

Development of a Modified FDM-Based Bioprinter with Optimized Extrusion Parameters for High-Fidelity Alginate Bioinks and Aortic Valve Scaffold Manufacturing

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Abstract: - Aortic valve disease is a major cause of cardiovascular deaths worldwide. Aortic stenosis increases the workload on the heart and severely alters hemodynamic function by impairing effective blood flow. In recent years, extrusion-based bioprinting technologies have made significant progress in producing patient-specific, biocompatible cardiovascular structures, opening up new opportunities for advanced therapeutic applications. In this study, a commercially available FDM-type 3D printer (ENDER-3 Pro) was mechanically and electronically modified into a fully functional, low-cost bioprinter capable of depositing alginate-gelatin hydrogels with high structural fidelity, despite their low viscosity. The redesigned system integrates a NEMA-17 driven syringe extrusion module equipped with a TR8X8 lead screw, enabling high-resolution pressure-controlled deposition of viscoelastic bioinks. A three-leaflet aortic valve geometry was digitally reconstructed and simplified using Fusion 360 to ensure printability while preserving essential anatomical features. The printing parameters were systematically investigated according to the alginate-based bioinks and set to a nozzle diameter (0.6 mm), layer height (0.6 mm), extrusion multiplier (0.02), and printing speed (5 mm/s). Quantitative shape fidelity assessment, including top-down geometric deviation analysis, confirmed that the bioprinted aortic valve constructs successfully maintained the nominal CAD boundaries. By dynamically optimizing slicing parameters, specifically the extrusion multiplier, rheologically driven deformations such as excessive lateral spreading were effectively mitigated. Although comprehensive evaluation of hemodynamic performance requires future in vitro experiments and coupled fluid–structure interaction (FSI) simulations, the present study establishes a robust and accessible biofabrication framework capable of producing highly complex cardiovascular geometries. Cost-effective and adaptable bioprinting platforms, derived from consumer-grade fused deposition modeling (FDM) systems, can achieve the geometric fidelity and structural stability necessary for advanced cardiovascular tissue engineering. This approach significantly enhances the accessibility of high-precision bioprinting technologies, thereby facilitating broader adoption in both research and translational applications.

Key-Words: - 3D Bioprinting, Extrusion-Based Bioprinter, Alginate-Gelatin Bioink, Shape Fidelity, Aortic Valve Scaffold, Cardiovascular Tissue Engineering.

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1 Introduction

Bioprinting has emerged as a key paradigm within advanced manufacturing, enabling the controlled fabrication of three-dimensional, cell-laden biological constructs with high spatial resolution

and architectural fidelity. Unlike conventional scaffold-based approaches, bioprinting facilitates the precise replication of complex tissue microarchitectures, supports patient-specific geometrical customization, and allows the

incorporation of living cells within biomimetic microenvironments. These capabilities position bioprinting as a critical enabling technology for translational research in regenerative medicine, particularly in the development of functional tissue substitutes tailored to individual clinical needs [1], [2].

The aortic valve is a highly specialized component of the cardiovascular system, with a complex hierarchical structure and anisotropic mechanical behavior that provides unidirectional blood flow from the left ventricle to the aorta. Cardiovascular diseases and aortic valve problems are the leading cause of death globally [3], [4]. Among these conditions, aortic stenosis is particularly critical because narrowing of the valve opening leads to increased left ventricular pressure, decreased cardiac output, and myocardial hypertrophy, ultimately impairing heart function [5].

Current clinical interventions for aortic stenosis primarily rely on mechanical and bioprosthetic valve replacements [6]. However, both approaches have their own limitations. Mechanical valves require lifelong anticoagulation therapy associated with increased risks of bleeding and thromboembolic complications. In contrast, bioprosthetic valves exhibit limited durability due to structural degeneration and calcification, as well as suboptimal integration with host tissues [7]. These challenges necessitate the development of next-generation solutions that can overcome the limitations of current prosthetic technologies.

Tissue engineering combined with three-dimensional bioprinting offers a promising strategy to produce patient-specific, biologically functional valve structures. Bioprinting enables the layer-by-layer deposition of cell-loaded hydrogels, allowing for the reconstruction of anatomically accurate and functionally relevant structures. Typically, a digital model generated via CAD/CAM platforms is processed through slicing algorithms to define the deposition path, after which bioink based on materials such as alginate or gelatin methacryloyl is extruded, sprayed, or polymerized using various techniques. As illustrated in Table 1, there are various layer-by-layer manufacturing techniques that differ according to the specific application areas of bioprinters. Among these methods, extrusion-based and laser-assisted bioprinting are widely used due to their versatility and applicability in a variety of tissue engineering scenarios.

Alginate-based hydrogels are widely used in bioprinting due to their biocompatibility, easily adjustable viscosity, and rapid ionic cross-linking

capabilities [8]. Their shear thinning behavior allows for stable extrusion under applied stress, while their rapid gelation upon precipitation promotes shape retention and structural integrity, making them ideal for producing geometrically complex structures such as heart valves. However, achieving optimum printing capability and structural accuracy requires precise control of key parameters including viscosity, gelation kinetics, extrusion pressure, nozzle diameter, and printing speed [9], [10], [11].

Recent studies (2024–2025) have highlighted the critical role of bioink composition in determining both printing capability and mechanical performance. It has been shown that optimizing alginate-gelatin ratios significantly affects rheological behavior and post-printing stability. For example, formulations such as the combination of 7% alginate and 8% gelatin have shown improved shear thinning properties during extrusion and improved shape accuracy in the final structures; furthermore, variations in extrusion flow rate have further regulated structural resolution and mechanical strength [12], [13].

Table 1. Bioprinting methods and applications

Method	Characteristics	Field of Applications
Inkjet Bioprinting	A construction method in which low-viscosity bioinks are deposited in the form of droplets.	Used in thin structures and applications requiring precise cell distribution
Laser-Assisted	It utilizes laser energy to transfer micro-droplets from a bioink layer onto a receiving substrate.	It is used for complex microstructures and applications requiring high resolution.
Extrusion-Based	In this printing method, the material is pushed forward from a syringe or similar reservoir through a nozzle.	It is suitable for high-viscosity bioinks, such as bone scaffolds and thick tissues.
Microvalve/ Piezo	In this method, bioink is released in a controlled manner via pressure pulses.	It is utilized in applications requiring specific cell patterning.

Source: created by the authors.

Current low-cost bioprinters derived from FDM platforms face certain unresolved challenges, particularly in ensuring extrusion accuracy and structural accuracy of low-viscosity hydrogels such as alginate, which are critical for copying thin sheet geometries and fabricating aortic valve structures. High-end commercial bioprinters are also too

expensive for many research laboratories, leading to increased interest in cost-effective systems that meet the engineering demands of complex tissue fabrication [14], [15], [16].

Commercial bioprinters designed for high-precision hydrogel deposition are often very expensive; therefore, there is a need for the development of low-cost, interchangeable, and open-source bioprinter platforms [17]. While previous studies have demonstrated the potential of FDM conversions, challenges remain in ensuring the necessary print resolution for complex geometries such as aortic valves, robust extrusion stability for low-viscosity, viscoelastic bioink, and precise control of deposition rates [18], [19]. However, extrusion stability, deposition rate control, structural deformation of viscoelastic materials, and achievable printing resolution require detailed engineering modifications and systematic optimization [20].

Although alginate is one of the most utilized bioinks in bioink-based additive manufacturing methods, these bioinks are employed in the fabrication of scaffolds designed using Computer-Aided Design (CAD) methods; therefore, it is crucial to compare the produced results with the intended scaffold designs. In its most accurate expression and definition, printability refers to the capability of a material to be printed under specific conditions to achieve the desired outcome [20]. Generally, printability is determined by three key properties: viscosity, liquid-to-solid transition, and biofabrication parameters.

During bioprinting, bioinks are subjected to high shear stress as they pass through the nozzle under extrusion pressure and return to their resting state immediately after exiting the nozzle. Consequently, the resulting change in viscosity (Figure 1) enhances the depositability of the bioink, and the reduction in shear stress at the nozzle exit improves printing accuracy [21].

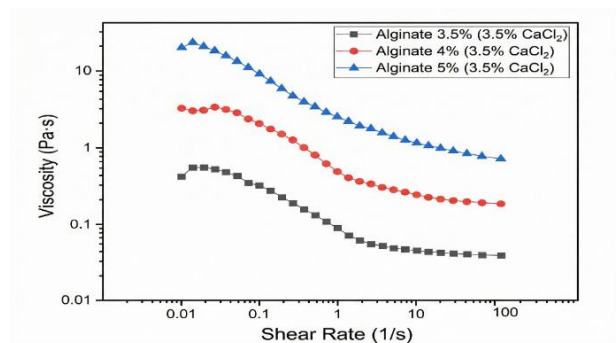


Fig. 1: Viscosity Change with Respect to Shear Rate

Source: [22]

This study introduces a novel engineering strategy to enhance the printability and structural fidelity of alginate-based hydrogels using a custom-modified, cost-effective bioprinting platform. This study uniquely integrates the following:

- (i) mechanical reconfiguration of a consumer-grade FDM printer for high-precision syringe-based extrusion,
- (ii) rigorous characterization of extrusion stability under varying rheological conditions, and
- (iii) quantitative printability assessment that correlates CAD-derived scaffold geometries with fabricated outputs using controlled alginate formulations. By directly addressing the limitations of extrusion precision, deposition homogeneity, and geometric reproducibility, this study established a robust framework for producing complex, patient-specific valve architectures that were previously unattainable using low-cost systems.

2 Method

2.1 Development of the 3D Bioprinter Platform

The bioprinting platform was developed by modifying a high-precision Fused Deposition Modeling (FDM) system (Ender 3 Pro) to accommodate a specialized bioextrusion assembly. The conversion process involved the removal of the thermal plastic extruder and the integration of a custom-designed syringe-pump system. This modification ensured that the X-, Y-, and Z- motion accuracy of the original printer was maintained while providing the necessary volumetric control for hydrogel deposition [23]. Among the various bioprinting techniques, extrusion-based methods are widely adopted because they offer flexible processing conditions. Their ability to handle a broad range of viscosities is uniquely suited for achieving the production speed required for clinical applications [24].

2.1.1 Design of Syringe-Based Extrusion Mechanism

The core of the bioprinter is the syringe-pump extrusion mechanism, which is designed to provide a constant and pulse-free flow of bioink. Unlike pneumatic systems that rely on air pressure, this mechanical extrusion system utilizes a high-torque NEMA 17 stepper motor coupled with a lead screw (8 mm pitch) to translate the rotational motion into

the precise linear displacement of the syringe plunger [23]. The custom-designed syringe-pump extrusion mechanism, which secures a standard 10 or 20 ml Luer-lock syringe within a 3D-printed PLA housing and utilizes a lead screw to drive the plunger, is detailed in Figure 2. This configuration ensures the high extrusion force necessary for viscous materials such as alginate blends.

The integration of this completed print head assembly onto the upper carriage of the modified Ender 3 Pro 3D printer platform is illustrated separately in Figure 3. Components found in the original printer but redundant for bioprinting, specifically the thermistor and heater cartridge, were removed from the design. To enable extrusion at room temperature, the software-defined safety threshold for the minimum extrusion temperature was disabled within the 'Configuration.h' file of Marlin firmware. No additional devices or tools are required for the bioprinter for bioinks that do not include any chemical materials cured by ultraviolet light.



Fig. 2: Syringe-Based Extruder Design
Source: created by the authors.

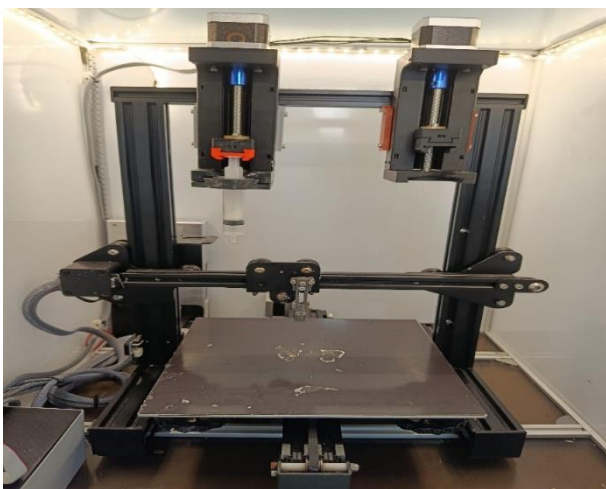


Fig. 3: Assembly of Dual Syringe-Based Extrusion System
Source: created by the authors.

2.2 Preparation of Bioinks

Three different bioinks were prepared according to their alginate-gelatin content. For 2% Alginate 8% Gelatin, (A2G8) 0.02 g/ml Merck 104078 gelatin (Sigma-Aldrich, Switzerland) was dissolved in 15 ml phosphate-buffered saline (PBS) at 40°C for 10 minutes. Then, 0.3 g of alginate (Sigma-Aldrich 71238, Switzerland) was added slowly to gelatin at room temperature and mixed for 15 min. The alginate/gelatin bioink was loaded into a syringe and stored in a 4°C fridge. Other bioinks, 3% alginate 7% gelatin (A3G7) were prepared with 0.03 g alginate, 0.07 g/ml gelatin, and 4% alginate 6% gelatin (A4G6) was prepared with 0.04 g alginate, 0.06 g/ml gelatin [25], [26], [27].

2.3 Spreading Ratio and Print Settings

When a polymer melt or solution is forced through an orifice or die, its diameter expands and tends to maintain this cross-sectional profile after the initial expansion; this phenomenon is known as die swell [28]. The swell ratio, defined as the ratio of the extruded filament diameter to the nozzle exit diameter, varies with the extrusion rate and is dependent on both the die geometry and the viscoelastic properties of the fluid [29]. Accordingly, the extrusion rate and extrusion multiplier were determined based on the specific die swell ratio of the selected biomaterial [11]. The volumetric speed of extrusion was determined using Equation (1), and set in the slicer:

$$MVS = \text{Layer Height} \times \text{Extrusion Width} \times \text{Speed} \times \text{Extrusion Multiplier} \quad (1)$$

However, this value is intended for printing plastic materials using conventional printers. For laminar flow within a cylindrical volume or a constant cross-sectional volume, the Hagen-Poiseuille equation provides the pressure drop, assuming that the flow is Newtonian or incompressible [30]. Assuming that bioink is a Newtonian fluid, the pressure difference created as the bioink is pushed from the syringe to the nozzle is calculated using the following formula:

$$\Delta p = \frac{8\mu LQ}{\pi R^4} \quad (2)$$

Equation 2 provides a basic theoretical approximation; however, the actual pressure requirements observed during extrusion turned out to be higher than predicted. Because the biomaterial is non-Newtonian, the pressure difference and flow behavior are more accurately represented by the Power-Law [24]:

$$model = \tau = K\dot{\gamma}^n \quad (3)$$

$$wall\ shear\ ratio = \dot{\gamma}_w = \frac{4Q}{\pi r^3} \quad (4)$$

Based on the rheological characterization of similar alginate-gelatin hydrogel blends in the literature, the fluid behaves as a shear-thinning pseudoplastic material ($n < 1$). For the mathematical modeling of the extrusion process, the flow behavior index (n) and consistency index (K) were approximated from established empirical power-law fits. A3G7 formulation at room temperature, the consistency index is estimated as $K \approx 35\ Pa.s^n$ and the flow behavior index as $n \approx 0.42$. Formulations with higher alginate content (A4G6) exhibit increased viscosity and stronger pseudoplasticity ($K \approx 45\ Pa.s^n, n \approx 0.38$), while lower alginate formulations (A2G8) show reduced consistency ($K \approx 25\ Pa.s^n, n \approx 0.45$). These parameters were utilized to evaluate the effective viscosity distribution within the nozzle during extrusion [22].

The effective viscosity was calculated using the following equation:

$$Effective\ Viscosity = \mu_{eff} = K\dot{\gamma}^{n-1} \quad (5)$$

In extrusion-based bioprinting, it is necessary to control the spreading rate of the extruded bioink to achieve high shape accuracy [10]. The spreading ratio is defined as the quotient of the actual printed filament width to the inner diameter of the nozzle and is calculated using Equation (6) [31].

$$SR = \frac{\omega_P}{D_n} \quad (6)$$

Viscoelastic hydrogels tend to spread across the substrate due to gravity, surface tension, and wetting phenomena before complete cross-linking or thermal gelling occurs. Minimizing this post-extrusion deformation is crucial, as a spreading ratio close to 1.0 indicates optimum print quality and structural resolution of the printed object. Variations in rheological properties, particularly yield stress and zero-shear viscosity, directly dictate the extent of spreading, necessitating the precise calibration of extrusion pressure and printing speed to ensure the dimensional accuracy of the final construct [10], [32].

2.3.1 Shape Fidelity Analysis

To quantitatively assess the printability and time-dependent shape fidelity of the bioink, the

spreading ratio was determined through morphological analysis. Single-line rectilinear strands, each 30 mm long, were extruded onto the printing substrate. To observe the effects of material accumulation and layer stacking on the spreading behavior, constructs consisting of two and three consecutive layers were fabricated. Immediately after extrusion, the structural deformation of the filaments was monitored using microscopy. To capture the dynamic spreading profile before complete stabilization, microscopic images were acquired at discrete time intervals of 30, 60, and 90 s after printing. The actual printed filament width (ω_P) was measured from these images using ImageJ software (National Institutes of Health, Bethesda, MD, USA), and the spreading ratio was calculated using Equation (6).

2.3.2 Slicer Settings

To create precise toolpaths for the bioprinting process, CAD models were machined using PrusaSlicer slicing software with parameters specifically adapted for hydrogel extrusion. A nozzle diameter of 0.6 mm was used, and both the primary and initial layer heights were set uniformly to 0.5 mm. To accurately assess the shape accuracy of single-line strips without structural intervention, the infill density was set to 0% (without infill pattern), and all auxiliary structures, including supports, skirts, and edges, were also disabled. The start position (seam) was configured to 'aligned' to maintain a consistent deposition across layers. The printing speed was maintained at 5 mm/s, a widely accepted parameter in the bioprinting literature to minimize shear stress on the biomaterial while ensuring continuous filament deposition [33].

Furthermore, given the ambient temperature requirements of the bioink and hardware configuration, both the nozzle and build plate heating mechanisms were disabled. Material retraction was completely turned off to prevent air bubble entrapment within the syringe barrel. Additionally, all default start, layer change, and end G-codes were removed to prevent any unwanted rapid movements of the extruder mechanism [31].

A critical parameter in this experimental setup was the extrusion multiplier (flow rate), which was initially configured to a very low value of 0.02. This drastic reduction is a mathematical necessity when adapting conventional FDM slicing algorithms, which natively calculate the volumetric flow based on a standard 1.75 mm solid thermoplastic filament, to the volumetric displacement of a syringe-based extrusion mechanism. The first-layer extrusion width was set

to the default automatic calculation to match the nozzle profiles. It is crucial to emphasize that this extrusion multiplier is not a universal constant; it must be empirically recalibrated for each hydrogel formulation. Variations in the rheological properties, specifically the zero-shear viscosity and yield stress of different bioinks, directly alter the required stepper motor displacement to achieve an ideal spreading ratio and uniform strand width of 0.6 mm.

2.4 CAD Design of Aortic Valve

Geometric modeling of the heart valve leaflets was performed using Autodesk Fusion 360 (Autodesk Inc., San Rafael, CA, USA) software, as illustrated in Figure 4. A compact and simplified three-dimensional solid model of the tri-leaflet was developed. The valve was designed with an outer diameter of 20 mm and a total length of 8 mm. Additionally, the clearance between the leaflets was set to 2 mm, and the uniform leaflet thickness was adjusted to 0.6 mm [34].



Fig. 4: Compact Simplified Aortic Heart Valve Leaflets

Source: created by the authors.

A significant limitation of the current study is the geometric simplification of the aortic valve model; this means that while successful fabrication is possible, the printed structures cannot fully replicate the complex biomechanical functions of natural valves. This simplification was necessary due to the inherent printing challenges of extrusion-based bioprinting and the low mechanical stiffness of alginate-gelatin hydrogels prior to cross-linking, which restricts the immediate functional assessment of the valve's hemodynamic performance. Hydrogel-based materials, particularly blends such as 3% alginate and 7% gelatin, exhibit low mechanical stiffness before crosslinking. Consequently, they cannot adequately support their own weight in highly curved, overhanging anatomical regions, such as native leaflet coaptation zones, and are highly prone to structural

collapse during layer-by-layer deposition [25]. By employing a compact and simplified geometric model, both printability and shape fidelity were significantly enhanced. Ultimately, this approach ensures that the fabricated structure functions as a mechanically viable scaffold, providing a stable microenvironment designed for subsequent cell attachment, proliferation, and extracellular matrix (ECM) deposition [35].

2.5 Computational Modeling of Extrusion Dynamics

To computationally evaluate the flow behavior and shear-thinning characteristics of the bioink during the printing process, a 2D axisymmetric numerical model was developed using Ansys Fluent. In the experimental setup, instead of a standard metal 3D printer nozzle, a disposable 200 μL conical plastic pipette tip was utilized.

The fluid domain was modeled according to the physical dimensions of the pipette tip, featuring an inlet diameter of 5.0 mm that linearly converges to a 0.6 mm outlet over a total length of 50 mm. A fine mesh as shown in Figure 5, was generated at the boundaries to accurately capture the high velocity gradients and boundary layer effects near the nozzle walls. The bioink flow was assumed to be steady, incompressible, and laminar. To model the non-Newtonian pseudoplastic nature of the alginate-gelatin blend, the Power-Law viscosity model was implemented using the approximated empirical constants for the A3G7 formulation $K \approx 35 \text{ Pa} \cdot \text{s}^n$, $n \approx 0.42$. A no-slip boundary condition was applied to the stationary conical wall. The pressure outlet was set to zero-gauge pressure (atmospheric condition). To match the experimental deposition speed of 5 mm/s at the 0.6 mm orifice, the corresponding mass-conserving inlet velocity was calculated as $7.2 \times 10^{-5} \text{ m/s}$ and applied at the velocity inlet boundary. The SIMPLE algorithm with second-order upwind schemes was used to solve the governing conservation of mass and momentum equations [11], [21], [22], [30].

A preliminary grid independence study was conducted by systematically refining the mesh, ensuring that the selected grid density provided computationally stable and mesh-independent results with less than a 2% variation in the maximum velocity compared to a finer grid.

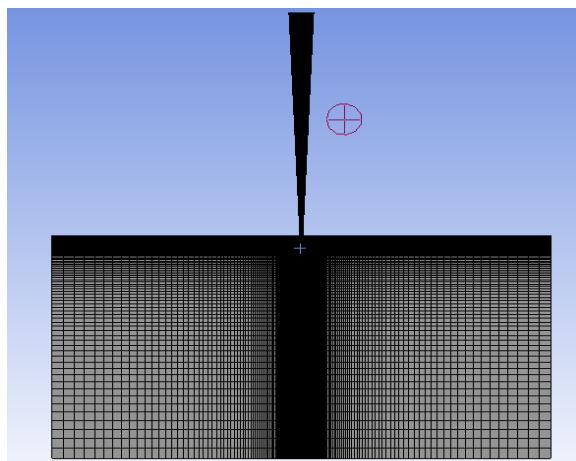


Fig. 5: Fine Mesh for Fluid Domain
Source: created by the authors.

3 Results

3.1 Spreading Analysis

In this section, the morphological characterization and quantitative assessment of the printability of the formulated hydrogels are discussed. To evaluate shape fidelity and post-extrusion structural stability, the spreading ratios (SR) of three distinct bioink compositions, [A2G8], [A3G7], and [A4G6], were systematically analyzed. Before evaluating the bioink samples, a 1 mm standard reference gauge was imaged under identical magnification settings. This reference image was imported into ImageJ to calibrate the spatial scale Figure 6 and Figure 7, thereby converting the pixel measurements into precise metric values for accurate morphological characterization.

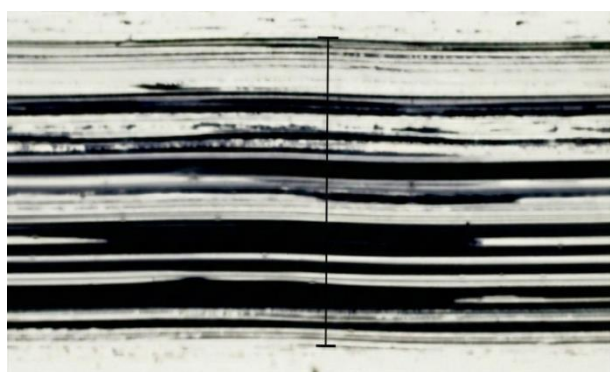


Fig. 6: Reference Measurement of 1 mm
Source: created by the authors.

As shown in Figure 8, Figure 9, and Figure 10, the lateral spread and geometric deformation of the extruded bioinks were captured by microscopic imaging. As previously mentioned, the spread ratio (SR) is a critical indicator of printing capability; an SR value approaching one ($SR \approx 1.0$) reflects an

optimal rheological equilibrium, indicating that the bioink can effectively support its own weight without significant structural collapse. Conversely, high SR values indicate excessive lateral flow, which is often associated with insufficient yield strength or delayed gelation. The comparative microscopic evaluation of these three bioinks and their corresponding spread profiles provides fundamental information about their structural integrity and suitability for multilayer scaffold fabrication.

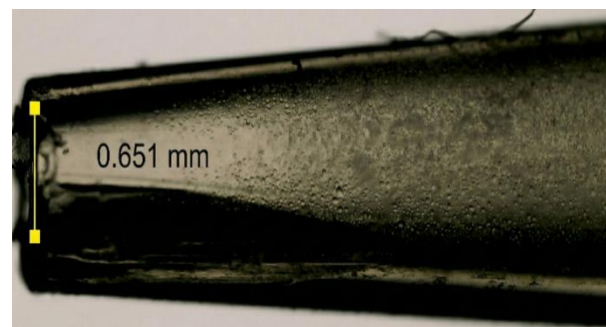


Fig. 7: Nozzle Diameter
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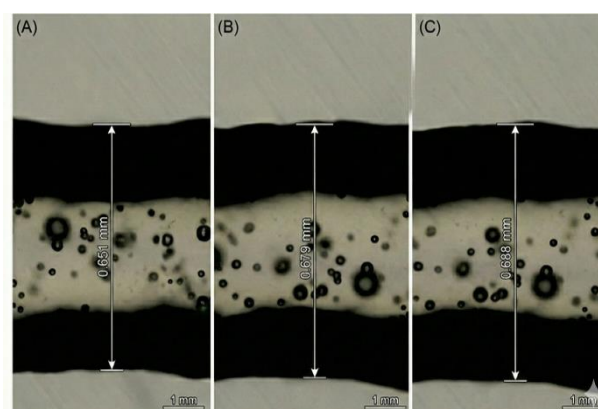


Fig. 8: 2 Layer Spreading for A2G8 A) 30 seconds
B) 60 seconds C) 90 seconds
Source: created by the authors.

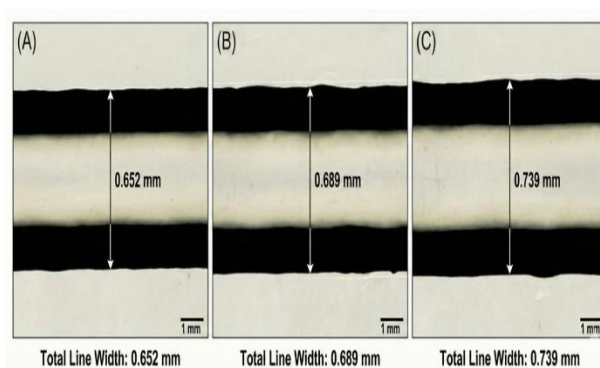


Fig. 9: 2 Layer Spreading for A3G7 A) 30 seconds
B) 60 seconds C) 90 seconds
Source: created by the authors.

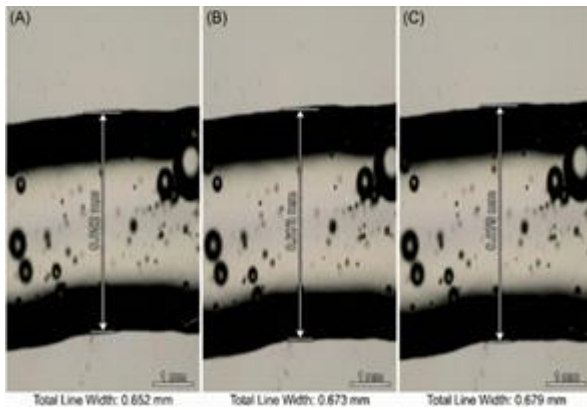


Fig. 10: 2 Layer Spreading for A4G6 A) 30 seconds B) 60 seconds C) 90 seconds

Source: created by the authors.

Time-dependent spread rates (SR) of A2G8, A3G7, and A4G6 bioink formulations were monitored over a 90-second interval after extrusion, and quantitative results are summarized in Table 2. Initially ($t=30$ s), all formulations achieved excellent shape accuracy with spread rates approaching one ($SR \approx 1.00$), indicating that the extrusion parameters were well calibrated for the nozzle diameter. However, significant changes in structural stability were observed over time.

The A3G7 group demonstrated the most pronounced spreading behavior, reaching an SR of 1.06 at 60 s and increasing to 1.14 at 90 s. In contrast, the formulations with modified concentrations exhibited superior dimensional retention values. The A2G8 group, with its higher gelatin content, maintained an SR of 1.06 after 90 s, likely due to the rapid thermal gelation mechanism of the gelatin network which provided immediate structural support. Similarly, the A4G6 group exhibited the highest shape fidelity, with a final SR of 1.05. This stability in the A4G6 formulation can be ascribed to the increased zero-shear viscosity and yield stress resulting from the higher alginate concentration, which effectively restricted the lateral flow under gravitational forces.

Table 2. Spreading ratio analysis

Spreading Ratio	2 Layers			3 Layers		
Time	A2 G8	A3 G7	A4 G6	A2 G8	A3 G7	A4 G6
30	1	1	1	1	1	1
60	1.04	1.06	1.04	1.06	1.08	1.03
90	1.06	1.14	1.05	1.07	1.15	1.05

Source: created by the authors.

3.2 Shape Fidelity Analysis

Following the single-line spreading ratio assessments, the macroscopic shape fidelity of the

bioink formulations was evaluated by fabricating multi-layered grid constructs. This analysis is crucial for determining a material's capacity to maintain interconnected pore networks and withstand the weight of successive layers without structural collapse. Figure 11 illustrates the top-down macroscopic views of the 3D bioprinted 18 x 18 rectangular scaffolds utilizing the A2G8, A3G7, and A4G6 formulations. Under the default slicing settings, the A3G7 formulation was unsuccessful, as excessive lateral spreading led to severe structural compromise and closure of the internal pores. Conversely, the A4G6 formulation yielded the best overall results, demonstrating superior shape fidelity and excellent preservation of designed macroporosity. In the case of the A2G8 formulation, although the overall grid pattern was successfully maintained, partial occlusion of certain internal pores was observed. Because material retraction was intentionally disabled during the printing process to prevent air bubble entrapment within the syringe, the default non-printing travel movements generated by the slicing algorithm resulted in continuous material oozing across the voids. Consequently, this observation highlights that custom optimization of the toolpath trajectories is necessary to mitigate unwanted cross-travel deposition and fully preserve the designed gap structures.

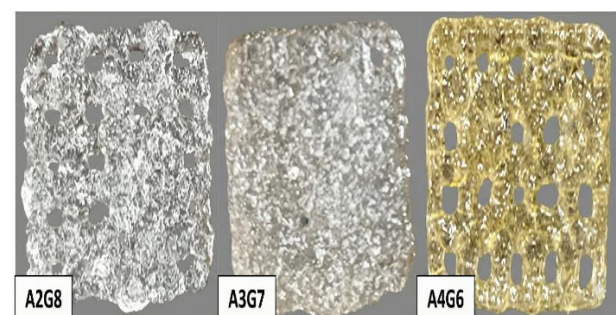


Fig. 11: 18x18 Grid Pattern Samples

Source: created by the authors.

To overcome the structural failure of the A3G7 formulation observed under default parameters, targeted optimization of the slicer settings was conducted. As shown in Figure 12, a highly defined grid construct with clear, interconnected macropores was successfully fabricated using the A3G7 bioink. This dramatic improvement in macroscopic shape fidelity was achieved by proportionally reducing the extrusion multiplier (EM) to compensate for the excess material volume, while concurrently fine-tuning the layer height. To mathematically justify this optimization, a predictive volumetric conservation model was

utilized. Assuming the deposited filament forms a roughly rectangular cross-section on the build plate before gelation, the volume of hydrogel deposited per unit length V_L is given by the product of the final filament width and the layer height. This volume is directly proportional to the Extrusion Multiplier (EM) and inversely proportional to the printing speed v_p :

$$w \cdot h = \frac{k \cdot EM}{v_p} \quad (7)$$

where, k is an experimental, machine-specific constant representing the displacement of the stepper motor. By rearranging this equation to find the spread ratio ($SR = w/D_n$), the direct relationship between slicing parameters and shape accuracy becomes:

$$SR = \frac{k \cdot EM}{v_p \cdot h \cdot D_n} \quad (8)$$

According to this predictive model, the initial A3G7 formulation effectively compensated for the higher spreading ratio ($SR = 1.14$) by volumetrically restricting the hydrogel deposited per unit length (Figure 12). It must be emphasized that modifying the standard E-step (extruder steps per millimeter) calibration within the printer's firmware is an impractical and fundamentally incorrect approach for addressing such material over-extrusion. E-step is a hardware-specific calibration metric for stepper motors; however, different types of bioinks have their own unique rheological profiles and swelling behaviors. Consequently, successful optimization requires dynamic adaptation of parameters such as extrusion multiplier and layer height within the slicing software to create material-specific printing profiles for each bioink formulation.



Fig. 12: A3G7 Optimized Print
Source: created by the authors.

3.3 Computational Fluid Dynamics of Nozzle Extrusion

To theoretically validate the successful extrusion of the high-viscosity alginate-gelatin bioink through a narrow orifice without clogging, a 2D axisymmetric Computational Fluid Dynamics (CFD) simulation was conducted using Ansys Fluent. The simulation modeled the flow of the A3G7 formulation through the conical geometry of the 200 μ L disposable pipette tip. The non-Newtonian behavior was characterized by using the Power-Law model with the previously defined empirical constants.

The resulting velocity magnitude contour as shown in Figure 13 illustrates the acceleration of the bioink as it approaches the 0.6 mm orifice. The maximum velocity is observed at the central axis of the nozzle tip, while the velocity at the walls is zero due to the no-slip condition.

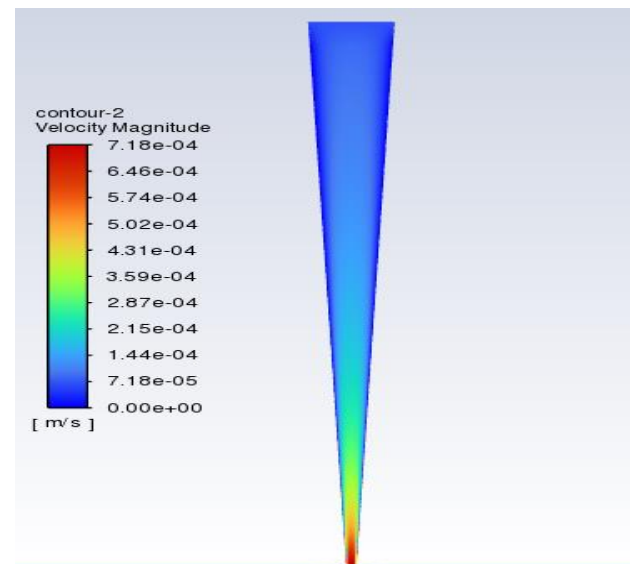


Fig. 13: Velocity Contour of A3G7 Bioink Through Nozzle

Source: created by the authors.

The effective molecular viscosity profile as shown in Figure 14 directly demonstrates the shear-thinning (pseudoplastic) mechanics of the bioink. Due to the high shear rates generated near the converging walls of the conical tip, the effective viscosity of the hydrogel drops significantly in these boundary regions. As the material exits the nozzle into the ambient environment, the shear stress abruptly decreases, leading to an immediate recovery of its high resting viscosity. This rapid viscosity recovery confirms the material's ability to maintain structural shape fidelity immediately upon deposition, supporting the stable fabrication of the multi-layered aortic valve construct.

3.4 Fabrication of a Simplified 3D Aortic Valve Three-Leaflets Model

After successfully establishing the fundamental rheological requirements and optimizing the processing parameters to achieve high shape fidelity in planar grid constructs, the investigation was advanced to the fabrication of anatomically inspired 3D structures. To demonstrate the translational potential of the optimized bioink formulations for cardiovascular tissue engineering applications, a simplified three-leaflet (trifoliate) aortic valve model was selected as a proof-of-concept. Unlike basic rectilinear scaffolds, the fabrication of this valve prototype presents significantly elevated challenges, necessitating the precise and continuous deposition of hydrogel filaments along complex curved trajectories to form leaflet-like structures without using sacrificial support materials. Consequently, the robust printing protocols validated in the preceding shape fidelity analysis were used to address these intricate geometric demands. A macroscopic top-down view of the successfully bioprinted freestanding aortic valve construct, which clearly demonstrates the preservation of the trifoliate geometry and central coaptation area, is shown in Figure 15.

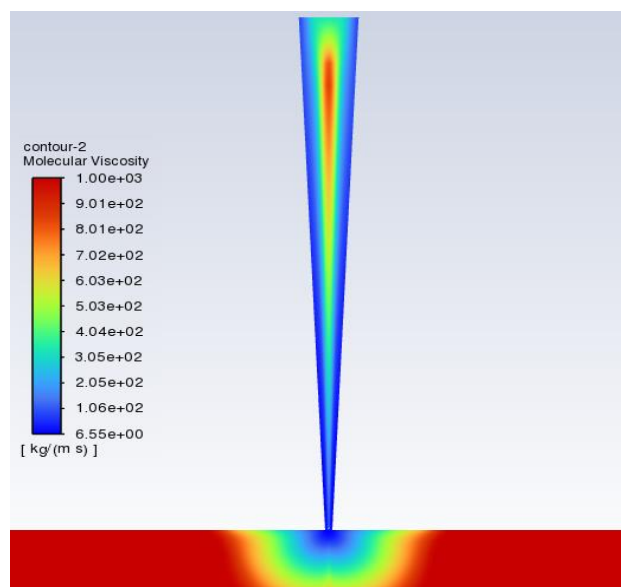


Fig. 14: Molecular Viscosity Contour
Source: created by the authors.

To quantitatively assess the macroscopic shape fidelity and dimensional accuracy of the bioprinted construct, a top-down geometric deviation analysis was performed. Figure 16 illustrates the superposition of the nominal CAD model boundary (black outline) over the fabricated aortic valve scaffold. As observed, the construction largely maintains the designed trifoliate geometry and

structural integrity. However, consistent with the single-line spreading ratio analysis ($SR > 1.0$), the extruded hydrogel exhibits minor lateral material spreading beyond the defined CAD borders. Furthermore, the surface tension and viscoelastic relaxation of the bioink before complete thermal gelation led to slight corner rounding at the central coaptation area, resulting in a reduction of the internal pore size. Despite these rheologically driven deviations, the optimized slicing parameters successfully prevented the structural collapse of the overhanging leaflets.



Fig. 15: Simplified Aortic Heart Valve Three-Leaflets
Source: created by the authors.

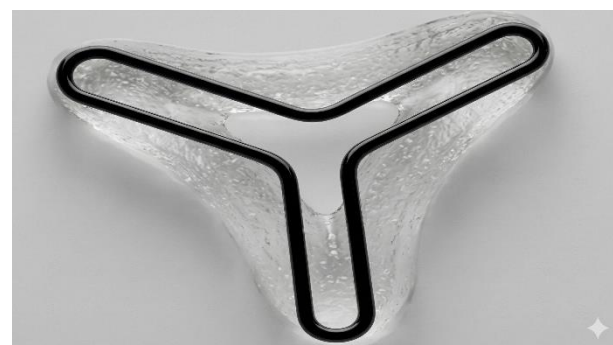


Fig. 16: Top-Down Geometric Deviation Analysis
Source: created by the authors.

4 Conclusion

This study successfully demonstrated the engineering conversion of a commercial, low-cost Fused Deposition Modeling (FDM) 3D printer into a highly capable, precision-driven bioprinting platform. By integrating a custom NEMA-17 syringe-pump extrusion mechanism, the modified system effectively deposited viscoelastic, low-viscosity alginate-gelatin bioinks with high volumetric control and continuous flow dynamics.

A critical outcome of this research is the establishment of a systematic protocol for printability assessment and defect mitigation. Through quantitative morphological analysis of the spreading ratio, it was proven that dynamic calibration of slicing parameters, specifically the

extrusion multiplier and layer height, can successfully compensate for material-specific rheological behaviors. This software-driven optimization approach prevented structural collapse and enabled the high-fidelity printing of complex geometries without necessitating costly hardware alterations.

The translational potential of the optimized bioprinter and bioink formulations was validated through the support-free fabrication of a simplified three-leaflet aortic valve scaffold. Geometric deviation analysis confirmed that the bioprinted structure largely retained the nominal CAD geometry and exhibited only minor, rheologically expected lateral spreading and corner rounding before full thermal gelation. While this study demonstrates geometric accuracy and initial structural stability, a significant limitation is the lack of a comprehensive evaluation of the mechanical properties (e.g., tensile strength, elasticity, and fatigue resistance) and long-term biological functionality (including cell viability, proliferation, and extracellular matrix production) of bioprinted aortic valves, as well as their hemodynamic performance. Consequently, the clinical applicability of these structures is not fully assessed based on the current findings. Further research is needed to focus on these issues.

Declaration of Generative AI and AI-assisted Technologies in the Writing Process

During the preparation of this work, the author(s) utilized artificial intelligence (AI) technologies solely for the purpose of linguistic refinement and grammatical correction. Following the application of these tools, the author(s) comprehensively reviewed, verified, and edited the resulting text. The author(s) maintain full accountability and responsibility for the accuracy, integrity, and final content of the publication.

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