

COMBINED SFF PATTERNING AND REPLICA MOLDING FOR MICROFABRICATION OF CELL-LADEN MICROFLUIDIC DEVICE

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Jessica Snyder¹, Chengyang Wang¹, Qudus Hamid¹, Wei Sun^{1,2}

¹Drexel University, PA 19104

²Tsinghua University, Beijing China 100084

1. Abstract

We report on a novel technique using additive manufacturing to fabricate a cell-laden polydimethylsiloxane (PDMS) microfluidic device by SFF processes and replica molding. We demonstrate concept feasibility and present results using single and multiple layer patterns. 3-dimensional channel architecture is achieved by CAD/CAM technology and tuning manufacturing process parameters. Our microfluidic device is fabricated in two stages (1) print negative mold by thermal extrusion of polycaprolactone (PCL) using layer-by-layer precision extrusion deposition then (2) casting PDMS. Cells and matrix are selectively assembled inside microchannels using multi-nozzle printing to demonstrate feasibility of chip as a culture environment. The objective of this work is to fabricate a cell-laden microfluidic device by combined solid freeform patterning and replica molding with direct cell writing into channels. This work has application as a 3D physiological model for *in vitro* pharmacokinetic study in space environment in preparation for long term manned missions.

2. Introduction

Cell-laden microfluidic devices recreate *in vivo* cell-to-cell interactions, a current challenge in drug screening, through reproducible quantifiable patterning of spatial and temporal gradients [1-3]. Conventional cell culture of a monolayer on petri dish is a well-established methodology which has been optimized for various drug's ADMES-Tox profiles in humans [4]. However, the fidelity of conventional models is limited due to local depletion of drug-metabolizing enzymes and transporters, lack of controlled cell seeding and static medium reservoir. Failure to predict drug toxicity by conventional culture methodology is considered the principle reason 90% of new drugs fail during Phase 1 preclinical tests [5]. Biomimetic extra-cellular cues, cell-to-cell contact and definable gradient of drug conditions are engineered into microenvironments for high throughput measurement of toxicity and pharmacokinetics [6, 7]. Cell-laden microfluidic devices improve fidelity of *in vitro* platforms by physiologically relevant surface area to volume ratio, mass transfer by diffusion, biomimetic microenvironment engineering, dynamic perfusion, and dual organ model [8, 9].

The advantage of drug discovery by cell-laden microfluidic devices is reproducible quantifiable control over the stimulation and patterning of biological material [10]. Micron-scale quantities of cell-laden matrix and microfluidic channel produce a cell to interstitial fluid volume ratio of approximately one, as is physiologically relevant [11]. The microfluidic length scale improves uniformity of temperature field and gas supply through cell laden matrix, induce laminar flow pattern and therefore mass transfer by diffusion. These factors reduce variability caused by proportions of experimental design. Hydrodynamic forces on the cells by extracellular fluid produce physical cues stimulate and direct cell function. Dynamic perfusion causes naturally occurring gradients of diffusing chemicals and produces controllable gradient of drug conditions. Cells are assembled in 3-dimensional biomimetic microenvironments to produce biomimetic morphology, cytoskeleton alignment, cell-to-cell contact, focal adhesion of cell to matrix and signaling [12]. Lab-on-a-chip bioanalytic micro-systems introduce multi-component co-cultures to simulate an array of cells by external factors and signals secreted by the cells themselves to engineer a functional tissue. A controllable gradient of drug captures downstream effects of metabolized drug on target organ and conditions in parallel for physiologically-based pharmacokinetic (PBPK) models [13].

Leading microfluidic devices use microfluidic systems and controlled cell seeding to produce cellular microenvironments to facilitate homotypic interaction by co-culture. Vasculature and metastatic prostate cancer microfluidic models use co-culture to understand specific cytokines and growth behavior mechanisms [14, 15]. The effect of stem cells on cancer apoptosis is evaluated in vitro by unidirectional perfusion of a microfluidic device [16]. Microfluidic co-cultures of hepatocytes with nonparenchymal and stem cells are used to study 3-dimensional capillary morphogenesis and drug toxicity [17, 18]. In these leading microfluidic devices, microfluidic channels are fabricated in an elastoployer by soft lithography with wet chemical etching or integrated on a polystyrene biochip and cell seeding is controlled by diffusion across matrix, semi-permeable membranes or time course introduction of cell types.

Key technology for methodological progress in microfluidic devices for drug discovery include microfabrication techniques and 3-dimensional cell patterning. Miniaturization of perfusion system by microfabrication produces biomimetic interconnected network of channels [19]. Current techniques include photolithography, etching, and molding. Controlled cell assembly is required to produce specific cellular microenvironments and heterogeneous cell-to-cell contact [20]. Design models use bottom-up tissue engineering to assemble modular cell environments to achieve biomimetic spatial and temporal interactions of soluble and biological cues. Current techniques include surface patterning by standard photolithography liftoff, photoreactive chemistry, microcontact printing with lamination, molding, and photo-polymerization [21]. Cell migration and separation after printing can be achieved by labeling cells with magnetic nanoparticles or gradient surface treatments to substrate. Cell deposition techniques such as syringe-based cell deposition, rapid prototyping of hydrogel structures, and inkjet-based cell printing include cells as part of the working material to directly assemble biologics in 3-dimensional space [22]. A pumping mechanism is required to precisely transport volumes of liquid to induce specific transient delivery of soluble cues and hydrodynamic strain.

A combined solid free form (SFF) patterning and replica molding process offers the following advantages over current techniques to create a cell-laden microfluidic device: All structures and components of a multi-component micro-systems is fabricated in a single step of micro-molding. The channels in the microfluidic network are printed as a 3D continuous pattern. An elastopolymer is cured against the printed pattern and then printed pattern is then cleared from channels thermally or chemically. The process includes direct cell writing to control distribution of the seeded cells in microfluidic device. Cells and matrix are printed directly into channel. The volume and cross-sectional geometry of cell-laden filaments are controlled by manufacturing process parameters. Cell to fluid volume ratio is also controlled by microfabrication and direct cell writing process. The homogenous curing technique of the elastopolymer does not restrict the surface area of the microfluidic device, unlike radiation or point source based exposure techniques. Additive manufacturing techniques increase height of printed mold without also changing width of the mold, allowing for high aspect ratio structures. The printed pattern can be removed from the elastomer microfluidic chip without damaging the channels, which allows for multilevel features and 3D profiles. The design to the finished device takes less than 24 hours to produce.

The objective of this work is to fabricate a cell-laden microfluidic device by combined solid freeform patterning and replica molding with direct cell writing into channels. This work has application for in vitro disease modeling, pharmacokinetic studies and drug screening. Pre-clinical determinations of drug toxicity to liver would greatly improve screening technology [23].

3. Method to Produce Cell-laden Channels in Microfluidic Device

The combined SFF patterning and replica molding process produces a cell-laden microfluidic device with channel designed to biomimic liver physiology and flow design. First, microfluidic channel pattern is designed from in vivo liver structure and hydrodynamics. Second, microfluidic pattern is printed using precision extrusion deposition. PDMS is cured against the PCL pattern in a single step of replica molding to produce PDMS substrate. PCL pattern is cleared from PDMS, which leaves interconnected microfluidic system. PDMS is plasma treated to decrease surface energy and produce homogenous wetting of the surface during printing. Cell-laden matrix is directly printed into the channels using direct cell writing system. Cell-laden microfluidic chip is sealed in microfluidic chip and nutrient is perfused through system by programmable syringe pump. Figure 1 diagrams the combined SFF patterning and replica molding for microfabrication of cell-laden microfluidic device process.

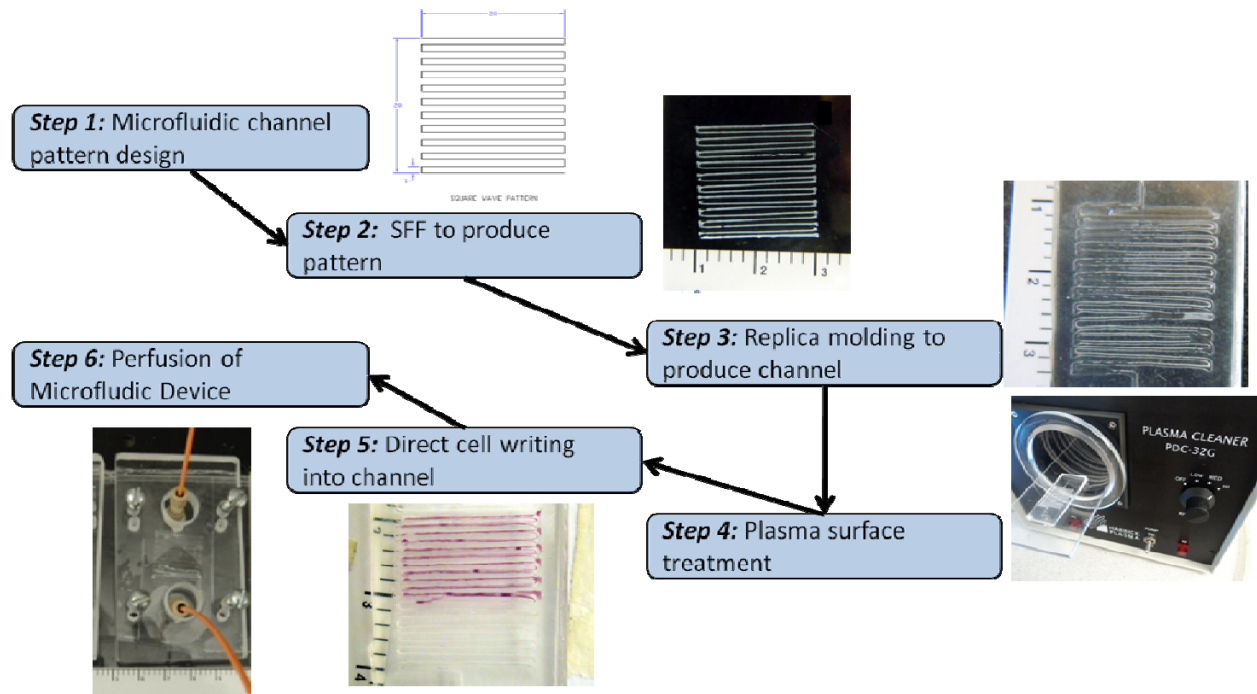


Fig 1. Combined SFF patterning and replica molding for microfabrication of cell-laden microfluidic device process diagram.

3.1. Step 1: Microfluidic Channel Pattern to Biomimic Liver Physiology and Flow Design

In the liver, diffusion through a 3-dimensional network of microvasculature and bile canaliculi network carries nutrients, waste, and oxygen to parenchymal cells, hepatocytes, and non-parenchymal cells, such as the Kupffer and stellate cells [24]. The relative volume of interstitial fluid to cell matrix is approximately one in liver. The microfluidic channel is designed to produce a network of channels to be 50% (v/v) cell-laden matrix and 50% (v/v) interstitial fluid. This ratio is controlled by microfabrication of the channel and direct cell writing process. The range of possible channel dimensions is limited by the manufacturing capability, whose threshold minimum feature size is to be defined by a limiting set of process parameters. The cell volume is controlled by direct cell writing process parameters, and is determined after microfluidic channel geometry is characterized. The microfluidic network is interconnected to allow for dynamic perfusion and controlled transient interaction of interstitial fluid and cell. The system flow rate and architecture cause capillary and hydrodynamic forces acting on the cells. These forces physically strain cell membrane and actin cytoskeleton to induce biomimetic environmental stimulation and cell-to-cell contact necessary to maintain phenotype stability [25]. The microfluidic

channel pattern for 3-dimensional network of microvasculature in liver is simplified to a square wave pattern and elaborated to a sinusoid wave pattern in this work.

3.2. Step 2: SFF to Produce Pattern

SFF by precision extrusion deposition (PED) produces explicitly designed 3-dimensional structures by layer-by-layer deposition of thermoplastic filaments [26-28]. The material delivery system is mounted to a high precision positioning system to print the extruded filament into explicitly designed 3-dimensional patterns. Design model inputs and manufacturing process parameters, excluding dispensing nozzle diameter, are defined by the operator through automation software Motion Planner (Parker Hannifin Corp). The nozzle is selected from a pre-fabricated set and fixed to the material delivery system prior to extrusion. The material delivery system feeds material into a liquefying chamber by a screw and extruded through interchangeable micron-scale nozzle. Figure 2 presents process information pipeline and system configuration for PED system. Polycaprolactone (PCL) (Sigma) in the form of pellets is loaded into precision extrusion deposition (PED) system's extruder and heated to 70°C. Once liquefied and automation software is run, PCL is extruded through 150µm or 350µm nozzle tip by pressure created by a turning screw at 30rpm. Remaining process parameters were derived from previous work using the PED and PCL to produce threshold minimum filament diameter, as it is the limiting case. Nozzle tip is positioned 0.30mm above level substrate. Single layer square wave pattern of dimensions 20mm x 20mm with struts spaced 1mm on center is produced, as presented in figure 3. Single layer sinusoid wave pattern of dimensions 20mm x 22mm with struts spaced 3mm on center and radius of 1mm is produced, as presented in figure 4. Nozzle is raised 0.30mm between subsequent layers for multi-layer patterns.

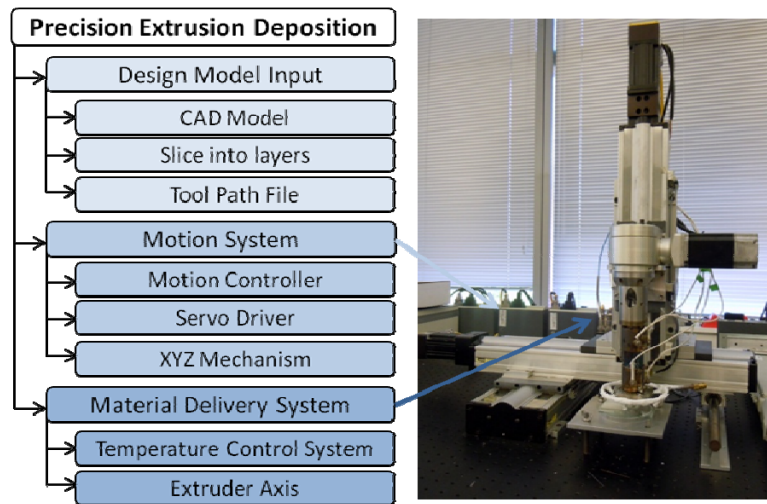


Fig 2. Process information pipeline and system configuration for PED system.

3.3. Step 3: Replica Molding to Produce Channels in PDMS

Elastopolymer solution is prepared from 10:1 ratio base to curing agent of polydimethylsiloxane (PDMS) Sylgard 182 silicone elastomer kit (Dow Chemical). PDMS is selected for the microfluidic device as a biocompatible, non-toxic, optically transparent, & highly gas permeable material [29]. Freshly mixed PDMS is poured over PCL patterns to final depth of 1mm and let cure for 8hrs on 40°C hot plate. PCL cleared from PDMS channel after curing by one of two methods (1) gently peeling PCL from channels using forceps or (2) dissolving PCL from channels by sonication in dichloromethane (Sigma) for 50 min at 40°C [30].

3.4. Step 4: Plasma Treatment

Plasma surface treatment oxidizes the PDMS substrate methyl groups to form silanol groups [31]. The wettability of cross-linking solution and printed alginate matrix is controllable and homogenous on

post-plasma treated surface. PDMS substrate is placed in RF plasma chamber (Harrick Plasma) for 90 seconds less than 2 minutes prior to printing.

3.5. Step 5: Direct Cell Writing Into Channel

SFF of biological material and matrix by direct cell writing produces explicitly designed 3-dimensional patterns of cell-laden matrix in microfluidic device [32, 33]. The material delivery system is mounted to a high precision positioning system to print the cells into microfluidic channels. Design model inputs and manufacturing process parameters, excluding dispensing capillary tip diameter, are defined by the operator through automation software Motion Planner (Parker Hannifin Corp). The nozzle is selected from a pre-fabricated set of EFD precision capillary tips and fixed to the material delivery system prior to extrusion. The material delivery system feeds cell-laden matrix through interchangeable micron-scale capillary tip by continuous pneumatic pressure regulated from reservoir of breathing quality compressed air (AirGas). Once pressurized and automation software is run, cell-laden matrix is extruded through 150 μ m capillary tip 0.5 inches in length by 15psi of air pressure. Capillary tip is position 0.10mm above bottom of channel. Cells are printed in square wave pattern of dimensions 20mm x 20mm with struts spaced 1mm on center to align with PDMS channel pattern. Figure 3 presents system configuration for direct cell writing system aligned to PDMS substrate to print cells into microfluidic channels.

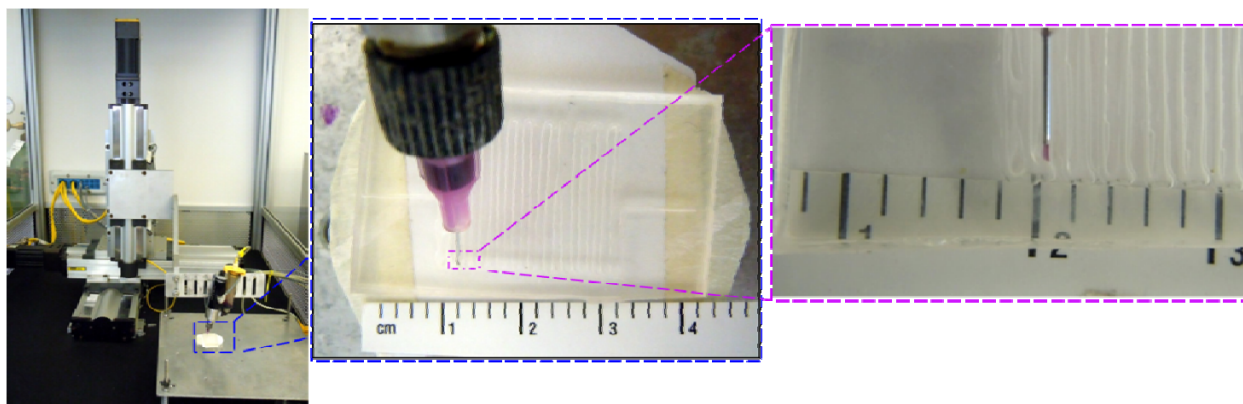


Fig 3. System configuration for direct cell writing system aligned to PDMS substrate to print cells into microfluidic channels.

3.6. Step 6: Perfusion of Microfluidic Device

Cell-laden PDMS microfluidic chip and PDMS cover fitted with nanoport assemblies (Upchurch Scientific) secured together by polycarbonate brace to form a microfluidic circuit. System perfused by programmable syringe pump (Next Era) and flow tested to 10 μ L/hr with media.

3.7. Cell Culture and Encapsulation

Human hepatocytes of the HepG2 cell line (ATCC) are cultured in Eagle's Minimum Essential Medium base medium (ATCC) supplemented with 10% (v/v) fetal bovine serum (FBS) (Invitrogen) and 1% (v/v) antibiotic/antimycotic (Invitrogen). Cell are maintained at 32°C and 5% CO₂ for less than 3 passages after thawing prior to experiment. Alginate matrix is prepared from 6.0% (w/v) alginic acid sodium salt from brown algae (Sigma) in deionized water and sterilized through aseptic serial filtration through 20 μ m mesh (VWR). Cells are mixed with alginate for final concentration of 2.0 \times 10⁶ cells/mL HepG2 cells. Prior to printing, channels are flooded with cross-linking solution as formed by 5.0% (w/v) calcium chloride (Sigma) in deionized water.

3.8. Fluorescent Determination of Cell Viability

Cell viability post-printing is qualitatively evaluated using fluorescence based Live/Dead Viability/Cytotoxicity Kit for mammalian cells (Molecular Probes). Two-color discrimination by the fluorescent probes ethidium calcein AM and ethidium homodimer-1 labels live cells as green and dead cells as red. Samples were analyzed using a DM RIB inverted microscope (Leica) with UV source. Images of all cells were captured electronically by Insight 4.0Mp Monochrome digital camera (Spot Insight) and manufacturer provided imaging software.

4. Results

Here we demonstrate the feasibility of a combined SFF and replica molding microfabrication process to produce a cell-laden microfluidic device. Having established control over microfluidic pattern and channel depth, we leverage additive manufacturing techniques to increase aspect ratio of channels and produce 3-dimensional networks of interconnected channels. Results demonstrate process control.

4.1. Printed PCL Patterns and PDMS Channels

Inspection of three designs for fabricated PCL pattern and PDMS channels was performed to demonstrate the pattern could be created, molded, and cleared from PDMS channel. Figure 4 presents design model input, printed PCL pattern produced by PED system, and PDMS cured against PCL pattern for single layer square wave pattern of dimensions 20mm x 20mm with struts spaced 1mm on center. Figure 5 presents design model input, printed PCL pattern produced by PED system, and PDMS cured against PCL pattern for single layer sinusoid wave pattern of dimensions 20mm x 22mm with struts spaced 3mm on center and radius of 1mm. Figure 6 presents design model input, printed PCL pattern produced by PED system, and PDMS cured against PCL pattern for single layer multi-compartment array of two square and one sinusoid wave arranged in series.

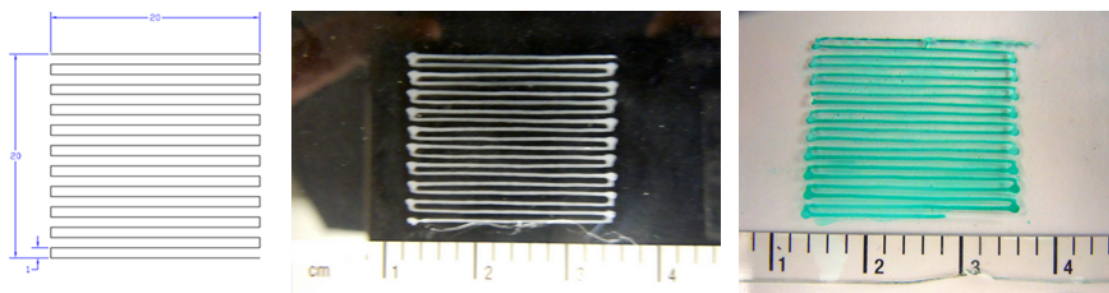


Fig 4. Single layer square wave pattern of dimensions 20mm x 20mm with struts spaced 1mm on center design model input (left) and printed PCL pattern produced by PED system (center) and PDMS cured against PCL pattern with channels flooded with green dye (right).

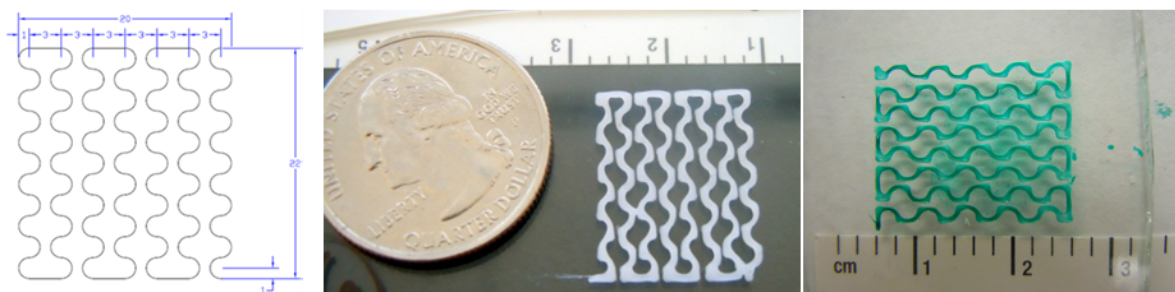


Fig 5. Single layer sinusoid wave pattern of dimensions 20mm x 22mm with struts spaced 3mm on center and radius of 1mm design model input (left) and printed PCL pattern produced by PED system (center) and PDMS cured against PCL pattern with channels flooded with green dye (right).

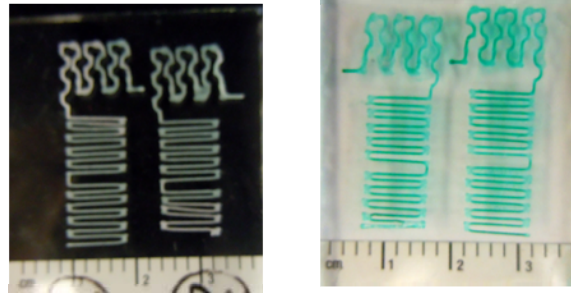


Fig 6. Single layer multi-compartment array of square and sinusoid wave design models printed PCL pattern produced by PED system (left) and PDMS cured against PCL pattern with channels flooded with green dye (right).

Inspection using phase contrast microscope and image analysis software was performed on fabricated samples to characterize PCL pattern and PDMS channel depth and width. Single layer square wave PCL pattern and PDMS chip were viewed from the top and then sliced through the center to inspect cross-section. Figure 7 presents top view and section view of printed PCL pattern and PDMS channels for square wave channel. PDMS curing against the PCL pattern yields a network of channels.

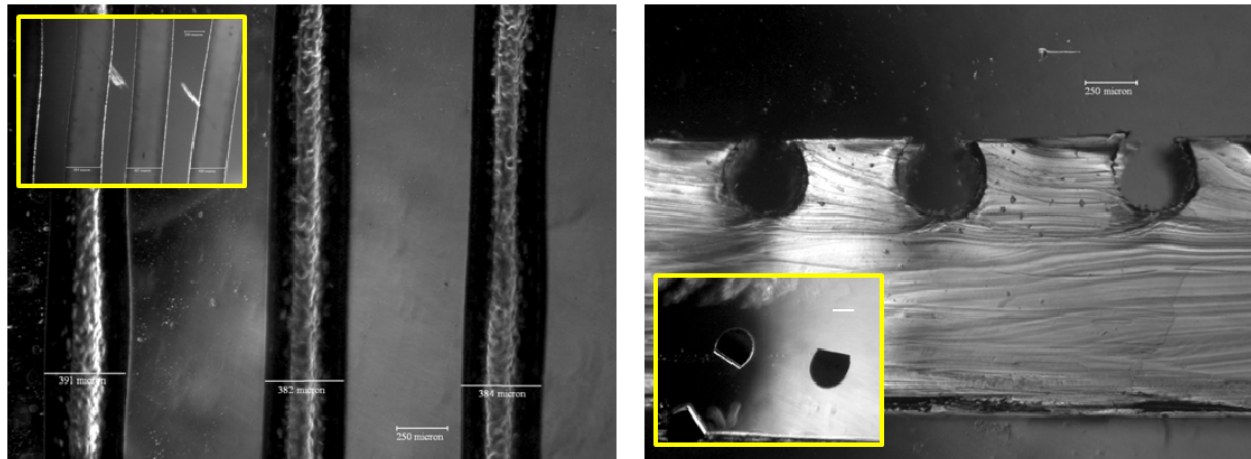


Fig 7. Top and section view of PDMS Channels printed using 350 μ m nozzle tip. Top view of PCL pattern inset.

Imaging confirmed PCL pattern produces PDMS channel network. PED produces continuous PCL patterns of controlled geometry. PDMS curing against PCL pattern produces a polymer replica with channel network. Micro-systems structures of all of the components are formed in a single step of micro-molding. PDMS channel width, depth, and cross-section is controlled by PCL pattern, which is controlled by PCL manufacturing process parameters. The process implements manufacturing control over PDMS channel size.

4.2. SFF to Incrementally Increase Channel Aspect Ratio

Having established PDMS channels can be produced by PCL patterns and the micro-system structures can be controlled by PED manufacturing process parameter, we next investigated the effect of

SFF to increase channel aspect ratio. Inspection using phase contrast microscope and image analysis software was performed on PDMS channel fabricated using 1, 2, 3, and 4 layers to characterize channel width, depth and aspect ratio. A scalpel is used to cut PDMS chip along its centerline to view cross-section of channels. The aspect ratio (AR) is defined as the channel depth divided by the channel width. Figure 8 presents cross section view of PDMS channels molded from multi-layer PCL patterns.

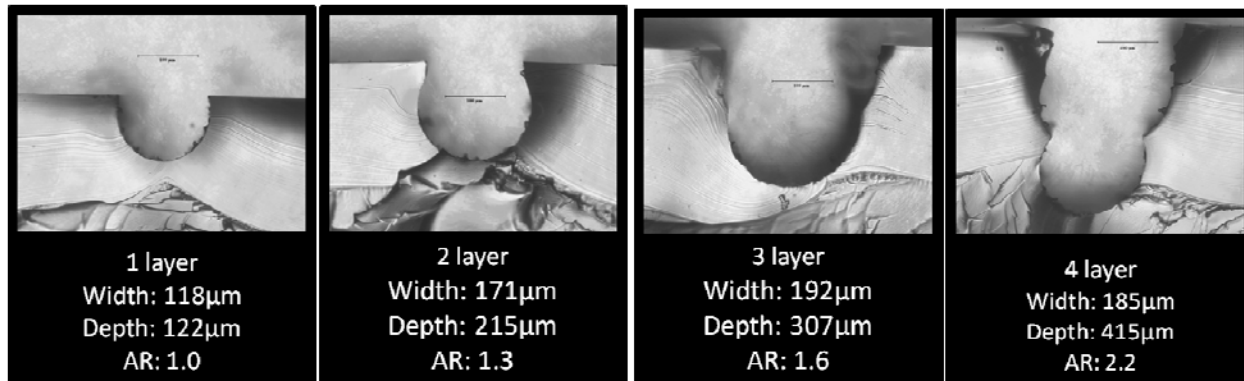


Fig 8. Cross section view of PDMS channels molded from multi-layer PCL patterns.

The depth of the channel is increased by printing additional layers on top of the first layer. The width of the channel is increased by printing additional layers on top of the first layer. One layer produced a 1.0 aspect ratio channel. Two layers increased the aspect ratio 30%, three layers increased the aspect ratio 60%, and 4 layers increased the aspect ratio 120%.

4.3. 3-D PCL Pattern Cleared from PDMS without Damaging Channel Network

Having established PDMS channels can be produced by multi-layer PCL patterns and the micro-system structures can be controlled by SFF manufacturing in 3-dimensions, we next investigated the effect of mutually orthographic layers to create interconnected network of channels. Inspection using phase contrast microscope and image analysis software was performed on PCL scaffold and PDMS channel fabricated using scaffold to characterize channel width, depth and orientation. Figure 9 presents printed 0-90 PCL scaffold pattern and PDMS channels after PCL cleared from channels.

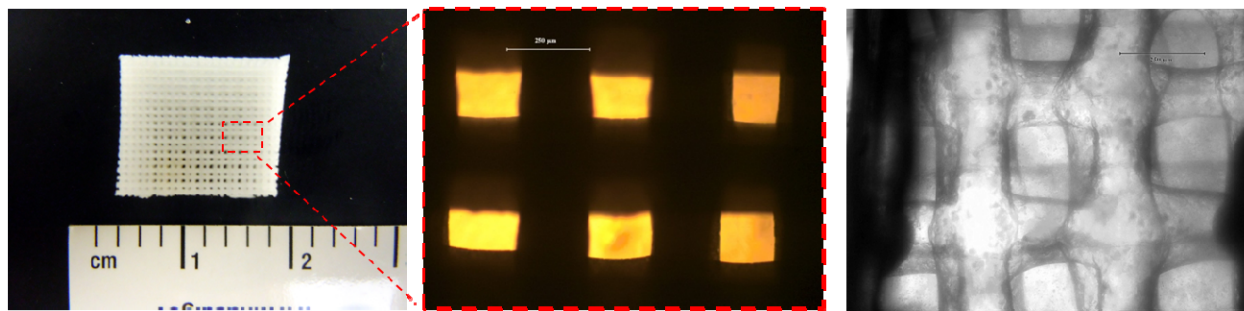


Fig 9. Printed 0-90 scaffold pattern using two layers as viewed from the top macroscopically and under 4x magnification using phase contrast microscope (B) from the top (A) and front (B). PDMS Channels after PCL is removed by sonication and the construct is viewed from the top under 4x magnification using phase contrast microscope (C).

A porous 3-dimensional PCL scaffold is produced by PED manufacturing. Two mutually orthographic layers are printed by continuous extrusion in the same z-plane. A second set of mutually orthographic layers are printed on top of the first set to produce a scaffold. PCL is continuously extruded

during entire printing episode to produce a continuous pattern. PDMS cures against PCL scaffold to pattern produce a polymer replica with channel network. PCL scaffold is cleared from PDMS chip without disturbing PDMS due to the difference in material melting temperature and solubility. After replica molding PCL is cleared by 50 minutes of sonication in an 80°C dichloromethane bath. A scalpel is used to cut PDMS chip along its centerline to view cross-section of channel network. Figure 10 presents cross section view of PDMS channels molded from PCL scaffold.

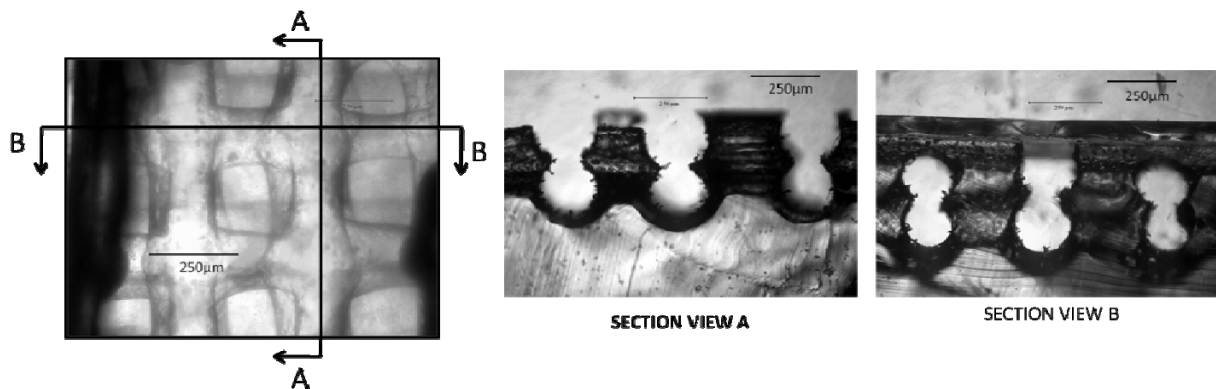


Fig 10. Continuous extrusion during PCL patterning creates an interconnected 3-D network for replica molding.

Figure 13 presents the top view of PDMS chip and two orthographic cross-section views. Two layers of channels are produced in PDMS chip. Two rows of pores are visible in both Figure 13 section view A and B. PED manufacturing is used to layer 2-dimensional PCL patterns to create 3-dimensional architecture. This architecture is replica molded and cleared from PDMS chip to produce a 3-dimensional microfluidic system. The depth of the channels from the top of the chip to the bottom of the lower row is 325-385µm.

Channels are produced in orthographic directions. The pores visible in the Figure 13 section view A is created by PCL filaments normal to the cutting plane, PCL cleared from the PDMS prior to imaging. Pores in the traverse direction are visible as circular cross-section in Figure 13 section view B. Orthographic orientation of PCL scaffold filaments produces PDMS channels in two directions. The 0-90 orientation of the two square waves in each layer produces a second pore orientation. The diameter of the pore is 185 +/- 17 µm in diameter.

4.4. Controlled Cell Seeding by Direct Cell Writing into PDMS Channels

Having established PDMS channels can be produced by PCL patterns and the micro-system structures can be controlled by SFF manufacturing in 3-dimensions, we next investigated the process of direct cell writing for controlled cell seeding into microfluidic channels. A set of fully defined direct cell writing process parameters produces a unique volume dispensed per unit surface area of substrate. Process parameters can be varied independent of printed pattern to produce patterns of extruded filaments of variable diameter. The volume dispensed per unit area of substrate is a function of the flow rate and speed of material delivery system. The flow rate through the nozzle is a function of the capillary tip diameter and length, material viscosity, and dispensing pressure. The matrix viscosity is a function of the material and cells concentration, which are selected for to support cell growth after printing. Dispensing tip is selected from a set of prefabricated tips and fixed to material delivery system before printing. Dispensing pressure and speed of material delivery system can be adjusted during printing and tuned to create variable filament diameters. Figure 11 presents matrix extruded through 150µm nozzle tip at 5psi with variable speeds of material delivery system.

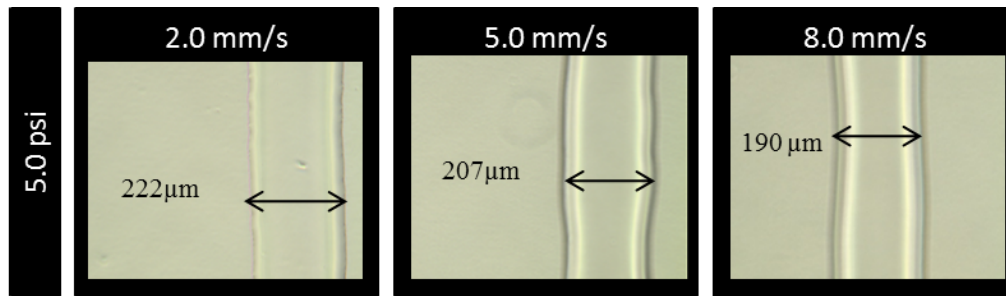


Fig11. Matrix extruded through 150 μ m nozzle tip at 5psi with variable speeds of material delivery system.

The diameter of the extruded filament, and therefore volume of dispensed material is controllable by manufacturing process parameters. The design input model for direct cell writing system is copied from PED model to produce PCL pattern to trace channel pattern during cell printing. Cell-laden matrix is prepared and loaded into direct cell writing system and printed. Cells are fluorescently labeled after printing to demonstrate their position. Inspection using phase contrast microscope and fluorescent microscopy was performed using image analysis software. Figure 12 presents hepatocytes in alginate printed into channels labeled with the Live/Dead stain and photographed under phase contrast (top row) and fluorescent(bottom row) microscope.

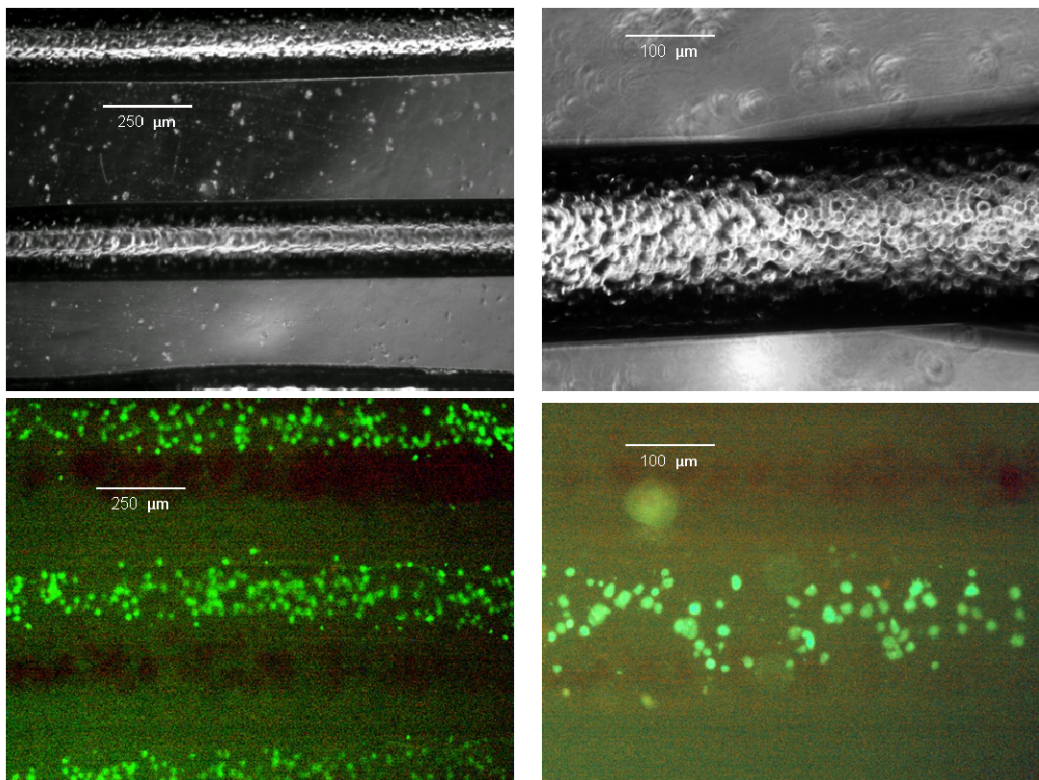


Fig 12. Hepatocytes in alginate printed into channels labeled with the Live/Dead stain and photographed under phase contrast (top row) and fluorescent(bottom row) microscope.

Fluorescent imaging confirmed viable hepatocytes were printed into microfluidic channels on PDMS chip. Direct cell writing process produces control over pattern of printed cell-laden matrix and flow rate

of extrusion. The process implements manufacturing control over placement and volume of cells dispensed.

5. Discussion

This study describes a novel process to produce microfluidic network based on liver physiology by combined solid freeform patterning and replica molding with direct cell writing into channels. The process is designed to provide engineering control over environmental cues in microfluidic device; such as microfluidic pattern and channel cross-section, cell to interstitial fluid volume, cell seeding, and perfusion flow rate.

Microfluidic pattern is produced by solid free form fabrication of a removable pattern. PED produces continuous PCL patterns of controlled geometry. PDMS curing against PCL pattern produces a polymer replica with channel network. Micro-systems structures of all of the components are formed in a single step of micro-molding. The design model input can be digitally adapted to physiologically relevant patterns and arrays of patterns. Continuous extrusion during the printing process creates an interconnected 3-dimensional network for replica molding. PDMS channels can be produced by multi-layer PCL patterns and the micro-system structures can be controlled by SFF manufacturing in 3-dimensions. SFF techniques during microfabrication process increase channel aspect ratio and embedded architecture in microfluidic system. An interconnected system of mutually orthographic channels was produced by replica molding of a porous scaffold. This capability can be used to introduce hierarchical modular architecture throughout the depth of the chip, in addition to the planar patterning.

PDMS channel width, depth, and cross-section is controlled by PCL pattern. PCL pattern is controlled by manufacturing process parameters. Process produces manufacturing control over PDMS channel size and surface area-to-volume ratio oxygen diffusion and thermal field. The diameter of the extruded filament is a function of the nozzle diameter, feed rate (screw speed), temperature, and travel speed of the material delivery system. In this work, we used two nozzle diameters, 150 and 350 μm . Remaining process parameters were derived from previous work using the PED and PCL to produce threshold minimum filament diameter, as it is the limiting case. The filament diameter can be increased by increasing the volume of material dispensed per unit area of substrate. This can be accomplished by increasing feed rate, increasing temperature, or decreasing travel speed of the material delivery system. The process allows for independent control over the filament cross-section and pattern.

Cell seeding is controlled by direct cell writing of cell-laden matrix into microfluidic channels. SFF manufacturing by direct cell writing traces channel pattern to dispense cell-laden matrix into channels. The process provides control over cell seeding. The direct cell writing process has multi-nozzle capability to pattern different types of biologics within a single microfluidic device during a single printing episode. The direct cell writing motion system has the capability to move in 3-dimensional space to layer and pattern biologics. Similar to the PED process, direct cell writing can be used to introduce hierarchical patterning of cell/biologics densities or cell types throughout the depth of the chip, in addition to the planar patterning.

Direct cell writing process produces manufacturing control of volume of printed material per unit area of substrate and therefore control of cell to interstitial fluid volume ratio. The diameter of the extruded cell-laden filament is a function of the nozzle diameter, feed rate (dispensing pressure), matrix viscosity, and travel speed of the material delivery system. In this work, we used 150 μm capillary tip. Remaining process parameters were derived from previous work printing alginate using direct cell writing process to produce threshold minimum filament diameter, as it is the limiting case. The filament diameter can be increased by increasing the volume of material dispensed per unit area of substrate. This can be accomplished by increasing dispensing rate, decreasing matrix viscosity, or decreasing travel speed of the material delivery system. Since the cross-section of the microfluidic channel has been characterized,

operator produce a cell to interstitial fluid ratio close to one by tuning direct cell writing process parameters. The process allows for independent control over the filament cross-section and pattern. Printed pattern and set of process parameters manufactures a unique 3-dimensional cell-laden construct.

Cells are part of the working material in direct cell writing process. The hydrodynamic forces caused by the flowing matrix during printing cause damage to cells. Cell viability in printed construct is a function of the process parameters. During process parameter selection, cell survivability is considered to preserve functionality and viable cell density in printed construct. The speed of the material system is programmable and does not affect hydrodynamic force in the nozzle tips. This means the diameter of the extruded filaments can be controlled without effecting hydrodynamic forces and cell viability. This is desirable, as it produces independent control over filament diameter and cell viability.

Perfusion flow rate is controlled by programmable syringe pump. Precise volumes of fluid in a microfluidic device are transported through microfluidic device through continuous network of channels for medium perfusion, delivery of drug compounds, generation of drug gradient, creation of specific microenvironments and connection of cell culture. Dynamic perfusion using syringe pump and was flow tested to 10 μ L/hr.

6. Conclusion

The objective of this work is to fabricate a cell-laden microfluidic device by combined solid freeform patterning and replica molding with direct cell writing into channels. Process includes SFF manufacturing by precision extrusion deposition (PED) and direct cell writing for independent process control of structural and cell-laden material patterning. Manufacturing process parameters provided control of PCL pattern and cell-laden filament diameter & cross-section. PED process parameters provide control over PCL pattern and therefore PDMS channel surface area-to-volume ratio, oxygen diffusion and thermal field. Direct cell writing process parameters provide control over cell to fluid volume ratio and diffusion. Precise volumes of fluid in a microfluidic device are transported through microfluidic device through continuous network of channels up to a flow rate of 10 μ L/hr. This work has application as a 3D physiological model for in vitro pharmacokinetic study in space environment in preparation for long term manned missions.

7. References

1. Shuler M L, Viravaidya K, and Sin A 2004 Development of a microscale cell culture analog to probe naphthalene toxicity. *Biotechnology Progress* **20**(1): p. 316-323.
2. Jensen K F, El-Ali J, and Sorger P K 2006 Cells on chips. *Nature* **442**(7101): p. 403-411.
3. Dickson M and Gagnon J P 2004 Key factors in the rising cost of new drug discovery and development. *Nature Reviews Drug Discovery* **3**(5): p. 417-429.
4. van Midwoud P M, Verpoorte E, and Groothuis G M M 2011 Microfluidic devices for in vitro studies on liver drug metabolism and toxicity. *Integrative Biology* **3**(5): p. 509-521.
5. Brandon E F A, Raap C D, Meijerman I, Beijnen J H, and Schellens J H M 2003 An update on in vitro test methods in human hepatic drug biotransformation research: pros and cons. *Toxicology and Applied Pharmacology* **189**(3): p. 233-246.
6. Wu M H, Huang S B, and Lee G B 2010 Microfluidic cell culture systems for drug research. *Lab on a Chip* **10**(8): p. 939-56.
7. Kim S and Marimuthu M 2011 Microfluidic cell coculture methods for understanding cell biology, analyzing bio/pharmaceuticals, and developing tissue constructs. *Analytical Biochemistry* **413**(2): p. 81-89.
8. Buyukhatipoglu K, Chang R, Sun W, and Clyne A M 2010 Bioprinted Nanoparticles for Tissue Engineering Applications. *Tissue Engineering Part C-Methods* **16**(4): p. 631-642.

9. Walker G M, Zeringue H C, and Beebe D J 2004 Microenvironment design considerations for cellular scale studies. *Lab on a Chip* **4**(2): p. 91-7.
10. Yeo L Y, Chang H C, Chan P P, and Friend J R 2011 Microfluidic devices for bioapplications. *Small* **7**(1): p. 12-48.
11. Whitesides G M, Siegel A C, Tang S K Y, Nijhuis C A, Hashimoto M, Phillips S T, and Dickey M D 2010 Cofabrication: A Strategy for Building Multicomponent Microsystems. *Accounts of Chemical Research* **43**(4): p. 518-528.
12. Bornens M, They M, Racine V, Pepin A, Piel M, Chen Y, and Sibarita J B 2005 The extracellular matrix guides the orientation of the cell division axis. *Nature Cell Biology* **7**(10): p. 947-U29.
13. Cheng K C, Maguire T J, Novik E, Chao P, Barminko J, Nahmias Y, and Yarmush M L 2009 Design and Application of Microfluidic Systems for In Vitro Pharmacokinetic Evaluation of Drug Candidates. *Current Drug Metabolism* **10**(10): p. 1192-1199.
14. Song J W, Cavnar S P, Walker A C, Luker K E, Gupta M, Tung Y C, Luker G D, and Takayama S 2009 Microfluidic Endothelium for Studying the Intravascular Adhesion of Metastatic Breast Cancer Cells. *Plos One* **4**(6).
15. Takayama S, Hsiao A Y, Torisawa Y S, Tung Y C, Sud S, Taichman R S, and Pienta K J 2009 Microfluidic system for formation of PC-3 prostate cancer co-culture spheroids. *Biomaterials* **30**(16): p. 3020-3027.
16. Kong B H, Song X Y, and Li D 2008 A new tool for probing of cell-cell communication: human embryonic germ cells inducing apoptosis of SKOV3 ovarian cancer cells on a microfluidic chip. *Biotechnology Letters* **30**(9): p. 1537-1543.
17. Cheng K C, Novik E, Maguire T J, Chao P Y, and Yarmush M L 2010 A microfluidic hepatic coculture platform for cell-based drug metabolism studies. *Biochemical Pharmacology* **79**(7): p. 1036-1044.
18. Kamm R D, Sudo R, Chung S, Zervantonakis I K, Vickerman V, Toshimitsu Y, and Griffith L G 2009 Transport-mediated angiogenesis in 3D epithelial coculture. *Faseb Journal* **23**(7): p. 2155-2164.
19. Becker H, Carstens C, Elbracht R, and Gartner C 2010 Opportunities and limits of cell-based assay miniaturization in drug discovery. *Expert Opinion on Drug Discovery* **5**(7): p. 673-679.
20. Khalil S and Sun W 2009 Bioprinting Endothelial Cells With Alginate for 3D Tissue Constructs. *Journal of Biomechanical Engineering-Transactions of the Asme* **131**(11): p. -.
21. Borenstein J T, Weinberg E J, Orrick B K, Sundback C, Kaazempur-Mofrad M R, and Vacanti J P 2007 Microfabrication of three-dimensional engineered scaffolds. *Tissue Engineering* **13**(8): p. 1837-1844.
22. Khalil S, Nam J, and Sun W 2005 Multi-nozzle deposition for construction of 3D biopolymer tissue scaffolds. *Rapid Prototyping Journal* **11**(1): p. 9-17.
23. Griffith L G, Sivaraman A, Leach J K, Townsend S, Iida T, Hogan B J, Stolz D B, Fry R, Samson L D, and Tannenbaum S R 2005 A microscale in vitro physiological model of the liver: Predictive screens for drug metabolism and enzyme induction. *Current Drug Metabolism* **6**(6): p. 569-591.
24. Neville C, Carraro A, Hsu W M, Kulig K M, Cheung W S, Miller M L, Weinberg E J, Swart E F, Kaazempur-Mofrad M, Borenstein J T, and Vacanti J P 2008 In vitro analysis of a hepatic device with intrinsic microvascular-based channels. *Biomedical Microdevices* **10**(6): p. 795-805.
25. Griffith L G, Powers M J, Domansky K, Kaazempur-Mofrad M R, Kalezi A, Capitano A, Upadhyaya A, Kurzawski P, Wack K E, Stolz D B, and Kamm R 2002 A microfabricated array bioreactor for perfused 3D liver culture. *Biotechnology and Bioengineering* **78**(3): p. 257-269.
26. Sun W, Shor L, Guceri S, Wen X J, and Gandhi M 2007 Fabrication of three-dimensional polycaprolactone/hydroxyapatite tissue scaffolds and osteoblast-scaffold interactions in vitro. *Biomaterials* **28**(35): p. 5291-5297.

27. Shor L, Guceri S, Chang R, Gordon J, Kang Q, Hartsock L, An Y H, and Sun W 2009 Precision extruding deposition (PED) fabrication of polycaprolactone (PCL) scaffolds for bone tissue engineering. *Biofabrication* **1**(1): p. -.
28. Wang F, Shor L, Darling A, Khalil S, Sun W, Guceri S, and Lau A 2004 Precision extruding deposition and characterization of cellular poly-epsilon-caprolactone tissue scaffolds. *Rapid Prototyping Journal* **10**(1): p. 42-49.
29. Mata A, Fleischman A J, and Roy S 2005 Characterization of polydimethylsiloxane (PDMS) properties for biomedical micro/nanosystems. *Biomedical Microdevices* **7**(4): p. 281-293.
30. Vazquez A, Luduena L N, and Alvarez V A 2007 Processing and microstructure of PCL/clay nanocomposites. *Materials Science and Engineering a-Structural Materials Properties Microstructure and Processing* **460**: p. 121-129.
31. Whitesides G M, Ng J M K, Gitlin I, and Stroock A D 2002 Components for integrated poly(dimethylsiloxane) microfluidic systems. *Electrophoresis* **23**(20): p. 3461-3473.
32. Sun W, Chang R, and Nam Y 2008 Direct cell writing of 3D microorgan for in vitro pharmacokinetic model. *Tissue Engineering Part C-Methods* **14**(2): p. 157-166.
33. Sun W, Chang R, Emami K, and Wu H L 2010 Biofabrication of a three-dimensional liver micro-organ as an in vitro drug metabolism model. *Biofabrication* **2**(4): p. 1-11.