

Microvascular Channel Bioprinter Shutoff Valve
Final Report

BME 402 Design

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Abstract

Tissue engineering is a rapidly advancing industry that grows and utilizes cells, tissues, and organs to address clinical and research needs. However, one persistent problem in the field is inadequate vascularization and perfusion of tissues. One solution uses chaotic printing to achieve highly ordered structures from biofluids. Kenics Static Mixers (KSMs) create an alternating pattern of bio- and fugitive ink, which is extruded in hydrogels using Continuously Extruded Variable Internal Channeling (CEVIC) technology. The hydrogel outputs mimic vasculature from the artery level down to the capillary level. However, challenges remain with instantaneously switching between KSM outputs to achieve sequentially smaller hydrogel resolutions. Therefore, an automatic shutoff valve is needed. A solution involves using a rotational element fitted within the CEVIC device that only allows output from one KSM at a time. Initial testing demonstrated effective switching between KSMs, and future improvements will focus on refining hydrogel resolutions through faster switching. This research allows tissue engineered 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 [1]. 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 [3]. 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 [4]. 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 Anatomy and physiology of vascularization

To achieve necessary perfusion, every cell in the body must be within 50-70 μm of a blood vessel [3]. 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]. Firstly, achieving the resolution of microvasculature remains a main issue. Capillaries are responsible for cell perfusion, and the smallest capillaries are 10 μm in diameter, and fabricating a channel that small is challenging for 3D bioprinting and other tissue engineering methods [2]. Secondly, the artificial microvasculature must be able to connect to and mimic the body's circulatory system, which includes progressive branching of arteries to arterioles to capillaries (as well as from capillaries to venules to veins).

1.3 Existing technologies

Conventional Extrusion 3D Bioprinting utilizes multi-material filaments to create complex tissue architectures while maintaining low leakage rates. However, this approach faces three significant limitations, including a resolution constraint of 100-200 μm risks damaging cells during printing due to extrusion pressure [5]. 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 [6]. While effective when tested in 800 μm diameter channels, their performance at smaller scales, such as 10 μm , remains unverified.

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 $\leq 0.043 \mu\text{L}/\text{min}$ at 330 mbar [7]. Their principal limitation is a lack of inherent sequential layering or branching capability, which necessitates design adaptation for complex systems.

1.4 Chaotic Printing and the CEVIC Device

Chaotic bioprinting is an extrusion 3D bioprinting technique that utilizes Kenics Static Mixers (KSMs) to produce channels of significantly higher resolution compared to other extrusion bioprinters [2]. KSMs work through the chaotic advection of two or more materials. Chaotic advection is the phenomenon where laminar flow fields produce complex, exponential stretching and folding of fluid elements [8].

The 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 the latter is vacated, leaves empty inner channels. These vacant channels serve as the scaffold for microvasculature within the hydrogel.

Multiple KSM sizes with increasing numbers of KSM elements produces the various microvasculature resolutions [2]. Seven sizes with 1 through 7 mixing elements produce between 8 (the largest resolution) and 512 (the smallest resolution) channels. See Figure 1 below depicting the different sized KSMs.



Figure 1: Five of seven different sizes of KSM, from 1 to 5 mixing elements (left) and a CEVIC (right)

The Continuously Extruded Variable Internal Channeling (CEVIC) device prints these resolutions sequentially to mimic vasculature of the human body. It 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 holds all of the KSMs. Sequential deposition of the different KSM outputs is controlled by a mechanical valve within a larger Voron 3D printing setup, which has been augmented for bioprinting purposes (Figure 2).

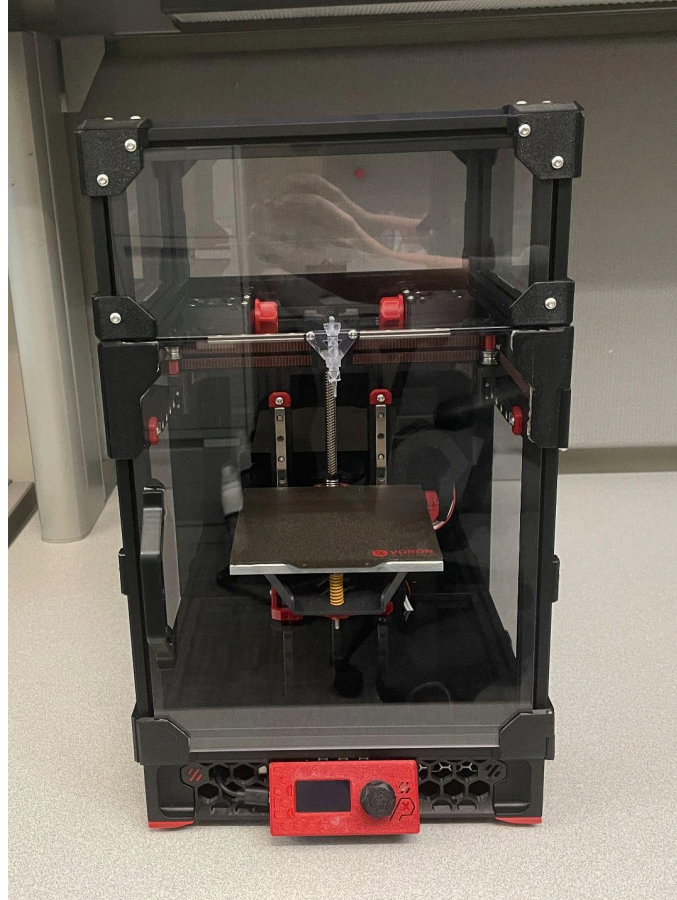


Figure 2: Voron 3D printer augmented to be a 3D bioprinter. See Figure 6 below which displays how the CEVIC and KSMs integrate into the Voron setup

The mechanical valves controlling which KSM the GelMA and fugitive ink divert to, however, present 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. A rotary valve controls 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.

1.5 The Integrated Rotary Element (IRE)

The Integrated Rotary Element (IRE) was devised to address the challenge of switching between microvascular channel resolutions. The IRE is an automatic, programmable valve to seamlessly shut off and switch between KSM outputs, and therefore multiple hydrogel resolutions. The new apparatus 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 which rotates a gear, and a holder to keep the Servo and CEVIC connected. Figure 3 shows the two halves of the modified CEVIC and how they lock together via the pin with the IRE in the middle. Figure 4 below displays all components together.

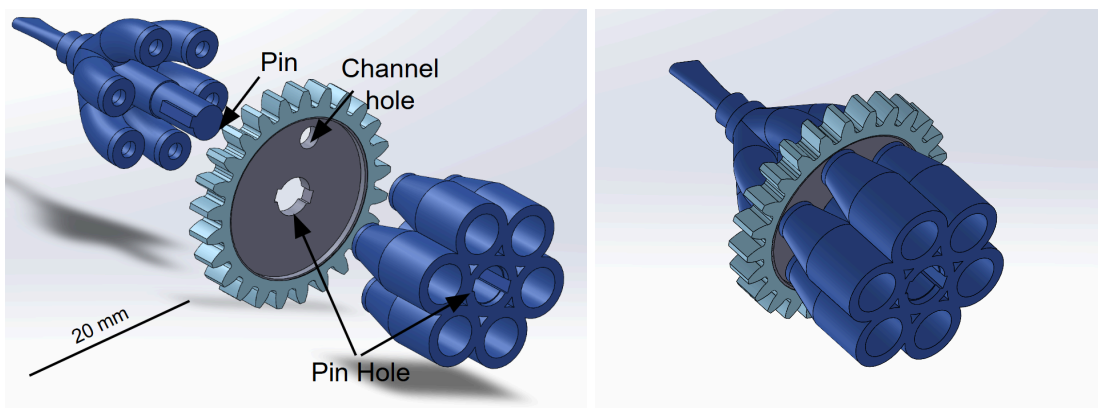


Figure 3: 3D model demonstrating how the CEVIC and IRE mate together

The IRE 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 175-250 μm for a total hydrogel length of 2-3 cm [2].

The IRE supports automated operation controlled via Arduino. For safety, CEVIC, IRE, and KSMS are removable for sterilization via UV, with ergonomic design to minimize injury risk per ISO 14971 and 62366. All materials are biocompatible per ISO

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10993-1 and function in standard lab environments (20-25 °C, 35-50% RH) while withstanding operational temperatures up to 70 °C [9]. The shutoff valve does not exceed 10% of total system weight and has a shelf life of 5 years [10]. Testing followed ISO 9001 and ISO/IEC 17025. The device can produce a 2 cm long hydrogel sheet within 5 minutes, enabling multiple prints per hour.

2 Materials and Methods

2.1 Materials

Mechanical Components

The main mechanical components of the design are as follows: the CEVIC (split in two halves with a locking pin), the IRE, a Gear, a high torque 35 kg Servo motor, and a holder [11]. The CEVIC and IRE were fabricated from Formlabs' BioMed Clear Resin to ensure biocompatibility and durability [12]. The Gear and holder were made from Bambu Labs PLA. A 35 kg high torque Servo motor, compatible with Arduino, was chosen to overcome large friction forces that arise on the IRE from the two CEVIC halves.

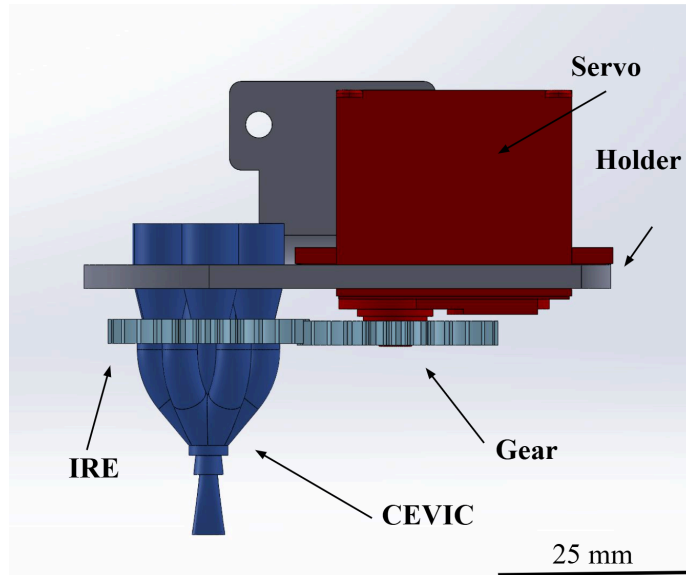


Figure 4: 3D model of assembled servo motor, gear, IRE, and CEVIC in holder

Electrical Components

The circuit consists of the Servo motor, breadboard with a button, and an Arduino. The system is coded to rotate the gear 60° when the button is pushed. Code used for testing can be found in the Appendix 7.1. Figure 5 displays the final assembly.

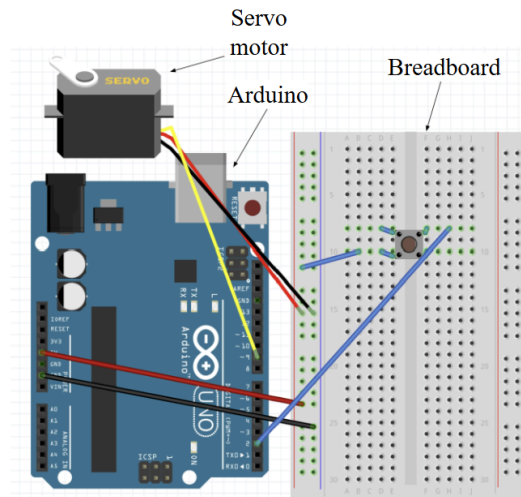


Figure 5: Fritzing diagram of the circuit used to test Servo functionality

2.2 Methods

The gear and CEVIC were modeled through a combination of OnShape and Solidworks. 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 channels. The center of the IRE has a hole with a pin mechanism to allow the CEVIC to lock into place while still permitting IRE rotation. The IRE and CEVIC were 3D printed using BioMed Clear Resin on a Formlabs printer (with one IRE printed out of Formlabs Flexible 80A Resin for testing purposes [13]). To assemble the IRE and the CEVIC, the CEVIC is pulled apart and the IRE is placed in between the two halves. The CEVIC is pushed together until the IRE is flush to the CEVIC on both sides. The device is twisted to lock the pin into a groove. This holds the two CEVIC halves in place relative to each other. The CEVIC and IRE are placed into the holder, which was printed on a Bambu labs printer, and pushed until firmly in place.

The final assembly is created by inserting the CEVIC, IRE, and Servo motor into the holder. The gear fits onto the shaft of the Servo via friction and interfaces with the gear teeth of the IRE. 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°, and because the IRE:gear tooth ratio is 1:1, the IRE also rotates 60°. Finally, the design integrates with the Voron 3D printer through two screws which secure it to the printhead. The assembled device is displayed in Figure 6 below.

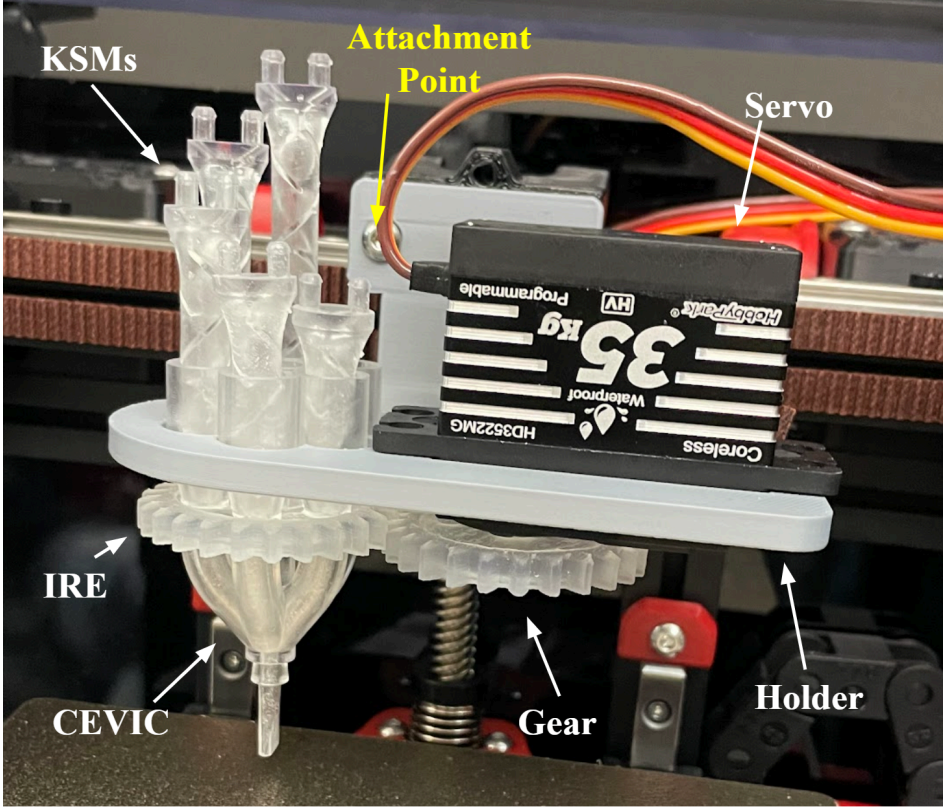


Figure 6: Final assembled device integrated with the Voron

3 Testing

3.1 Functionality Testing

Preliminary functionality testing was conducted using water. Then, gelatin hydrogels of two different colors to demonstrate device feasibility prior to integrated GelMA/HEC testing (described below), where one syringe contained the GelMA/alginate mixture and the second syringe contained the HEC fugitive ink. Initial functionality testing was performed using water in two syringes with the IRE in the closed position, followed by testing with the IRE in the open position by manually aligning the holes on the IRE and CEVIC. The closed position refers to when the IRE and CEVIC openings are not aligned, preventing fluid flow. The open position refers to when the IRE and CEVIC openings are aligned, allowing fluid to flow through the device. A syringe pump controlled the speed and volume of the hydrogels of the syringes. Subsequent trials repeated both configurations using two differently colored gelatins to better replicate the intended hydrogel system.

Integrated testing was conducted connecting the circuit to the IRE and CEVIC and utilized the GelMA and HEC hydrogels. When the button in the circuit was pushed, the IRE rotated 60°. During each trial, device performance was evaluated by documenting automatic shutoff valve switching behavior and accuracy (i.e., rotating 60°), noting any leakage events and their location, and assessing whether distinct alternating channels could be observed within the resulting hydrogel constructs. The protocol for functionality and integrated testing can be found in Appendix 7.2.

3.2 Durability Testing

Durability testing evaluated the device's ability to satisfy design requirements, including continuous operation of five minutes per hydrogel sheet and production of multiple sheets per

hour. The test assessed whether the IRE maintained strength and functionality under repeated use, providing an estimate of its service lifetime. Five samples were subjected to a simulated life-in-service protocol consisting of one hundred cycles, where one cycle was defined as a rotation of the IRE 180° and back to its original position. Samples were considered to pass if minimal visible damage to the IRE was observed following testing. Durability testing protocol can be found in Appendix 7.1.

3.3 Electronic Testing

Electronic testing evaluated whether the Servo motor, when powered by 5V from an Arduino, could reliably rotate the IRE and return it to the reference position. Five trials were completed in which the pushed button rotated the IRE 60° three times consecutively from the reference position, for a total of 180° per trial. The number of degrees per gear tooth was calculated by dividing 360° by the total number of teeth on the gear (25), so each tooth represents a rotation of 14.4°. The number of teeth rotated were counted per button push to estimate rotation angle. The main goal of this testing was to verify accuracy between the code and the Servo motor and consistency in rotation between the trials. The protocol for electronics testing can be found in Appendix 7.4.

3.4 Leak Testing

Leak testing was performed using the fully assembled syringe pump setup to assess the combined performance of the device under flow conditions. Ten leak-testing trials were completed for each iteration of the IRE, including BioMed Clear Resin (control), Sanded BioMed Clear Resin, and Flexible 80A Resin, which are depicted in Figure 7.



Figure 7: *The three IRE materials tested: Control (left), Sanded (center), and Flexible (right)*

During each trial, the initial and final volumes of water in two 20 mL syringes in a syringe pump were measured to quantify the total expected volume of fluid flow through the system. The output from the CEVIC extruder was collected in a 10 mL graduated cylinder with tape on the top to only allow output from the CEVIC, which is shown in Figure 8 below. The graduated cylinder volume measured how much fluid actually flowed through the system. The difference between the expected and collected volumes gives the total amount of liquid that leaked out of the system during the test. Protocol for leak testing can be found in Appendix 7.3

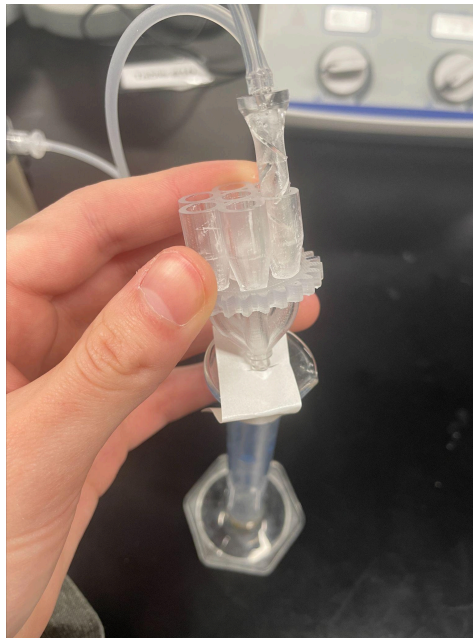


Figure 8: *CEVIC extruder placed into a graduated cylinder to collect and measure volume, with tape on top to prevent leakage from entering*

4 Discussion

4.1 Functionality/Integration Testing Results

Initial functionality testing successfully fabricating three gelatin hydrogels that appeared to exhibit striated microvascular architectures, as seen in Figure 9. While this demonstrates promising device functionality, these patternings were not verified with microscope imaging. Additionally, gelatin backflow was observed during operation. Therefore, improved fluid management strategies to redirect flow and ensure proper hydrogel patterning and resolution are necessary.

Backflow is a recognized limitation in extrusion-based bioprinting and is commonly associated with poor component sealing, low-viscosity bioinks, and system complexity. A 2021 study by Wang *et al.* introduced a novel valve-based consecutive bioprinting strategy incorporating pressure-driven control and microfluidic micromixers to improve flow regulation [14]. This approach used a two-way pinch valve to enable continuous bioink flow while seamlessly switching between bioinks during fabrication of vascularized muscle constructs. Design frameworks incorporating active flow regulation mechanisms may be integrated into future iterations of the CEVIC/IRE configuration to regulate fluid flow through KSM outputs.



Figure 9: Testing pushing two different colored gelatins through the CEVIC and IRE (left) and the resulting hydrogels (right)

Following initial proof of concept, integrated testing of the CEVIC, IRE, Servo, and syringe pump found that the IRE consistently achieved the intended angular displacements matching the Arduino programming and motor operation, enabling rotation to the appropriate KSM to deposit sequential resolutions to create continuous branching hydrogels. These results support successful integration and control of the motor, demonstrating the device's capability to produce patterned hydrogels with spatial precision. However, verification of angular displacement was limited by the use of a manual protractor, introducing potential measurement error. Additionally, during initial functionality testing, microvasculature patterns were visible macroscopically, but were not verified using a microscope because the GelMA and HEC require cross-linking and UV curing. See Figure 10 for the integrated functionality testing setup.

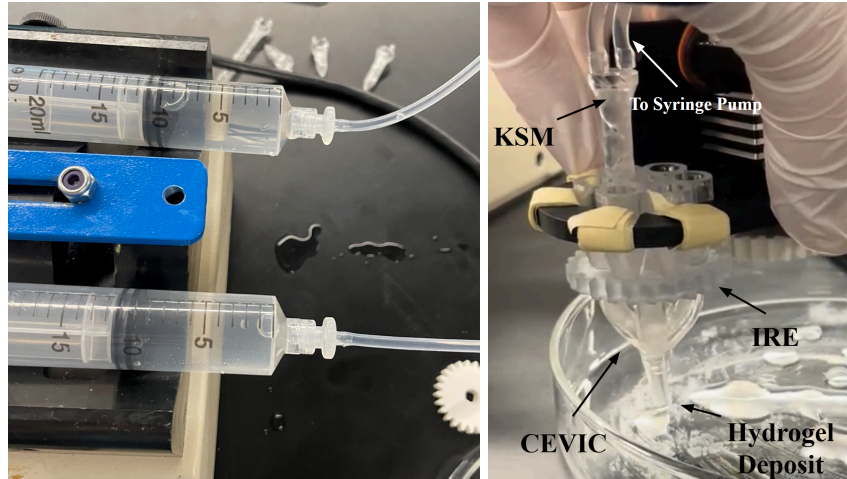


Figure 10: Syringe pump with two 20 mL syringes containing GelMA and HEC (left), which is connected to a KSM to print a hydrogel using the CEVIC, IRE, and Servo motor

4.2 Durability Testing Results

Durability testing results consisted of qualitative visual inspection. During testing, scratching of the IRE was observed immediately upon operation. However, no additional visible damage accumulated after the initial cycles. This observation is likely due to the friction between the rotating components combined with the material properties of the BioMed Clear Resin. The observed surface scratches were minor relative to the overall thickness of the IRE and therefore were not expected to compromise the structural integrity of the IRE. Figure 11 illustrates the extent of surface wear before and after the simulated life-in-service durability test of 100 cycles.

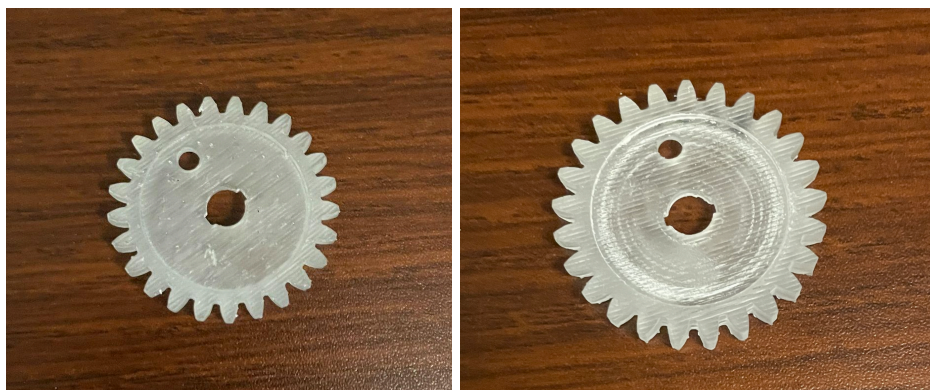


Figure 11: Images of the IRE pre (left) and post (left) testing, with scratches on IRE post testing

4.3 Electronics Testing Results

Figure 12 displays the results of electronics' angular displacement testing, where the average measured displacement was 207.8° with a standard deviation of 6.0° and $p < 0.05$. Each trial consisted of three consecutive rotations coded for 60° , and these results were summed to find the final rotation. The purpose of this design was to evaluate angle displacement errors over time. The average total displacement was nearly 30° greater than the target of 180° , or an error of 15.33%. This error is too great to align the hole in the IRE precisely with the CEVIC channels. IRE angular displacement errors introduced misalignment (partial or total blockage of the hole) between the IRE and CEVIC. These errors could impact the final hydrogel resolution and introduce leakage on other system components. Residual drift from the motor may be one contributing factor that impacted IRE angular displacement.

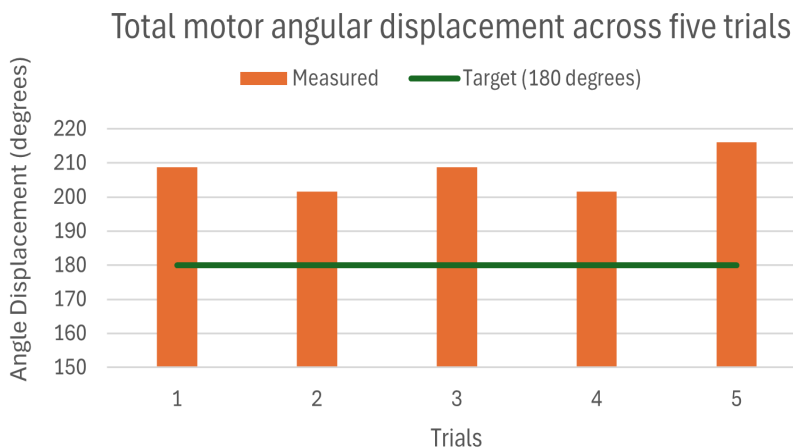


Figure 12: Bar chart displaying the results of electronics motor angular displacement testing

Electronics testing also investigated whether, after rotating 180° , the motor would return to its original position or vary from the starting position. Testing found that the average difference between the original and final position was $23.0^\circ \pm 9.4^\circ$. This means that the percent error for return rotation is 12.78%, which is again above tolerance.

However, the aforementioned integration and functionality testing did not experience significant error in the rotation angles. Pressing the button to rotate the Servo gear resulted in the IRE lining up accurately when simulating hydrogel printing. This implies that for the electronics testing, there was likely an error in either the method of angle measurement or within the code. Limitations in using manual methods such as protractor or counting gear teeth to quantify angular displacement could have exacerbated the percent error. Future testing iterations and data analysis should utilize ImageJ or similar tools to measure angular displacements more precisely.

4.4 Leak Testing Results

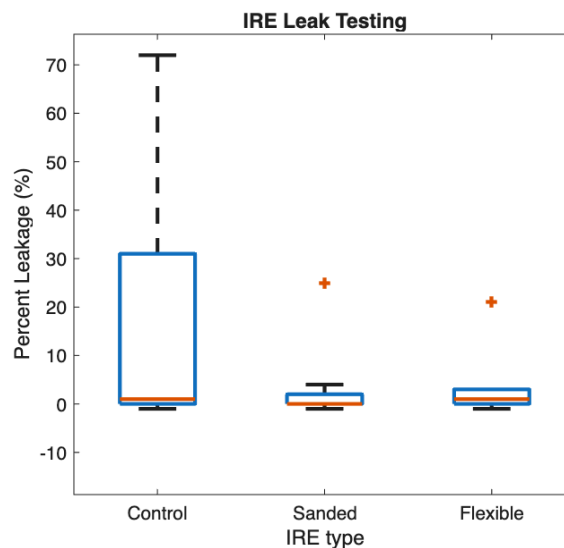


Figure 13: Box plot displaying the percent leakage per IRE type

Figure 13 above documents the leak testing results. The averages and standard deviations for the Control Sanded, and Flexible IREs were $16.3 \pm 26.9\%$, $3.1 \pm 7.8\%$, and $3.1 \pm 6.4\%$ respectively. A one-way ANOVA test was done and found a p-value of $0.14 > 0.05$, meaning the null hypothesis can not be rejected. While the Control group appears to have a larger percent leakage, the different IRE groups can not be considered statistically significant. This is likely due to the fact that the medians of all three trials are very similar (as denoted by the orange horizontal lines in Figure 12), or that the data is not normally distributed. The three IREs all experienced

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leakage for 3 out of 10 trials, however, the Control experienced significantly larger leak volume when it happened. The greatest percent leakages for the Sanded and Flexible IREs were 25% and 21% respectively, whereas the maximum value for the Control was 72%. More data is required to determine if the Sanded and Flexible IREs are truly more leak resistant, however, recommendations include moving forward with the Sanded IRE because a smooth surface reduces friction forces and abrasion to the CEVIC device. The Flexible IRE was not continued, for while it appeared to perform best in the leak trials, it required too much torque to rotate when clamped between the two halves of the CEVIC.

5 Conclusions

Tissue engineering is a growing field with broad potential. However, 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 resolutions as low as 10 μm , but a problem remains in switching between these resolutions while printing to imitate how arteries (1-6 mm) decrease in diameter to capillary size (10 μm). The IRE integrated with CEVIC bioprinting framework through alternating between KSM resolutions, and minimizing excess bioink deposition from other KSMs. All materials used were fully biocompatible and do not pose toxic or degradable effects to cells that will be incorporated into the seeding process.

Overall, the IRE provides a competitive advantage due to its ability to harness output from one KSM at a time. Additionally, the tight seal between the CEVIC and IRE blocks excess fluid buildup in other KSMs, while enabling precise control over the KSM depositing its fluid at a given time. However, several improvements can be made to strengthen the overall performance, reliability, and usability of the system. While mechanical integration of the CEVIC, IRE, and Servo via holder was a success, one priority would be fully integrating the software into the existing Voron 3D printer setup. Bringing these components together into a unified system would make the device easier to operate during testing and reduce variability.

Future work includes adjusting the gear ratio to 2:1 (IRE:gear respectively) to allow the IRE to rotate 360°, as the Servo motor can only rotate 180°. Implementing negative pressure could help pull any excess GelMA/HEC back into the tubing and prevent it from leaking out of the system. This would decrease leak interference with the hydrogel output and improve cleanliness. Additional testing is needed to determine the negative pressure implementation and to ensure that negative pressure does not interfere with printing. Incorporating the rotary valve

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would assist with automated diversion of hydrogel between KSMs. Developing a graphical user interface and relay-based system would make the design more user-friendly by allowing the operator to enter key parameters and have the valves switch automatically based on those inputs rather than button press, reducing the chance of user error and improving repeatability.

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7 Appendix

7.1 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°. 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:

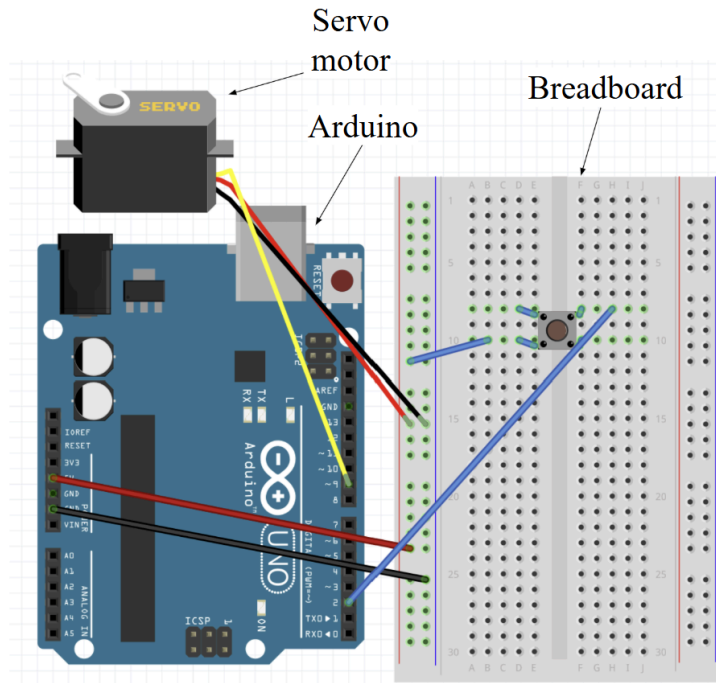


Figure 1: Breadboard schematic for the servo sweep functionality

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° and 180° 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

7.2 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.

Functionality testing evaluates the following client specifications and product specifications:

-

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 70°C. 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

7.3 Leak Testing

Purpose

The purpose of this testing is to evaluate the performance of different materials against leakage.

Equipment and Materials

This test uses the following equipment:

- 50 mL beaker
- 10 mL graduated cylinder
- CEVIC and IRE
- KSM
- 2x 20mL Syringes
- Syringe pump
- Tubing
- Razor blade or x-acto knife

This test using the following materials:

- DI Water

Procedure

Preparation

1. Plug in and turn on the syringe pump
2. Ensure the syringe pump is set to 20 mL syringe size and 1.5 mL/min speed
3. Attach tubing to the ends of the 20 mL syringes
4. Fill a 50 mL beaker with DI water
5. From the 50 mL beaker, fill the syringes with 20 mL of water each
6. Place and secure both syringes into the syringe pump cradle
7. Tape the top of the graduated cylinder and cut a small slit in the top, just long enough for the bottom of the CEVIC to fit through

Testing

1. Select the first IRE sample (control, flexible, or sanded)
2. Prepare the CEVIC and IRE in the “open” position, where the holes in the CEVIC align with the hole in the IRE
3. Connect the tubing from the syringe pump to the KSM, then fit the KSM into the “open” slot in the CEVIC
4. Prime the CEVIC by pressing “run” on the syringe pump until all air is pushed out of the system
5. Press “stop”
6. Measure the initial volume of the 20 mL syringes. Record on the data sheet
7. Fit the end of the CEVIC into the slit on top of the graduated cylinder
8. Press “run” on the syringe pump and allow approximately 5 mL from each syringe to flow
9. After above 5 mL, press “stop”

10. Record the final volume of the 20 mL syringes. Record the collected volume in the graduated cylinder
11. Repeat for a total of 10 trials per sample
12. When finished, clean benchtop and return all lab equipment

7.4 Electronics Testing

Goals: Protocols aim to verify servo motor reliability, alignment accuracy, torque output, and cycle consistency in a chaotic bioprinting setup with rotating intermeshing rotors (IRE) driven by micro servo pulses.

All testing objectives will be with vs without button

Test 1: Alignment Drift Over Cycles

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

+/- 60 degrees / cycle

+/- 180 degrees / cycle

Convert degrees to radians.

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 \approx 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: $60^\circ \pm 2^\circ$).
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).

BME Design: 200, 201, 300, 301, 400 and 402

Test 3: Starting Point Stability

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

Steps:

Perform one cycle and then measure the frequency that the ending position aligns where it is supposed to be.

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