) as an effective, low-cost, and high-throughput mechanism for creating homogeneous microspheres.

Background

Microfluidics

The field of microfluidics concerns itself with the properties, applications, and control of fluidic systems characterized by micro-scale dimensions and volumes. Microfluidics was born in the 1980s from analytical methods and detection systems designed to operate on tiny amounts of sample material. The application of these techniques to chemical and biological weapons detection, molecular biology and genomics analysis, and microelectronics or microelectromechanical systems (MEMS) has since driven the development of the field (Whitesides 2006). Numerous journals highlighting microfluidic systems and results obtained thereby have arisen, including Lab-on-a-Chip, Sensors and Actuators, Microfluidics and Nanofluidics, and Analytical Chemistry. Meanwhile, improvements in available materials, fabrication techniques, and embeddable subsystems have allowed for the advent of comprehensive “lab-on-a-chip” solutions featuring valves, pumps, actuators, switches, sensors, dispensers, mixers, filters, separators, and more (Stone et al. 2004).

Microfluidic droplet generators, or MDGs, comprise a specialized class of microfluidic device that leverages flow-focusing geometry and the fluid properties typically observed in microfluidics to create emulsions (Anna et al. 2003). While other methods for creating less monodisperse emulsions exist, the strength of MDGs lies in their ability to produce highly monodisperse microspheres at a broad range of sizes, while supporting various fabrication techniques and a broad range of features and components. MDGs are capable of creating oil-in-water and water-in-oil emulsions, as well as double, triple, and even quadruple emulsions (Abate & Weitz 2009). MDGs retain their capability to produce homogeneous microspheres from fluids with a broad range of properties, yielding microspheres with diameters of 1 – 500 µm (Nie et al. 2008; Dendukuri & Doyle 2009; Weibel & Whitesides 2006). Polymer solutions can be dispersed, formed into special shapes, then crosslinked in situ using either continuous or stop-flow lithography, or chemical crosslinking (S. Xu et al. 2005; Hu et al. 2012). MDGs are also capable of encapsulating living cells in polymeric microspheres with high rates of cell viability, a property crucial to the EBRL’s eventual research goals (Choi et al. 2007; Martinez et al. 2012).

Types of Droplet Generators

Three main types of droplet generators exist, and are characterized by the geometry they employ for droplet generation: T-Junction, Co-Flow, and Flow-Focusing. T-Junction droplet generators feature two orthogonal channels where the dispersed fluid is carried by the incident, truncated channel. As fluids are pumped through the junction, break-up of the dispersed fluid occurs as the result of combined shear forces and capillary pressure exerted by the continuous phase (Dendukuri & Doyle 2009).
Co-flow droplet generators generally feature three coaxial channels, where two cylindrical pipettes with tips pulled to a desired diameter are enclosed in a third square tube. The fluid to be dispersed is pumped through one cylindrical pipette, while the continuous fluid is pumped into the enclosing tube. As the two immiscible phases are pushed into the empty pipette tip, shear forces exerted by the continuous phase pinch the dispersed phase into a fine cylinder. Eventually, the surface energy of the thread becomes too great, and the thread splits into droplets (Baroud et al. 2010; Geschiere et al. 2012). Co-flow droplet generators are capable of producing multiple emulsions, although the simplest co-flowing geometries feature a capillary tube ejecting fluid into a second, flowing, immiscible phase to create a single emulsion (Martinez et al. 2012; Fu et al. 2012).

Flow-focusing droplet generators display greater versatility than co-flowing droplet generators, and have three or more distinct configurations. These, as shown in Figure 1, include 1) equidimensional continuous, dispersed, and outflow channels, 2) large continuous and dispersed channels feeding into a small outflow orifice and channel, and 3) small continuous and dispersed channels feeding into a small orifice, which then opens to a wider outflow channel (Abate, Poitzsch, et al. 2009a).

All three designs feature co-axial dispersed and outflow channels, with the continuous phase supplied on either side by channels perpendicular to the dispersed and outflow channels. While shear forces and capillary pressure tend to govern droplet breakup, the exact mechanism of breakup is highly dependent on the fluid properties and flow characteristics (Anna et al. 2003; Fu et al. 2012). We chose to focus on flow-focusing microfluidic droplet generators, specifically the first type with equidimensional channels, because 1) they are capable of producing droplets in a wide range of sizes, 2) they are relatively inexpensive to fabricate, and 3) their operating parameters are well understood and highly tuneable (McDonald et al. 2000; Cubaud & Mason 2008).

**RELEVANT FLUID PARAMETERS**

Biphasic microfluidic dynamics in a flow-focusing device are governed by a set of parameters calculable from the properties of the two working fluids and the dimensions of the device junction. These are $\mu_c$ and $\mu_d$, the dynamic viscosities, $\rho_c$ and $\rho_d$, the densities, $Q_c$ and $Q_d$, the flow rates, $P_c$ and $P_d$, the imposed pressures, and $\gamma$, the interfacial tension of the two fluids. The subscripts $c$ and $d$ denote the continuous and dispersed phases, respectively. Due to the various fabrication methods most often used, microfluidic channels are often of uniform height, giving us $h$, the height of the channel, and $w_c$ and $w_d$, the channel widths. From these, the mean velocity of a flow can be calculated as $U = Q/A$ (Christopher & Anna 2007). Finally, the ratios of volumetric flow rates, viscosities, and channel widths will be denoted as $\varphi = Q_d/Q_c$, $\lambda = \mu_d/\mu_c$, and $\chi = w_d/w_c$, respectively (Baroud et al. 2010; Christopher & Anna 2007).

From the basic parameters above, a set of dimensionless groups can be calculated to describe fluid behavior inside the device. The first of these is the Reynolds number, which describes the ratio of inertial forces to viscous forces acting on a fluid flow, and is often used to predict the characteristics of flow regimes (e.g. laminar, mixed, turbulent). For closed-pipe flows with rectangular cross-sections, the Reynolds number is formally defined as $Re = \rho D h U / \mu$. The characteristic length is taken as the hydraulic diameter, $D_h = 4A/P$, where $A = wh$ and $P = 2w + 2h$ for a given channel (White 2010).

The second dimensionless group of concern is the Weber number, which describes the ratio of inertia to surface tension. The Weber number is found from $We = \rho U^2 d / \gamma$, where $\gamma$ is the interfacial tension between the two working fluids, and $d$ is the characteristic diameter of the dispersed phase as it penetrates into the continuous phase. The third dimensionless group to consider is the capillary number, which describes the ratio of viscosity to surface tension, or the ratio of local shear stress to capillary pressure. For a given flow,
the capillary number is calculated by \( Ca = \frac{\mu U}{\gamma} \) or \( Ca = \frac{\rho Q}{\mu y} \) (Nunes et al. 2013; White 2010). As inertial effects are often negligible in microfluidic flows, the capillary number is often used to delineate microfluidic flow regimes.

The pronounced influence of device geometry in microfluidics gives rise to slight modifications in the definitions of relevant dimensionless groups. The Reynolds number is often calculated using channel height as the characteristic length, such as \( Re = \frac{\rho Q h}{\mu w} \) or \( Re = \frac{\rho Q}{\mu w} \), as for a given device, channel height tends to be more consistent than width (Anna et al. 2003). Furthermore, the capillary number has been defined as \( Ca = \frac{\mu_c G w_d}{2\gamma} \), where \( G = \frac{\Delta V}{\Delta Z} \), \( \Delta V \) being the difference in velocity between the flow-focusing junction and the upstream velocity, and \( \Delta Z \) being the distance between the end of the dispersed channel to the flow-focusing orifice (Anna & Mayer 2006).

The language of dimensionless numbers can greatly facilitate comparison between devices and generalization of results. However, the calculation of relevant fluid parameters, such as interfacial tension, and the measurement of micro-scale internal channels are challenging tasks on their own. The complete characterization of a microfluidic device is a decidedly non-trivial process.

**Flow-Focusing Dynamics**

As many as five distinct regimes of flow behavior in equidimensional, type 1 microfluidic flow-focusing devices have been identified (threading, jetting, dripping, tubing, and displacement); predicting the transitions between these five is best done using the capillary number (Cubaud & Mason 2008). As flows in microfluidic devices tend to be strongly laminar, with Reynolds numbers rarely increasing above \( Re = 10 \), microsphere production relies on the occurrence of natural instabilities (Stone & Kim 2001; Tang & Whitesides 2009). The two flow regimes known to produce monodisperse droplets are the jetting and dripping regimes – in the remaining three flow regimes, no droplets, or polydisperse droplets, are produced.

**The Jetting Regime**

In the jetting regime, shear forces exerted by the continuous phase drive a cylindrical thread of the dispersed fluid through the flow-focusing junction. Monodisperse droplets form downstream of the junction due to axisymmetric capillary instabilities that occur in the thread (Eggers 1997; Geschiere et al. 2012). Jetting behavior occurs for \( Ca_d > 0.1 \). During operation, the capillary thread characteristic of the jetting regime reaches a critical length \( L_c \) where droplets break off, such that

\[
L_c \approx \frac{\mu_d}{\gamma} \frac{}{\pi h Ca_{crit} \left( \frac{Q_d Q_c}{2} \right)^0.5}
\]

where \( Ca_{crit} \) represents the threshold capillary number, above which convective instabilities drive droplet breakup. Furthermore, the diameter of the droplets produced can be predicted by the relation

\[
\frac{d}{h} \approx 2.192 \varphi^{0.5}
\]

revealing the dimensionless droplet size to be a function of the flow rates of the fluids. This relation holds regardless of the relative size of the droplets in their channel, and it is noted that droplets smaller than their enclosing channel are produced when \( \varphi < \varphi_{crit} \), where \( \varphi_{crit} \approx 0.21 \) (Cubaud & Mason 2008). Fu et al propose a more nuanced relation governing droplet size in the jetting regime:

\[
\frac{d}{w_d} \approx \begin{cases} 1.23 \varphi^{0.41}; & 1 < \lambda < 12 \\ 13.64 \varphi^{1.07}; & \lambda \approx 112 \end{cases}
\]

where the second condition \( \lambda \approx 112 \) is mentioned in the literature simply as “high viscosity contrast” (Fu et al. 2012).

**The Dripping Regime**

In the dripping regime, a hemispherical cap of the dispersed fluid protrudes from the dispersed channel until it begins to obstruct the outflow orifice, at which point capillary pressure and shear forces from the continuous phase pinch the cap of the dispersed phase into a droplet, and the dispersed phase retracts to the opening of its channel (Nunes et al. 2013; Garstecki et al. 2005). Dripping behavior occurs for \( Ca_d < 0.1 \) and
cut tubing is inserted into the new device

3) the slab is peeled from the mold and bonded to a glass slide or a second PDMS sheet, and 4) holes are cut and tubing is inserted into the new device (Duffy et al. 1998). The simplicity of this method allows for fabrication of microfluidic devices in a variety of methods (Martinez et al. 2012; Tang & Whitesides 2009). The first generation of microfluidic devices were fabricated using techniques developed for microelectronics, and consisted of photolithography or electron-beam lithography in silicon and glass. Although these methods produce dimensionally accurate and chemically inert devices, they have been critiqued for being expensive, inflexible, dependent on specialized equipment, and useful only for planar geometries – silicon or glass devices are better suited for factory production than lab work (Tang & Whitesides 2009; Whitesides & Stroock 2001).

Researchers in the field sought to develop faster, cheaper, and more versatile fabrication systems that retained the dimensional stability and precision of the silicon and glass devices. As of 2008, silicon, silica, or glass microfluidic devices were still the most used in publications, followed by poly(dimethylsiloxane) (PDMS), then thermoplastics, such as polymethylmethacrylate (PMMA), polycarbonate (PC), copolymers and cyclic olefin polymers (COC and COP), among others (Tsao & DeVoe 2008). Various methods exist for fabricating microfluidic channels from plastics, including hot embossing (Martynova et al. 1997), cold embossing (J. Xu et al. 2000), injection molding (McCormick et al. 1997), microthermoforming (Giselbrecht et al. 2006), and CO2 laser machining/IR laser engraving (Romoli et al. 2011; Huang et al. 2006). While thermoplastics are cheap and easily etched, engraved, or through-cut using a laser cutter, bonding multiple layers of engineering plastics can become problematic: resins and other glues tend to result in fouling of the channels, while direct bonding by thermal fusion requires intimate thermal control during the bonding process, and like bonding via solvent addition, tends to result in poor dimensional stability or channel collapse. However, welding via laser or ultrasonic energy, especially if enhanced by surface modifications to the bonded plastics, offer promising alternatives and open the door for high-volume production (Tsao & DeVoe 2008).

Of all the materials discussed above, PDMS is by far the most flexible, featuring a Young’s modulus of 0.750 MPa < E < 1.0 MPa, and supporting a broad array of geometries and integratable components (Dendukuri et al. 2007; Unger 2000). Planar PDMS devices are most commonly formed in a process known as soft-photolithography, whereby 1) a mold is made by hardening a photoresist under light through a photomask, 2) liquid PDMS and a curing agent are poured over top of the mold and allowed to solidify into a slab, 3) the slab is peeled from the mold and bonded to a glass slide or a second PDMS sheet, and 4) holes are cut and tubing is inserted into the new device (Duffy et al. 1998). The simplicity of this method allows for
rapid prototyping from CAD files (McDonald et al. 2000). The bonding step is crucial and can be performed reversibly via Van der Waals contact with a second surface, or irreversibly, by activating the PDMS surface with oxygen plasma and allowing Si-O-Si bonds to form with glass, resulting in a bond capable of withstanding pressures up to 207-345 kPa (McDonald et al. 2000). In addition to the stamping/casting process described above, PDMS can also be formed via wet etching, dry etching, or a combination of the two (Garra et al. 2002; Balakrisnan et al. 2009).

This bonding technique enables the stacking of multiple layers of naturally elastomeric PDMS for the creation of complex, three-dimensional mixers (Jo et al. 2000), pneumatic/hydraulic “Quake” microvalves (Wang & Lee 2013), screw and solenoid microvalves (Hulme et al. 2009), pneumatic/hydraulic micropumps (Unger 2000), prefabricated heaters (Erickson & Li 2004), and heaters fabricated in situ by cooling liquid solder in microfluidic channels (Tang & Whitesides 2009). The resulting complexity of microfluidic devices is impressive. Examples include devices that use pneumatic valves to change the size and frequency of droplets during production (Abate, Romanowsky, et al. 2009b), devices featuring one to five sequential, alternatively-wetting flow-focusing junctions for single- to quintuple-emulsions (Abate & Weitz 2009), and a flow-focusing droplet generation and sorting device featuring embedded, computer-controlled laser-detection and a pressure-actuated valve to separate light from dark droplets at rates of up to 250 Hz with < 0.01% error (Abate et al. 2010).

While glass may retain the best dimensional stability and chemical inertness and engineering plastics may be cheaper and faster to fabricate or mass-produce, the moderate cost, low turnaround time, and incredible flexibility of PDMS microfluidic devices make them optimal for our somewhat open-ended applications.

**OBJECTIVES**

Accurate and reliable protocols will be developed for the fabrication, evaluation, and testing of PDMS microfluidic devices, with the ultimate goal of producing homogeneous microspheres between 1 and 200 µm in diameter using fluids relevant to the EBRL’s research aims. Using the developed protocols, one or more microfluidic flow-focusing devices will be fabricated, evaluated, and tested. Data collected will be displayed analytically, and, if possible, statistically. Its dimensions will be characterized and compared to the intended design. The device will be filmed under operation using high-speed videography.

**METHODS**

The bulk of the time spent on this research went into developing and refining the various steps of the fabrication procedures for PDMS microfluidic devices. Due to the number of steps in the fabrication process, adequate completion of a step often becomes more important than perfection (for example, the necessity for spin-coating to produce an even film is crucial, but producing a film within +/- 1 µm of the intended thickness is secondary). Early devices suffered due to problems with nearly every step in the fabrication process. Protocol modifications were sometimes suggested by literature, but groups publishing results obtained with microfluidic devices tend not to reveal the details of their fabrication methods.
DEVICE FABRICATION PROCEDURE

OVERVIEW

PDMS microfluidic devices are made in a multi-step process that increases in complexity with the design intent. For planar microfluidic devices, a casting/stamping procedure is used. First, a mold is made. This is accomplished by applying an even film of photoresist to a substrate (glass or silicon) then selectively hardening the photoresist with light through a photomask, sometimes called a transparency. The excess unhardened photoresist is washed off of the substrate to create the mold, referenced in Figure 2 as a master. To create the device, PDMS is then mixed, poured over the mold, and hardened (note: PDMS curing time varies with composition, thickness, and thermal properties of the surrounding materials). Once cured, the PDMS slab is peeled from the mold and bonded to a microscope slide or a second slab of PDMS to create microchannels. Finally, if necessary, tubing is fitted to the new device, allowing for fluids to be pumped through.

MAKING THE MOLD

To make a microfluidic device, a mold must be made by selectively hardening a photoresist on a glass slide. SU-8 2075 (MicroChem) is a negative photoresist known for its ability to create features with high aspect ratios. First, a glass slide (75mm x 25mm, Fisher Scientific) is cleaned and dried, then placed on the vacuum head of a spin coater (Laurell Technologies WS-400BZ-6NPP/LITE). SU-8 is deposited atop the glass slide then spun to produce a film of uniform thickness. Film thickness varies inversely with rotational velocity: SU-8 2075 produces a thickness of ~240 µm at 1000 rpm, and ~60 µm at 4000 rpm (MicroChem 2006). This step of the process is highly sensitive to contamination; small dust particles can result in non-uniform films of SU-8. Optical examination immediately after spin coating reveals the uniformity of the SU-8 film, as shown in Figures 3 and 4.

Immediately after spin coating, the coated slide is placed on a hot plate (VWR Int’l. 11301-068) and undergoes a pre-exposure bake, in which it is heated to 65 °C for five minutes, cooled to room temperature, then heated to 95 °C for 10 minutes. This evaporates remaining solvents in the SU-8, and can help smooth wrinkles that may have appeared during coating. A custom photomask, designed in Solidworks and fabricated by (PhotomaskPORTAL) is then applied (see Figure 8). Exposing the SU-8 requires between 150-350 mJ/cm² of
near-UV light (IntelliRay 400 W) and correlates to film thickness. For films of this thickness, 4-5 seconds of exposure hardens the photoresist in the necessary shapes. After curing, a post-exposure bake is performed, where the slide, now bearing both hardened and uncured SU-8, is heated to 65 °C for two minutes, cooled to room temperature, then heated to 95 °C for 10 minutes. All uncured SU-8 is washed off by submerging the slide in ethyl lactate and gyrating (VWR Int’l OS-500) for eight minutes, twice.

At this point, the SU-8 molds are evaluated under an optical microscope (Fisher Scientific, 12-561-330) to ensure that they are free of defects. The critical x-junction at the center of the mold is of particular interest. Figures 5 through 7 show high quality mold features at varying levels of magnification. The verticality of the sidewalls and the smoothness of the top of the features are evaluated. The creases, visible in Figures 6 and 7, are likely due to thermal stresses, but have not been shown to affect the resulting device. A mold that fails to pass optical inspection is discarded.

Finally, the finished mold is placed in a vacuum chamber (Cole-Parmer Instrument Co., P-79202-00) with 200 µL (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane (Sigma-Aldrich). Inside the vacuum chamber, the silane is vaporized and evenly coats the glass and SU-8 features, preventing the PDMS from sticking to the mold. A finished mold is visible in Figure 9.

![Figure 5: X-Junction, 10x](image1)
Mold used to make device 1, 10x magnification

![Figure 6: X-Junction, 20x](image2)
Mold used to make device 1, 20x magnification

![Figure 7: X-Junction, 40x](image3)
Mold used to make device 1, 40x magnification

![Figure 8: Photomask on SU-8](image4)
Applying a photomask to a slide coated in uncured SU-8 photoresist

![Figure 9: Finished Mold](image5)
Exposed, rinsed, and silanized, a mold with visibly raised features reposes in its custom-made curing dish is ready for PDMS

### Making a Device

Polydimethylsiloxane (PDMS) (Dow Corning, Sylgard 184) is used to create the device. PDMS is a two-part product. It is thoroughly mixed in a 10:1 ratio of elastomer base and curing agent, then centrifuged at 4000 rpm to accelerate degassing. About 22 mL of PDMS are necessary to make one device. The mold is placed into a custom curing plate, designed in Solidworks and fabricated in-house, which also contains the PDMS as it cures on a hot plate. Centrifuged PDMS is poured into the curing plate over the mold, as seen in
Figure 10. To eradicate the bubbles that inevitably form in concave corners of the mold features, the full curing dish is placed back in the vacuum desiccator, and a vacuum is cyclically pulled and released until all bubbles rise to the surface of the PDMS. This done, the curing dish is placed on a hot plate, and cured at any temperature between 70 °C and 150 °C (leading to approximate curing times of two and a half hours and half an hour, respectively). Devices cured at lower temperature show less thermal distortions and fewer air bubbles than devices cured at high temperature.

Once cooled, the now hard PDMS slab is gently peeled from its mold and curing dish. The raised features on the mold are now impressed into the underside of the PDMS slab. With a razor blade, the slab is trimmed to fit on a new 75mm x 25mm slide. A 1.5mm biopsy punch (Fisher Scientific) is used to create holes for three 0.0625" OD teflon tubes (McMaster-Carr). In order to create closed microfluidic channels capable of withstanding high pressure without bursting, PDMS and glass are chemically bonded. Air plasma, visible in Figure 11, is generated using a corona wand (Electro-Technic Products, BD20AC). The plasma alters the surface chemistry of the new glass slide and the new PDMS slab, allowing the surfaces to bond on contact. The resulting covalent siloxane (Si—O—Si) bonds that form at the interface are stronger than the PDMS itself (Duffy et al. 1998). Unfortunately, this treatment also makes the microfluidic channels temporarily hydrophobic, which is unfavorable for creating water-in-oil emulsions. Finally, the teflon tubes are inserted, as is visible in Figure 12, and the device is ready for testing.
DEVICE EVALUATION

Microfluidic devices fabricated using the above procedure were evaluated using a variety of methods. Due to the time-intensive nature of PDMS microfluidic device fabrication, some level of evaluation occurred at each stage of fabrication to ensure that the resulting device would not be defective. Spin-coated glass slides are observed using the naked eye to ascertain film flatness, as seen in Figures 2 and 3. Hardened molds are evaluated for obvious defects using an optical microscope, as seen in Figures 5 through 7. Lastly, the x-junctions of hardened molds are measured using a calibrated optical microscope (Beuhler, Micromet II), as seen in Figure 13. The calibrated vertical bars are moved together or apart, and the gap between the two can be read off a dial on the apparatus. Channel width in each direction at the x-junction is measured three times consecutively for statistical power.

Once deemed viable, a mold is silanized and used to create a device. Upon peeling a PDMS slab from the mold, the slab and mold checked to ensure that no chunks of hardened SU-8 are stuck in the PDMS channels – any stuck chunks are gently removed. The slab is then bonded to a glass slide as described above. Evaluation under a microscope is often ineffective, as bonding problems will only present themselves once the device is pressurized.

To characterize the dimensions of microfluidic channels at higher resolution, the device is pumped full of a red fluorescent stain (Acti-Stain 555). A confocal microscope (Zeiss, LSM 510 META) is used to obtain a set of two-dimensional images in the x-y plane with varying focal depth. This set, known as a z-series, is then reconstructed in specialized software (Volocity) to form a measurable three-dimensional rendering. Sample results from confocal microscopy are visible in Figure 14. The viscosities of the working fluids were evaluating using a shear-rate sweep on the EBRL’s rheometer (TA Instruments, AR2000).

DEVICE TESTING

From an understanding of the device dimensions, the properties of the working fluids, and relationships proposed by previous literature, a range of potentially viable flow rates to test is determined (Nie et al. 2008; Cubaud & Mason 2008). To ascertain whether or not a device will function, preliminary tests are performed under an optical microscope (see Figure 15). Once a device is shown to be operational through preliminary testing, high-speed videography is used to analyze the size, dynamics, and rate of formation of droplets produced by a device, as shown in Figure 16.
Visible in **Figure 16**, but not in **Figure 15**, is the pair of syringe pumps (Harvard Apparatus, PHD-2000), used to feed fluids to microfluidic devices. As seen in **Figure 16**, high-speed videography is performed with a high-speed camera (Phantom, v310), which is equipped with an objective microscopic lens (Mitutoyo, Plan Apo 20x) and an in-line fiber optic light source (Edmund Industrial Optics, 21AC). Specialized software is used to control the high-speed camera (Vision Research, Phantom Camera Control), while more standard software is used to convert the high-speed videography output into videos (Apple, Quicktime Pro). High-speed videography data was processed using Matlab routines developed for monopropellant microthruster research.

**RESULTS AND DISCUSSION**

**MOLD CHARACTERIZATION**

The above fabrication protocol was used to fabricate four molds during the fall of 2014. The x-junctions of these molds were measured in two dimensions as described above using a calibrated optical microscope tester, as in **Figure 12**, and the resulting data is plotted below. Note: channel height data was obtained via confocal microscopy on devices resulting from the molds measured using the calibrated optical microscope tester.

**Figures 17 and 18** show the mean width of the longitudinal and transverse channels at the x-junction of each of the four molds. Each channel is measured three times. **Figure 17** displays the standard deviation of each set of three measurements, while **Figure 18** shows the upper and lower control limits of the data \((UCL = 80.37 \mu m \text{ and } LCL = 78.02 \mu m, \text{ respectively})\). It is noteworthy that the control limits shown in **Figure 18** are artificially small due to small sample size – only three samples comprise each data point in this
Both plots show the global mean channel width, \( \mu = 79.20 \, \mu m \), very close to the intended value of 80 \( \pm \) 5 \( \mu m \). This tolerance comes from the fabricator of the photomask, who claims 5000 dpi, or 5.08 \( \mu m \) resolution.

<table>
<thead>
<tr>
<th>Device</th>
<th>Mean (Width, 1)</th>
<th>Mean (Width, 2)</th>
<th>Std Dev (Width, 1)</th>
<th>Std Dev (Width, 2)</th>
<th>Range (Width, 1)</th>
<th>Range (Width, 2)</th>
<th>Mean 2 Minus Mean 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>86.800</td>
<td>89.033</td>
<td>0.819</td>
<td>0.751</td>
<td>1.600</td>
<td>1.500</td>
<td>2.233</td>
</tr>
<tr>
<td>2</td>
<td>81.500</td>
<td>84.633</td>
<td>0.608</td>
<td>0.231</td>
<td>1.100</td>
<td>0.400</td>
<td>3.133</td>
</tr>
<tr>
<td>3</td>
<td>66.100</td>
<td>67.800</td>
<td>0.954</td>
<td>0.300</td>
<td>1.700</td>
<td>0.600</td>
<td>1.100</td>
</tr>
<tr>
<td>4</td>
<td>78.467</td>
<td>79.233</td>
<td>0.681</td>
<td>0.503</td>
<td>1.300</td>
<td>1.000</td>
<td>0.767</td>
</tr>
<tr>
<td>Means:</td>
<td>78.217</td>
<td>80.175</td>
<td>0.765</td>
<td>0.446</td>
<td>1.425</td>
<td>0.875</td>
<td>1.958</td>
</tr>
</tbody>
</table>

* Figure 19: Table of Statistical Values from JMP

**Figure 19** supports the above graphs with numerical data. All devices feature longitudinally oriented channels wider than transversally oriented channels by 1.968 \( \mu m \), on average, but the difference in channel width ("Mean 2 Minus Mean 1" in **Figure 19**) does not correlate with the magnitude of the channel widths. This value is within the tolerance of the photomask, and as it is relatively consistent across all devices, it can be attributed to error in the fabrication of the device. The low standard deviations observed for set of channel measurements show relatively precise data collection.

**DEVICE CHARACTERIZATION**

From the confocal microscopy data obtained of Devices 1 and 2, UVM’s Microscopy Imaging center returned still images, three-dimensional reconstructions and fly-through videos, and partial dimensions of Devices 1 and 2. Still images are included below in **Figures 20** through **27**.

* All values in \( \mu m \)
The images obtained via confocal microscopy reveal microfluidic channels of mixed quality. It is immediately visible that the sidewalls of channels in both devices are not vertical. The effect is not visible only in the wide sections of the microfluidic channels, but also in the critical, choked x-junction. This could be due to the scattering of UV light as the photoresist is hardened, but it is more likely that the tapering be the result of deformation as the PDMS slab is “rolled” onto its glass slide after plasma treatment. These problems can be solved by ensuring that the photomask is pressed tightly to the surface of the SU-8 photoresist before hardening, and by deforming the PDMS slab less as it is affixed to the glass slide, respectively.

As can be seen in the above images, the channels of Device 1 are higher than those of Device 2. The height of Device 1’s channels at the x-junction was estimated by the MIC at 71.775 µm, while the height of Device 2’s
channels was at the x-junction was estimated at 45.675 µm. It is unclear exactly why this difference occurred, as the two molds were created using identical procedures. As mentioned before, minor impurities can have dramatic impacts on the uniformity of the photoresist film, thus, to help eradicate this inconsistency, slides should be treated with extreme care from initial cleaning to after the photoresist is hardened.

**VIDEOGRAPHY DATA**

A microfluidic flow-focusing device, fabricated using the above methods from mold 4 (see Figures 17 through 19) was tested using the setup shown in Figure 16. 2% sodium-alginate in deionized water dyed with Trypan blue (Sigma-Aldrich, CAS: 72-57-1) was pumped into the device as the dispersed phase, while light mineral oil (Sigma-Aldrich, CAS: 8042-47-5) was pumped into the device as the continuous phase. A typical viscosity profile for 2wt% sodium-alginate is included in Figure 28. The Na-alginate solution exhibits slight shear-thinning behavior, decreasing in viscosity from 0.7 to 0.3 Pa*s over the range of shear rates tested. The light mineral oil is considered Newtonian with a viscosity of .03 Pa*s (Sigma-Aldrich 2011). Code used to produce Figure 28 can be found in the appendix.

During videography testing, data was recorded at 2000 frames per second, and saved either as individual .jpg files, or as .mov files for ease of use. The test protocol described in Figure 16 was applied directly, with two syringe pumps delivering one fluid each at finely controllable flow rates. To finely tune the position of the camera, the device was placed on a stage that can be adjusted in three dimensions.

Device 4, visible in Figures 12 and 16, displayed three of the five behaviors seen in microfluidic flow-focusing devices: tubing, dripping, and jetting. Although tubing is a curious behavior to observe, it is secondary to the research objectives and was not recorded. Dripping was the most commonly observed behavior, occurring at lower total flow rates, but at a broad range of flow-rate ratios. Jetting behavior was also achieved, despite its relative difficulty.

**Dripping Behavior**

The following sequence of still images showcases the dripping behavior of Device 4. At 2000 fps, each frame represents a 0.5 ms advancement in time from the previous. Oil enters through the left and right channels, while 2wt% Na-Alginate enters through the top channel and protrudes into the x-junction. The net direction of the flow is downwards.
Over the course of the 26 frames above, one droplet of 2wt% Na-Alginate is produced. Roughly 12.5 ms transpire; during this period of steady state operation, droplets were produced at about 80 Hz. The droplet breakup dynamics characteristic of the dripping regime are showed step-by-step in this sequence: the cycle begins with the dispersed phase protruding out into the junction from the dispersed channel. As it blocks more and more of the outflow orifice, capillary pressure and shear force from the impinging oil pinch the dispersed phase until a new droplet breaks off. Although the new droplet in D.26 shares its position with the new droplet in D.1, creating the impression that a whole cycle has occurred, care must be taken to comprehensively evaluate the flow. A glance downstream at the droplets populating the efflux channel in D.26 shows that they are on opposite sides of a hypothetical centerline of the device. Thus, it is more correct to conclude that over 25 ms two droplets are produced. Using the width of the x-junction, found to be measure roughly 79 µm, the diameter of the droplets produced can be estimated at roughly 70 µm.

**Jetting Behavior**

The following sequence of images shows Device 4 displaying jetting behavior. The video data was recorded at 2000 fps, but in this sequence, only half the frames are displayed, yielding an effective frame rate of 1000 fps and 1 ms time advancement between frames. The orientation of the device is the same as in the previous sequence of images.
In this sequence, one microsphere is produced over the course of 38 ms, giving a frequency of approximately 26.3 Hz. Classing dripping regime droplet breakup dynamics are showcased in this sequence. In contrast with the previous segment, droplet breakup occurs downstream of the x-junction. Increased shear forces pull the dispersed phase into a capillary thread as it passes through the x-junction. Plateau-Rayleigh instabilities appear as axisymmetric ripples in the dispersed phase thread in the otherwise highly regular flow. Eventually, as predicted, the surface energy becomes too high, and the capillary thread breaks off another microspheres.

**SUMMARY**

Over the course of this research, an understanding of the fluid mechanics driving microfluidic droplet generation through flow-focusing was obtained through a study of extant literature. Techniques for the fabrication of PDMS microfluidic devices were developed and refined, then codified as procedures for laboratory use. Various methods for characterizing the dimensions of a PDMS microfluidic device were investigated and used to inform design decisions and support modifications to fabrication procedures. Protocols for testing microfluidic devices were created, and the performance of microfluidic flow-focusing devices was demonstrated through high-speed videography.

**CONCLUSIONS**

The groundwork laid by this research was sufficient to produce a PDMS microfluidic flow-focusing device capable of generating microspheres of sizes useful to the Engineered Biomaterials Research Laboratory's research aims.
Future Work

While previous improvements in procedures could be made based on largely subjective assessments, statistical analysis is required to assess the most important next steps forward. Thus, the first step to take is to make at least 30 more devices using the above procedures. Once the consistency of the fabrication methods is assessed quantitatively (and not qualitatively or with too few samples) devices with different dimensions should be fabricated, evaluated, and tested in order to begin making microspheres of smaller size. This done, the EBRL will be able to apply this research to its various projects requiring microspheres, including photocrosslinked alginate microspheres, osteochondral repair systems, and pleural sealant patches.

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APPENDIX

Relevant video data not included will be shown and distributed during defense.

MATLAB CODE

**CHANNEL MEASUREMENT ANALYSIS AND PLOTTING**

```matlab
% Device dimensions
clear all;
dims = [85.9 89.0 81.8 84.5 66.6 67.5 77.7 78.7; 87.5 88.3 80.8 84.9 65.0 67.8 79.0 79.3; 87.0 89.8 81.9 84.5 66.7 68.1 78.7 79.7];
molds = {'Mold 1, Tr' 'Mold 1, Lo' 'Mold 2, Tr' 'Mold 2, Lo' 'Mold 3, Tr' 'Mold 3, Lo' 'Mold 4, Tr' 'Mold 4, Lo'};
means = [mean(dims(:,1)) mean(dims(:,2)) mean(dims(:,3)) mean(dims(:,4)) mean(dims(:,5)) mean(dims(:,6)) mean(dims(:,7)) mean(dims(:,8))];
specDifs = [abs(means(1) - means(2)) abs(means(3) - means(4)) abs(means(5) - means(6)) abs(means(7) - means(8))];
meanDifs = mean(specDifs);
stds = [std(dims(:,1)) std(dims(:,2)) std(dims(:,3)) std(dims(:,4)) std(dims(:,5)) std(dims(:,6)) std(dims(:,7)) std(dims(:,8))];
bar(means)
hold on
errorbar(means,stds,'r.'
plot([0 9],[mean(means) mean(means)],'gs--')
set(gca,'XTickLabel',molds)
str1 = 'Global Mean: ';
str2 = num2str(mean(means));
str3 = '\mu';
str4 = 'm';
text(5,82,strcat(str1,str2,str3,str4))
xlabel('Molds 1-4, Widths 1 and 2')
ylabel('Channel Width, um')
title('MFFD x-junction Dimensions')
print(gcf,'ChannelDims','-r1000','-djpeg')
```

**VIDEO ANALYSIS AND PLOTTING**

```matlab
format short
FrameRate = 20000;
MaxFrame = 2461;

ImageData = cell(1,MaxFrame);

for FrameNumber=1:MaxFrame
    if FrameNumber<10
        FrameName = ['000' num2str(FrameNumber) '.jpg'];
    elseif FrameNumber<100
        FrameName = ['00' num2str(FrameNumber) '.jpg'];
    elseif FrameNumber<1000
        FrameName = ['0' num2str(FrameNumber) '.jpg'];
    else
        FrameName = [num2str(FrameNumber) '.jpg'];
    end
    ImageData{FrameNumber} = imread(FrameName);
    [nrow,ncol,ndim] = size(ImageData);
    ImageData{FrameNumber} = ImageData{FrameNumber}(24:48,32:793,:);

    % The numbers help to zoom in on the dimensions of the channel background = imopen(ImageData{FrameNumber},strel('disk',15));
    ImageData{FrameNumber} = ImageData{FrameNumber} - background;
    level = graythresh(ImageData{FrameNumber});
    bw = im2bw(ImageData{FrameNumber},level);
    bw = bwareaopen(bw, 50);
end
PixData = ImageData{FrameNumber}{(:,1,1)};
PixData = mean(PixData,1);
PixData = double(double(PixData) - mean(PixData));
```

% Plot the pixel intensity data
figure;
plot(PixData);

%% This just shows the average peaks and valleys that the FFT is
%% based upon. If it's not giving you roughly the same profile as
%% you're seeing in your images, then the image thresholding may not
%% be working properly.

%% Compute and plot power spectrum for intensity sequence

NPixData = length(PixData);
MyFFT = fft(PixData);  % compute FFT

MyPow = MyFFT.*conj(MyFFT);  % compute power spectral density
MyPow = MyPow/max(MyPow);  % normalize spectral data

freqrange = linspace(0,0.5,round(NPixData/2))*FrameRate;
figure;
semilogx(freqrange,MyPow(1:NPixData/2));
xlabel('Frequency(HZ)');
ylabel('Normalized Spectral Density');

fid = fopen('pixdata.txt', 'w');
for f = 1:NPixData
fprintf(fid, '%6.2fn', PixData(f));
end
fclose(fid);

fid1 = fopen('powdata.txt', 'w');
for f = 1:NPixData/2
fprintf(fid1, '%6.2fn', MyPow(f));
end
fclose(fid1);

clc;clear all;
filename = '2wt% AAMA in 1xPBS visco 1-0007f exp.txt';
fid = fopen(filename);
data = textscan(fid, '-f %f %f %f %f %f %f %f %f;','HeaderLines',7)

normalForce = data{1};
shearRate = data{2};
time = data{3};
velocity = data{4};
viscosity = data{5};
handie = figure;
plot(shearRate,viscosity,'bo')
set(gca,'FontName','Sans Serif','FontSize',6)
title('2wt% AAMA Viscosity vs. Shear Rate')
xlabel('Shear Rate (1/s)')
axis([0 1.1*max(shearRate) 0 1.1*max(viscosity)])
filename = filename(1:end-4);
filename = strcat(filename,'.jpg');
print(handie,filename,'-r800','-djpeg');
BIBLIOGRAPHY


Hu, Y. et al., 2012. Shape controllable microgel particles prepared by microfluidic combining external ionic crosslinking. Biomicrofluidics, 6(2).


