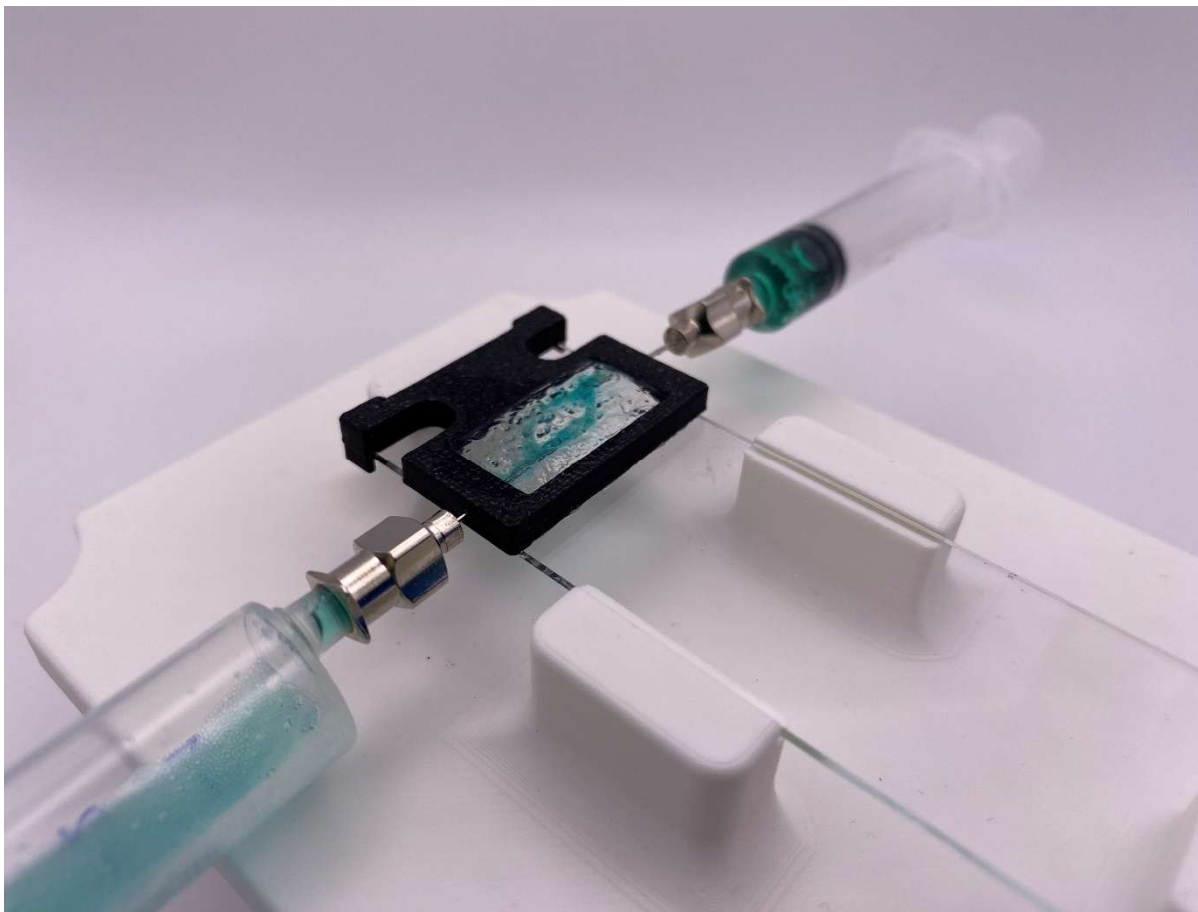


Development of a Bioprinted Perfusable 3D Cell Culture Chip

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Abstract

This study details the development and characterization of a bioprinted perfusable chip system designed for advanced 3D cell culture models. Utilizing a sacrificial printing approach, channels were integrated into a cell-compatible hydrogel matrix. Pluronic F127 (30%) served as the sacrificial material, while Red Drop Gelatine (5%) constituted the main body material. The chip's structural frame was fabricated from PLA, secured to a microscope slide, and equipped with 25G straight metal nozzles for perfusion. The elevated Black Drop template facilitated thin chip design and accessibility of connectors during printing and while perfusion. Post-printing, channels were successfully flushed with a cold flushing solution, demonstrating the system's perfusable nature. This platform offers a tool for creating complex vascularized tissue models in a controlled, perfusable environment.

Introduction

The study of complex biological phenomena, such as tissue development, disease progression, and drug response, often necessitates physiologically relevant *in vitro* models. Traditional 2D cell cultures frequently fail to recapitulate the intricate cellular microenvironment, including cell-cell and cell-matrix interactions, and nutrient/waste transport. Three-dimensional (3D) cell culture models offer a significant improvement, providing a more accurate representation of *in vivo* conditions. However, a critical limitation in many 3D models is the lack of a functional vascular network, which is essential for maintaining cell viability and function over extended periods and for studying processes like angiogenesis and perfusion-dependent cellular responses. Bioprinting technology has emerged as a powerful tool for fabricating sophisticated 3D tissue constructs with precise architectural control. This work focuses on developing a bioprinted perfusable chip system capable of supporting 3D cell models under controlled perfusion, thereby providing a versatile platform for studying vascularization, drug delivery, and cell-environment interactions in a more physiologically relevant context.

Materials and Methods

The bioprinted perfusable chip system was constructed using a combination of materials and fabrication techniques. The main body material for the 3D cell culture environment is 5% Red Drop Gelatine. For creating perfusable channels, Pluronic F127 hydrogel at a concentration of 30% was employed as a sacrificial material. The Pluronic F127 was colored to enhance visibility during the flushing process. A structural frame for the chip was produced using polylactic acid (PLA) via 3D printing, designed to integrate with standard microscope slides. Bioprinting was performed using the Black Drop Regenate 3D bioprinter. The Red Drop Gelatine was deposited using a 25G straight metal nozzle, while the Pluronic F127 sacrificial ink was extruded through a 0.15 mm conical nozzle to ensure precise channel geometries. To facilitate a thin chip design and ensure access to perfusion connectors, the Elevated Black Drop template was utilized to fix the chip onto the printbed during the bioprinting process. After hotmelt 3D printing, the PLA frame was securely glued to a microscope slide. Perfusion nozzles, also 25G straight metal nozzles, were integrated into the PLA frame. The Black Drop Superfill Software was used to create a printing path based on an STL-File, allowing precise bioink deposition inside the frame. Height of Well was set to 7 mm ensuring collision avoidance. Following the bioprinting process, the entire chip assembly (PLA + bioinks) was placed in a refrigerator for 15 minutes to allow for the complete solidification of the Red Drop Gelatine hydrogel. Subsequent to solidification, the sacrificial Pluronic F127 channels were removed by flushing cold flushing solution from both sides of the chip using the integrated connections in the PLA frame. The flushing solution was colored green to visually confirm the successful removal of the sacrificial material and the patency of the created channels.

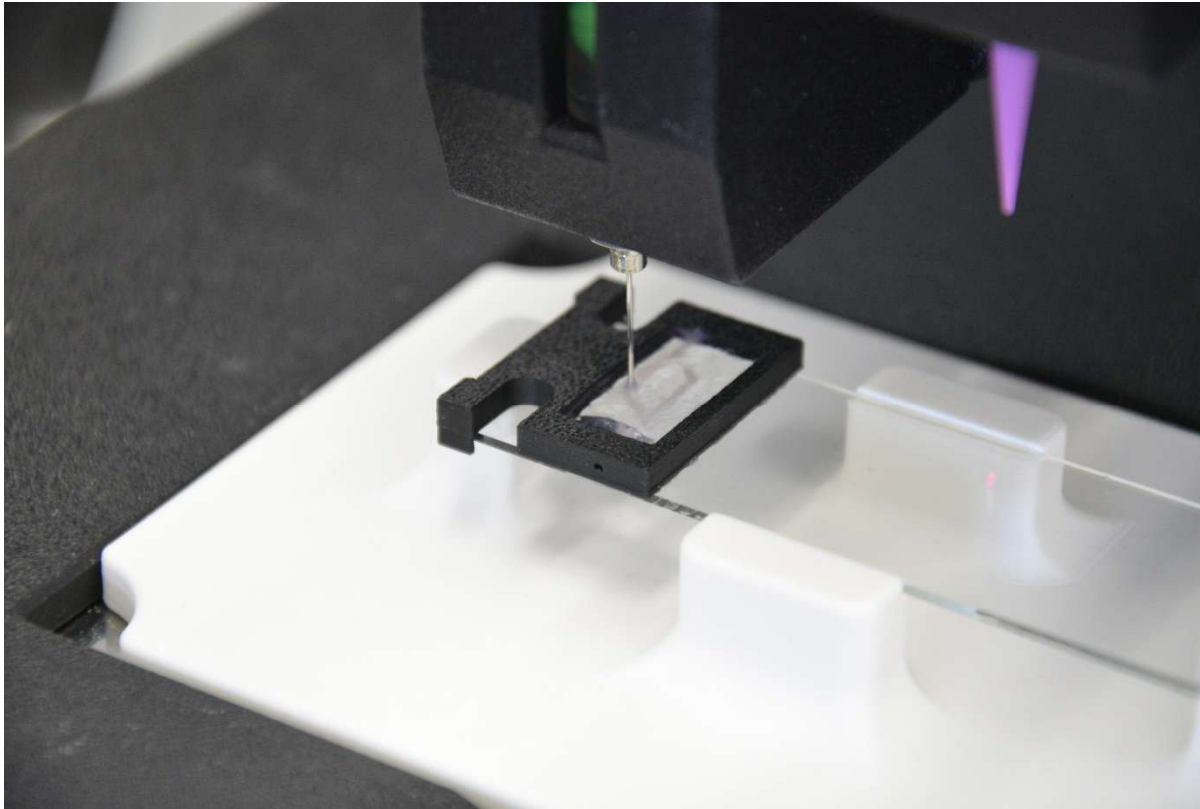


Figure 1: Close-up view of the bioprinting process, showing the printing of Red Drop Gelatine into the PLA frame, which is fixed to the printbed using the Elevated Black Drop template (white).

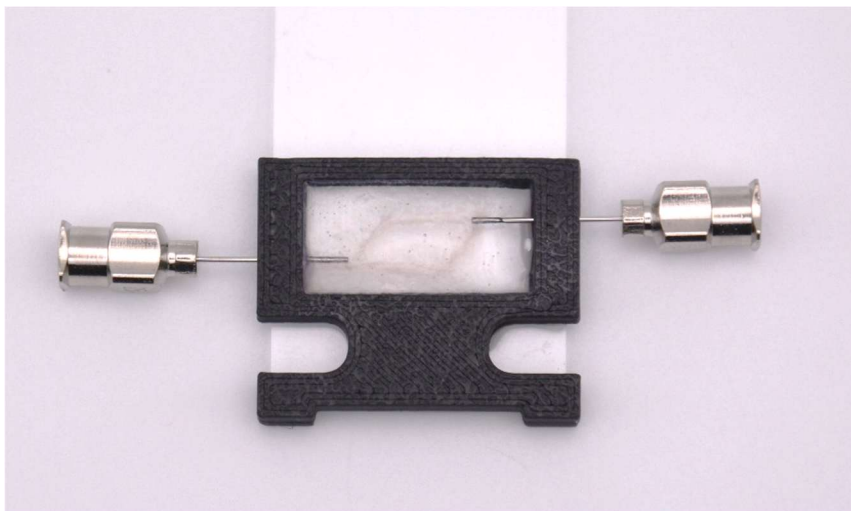


Figure 2: Top-down view of the fully bioprinted chip with 25G straight metal perfusion nozzles inserted into the PLA frame, ready for flushing and connection to a fluidic system.

Results

The bioprinting process successfully yielded a structurally sound and perfusable chip system. The use of a 25G straight metal nozzle for the Gelatine body material and a 0.15 mm conical nozzle for the sacrificial Pluronic F127 allowed for the precise deposition of materials, creating well-defined channels within the hydrogel matrix. The PLA frame effectively secured the hydrogel construct to the microscope slide and provided robust connection points for the perfusion nozzles. Post-printing solidification of the Gelatine in the refrigerator ensured the structural integrity of the chip before channel clearance. The subsequent flushing step, employing a cold, green-colored solution, visually confirmed the complete removal of the sacrificial Pluronic F127. The green flushing solution was observed to flow freely through the designed channels, entering from one side and exiting the other, thereby demonstrating the successful creation of patent and perfusable microchannels within the bioprinted hydrogel. This outcome confirms the feasibility of creating intricate, perfusable vascular networks within 3D tissue models using this bioprinting approach, enabling future studies on cellular responses to flow and vascularization.

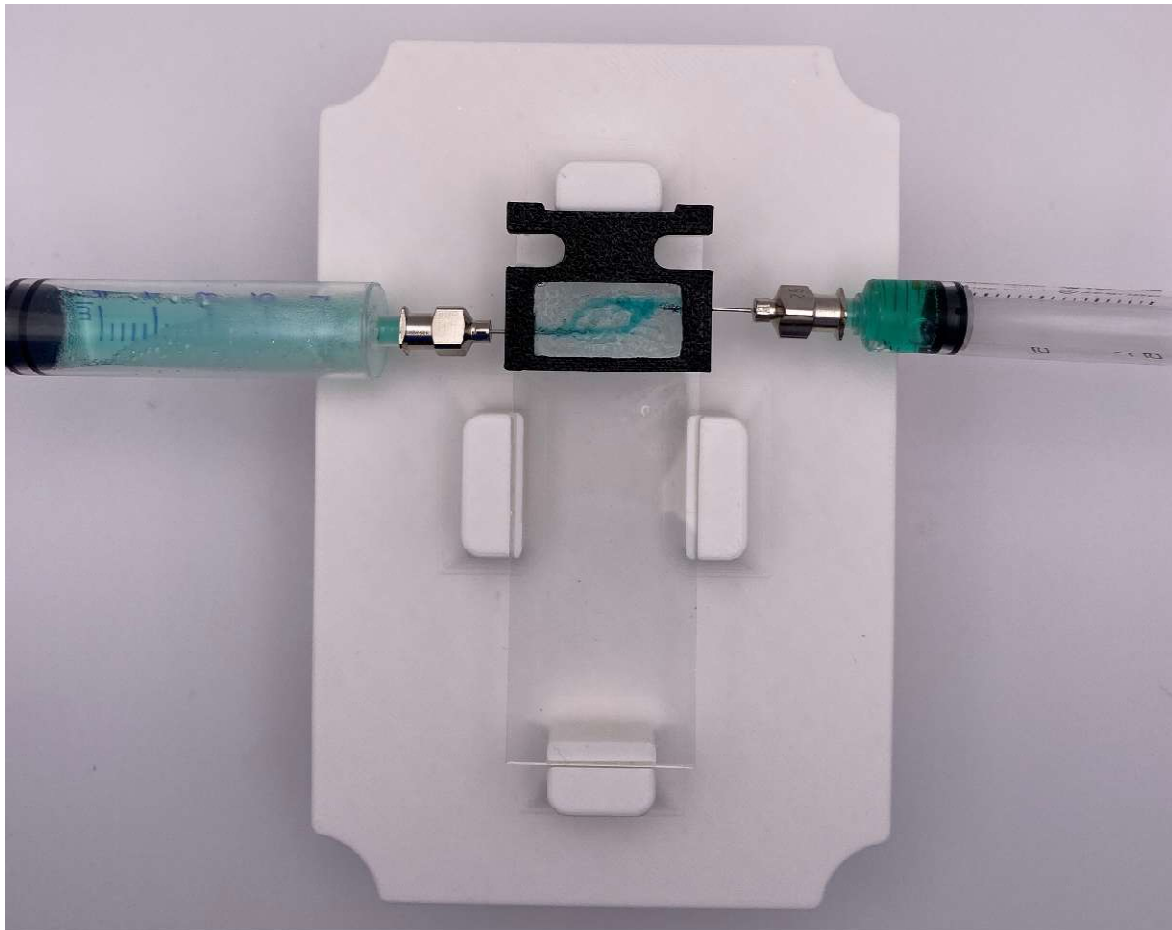


Figure 3: The bioprinted chip system could be easily connected to syringes for the flushing procedure. The green flushing solution could be actively circulated through the microchannels without leakage, confirming their functionality.

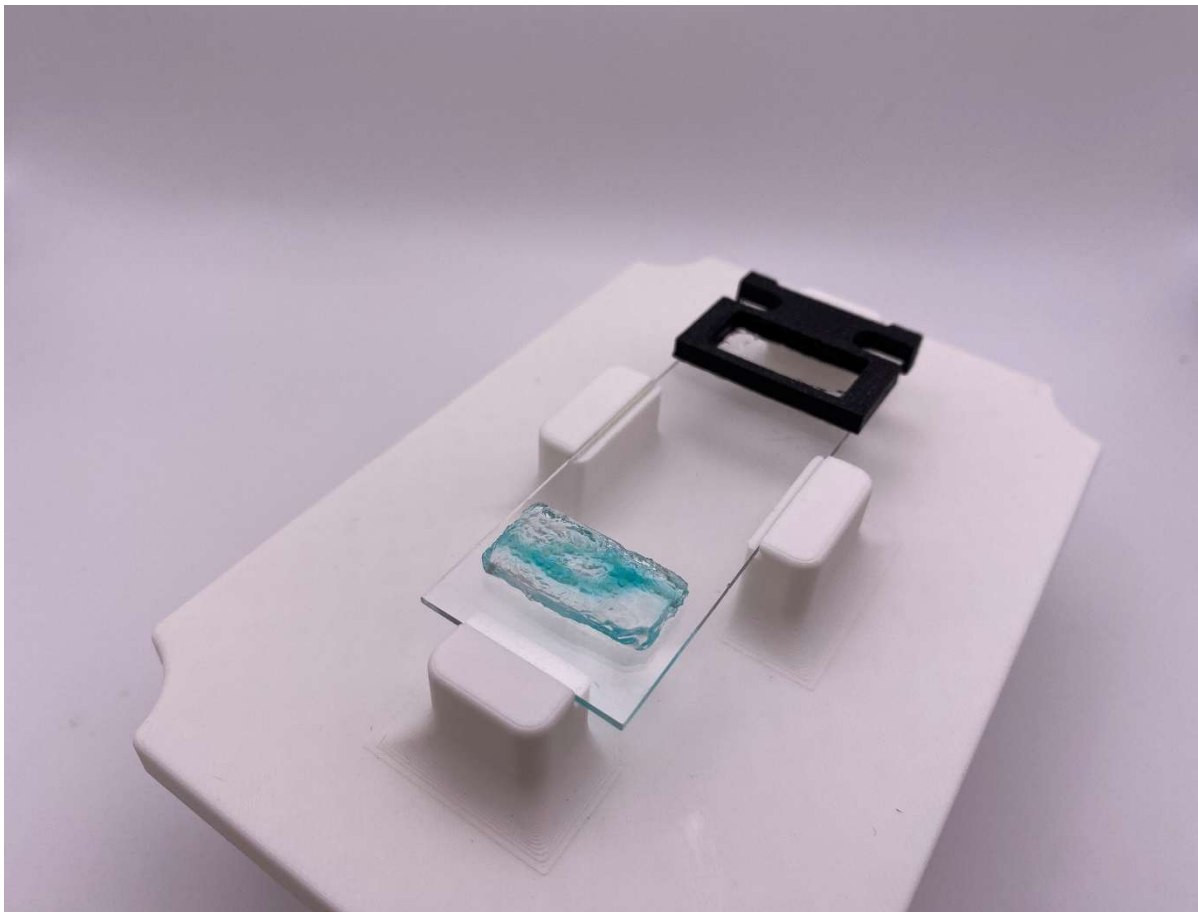


Figure 4: The bioprinted perfusable chip could be gently removed from the PLA frame with a spatula without severe damages.

Discussion

This study demonstrates the automated and scalable fabrication process of a perfusable, bioprinted chip system that could be used for developing advanced in vitro models. The chosen combination of a Gelatine body material and a Pluronic F127 sacrificial ink proved effective for creating stable hydrogel constructs with embedded microchannels at room temperature. Gelatine offers a biocompatible and mechanically stable environment suitable for various cell types, while Pluronic F127's thermosensitive properties make it an ideal sacrificial material, easily removed by cold flushing without damaging the surrounding hydrogel. Nevertheless, the shown model cannot be directly transferred to long-term cell-culture research since Red Drop Gelatine is not stable at 37 °C. To bioprint cell-laden and cell-culture-stable model, photocrosslinkable recombinant CureDrop Hydrogel or Gelatine Methacrylate (GelMA) would need to be used. For acellular cell-culture-stable models, the described process and materials could be kept but expanded by an additional crosslinking step with Transglutaminase to crosslink the Gelatine. After thorough cleaning, this can provide a cell-culture stable and cell friendly perfusable matrix that can be used for vascular models.

The incorporation of a PLA frame glued to a microscope slide provides a viable platform compatible with microscopy, crucial for real-time observation of cell behavior and vascularization processes. The Elevated Black Drop template allowed for a thin chip design, maximizing optical clarity and minimizing

material usage, while maintaining accessibility for external connections. The confirmed patency of the flushed channels validates the design and fabrication process, ensuring that the system can effectively deliver nutrients, remove waste, and apply shear stress to cultured cells, mimicking physiological perfusion. This perfusable system holds immense potential for studying complex biological questions, including angiogenesis, vasculogenesis, and the effects of fluid shear stress on cellular differentiation and function. It provides a more robust and controllable environment than static 3D cultures, moving closer to in vivo conditions for drug screening and disease modeling.

Conclusion

We have successfully developed bioprinting process to automate and scale the production of perfusable chip systems designed for studying 3D cell models under dynamic perfusion. The use of Gelatine- as the body material and Pluronic F127 as a sacrificial ink, combined with precise bioprinting techniques and a custom PLA frame, enabled the creation of stable hydrogel constructs with patent, perfusable microchannels. The ability to visually confirm channel clearance through colored flushing solution underscores the system's reliability. This process platform provides a versatile and physiologically relevant tool for researchers to investigate vascularization, cell-fluid interactions, and other complex biological processes, paving the way for more accurate in vitro models and drug discovery efforts.