Scalable Approach for Extrusion and Perfusion of Tubular, Heterotypic Biomaterials

by

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A thesis submitted in conformity with the requirements for the degree of Master of Applied Science

Institute of Biomaterials and Biomedical Engineering
University of Toronto

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2014

Abstract

Soft material tubes are critical in the vasculature of mammalian tissues, forming networks of blood vessels and airways. Homogeneous and heterogeneous hydrogel tubes were extruded in a one-step process using a three layer microfluidic device. Co-axial cylindrical flow of crosslinking solutions and an alginate matrix is generated by a radial arrangement of microfluidic channels at the device’s vertical extrusion outlet. The flow is confined and begins a sol-gel transition immediately as it extrudes at velocities upwards of 4 mm/s. This approach allows for predictive control over the dimensions of the rapidly formed tubular structures for outer diameters from 600 µm to 3 mm. A second microfluidic device hosts tube segments for controlled perfusion and pressurization using a reversible vacuum seal. On-chip tube deflection is observed and modeled as a measure of material compliance and circumferential elasticity. I anticipate applications of these devices for perfusion cell culture of cell-laden hydrogel tubes.
Acknowledgments

I would like to acknowledge Dr. Axel Guenther for his assistance, support and supervision towards the completion of this research thesis. Many thanks must also be extended to all of the members the Guenther lab for their motivation, advice and happy spirits throughout my degree.
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1 Introduction

1.1 Background

The vasculature of most life is constructed from tubular structures of various sizes, functions and mechanical properties. Vital transport throughout mammalian tissues critically relies on soft material tubes that build networks of blood vessels (capillaries, veins and arteries) and airways. Numerous approaches have been established to form synthetic tubular structures or embedded channels that match the size and properties of mammalian tissues. Doping these structures with different cell types or modeling channels to resemble tissue vasculature has led to artificial tissues that replicate in-vivo architectures and 3D cell culture that closely resembles physiological conditions.

1.1.1 Tubular Structures

The structure of tubular architectures in animal and human life is highly specialized with respect to their function and may even adapt over time. Blood vessels are composed of different extracellular matrix molecules (e.g., fibrils collagen, and elastin fibers) and embedded cells (e.g., endothelial cells, smooth muscle cells, and pericytes) and vary with genetic background and position within the vascular tree. Arteries and veins have internal diameters that vary from 2-3 mm to 20 µm [1, 2]. Arteries are divided into two types: resistance vessels controlling flow and large elastic vessels with substantial compliance over a large range of transmural pressures, whereas veins can be considered capacitors, capable of storing large volumes without a significant pressure buildup. The mean arterial pressure is adults in 70-105 mmHg; normal systolic pressure is <120 mmHg and diastolic <80 mmHg. Capillaries have very small diameters comparable with the size of red blood cells to increase their total surface area for the rapid delivery and removal of nutrients and waste materials from organ tissue. Furthermore, their small diameter, approximately 5 µm [2], and abundant distribution decreases the diffusion distance between the blood they carry and tissue. The diffusion distance and, hence, the separation of adjacent capillaries is characterized by the Krogh length [3], \( \lambda_k = \sqrt{D_S C_0/R} \) where \( D_S \) is the molecular diffusivity of the solute, \( C_0 \) is the solute concentration and \( R \) is the consumption rate of the surrounding tissue. The ability to obtain replacement structures that accurately resemble
the architecture, heterotypic composition and important functional characteristics of large blood vessels is still very limited. Existing approaches towards tissue-engineered blood vessels (TEBVs) are described in terms of technique and culture time: fibroblast sheets wrapped around a steel support and mature, over 10 weeks, into homogeneous cylindrical tissue [4]; 250-500 µm cell-enclosing alginate fibers were extruded by co-axial flow and encapsulated in collagen solution, containing alginate lyase, that gels over 4 hours creating perfusable channels after 6 days of incubation [5]; and acellular collagen-elastin sheets are produced over two days and then dried and rolled into tubular constructs of 1.3 mm or 4 mm inner diameter [6].

With the rise in interest of tissue engineering, and the need for vascular grafts as replacements for traditional allografts and autografts, numerous approaches have emerged for the formation of vessel-scale fibers and tubes. Microscale production techniques range from scaled-down wet/gel-spinning [7, 8] to direct printing [9, 10] and layered sheet rolling [11].

Several microfluidic platforms have been presented for the continuous formation of fibers and tubes with diameters smaller than 1 mm that are composed of different materials. Examples of vessels that are of interest beyond human tissues are rat and mouse aorta, 1.3/1.55 mm and 500/750 µm inner/outer diameter, respectively. Techniques for fiber formation took advantage of laminar coaxial sheath flows [12] generated through concentric needles [9, 13], pulled glass capillaries [14-16] or poly(dimethylsiloxane) (PDMS) channels (rectangular and circular) [17-21]. The underlying principle is the confinement of a polymer solution between surrounding liquid streams and the confined polymer solution undergoing a sol-gel transition that results in a cylindrical fiber or hollow tube. Takeuchi et al. employed double-coaxial flow to create meter-long fiber cores consisting of cell populated extra cellular matrix (ECM) proteins surrounded by a rapidly cross-linked calcium alginate cladding. The cladding retained the fiber geometry while the slower gelation of the core progressed. The authors populated the core with several cell types, including fibroblasts, myocytes, endothelial and epithelial cells, that were cultured for several days before they digested the outer cladding with alginate lyase. Tubes and fibers produced with coaxial flows had outer diameters between less than 5 µm and 1.2 mm [12, 16, 17, 20, 22-24]. Other approaches have also been implemented for the preparation of larger diameter (0.1-2 cm), multi-layered tubes (up to three layers) [11, 25, 26]. In a multi-step approach [11], different cell types were attached in rows along the length of a partially cured and longitudinally pre-stressed PDMS membrane. When the stress was relieved from one end of the sheet, a stress-
induced shape transformation took place and resulted in the sheet rolling up to become a multilayered cylinder. The authors demonstrated tubes composed of up to three layers with outer diameters of 1-5 mm and inner diameters of 100-750 µm. A bioprinting system was used to programmably deposit long agarose template rods and multicellular spheroids or cylinders (300-500 µm) that built up to a tubular assembly and upon culture for 5 to 7 days fused into a monolythic tube. Single and double layered tubes were constructed with outer diameters ranging from 0.9 to 2.5 mm [26].

However, to date, no scalable platforms for the extrusion of multilayered tubular structures with predictable dimensions over a wide range (multiple millimeters to hundreds of microns) has been developed.

Arianna McAllister in the Guenther lab has previously developed a microfluidic approach for the extrusion of hydrogel tubes, but this approach was limited in its output range and control [27]. The platform used a multilayer device to deliver coaxial flow of an aqueous alginate solution and a calcium chloride cross-linking solution. The vertical extrusion of the sheathed (with crosslinker) alginate biopolymer allowed a high production rate of soft material tubes. This approach was developed from a similar device used to continuously produce hydrogel sheets of the same material [28]. Leng et al., of the Guenther lab, produced planar soft material, 3 mm wide and of controllable thickness (between 150-350 µm), with heterotypic patterning in a number of different biopolymers and incorporating different cell types.

Here, I present a further development of this microfluidic approach for the one-step rapid formation of homogeneous and heterogeneous soft material tubular structures across a wide range of predictable and customizable dimensions. The improvements of this approach include changes to the device design and critical features sizes, and a full parameter study to improve the robustness, reusability and yield of device fabrication and tube extrusion.

Moreover, a significant effort has been put towards the construction of replicate vascularized tissues with embedded fibers or tubes, or degradable templating. Bulk tissue constructs have been formed in gelatin methacrylate (GelMA) [10], star poly(ethylene glycol-co-lactide) acrylate (SPELA), poly(ethylene glycol) diacrylate 4000 (PEGDA) and poly(ethylene glycol) dimethacrylate (PEGDMA) [29], and alginate and chitosan hydrogels [9]. Microchannels are created by direct printing a desired geometry or pattern with a sacrificial or fugitive material,
which is encapsulated by the bulk medium. After removal of the “channel” material, the channel pattern can be perfused and have even been lined with human umbilical vein endothelial cells (HUVEC) [10, 30]. Alternatively, a coaxial nozzle assembly mounted on a robotic printer is able to print a long pattern of alginate tube (crosslinked inside and outside) onto a thin layer of gelled alginate. Multiple layers can be built up by repeatedly layering tubes and pure alginate. Cells, encapsulate in the printed hydrogel tube walls, were kept alive and viable by perfusing the bulk material with culture media [9].

1.1.2 Elastic Properties of Blood Vessels

Matching the mechanical properties of blood vessels and tissues in the body is vital for any surgical procedures or tissue engineering. The elasticity of each type of blood vessel is a function of its role, and the stress-strain relationship for these tissues is typically nonlinear and, furthermore, some vessels are anisotropic [31]. The stiffness and integrity of a human vessel depends on the relative amounts of elastin and collagen in the tube wall [32, 33]. However, measuring the elasticity of in vivo blood vessels is not a simple task and can be reliant upon the method used.

The first approach for the measurement of the arterial elastic modulus was discovered by Adriaan Isebree Moens [34] by measuring pulse wave velocity through elastic tubing. Moens equation [35, 36] and eventually the Moens-Korteweg equation [37, 38] relate the pulse wave velocity (PWV) through a tube to the elastic modulus (E) of the tube: 

\[ PWV = \sqrt{\frac{Eh}{2\pi r\rho}} \]

where h is the wall thickness, r is the vessel radius and \( \rho \) is the blood density. This relationship was originally shown to function correctly for all types of elastic tubing and has since been applied to the approximation of the elastic modulus of blood vessels. Presently, PWV is used as a highly reliable measurement for cardiovascular health. Pressure catheters, placed a known distance apart, track and time the movement of a pressure wave, generated as blood is ejected from the left ventricle. The velocity of the wave is dependent on the arterial compliance [39]. Published works have demonstrated this technique being used in animals and humans, and compared to other measurement techniques [40, 41].

Some of these other techniques for estimating blood vessel stiffness include using ultrasound technology to track changes in pulse pressures or using an angioplasty balloon catheter to deform
a vessel at a known pressure. Gamble et al. [42] used a B-mode ultrasonography to locate the carotid artery and then M-mode cursor perpendicular to the artery wall recorded multiple cardiac cycles. The recorded data was digitized and analyzed to measure the distance between successive R waves. With this data, the distensibility, compliance and Young’s modulus were calculated. Finally, Ilic et al. [43] developed a method of using an angioplasty balloon catheter, used in interventional radiology procedures, to measure the modulus of elasticity of an artery. The pressure inside the balloon and the volume of liquid injected into it are carefully monitored and used to calculate the compliance of the vessel, assuming the outer wall of the balloon matches the inner wall of the vessel. This was shown to be a successful technique via the analysis of pressure-volume curves for a polyvinyl alcohol (PVA) model.

The mechanical properties of tissue engineered blood vessels are of vital importance when attempting to replicate real tissues and their function in vivo. Ultrasound elasticity imaging has already been used on arterial constructs, recognizing the importance of this parameter [44]. It will be important to have a system in place to readily measure the stiffness of synthetic, tissue-engineered blood vessels. Furthermore, a technique for the calculation of the circumferential elasticity would be even more valuable and is not easily estimated otherwise.

### 1.1.3 Microfluidic Cell Culture and Organs-on-Chip

In vivo, cellular niches are characterized by multiple cell types possessing a particular organization with respect to each other, experiencing paracrine and autocrine signals amongst other chemical cues, as well as forces caused by fluid shear stress or the extracellular matrix they are imbedded in [45]. Furthermore, cell growth and culture was shown to respond directly to the stiffness of their substrate [46]. Microfluidic devices have during the past ten years become increasingly capable in recapitulating aspects of these in vivo conditions in ways that far exceed the realities of 2D cell culture on stiff substrates. For instance, Kamm et al. [47] analyzed angiogenesis in 3D co-culture of liver and vascular cells on a collagen gel scaffold in a microfluidic channel. The authors demonstrated increased migration of cells under consistent reverse flow compared to static conditions.

Perfusion culture systems have already been developed; enhancing nutrient transport and providing mechanical stimulation via shear to cell networks, and used to fabricate tissue-engineered grafts [48, 49]. Microfluidic devices made in poly(dimethylsiloxane) (PDMS) and
poly(methyl methacrylate) (PMMA) have been used for the perfusion culture of many cell types, including fibroblasts, neural stem cells and HeLa cells, under physiological conditions and flow rates varying from 0.1 µL/min to 5 µL/min [50-53]. The use of a hydrogel tubular structure as support for perfusion cell culture under physiologically relevant environments is, however, novel. Furthermore, Ingber et al. developed gut-on-a-chip [54] and lung-on-a-chip [55] fluidic platforms for 2D culture under cyclic physiological strain to imitate the tissue-tissue interfaces that are critical for organ function. A microdevice with a thin porous membrane coated in ECM and epithelial cells was perfused with culture media to reproduce the peristaltic motions of human intestines (shear stress ~0.002-0.08 dyne/cm²). Many approaches allow 2D or 3D perfusion culture in the presence of physiological levels of fluid shear stress [56]. Osteogenic responses in particular have been shown to react to fluid flow shear stresses in vitro [57, 58]. Other perfusion bioreactor systems demonstrated the importance of shear stresses on human mesenchymal stem cells (hMSC) construct development within 3D matrices perfused at 0.1 and 1.5 mL/min [59]. Platforms that allow co-localized cell populations to be studied in a 3D format and exposed to physiological strain levels have not yet been demonstrated. Here, I present a platform for 3D cell culture in a tubular substrate with desirable dimensions and stiffness that can be controllably subjected to cyclic physiological strain rates.

1.1.4 Reversibly Sealed Microfluidic Devices

A device for on-chip hosting of hydrogel tubes for perfusion experimentation could benefit from the reusability and practicality of reversibly sealed devices compared to typical permanent bonding solutions.

A permanent bonding technique is generally used to cap substrates with microchannel networks at the end of the fabrication sequence. Such bonds provide very high sealing forces and can support maximum pressures in the range of 210-345 kPa [60] for PDMS-to-PDMS bonds and even higher for microfluidic devices that are fabricated in either glass or silicon substrates. Surface modification via plasma oxidation forms an irreversible bond on contact. However, permanently bonded devices often face a number of practical disadvantages when applied to the perfusion culture of cells, microtissues and organs. To overcome some of these limitations, reversible sealing techniques are often used for microfluidic perfusion culture of cells as they reduce the device manufacturing cost by eliminating a bonding step, and promote the reusability
of devices. Different reversible sealing approaches have been demonstrated [61]: conformal contact establishing a bond due to the self-adhesive properties of bonding materials [30, 62, 63], magnetic seals [64, 65] and vacuum seals [66-68].

The simplest and earliest technique for creating a reversibly sealed assembly is through conformal contact between smooth surfaces [61]. This process requires no surface modification. Elastomeric PDMS forms a tight seal to smooth or non-planar surfaces, established by van der Waals forces. However, this self-adhesion between like or different materials was found to only withstand pressures up to 35 kPa [62] and flow rates of approximately 1 µL/min [30]. Improvements to the seal to reduce leakage include drawing liquids through the device via negative pressure, and clamping around the device edges.

Magnetic forces were used to establish a stronger seal and, permitted higher pressures within macro- and microfluidic devices [64, 65]. This sealing mechanism is generally applicable, but requires the addition of magnets or doping with magnetic materials. D. Auguste et al. [64] demonstrated a magnetic seal for microfluidic devices in PDMS that contained simple channels, 7.5 mm wide, 1.6 mm deep and 70 mm long. Their approach used large neodymium bar magnets distributed around the channel, on either side of the substrates to be bonded. Successful seals were formed with PDMS, agarose, poly(methyl methacrylate) (PMMA), polyhydroxyethylmethacrylate (PHEMA) and alginate, supporting a maximum pressure of 145 kPa. This was later adapted and characterized for microfluidic channels with more complex geometries, and applied to cell culture experiments by Moretti et al. [65]. During fabrication cavities are introduced and doped with a mixture of iron powder and wet PDMS to create a device that can be reversibly bound to glass by placing a neodymium magnet under the glass. Static and dynamic sealing tests showed a strong seal up to 60 kPA and 950 µL/min.

The most popular and consistent means of reversibly sealing microfluidic devices is a vacuum seal. This bond is easily formed between a micropatterned substrate (containing the microchannel features) and a flat substrate and provides sealing forces significantly exceeding van der Waals forces. Reversible seals have been used in many microfluidic applications including surface functionalization [67] and cell culture techniques [66, 68]. Vacuum is applied to a complex network of microchannels arrayed around the working fluidic channels. Reportedly 1-10 torr vacuum pressures [66] were sufficient to maintain a seal, though the value may vary
depending on device design and experimental application. Working pressure is limited by the strength of the vacuum, but pressures up to 100 kPa have been demonstrated in functional channels [61]. Sealing by vacuum creates an easy, but sturdy bond between elastic materials and any flat surface, which can be removed and reapplied repeatedly. Flow through the sealed working channels is preferably accomplished in the withdraw mode and special care must be taken to avoid particles that may compromise the effectiveness of the reversible seal.

Pressure values and flow rates for each type of reversible sealing should not be compared directly as these are dependent on channel geometry.

### 1.2 Objectives

1. Consistent extrusion of homogeneous and heterogeneous hydrogel tubes with controllable dimensions.

   There is a lack of techniques available for high throughput formation of tubular structures that can be readily handled and perfused after production. This thesis research aims to fulfill this need by developing a microfluidic platform for single-step extrusion of tubular structures with diameters ranging from multiple millimeters to hundreds of micrometers. Such a platform should be simple to use and require no specific expertise or complex equipment to run. Furthermore, devices should be reusable and the technique scalable. This dimensions of the extruded structure, diameters and wall thickness, should be controllable and predictable. Soft material tube extrusion will be analytically modeled to calculate the diameter and wall thickness of a tube formed with known input flow rates of the biopolymer and a crosslinking solution. Experimental and analytical results can be compared for agreement between each method.

2. Hosting and perfusion of hydrogel tubes on-chip as a characterized platform under conditions relevant for perfusion culture of tubular microtissues.

   The ability to perfuse and pressurize these rapidly formed hydrogel tubes is a novel concept and a valuable one for applications such as perfusion cell culture and circumferential tensile tests of tubular structures. Herein, describes a second microfluidic device for the perfusion of hydrogel
tubes, produced with the first device. This device takes advantage of a reversible seal via vacuum pressure to improve ease of use and reusability, and reduce the risk of damaging or collapsing tube segments during loading. Reversibly holding a soft material tube on-chip will allow cell-lined or cell-laden tube segments to be controllably perfused under physiologically relevant perfusion culture conditions; culture media, flow rate, temperature and cyclic strain rates, generated by altering the transmural pressure. Pressurizing a mounted tube also has the benefit of allowing an on-chip measurement of material circumferential elasticity, a property that is otherwise difficult to measure and can be compared to off-chip measurements of the axial elasticity.

2 Contributions

I acknowledge the contributions and work done in collaboration towards the completion of this thesis report and the results herein discussed.

Arianna McAllister first demonstrated the extrusion of hydrogel tubes with a microfluidic device similar to the one discussed here. Some details of her approach were described earlier and are given in more depth in her Master’s thesis [27]. I began my work with Arianna and sought to develop a system that could predictably control the dimensions of printed tubes by altering the platform and modeling hydrogel extrusion.

Collection and formation of tubular structures with predictable dimensions was done in collaboration with Haotian Chen. Sections 3.2.1, 3.4.1, 3.4.2 and 3.4.3, as well as, Appendices A1, A3, A9 and A11, contain experimental data of tubular structure formation that were collected collaboratively. These include plots and images in Figures 7, 8, 9, 10, A1, A3, A7 and A9. Furthermore, device modifications allowed us to form multilayer and patterned tubes in a similar fashion (Section 3.4.3). Some of this experimental data may be included in Haotian Chen’s thesis report.

The unique contributions of this thesis include the numerical modeling (Section 3.3) of the controlled extrusion of hydrogel tubular structures, and the entirety of Section 4 on the on-chip fixation of soft material tubes. The results of the model are compared to experimental data in
Section 3.4.2. Supporting data to these contributions is found in Appendices A6, A7, A8, A10, A11, A12 and A13.

The tube formation process and tube hosting device described in this thesis report are protected under US Patent PCT/CA2014/050413 [69].

3 Single Step Hydrogel Tube Formation

Very few approaches exist that allow the replication of the 3D tissues that are abundant in nature. There is particular interest in the formation of synthetic, biocompatible, perfusable tubes as a scalable approach for the generation of tissue engineered blood vessels or cell-encapsulation. Here is described a single step technique for the extrusion of hydrogel (soft material) tubular structures. A multilayer microfluidic device is used to spatially organize the biopolymer as it exits a vertical outlet and immediately begins crosslinking, solidifying, into a tube that can be promptly handled. The dimensions of these tubes can be predictably controlled and have been justified experimentally and with an analytical model. Changes to the device and operating parameters allow multilayer tubes to be formed.

Figure 1. Single Step Hydrogel Tube Formation. (a) Microfluidic device for single step, continuous formation of hydrogel tubular structures. (b) Single continuous strand of tube collected and stored into a Petri dish. (c) Confocal scan of the cross-section of a soft material tube.
3.1 Methods and Materials

The one-step extrusion of tubular hydrogels is a rapid process requiring a vertical tube extrusion device and a simple experimental setup. The multilayer microfluidic device, with PEEK tubing epoxied at its inlets, is placed into a shelved, liquid-filled reservoir that the hydrogel will be extruded into. The device inlets are fed by syringe pumps (Cetoni NeMESYS and Harvard Instruments pumps) containing biopolymer or crosslinking solutions that allow precise and individual control of the flow rates to each layer. By changing these input flow rates the inner and outer diameters (\(D_I\) and \(D_O\)), and hence, wall thickness \(\delta\), can be actively varied. The channels within the device arrange the flows around the common outlet such that there is a cylindrical flow of the biopolymer solution sheathed, inside and out, by the appropriate crosslinking solution. Upon meeting and exiting the device, the flows interact and the biopolymer begins to quickly transition from a liquid to a gel. A confining tube, fit to the same size as the device outlet (for most cases), maintains concentric cylindrical flow outside of the device and as it enters the reservoir.

The reservoir is filled with a solution of the hydrogel’s crosslinking agent (in this case, an alginate hydrogel, which crosslinks in an aqueous calcium chloride solution) such that the microfluidic device sits on the reservoir’s shelves and has its outlet submerged. The calcium chloride in the reservoir aids in the continuous crosslinking and strengthening of the hydrogel tube. The reservoir solution is of a lower density than the input fluids (which themselves are density matched) so that the only factors affecting the tube’s extrusion velocity is the input flow rates and the effect of gravity; there are no other external pulling effects. Data was primarily collected for this reservoir solution, but tubes can also easily be formed with the reservoir solution also being density matched to the input solutions, this creates an extrusion setup where only the supplied flow rates affect extrusion velocity, a plot of tubes extruded in density matched, and aqueous solutions can be found in Appendix A1. Flow is begun on all three syringe pumps (for the simplest device) and tube formation is seen within seconds of the three fluids reaching the punched outlet. Occasionally, some gas bubbles, from the reservoir or fluid lines, become trapped in the outlet hole and have to be carefully removed with tweezers, but afterwards, extrusion is rapid and continuous. After tube formation is establish, and without
stopping the flow, the outlet is cleared and the device is pressed on to the separate confinement piece and aligned with the confining tube. Extrusion continues, now through the confinement tube. The solidifying soft material structure descends approximately 8cm to the bottom of the reservoir, where it forms a steady helical coil as described by [70]. By the time the tube has reached the bottom of the reservoir it is already sufficiently gelled to be carefully handled, cut or manipulated. Long segments of intact tube are then collected into small dishes with reservoir fluid and labeled with the appropriate flow rates. This extrusion process is continuous and can produce very long tubes, so long as inlet fluids are being supplied. Figure 2a-c are assembled demonstrations of the experimental setup, fluidic connections and the microfluidic device.

In order to visualize tube extrusion the reservoir is set up below a stereomicroscope and a camera is positioned beside the tank. With this arrangement, the device outlet can be imaged from above and the flows through, and out of, the confinement can be imaged from the side with a Q-Imaging ExiBlue camera and QCapture Pro 6 software (Surrey, B.C. Canada), see Figure 2d. This allows for in-time observation of tube formation and the effects of varying syringe pump flow rates.
Figure 2. Experimental setup. (a) Isometric view of experimental setup. Confinement piece is submerged in reservoir and positioned on side supports; outlet of microfluidic device is aligned with confinement tube and pressed against support with 500 g weight. Scale bar: 3 cm. (b) Experimental setup with fluidic connections via syringe pump ($Q/\dot{Q}_m/\dot{Q}_o$). The three fluids pass through confinement in co-axial organization while biopolymer layer gels. Optical access provided at confinement exit. Scale bar: 4 cm. (c) Photograph of tube extrusion device with dye-labeled channels; colors match labels in (b). Scale bar: 1 cm. (d) Photograph taken perpendicular to direction of extrusion, demonstrating a tube passing through confinement. Scale bar: 2 mm.

Appendix A2 contains a photograph of the experimental setup with cameras mounted for side and top-view imaging.
In an attempt to add another level of control to the extrusion of tubes a special reservoir was machined and fitted with a motor-controlled drum to collect the falling tube. Control of the motor’s speed allowed a pulling force to be applied to the tube, controlling its extrusion velocity. It was difficult to properly wrap the tube around the drum so it would begin pulling, but when achieved the tube was seen to collect around the spindle as expected. However, the elastic tube easily collapsed under the pulling force of the drum, and as more and more tube length coiled on top (see Appendix A3). Furthermore, as the collected hydrogel piles up, the effective radius of the rotating drum changes, so the pulling velocity imposed on the extrusion is not consistent over time. For these reasons, experiments are run without the collection drum and the tube is retrieved as described above.

### 3.1.1 Flow Confinement

Upon reaching the exit of the microfluidic device, the crosslinking fluids and the polymerizing matrix are guided through a confining plastic tube (confinement) that maintains cylindrical flow outside the device, as the hydrogel solidifies. The confinement has a 3.175 mm diameter to match that of the extrusion hole and is modeled in Figure 2a. The minimum flow rates (minimum total flow rate) are then calculated for the extrusion hole/confinement diameter such that there is no backflow into the exit of the confinement tube. The confinement is 2.8 cm long and leads into a crosslinker filled reservoir. The size of the confining tube is altered with the diameter of the punched extrusion hole so there is no constriction or possible expansion of the flow arrangement outside of the device’s outlet. The confinement aids in the formation of predictable tube dimensions, but is not necessary to make intact tubes; without confining the flow it is more difficult to model and predict the extruded dimensions and backflow or other disturbances around the flow may disrupt proper formation.

### 3.1.2 Biopolymer Matrix

Forming solid tubular structures with predictable and customizable dimensions requires a solution that undergoes rapid gelation. Hydrogel polymers begin as an aqueous colloidal solution and gel into a solid network when polymerization or cross-linking is initiated. This formation of a three-dimensional network can be permanent, but the process can be reversed for many stimuli-sensitive hydrogels. These hydrogels can undergo a reversible sol-gel phase transition in response to many external physical or chemical stimuli, including temperature, pH, solvent
composition, pressure and ionic interaction. Hydrogels, including biocompatible ones, are widely used in drug delivery mechanisms, cell encapsulation and bio-sensing [71].

Sodium alginate is a hydrogel that transitions from solid to liquid by ionic cross-linking. It is already commonly used in the formation of soft material sheets, fibers and tubes due to its rapid gelation process [9, 14, 24, 28]. For these same reasons it is an ideal candidate material for the one-step formation of tubular structures. Alginate has a block copolymer structure composed of two monomers, α-L-guluronic acid (G) and β-D-mannuronic acid (M), whose proportions, distribution and relative lengths determine the chemical and physical properties of the alginate material. Amounts of GG, MM or alternating MG blocks affect the gel-forming ability and flexibility of the molecules [72, 73]. Alginate has a selective affinity towards divalent calcium ions Ca\(^{2+}\) that allows them to cooperatively bind and gel [72]. Specifically, the calcium ions bind between aligned GG blocks of two alginate chains. The gelation is an almost instantaneous process that is governed by the concentration of Ca\(^{2+}\) and alginate G blocks, and the diffusion of the ions into the gelling zone [74, 75]. The gelation of alginate hydrogels requires no other environmental stimuli, such as photopolymerization or temperature.

3.1.3 List of Materials and Equipment

With reference to Figure 2, a three layer microfluidic device, fabricated in PDMS, is fed by two solutions. The biopolymer that fills the matrix layer is a 2% aqueous alginate sodium salt solution (Sigma-Aldrich, ON, Canada), with glycerol (BioShop Canada Inc.). The inner and outer flow layers contain a crosslinking solution of 100 mmol aqueous calcium chloride dihydrate (Molecular Biology Grade, >99.5%, Bioshop Canada Inc.) in glycerol. The two solutions are made with glycerol to match their densities; ρ=1.12 g/mol. These solutions extrude from the device into a polycarbonate (PC) reservoir (MIE machine shop) containing 100 mmol calcium chloride with a density of 1.08 g/mol. Before entering the reservoir the flows pass through confinement made from PC (MIE machine shop) with a diameter of 3.18/1.98/1.59 mm. The flow rates of the three input solutions are controlled via syringe pumps: neMESYS high precision pump (Cetoni GmbH, Korbussen, Germany) and Harvard PHD 22/2000 syringe pump (Harvard Apparatus, Holliston, MA, USA). Tube formation is recorded with a Q-Imaging ExiBlue camera (Q-Imaging, Surrey, BC, Canada).
3.2 Tube Extrusion Device Design

The presented method for extruding single-layer homogeneous tubes uses a three-layer microfluidic device made from PDMS. The device, in Figure 3a, is designed to deliver a liquid biopolymer and its crosslinking solution to a common outlet as a complex fluid that yields a soft material tubular structure when gelled. The three fluidic layers are arranged such that a single biopolymer matrix layer is surrounded, top and bottom, by crosslinker solution. An assembly of the layers is shown in Figure 3b. All of the layers are nearly identical for the most basic device, as shown in Figure 3c; the biopolymer layer differs from the crosslinker layers in its width only, ensuring that adjacent layers have as little channel overlap as possible. Channels are fabricated to be 150 µm deep with 100 µm thickness in between each layer. This small separation of layers is achieved by spin-coating the masters of the two bottom layers, instead of casting, and ensures there is minimal separation of the reagents at the exit of the device. Within each layer, the flow is divided into three channels that further bifurcate until there is a total of twenty-four channels at the outlet, each 300 µm in width. These outlets are arrayed in a circular configuration with an outlet every 15 degrees, forming a ring of outlets that is 4 mm in diameter. This even distribution of channels around the exit and the total channel geometry ensures the total resistance to each outlet is the same and, therefore, the layer’s solution is delivered from all outlets equally. This circular configuration of channels is the same on each layer and must be very carefully aligned during fabrication. The extrusion outlet is punched in the center of this configuration with a 3.175 mm (1/8”) punch and is the first place the flow of each layer meets (see Figure 3d). A vital change to early iterations of the tube formation device was to make the outlet configuration have a larger diameter than the punched hole. Before this change, slight misalignment of the punched hole meant resistance channels would be cut and the flow distribution around the outlet would not be even; poor flow distribution would often lead to incomplete tube circumference and unusable devices. Increasing the diameter of the outlet configuration significantly reduced the chance of severing any important features and created a small plateau region around the outlet where the flow could spread out, even if the two features (outlet hole and outlet channel configuration) are not perfectly concentric. These aspects of the design make the device robust, reusable and resistant to small variations in layer alignment.
Figure 3. Microfluidic device employed for hydrogel tube formation. (a) 3D sectioned model of the microfluidic device at the extrusion hole during biopolymer flow, through a confining tube (light blue) of length $L_c$. The inner fluid, biopolymer and outer fluid layers are colored orange, blue and green, respectively. Scale bar: 1mm. (b) Assembly schematic of the three layers and confinement piece; device footprint is 3x4”. Scale bar: 1.5 cm. (c) Layout of the extrusion features. Scale bar: 1 cm. Insert: close-up view of outlet channel configuration for radial media supply and concentric cylindrical flow. Scale bar: 2 mm. (d) Photograph of resistance channels at the extrusion outlet, imaged from above. Scale bar: 1 mm.

From the top down the layers are arranged: inner crosslinking, biopolymer matrix and outer crosslinking fluid (or inner, matrix and outer fluids, for short). At the outlet, this creates a complex fluid with concentric rings of outer and matrix fluid, and inner fluid at the center. Even while still inside the device, where the crosslinker meets the biopolymer, the hydrogel begins to gel. Gelation is immediate and rapid at the inner and outer surfaces of the ring of matrix flow. This cylindrical flow, still in transition from liquid to solid, descends vertically from the device and allows continuous extrusion of homogeneous hydrogel tubes.

The range of tube formation diameters that can be extruded with a given device can be expanded by punching a smaller hole through the channel outlet configuration.
3.2.1 Device Modifications

Modifications to the device design allow the range of tubular dimensions to be varied even further and more complex, heterogeneous structures to be formed. By simply changing the diameter of the outlet channel configuration by moving the twenty-four outlets in or out, and making sure all the channel resistances remain the same as each other, smaller or larger tubes can be produced, see Figure 4a-b. By increasing the number of vertically-aligned matrix layers during fabrication, multilayered (multi-material) tubular structures can be created. For example, aligning \( n \) matrix layers in between the crosslinking layers permits the formation of \( n \)-layered tubes with a different compatible material for each layer; Figure 4c is the design for making a three layer tube. Each of these extra layers’ flow rates, and therefore resulting tubular layer thickness, can be controlled independently to extrude multilayer heterogeneous tubes of predictable dimensions (similar to the homogeneous case). Each additional layer that is added during fabrication, as well as the complexity of said layer, is increasingly difficult to align; even under microscope optical access is reduced as the device grows thicker. A design for the simultaneous extrusion of ten hydrogel tubes is included in Appendix A4.

![Multilayer, microfluidic device designs](image)

**Figure 4. Multilayer, microfluidic device designs.** (a) Three layer device for single layer, homogeneous tube formation. (b) Modified outlet feature, expanded to print tubes up to 6mm in diameter. (c) Five layer device with three independent matrix layers for the formation of trilayer structures. 5-layer device tested with Haotian Chen. All scale bars: 1 cm.
3.2.2 Device Fabrication

The multilayered microfluidic, single-step tube extrusion device and on-chip tube hosting device are fabricated using a previously described technique called multilayer soft lithography. AutoCAD 2013 (Autodesk) is used to design the device channel geometry, which is printed on to a transparent photomask at 25400 DPI (CAD/ART, OR, USA). The photomask is used to expose a desired thickness of spin-coated SU-8 2050 or SU-8 100 (MicroChem, Newton, MA, USA), a negative photoresist; the thicker and more viscous SU-8 100 allows for thick layers to be spun and, therefore, deeper channels in the final device. Once baked and developed, the remaining photoresist functions as a master mold for poly(dimethylsiloxane) (PDMS) casting. Master thickness and uniformity across the whole surface is verified by measurement with a profilometer.

The tube extrusion device is fabricated using a combination of cast and spin-coated PDMS layers, in a process known as multilayer soft lithography [76]. PDMS kits (Sylgard 184 Silicone Elastomer Kit, Dow Corning, Midland, MI, USA), purchased from Ellsworth, contain a base and curing agent that must be mixed and cured; for all the described devices PDMS was mixed at a 10:1 base to curing agent ratio. A 2mm layer of PDMS is cast over the topmost layer of the device (inner streaming layer), the matrix and outer streaming layers’ masters are spin-coated with approximately 250 µm of PDMS, and, finally, a very thin layer of PDMS is poured into a dish as a seal to the outer streaming channels. Working from the top down, two layers are partially cured in an 80°C oven, carefully peeled off their masters. These layers are then carefully aligned under microscope and pressed together; even small misalignment of the outlet geometry between feature layers can cause the device to fail, by rapidly clogging, delaminating under pressure or not forming a full tube circumference. When baked this forms a permanent bond between layers. This process is repeated for subsequent layers until the bottom is sealed. Finally, the device is fully cured and the extrusion outlet is punched by hand. Figure 5 is an exploded view of the final device assembly, with all three features layers, the bottom sealing layer, outlet hole and PEEK tubing epoxied at the three inlets as fluidic connections (created in SolidWorks). Appendix A5 contains detailed protocols for master and device fabrication.
**Figure 5. Fabrication of tube extrusion device.** An exploded view of the single layer tube extrusion device assembled in SolidWorks. The device contains three features layers and an empty bottom PDMS layer to seal the "Outer" layer's channels. PDMS for each layer is cast over an SU-8 master, partial cured and carefully bonded together, from the top down. Scale bar: 10 mm.

### 3.3 Modeling Tube Extrusion

An analytical model is used to predict the dimensions (inner and outer diameter) of a tube extruded under given conditions and with a given device. The model allows the required inner, matrix and outer flow rates to be calculated for the extrusion of a desired tube size, through a given device and confinement.

As the matrix and, inner and outer crosslinking fluids arrive at the device’s exit they are arranged into the desired configuration of a biopolymer ring, see Figure 6. The relative flow rates of these three flows govern the volume ratio of the concentric configuration. Both inner and outer surfaces of the hydrogel ring are in contact with calcium ions in the crosslinking solutions and the walls of the tubular construct solidify almost instantly. This allows a model to be written to estimate the dimensions of the final tube structure, based on three assumptions.

First, it is assumed that the entire thickness of the matrix flow gels immediately upon contact
with the crosslinking solutions and the matrix can be considered a solid moving wall. This defines the flow geometry at the outlet of the device and maintains it through the length of the confinement piece. This assumption implies a constant velocity profile across the width of the alginate walls, and with no external forces acting on the tube, the velocity (and therefore, flow rate) remains constant. Furthermore, the fluid/solid boundary between the inner fluid and the matrix, and the matrix and the outer fluid implies a no-slip condition at the interface; the velocity of the inner and outer fluid will be equal to that of the wall at their boundaries.

The second assumption made is that there is no pressure gradient difference between the inner and outer fluids. If gelation of the matrix solution was ignored, when the three liquids entered the confinement tube the area of the matrix would increase or decrease such that equilibrium was reached where the pressure gradient through the confinement and across all liquids was equal (see Appendix A6 for the extrusion system modeled in this fashion). However, in actuality the matrix solidifies, as experimentally observed, affecting the final diameters of the fabricated tube. The solid wall that forms in between the crosslinking fluids can generate a pressure gradient difference between the inner and outer fluids, $G_i \neq G_o$. In order to estimate the inner and outer diameter of the printed hydrogel tube, the second assumption of the model states $G_i = G_o$.

Finally, the pressure gradient within the matrix fluid (acting as a rigid body) is assumed to be the mean of the pressure gradients of the inner and outer fluids.

The following boundary conditions can be written for modeling the extrusion of a hydrogel tube controlled by the flow rates of the biopolymer and crosslinking solutions.

**Boundary Conditions:**

\[
\begin{align*}
    r = 0 & \quad \frac{dV_i}{dr} = 0 & (1) \\
    r = r_i & \quad V_i = V_m & (2) \\
    r = r_o & \quad V_o = V_m & (3) \\
    r = r_c & \quad V_o = 0 & (4) \\
    G_i = G_o & & (5)
\end{align*}
\]
Where \( V_{i,m,o}(r) \) are the velocity profiles of the inner, matrix and outer fluids respectively. And \( r_{i,o,c} \) are the radii of the inner and outer walls of the tube, and of the confinement, respectively.

**Figure 6. Modeling tube extrusion.** Cross-sectional view of the tube extrusion device at its outlet. Fluid flows \( Q_i, Q_m \) and \( Q_o \) meet and start forming a tube of outer diameter \( D_O \) and inner diameter \( D_I \). The tube (blue) begins crosslinking immediately as it extrudes along \( x \) at a velocity \( V \), through the confinement of diameter \( D_c \) and length \( L_c \).

With these assumptions and boundary conditions, equations can be written for the three applied flow rates: \( Q_o, Q_m \) and \( Q_i \). Solving for the velocity profile across the region of each fluid, the flow rate can be determined using Equation (7).

\[
Q = \int_{r_1}^{r_2} V(r) \cdot 2\pi r \, dr 
\] (7)
**Inner Flow Rate**

Beginning with a general solution to the Navier-Stokes equation for incompressible flow, the inner flow rate can be derived using boundary conditions Eq. (1) and (2) for \( r \leq r_i \).

\[
Q_i = \pi r_i^2 V_m + \frac{G_i \pi r_i^4}{8 \mu_i} \tag{8}
\]

Where \( \mu_i \) is the viscosity of the inner fluid. See Appendix A7 for data collected with rheometer for the viscosity of the matrix and crosslinking solutions.

**Matrix Flow Rate**

The matrix fluid is assumed to be solid so the velocity profile is a constant, \( V_m \). Hence, the flow rate from \( r_i \) to \( r_o \) is:

\[
Q_m = \pi (r_o^2 - r_i^2) V_m \tag{9}
\]

This is equal to the volumetric flow rate of steady flow.

**Outer Flow Rate**

Using Eq. (3) and (4), and integrating the resulting velocity profile between \( r_o \) and \( r_c \) yields:

\[
Q_o = \frac{G_o \pi}{8 \mu_o} (r_c^2 - r_o^2)^2 + \frac{\pi [4 \mu_o V_m - G_o (r_c^2 - r_o^2)]}{2 \mu_o \ln \left( \frac{r_o}{r_c} \right)} \left[ \frac{r_o^2 - r_c^2}{4} - \frac{r_o^2}{2} \ln \left( \frac{r_o}{r_c} \right) \right] \tag{10}
\]

Where \( \mu_o \) is the viscosity of the outer fluid. See Appendix A7 for data collected with rheometer for the viscosity of the matrix and crosslinking solutions.

A force balance on the hydrogel in the radial direction yields a relationship between the pressure gradients, \( G \), and the matrix velocity, \( V_m \). The shear forces on each side of the wall must be balance by the normal forces.

\[
2 \pi r_i \mu_i \frac{dV_i}{dr} (r = r_i) - 2 \pi r_o \mu_o \frac{dV_o}{dr} (r = r_o) = \pi (r_o^2 - r_i^2) \frac{G_i + G_o}{2} \tag{11}
\]

With Eq. (5) this equality can be simplified to an equation for \( G (= G_i = G_o) \):
Substituting Eq. (12) for $G_i$ and $G_o$ in Eq. (8) and (10), respectively, allows them to be simplified:

$$Q_i = \pi r_i^2 V_m \left( 1 + \frac{\mu_o r_i^2}{2 \mu_i (r_c^2 - r_o^2)} \right)$$  \hspace{1cm} (13)

$$Q_o = \frac{\pi (r_c^2 - r_o^2) V_m}{2}$$  \hspace{1cm} (14)

For a given set of input flow rates, the three unknowns ($r_i$, $r_o$, $V_m$) can be calculated by solving Equations (9), (13) and (14). This model allows the inner and outer diameters and therefore, wall thickness, to be controlled by varying the inner, matrix and/or outer fluid flow rates. A more detailed work through of these equations and how they were derived is given in Appendix A8.

Appendix A6 contains the boundary conditions and details for modeling tube extrusion if gelation of the alginate were to be ignored and all fluids remain that way throughout. In this scenario the pressure gradient in the inner, matrix and outer fluids is equal. Included are the results of this analytical model compared to experimental results collected and measured at the same combinations of flow rates.

### 3.4 Tube Formation

With the experimental setup described in Section 3.1 hydrogel tubes can be rapidly extruded in a single step. The biopolymer and crosslinker flow rates are controlled independently via syringe pumps, and when all are running tube formation begins within seconds of the solutions reaching the device’s outlet. Occasionally, some gas bubbles, from the reservoir or fluid lines, become trapped in the narrow channels around the outlet and in the outlet itself, and have to be carefully removed with tweezers and light pressure, but afterwards extrusion continues unimpeded.

Flow and tube extrusion is typically first established by supplying flow rates of 650 µL/min, 200 µL/min and 650 µL/min to the inner, matrix and outer layers, respectively. Once formation has
begun, the flow rates can be varied over a very wide range of values before the tubes become too large for the confinement tube or so small that they collapse. The tubes extrude at approximately 4-5 mm/s and can be immediately handled. Extrusion is continuous making this platform suitable for high-throughput formation of soft material tubes. Measuring and comparing the size of these tubes to the model predictions allows for dimensional control of tubular structures. More complex, multilayered tubes can be extruded just as easily by making some modifications to the device.

Experimental data in the following sub-sections (3.4.1-3.4.3) was collected in collaboration with Haotian Chen.

3.4.1 Extrusion Parameter Space

To explore the full range of flow rates that would yield continuous tube formation a parameter study was done with a simple three layer device with a 3.2 mm ±0.1 mm outlet extruding through a 3.175 mm (1/8") confinement piece. At a constant matrix solution flow rate of 200 μL/min, the inner and outer flow rates were varied over multiple orders of magnitude. Figure 7 is a log-log plot of this parameter space, with flows ranging from 5 μL/min to over 3000 μL/min. The green circles represent combinations of inner and outer flow rates that successfully produce tubes, whereas the red circles identify conditions where they did not. Tubes start to collapse at inner flow rates around 10 μL/min, but still maintain their shape at very low outer flow rates. At very high flow rates the tube begins touching the walls of the confinement, restricting its size and causing some stuttering in its descent. This parameter study was only done for single layer, homogeneous alginate tubes.
Figure 7. Operating envelope for tube formation. $Q_M = 200 \, \mu$L/min, 2% alginate solution and 100 mmol calcium chloride solution. The inner flow rate, $Q_i$, is plotted against outer flow rate, $Q_o$, in a parameter study illustrating the wide range of flow conditions permissible with the successful formation of homogeneous alginate tubes. Green circles: forms tube; Red circles: does not form tube. Experimental data collected in collaboration with Haotian Chen.

3.4.2 Predictable Homogeneous Tubular Dimensions

The same device (single layer tube, 3.175 mm extrusion hole and confinement) was used to create tubes to characterize the effect of changing the inner and outer flow rates on the inner and outer diameters, whilst keeping the total and matrix flow rates constant; 1500 $\mu$L/min and 200 $\mu$L/min, respectively. The alginate tubes, extruded with 5% v/v 0.1 $\mu$m fluorescent microspheres (Fluosphere, Life Technologies, California, USA) in the matrix solution, are allowed to collect at the bottom of the reservoir for two to three minutes, at a given set of flow rates, before they are collected into a small petri dish filled with a calcium chloride solution (crosslinker). Changes in
the flow rate occur rapidly, and hence, so do changes in the tubes shape, but to ensure extrusion is stable at the new flow rates at least one minute’s worth of tube is discarded after any change. To measure the dimensions of the tube segments they are imaged with a confocal microscope (Nikon Eclipse Ti and A1R confocal controller, Tokyo, Japan) and NIS-Elements AR software. A thin cross section of the tube is carefully cut and situated on the objective stage submerged in a small imaging dish. Figure 8a-d shows examples of the tubes’ cross sections imaged with the confocal microscope, from which the diameters, thickness and cross-sectional area can be measured. The inner, matrix and outer flow rate combinations for the tubes imaged in Figure 8a-d are 300/200/1000 µL/min, 600/200/700 µL/min, 900/200/400 µL/min and 1200/200/100 µL/min, respectively. Measurements are done post imaging with ImageJ (National Institutes of Health, Public Domain). The inner and outer diameters are calculated by measuring the cross-sectional areas of the lumen and wall, respectively, and dividing by Pi. This assumes a circular tube, as seen in Figure 8a-d, and avoids inconsistencies in a tube segment that is not sitting perfectly in the imaging dish and makes the measurement more reliable. The wall thickness is simply the difference between the outer and inner tube diameters. Figure 8e shows the behavior of the inner (red circles) and outer (blue squares) diameters, as well as wall the thickness (green triangles), as the inner and outer flow rates varying between 100 and 1200 µL/min. Data for this plot was collect from three different devices (n=3) over the same flow rate combinations. With each device, the full range of flow combinations was tested and three replicate measurements were taken at each condition (nine total measurements per point). At a given set of conditions, the three replicate sets of wall thickness, and inner and outer diameters measurements were averaged, and then these were averaged across all three devices. The variation of tube dimensions between devices is demonstrated by the standard deviation of the measurements across devices; standard error or confidence intervals are less informative for such a small number of experiments (n). The error bars in Figure 8e represent the mean of the three devices (three replicate measurements at each point with each device) plus or minus one standard deviation. The variability across the microfluidic devices, when the n value is small, could also be accurately represented by simply plotting all data points for each device without error bars [77]. Here, error bars are used to improve the readability of the plot, when comparing the experimental results to theoretical predictions. Appendix A9 contains a scatter plot of all of the raw data points.
Increasing the inner flow rate and decreasing the outer flow rate increases the outer diameter of the tube, but results in a thinner wall. Over this range of flow combinations, the outer diameter of the tubes were seen to change from approximately 1100 μm to 3000 μm, and the respective wall thicknesses from 400 μm to 80 μm. Smaller tubes can be produced by decreasing the inner flow rate further (refer to parameter space in Figure 7), or by punching a smaller outlet hole in the PDMS device and, similarly, using a smaller confinement. Tubes as small as 600 μm (OD) were fabricated.

Figure 8. Formation of homogeneous tubes. Alginate tubes, dyed with a 5% v/v yellow-green 0.1 μm fluorescent particles, extruded in a single step with the microfluidic device at the following flow rates ($Q_i/Q_m/Q_o$): (a) 300/200/1000 μL/min; (b) 600/200/700 μL/min; (c)
900/200/400 µL/min; (d) 1200/200/100 µL/min. Scale bars: 500 µm. (e) Plot of the diameters ($D_O$ and $D_I$) and wall thickness ($\delta$) of a soft material tube printed at a given inner and outer flow rate. Analytical model predictions of the same dimensions are included as solid lines. Blue: $D_O$; Red: $D_I$; Green: $\delta$. n=3 devices. Error bars represent one standard deviation. Experimental data collected in collaboration with Haotian Chen.

Comparing the experimentally extruded and measured hydrogel tubes to the analytical model shows favorable agreement; see the solid lines plotted in Figure 8e. This agreement strongly suggests that the dimensions of extruded tubes are predictable via independent flow rate control. A residuals plot, illustrating the differences between the experimental and theoretical results, is attached in Appendix A10. The prediction of the analytical solution falls in line with the experimental results for most of the conditions tested, except for the high and low end $Q_o/Q_i$ ratios. We see the effects of crosslinking occurring immediately at the inner and outer surfaces of the hydrogel fluid and defining the tube shape, especially when taken to very high or low flow rates. At high $Q_o/Q_i$ ratios, the instant formation of the inner surface of the tube inside the device defined the inner circumference of the tube. While the cross sectional area occupied by inner fluid decreases due to low flow rate, the inner wall of the tube buckles in order to accommodate the shrinkage. Furthermore, this buckling can most likely be attributed to the presence of a pressure gradient difference from the inner to outer fluids, an effect that is not modeled. This results in the higher than predicted wall thicknesses seen at the left side of Figure 8e. At the low $Q_o/Q_i$ ratio, the rapid gelation of the outer wall of the tube prevents the diameter of the tube from expanding to reach the predicted result. Due to this limiting factor, the diameters of the tube formed are smaller than approximated by the model. These effects cause the residuals seen between the experimental and model results; larger differences at low and high inner flow rates (Appendix A10).

In modeling the tube extrusion in this fashion, the assumption that the pressure gradients were equal in the inner and outer fluids had to be made. Due to the gelation of the matrix fluid in between these two fluids, this assumption is most likely not true. Using the experimentally collected outer tube diameters, the Equations (8), (9), (10) and (11) can be solved to predict the inner diameter, $r_i$ (whilst simultaneously calculating $G_i$, $G_o$ and $V_m$). The resulting solutions for $r_i$ agree closely with the experimental data and fit better than the simplified model predictions at high inner flow rates. Appendix A11 contains a plot of the experimental data for inner and outer
diameters and the predicted inner diameters, as calculated with $G_i \neq G_o$. The numerical analysis of the extrusion matches closely with the experimental results.

It was suspected that the inner fluid acts as a template during tube extrusion; the presence of the flow helping to maintain the shape of the tube during extrusion and gelation. To verify this query, the system was run without calcium chloride in the inner fluid solution. In the absence of calcium ions, the inner surface of the tube will not gel until the ions can diffuse through the matrix from the outer flow. Tubes were successfully formed and seen to maintain their expected shape (as compared to previous results with calcium chloride) for the majority of the flow rates tested. With this technique, the experimental results do not match the model as well because the inner wall does not solidify as quickly as before, hence the assumption that the tube wall acts as a rigid body is not as applicable, see Figure 9a. The outer wall is crosslinked as before, but the inner wall is less well defined, despite still being mostly circular as seen in Figure 9b-d. However, the inside of the tube is crosslinked and the tube can be handled immediately after extrusion without serious damage. This confirms that the calcium ions were able to diffuse through the hydrogel to solidify the inside and that the fluid inside the tube acts as a guiding template for the tube’s formation. Unfortunately, without the central crosslinking the tube’s dimensions are more difficult to control and do not follow the model’s predictions as closely. Furthermore, small imperfections may appear on the inner surface (see Figure 9d) due to the slower gelling and at low $Q_o/Q_i$ ratios the flows become too unstable and tube formation is not possible. This demonstrates that, though possible, it is far superior to extrude alginate tubes with a calcium solution in the inner and outer flows; tubes gel faster, their dimensions can be more accurately predicted, the range of flow conditions is greater and there are fewer imperfections in the tube wall.
Figure 9. Dimensions of tube extruded in absence of internal crosslinking. (a) Tube’s dimensions when lacking crosslinking ions in inner fluid. Blue: $D_O$, Red: $D_I$, and Green: wall thickness, and for comparison, results with cross linker are included as semi-transparent markers of the same color. (b)-(d) Confocal images of tubes extruded with no inner crosslinking, at $Q/Q_m/Q_o = 300/200/1000$ µL/min, 600/200/700 µL/min, and 900/200/400 µL/min, respectively. Tube formation was unsuccessful at higher inner flow rates. All scale bars 500µm. Experimental data collected in collaboration with Haotian Chen.
3.4.3 Tube Morphologies and Heterogeneities

Specific manipulation of the flow conditions or the device itself can yield some very interesting and reproducible changes to a tube’s geometry.

By changing the inner flow rate to very high (over 3000μL/min) or low (under 200μL/min) the inner wall and tubular cross-section is seen to change quite drastically. These consistent effects occur due to the exit size and geometry of the microfluidic device and the rapid gelation of the alginate solution. When the inner crosslinking fluid meets the hydrogel, still within the outlet, an inner circumference is defined, which is dependent on the input flow rates. However, at the extremes of the inner flow rate, the circumference is too small or large to accommodate the high or low flow. At very high inner flow rates, the inner tube wall is forced to zig-zag back and forth, varying the thickness of the wall, to account for the large transport of liquid. The result is the tube seen in Figure 10a; the zig-zagging tube also has a large outer diameter, as is expected of high inner flow rates. At inner flow rates below 200 μL/min, the area of this flow (at the extruding velocity) is smaller than the inside of the already surface gelled hydrogel. This forces the inner wall to buckle inwards to fill the area, creating an irregular wall thickness, often similar to a star-like pattern, as seen in Figure 10b. Under both of these conditions, the outer diameter of the tube is unaffected, and still circular.

Devices fabricated with multiple and/or modified matrix layers can be used to controllably produce heterogeneous tubes. Increasing the number of matrix biopolymer layers, in between the inner and outer fluid layers, increases the number of concentric rings of flow being extruded through the confinement. Like with homogeneous tubes, the calcium ions in the inner and outer solutions diffuse through the inner and outer boundaries of the matrix flow allowing each layer of biopolymer to crosslink into distinct layers (of the tube’s wall). The tube produced is multilayered, with the number of alginate layers equaling the number of microfluidic matrix layers. Figure 10c-d shows the cross-section of a three layer hydrogel tube that was produced by supplying 2% alginate solution to each layer; each layer is labeled with a different color dye: yellow-green, blue and red, from the inside out. The sum of the matrix flow rate was kept the same as that for single layers tubes (200 μL/min), such that the dimensions are consistent with those of single layer tubes extruded under the same conditions. Similarly, the diameters of the
multilayer tube can be predicted with the same numerical model as for homogeneous tubes. The relative flow rates for each colored layer controls the relative thickness of each layer, whereas the total wall thickness depends on the total matrix flow rate (as well as the inner and outer crosslinker flow rates). Tubes up to five layers thick have been extruded with this method, but fabrication and tube yield decreases with these more complex devices. This robust technique could enable the fabrication of tubes of multiple layers of different materials and allow slow gelling materials, enclosed in a rapidly crosslinked material, to solidify.

**Figure 10. Different morphologies heterogeneous tubes produced by altering flow rates or device features.** (a) Cross-section of a tube produced at low inner flow rate, inner wall buckles, imaged with confocal microscope at 4X. Flow regime: $Q_i=100 \ \mu$L/min, $Q_m=200 \ \mu$L/min and $Q_o=1200 \ \mu$L/min. Scale bar: 500 µm. (b) Brightfield image of tube being extruded at $Q_i=3500 \ \mu$L/min, $Q_m=200 \ \mu$L/min and $Q_o=5 \ \mu$L/min. Tube inner circumference buckles during formation. Scale bar: 3 mm. (c) Three layer alginate tube imaged at 4X magnification, $Q_i=1200 \ \mu$L/min, $Q_m=3 \times 67 \ \mu$L/min and $Q_o=100 \ \mu$L/min. Scale bar: 500 µm. (d) Same 3-layer tube imaged with
On-Chip Fixation of Hydrogel Tubes

The ability to perfuse and pressurize these rapidly formed hydrogel tubes is a novel concept and a valuable one for applications such as perfusion cell culture and circumferential tensile tests of tubular structures. Supported by the success of previous works by the Guenther lab [78] a platform has been developed to host and perfuse biopolymer tubular constructs. This platform, another microfluidic device, employs a reversible seal to temporarily fix, perfuse and superfuse tube structures, such that the device may be reused for multiple samples or experiments. Continuous flow can be perfused for upwards of 24 hours. The device design permits the luminal and abluminal microenvironments to be controlled, generating a transmural pressure. The effect of this transmural pressure can be modeled and experimentally evaluated. The resulting alteration of the on-chip dimensions is an indicator of the compliance, and therefore elasticity, of the soft material tubes.

4.1 Methods and Materials

Soft material, hydrogel tubes that are pre-extruded can quickly and simply be inserted into the on-chip perfusion device. The tubes should be stored in a calcium chloride solution immediately after fabrication and then 5-7 mm segments are cut in preparation for on-chip hosting. Before mounting the tube, the microfluidic device is cleared of dust with adhesive tape and all fluidic connections are made for all lines: perfusion and superfusion inlets and outlets, and fixation and vacuum out-flow. For perfusion and/or superfusion of the tubular structure syringe pumps are used to control the media flow rate through and around each side of the tube. Four fixation channels provide a negative pressure to the ends of the tube to hold it open. All four are fed by a single inlet that is connected to a water column reservoir that is suspended below the device. The vacuum line creates a sealing force between the open device channels and a coverslip, and is connected to a negative pressure head or small diaphragm vacuum pump, depending on the positive pressures being studied on-chip.

The hydrogel tube is carefully handled with tweezers as it is placed into the device’s perfusion channel and aligned with the fixation channels. A drop of deionized water is placed on top of the
tube and chip, and then a coverslip is centered and set on top of the elevated features. The coverslip is gently pressed down to form a contact seal, careful to not trap bubbles inside, before negative pressures are applied to the fixation and vacuum channels. The vacuum assists in strengthening the reversible seal that contains the tube and all liquids. The seal is robust enough that the device can be safely inverted without leakage, as long as the device or coverslip is not bent, which could cause a gap in the seal. The vacuum seal is strong enough with a vacuum pump that if the pump is disabled (after the seal is established) the seal will still remain, until the coverslip is disturbed or removed. For the performed experiments, however, the negative pressure was continually supplied. With the coverslip at the bottom of the device, it can now be mounted on the stage of an inverted microscope for imaging and micro-particle image velocimetry (µPIV).

4.1.1 Perfusion and Superfusion Setup

With a hydrogel tube mounted on-chip the perfusion and superfusion flow rates can be set to controllably flow media through and around the hosted tube. The tube should first be pressurized with a hydrostatic head at the outlet of the perfusion or superfusion to seal the device at the fixation features and limit leaks. Imaging techniques allow the coverslip and fixation seals to be monitored for leakage. The perfusate, superfusate and any leakage into the fixation lines can be collected as a measure of efficiency. Severe leakage, such as that into the vacuum area of the chip, is immediately obvious from visual inspection alone. For long term, continuous perfusion the flow rate can be set via a hydrostatic head pressure on a large reservoir. Figure 11 shows a tube hosting device sealed and inverted on the stage of an inverted microscope for imaging of a tube sample as it is perfused/superfused.

As a system to demonstrate on-chip perfusion of tubular constructs, the flow rates, pressures and media are kept consistent with typical perfusion culture approaches. Dulbecco’s modified eagle medium (DMEM) is used as a perfusate/superfusate due to its relevance and common usage as a culture media. DMEM is one of the most common medium modifications and is less complex than others, which are more specialized for specific cell types. For these reasons it is suitable to use DMEM as the media for the on-chip perfusion of fabricated biopolymer tubes.
Figure 11. Tube hosting microfluidic device mounted on inverted microscope. A device with a 900 µm wide perfusion channel is reversibly sealed to a plastic coverslip by vacuum and fluidic connections are made via the distribution layer.

4.1.2 Setup for On-Chip Tensile Measurements

An on-chip measurement of the hydrogel tube’s tensile properties can be done on the same microfluidic device as tube perfusion. A tube is mounted as above and the device is reversibly sealed, but the perfusion and superfusion inlets are fed by reservoirs. By drawing from reservoirs and plugging the outlets of the perfusion and superfusion a controlled pressure head can be applied to the inlets, dictating the pressure inside and outside the tube, instead of flow through and around the tube. This generates a pressure difference across the wall, a transmural pressure, which will cause the elastic material to deform. The change in width of the tube as measured on-chip after pressurization with a given pressure difference is a measure of the tubular material’s compliance. The compliance of the material is inversely proportional to its elastic modulus. Hysteresis effects can be identified by periodic stretching of the material, again under
physiologically relevant strain rates. Furthermore, the elasticity can be measured repeatedly over time to study the degradation of the material as it remains hosted and pressurized or perfused.

4.1.3 List of Materials and Equipment

Figure 12 is a schematic representation of the experimental setup for the perfusion/pressurization of a short tubular hydrogel section. A two-layer microfluidic tube hosting device, fabricated in PDMS, is loaded with a pre-extruded hydrogel tube segment 5-7 mm long. Fluidic connections link the perfusion and superfusion inlets to a neMESYS syringe pump and outlets to open water-filled reservoirs as positive hydrostatic pressure levels. The fixation and vacuum inlets are connected to negative pressures with a reservoir suspended below the device and a CTS micro-diaphragm vacuum pump (9 VDC, coreless, Parker, USA), respectively. The vacuum line is run through a water trap and small particulate filter to prevent damage. A 0.2 mm thick plastic, Thermanox™ coverslip (Ted Pella, CA, USA) covers the open features and is reversibly sealed to the PDMS with vacuum. The sealed device is inverted and placed on the stage of a Nikon inverted microscope with ExiBlue camera (Q-Imaging, BC, Canada) and SoloPIV III system (NewWave Research, USA). DMEM is a culture-relevant media for perfusion and superfusion.
Figure 12. **On-chip tube hosting setup.** Microfluidic tube hosting device (extended above is bottom view of hosting and distribution layer overlaid) is loaded with a short tube segment and reversibly sealed to a coverslip by vacuum pump. Tube is pressurized and fixed in channel with perfusion, superfusion and fixation pressures: $P_{in}$, $P_s$, $P_{fix}$, respectively. Perfusion and superfusion flow rates, $Q_p$ and $Q_s$, adjusted by syringe pump. Device is loaded onto inverted microscope stage for imaging. Not to scale.

### 4.2 Tube Hosting Device Design

The microfluidic device for tube hosting contains two fluidic layers fabricated from PDMS. The layers are distinguished as the tube hosting layer, Figure 13a-b, and the flow distribution layer, Figure 13c. The distribution layer is permanently sealed to the reverse of the hosting layer, closing in the distribution channels. Oppositely, the hosting layer’s channels are left open, giving access to insert and reversibly fix short tube segments. A color-labeled drawing of the two layers is illustrated in Figure 13d.
The distribution layer consists of simple channels for the delivery of flow to the tube hosting layer. For tube fixation, perfusion and pressurization, the channels feed the inlets and outlets of perfusate and superfusate media, as well as four fixation locations and a vacuum pressure channel for reversible sealing. All channels are 150 µm deep and the widths are designed such that the resistance is identical for each type of flow. All of the layer’s inlets and outlets are distributed on the same side of the device for optical access of the hosted tube and ease of compatibility with microscope stage. The 1 mm diameter inlets and outlets are fluidic connected via metallic pins.

The tube hosting layer contains the crucial features for perfusion and on-chip analysis of hydrogel materials. The deep, 600 µm, channels must be fabricated by a micro-mill and allow the tubular structures to be held on-chip. The width of the main perfusion channel controls the size of the tubes that can be hosted on a given device and must be set so the tube touches all four sides of the channel without the walls collapsing. Two different devices were fabricated with perfusion channel widths of 900 µm and 1200 µm. These channels are for tubes of approximately 750 µm and 950 µm outer diameter, respectively, to be hosted. Fixation channels provide a negative pressure to both sides of the tube, at both upstream and downstream ends, and stretch the elastic tube walls to the peripheries, holding the tube open and perfusable. The width of the fixation channels was designed based on estimates of the wall deflection under a fixation pressure. The tube must not deflect more than one and a half times the wall thickness into the fixation channels. Appendix A12 contains a plot of the tube wall deflection under various fixation pressures against the width of the fixation features. This is modeled for the typical wall thickness of 100 µm and for fixation pressures varying from small pressure heads to a vacuum pressure, established with a small diaphragm pump. Independent superfusion channels on either side of the tube control the abluminal environment of the tube. The location where the superfusion channels open into the perfusion channel is referred to as the inspection area. The width of the channel is increased here to allow the tube to elastically deform when pressurized. All of the channels and inlets are surrounded by a narrow wall, whose top surface is used to reversibly seal and isolate the tube and media from the vacuum area. All the features are enclosed by a 500 µm wide ring that is 12 mm in diameter and serves as the outer boundary of the vacuum area. The diameter of this feature was chosen with device scalability in mind.
When all fluidic connections have been made, a tube segment 5-7 mm long is placed into the perfusion channel, ensuring that both ends extend past the fixation forks, as in Figure 13b. Once in place, a fixation pressure is applied to the fixation channels to secure the tube and hold the lumen open. The device can then be reversibly sealed using the vacuum area that surrounds all the fluidic channels.
Figure 13. **On-chip tube hosting device.** (a) Tube hosting layer and device footprint, with color coded channels and features: perfusion (green), superfusion (red), fixation (light blue), vacuum (white) and sealing surfaces (grey). Colors apply to all panels of figure. Scale bar: 2 mm. (b)
Close-up of insert indicated in (a) with a hydrogel tube (blue) held in the perfusion channel, in between the fixation forks. Expansion of the tube when pressurized is designated by a dashed line. Scale bar: 1 mm. (c) Distribution layer. Scale bar: 2 mm. (d) Overlay of the hosting and distribution layers. Hosting layer is semi-transparent to highlight channel alignment. Scale bar: 2 mm. (e) Exploded view of the microfluidic device with fluidic connection pins (orange) and thin plastic coverslip for reversible sealing. Scale bar: 5 mm.

4.2.1 Device Fabrication

Fabricating the tube hosting device is more difficult than the tube extrusion device due to large feature depths of the hosting layer; the achievable aspect ratio is a limiting factor for feature sizes. The device masters can be made using standard soft lithography as explained for the previous device [76] or with micro-milled molds. Both approaches are then used in conjunction with PDMS to form a flexible microfluidic device.

Fabricating the deep channels required for the on-chip tube hosting master is a lengthy process and means spinning many consecutive layers of SU-8. Furthermore, a uniform thickness across all features is very important to form a proper seal between PDMS and coverslip. The PDMS device can be fabricated by casting each layer and then bonding them in a similar fashion to the extrusion device. Alternatively, the more traditional technique of plasma treating the two cast surfaces to form an irreversible bond can be used. The latter technique is, perhaps, more beneficial as it is a “cleaner” process and foreign particles, which may interfere with the device’s reversible seal, can be removed, with adhesive tape, at each step.

In order to create a more reliable feature depth and flatness across the entirety of the hosting features, the same masters can be micro-milled. Both the MIE machine shop at the University of Toronto and Z-Microsystems (Austria) machined molds out of aluminum and brass, respectively. Feature aspect ratio and roughness are important and must be tailored to each manufacturer, depending on their capabilities. The MIE machine shop is limited in channel depth by the size of the mill bit; a smaller radius bit cannot cut as deep. Two hosting designs (900 µm and 1200 µm perfusion channels) were milled at a depth of 600 µm into a single slab of aluminum, with a surface roughness finish of 0.8 µm (32 microinches). All inset corners are filleted to at least a 0.0055” radius (0.1397 mm). The machined features were inspected with a profilometer and the feature depth was actually 625 ±3 µm, and the surface roughness 0.53 µm, better than the
proposed finish. Appendix A13 contains profilometry scan data for the surface roughness of the milled piece and a partial, stitched depth scan for the 900 µm channel design. An almost identical piece was ordered and milled by Z-Microsystems, the only difference being the radius of fillets, which has been reduced to 0.03 mm. Z-Microsystems claims a mirror surface finish, approximately 25 nm roughness, on milled features with a multi-step machining procedure. The master part design used for both orders is shown in Figure 14. The PDMS casting, curing and bonding steps for aluminum and brass parts are the same as those previously described with SU-8. These micro-milled masters are for the tube hosting layer only, whereas the distribution layer, requiring a depth of 150 µm, is made using traditional photolithography with SU-8; Appendix A5.

![Figure 14. Fabrication tube hosting devices.](image)

(a) Rendered image of micro-milled master accommodating two designs with 900 µm and 1200 µm wide perfusion channels, respectively. Scale bar: 5 mm. (b) SU-8 master of the distribution layer for the tube perfusion device. Scale bar: 5 mm.

### 4.3 Reversibly Sealing the Device

This microfluidic device uses a reversible seal to close the channels of the tube hosting layer so hydrogel tube segments can be directly placed and accurately aligned in the perfusion channel. Furthermore, the ease of disassembly means a single device can be reused for multiple tube segments or experiments without fear of clogging. Similar to some of the approaches described previously this device is reversibly sealed by a vacuum pressure. Once a tube is positioned in the
device and the negative fixation pressure has been applied a plastic coverslip (Ted Pella) is placed over the microfeatures and lightly pressed to form a seal. A vacuum pressure is then fed to the vacuum area of the chip design to strengthen the seal. Unlike most microfluidic devices in PDMS, the features that define the channels are elevated from the surface, as opposed to being cut into it. These elevated features form walls around the channels and reduce the area of contact between the PDMS and coverslip. The reduced interaction area reduces the chance of non-uniform planarity or foreign particles affecting the quality of the seal and leading to leakage. Surface roughness and feature planarity is an important consideration when fabricating this device and in order to achieve acceptable values for 600 µm features they had to be micro-milled. The region between the channel walls and outer ring forms the vacuum area that holds the coverslip, which seals the channels, in place. The compliance of the 0.2 mm thick coverslip and the PDMS device itself further assist in creating a reliable seal that is less susceptible to small flatness irregularities. An exploded view of the coverslip and device layers, with a hosted tube, is included in Figure 13e.

4.3.1 Reversible Seal Characterization

The microfluidic tube hosting device is reversibly sealed to a thin coverslip by applying a negative pressure to the vacuum area of the device. In order to create a proper seal, where no air leaks past the outer boundary wall and none of the perfused or superfused media drains into the vacuum area, the force acting on the coverslip generated by the vacuum must be greater than the force attempting to separate the PDMS and coverslip. The forces acting to break the seal are the pressure inside the fluidic channels (all assumed to be at the same pressure for calculation) and the weight of the coverslip and liquid. If this is obeyed then there should be no leakage across any of the PDMS support walls. The force generated by each pressure is the product of the applied pressure and the surface area of the respective vacuum region or fluidic channels. The area of the vacuum region is by design larger than the enclosed channel areas such that the reversible seal holds with small negative pressures. Figure 15 contains a plot of the force balance, where a negative value indicates that the vacuum pressure dominates and the coverslip should be held tight against the PDMS. In this case, the pressure inside all of the channels is assumed to be equal, when in actuality the fixation channels will be operated at a negative pressure, which would only serve to strengthen the seal. Theoretically, the reversible seal should hold for all the shown positive channel pressures at vacuum pressures as small as -3500 Pa,
approximately a 36 cm water column. For each case, the absolute value of the vacuum pressure can be smaller than the channel pressure and the seal will remain intact.

Figure 15. Force balance for reversible seal. Reversible seal intact if the net force is negative; the force exerted on the coverslip by the vacuum is greater than the opposing forces. Here the force balance is plotted against decreasing vacuum pressure. Each curve represents a different positive pressure in the channels: Pink, 20000 Pa (120 mmHg); Blue, 8000 Pa (60 mmHg); Green, 3920 Pa (40 cm H₂O); Red, 1960 Pa (20 cm H₂O); and Purple, 490 Pa (5 cm H₂O).

A tube segment was loaded into the hosting device and the coverslip placed over the channels. A vacuum pump applied a negative pressure to the vacuum area of the device and the region was aspirated of all liquid, without disturbing the fluid inside the fluidic channels. With the device sealed it can be inverted and mounted on an inverted microscope to be imaged without leakage. A 20 cm water column pressurizes the fluidic channels as the vacuum channel pressure was decreased towards zero. The seal was reliable and consistent for any positive pressure so long as the vacuum pump (approximately -70 kPa) was used to provide the vacuum, but as the negative pressure was brought towards zero the seal broke below a -60 cm water column (5890 Pa). Experimentally, the seal broke before predicted and leakage was seen into the vacuum area and beyond, causing the coverslip to dislodge. The unexpected leak is most likely due to flexibility of
the two materials and the presence of foreign particulate where there is contact. Bending or local
imperfections in the materials or particulate on the walls can create small gaps in between the
PDMS and the coverslip that grow large enough to allow liquid to leak through, when the
difference between the sealing and fluidic pressures decreases. Hence, a small diaphragm
vacuum pump can be used to ensure a reliable and consistent reversible seal for the hosting of
hydrogel tubes, as seen in Figure 16.

![Image of reversibly sealed device](image)

**Figure 16. Photograph of reversibly sealed device.** A Thermanox coverslip is reversibly sealed
to the features of a tube hosting device. A short tube segment is fixed in the device and perfused
with water dyed blue with food coloring. A vacuum is applied to prevent leakage from the fluidic
channels. The PDMS boundary and the fluidic channels are clearly isolated from the excess
liquid. Any liquid in the vacuum area is sucked into a water trap during sealing. Some small
pockets of water are trapped in the vacuum area, but no leakage is seen across the PDMS walls.
4.4 Pressurization of Hosted Tubular Structures

When a hydrogel tube is mounted on-chip it is deformed by fixation and perfusion pressures to hold the segment in place. A model was generated to understand how a hydrogel tube, confined to the perfusion channel geometry, deforms when pressurized. When reversibly sealed to a coverslip the tube is confined vertically to the top and bottom of the channel (as seen in Figure 18a-b), but not laterally. The expansion of the tube walls in this direction, from \( w_0 \) to \( w_p \) (initial and pressurized widths, respectively), can be predicted for a given pressure difference for a tube of circumferential elasticity, \( E \). It is assumed that the axial elasticity of a hydrogel tube is comparable to its circumferential elasticity. The axial elasticity of tubes could be measured off-chip with an Instron tensile machine.

4.4.1 Off-Chip Measurement of Axial Tube Elasticity

To measure the tensile properties of alginate tube samples a short, 2-3 cm, segment of tube was mounted into the vertical stretching apparatus, of the Instron machine, by clamping 0.5-1 cm of each end. After zeroing the force balance and position, the tube is controllably stretched and the associated force is tracked. The tube is stretched at a rate of 5 mm/min until the tube fractures. The force and displacement are output and used to generate a stress and strain plot by comparing the original sample length to its extension and dividing the force by the cross-sectional area of the given tube. The slope of this plot is a measure of the material’s elasticity = stress/strain. Figure 17 is a stress vs. strain plot for four different tube samples of different outer diameter and wall thickness. Included in the plot is the ultimate stress each sample was able to endure, which is indicated by a sudden decrease in the stress at a given extension. Fitting a straight line to the data, the slope is equal to the elastic modulus, \( E \), of the tube and they are: Green, 1910 ±70 µm OD, 90 ±8 µm wall, \( E = 82400 \) Pa; Red, 2060 ±40 µm OD, 100 ±4 µm wall, \( E = 109000 \) Pa; Blue, 2000 ±60 µm OD, 150 ±20 µm wall, \( E = 153000 \) Pa; and Black, 2520 ±40 µm OD, 240 ±50 µm wall, \( E = 88100 \) Pa. The sudden change in slope of the green curve is most likely due to the tube drying out and stiffening before the experiment is complete. Comparable elasticity values have been demonstrated in 2% alginate sheets [28].
Figure 17. Tensile properties of extruded tubes evaluated in axial direction (Instron). Initial tube segment lengths varied from 7-15 mm and were stretched until failure, represented by sudden fall in stress value. Straight lines were fit to the data to solve for the elastic modulus. Green, 1910 ±70 µm OD, 90 ±8 µm wall, E = 82400 Pa; Red, 2060 ±40 µm OD, 100 ±4 µm wall, E = 109000 Pa; Blue, 2000 ±60 µm OD, 150 ±20 µm wall, E = 153000 Pa; and Black, 2520 ±40 µm OD, 240 ±50 µm wall, E = 88100 Pa.

4.4.2 Modeling On-Chip Tube Pressurization

The expected change in structure when a tube is pressurized can be estimated for a cylindrical solid and then related to the cross-section of a hosted (confined) tube and expansion in the lateral direction. It is assumed that in the inspection area, where the tube is not confined at its side, the tube assumes a rectangular cross-section, with width \( L_0 \), and circular ends (with diameter equal to the depth of the channel, \( d \)) where it is not confined, as represented in Figure 18b. The elasticity of the hydrogel structure is assumed to be 110 kPa, based on axial tensile measurements of alginate tubes (Figure 17), and the tube itself acts as a thick-walled cylinder. Simple approximations for stresses in thin-walled cylinders cannot be used because the radius of the fabricated tubes is not ten times greater than their wall thickness. With this geometry the
circumferential, or hoop, stress along the radius of the tube wall can be calculated for any transmural pressure difference. Young’s modulus compares the stress to the strain in the walls. The longitudinal stress is negligible in this case because the ends of the tube are open and, therefore, the entirety of the strain, or deformation, translates as a change in the diameter of the tube. The change in diameter of a circular cross section tube can then be equated to a change in the width of the rectangular portion of the tube’s cross. The total change in width when an alginate tube is pressurized at an applied pressure can then be predicted.

Lamé’s equations state that the hoop stress, $\sigma_{\theta}$, varies along r-direction through the wall of a thick-walled cylinder as follows:

$$\sigma_{\theta} = \frac{r_i^2 P_{in} - r_o^2 P_{o}}{r_o^2 - r_i^2} + \frac{(P_{in} - P_{o})r_i^2 r_o^2}{(r_o^2 - r_i^2)r^2}$$  \hspace{1cm} (15)

Where $P_{in}$, $P_{o}$, $r_i$ and $r_o$ are the internal pressure, external pressure, and the initial inner and outer radii, respectively.

The elasticity, $E$, expresses the relationship between stress, $\sigma$, and strain, $\varepsilon$:

$$E = \frac{\sigma}{\varepsilon} = \frac{\sigma_{\theta}}{\Delta r/r}$$  \hspace{1cm} (16)

On chip, the observed change will be in the outer dimension of the tube, so consider only the stress and strain at $r=r_o$, and hence, a change in the outer radius:

$$\therefore \Delta r = \frac{r_o}{E} \left[ \frac{r_i^2 P_{in} - r_o^2 P_{o}}{r_o^2 - r_i^2} + \frac{(P_{in} - P_{o})r_i^2 r_o^2}{(r_o^2 - r_i^2)r^2} \right] = \frac{r_o}{E} \left[ \frac{2r_i^2 P_{in} - P_{o}(r_o^2 + r_i^2)}{r^2 - r_i^2} \right]$$  \hspace{1cm} (17)

And $\Delta r = r_p - r_o$  \hspace{1cm} (18)

Where $r_p$ is the radius of the pressurized tube.

Finally, after solving for $r_p$ the area of a circle and the tube can be equated to solve for the change in the horizontal width, $\Delta w$, and estimate the expansion that will be seen on-chip.

$$\Delta A = \pi r_p^2 - \pi r_o^2 = L_p d + \frac{\pi d^2}{4} - (L_o d + \frac{\pi d^2}{4})$$  \hspace{1cm} (19)
\[
\therefore \Delta L = \frac{\pi (r_p^2 - r_o^2)}{d} \tag{20}
\]

The observed change of the width of a hosted hydrogel tube when pressurized at a given luminal and abluminal pressure will be \(\Delta L\). The width of the tube will have changed from \(L_0+d\) to \(L_0+\Delta L+d\). The relative change in the hosted tube width can be calculated by considering the area of the assumed geometry and the initial width, \(w_0\), which is defined by the initial outer radius of the circular tube.

\[
w_0 = L_0 + d, \tag{21}
\]

and

\[
L_0 = \frac{\pi r_o^2 - \pi d^2}{4d} \tag{22}
\]

\[
\therefore \quad \frac{\Delta w}{w_0} = \frac{\Delta L}{L_0 + d} = \frac{\Delta L}{\left[ \frac{\pi \left( r_o^2 - \frac{d^2}{4} \right)}{d} \right] + d} \tag{23}
\]

Figure 18c shows a plot of the percentage width change of a hosted tube as the internal pressure is increased. Also shown is the effect of varying tube outer diameter and wall thickness. Strains are only plotted for up to 20 kPa transmural pressure because beyond this the largest tubes are approaching failure (based on axial stress-strain plot). Furthermore, this already allows the expected dimension change to be compared to artery expansion at physiological pressures: 8 kPa (60 mmHg) and 16 kPa (120 mmHg).
Figure 18. Modeling width of pressurized tubular structures. (a) Top-view schematic of hydrogel tube hosted on-chip with initial width $w_0$. When internal pressure, $P_{in}$, is increased relative to the outer pressure, $P_{out}$, the tube width increases to $w_p$. Not to scale. (b) Cross-sectional view of chip-hosted tube located between the PDMS device and reversibly sealed coverslip. Assumed cross-sectional geometry of pressurized tube: rectangle of length $L_0$ or $L_p$ with circular ends of diameter $d$ (channel depth). When pressurized the tube undergoes a change $\Delta L$, but the round ends do not change curvature. Not to scale. (c) Percentage change in width of chip hosted tube, $E = 110$ kPa, of $D_O$ versus increasing transmural pressure. $P_{out} = 0$ (gauge) for all curves. $\delta = 100$ µm (solid line); $\delta = 200$ µm (dotted line). Purple: $D_O = 1000$ µm (purple); 900 µm (green); 800 µm (blue); 700 µm (red).
4.5 Characterization of Fixation Seal

To establish a separation of the perfusion and fixation channels and secure the tube axially along the channel, the elastic tube must be deformed to conform to the perimeter of the microfluidic channel. The pressures in the perfusion and fixation lines can control the pressures inside and outside of the tube, respectively. It is this transmural pressure that will cause the diameter of the tube to expand locally at the fixation features; a positive perfusion pressure on the lumen and a negative fixation pressure on the abluminal side. By design, when the fixation is applied to the tube walls the walls should deflect slightly into the forked fixation feature, sealing flow from the perfusion into the fixation. Figure 19a depicts the deflection of a tube, on both sides, when submitted to a fixation pressure, $P_{fix}$, and perfusion pressure, $P_{in}$. Furthermore, for a tube of outer radius $r_o$ to expand to fill the perimeter of the perfusion channel a minimum transmural pressure must be applied, dependent on the elasticity of the tube. Figure 19c is a plot of the transmural pressure necessary for tubes of radius $r_o$ to conform to the peripheries of the perfusion channel for the 900 µm (Red) and 1200 µm (Green) devices. The elasticity is estimated to be 110 kPa taken from axial measurements of 2% alginate tubes (Figure 17) and can be related to the tube compliance to determine the cross-sectional area change when pressurized. The tube size for each device is limited by the size of the perfusion channel – if a tube’s circumference is greater than the channel perimeter the tube will wrinkle and possibly collapse in order to fit – and the maximum strain the tubes can sustain before breaking (Figure 17).
Figure 19. Characterizing the fixation seal. (a) Schematic of a tube of diameter $D_O$ hosted in a channel with a fixation pressure, $P_{fix}$, and an internal pressure, $P_{in}$, applied to it and a perfusion flow rate $Q$. This transmural pressure causes the walls of the tube to expand to the edges of the channel and deflect into the fixation feature. (b) Brightfield image of a chip-hosted tube whose wall is deflecting into the fixation channel when a negative pressure is applied. Scale bar: 200 µm. (c) Plot of the transmural pressure necessary to deform a tube of a given outer radius to the peripheries of the perfusion channel. Red: 900 µm channel device; Green: 1200 µm channel device.

The transmural pressures plotted in Figure 19c sets a threshold value for hydrogel tubes hosted on the perfusion device. To properly seal the perfusion fluid from the fixation lines this
minimum pressure difference must be applied. This limits the range over which a tube can be effectively deformed without leakage.

### 4.6 Measurement of Hydrogel Tube Elasticity

The two-layer microfluidic device, previously described, for the on-chip hosting and perfusion of hydrogel tubular constructs can also be used to measure the circumferential elasticity of the mounted tube. The change in cross-sectional area of a hosted tube when subjected to a known transmural pressure can be related to the compliance and, therefore, the elasticity of the material. The strain being in the circumference of the tube as it expands outwards gives a measure of the circumferential elasticity that, compared to the axial elasticity, cannot be easily quantified off-chip.

When a tube is pressurized, by generating a pressure difference between the perfusion and superfusion lines, the tube will expand, or contract, in the 2mm inspection area (where the wall are not confined on all sides), see Figure 18a. With optical access to the inspection zone the width of the tube, as seen from above, can be measured before and after a pressure change. This dimension is not the diameter of the tube because the height of the hosting channel is smaller than the original outer diameter. The tube is confined at the top and bottom and must, instead, deform laterally to conform to the channel geometry. Assuming the same geometry as in Figure 18b suggests that any change in the size of the tube is strictly in the horizontal direction and it is assumed that the curvature of the circular sides of the geometry does not change. To model the expansion of the confined tube it is assumed that there is no friction in between the tube walls and the top and bottom of the channel (coverslip and PDMS surfaces, respectively). Hence, the change in the observed width is created by a change in the width of the rectangular portion of the cross-section. The initial width, \( w_0 = L_0 + d \), and the final width, \( w_p = L_p + d \), are experimentally measured on-chip and used to calculate a change in cross-sectional area of the tube for a given change in pressure; where the \( p \) subscript describes the pressurized state. This in turn can be related to the compliance of the alginate tube. Because the induced dimensional change is in the circumference of the tube, the compliance is inversely proportional to the circumferential elasticity [79].
\[ C = \frac{\Delta A}{A_0 \Delta P} = \frac{1}{E} \]  

(24)

Where \( A \) is the cross sectional area of the tube, \( E \) is the elasticity of the material and \( \Delta P \) is the transmural pressure.

\[ A_0 = L_0 d + \frac{\pi d^2}{4}, \]

(25)

\[ \therefore \frac{\Delta A}{A_0} = \frac{L_p d + \frac{\pi d^2}{4} - (L_0 d + \frac{\pi d^2}{4})}{L_0 d + \frac{\pi d^2}{4}} = \frac{(L_p - L_0)d}{L_0 d + \frac{\pi d^2}{4}} \]

(26)

Therefore, with equations (24) and (26):

\[ E = \frac{(L_0 + \frac{\pi d}{4}) \Delta P}{L_p - L_0} \]

(27)

Where \( L_p = w_p - d \), \( L_0 = w_0 - d \), and \( w_p \) and \( w_0 \) are measured experimentally.

The circumferential elasticity calculated on-chip, by measuring the width of the tube before and after a pressure change, can be compared to the off-chip axial measurement of elasticity done with an Instron machine.

The circumferential elasticity is an important tensile property for tubular structures that is not easily measured, particularly for soft-material tubes. This platform will provide a unique way of in-situ tracking of the material properties and how they degrade over the course of an experiment. This can be extended to compare the properties of heterogeneous tubes and alternate material compositions (e.g. alginate and collagen tubes shown here). Demonstrating the periodic stretching of a cell-compatible material tube – by controlled fluctuations of the transmural pressure – would allow physiologically relevant strain rates to be generated in the tube wall. This would allow the controlled stretching of an elastic structure that is amenable to 3D cell culture.
4.7 Perfusion of Chip-hosted Tubular Structures

When a tube segment was loaded into the hosting device and fixed in place with a fixation pressure and reversibly sealed coverslip it was controllably perfused with a syringe pump. A transmural pressure was established between the perfusion and fixation channels to expand the tube cross-section to the channel dimensions and preventing the perfusate from flowing directly into the fixation channels. Descriptions of preliminary experimentation are given below.

For most tests, a large amount of fluid was drawn into the fixation channels, as leakage flow, taking away from most or all of the liquid that should have flowed through the tube to the perfusion outlet. It appears as if, despite there being some deflection of the tube wall into the fixation channels, the tube is not deformed enough to isolate the perfusate from the fixation channels. Considering the geometry of the tube and the channel it is most probable that the corners of the rectangular channel are open and allow flow around the tube. These open “channels” along with the negative pressure present at the fixation channels draw a significant amount of the input flow.

A successful perfusion seal was obtained occasionally, but was difficult to reproduce. An 800 µm outer diameter tube section that was 6 mm long was loaded into the 900 µm device and a negative pressure head of -5.4 kPa was established at the fixation inlet. A positive pressure of 1.2 kPa was connected to the perfusion inlet and allowed to flow through the tube, see Figure 20a-b. The drain from the perfusion outlet was collected and weighed, and the movement of a meniscus in the fixation line was tracked. After approximately ten minutes, the volume of water measured at each outlet was compared and of the total volume 4.9% was lost to the fixation line (leakage). Small amounts of dye were seen to pass into the superfusion area where the tube sides are not confined, proving that flow is travelling around the tube and not just through it. Perfusion was also demonstrated using a syringe pump. A collagen tube, extruded with the same tube formation device with a polyethylene glycol (PEG) crosslinking solution and an extended confinement tube, was hosted in the 1200 µm wide device (Figure 20c-d) and fixed with -5.4 kPa and perfused at 40 µL/min. After 8 minutes 0.315 g of water, 315 µL, was collected at the perfusion outlet, 98.4% of the volume supplied at the inlet.
Figure 20. On-chip perfusion of soft material tubes. (a) 800 µm OD alginate tube, chip-hosted and perfused with a green dye. Superfusion lines filled with red dye and the superfusion outlets are capped. Leakage is evident by the colored liquids being drawn into the fixation channels. Scale bar: 5 mm. (b) 1X magnification photograph of an 800 µm OD alginate tube being perfused. The walls of the tube are deflecting slightly into the fixation channels. Scale bar: 0.5 mm. (c) A collagen tube being perfused with a blue dye. The tube was slightly too large for the channel and is partially collapse leading to leakage into the superfusion and fixation channels. Scale bar: 5 mm. (d) Collagen tube perfused and pressurized with a 12 cm water column imaged at 1X. Scale bar: 1 mm.

Following the characterization of the fixation seal, it was estimated, in Figure 19c, that an 800 µm OD tube must be exposed to a 8.2 kPa transmural pressure (or the equivalent of an 84 cm...
water column) to force it to conform to the shape of the perfusion channel. The pressure difference between the fixation and perfusion channels in the above test was only 6.6 kPa and leakage was observed. The tube is not able to fill the channel and there is a significant flow rate around the tube, further accentuated by the presence of a negative pressure, which instead of stretching the tube is drawing large amounts of flow. To improve on this preliminary data, a larger perfusion pressure should assist in increasing the flow rate through the tube and, in the future, a syringe pump should be used in combination with a reservoir, forcing flow against the defined hydrostatic pressure head, as illustrated in Figure 12. Figure 20a-b are promising evidence that perfusion and superfusion of alginate tubes is possible. A slightly larger tube that is required to deform less, and therefore requires a lower transmural pressure, should improve the tube’s seal. It is expected that by leaving the superfusion at atmospheric and pressurizing the perfusion with a fixed flow rate of approximately 50 µL/min there should be little to no leakage into the superfusion area. Additionally, the length of the hosted tube segment should be maximized (approximately 7 mm) to increase the resistance, and decrease, through any corner gaps between the tube and the channel wall.

Experiments were run with the reversibly sealed device inverted on a stereomicroscope where close inspection of the flow could be done. Once reliable perfusion/superfusion has been established an aqueous solution of 1 μm fluorescent latex beads (5% v/v) can be fed into the perfusion inlet and their fluorescent emission imaged by particle image velocimetry (PIV). A PIV system incorporated into the stereomicroscope uses a pulsed 532 nm Nd-YAG laser (SoloPIV III, NewWave Research, USA) to send a laser pulse at the flow. The emission is captured in two frames in rapid succession (dt), by a pco 1200hs high speed CCD camera (pcoImaging, Germany). The displacement of particles between the two frames over time dt can be correlated with DaVis 7.2 software (LaVision, Germany) to calculate the flow velocity. PIV measurements taken at the perfusion outlet or in a fixation channel would allow one to compare the input flow rate to the flow rate beyond the tube or measure the leakage flow rate, respectively.
5 Summary

Soft material tubular structures are critical in mammalian architecture and the ability to synthetically produce replacements that accurately resemble their architecture, composition and physical characteristics is limited. To this end a platform was developed for the one step formation of hydrogel tubes. A three layer microfluidic device, fabricated in PDMS and featuring a specific radial organization of channels around a common outlet, vertically extrudes intact hydrogel tubes whose dimensions can be predictably controlled. The outlet channel configuration arranges the three fluids as cylindrical flow with inner and outer crosslinker flows forming a sheath around the biopolymer fluid, which undergoes a sol-gel transition when in the presence of a crosslinker. Sodium alginate rapidly gels by ionic cross-linking with calcium ions and the resulting tubes can immediately be handled. The extrusion process is almost entirely automated and is continuous so long as solutions are provided. Tubular formation rate is 4-5 mm/s and the inner and outer diameters can be tailored by configuring the input flow rates. An analytical model to predict the dimensions for given crosslinker and alginate matrix flow rates was shown to strongly agree with experimental results for tubes extruded between 600 and 3000 µm outer diameter and 80 to 400 µm wall thickness. Inner diameters varied from 2.7 mm to 500 µm, a range comparable to the dimensions of human arteries. Simple modifications to the device design allowed multilayer tubes to be formed with equally controllable dimensions. The developed platform is compatible with other materials and is suited for the extrusion of cell-laden soft material tubes. A single device is capable of controlled and rapid extrusion of a very wide range of homogeneous and heterogeneous tube sizes.

A second microfluidic device was designed to perfuse and measure the circumferential elasticity of the extruded alginate tube. This two layer device uses a reversible vacuum seal to hold a 5-7 mm tube segment and controllably pressurize it. The reversible seal allows a device to be reused for numerous tube samples and makes loading very easy. The vacuum seals the open PDMS channels to a plastic coverslip, preventing any leakage beyond the channel walls. The tube is held in place and open for perfusion by four forked fixation channels, two at each end of the tube, set to a negative pressure with a hydrostatic head. The reversible and fixation seals are each characterized in terms of operational transmural pressures and hosted tube size. Pressurizing an elastic tube, confined to the perfusion channel of the device, causes a strain and deformation in the tube wall. Based on the channel geometry and the effective transmural pressure the expected
change in on-chip tube width was modeled for an elastic modulus that was measured in the axial direction off-chip. Tubes pressurized to physiologically relevant pressures will deform and this deformation can be used to interpret the compliance of the tubular alginate structure. The compliance is inversely proportional to the circumferential elasticity of the tube, which can be compared to axial measurements. When a tube is pressurized, syringe pumps can be used to perfuse or superfuse them at a defined flow rate. Experimentally, there was significant leakage past the tube end and into the fixation lines, from the perfusion inlet and so proper validation was not possible. This was most likely attributed to the transmural pressure, and specifically the internal pressure, not being large enough for the tube’s cross-section to conform to the channel’s dimensions. Alginate and collagen tube sections were shown to be hosted and perfused with minimal leakage, but the experimental setup and procedure was not well enough established for a consistent and reliable seal or perfusion. The feasibility of on-chip perfusion and pressurization towards an in-situ measurement of the circumferential elastic modulus was shown and modeled, but further work must be carried out to generate statistical data for comparison to microfluidic cell culture platforms and known hydrogel material properties.

The application of these two devices towards on-chip perfusion cell culture of cell-laden or cell-lined soft material tubes is an exciting prospect. It is expected that multi-material, multilayered tube compositions can be extruded over a wide range of dimensions (multiple millimeters to hundreds of microns) and that these tubes can be chip hosted and perfused. Furthermore, the tensile properties of tube compositions and sizes can be compared with in-situ measurement of the circumferential elasticity and material degradation.
References


27. McAllister, A., *Continuous Extrusion of Homogeneous and Heterogeneous Hydrogel Tubes*, in Institute of Biomaterials and Biomedical Engineering. 2014, University of Toronto.


Appendices

Appendix A1 - Tube Extrusion in Water

Figure A1. Dimensions of tubes extruded with water based solutions. Plot of the outer (blue) and inner (red) diameters, and the wall thickness (green) of tubes extruded in the absence of any glycerol. When the crosslinker, matrix and reservoir solutions are all density matched by mixing with water only, the only parameters affecting the extrusion velocity are the input flow rates. Data collected in collaboration with Haotian Chen.
Appendix A2 - Experimental Setup for Tube Extrusion

**Figure A2. Photograph of tube extrusion setup.** Key elements are labeled: 1) neMESYS pump for inner and outer crosslinker flows; 2) in-line light source; 3) reservoir containing crosslinker solution; 4) microfluidic device (location indicated by black outline) with confinement piece; 5) side-view camera for imaging extrusion at outlet of confinement piece; 6) Harvard Instruments pump for matrix flow; and 7) top-view camera mounted to stereomicroscope.
Appendix A3 – Tube Collection by Pulling

**Figure A3. Hydrogel tube being pulled and collected on a rotating drum.** Tubes collected in this fashion collapsed as they were rolled onto the collection drum. Experiment conducted with Haotian Chen.
Appendix A4 – Ten Tube Extrusion Device

A design was developed for the simultaneous extrusion of ten hydrogel tubes, as seen below. However, it is significantly more difficult to fabricate, compared to single tube designs, due to the number of features that have to be aligned over a large area. The device is designed to fit a 3x4” footprint. Micro-milling or injection molding techniques with stiff substrates and more accurate alignment capabilities would be a more reliable approach.

Figure A4. Channel layout for the simultaneous extrusion of ten homogeneous hydrogel tubes. Green: outer fluid; Blue: matrix fluid; Orange: inner fluid.
Appendix A5 – Fabrication Protocols

Mask writing:

1. Mask design is made in AutoCAD (AutoDesk, California, USA)

2. Transparency masks printed at 23400 DPI and delivered by CAD/Art Services (OR, USA)

Master fabrication – 150 µm deep features:

1. A 3x4” glass slide (Ted Pella, California, USA) is rinsed with isopropyl alcohol (IPA) and acetone, and then dehydrated for 30 minutes on a hot plate (model HP30A, Torrey Pines Scientific, CA, USA) at 200°C.

2. Oxygen plasma treat (model PDC-32G, Harrick Plasma, NY, USA) the slide for 1 minute.

3. Spin a thin (25 µm) seed layer of negative resist, SU-8 25 (MicroChem, MA, USA) with SU-8 spin-coater (model SCS G3 spin coater, Specialty Coating Systems, IN, USA).
   a. Recipe: speed/dwell/ramp; Step 1: 500/10/5, Step 2: 2000/30/5.

4. Bake at 65°C/95°C for 4/6 minutes.

5. UV expose seed layer for 13 seconds (Mask Aligner Model 200, OAI, CA, USA).

6. Bake at 65°C/95°C for 4/6 minutes.

7. Spin 75 µm layer of SU-8 2050 (MicroChem, Massachusetts, USA)
   a. Recipe: Step 1: 500/5/5, Step 2: 1900/30/5.

8. Bake at 65°C/95°C for 5/20 minutes.

9. Repeat step 7.

10. Bake at 65°C/95°C for 15/50 minutes.
11. Align mask on cured SU-8 slide and expose at 250 mJ/cm$^2$ with UV source.

12. Post exposure bake for 15 minutes on 95°C hotplate.

13. Develop the resist in a bath of SU-8 Developer (MicroChem, MA, USA) for ten minutes.

14. Rinse with Developer and IPA.

15. Place in 80°C oven for 60 minutes.

Multilayer device fabrication:

A tube extrusion device contains 4 layers of PDMS; from the top down they are the inner flow, matrix flow, and outer flow and bottom layers. A combination of casting and spin-coating is used to control layer thickness and separation.

1. Prepare a 10:1 (bulk:curing agent) mixture of PDMS (Ellsworth, WI, USA)

2. In an empty dish, cast a thin (0.5-1 mm) layer of PDMS. This is the bottom layer.

3. Place the inner flow master in a dish and cast it with approximately 2 mm of PDMS.

4. Place approximately 5 mL of PDMS on each of the matrix and outer flow masters and spin-coat each one.
   a. Recipe: Step 1: 200/5/5, Step 2: 400/30/5.

5. Degas all four layers and any remaining PDMS for 90 minutes.

6. Move all layers to an 80°C oven:
   a. Inner flow layer is fully cured; 25 minutes.
   b. Matrix layer is partially cured so it is still slightly sticky but does not pull away from the surface; 10-13 minutes (checking every minute after the first 10).
   c. Outer flow layer is partially cured; 10-13 minutes.
   d. Bottom layer is partially cured; 10-13 minutes.
7. Carefully peel the inner flow layer away from its master, align the central features with those of the matrix layer (still on its master) under a stereomicroscope and press the two layers together. Push any trapped bubbles out with the back of a scalpel.

8. Coat the edges, where the layers meet with a small amount of PDMS.

9. Fully cure the two layers, 15-20 minutes.

10. Peel the two layer device away from the matrix layer master and repeat steps 7-9, but with bonding to the outer layer now.

11. Peel the now three layer device away from the outer layer master.

12. Using a 5/64” manual punch, make inlet holes for the inner, matrix and outer layers.

13. Press the layers onto the partially cured (empty) bottom layer and bake for 15-20 minutes.

14. Cut the fully sealed device out of the dish and carefully punch the central outlet hole through the bottom of the device. Use a 1/8” punch and punch through all layers.

15. Cover the bottom of the device in adhesive tape so that nothing may enter the extrusion outlet from the bottom of the device.

16. Cut a small square of PDMS from the remaining bottom layer material and use it to cover the outlet hole on the top of the device. Seal the edges with PDMS.

17. Cut three 3 cm long pieces of 1/16” OD PEEK tubing (UpChurch Scientific, Washington, USA) and insert them into the inlet holes.

18. Fix the inlet tubing in place with a quick setting epoxy. Wait for the epoxy to dry.

19. Place the device in a dish and cover with PDMS.

20. Bake overnight in 80°C.
Appendix A6 – Modeling Tube Dimensions in Absence of Crosslinking (Liquid Model)

A second model was considered wherein the flow rate and tube dimensions are estimated before the hydrogel is crosslinked; all fluids are considered liquid throughout [16]. For this model, the following assumptions are made:

- All solutions are considered as liquids (prior to crosslinking of biopolymer),
- Viscosity of each solution is constant in axial and radial directions,
- \( P(r) \) is constant and \( dP/dz \) is the same for each solution,
- Solutions are density matched.

Beginning with the Navier-Stokes equation:

\[
\frac{\partial P}{\partial z} = \mu \left[ \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial V_z}{\partial r} \right) \right] + f(z) + g(r)
\]  

(28)

Assuming \( f(z) \) and \( g(r) \) are constants and ignored.

General solution for inner, outer and matrix velocity:

\[
V_{i,m,o} = \frac{1}{4\mu_{i,m,o}} Ar^2 + B_{i,m,o} \ln r + C_{i,m,o}
\]  

(29)

\[
\frac{dV_{i,m,o}}{dr} = \frac{A}{2\mu_{i,m,o}} r + \frac{B_{i,m,o}}{r}
\]  

(30)

Where \( A = dP/dz \) and is equal for inner, matrix and outer flow throughout the radius of the confinement tube.

Apply the following boundary conditions for annular flow in a pipe:

- \( r=0 \)  \quad \text{dVi/dr} = 0
- \( r=r_i \)  \quad V_i = V_m;  \quad \mu_i, dV_i/dr = \mu_m, dV_m/dr
- \( r=r_o \)  \quad V_m = V_o;  \quad \mu_m, dV_m/dr = \mu_o, dV_o/dr
- \( r=r_c \)  \quad V_o = 0
Where \( r_i, r_o \) and \( r_c \) are the inner and outer radii of the matrix flow and the radius of the confinement tube, respectively.

Solving for B’s and C’s:

\[
B_{i,m,o} = 0;
\]

\[
C_i = \frac{A}{4}[\frac{r_i^2}{\mu_m} + \frac{r_o^2}{\mu_o} - \frac{r_c^2}{\mu_o} - \frac{r_i^2}{\mu_i}] ;
\]

\[
C_m = \frac{A}{4}[\frac{r_o^2}{\mu_o} - \frac{r_c^2}{\mu_o} - \frac{r_o^2}{\mu_m}] ;
\]

\[
C_o = -\frac{A}{4\mu_o}r_c^2 .
\]

Subbing these coefficients into \( V_{i,m,o} \) and solving \( Q_{i,m,o} = \int V_{i,m,o}(r) \cdot 2\pi r \, dr \) yields equations for the three flow rates:

\[
Q_i = \frac{\pi}{4} \frac{dP}{dz} \left[ -\frac{r_i^4}{2\mu_i} + \frac{r_i^2(r_i^2 - r_o^2)}{\mu_m} - \frac{r_i^2(r_c^2 - r_o^2)}{\mu_o} \right] ;
\]  

\[
Q_m = \frac{\pi}{4} \frac{dP}{dz} \left[ \frac{r_o^4 - r_i^4}{2\mu_m} + \frac{(r_o^2 - r_i^2)(r_c^2 - r_o^2)}{\mu_o} - \frac{r_o^2(r_o^2 - r_i^2)}{\mu_m} \right] ;
\]  

\[
Q_o = -\frac{\pi}{8\mu_o} \frac{dP}{dz}(r_c^2 - r_o^2)^2 ;
\]

These can be simplified into two equations that can be used to solve for \( r_i \) and \( r_o \) for a given set of flow conditions \( (Q_o, Q_i, Q_m) \):

\[
\frac{Q_i}{Q_o} \cdot \frac{(r_c^2 - r_o^2)^2}{2\mu_o} - \frac{r_i^4}{2\mu_i} + \frac{r_i^2(r_i^2 - r_o^2)}{\mu_m} - \frac{r_i^2(r_c^2 - r_o^2)}{\mu_o} = 0 ;
\]

\[
\frac{Q_m}{Q_o} \cdot \frac{(r_c^2 - r_o^2)^2}{2\mu_o} - \frac{(r_i^2 - r_o^2)^2}{2\mu_m} - \frac{(r_o^2 - r_i^2)(r_c^2 - r_o^2)}{\mu_o} = 0 ;
\]

\( \mu_{i,m,o} \) is the viscosity of each of the fluids; calculated in lab with a rheometer: \( \mu_{i,o} = 0.004 \) Pa s, and \( \mu_m = 0.647 \) Pa s at a shear rate of 10.1 s\(^{-1}\) (at approximately 4 mm/s extrusion velocity).
Solving these two quadratic equations for the same combinations of flow rates that were used experimentally, the inner and outer radii of the extruded tube can be estimated. Figure A5 is a plot of the analytical results using this non-crosslinking model, compared to the experimental results collected from confocal images of tube segments. There are significant differences between the experimental and theoretical results that are most likely attributed to the assumptions that had to be made when modeling this fluidic system. A strong effect is due to the assumption that there is no crosslinking occurring, while from experimentation it is obvious that it occurs and that it occurs quickly. Furthermore, the viscosities of each fluid should not be considered to be constant throughout the system. Large differences are indicative of the rapid crosslinking that takes place when the inner or outer crosslinker flow comes into contact with the hydrogel. Their distribution within the extrusion hole is not yet how the model would predict, but due to the ionic polymerization that quickly gels the biopolymer at its inner and outer surfaces the diameters are fixed larger (at low inner flow rates) or smaller (at high inner flow rates) than (theoretically) expected. The under estimation of the model becomes less and less significant as the inner flow rate is increased and the outer flow rate is decreased.
Figure A5. Model results for non-crosslinked case. Experimental (markers) and theoretical (solid lines) results for the dimensions of a 2% alginate tubes printed at constant $Q_m = 200 \mu\text{L/min}$, as $Q_i$ and $Q_o$ are varied. The total flow rate is kept at a constant 1500 µL/min. The inner (red) and outer (blue) diameters of a hydrogel tube are modeled under the assumption of concentric, annular flow in the absence of any crosslinking.
Appendix A7 – Rheometer Data

Figure A6. Viscosity measurements of matrix and crosslinker solutions. Samples of 2% alginate with glycerol and 100 mmol CaCl₂ with glycerol were tested with a plate and plate, rotational rheometer. Viscosity measurements were recorded as the shear rate was increased from 0 to 200 s⁻¹. The viscosity of the inner and outer fluid is the same as they use the same solution.
Appendix A8 – Model Conditions and Equations

The following is a detailed description of the assumptions, conditions and equations used to model the extrusion of an alginate tube when the hydrogel is assumed to gel instantly.

Assumptions:

- Walls solidify at interfaces $r=r_i$ and $r=r_o$ (inner and outer radii of tube/biopolymer flow) almost instantly so flow geometry is defined at outlet and is maintained.
  - Matrix fluid acts as rigid body with constant velocity.
- There is no pressure gradient difference between the inner and outer fluids.
- The pressure acting within the matrix fluid is equal to the mean of the inner and outer fluids’ pressure gradients.
- During extrusion there are zero net forces acting on the hydrogel tube.

Boundary Conditions:

$$r = 0 \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \frac{dV_i}{dr} = 0 \hspace{1cm} \text{(36)}$$

$$r = r_i \hspace{1cm} V_i = V_m \hspace{1cm} \text{(37)}$$

$$r = r_o \hspace{1cm} V_o = V_m \hspace{1cm} \text{(38)}$$

$$r = r_c \hspace{1cm} V_o = 0 \hspace{1cm} \text{(39)}$$

$$G_i = G_o \hspace{1cm} \text{(40)}$$

$$G_m = \frac{G_i + G_o}{2} \hspace{1cm} \text{(41)}$$

**Inner Flow Rate**

Beginning with a general solution to the Navier-Stokes equation:

$$V_i(r) = -\frac{G_i}{4\mu_i}r^2 + B_i\ln(r) + C_i \hspace{1cm} \text{(42)}$$

$$\frac{dV_i(r)}{dr} = -\frac{G_i}{2\mu_o}r + \frac{B_i}{r} \hspace{1cm} \text{(43)}$$

With Eq. (36):
With Eq. (37):

\[- \frac{G_i}{2\mu_i} (0) + \frac{B_i}{0} = 0 \Rightarrow B_i = 0\]  

(44)

With Eq. (37):

\[- \frac{G_i}{4\mu_i} r_i^2 + C_i = V_m \Rightarrow C_i = V_m + \frac{G_i}{4\mu_i} r_i^2\]  

(45)

\[\therefore V_i(r) = V_m + \frac{G_i}{4\mu_i} (r_i^2 - r^2)\]  

(46)

Solve for the flow rate using Eq. (7):

\[Q_i = 2\pi \int_0^{r_i} V_m r + \frac{G_i}{4\mu_i} (r_i^2 - r^2) r \, dr\]  

(47)

\[Q_i = \pi r_i^2 V_m + \frac{G_i \pi r_i^4}{8\mu_i}\]  

(48)

**Matrix Flow Rate**

Acts as rigid structure with constant velocity, \(V_m\).

\[Q_m = 2\pi \int_{r_i}^{r_o} V_m r \, dr = \pi (r_o^2 - r_i^2) V_m\]  

(49)

**Outer Flow Rate**

Using the same solution forms as for the inner fluid:

\[V_o(r) = - \frac{G_o}{4\mu_o} r^2 + B_o \ln(r) + C_o\]  

(50)

With Eq. (38):

\[- \frac{G_o}{4\mu_o} r_o^2 + B_o \ln(r_o) + C_o = V_m\]  

(51)

With Eq. (39):
\[-\frac{G_o}{4\mu_o} r_c^2 + B_o \ln(r_c) + C_o = 0 \quad (52)\]

Subtract Eq. (52) from Eq. (51):

\[\frac{G_o}{4\mu_o} (r_c^2 - r_o^2) + B_o \ln\left(\frac{r_o}{r_c}\right) = V_m \quad (53)\]

\[B_o = \frac{V_m - \frac{G_o}{4\mu_o} (r_c^2 - r_o^2)}{\ln\left(\frac{r_o}{r_c}\right)} \quad (54)\]

Eq. (54) in Eq. (52):

\[-\frac{G_o}{4\mu_o} r_c^2 + \frac{V_m - \frac{G_o}{4\mu_o} (r_c^2 - r_o^2)}{\ln\left(\frac{r_o}{r_c}\right)} \ln(r_c) + C_o = 0 \quad (55)\]

\[C_o = \frac{G_o}{4\mu_o} r_c^2 - \frac{V_m - \frac{G_o}{4\mu_o} (r_c^2 - r_o^2)}{\ln\left(\frac{r_o}{r_c}\right)} \ln(r_c) \quad (56)\]

\[B_o \text{ and } C_o \text{ in Eq. (50)}:\]

\[V_o(r) = \frac{G_o}{4\mu_o} (r_c^2 - r^2) + \frac{4\mu_o V_m - G_o (r_c^2 - r_o^2)}{4\mu_o} \frac{\ln(r/r_c)}{\ln\left(\frac{r_o}{r_c}\right)} \quad (57)\]

Eq. (57) in Eq. (7):

\[Q_o = 2\pi \int_{r_o}^{r_c} \left[\frac{G_o}{4\mu_o} (r_c^2 - r^2) + \frac{4\mu_o V_m - G_o (r_c^2 - r_o^2)}{4\mu_o} \frac{\ln(r/r_c)}{\ln\left(\frac{r_o}{r_c}\right)}\right] r \, dr \quad (58)\]

\[\therefore Q_o = \frac{G_o \pi}{8\mu_o} (r_c^2 - r_o^2)^2 + \frac{\pi [4\mu_o V_m - G_o (r_c^2 - r_o^2)]}{2\mu_o \ln\left(\frac{r_o}{r_c}\right)} \left[\frac{r_o^2 - r_c^2}{4} - \frac{r_o^2}{2} \ln\left(\frac{r_o}{r_c}\right)\right] \quad (59)\]

**Force Balance on Tube Wall**

Shear forces balance normal force from solid walls:
Simplifies to the following when the derivative of Eq. (46) and (57) are evaluated at \( r_i \) and \( r_o \), respectively:

\[
-\frac{G_i r_i^2}{2} + G_o r_o^2 - \frac{4\mu_o V_m - G_o (r_c^2 - r_o^2)}{2 \ln\left(\frac{r_o}{r_c}\right)} = \left(\frac{r_o^2 - r_i^2}{2}\right) \frac{G_i + G_o}{2} \tag{61}
\]

With Eq. (40) this becomes:

\[
G = \frac{4\mu_o V_m}{r_c^2 - r_o^2} \tag{62}
\]

Substituting Eq. (62) into Eq. (48) and (59) gives:

\[
Q_i = \pi r_i^2 V_m \left(1 + \frac{\mu_o r_i^2}{2\mu_i (r_c^2 - r_o^2)}\right) \tag{63}
\]

\[
Q_o = \frac{\pi (r_c^2 - r_o^2) V_m}{2} \tag{64}
\]

The system of equations (49), (63) and (64) can be solved for a given set of flow rates to calculate the inner and outer radii and the extrusion velocity.
Figure A7. Scatter plot of the raw data collected from tube extrusion experiments. Supplementary to Figure 8e, here the raw data is plotted for the inner (red) and outer (blue) diameters, and wall thickness (green) of hydrogel tubes, printed from three different devices, as $Q_i$ and $Q_o$ were varied. $Q_m = 200\mu$L/min and $Q_{Total} = 1500\mu$L/min. Each data point represents triplicate measurements along the length of a tube segment collected with a single device. Circles: Device 1; Squares: Device 2; Triangles: Device 3. (n=3). Data collected in collaboration with Haotian Chen.
Appendix A10 – Residual Plot of Measured and Theoretical Tube Dimensions

Figure A8. Residual differences between experimental and theoretical tube dimensions. Residual calculated for Figure 8e, in Section 3.4.2, dimensions (outer and inner diameter, and wall thickness) of homogeneous alginate tubular structures. The residual is the difference between the experimental dimension and the predicted value, as calculated by an analytical model of the tube extrusion system. Blue: $D_o$; Red: $D_i$; Green: $δ$. The downward sloping trend of the outer diameter is explained in the main body and is an effect of the rapid solidification of the hydrogel as it comes into contact with the crosslinking ions.
Figure A9. **Inner tube diameter predicted by experimental results.** The experimental values of $D_o$ are used with Eq. (8)-(11) to predict $D_i$ without assuming the pressure gradient in the inner and outer fluids is equal throughout extrusion. Experimental: $D_o$ (Blue squares), $D_i$ (Red circles). Predicted: $D_i$ (Red line). $n=3$. Error bars on inner diameter represent one standard deviation. Experimental data collected with Haotian Chen.
Figure A10. Tube deflection into fixation channels. Plot of wall deflection against the width of the fixation channels. Four lines are plotted for different fixation pressures ranging from a 40cm water column to that generated by a small diaphragm vacuum pump: Red 65 kPa, Blue 19.6 kPa, Green 9.8 kPa and Pink 4.9 kPa. The final fixation width was chosen based on a maximum deflection of one and a half times the wall thickness. The thinnest tubes tested have a wall thickness of 100 µm and hence, a fixation channel width of 200 µm was chosen (marked by dashed black line).
Appendix A13 – Profilometry Scans of Milled Parts

Figure A11. Surface roughness of MIE machined shop molds. (a) Surface roughness scan on the bottom surface of the MIE milled mold, on the wall between the superfusion and fixation lines. Top: a topographical scan of the surface that will be reversibly bonded to once fabricated in PDMS. Bottom: X and Y profiles of changes in the surface height. (b) 3D scan of the wall’s surface in between the two superfusion channels.
Figure A12. Profilometry, depth scan of tube hosting device master. (a) Partial, stitched scan of the depth of the tube hosting device master, with tilt adjustment. Features are approximately 625 μm ±5 μm. The black markings represent regions where the incident light was reflected through a large angle and a depth measurement could not be taken. (b) 3D representation of the same device scan.