Microvascular Channel Bioprinter Shutoff Valve

Final Report

BME 400 Design

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Abstract

Tissue engineering is a rapidly growing industry that grows and utilizes cells, tissues, and organs to address clinical and research needs [1]. However, one persistent problem in the field is vascularization of these tissues. One potential solution lies within chaotic bioprinting. Kenics Static Mixers (KSMs) create an alternating pattern of bio- and fugitive ink [2]. Continuously Extruded Variable Internal Channeling (CEVIC) technology, created by the Dean Lab, prints hydrogels in flat sheets rather than filaments [2]. The hydrogel outputs are intended to mimic vasculature from the artery level down to the capillary level. However, a difficulty arises in instantaneously switching between the different KSM outputs in order to have sequentially smaller hydrogel resolutions. Therefore, an automatic shutoff valve is needed. A solution was identified which utilizes a rotational element fitted within the CEVIC device that only allows output from one KSM at a time. This design was fabricated and tested in order to resolve the hydrogel resolution and switching problem and allow vascularization and life-saving research to advance.

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1 Introduction

1.1 Motivation

On any given day, over 100,000 people in the United States are waiting for a life saving organ donation [3]. This statistic highlights a critical gap between the supply and demand of donor organs, a disparity that leads to mortality and underscores the limitations of the current donor-based system. Bioprinting has emerged as a disruptive technology with the potential to address this organ availability crisis by enabling the fabrication of functional tissues and organs [2]. The potential to create patient-specific organs on demand could one day eliminate transplant waiting lists and the complications associated with organ rejection. The applications of bioprinting extend to in-ex-vivo testing and support high-risk surgical procedures. A critical barrier that still limits the clinical translation is vascularization. Over 90% of engineered tissue constructs fail to sustain long-term function because they lack a functional vascular network capable of delivering oxygen and nutrients [4]. This vascularization bottleneck severely limits the size and complexity of printable tissues. Current constructs rarely exceed 2 mm in thickness before central regions become hypoxic and necrotic [5]. The development of systems that can fabricate microchannels at physiological scales is essential to bridging the gap between laboratory prototypes and clinically viable tissues.

1.2 Existing devices

Conventional Extrusion 3D Bioprinting serves as a foundational method in the field, characterized by its ability to utilize multi-material filaments to create complex tissue architectures while maintaining low leakage rates. However, this approach faces three significant limitations, including a typical resolution constraint of 100-200 μm and the risk of damaging sensitive cells during printing due to extrusion pressure [6]. A specific application of this printing technology is seen in 3D Printed Microfluidic Multiport Valves. These devices provide precise automated switching via stepper motor control, demonstrating no leakage in static tests and less

than 0.5% leakage in dynamic use [7]. While effective in tested 800 µm channels, their performance at smaller scales, such as 10 µm, remains unverified.

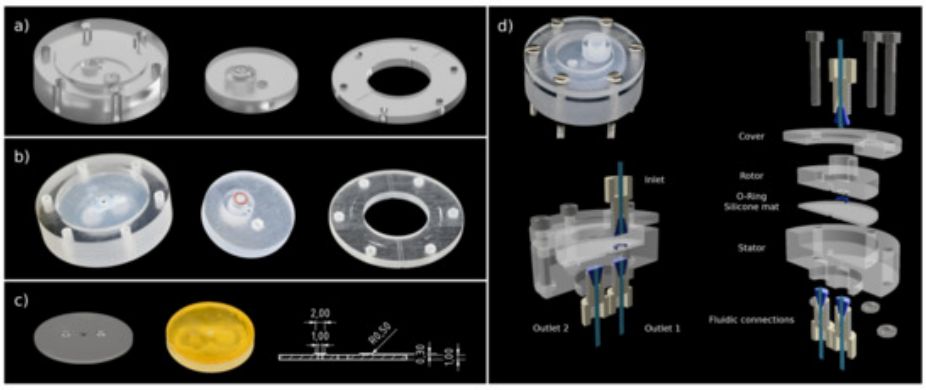

Figure 1: *Test valve CAD design for 3D-printed microfluidic multiport systems* [7]

In a separate technological approach, On-Chip Liquid-Metal Microvalves offer exceptional performance in fluid control. They achieve precise directional control with no leakage up to 320 mbar and a minimal leak rate of ≤ 0.043 µL/min at 330 mbar [8]. Their principal limitation is a lack of inherent sequential layering or branching capability, which necessitates design adaptation for complex systems.

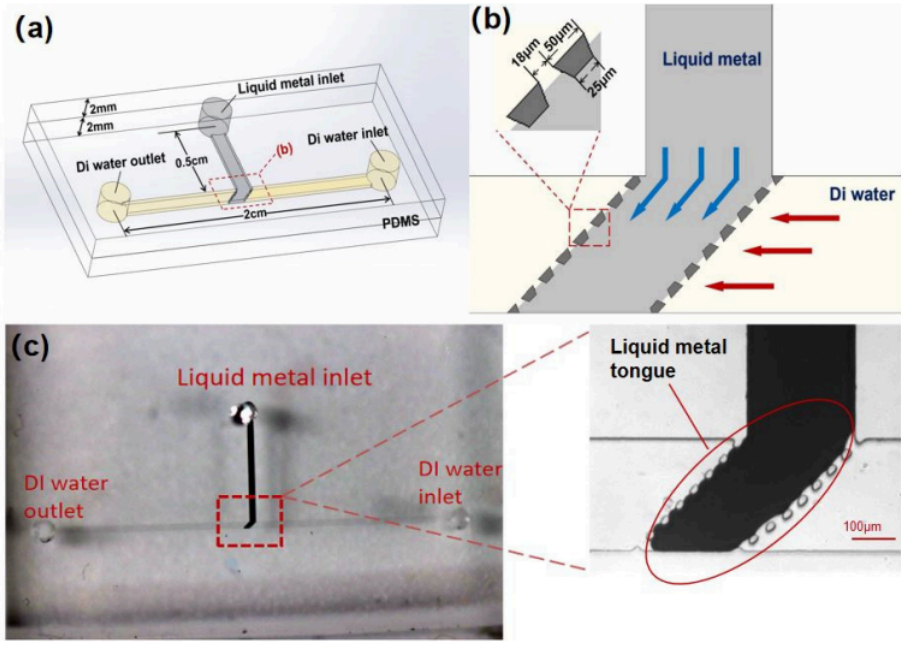

Figure 2: *Structural diagrams of the on-chip liquid-metal microvalve* [8]

2 Background

2.1 Anatomy and Physiology

To achieve necessary perfusion, every cell in the body must be within 50-70 μm of a blood vessel [5]. In a laboratory setting, researchers can supply cells and fabricated tissues with the necessary oxygen and nutrients and remove waste. However, this is not possible when transferring these engineered tissues from in vitro to in vivo. Therefore, engineers and researchers must find a way to vascularize tissue engineered tissues, and it is considered one of tissue engineering's current key challenges [4]. One main challenge remains in achieving the resolution of small arteries and capillaries. The smallest capillaries are 10 μm in diameter, and fabricating a channel that small presents a challenge for 3D bioprinting and other tissue engineering methods [2]. Secondly, the artificial vasculature must be able to connect to and mimic the circulatory system in the body, which includes the progressive branching of arteries to arterioles to capillaries (as well as from capillaries to venules to veins). It is from capillaries that the surrounding cells are perfused. Figure 3 depicts the transitions between the various blood vessels.

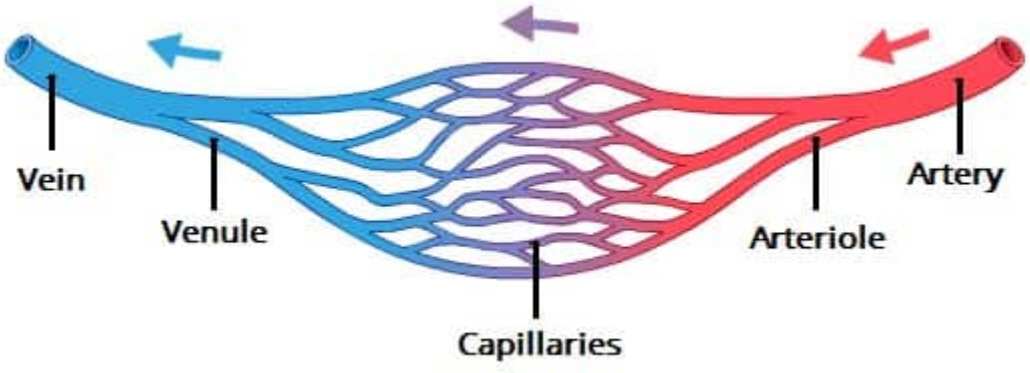

Figure 3: *Diagram depicting the circulatory system branching into smaller channels from arteries* [9]

Researchers at the Dr. Dean Lab have devised a way to address both these challenges of tissue engineering through chaotic bioprinting. Chaotic bioprinting is a patent-pending, extrusion 3D bioprinting technique that utilizes Kenics Static Mixers (KSMs) to produce channels of significantly higher resolution as

compared to other extrusion brioprinters [2]. KSMs work through the chaotic advection of two or more materials. Figure 4 below provides a visualization of the mixing process and how it results in high resolution filaments [2].

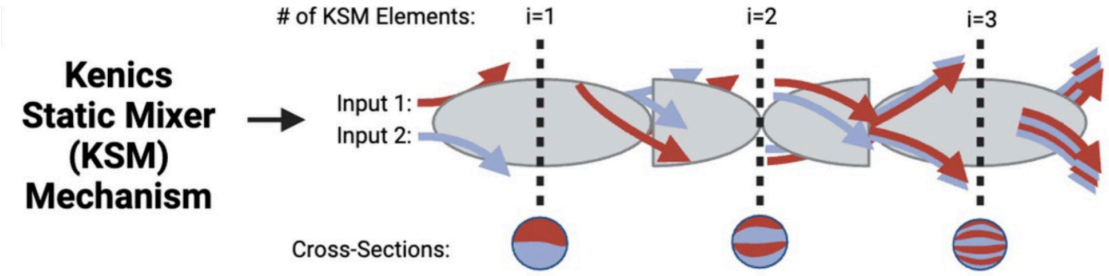

Figure 4: *Kenics Static Mixer chaotic advection process* [2]

In the Dean Lab, KSMs are used to create alternating channels of cell seeded bioink and fugitive ink. The bioink hydrogel is 3% Gelatin Methacryloyl (GelMa), 2% Sodium Alginate (SA), and 0.1% lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP), and can be seeded with cells such as pericytes (hPCs) [2]. The GelMA promotes cell adhesion and the SA is crosslinked via calcium chloride to create the hydrogel. The fugitive ink is a 0.8% solution of hydroxyethyl cellulose (HEC), and can also be seeded with cells of either the same or different type as the GelMa (such as endothelial HUVEC cells) [2]. After being mixed in a KSM, the resulting filament has alternating rows of the GelMa hydrogel and HEC fugitive ink, which when vacated, leaves empty inner channels. These vacant channels serve as the scaffold for microvasculature within the hydrogel. See Figure 5 below.

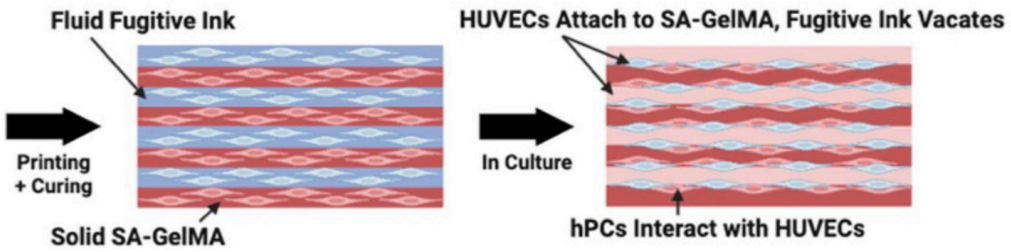

Figure 5: *Schematic depicting how the fugitive ink vacates and provides a scaffold to serve as a base for microvascularization* [2]

Multiple KSM sizes with increasing numbers of KSM elements produces the various artery/vein, arteriole/venule, and capillary resolutions [2]. Seven sizes with 3-9 mixing elements produce between 8 (the largest resolution) and 512 (the smallest resolution) channels. See Figure 6 below depicting the different sized KSMs.

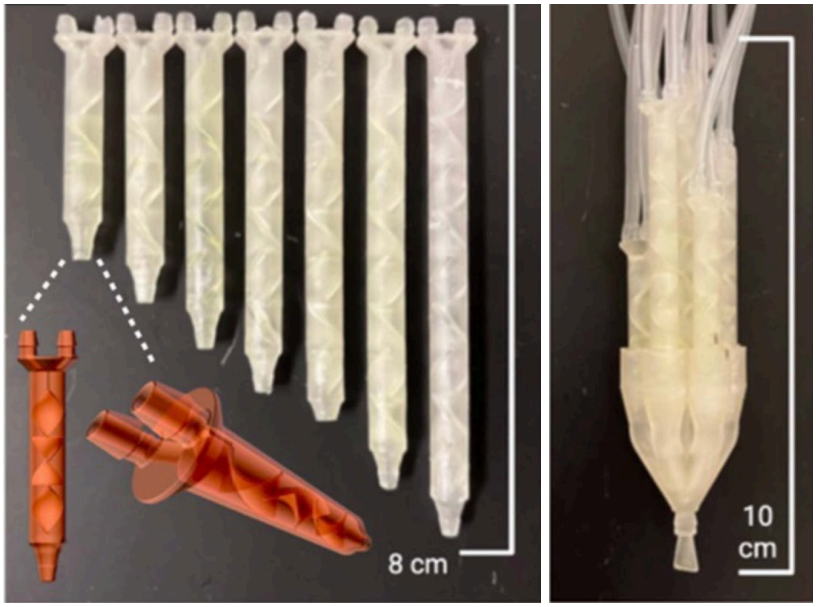

Figure 6: *The seven different sizes of KSM, from 3 to 9 mixing elements (left) and a CEVIC with the KSMs loaded into it (right)* [2]

In order to print these resolutions sequentially to mimic vasculature of the human body, the Dean Lab uses a Continuously Extruded Variable Internal Channeling (CEVIC) device. It is a novel, patent-pending invention that can achieve the aforementioned vasculature patterns and maintains the alternating channeling and resolution while converting the KSM outputs into a flat hydrogel sheet [2]. The CEVIC device (depicted in Figure 6 above) holds all of the KSMs. Sequential deposition of the different KSM outputs is controlled by a mechanical valve within the larger 3D printing Dean Lab setup (Figure 7 below).

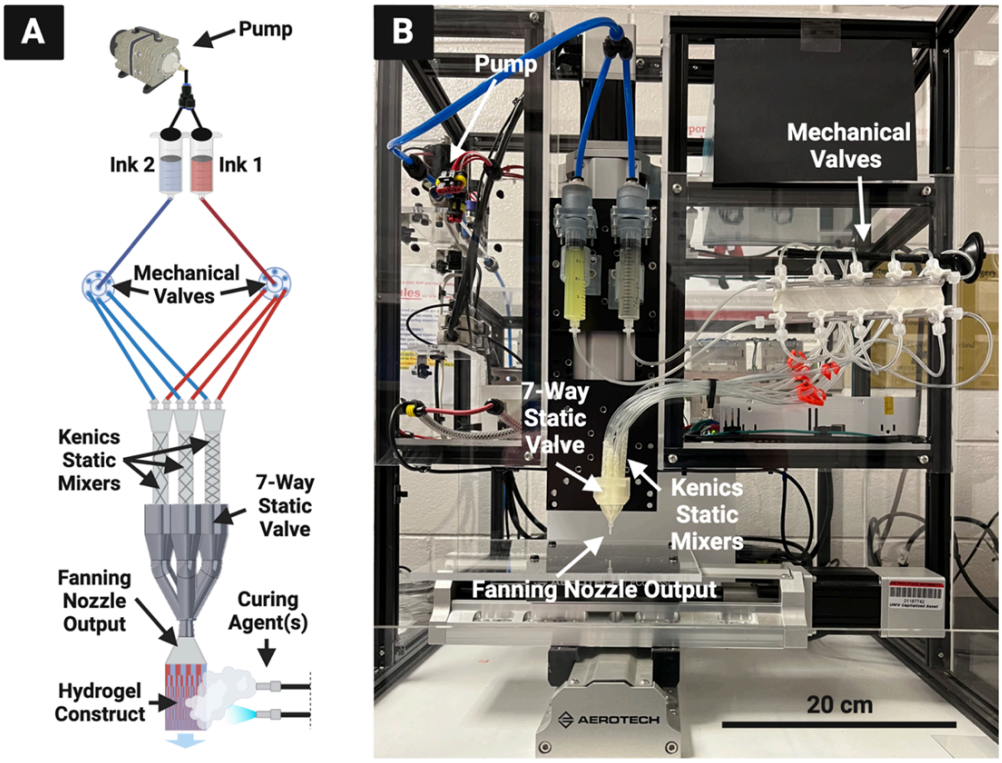

Figure 7: *Schematic of the chaotic printing setup (left) and the 3D bioprinting setup (right)* [2]

The mechanical valves controlling which KSM the GelMa and fugitive ink divert to, however, presents another challenge. In order for the sequence from larger channels to smaller and back to be seamless, the switch between inputs should be nearly instantaneous, and this is not achievable through a mechanical valve. The lab has also utilized a rotary valve to control this switch, however, the KSMs experience leakage due to the hydrogel material remaining in the tubing between the rotary valve and KSMs. Therefore, a seamless, automatable shutoff and switching mechanism is needed.

2.2 Problem Statement

To create an automatic, programmable valve to seamlessly shut off or switch between KSM outputs, and therefore multiple hydrogel resolutions.

2.3 Client Information

Dr. David Dean is a professor at the University of Wisconsin-Madison in the Department of Biomedical Engineering, whose lab focuses on surgical reconstruction or regeneration of skeletal structures [10]. He acquired a PhD in 1993 from City University of New York. His lab uses Computer Aided Design (CAD) to generate patient specific devices and implants, and then 3D prints them. He focuses on the 3D printing of resorbable tissue engineered bone scaffolds. To do this, the lab seeds cells, such as Mesenchymal Stem Cells (MSCs) or vascular progenitor cells onto 3D printed hydrogels, which is what this project pertains to. At the University of Wisconsin-Madison, he teaches several advanced Biomedical Engineering courses. He has made ten publications in the last two years, and is the recipient of the 2024 CIRP BioM Best Paper Award [10].

2.4 Design Specifications

The CEVIC device enables the fabrication of hierarchical, branching channels with continuous geometric and material gradients to mimic natural microvascular networks [2]. The team's shutoff valve must maintain the high precision of the system, extruding between 8 and 512 channels within 10 and 30 μm in diameter. Performance requires a less than 10% error between theoretical and measured channel widths, and transition lengths between channel regions are currently around 1 cm [2].

The device should support automated operation controlled via LabVIEW. For safety, valves must be removable for sterilization via UV, with ergonomic design to minimize injury risk per ISO 14971 and 62366. All materials must be biocompatible per ISO 10993-1 and function in standard lab environments (20-25°C, 35-50% RH) while withstanding operational temperatures up to 70°C [11]. The shutoff valve must not exceed 10% of total system weight and have a shelf life of 5 years [12]. Production will involve 5 prototype units with a total budget of $500. Testing will follow ISO 9001 and ISO/IEC 17025. The device is expected to produce a hydrogel sheet in 5 minutes, enabling multiple prints per hour.

3 Preliminary Designs

3.1 Design 1 - Clamp

The Clamp Design is an external feature intended to regulate fluid flow throughout the client's system. The clamps are designated to a certain KSM, and after the KSM is switched, the clamps on the tubing of the KSM that was previously operating is triggered to close, and the clamps on the tubing to the KSM that is now operating is opened. The clamps are placed directly on the tubing between the rotary valve and the KSMs. When necessary, the clamp will shut off to prevent wasted fluid output, as there is currently no mechanism to immediately stop fluid flow once the hydrogel printing is finished. One weakness of the design is that the clamps may introduce mechanical tubing degradation due to continuous pressures exerted by the clamps. One feature that is important for consideration when selecting the Clamp Design is its ability to be integrated into the client's framework, both electronically and mechanically.

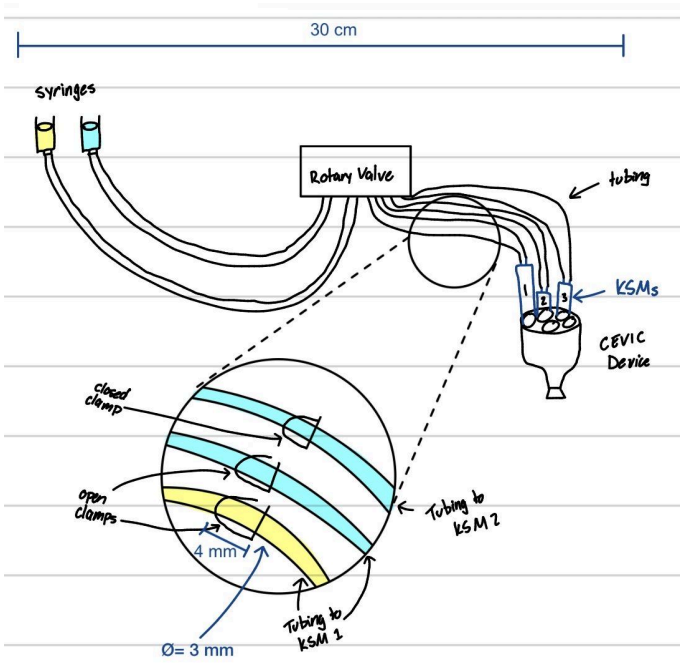

Figure 8: *Clamp design including syringes, rotary valve, tubing, and CEVIC device. Enlarged section of tubing shows Clamp Design.*

3.2 Design 2 - Integrated Rotary Element (IRE)

The IRE is a circular, uniform, disk of 25 mm. One feature of this design is its ability to rotate itself to allow one KSM at a time to deposit its fluid output (as evidenced by the singular hole depicted in Figure 9). If fluid output is needed from another KSM, the IRE can rotate to that KSM. This design excels in automating which KSM deposits fluid, which allows the client to print unique vascular patterns. The IRE design is also customized with gear teeth for integration with a Servo Motor and gear which can be programmed through an Arduino for automation. However, the continual rotation of the IRE within the CEVIC raises initial concerns about potential internal shear degradation to the CEVIC. Additionally, due to tight space restrictions in the CEVIC, the CEVIC may have to be redesigned to allow the IRE to fit and function properly with gears.

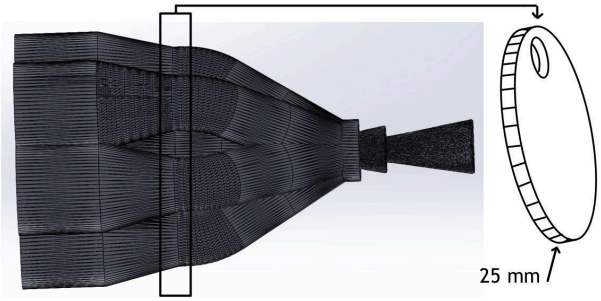

Figure 9: *Integrated Rotary Element design within the CEVIC Device*

3.3 Design 3- Flow Diversion System (FDS)

This device uses a manual 3-way valve between the rotary element and KSMs. Each valve has 2 positions, directing fluid from input to either waste or the KSMs. These two positions enable the operator to direct the system to control how fluid is utilized. For example, if the fluid did not have an appropriate pattern or contains bubbles, the FDS can redirect that undesirable fluid to waste while preserving the remaining fluid. One benefit of this system is its ability to precisely control fluid direction. Another benefit of this system is that it includes transparent tubing which enables visual monitoring of the hydrogel fluids. However, switching the valve consistently may lead to diminished quality of printed hydrogels due to disruptions in the fluid flow when

fluid needs to be redirected. Additionally, fabricating the valve to work with small volumes might be challenging.

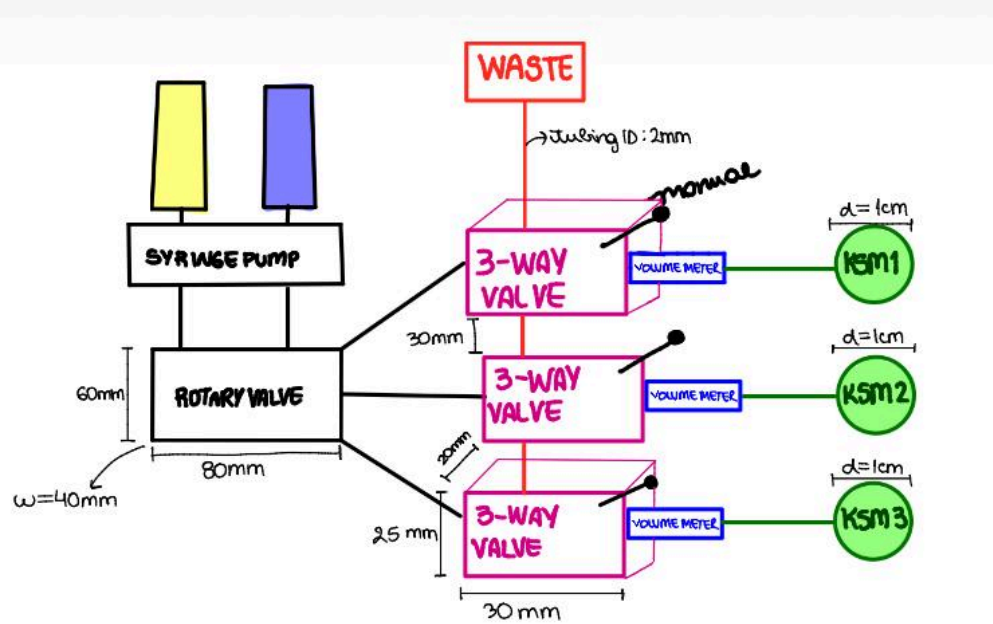

Figure 10: *Flow Diversion System schematic*

4 Preliminary Design Evaluation

4.1 Design Matrix

Table 1: *Design Matrix - Compares all three design ideas scored across six categories with the weight of each determined by the client's need and team's values.*

Criteria (weight)	Concept A: Clamp		Concept B: Integrated Rotary Element (IRE)		Concept C: Flow Diversion System (FDS)	
Maintain Pattern & Resolution (25)	*5/5*	25	*4/5*	20	*4/5*	20
Automatable (20)	*3.5/5*	14	*5/5*	20	*4/5*	16
Durability (15)	*3/5*	9	*3/5*	9	*3/5*	9
Ease of Fabrication (15)	*4/5*	12	*4/5*	12	*3/5*	9
Workflow Maintenance (15)	*4.5/5*	13.5	*3/5*	9	*4.5/5*	13.5
Safety (5)	*4.5/5*	4.5	*5/5*	5	*4.5/5*	4.5
Cost (5)	*4/5*	4	*5/5*	5	*5/5*	5
Total (100)	**Sum**	82	**Sum**	81	**Sum**	77

4.2 Summary of Design Matrix

The design matrix measured the categories of maintaining pattern and resolution, the automatable capabilities of the design, durability, ease of fabrication, workflow maintenance, safety and cost. The category that ranked the highest was the ability of the design to maintain pattern recognition due to this category being

one of the client's design specifications because achieving microvascular patterning and resolution is the goal of the client's research. This category serves as a quality control check to also choose a design that would minimize quality issues in the final hydrogel output (i.e. presence of bubbles, fluid leakage or backward flow, disrupted vasculature, etc.). Another important category is the automobility of the device because automating the design improves upon the client's current setup and will result in more distinct and accurate microvascular patterning.

The Clamp design scored highest in maintaining pattern and resolution and maintenance of workflow, since the clamping mechanism works upstream of the KSMs and CEVIC, and therefore would not impact the microvascular patterning or resolution. The Clamp design, however, is more difficult to automate than the IRE because each tube connecting to the KSMs would need its own clamp, and potentially its own motor, whereas the IRE only requires one motor total.

4.3 Proposed Final Design

Initial scoring of the Design Matrix concluded that the team should move forward with the clamp design, however, the two semester timeline and the nearly identical scores of the Clamp and IRE prompted the team to prototype both designs. Initial prototyping demonstrated the IRE as more feasible and first semester work focused on this design. The second semester will see the continuation of both the Clamp and the IRE design to ensure an optimal solution that meets all client and design requirements is reached.

The proposed final design of the Integrated Rotary Element includes the IRE itself complete with teeth to interface with a gear, a CEVIC device modified into two parts that interlock around the IRE, a Servo motor, and a holder to keep the Servo and CEVIC connected. Figure 11 shows the two halves of the modified CEVIC and how they lock together via the pin with the IRE in the middle.

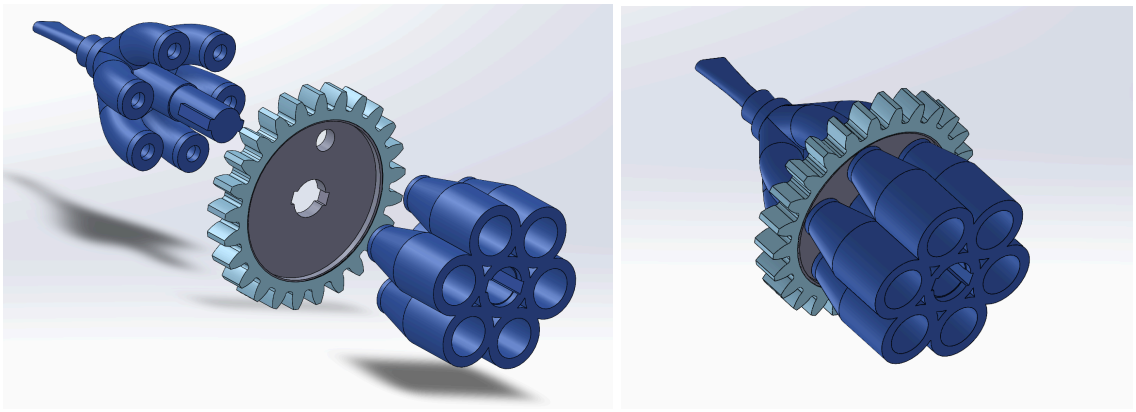

Figure 11: *3D model demonstrating how the CEVIC and IRE mate together*

5 Fabrication

5.1 Materials:

Mechanical Components

A servo motor is used to rotate a gear which rotates the IRE within the CEVIC. The servo motor was chosen because it was available at the University of Wisconsin-Madison's makerspace [13]. Also, it is compatible with an Arduino, which was accessible from past classes. The gear directly fits onto the shaft of the gear by friction. The gear was modeled in Onshape and printed with PLA so that it is sturdy, and the material is cheaper than printing out of Biomed Clear resin. The CEVIC and IRE are both 3D printed using Biomed Clear Resin at the University of Wisconsin-Madison makerspace to ensure biocompatibility and durability [13]. The IRE is secured in the middle of the CEVIC device using a pin mechanism that holds the CEVIC still while allowing the IRE to rotate. The sample holder was modeled on Solidworks and printed with PLA. The final assembly can be seen in Figure 12.

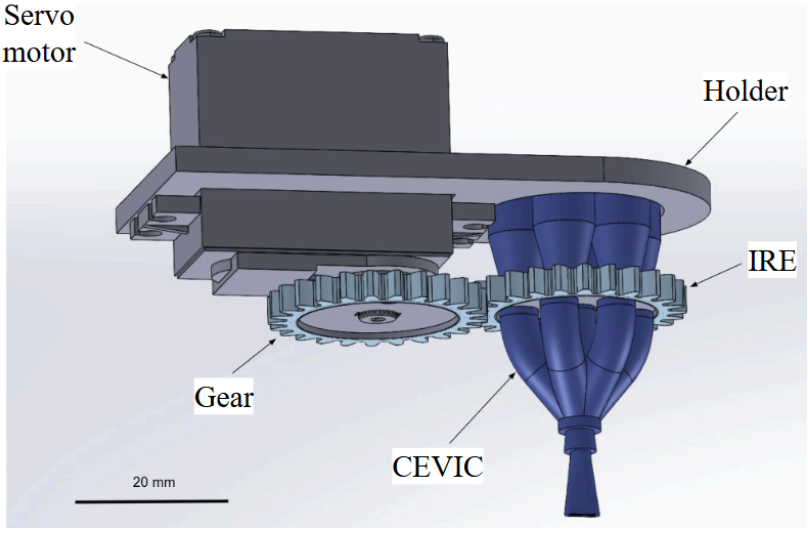

Figure 12: *3D model of assembled servo motor, gear, IRE, and CEVIC in holder*

Electrical Components

The circuit used for testing consists of a motor connected to a breadboard that has a button, and an Arduino. The Arduino executes code that sends a control signal to the servo to rotate 60 degrees when the button is pushed. Code used for testing can be found in the Appendix. The final circuit can be seen in Figure 13.

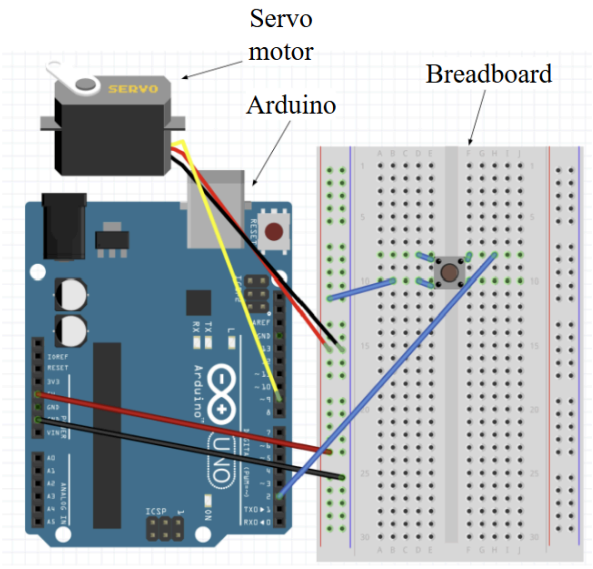

Figure 13: *Fritzing diagram of the circuit used to test Servo functionality*

5.2 Methods:

Integrated Rotary Element (IRE)

The IRE was modeled in Solidworks and consists of gear with a 3.5 mm hole the same diameter as the hollow inside of the CEVIC. The middle of the IRE has a hole with a pin mechanism to allow the CEVIC to lock into place while allowing the IRE to still rotate. To assemble the IRE and the CEVIC in the holder, pull the CEVIC apart and place the center of the IRE in between the two halves. Push the CEVIC together until the IRE is flush to the CEVIC on both sides. The CEVIC and IRE can be placed into the holder and pushed until firmly in place.

Assembly

The final assembly is created by inserting the CEVIC and IRE into the sample holder. Then, the gear is attached to the Servo motor, which is then inserted into the sample holder. The wires from the servo motor are then connected to the corresponding parts of the circuit (power, ground, and PWM). The circuit is assembled and code uploaded to the Arduino so that when the button is pressed, the servo turns the gear 60 degrees, and because the teeth on the gear and the IRE are the same, the IRE also rotates 60 degrees.

Final Prototype

The final prototype consists of the CEVIC and IRE printed out of Biomed Clear resin, placed in the holder printed out of PLA, and the servo with a PLA gear also inserted into the holder. The servo is connected to a circuit on the breadboard, which executes code uploaded to an Arduino. See Figure 12 for a labeled assembled Servo motor, gear, IRE, and CEVIC in the holder. See the fabricated prototype in Figure 14 below.

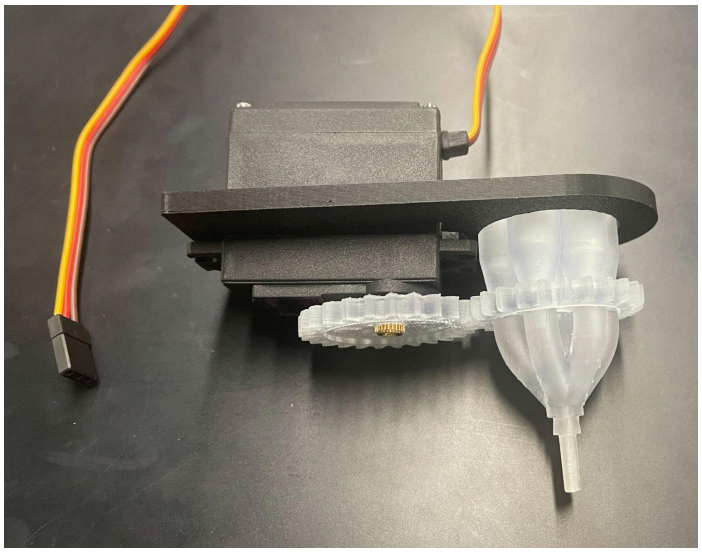

Figure 14: *Final assembled prototype*

6 Testing

Preliminary functionality testing utilized gelatin hydrogels of two different colors in order to save on costs and prove feasibility before continuing testing. In the future, one syringe will be filled with the GelMA/alginate mixture, while the second syringe will contain the fugitive ink. This first test was a control using water in both syringes with the IRE in the closed position, and the second test used water with the IRE in the open position. The third and fourth trial repeated the open and closed position of the IRE, but used the two colored gelatins to more closely replicate the hydrogels that will be used. “Closed position” refers to when the hole in the IRE does not line up with the hole in the CEVIC, meaning fluid should not be able to flow through. “Open position” refers to when the IRE hole is aligned with the CEVIC to allow fluid to pass through. For each of the tests, the researcher documented any difficulties related to the automatic switching of the shutoff valve and made detailed notes on any leakage observed, including when it occurred and the specific locations where it appeared. The researcher also evaluated whether or not alternating channels could be discerned from the resulting gelatin hydrogel.

Durability testing evaluated the device’s ability to meet the following client and design requirements: The device must be able to operate for 5 minutes per hydrogel sheet and print multiple hydrogel sheets per hour. This test assessed the ability of the IRE to maintain its strength and functionality over repeated use and therefore estimated the life in service of the IRE. Five samples were subjected to a simulated life-in-service test consisting of one hundred cycles, where each cycle is defined as rotation of the IRE 180° and back. Samples are considered to pass if there is minimal visible damage to the IREs.

Electronic testing evaluated the device’s ability to meet the client and design requirements associated with automated operation of the shutoff valve and the IRE. Testing verified that the shutoff valve can switch between KSMs without human intervention and that the servo motor reliably rotates the IRE by the required 60° for each cycle. Additional measurements were taken to assess servo accuracy and timing. Using Arduino, the time required for the IRE to rotate 60°, 120°, and 180° was recorded, and the corresponding angle deviations

were measured. A protractor was used to confirm that the IRE consistently returned to the same starting position between cycles. Refer to the Appendix for detailed functionality, durability, and electronic testing protocols.

7 Discussion of Results

During durability tests, the IRE experienced immediate scratches, however, after the first couple of cycles, no new scratches began to appear. Many factors led to this outcome, including the friction between the rotational elements and the material properties of Biomed Clear Resin. The surface scratches were negligible when compared to the thickness of the IRE, and therefore the strength of the IRE was not impacted. See Figure 15 for an image displaying the extent of the scratches.

Figure 15: *Scratches on IRE post testing*

Additionally, the IRE rotated with the appropriate degrees consistent with the arduino code and motor operation. As a result, the IRE can rotate to the appropriate KSM to dispense the GelMA and HEC. This result validates effective integration and control of the motor to make precise hydrogels. However, methods to confirm the angular displacement were limited with the use of a protractor, contributing to error. Furthermore, the IRE and CEVIC were able to print 3 hydrogels with striated patterns mimicking the vasculature. Although it is promising that the system can output hydrogels, it is worth noting that bioink backflow was observed during the process. This suggests a need to divert the fluid in the system to avoid system failure and diminished hydrogel quality. See Figure 16 for images of functionality testing.

Figure 16: *Testing pushing two different colored gelatins through the CEVIC and IRE (left) and the resulting hydrogels (right)*

Backflow in bioprinting methods is a known risk factor in extrusion-based bioprinting and can be attributed to poor sealing of components, low viscosity bioinks, and complex setups. A 2021 paper by Wang *et al*. introduced a novel valve-based consecutive bioprinting method that incorporates pressure-driven controls and microfluidic micromixers [14]. The main feature of this method involved a two-way pinch valve that enabled a continuous flow of bioinks that switched between bioinks in a seamless fashion to print vascularized muscle constructs. Frameworks such as these can be implemented in future iterations of the CEVIC/IRE configuration to regulate fluid flow through KSM outputs.

From an ethical standpoint, this research is conducted with a basis of accelerating tissue development. In 2021, over 100,000 individuals awaited an organ transplant and over 6000 individuals died while waiting [15]. The shortage of organ donors, combined with the risk of rejection from transplants, invites room to develop novel tissue engineering approaches to accelerate progress in this field [16].

8 Conclusions

Tissue engineering is a growing field with broad potential. However, many challenges remain to vascularize engineered tissues for necessary perfusion. “Chaotic printing” of hydrogels using KSMs and a CEVIC device can print hydrogel sheets down to specific resolutions, but a problem remains in switching between these resolutions while printing to imitate how arteries decrease in diameter to capillary size. For this reason, two designs were brainstormed, the Clamp Design and the Internal Rotary Element device. The IRE element showed early promise of meeting the Design Specifications and will continue to be modified during the Spring 2026 semester. More specifically, the IRE’s material choice and durability will be further evaluated to mitigate early damage during testing.

The team will conduct performance tests to evaluate the Shutoff Valve functionality to determine if it meets the following client requirements:

a. The shutoff valve can seamlessly switch between KSM resolutions, the shutoff valve can switch between KSMs without human intervention
b. The resulting vascular networks maintain their resolutions and alternation pattern
c. The shutoff valve limits dead space.

This test simulates printing a hydrogel using manual syringe methods and dyed gelatin rather than the 3D Bioprinter and the GelMa and HEC hydrogels. Performance testing includes four tests printing five hydrogel sheets, employing two 20 mL syringes mounted on a syringe pump, a CEVIC, the shutoff valve, and up to six Kenics Static Mixers (KSMs).

Additionally, the team is interested in methods to control fluid flow throughout the CEVIC and have started to brainstorm potential solutions. Fluid control will be an integral component of testing during the Spring 2026 semester.

9 References

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10 Appendix

10.1 Product Design Specifications

Function

On any given day, over 100,000 people in the United States are waiting for a life saving organ donation [1], [2]. The need for organs far exceeds the amount of organs donated per year. Because of this, many researchers are looking towards tissue engineering to fill the demand for transplants, as well as tailor them to a patient's specific needs. One of these methods is known as "bioprinting," which is the use of viable cells, biomaterials, or biomolecules in a 3D printer [3].

Within bioprinting there exist several methods, one such is "chaotic printing." Chaotic printing is a bioprinting strategy utilizing a kenics static mixer (KSM) to produce alternating channels of high resolution filament (less than 10 μm in width). These KSMs can be combined with a Continuously Extruded Variable Internal Channeling, or CEVIC, device to extrude these high resolution hydrogels into sheets while maintaining the alternating channel structure of chaotic printing. It should be noted that both the KSM and CEVIC devices are patent-pending [3].

Currently, the CEVIC devices can autonomously print hydrogel sheets of one resolution. If multiple resolutions from multiple KSMs are needed, the inputs must be manually changed. This takes time for the researcher and does not allow for a seamless transition between hydrogel channel resolutions. Therefore, the purpose of this project and the function of the device is to be an automatic valve to seamlessly shut off or switch between KSM outputs, and therefore hydrogel resolutions, ideally programmed so as to not need an operator.

Client requirements

The client has the following requirements:

- The shutoff valve can seamlessly switch between KSM resolutions.
- The shutoff valve can switch the KSM fluid without human intervention.
- The resulting vascular networks must maintain their resolutions (from 10 micrometers at the smallest to 1 millimeter at the largest).
- The resulting vascular networks must maintain their alternating pattern.
- The shutoff valve must limit dead space.
- The shutoff valve must be low shear on the cells passing through.
- The shutoff valve must be sterilizable via UV and autoclave and must withstand the following for 15 minutes:
    - 121^{o}C [4]
    - 15 psi [4]
    - 100% humidity [4]
- The shutoff valve material must be biocompatible.

Design requirements

1. **Physical and Operational Characteristics**
    a. **Performance requirements:** The CEVIC device enables the fabrication of hierarchical, branching channels with continuous gradients in geometry and materials, effectively mimicking natural microvascular networks. Its main advantage is precise structural control, but the microvalve's slow opening (around 9.2 seconds for a 30 degree valve) due to pressure drop and oxide buildup limits rapid, dynamic switching in bioprinting [3]. The syringes must operate at a rate of 1 milliliter per minute and 0.5 bar. The kenics static mixers should be able to mix GelMA and HEC fugitive ink to ultimately form a consistent, cohesive striated composition pattern that can be printed. The KSMs should also be able to maintain a watertight seal when attached to the valve rotary. The valves near the KSMs should be able to shut on/off to allow the appropriate mixture compositions to be printed. The final fluid mixture is

then transported to the nozzle output and should be able to extrude between 8 to 512 channels between 10 and 30 micrometers.

Printing can occur via an automated or manual process. In the automated version, pumps operate at a rate of 3.3 millimeters per second. In this setup, LabVIEW controls 2 rotary valves that switch between KSMs to control printing time and overall fluid composition. The automated electric rotary valve should be able to direct fluid to its appropriate KSM. In the manual process, the user must manually operate a stopcock on the valves to maintain hydrogel flow between KSMs without interruption.

b. **Safety:** The valve should be removable for sterilization purposes after each use. The manually operated valves should prioritize ergonomic design to ensure safe clamping and minimize potential injury risks during use. To reduce risk, the design should minimize direct contact with device components. A programmed pause feature can also be incorporated, allowing the user to safely adjust the equipment without concern of automated parts moving. The components should also be able to withstand autoclave temperatures. Proper precautions should be implemented to minimize safety risks in accordance with ISO 14971, 62366.

c. **Accuracy and Reliability:** The system should be able to operate with a high degree of precision and accuracy, given that capillary sizes are small (μm range) the morphology of the printed biomaterial is directly correlated to its function. The hydrogels undergo a sodium alginate and $CaCl_2$ reaction to cure the hydrogel, ensuring its structural integrity and durability.

   A 10 percent error between measured and theoretical channel widths, which stresses the importance of incorporating valve shutoff mechanisms to avoid depositing too much/little bioprint fluid. Additionally, it is critical to expose the hydrogel to $CaCl_2$ while it prints to help improve thickness uniformity and minimize disruptive channel flows.

   Finally, transition lengths between varying channel number regions were around 1 centimeter [3]. However, decreasing the fluid output rate and automating the valve switch could help achieve reduced transition lengths which would enhance the reliability of the hydrogel.

d. **Life in Service:** The device must be able to operate for 5 minutes for each hydrogel sheet. Over time the device should be able to print multiple hydrogels per hour.

e. **Shelf Life:** The shutoff valve is expected to maintain reliable performance for approximately 5 years.

f. **Operating Environment:** Materials must be biocompatible in accordance with ISO 10993 and capable of withstanding operating temperatures up to 70 degrees celcius. Standard laboratory conditions will apply during use, with ambient temperatures maintained between 20 and 25 degrees celsius and relative humidity in the range of 35–50 percent relative humidity, as recommended for controlled laboratory environments [5].

g. **Ergonomics:** Valves should be designed to minimize shear stress on the materials passing through and must allow intuitive manual operation with a controllable flow rate as low as 1 milliliters per minute [3]. The motors controlling the valves should be able to be neatly and compactly integrated within the device to prevent interference with biological samples and to isolate electrical components from user contact.

h. **Size:** The KSMs are about 12 centimeters in height and 1 centimeter in diameter, with channel flow rates ranging from 1 to 1.5 milliliters per minute. It is important to account for physiological dimensions, including artery and capillary diameters, as well as the distance between capillaries and cells in the body. The smallest arteries measure roughly 150 microns in diameter, while the smallest capillaries are approximately 10 microns [3]. A capillary must be within 50 to 70 microns of every cell in the body in order for the cell to have sufficient blood flow. Accordingly, the CEVIC device must be able to print within these dimensional constraints. The current manual valve to select the channel the bioink flows through is 16 centimeters in width and the current automatic valve to select the KSM is 5 centimeters in diameter [3].

i. **Weight:** The shutoff valve, positioned around either the manual or automatic rotary valve, should not exceed 10% of the total system weight. This restriction ensures that the valve remains sufficiently lightweight to operate effectively without compromising the performance of the mechanical components [6].

j. **Materials:** The CEVIC device and KSM are fabricated using clear biocompatible resin. The material selected for the shutoff valve must be chemically compatible with this resin, ensuring that it does not cause degradation or alter its properties. A solution of 3 percent GelMA and 2 percent sodium alginate is usually heated to allow it to flow through the KSMs [3].

k. **Aesthetics, Appearance, and Finish:** The shutoff valve should integrate seamlessly with CEVIC machine and any relevant connected components without causing excessive wear or interfering with functionality.

2. **Production Characteristics**

a. **Quantity:** The client requires only 1 to 2 units. For testing purposes, 5 units will be produced to support the testing requirements. The testing protocol will involve repeating experiments with the same prototype across multiple runs to ensure reliable and statistically meaningful results.

b. **Target Product Costs:** The total budget is $500, with a target cost of $15 per unit. Most expenses are expected to arise from 3D printing materials, with some additional electronic components purchased separately. The actual production cost for 5 units, estimated at $75, is projected to remain well below the allocated budget.

3. **Miscellaneous**

a. **Standards and Specifications:** Production and testing will follow established standards to ensure accuracy, safety, and regulatory compliance. The protocols and reference materials described below define the requirements for fabrication, measurement, and validation of the prototypes. Fluidic resistance measurements follow standard protocols using pressure steps (5 and 10 milibar) with controlled flow intervals (30 seconds), employing ISO-calibrated instrumentation such as the Druck DPI520 pressure controller and Sartorius MC1 LP620P scale, ensuring traceability and compliance with ISO 9001 and ISO/IEC 17025. EN-ISO 10993-1:2009/AC:2010 (Class I Biocompatibility) is referenced for guaranteeing biocompatibility and safety for direct or indirect biological contact, which is critical for cell culture applications and potential medical device use.

b. **Customer:** The client prefers that the valve be programmable to run for different combinations KSM, thereby reducing operator time and effort.

c. **Patient-related concerns:** This device is not patient contacting, therefore there are no patient-related concerns. This device does not store any patient data. However, this device may come into contact with a cell-seeded bioink. Therefore, the material and finish of the device should be biocompatible, non-toxic and low-shear to prevent unnecessary cell death and cell rupture.

d. **Competition:** There are numerous bioprinting valve techniques that have demonstrated low leakage rates and adaptability for systems operating at resolutions as fine as 10 micrometers. The Continuous Chaotic Bioprinting of Skeletal Muscle-like Constructs produces multi-layered, multi-material filaments with microvascular channels at resolutions down to 10 micrometers, demonstrating strong potential for complex tissue architectures [7]. The chosen material, alginate hydrogels, presents a challenge as it may not be optimal for clinical translation [7]. Configurable 3D Printed Microfluidic Multiport Valves with Axial Compression use stepper motor control for precise, automated switching, with no leakage in static tests and less than 0.5 percent in dynamic use [8]. The testing configuration used 800 micrometers channels, which are relatively large, raising uncertainty about performance at the smaller sizes needed [8]. The novel on-chip liquid-metal microvalve enables precise directional control of fluid flow, with no leak detected at pressures up to 320 millibar and a leak rate of less than or equal to 0.043 microliters per minutes at 330 millibar [9]. The method does not address sequential layering or branching between K mixers, requiring adaptation for applications involving complex fluid routing [9].

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10.2 Material and Expense Report

Item	Description	Manufac-turer	Mft Pt#	Vendor	Vendor Cat#	Date	#	Cost Each	Total	Link
Category 1										
	3D Printed CEVIC & 5 KSMs	N/A (3D Printed)	N/A	N/A	N/A	09/19	1		$3.48	
	3D Printed CEVIC and 8 KSMs					10/20	1		$12.60	
	3D Printed CEVIC and gear					11/10	7		$7.63	
	3D Printed 10 IRE gears for testing samples					11/18			$12.65	
	3D printed connector for CEVIC and Servo Motor					11/20			$0.14	
Category 2										
	Purchase makerspace Servo	Smraza				10/24	2		$5.28	
	PLA 3D printing						3		$0.27	

	Rotational Element									
	Additional Gear						3		$0.15	
	Makerspace 3D printed					10/24	1		$1.83	
								TOTAL:	**$44.20**	

10.3 Fabrication Protocols

Functionality testing

Purpose

The purpose of this testing is to evaluate the functionality of the complete assembled Shutoff Valve. This test simulates printing a hydrogel using manual syringe methods and dyed gelatin rather than the 3D Bioprinter and the GelMa and HEC hydrogels.

Equipment and Materials

This test uses the following equipment:

- Hot plate
- 2x 50 mL beaker
- 2x stir bars
- 2x weight boats
- Graduated cylinder
- CEVIC-Servo complex
- Up to 6 Kenics Static Mixers (KSMs)
- 2x 20mL Syringes

This test using the following materials:

- 2 food dyes of contrasting colors
- Gelatin
- DI Water

Procedure

Gelatin Preparation

1. Acquire all materials
2. Measure 20 mL of DI water using a graduated cylinder, then pour into 50 mL beaker
3. Repeat step two for the second beaker
4. Measure out 4 grams of gelatin (2 g per beaker), then add to the beakers. Swirl gently to mix
5. Let gelatin bloom in the cool water for 5-10 minutes
6. Add a stir bar to each beaker, then place them on a hot plate turned on to 70ºC. Heat and stir until the gelatin dissolves (approximately 10 minutes). Add one drop of food coloring near the end of the mixing process
7. Remove beakers from hot plate(s) and allow to cool to desired consistency

Testing

8. Prepare the CEVIC in the “closed” position (where the hole in the IRE is NOT lined up with any of the CEVIC holes
9. Place one KSM into the CEVIC
10. Fill up two syringes with water and attach to the two ports on the KSM
11. Simultaneously and slowly push syringes to flow water through the KSM. Make notes of when and where leakage occurs on the KSM
12. Repeat the water test with the CEVIC and IRE in the “open” position. Make notes on if leaks are present and where they occur, as well as how the water flows through the KSM and CEVIC
13. Keep the CEVIC and IRE in the “open” position. Repeat the test with the two colored “hydrogels.” Make notes on any leakage and flow observations.
14. Finally, return the CEVIC and IRE into the “closed” position. Repeat the test with the two colored “hydrogels.” Make notes on any leakage, and when and where it occurs
15. Clean up and put away all supplies

Durability Testing

Purpose

The purpose of this testing is to evaluate the durability of the Integrated Rotary Element (IRE) and therefore the life in service. It verifies that the strength and functionality of the IRE do not diminish with repeated use. The test will first run five samples through a simulated life in service of one hundred cycles to and from 180^{o}. These five samples will be visually inspected for wear/cracks/etc. Then, the five test samples and five control samples will be loaded onto the MTS testing machine and compressed until failure. The samples will pass if there is no statistically significant difference between the test samples and the controls, and if there is minimal visible damage to the IREs.

Durability testing evaluates the following client specifications and product specifications:

- Life in service: The device must be able to operate for five minutes per hydrogel sheet and print multiple hydrogels per hour

Equipment and Materials

This test uses the following equipment:

- MTS Universal Testing Machine
- 10 kN Load Cell

This test uses the following materials:

- CEVIC and IRE
- Servo motor
- CEVIC-Servo connector
- Breadboard
- Arduino
- Standard wires

Procedure

Setup

1. Label each of the ten test samples with a unique number/color. Avoid the center of the IRE as the label may be scraped off during testing. Label five of them as test samples and five of them as control samples.

2. Assemble the Arduino, breadboard, and wires, in the following configuration, with the red wire connected to 5V, the black/brown wire connected to GND, and the yellow/orange wire connected to digital pin 9:

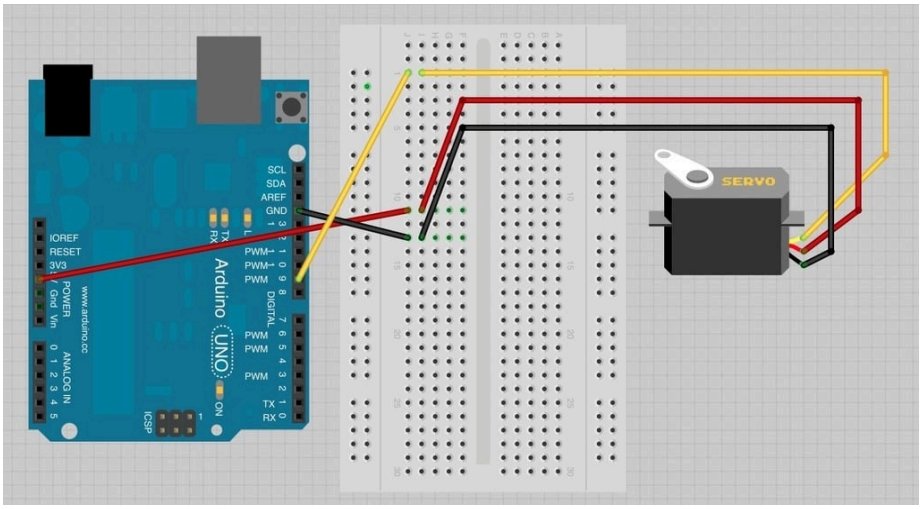

Figure 1: Breadboard schematic for the servo sweep functionality [1]

3. Open the Arduino IDE software on a computer and connect the computer to the Arduino using a USB Type-A to USB Type-B cord.
4. Select “File” -> “New” to create a new sketch
5. Copy and paste the following code into the new sketch

```
#include <Servo.h>

Servo myservo;  // create servo object to control a servo
// twelve servo objects can be created on most boards

int pos = 0;    // variable to store the servo position
int i = 0;

void setup() {
  myservo.attach(9);  // attaches the servo on pin 9 to the servo object
}

void loop() {
  for (i = 0;i <= 250; i++){
    myservo.write(0);
    delay(250);
    myservo.write(180);
    delay(250);
  }
 Serial.print("I'm done");
}
```

6. Be sure to select “Tools” -> “Board” to ensure the correct board and output are selected
7. Compile the sketch and confirm there are no errors

Motor Testing

8. Place a gear onto the Servo Motor
9. Place the first test sample IRE into CEVIC and press the two halves together into a snug fit
10. Place the CEVIC-IRE and the Servo motor into the CEVIC-Servo connector in such a way that the two gears interface
11. Upload the code onto the Arduino. The Servo motor should rotate between 0^o and 180^o and then back again one hundred times to simulate life in service. Make note of any observations during testing
12. Once completed, remove the IRE from the CEVIC. Make note of any visual observations about the IRE. Take an image next to a reference (such as a ruler).
13. Return the IRE to the CEVIC-Servo. Run the test for another two hundred fifty cycles.
14. Repeats steps 12-13 for a total of one hundred cycles
15. Repeat steps 9-14 for the rest of the test samples. Let the motor rest for 5-10 minutes minimum between each 100 reps. (problems with it slowing down/maybe overheating). Do NOT test the control samples. Set those aside for MTS testing

MTS Compressive Testing (See 1002 Universal Testing System documentation for guidance)

16. Attach the 10 kN Load cell to the crosshead of the Instron
17. Attach the 3-point bending fixture to the upper and lower clevis of the MTS machine. Insert the pin, then tighten the collar
18. Set the safety stops in such a way that the top and bottom fixtures will not collide
19. Open TestSuite on the computer. Select “file new” -> “test” -> “from template” -> “BME 315 Compressive Failure Testing”
20. Enter parameters, then Zero the load
21. Prepare samples in the center of the 3-point bend fixture by centering and securing using the small pins and the rubber bands
22. Unlock the crosshead on the handset and move it towards the sample until it just barely loads
23. Zero the system on the MTS computer screen
24. Select “Play” on the software and run until failure
25. Stop the tester once

Reference:

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Electronics Testing

Test 1: Alignment Drift Over Cycles

Objective: Measure how long (number of turns/cycles) it takes for the IRE hole alignment to misalign

Parameters: Test pulse width corresponding to 60° rotation (expected pulse: 1.5 ms high from 1-2 ms neutral range; confirm via datasheet for SG90-like micro servo).

- Perform cycles until 1 cycle returns to normal displacement (indicating reset).

- Check: Calculate expected pulse width modulation (PWM) for precise 60° rotation (e.g., 50 Hz signal, 1.5 ms pulse ≈ 60° from neutral).
- Record number of cycles to **50% misalignment**

Test 2: Mechanical Torque Measurement

Purpose: Confirm servo functionality and ability to rotate IRE by exactly 60° per cycle using force sensor or protractor. Quantify torque via arm displacement and load.
Steps:

1. Run standard Arduino code (pneumatic extrusion at >1 m/min with KSM chaotic mixing).
2. Attach servo arm to IRE; measure angular displacement with digital protractor (target: 60° ±2°).
3. Repeat for 10+ cycles, logging delay periods where rotation occurs at unknown speed.

Key Observations:

- 3-prong IRE: Reaches 50% non-alignment after ~10 prongs (3 KSM rotations, traveled 3 KSM distances over 3 trials).

Test 3: Starting Point Stability

Objective: Verify if starting alignment position drifts before cycle completion.
Steps:

1. Initiate cycle and interrupt mid-rotation (before 60° finish).
2. Measure starting point reference (e.g., via optical sensor or marking).

Results: Starting point remains unchanged (no drift observed; n= trials confirm stability).

Updated Recommendations for 20 Nov:

- Integrate force-torque sensor (e.g., HX711 load cell)
- refine PWM to 1.45-1.55 ms for 60°,
- Perform more cycles with 30 ms delays.
- Estimate velocity to travel from 1 KSM to the other