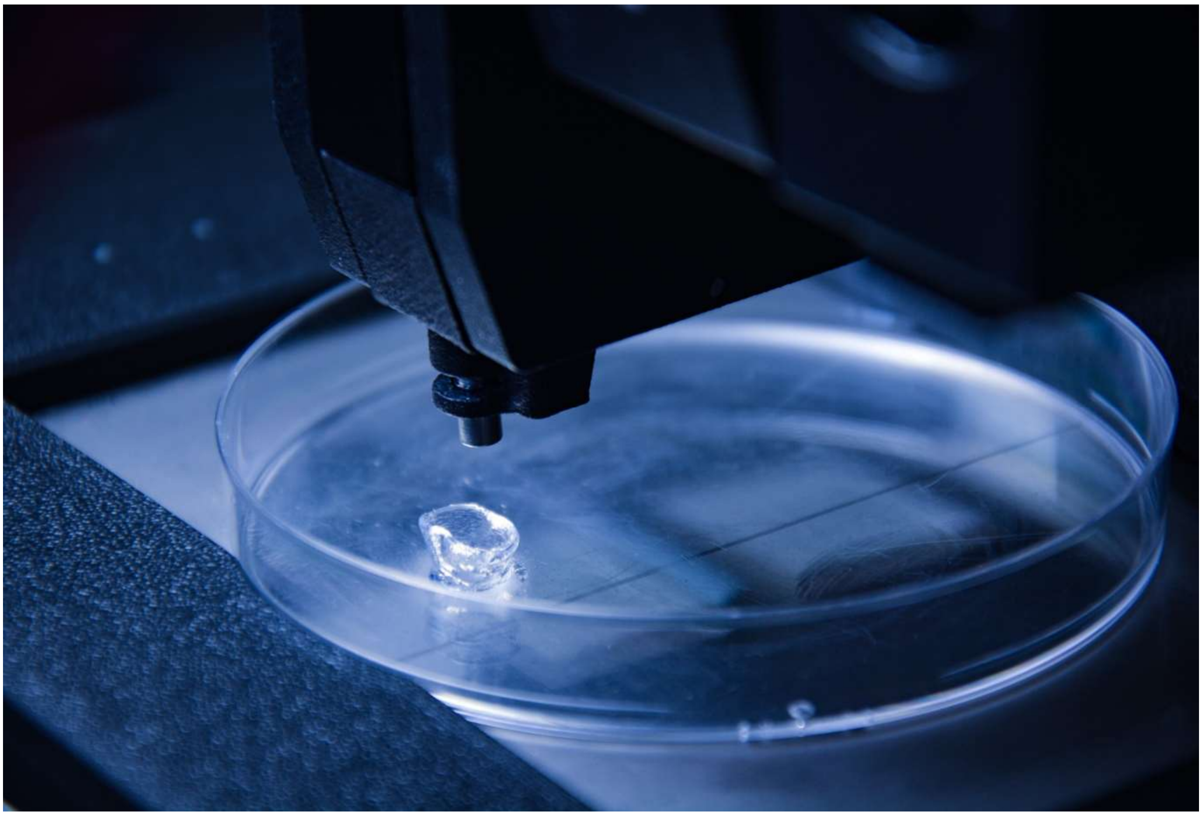
Optimization of Drop-on-Demand Bioprinting for High-Fidelity Human Bifurcation Models

Black Drop Biodrucker GmbH
Robert-Bosch-Str. 7, 64293 Darmstadt, Germany



Abstract

Advanced bioprinting techniques are crucial for creating complex 3D tissue models. This study focuses on optimizing Drop-on-Demand (DoD) bioprinting parameters for a human vascular bifurcation model. A human-sized bifurcation model was 3D bioprinted using 3% Blue Drop Agarose bioink with the Black Drop Regenate Bioprinter. Different printing orientations, were investigated, with a polycarbonate petri dish consistently used as the substrate. A slicing height of 0.2 mm was employed. Our findings revealed that printing the bifurcation upside-down into the petri dish yielded superior structural fidelity and integrity. Conversely, upright printing resulted in significant structural deviation. The optimally bioprinted structures were successfully filled with cell media and demonstrated leak-proof characteristics, indicating their potential for functional applications in tissue engineering and vascular modeling.

Introduction

Bioprinting has emerged as a transformative technology in tissue engineering, enabling the precise fabrication of 3D biological constructs with architectural complexity. The ability to create functional vascular networks is a critical challenge in developing viable organ-on-chip systems, in vitro disease models, and ultimately, regenerative medicine applications. Drop-on-Demand (DoD) bioprinting offers a versatile and gentle approach for depositing cell-laden bioinks, making it suitable for delicate biological materials. However, fabricating structures with intricate geometries, such as bifurcating vascular systems, often presents challenges related to structural stability and fidelity, particularly when dealing with gravity-induced deformation of softer hydrogel bioinks. This study aims to optimize the DoD bioprinting process for a human-scale vascular bifurcation model, focusing on the impact of printing orientation on structural integrity and functionality, thereby contributing to the advancement of complex tissue constructs.

Materials and Methods

A human vascular bifurcation model was designed from an STL file, featuring an inner diameter of 4 mm, a total height of 15 mm, and a wall thickness of 1 mm. The Regenate Bioprinter (Black Drop Biodrucker GmbH) was used with a DoD printhead and a 300 µm nozzle. The bioink used was 3% Blue Drop Agarose. The bioprinting platform was maintained at a controlled temperature of 10°C throughout the printing process. A standard 90 mm polypropylene petri dish served as the substrate for all experiments and was securely positioned on the printbed using the Black Drop petri dish template to ensure precise alignment and stability. The slicing height for all print files was set to 0.2 mm. Two primary printing orientations were tested for the bifurcation model: upright and upside-down. In the upright orientation, the base of the bifurcation was printed first, followed by the diverging branches. In the upside-down orientation, the diverging branches were printed first, allowing the structure to gradually build towards the wider base. Structural integrity and fidelity were assessed post-printing.

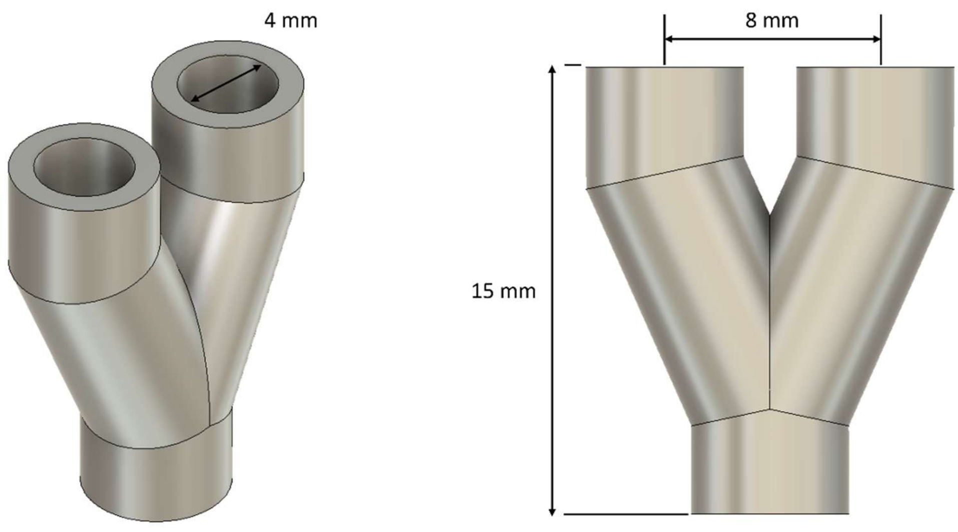


Figure 1: Dimensions of the STL file of the human sized bifurcation shown in upright position.

Results

The bioprinting experiments demonstrated a significant impact of printing orientation on the structural integrity of the human bifurcation model. Upright printing resulted in considerable deviation from the intended geometry, with noticeable sagging and lack of structural support, particularly in the branching regions, leading to compromised fidelity. In contrast, the upside-down printing orientation, where the structure was built from the diverging branches towards the main vessel, yielded significantly superior results. This method consistently produced bifurcation models with excellent structural integrity, closely matching the original CAD design. The optimized upside-down printed structures, fabricated directly into a petri dish, exhibited robust walls and clear lumens, as shown in the figures. Furthermore, functional assessment of these models involved filling them with cell culture media, confirming that the bioprinted bifurcations were entirely leak-proof, a critical requirement for their potential use in fluid dynamic studies and in vitro cell culture applications.

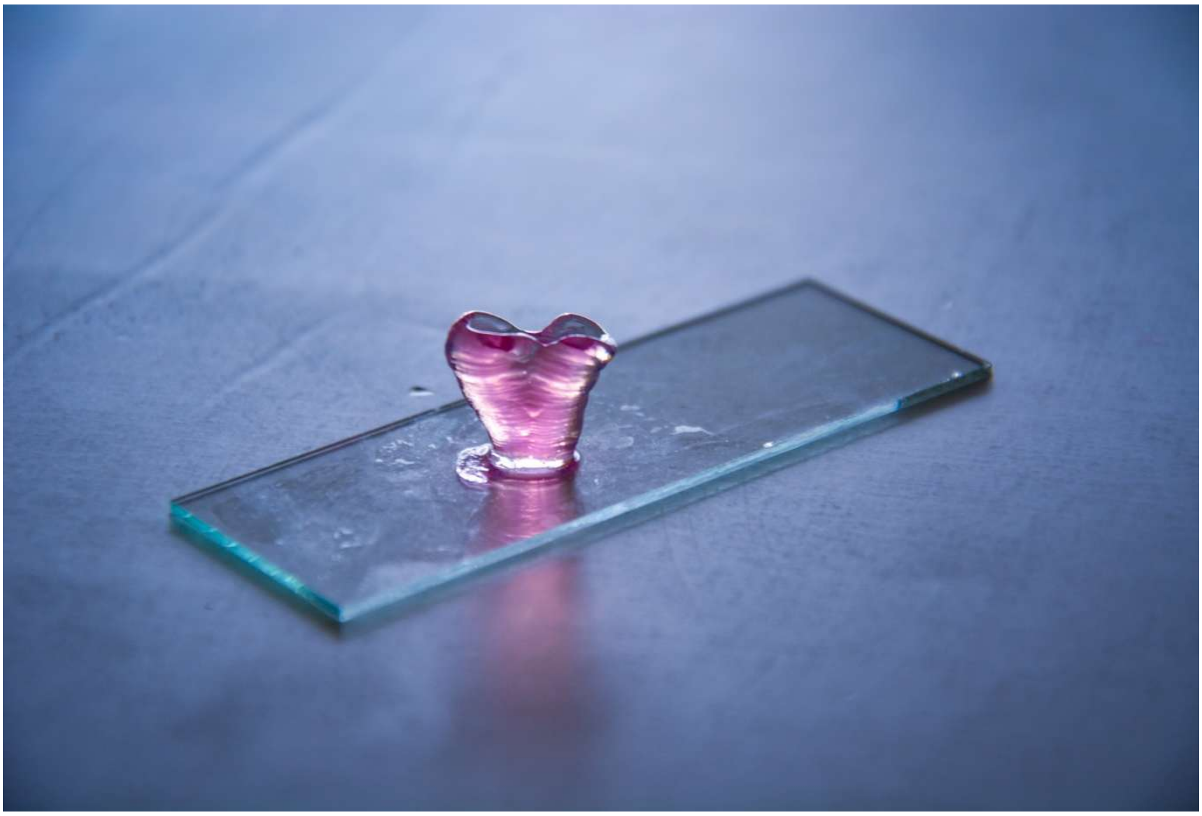


Figure 2: Completed human bifurcation model bioprinted in upright position.

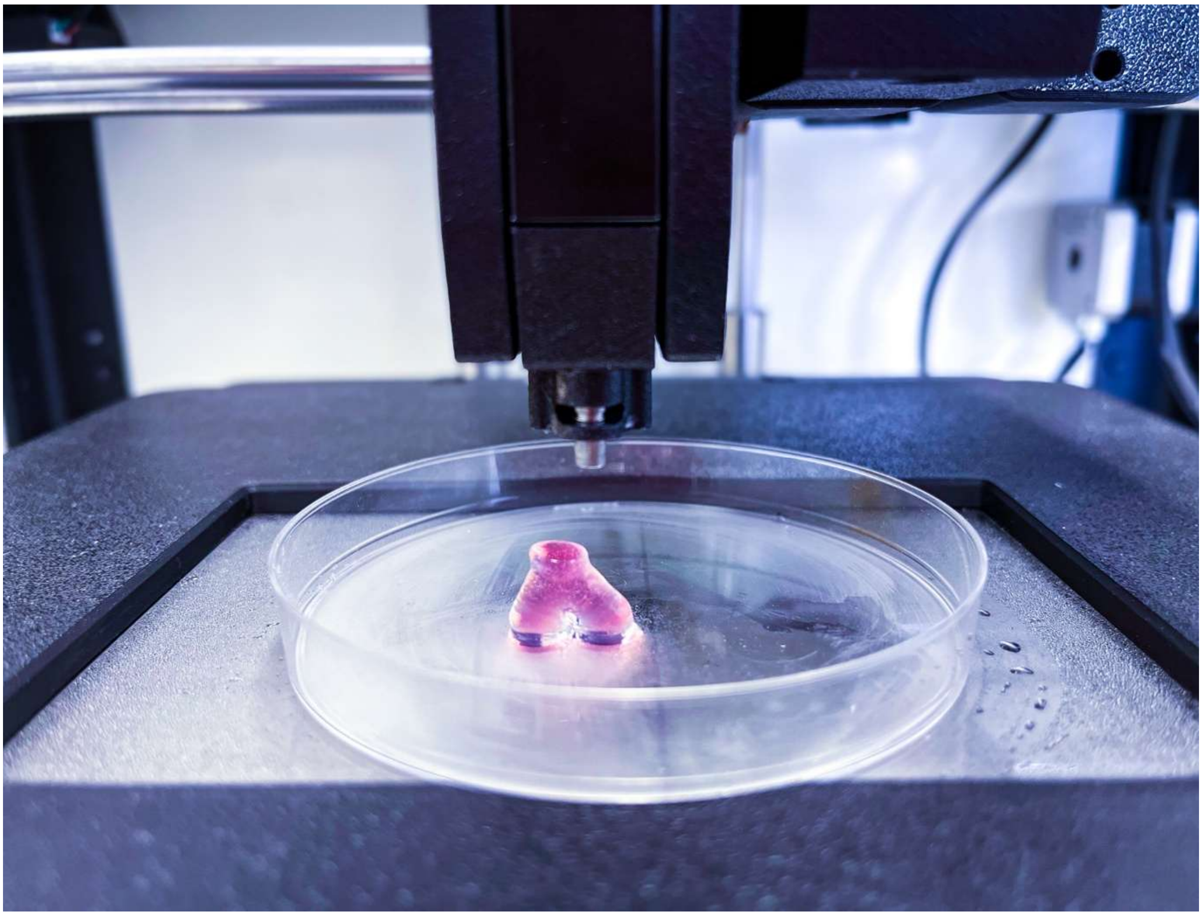


Figure 3: Completed human bifurcation model, bioprinted in upside-down orientation. The structure was filled with cell media post printing to assess potential leakage.

Figure 4: Further iteration of the construct with optimized printing parameters.

Discussion

The observed success of the upside-down printing orientation can be primarily attributed to enhanced gravitational support and improved layer-by-layer stability. When printing in an upright fashion, the unsupported overhangs and nascent branches are susceptible to deformation under gravity before the hydrogel fully consolidates, leading to structural collapse and deviation. By inverting the model, each successive layer receives more substantial support from the already printed, solidified layers below, mitigating the effects of gravity and enabling the faithful reproduction of complex geometries. The use of a cooled platform (10°C) also played a crucial role in promoting rapid gelation of the agarose bioink, further stabilizing each deposited drop and preventing slumping. The ability to produce a leak-proof bifurcation model is a significant step forward, validating the structural integrity necessary for applications such as fabricating vascular grafts or developing patient-specific disease models for studying hemodynamics. Future work could involve incorporating different cell types into the agarose bioink to create functional cellularized vascular models and evaluating their long-term viability and physiological response under flow conditions.

Conclusion

This study successfully optimized the Drop-on-Demand bioprinting of a human vascular bifurcation model using 3% Blue Drop Agarose. By employing an upside-down printing orientation on a cooled platform, we achieved high-fidelity, leak-proof structures that closely replicated the designed geometry. This optimized methodology represents a significant advancement in fabricating complex anatomical structures for applications in regenerative medicine, in vitro disease modeling, and pharmaceutical research, paving the way for more sophisticated bioprinted tissue constructs.