

Development of a PDMS Tissue Chip and Media Circulation System for *Xiphophorus* Biomedical Research Using Additive Manufacturing

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Abstract

Xiphophorus fish are a critical model for studying cancer resilience and disease progression, but traditional research methods pose ethical and practical challenges. A cost-effective tissue chip system with integrated media circulation was developed to support *Xiphophorus* cell and tissue culture. Microfluidic channel molds were designed and fabricated using additive manufacturing with optimized dimensions of 1.8 mm width \times 1.0 mm depth to balance unobstructed flow and structural integrity, and the chip was cast from polydimethylsiloxane (PDMS). A miniature peristaltic pump, integrated with a microcontroller for flow control, demonstrated stable circulation at 0.2–0.8 mL/min during water-based testing. Components were selected for ease of integration and scalability. While secure tubing-to-chip connections remain a challenge, proposed solutions include embedded tubing and standardized connectors. This platform offers an affordable, scalable alternative to traditional in vitro models, advancing disease research and therapeutic development while reducing dependence on live animal studies.

1. Introduction

Understanding how diseases like cancer develop and how some animals naturally resist them is a key focus in biomedical research. One important model for this work is the *Xiphophorus* fish. These small freshwater fish have helped scientists study melanoma (a type of skin cancer) for decades because of their unique genetic makeup [1], [2]. However, conducting research with live *Xiphophorus* fish often requires maintaining breeding populations, which can be expensive, time-consuming, and ethically complex.

To make this kind of research more accessible, scientists are turning to new tools called organ-on-a-chip or tissue chip systems. These small devices replicate organ-level functions by growing cells in controlled microenvironments. They allow researchers to study cells in realistic conditions, without relying on live animals [3], [4]. Many of these chips are made using PDMS (polydimethylsiloxane), a silicone material known for being safe for cells, easy to mold, and transparent for observation [5].

These innovations are gaining official recognition. In fact, the U.S. Food and Drug Administration (FDA) recently updated its policies to support non-animal testing models, including organ-on-chip systems, through the FDA Modernization Act 2.0 [6]. Still, most of these

technologies have been developed for human cells, leaving a gap in support for non-human models like *Xiphophorus*.

Evolutionary mutant models (EMMs), including *Xiphophorus*, axolotls, and cavefish, possess unique traits such as natural disease resistance and tissue regeneration. These traits, absent in human biology, make EMMs valuable for discovering novel mechanisms of disease resilience [7]. Unfortunately, many labs don't have the resources to maintain live populations of these animals [1], [8].

This project addresses the challenge by developing a low-cost, stand-alone tissue chip system specifically tailored for culturing *Xiphophorus* cells. Molds for casting PDMS microchannels, capable of housing and supporting these cells, were designed and fabricated using additive manufacturing (3D printing). This approach enables rapid design iteration and the efficient production of custom chip geometries at a low cost [9] [10].

To sustain cell viability, the system also incorporated continuous circulation of liquid culture media through the chip. A commercially available miniaturized pump was integrated with a microcontroller-based system to regulate flow rates. The decision to use an off-the-shelf pump, rather than developing a custom solution, streamlined the integration process and replicated the fluid dynamics found in more advanced microfluidic platforms [11], [12].

By combining low-cost materials, 3D printing, and modular components, the proposed system introduces a practical tool for researchers working with *Xiphophorus* and other evolutionary mutant models (EMMs). This approach has the potential to reduce dependence on live animal studies and broaden access to disease modeling tools in resource-limited laboratory environments.

2. Design and Fabrication

To develop a functional and accessible tissue chip system tailored for *Xiphophorus* research, the methodology was structured around two interconnected components: a PDMS-based microfluidic chip and a compact, cost-effective circulation system for nutrient media. This section describes the conceptualization, fabrication, and integration of both subsystems using an iterative, hands-on engineering approach.

2.1 Design Ideation and Modeling Process

The design process was initiated with preliminary sketches of the overall system. Early concept illustrations included methods for chip fabrication via photolithography and layout designs for one-channel and two-channel PDMS chips. A structured ideation process was conducted using the 6-3-1 method [13], wherein each concept was initially sketched within six minutes and subsequently refined through three- and one-minute iterations by others. This method led to the emergence of three distinct design directions: a flip-to-open housing modeled after a sunglasses case, a drawer-style chip with integrated flow bypass, and a cascade-flow configuration that allowed media to circulate over cells and drain for recirculation. These conceptual designs,

presented in Figure 1, reflect early considerations of chip accessibility, fluid control, optical transparency, and spatial arrangement from both top-down and cross-sectional perspectives.

Each concept offered distinct benefits and limitations. The flip-open design provided structural protection but restricted visual access to the cultured cells. The drawer-style layout introduced internal compartmentalization and partial flow control but required disassembly for inspection. The cascade-flow model allowed for external observation via a transparent face; however, it posed challenges in achieving controlled and consistent flow dynamics.

After careful evaluation of the initial design sketches and consideration of their functional viability, it was determined that none of the proposed concepts could independently fulfill the combined requirements of cell visibility, accessibility, and fluidic integration. Consequently, the design strategy was redirected toward a more modular and fabrication-friendly approach, specifically, the development of a molded PDMS tissue chip utilizing 3D-printed inverse molds. This alternative was selected to enhance precision, scalability, and compatibility with biocompatible materials.

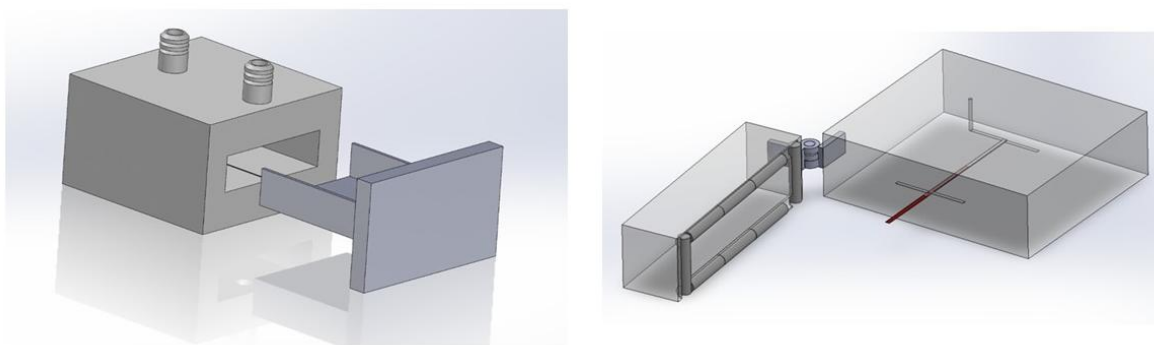


Figure 1. CAD designs developed from Primary Sketches

Following the completion of initial sketches through the 6-3-1 ideation technique, the conceptual designs were translated into CAD models based on dimensional parameters appropriate for tissue chip applications. This transition from analog sketches to digital representations enabled more accurate analysis of component geometry and assembly constraints. As shown in Figure 1, two CAD configurations were created to reflect the most promising concepts: a flip-to-open housing resembling a lighter, and a hybrid drawer-style housing incorporating a cascading media flow design.

The flip-open model was intended to simplify access to the chip interior; however, it lacked provisions for in-situ observation of cultured cells and did not incorporate an effective method for inserting or securing tissue samples. The drawer-cascade hybrid, although offering more intricate flow dynamics, also lacked transparency and introduced mechanical complexities related to fluid recirculation and sealing. These limitations were revealed during the digital modeling stage, as practical constraints associated with real-world fabrication and use began to emerge.

Through iterative analysis of the CAD models, it was determined that neither design adequately fulfilled the project's functional requirements, specifically, ease of cell observation,

compatibility with microfluidic flow, and feasibility of fabrication using available resources. In response to these shortcomings, the design approach was redirected toward a mold-based PDMS casting method in which an inverse mold could be produced through additive manufacturing. This transition allowed for enhanced control over microchannel geometry, improved surface quality, and increased modularity, while also streamlining the overall fabrication process.

The CAD modeling phase proved instrumental in illuminating both the conceptual diversity of the initial designs and the practical limitations that necessitated a shift toward a more biologically compatible and fabrication-efficient platform. A summary of these early design concepts is provided in Table 1.

Table 1. Composition of Early Tissue Chip Design Concepts

Design Style	Advantages	Limitations
Flip-to-Open Case	Protective enclosure; portable	Cells are not visible when closed; poor accessibility
Drawer System	Easier to insert components; modular flow	No easy visual access; more complex internal geometry
Cascade Flow Chip	Visual access to cells; the recirculation concept	Difficult to control flow; more complex to fabricate

Finally, as shown in Figure 2, a double-channel microfluidic mold was modeled in SolidWorks, with each channel dimensioned at 1.8 mm in width and 1.0 mm in depth. These dimensions were selected based on empirical observations and informed by relevant literature [9], [11]. Polydimethylsiloxane (PDMS) was selected as the chip material due to its well-established biocompatibility, optical transparency, and widespread application in organ-on-chip systems [5], [14].

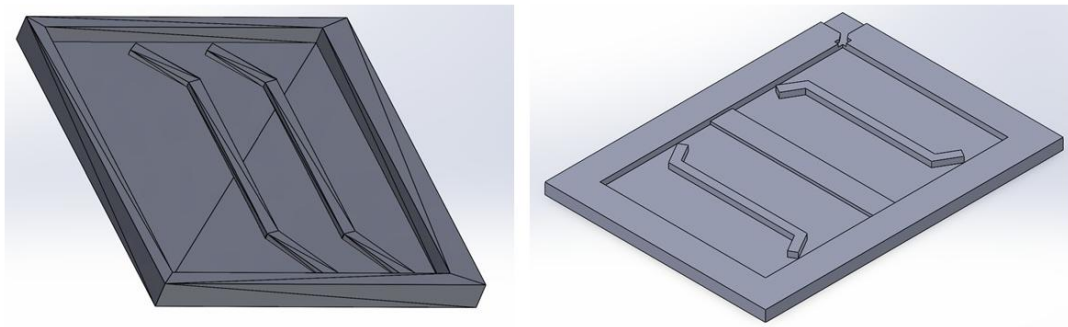


Figure 2. CAD designs of double channel microfluidic mold

2.2 Mold Fabrication via Additive Manufacturing

Initial prototypes were fabricated using material extrusion on the CraftBot XL printer. These early prints exhibited high porosity, visible layer lines, and a lack of surface uniformity, which can be seen from Figure 3. Such surface imperfections led to inconsistent PDMS curing and

significant difficulty during demolding. The surface roughness caused excessive adhesion, often resulting in the tearing or distortion of the PDMS structures upon removal.

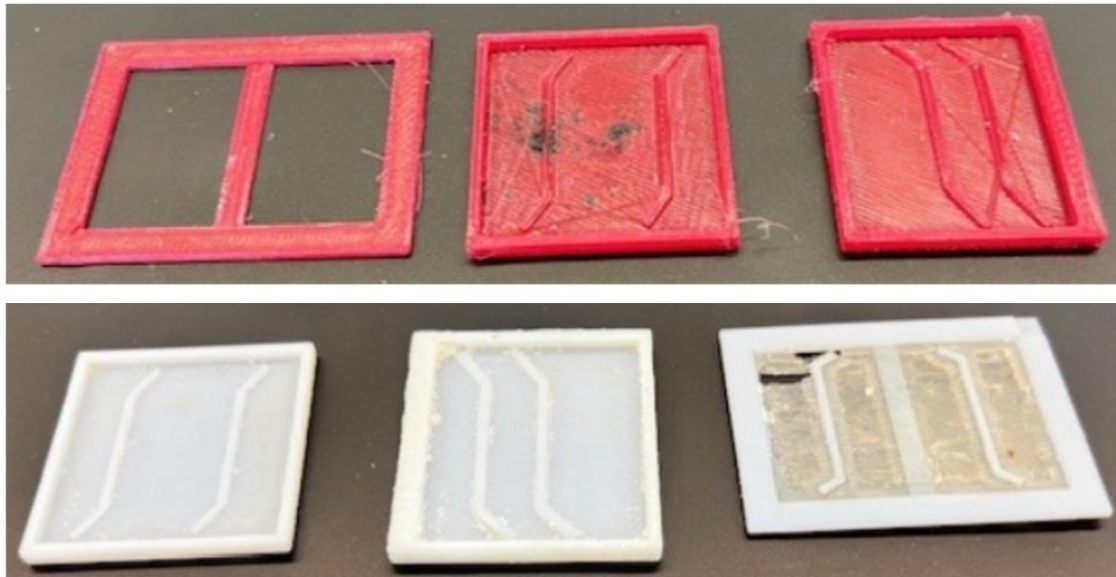


Figure 3. Top – Molds Printed with CraftBotXL. Bottom – Early Molds Printed with Connex Objet 260.

To address these deficiencies, a transition was made to the Connex Objet 260 printer, which employs a high-resolution resin jetting process. This method substantially improved both surface smoothness and dimensional accuracy. Nevertheless, casting PDMS directly onto Connex-printed molds continued to present challenges. The PDMS adhered strongly to the resin surfaces, causing incomplete or damaged chip extractions, thereby indicating the need for an intermediate molding interface (Figure 3, bottom).

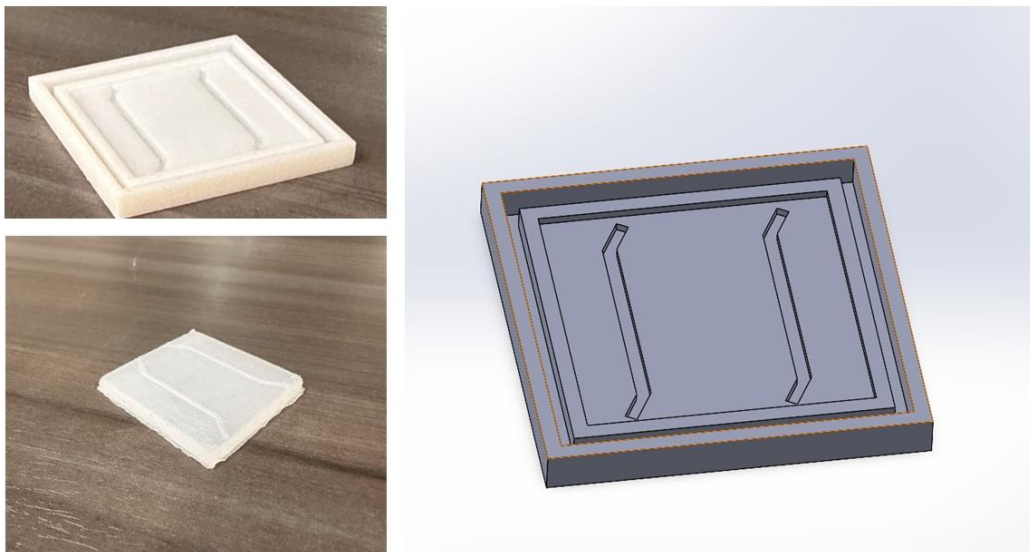


Figure 4. Inverse mold development workflow for the double-channel PDMS chip. Right: CAD model showing recessed mold cavities for channel shaping. Top Left: High-resolution print of the mold using resin jetting. Bottom Left: Final PDMS chip cast from the mold, demonstrating successful replication of channel geometry and clean demolding

A two-step molding process was subsequently adopted to overcome these limitations. Inverse molds were first printed using the Connex printer, then cast in silicone rubber to create a reusable mold with PDMS-compatible properties. Figure 4 shows that this strategy produced the most successful outcomes, as the silicone molds exhibited the flexibility and non-stick characteristics required to release PDMS chips with intact geometry and clean surface features.

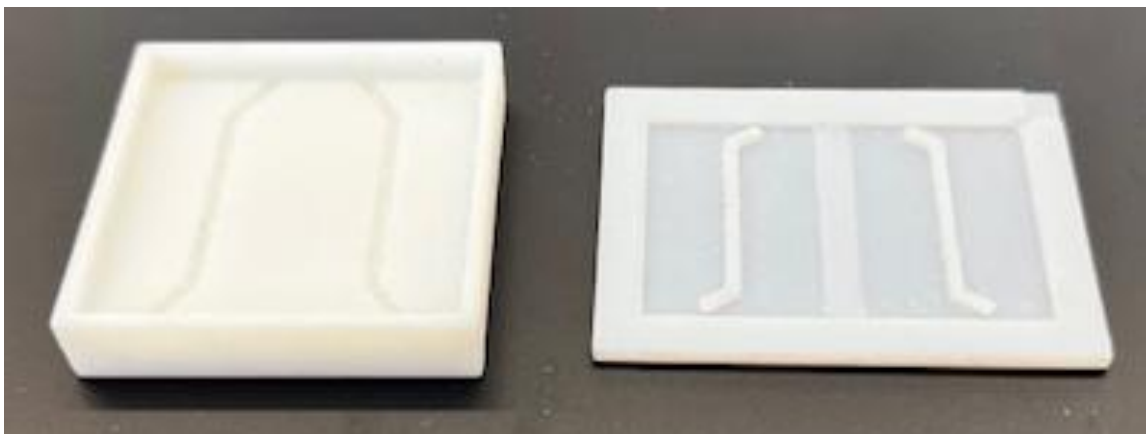


Figure 5. Fabricated models of the double-channel PDMS chip design printed using high-resolution material jetting

After multiple fabrication trials, the optimal dimensions for the microchannels were determined to be 1.8 mm in width and 1.0 mm in depth. Channel dimensions of 1.8 mm width and 1.0 mm depth were selected based on a combination of literature benchmarks and preliminary fabrication trials. Prior studies on microfluidic tissue chips indicate that channel widths below 2 mm provide a balance between laminar flow stability and structural robustness [9], [11]. Our sizing tests further confirmed that these dimensions supported unobstructed fluid flow without wall collapse or deformation during molding and demolding.

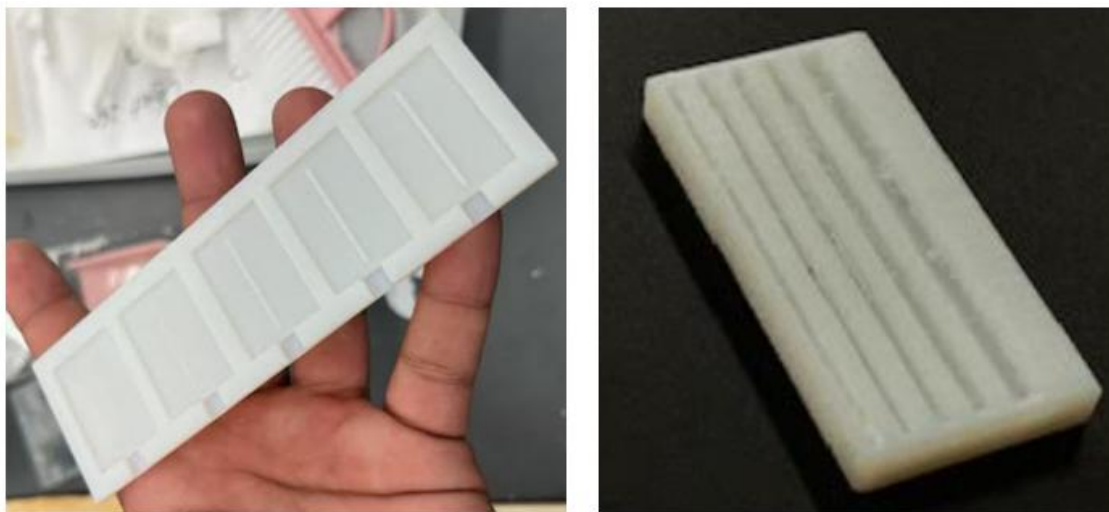


Figure 6. Example sizing models for depth (Left) and width (Right)

As illustrated in Figure 6, example sizing models were used to evaluate channel performance. These dimensions were found to offer an effective balance between unobstructed fluid flow and structural stability. Through a series of sizing tests, it was confirmed that these parameters could sustain consistent media circulation without compromising the integrity of the channel walls. A total of 12 mold-casting trials were conducted across the three fabrication methods. CraftBot XL molds showed only 1 of 5 successful chip extractions (20% yield), while Connex resin jetting molds improved to 3 of 4 successful extractions (75% yield) but suffered from PDMS adhesion. In contrast, silicone rubber intermediate molds achieved 100% clean demolding across three trials, confirming their reliability for reproducible PDMS casting.

2.3 PDMS Casting and Cure Behavior

Casting of PDMS into the finalized silicone molds yielded cleaner and more reproducible channel structures. The curing process was carried out in a temperature-controlled environment (approximately 30°C), with complete polymerization achieved within 3–4 hours. However, further exploration of resin-based mold alternatives was constrained due to limitations in available UV curing infrastructure. The existing 10W UV source proved insufficient, as reliable curing of epoxy molds typically requires 30–50W of power. Consequently, silicone was retained as the preferred material for reusable mold fabrication.



Figure 7. Molding Process chip. Left: Curing Resin parts in the mold. Middle Semi-cured resin molds: High-resolution print of the mold using resin jetting. Right: Cured PDMS ripped from mirrored mold.

Figure 7 illustrates key stages of the molding process and highlights several of the issues encountered. The left image shows resin parts curing within the mold, an early attempt at producing reusable molding surfaces. The middle image depicts semi-cured resin molds fabricated via high-resolution resin jetting; although geometrically accurate, these often failed to fully cure due to limitations in UV exposure. The right image captures a cured PDMS sample being removed from a mirrored mold. As shown, tearing and material sticking were common issues when PDMS was cast directly onto Connex-printed molds without an intermediate release layer, underscoring the need for a more compatible molding material such as silicone.

2.4 Pump System Integration and Control

To maintain continuous media flow through the microfluidic chip, a commercially available 3–5V mini peristaltic DC pump was selected based on cost-effectiveness, availability,

and compatibility with microcontroller-based control systems. Instead of developing a custom pump, efforts were directed toward integrating the selected pump into a programmable system utilizing an ESP32 Feather V2 microcontroller in conjunction with an Adafruit DC motor driver. Power was supplied via a benchtop variable power source, with all components connected using jumper wires. The ESP32 was programmed within the Arduino development environment to enable digital control over motor start/stop functions and rotational speed, which directly governed the fluid flow rate. During preliminary trials, water was used in place of culture media to evaluate operational stability. The system successfully demonstrated consistent performance across a range of speeds, indicating its potential suitability for future integration with nutrient-rich media in biological experiments. Despite successful pump operation, a critical limitation was encountered: a secure and leak-proof interface between the tubing and PDMS chip channels could not be established. This constraint prevented full circulation testing within the microfluidic platform and remains a key area for continued development and functional validation.

3. Results and Evaluation

The design, fabrication, and integration phases of the tissue chip system resulted in both encouraging progress and critical challenges. This section presents the outcomes from key development stages, including mold creation, PDMS casting, and pump system testing, while emphasizing the insights gained through iterative prototyping and evaluation.

3.1 Mold Surface Analysis

This experiment focused on analyzing the surface quality and dimensional accuracy of the molds used for PDMS chip fabrication. The initial molds produced via material extrusion (MEX) on the CraftBot XL exhibited significant surface roughness and poor dimensional fidelity, which caused PDMS adhesion and tearing. In contrast, molds printed using the Connex Objet 260 resin jetting printer provided improved surface smoothness and precision. Despite this, PDMS still adhered to the resin mold surfaces, leading to incomplete or damaged chip extractions. To address these issues, a two-step molding strategy using silicone rubber molds was adopted, which successfully overcame the adhesion problem and allowed for clean demolding. Repeated casting–demolding cycles demonstrated strong wall fidelity: after five consecutive castings, channels retained >95% dimensional accuracy, with average measured width of 1.82 ± 0.03 mm and depth of 1.01 ± 0.02 mm compared to design specifications. No wall collapse or permanent deformation was observed under optical microscopy.

3.2 PDMS Casting and Cure Behavior

Casting PDMS into silicone molds yielded clean and reproducible channel structures. The curing process was carried out at 30°C, with complete polymerization achieved within 3–4 hours. However, further exploration of resin-based mold alternatives was constrained due to limitations in available UV curing infrastructure. The existing 10W UV source proved insufficient, as reliable curing of epoxy molds typically requires 30–50W of power. Consequently, silicone was retained as the preferred material for reusable mold fabrication. Some other challenges arose during the process, particularly the difficulty in securing leak-proof tubing ports for fluidic interfaces. While manually drilled ports allowed for some level of connection, they were not sufficient for closed-loop testing.

3.3 Pump System Performance

This experiment evaluated the functionality and performance of the mini DC pump integrated with the tissue chip system. The pump demonstrated stable and adjustable flow rates across a range of motor speeds, controlled by the ESP32 microcontroller programmed through the Arduino IDE. The performance of the pump was tested with water, and continuous flow was confirmed under various voltage conditions, showcasing its potential for media circulation in the system. Flow rate characterization was performed by varying the supply voltage to the mini DC pump. At 3.0 V, the average flow rate was 0.21 ± 0.02 mL/min; at 4.0 V, it increased to 0.47 ± 0.03 mL/min; and at 5.0 V, the maximum stable flow was 0.82 ± 0.04 mL/min. Across all conditions, the pump maintained continuous circulation without flow interruptions for >60 minutes. The pump system exhibited high responsiveness and stability over time, making it suitable for integration into low-cost biomedical chip systems.

3.4 System Integration and Testing

The final experiment integrated the pump system with the PDMS chip for full system-level testing. The pump operated reliably and delivered stable water flow, confirming the feasibility of closed-loop circulation within the system. However, establishing a fully sealed connection between the tubing and PDMS chip remains a key challenge. Leakage testing showed that manually drilled ports tolerated back pressures of up to 5–7 kPa before microleakage occurred, providing a benchmark for future improvements such as Luer-lock connectors or embedded tubing interfaces.

Table 2. Overall Specification of Experiment

Component	Outcome	Challenges/Limitations
CraftBot Molds	Low accuracy, poor surface finish	High porosity, PDMS tearing
Connex Molds	High-resolution, smooth surface	PDMS stuck to mold without protective layer
Silicone Molds	Good release properties, reproducible shapes	Requires multi-step casting
PDMS Casting	Successful with silicone molds	Tubing interface still undeveloped
Pump System	Stable, adjustable flow via ESP32 controller	No secure tubing-to-chip connection
UV Curing (Resin)	Incomplete due to insufficient UV power (10W vs 30–50W needed)	Resin molds not viable within current setup

Overall, the project successfully demonstrated reproducible PDMS mold fabrication and stable pump operation, laying a solid foundation for continued development. While integration of the two subsystems is still in progress, the results highlight clear pathways for refinement and bring the system closer to supporting biological media circulation and eventual cell culture. A consolidated summary of outcomes and limitations is presented in Table 2, underscoring both the progress achieved and the remaining challenges to full system integration.

Table 3. Quantitative Performance Metrics

Parameter	Result
Channel width (designed)	1.80 mm
Channel width (measured)	1.82 ± 0.03 mm (n=5)
Channel depth (designed)	1.00 mm
Channel depth (measured)	1.01 ± 0.02 mm (n=5)
Casting yield (CraftBot)	20% (1/5 successful demoldings)
Casting yield (Connex)	75% (3/4 successful demoldings)
Casting yield (Silicone)	100% (3/3 successful demoldings)
Flow rate @ 3.0 V	0.21 ± 0.02 mL/min
Flow rate @ 4.0 V	0.47 ± 0.03 mL/min
Flow rate @ 5.0 V	0.82 ± 0.04 mL/min
Max back pressure (ports)	5–7 kPa before leakage

To summarize the key quantitative outcomes from mold fabrication, channel sizing, and pump testing, performance metrics are compiled in Table 3. The results confirm that the optimized channel dimensions (1.8 mm × 1.0 mm) were reproduced with high fidelity (1.82 ± 0.03 mm width, 1.01 ± 0.02 mm depth, n=5) across repeated castings. Casting yield varied significantly between fabrication methods, with CraftBot molds yielding only 20% success, Connex jetting molds 75%, and silicone intermediate molds achieving 100% clean demolding. Pump characterization demonstrated stable and tunable flow rates ranging from 0.21 to 0.82 mL/min under 3–5 V input. However, leakage testing of drilled tubing ports showed a maximum back pressure tolerance of only 5–7 kPa, underscoring the need for improved fluidic interfaces.

4. Conclusion & Future Work

This project demonstrated the feasibility of developing a low-cost, modular PDMS tissue chip system with integrated media circulation for Xiphophorus research. Using additive manufacturing and open-source electronics, the work established a reproducible method for mold fabrication and confirmed stable, controllable fluid flow with a mini DC pump. The primary challenge that remains is achieving a reliable, leak-proof tubing-to-chip interface, which limits full biological validation. Future iterations will address this through solutions such as Luer-lock connectors or embedded tubing during PDMS casting, along with improvements in mold curing and release strategies.

Future Work

Future development should focus on several key areas to overcome the current limitations. Such as:

- Upgrading the UV curing system to a higher-powered unit (30–50W) for complete polymerization of resin molds.
- Developing sealed fluidic interfaces using options like Luer-lock connectors or embedded tubing ports to ensure leak-proof and stable media circulation.
- Explore stereolithography (SLA) for higher-resolution mold fabrication.

Once these improvements are implemented, the system can progress toward biological validation by culturing *Xiphophorus* cells in-chip, serving as proof-of-concept for a fully functional, non-human organ-on-chip platform. Ultimately, this work contributes toward more ethical, scalable, and species-specific tools for cancer and evolutionary biology research.

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