

Modular, 3D-Printed Organ-on-Chip Platform with Integrated Optical Sensing for Real-Time Monitoring of the Tumor Microenvironment

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Abstract

Conventional two-dimensional (2D) cancer culture systems fail to reproduce key aspects of the tumor microenvironment (TME), including oxygen gradients, biomechanical cues, and three-dimensional (3D) architecture. Although 3D spheroids and organoids provide improved physiological relevance, their static culture conditions limit dynamic perfusion and hinder real-time environmental monitoring. Existing microfluidic Organ-on-chip (OoC) platforms address these limitations but remain inaccessible to many laboratories due to high fabrication costs, reliance on cleanroom facilities, and lack of intermediate access to cultures. To overcome these challenges, we present a modular, reusable OoC platform fabricated using stereolithography (SLA) 3D printing. This approach enables rapid, cost-effective production of a perfusion system without specialised infrastructure. The system incorporates plug-and-play elements, including a screw top, polydimethylsiloxane (PDMS) membrane support, and central perfusion chamber, allowing easy assembly, sterilisation, and direct access to cultures during experiments. An integrated optical oxygen sensor enables continuous, non-invasive monitoring of oxygen partial pressure (pO_2), providing quantitative insight into metabolic activity and hypoxia formation under perfusion. This platform supports long-term perfusion culture of 3D cancer organoids, enhances environmental monitoring, and improves accessibility of advanced in vitro models. It represents a significant step toward scalable, instrumented systems for cancer research and drug screening.

Keywords: Organ-on-Chip, Dynamic Culture Systems, Stereolithography 3D Printing, Oxygen Sensing, Modular Biofabrication

Introduction

Cancer remains one of the leading causes of mortality worldwide, underscoring the urgent need for improved strategies in drug development and translational oncology [1, 2]. Traditional 2D cell culture models, while widely employed in early-stage compound screening, fail to recapitulate the complexity of tumor biology observed in vivo. Tumor cells in the human body are exposed to dynamic gradients of nutrients and oxygen, interact with a heterogeneous extracellular matrix (ECM), and form 3D structures that influence their growth, metabolism, and drug sensitivity [3].

Over the past decade, 3D cell culture models, including multicellular tumor spheroids, patient-derived organoids, and engineered tissue constructs, have gained considerable prominence due to their capacity to reproduce key aspects of tissue architecture and function [4]. These models offer improved physiological relevance compared to monolayer cultures,

enabling investigation of tumor growth, invasion, and drug response under conditions that better reflect the in vivo TME. Despite these advantages, most 3D models are maintained under static conditions, which limits nutrient and oxygen exchange and creates poorly controlled concentration gradients [5].

In response to these limitations, microfluidic OoC platforms have emerged as powerful tools to introduce controlled perfusion, dynamic shear, and precise transport of solutes [6]. By integrating peristaltic or pressure-driven flow, these systems create physiologically relevant environments, reduce diffusion-limited gradients, and extend the viability of 3D constructs over prolonged culture periods. Furthermore, the integration of real-time sensing technologies, most notably luminescence-based optical oxygen sensors, enables continuous monitoring of metabolic parameters critical to tumor biology, including hypoxia-induced signaling and drug resistance mechanisms [7].

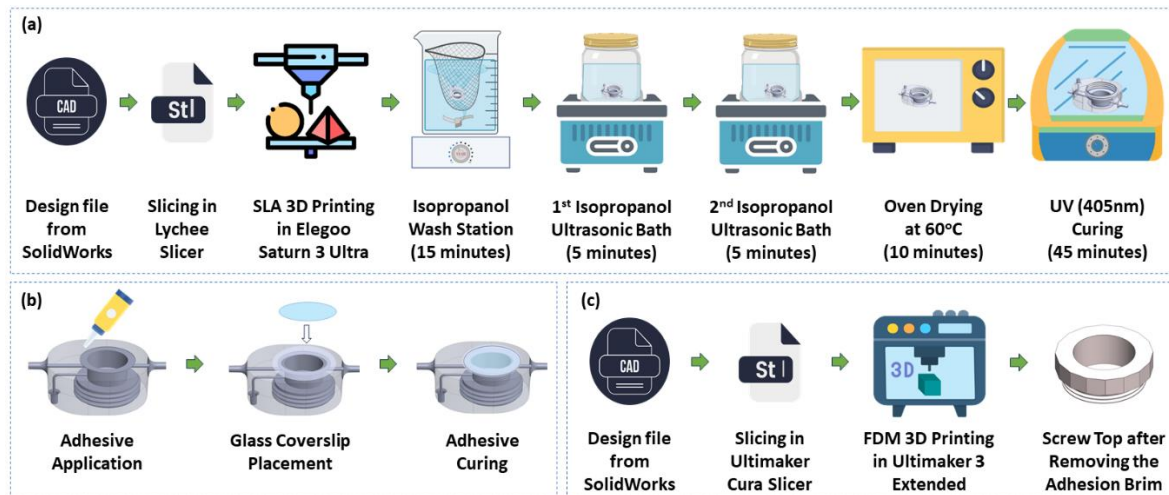


Fig. 1: Fabrication workflow for the 3D-printed perfusion chamber components. (a) SLA 3D printing of the chamber base, (b) Coverslip bonding process, (c) Fused Deposition Modeling (FDM) 3D-printing of the screw top. (Icons from <https://www.freepik.com/>).

However, conventional OoC platforms fabricated via soft photolithography are hindered by high costs, specialised cleanroom requirements, and irreversible sealing techniques that prevent intermediate access to cultures [8]. These barriers significantly restrict adoption in standard biological laboratories. There is, therefore, a pressing scientific and technical need for a simple, low-cost, reusable platform that combines the accessibility of conventional methods with the analytical power of dynamic perfusion and integrated biosensing. This paper presents the design, fabrication, and experimental validation of a novel modular OoC system that addresses these challenges. The platform leverages SLA 3D printing for rapid and cost-effective production of the core fluidic components, incorporates plug-and-play assembly for ease of use, and embeds an optical pO_2 sensor for real-time, non-invasive environmental monitoring. The system is validated using Hepatoblastoma G2 (HepG2) hepatocellular carcinoma 2D monolayers and 3D spheroids embedded in Basement Membrane Extract (BME), with continuous oxygen and viability tracking over up to 15 days.

Platform Architecture and Component Overview

The perfusion chamber consists of four primary components as shown in Figure 2: (1) the chamber body, (2) a thin PDMS gas-permeable membrane, (3) a polypropylene (PP) screw-top lid, and (4) a borosilicate glass coverslip affixed to the base.

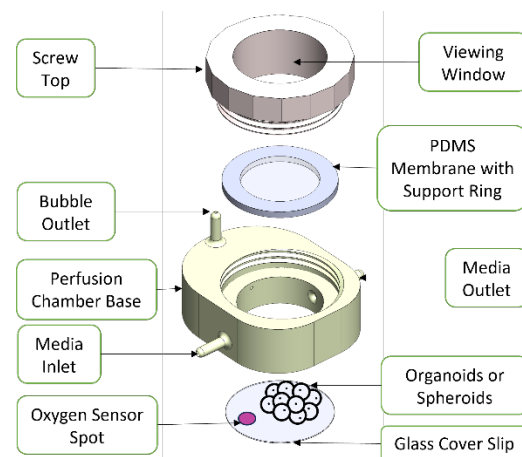


Fig. 2: Exploded view of the 3D-printed perfusion chamber assembly showcasing all the features and components.

Chamber Body Design and SLA Fabrication

The chamber body was designed in SolidWorks 2023 as a cylindrical well with an inner diameter (ID) of 16 mm and a height of 8 mm, yielding a culture volume of approximately 1.61 mL. Above the well, a flat annular surface was modelled to seat the PDMS membrane, with M27 × 2.0 metric threads incorporated into the surrounding side walls to accept the PP screw top. Inlet and outlet microchannels (1.5 mm ID) were positioned 2 mm from the chamber floor to facilitate efficient media exchange. An integrated bubble-outlet vent was incorporated to permit removal of trapped air via syringe aspiration without disrupting the fluidic circuit.

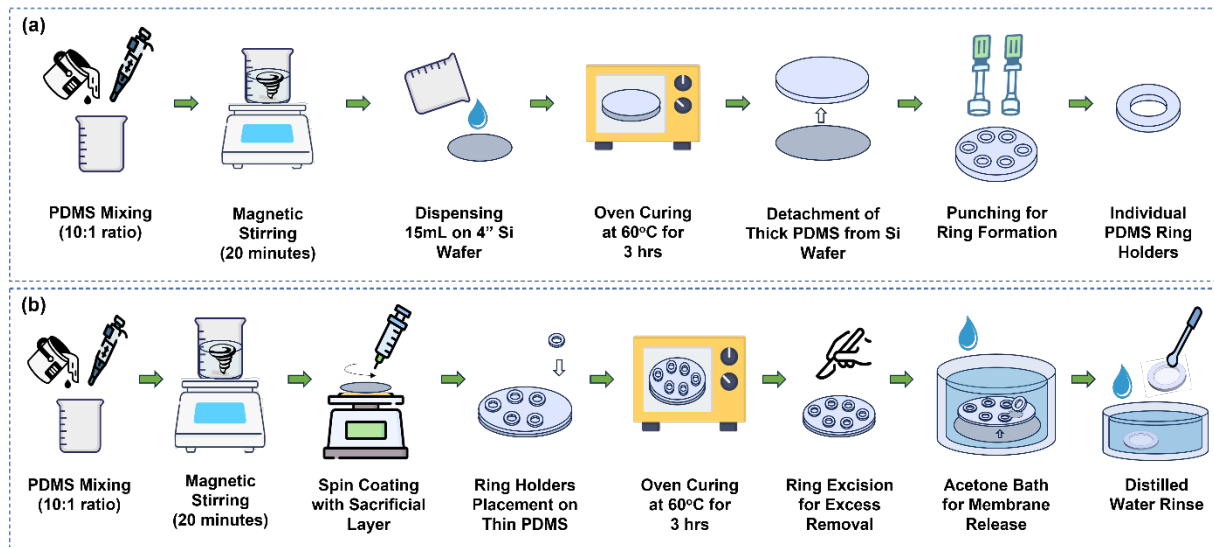


Fig. 3: Schematic representation of the PDMS membrane fabrication process. (a) PDMS ring holder and (b) composite thin membrane. (Icons from <https://www.freepik.com/>).

The chamber body was fabricated from Liqcreate Bio-Med Clear photopolymer resin using an SLA printer (Elegoo Saturn 3 Ultra, China). Design files were exported as STL and imported into Lychee Slicer 6.0.2. Models were oriented at approximately 45° to the build plate to minimise stair-stepping artefacts on critical surfaces. Following printing, parts were washed sequentially in three isopropanol (99%) baths, two of which included 5-minute ultrasonic cleaning cycles then dried at 60°C for 10 minutes, and finally ultraviolet (UV) cured for 45 minutes in an Elegoo Mercury X curing station. The process is summarized in Figure. 1.

PDMS Membrane Fabrication

PDMS membranes were prepared from Sylgard 184 silicone elastomer (Dow, USA) mixed at a 10:1 (base:curing agent) mass ratio as shown in Figure. 3. The mixture was stirred at 500 rpm for 20 minutes before use. A thin membrane (~22 µm) was produced by dispensing PDMS onto a 2-inch silicon wafer coated with AZ1512 photoresist as a sacrificial release layer, followed by spin-coating at 1800 rpm for 120 seconds (POLOS SPIN150i, SPS Netherlands). A pre-punched thick PDMS ring-holder (Outer diameter (OD): 22 mm, ID: 15 mm, ~1.5 mm thick) was placed concentrically over the uncured thin membrane to provide structural support during handling. The assembly was cured at 60°C for 3 hours. After curing and trimming the outer edges of the holder, the wafer was immersed in acetone to dissolve the sacrificial photoresist layer, releasing the composite membrane. Membrane thickness was verified by spectrophotometry (Filmetrics F20-UVX, USA).

Assembly and Sterilisation

The glass coverslip was bonded to the chamber base using SILPURAN® 4200 (Wacker Chemie, Germany), a biocompatible silicone adhesive, applied via a precision dispensing needle (Nordson EFD, USA, 1.5 mm, 14-GA) and cured at room temperature for 48 hours. Assembly was performed in a laminar flow hood; all reusable components were autoclaved prior to use, while the self-adhesive oxygen sensor spot (PreSens, Germany) was applied immediately before the experiment. The plug-and-play screw-top design allowed the chamber to be opened, accessed, and resealed between experimental time points without compromising the circuit's integrity.

Perfusion System Configuration

The perfusion circuit comprised a DURAN® GL 45 glass bottle as the media reservoir, Tygon® LMT-55 tubing (Saint-Gobain, France, 3 mm OD, 1.6 mm ID), a 3D-printed bubble trap, and a digital peristaltic pump (Ismatec Masterflex, Germany) and tubing (Ismatec Masterflex pump tubing, 0.25 mm ID, orange and blue, three stoppers). The media bottle was equipped with a three-port cap: two GL14 ports served as inlet and outlet (fitted with 3 mm OD tubing inserts), and the third port was connected to a 0.22 µm PTFE membrane filter (Sartorius, Germany) for sterile gas exchange. Figure. 4 depicts the assembly of the perfusion system. A standard flow rate of 50 µL/min was established and maintained continuously. A 3D-printed incubator door adaptor routed tubing without kinking, allowing the pump to remain outside the

incubator while the chamber, bubble trap, and media reservoir were maintained at 37°C, 5% CO₂, and 95% relative humidity (Thermo Scientific BBD 6220, USA).

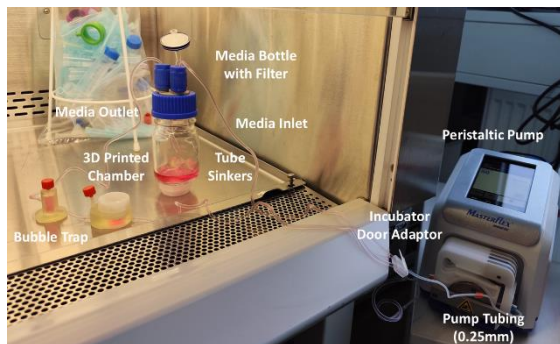


Fig. 4: Assembly of the perfusion system under the laminar flow hood.

Integrated Optical Oxygen Sensing

Dissolved oxygen was measured non-invasively using optical sensor spots operating on the principle of dynamic luminescence quenching (Stern-Volmer relationship):

$$I_0/I = \tau_0/\tau = 1 + K_{SV} [O_2] \quad (1)$$

where I_0 and τ_0 are the luminescence intensity and lifetime in the absence of the quencher, I and τ are the respective values in the presence of molecular oxygen, K_{SV} is the Stern-Volmer quenching constant, and $[O_2]$ is the local oxygen concentration [9].

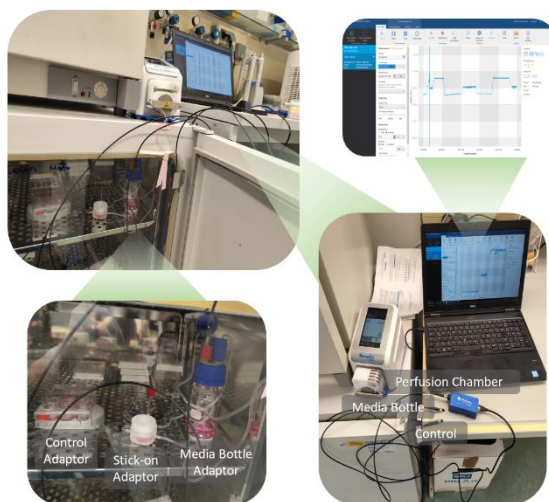


Fig. 5: Experimental oxygen measurement setup from different viewing angles.

Two sensor types were used: SP-Pst3-YAU (autoclavable glass-backed sensor) for the media reservoir, and SP-Pst3-SA (single-use self-adhesive polyester sensor) adhered to the culture chamber coverslip and to a control well

in a 12-well plate. Measurements were performed using an OXY-1-SMA single-channel fiber optic reader (PreSens, Germany) connected to PreSens Measurement Studio 2 software, which provided real-time graphical display, event annotation, and data export [10]. Custom 3D-printed adaptors maintained stable and precise alignment of fiber optic cables beneath each sensor spot, minimising measurement drift (Figure. 5).

Cell Culture and 3D Spheroid Formation

HepG2 human hepatocellular carcinoma cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) or Roswell Park Memorial Institute (RPMI) medium supplemented with 10% Fetal Calf Serum (FCS) and 1% penicillin/streptomycin at 37°C and 5% CO₂. Following routine 2D culture and at least three passages, cells were harvested via trypsinization and prepared for 3D spheroid formation using the hanging-drop method. Specifically, 10 µL drops containing 5,000-50,000 cells were resuspended in a mixture of 20% FCS, 40% DMEM, and 40% methylcellulose before being pipetted onto inverted Petri dish lids and incubated for three days. Once formed, individual spheroids were harvested, washed, and embedded in 10 µL of BME on 13 mm plastic coverslips (Sarstedt, Germany) [11]. After polymerization at 37°C for 30 minutes, the coverslips were transferred into 3D-printed perfusion chambers for downstream applications

Design Validation

The SLA-fabricated chamber body demonstrated excellent dimensional fidelity, with channel geometries and thread profiles matching design specifications. The M27 × 2.0 screw-top interface provided a repeatable, leak-free seal across multiple assembly/disassembly cycles, confirming the reusability of the platform. The SILPURAN® 4200-bonded coverslip showed no signs of delamination or media leakage throughout extended culture periods of up to 15 days under 50 µL/min perfusion, validating the adhesive selection for biocompatible sealing.

The spin-coated PDMS membranes achieved a mean thickness of 22 ± 2 µm as verified by spectrophotometry, sufficient to support passive gas exchange while maintaining mechanical integrity under the applied flow pressures. The plug-and-play design enabled assembly in under 10 minutes by a trained operator within a laminar flow hood, with no cleanroom access required, a considerable practical advantage over conventional photolithography-based OoC platforms [8].

HepG2 2D Monolayer Perfusion: Oxygen Dynamics and Flow Rate Effects

In the HepG2 2D monolayer experiment illustrated in Figure. 6, oxygen dynamics were observed over the course of two days in the perfusion chamber. During the initial perfusion phase, the system was maintained at a standard flow rate of 50 $\mu\text{L}/\text{min}$, where dissolved oxygen levels stabilised as a steady balance was reached between continuous oxygen delivery and cellular consumption. However, when the flow rate was momentarily increased to 600 $\mu\text{L}/\text{min}$, sharp upward spikes in pO_2 occurred due to the rapid replenishment of oxygenated media. While this intervention led to an increase in oxygen levels, the high-shear environment proved detrimental, causing significant cell detachment. This resulted in an unexpected rise in baseline oxygen levels by day two, reflecting a decrease in metabolic demand from the reduced cell population.

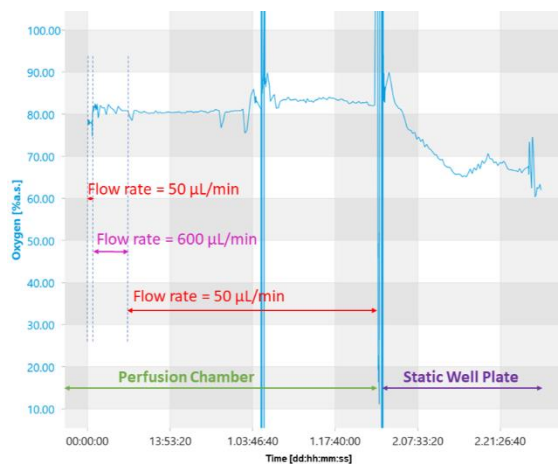


Fig. 6: Impact of flow rate on oxygenation and the variation in oxygen levels between the static control and perfusion chamber with HepG2 monolayer culture.

After two days, the input to the fiber optic oxygen meter was transitioned from the perfusion chamber to a static well plate control to observe the effects of stagnant conditions. Following the transition to the static environment, a loss of previous stability was observed, accompanied by a steady and monotonic decline in oxygen levels. By the final day of measurement, the pO_2 had reached near-depletion levels, as the dense, confluent HepG2 monolayer had exhausted the available oxygen without the benefit of active replenishment. This sharp contrast confirms that static culture conditions are insufficient to sustain the high metabolic demands of dense cell populations over time [5].

HepG2 3D Spheroid Culture: Long-Term Viability and Oxygenation

The HepG2 spheroid experiment represented the longest culture period in this study (15 days). As illustrated in Figure. 7, during the early phase (days 0–3), pO_2 in the perfusion chamber stabilised at approximately 85–86% air saturation, while the static control maintained $\sim 90\%$. In the intermediate phase (days 3–10), both conditions remained relatively stable. By the late phase (days 10–15), static well pO_2 declined sharply to $\sim 65\%$, indicative of oxygen depletion driven by the high metabolic activity of a densely packed spheroid culture with no active media replenishment. In contrast, the perfusion chamber maintained a stable $\sim 86\%$ pO_2 , confirming that continuous media exchange effectively prevented hypoxic depletion. The media reservoir consistently exhibited the highest oxygen levels (96–97%), confirming that observed variations arose from cellular consumption rather than environmental fluctuations.

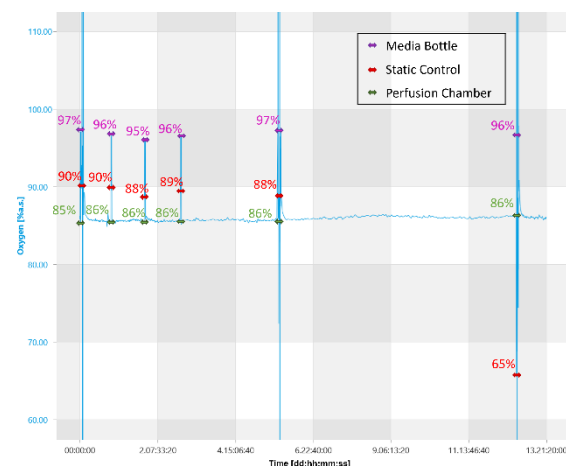


Fig. 7: Oxygenation differences among the static control, media bottle, and perfusion chamber in HepG2 spheroid culture over 15 days.

These findings highlight the platform's capability to distinguish between oxygen depletion regimes in real time, a capability not available in static systems and to sustain physiological oxygen tensions within 3D tumor models over extended culture periods. The approximately 4–6% difference in oxygen saturation between perfused and static conditions may appear modest but can be physiologically significant: even mild hypoxia can shift cellular metabolism, alter transcriptional programmes, and modulate drug sensitivity in tumor cell lines [12].

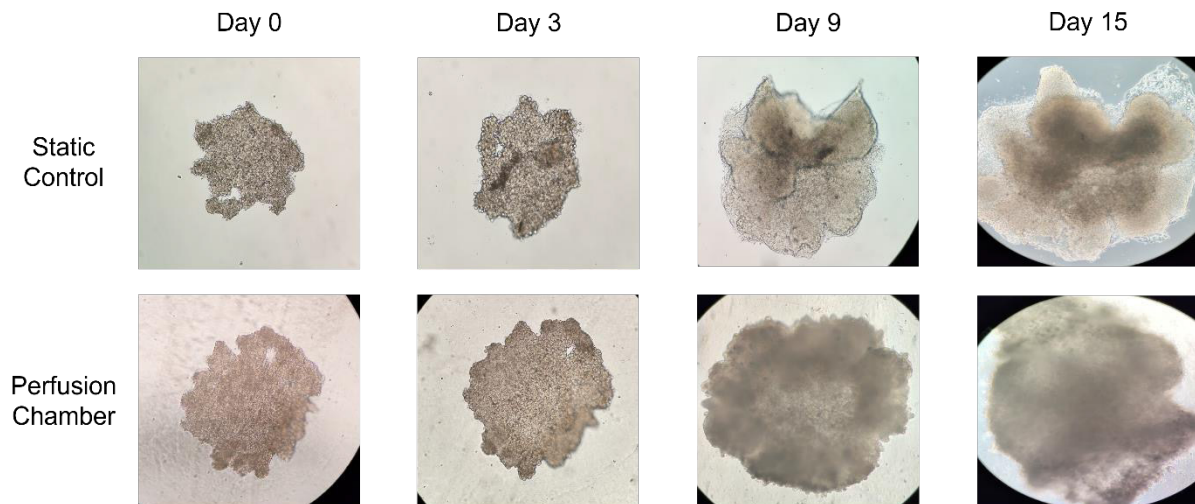


Fig. 8: Microscopic images of HepG2 spheroids in the static and perfusion conditions over the experimental period.

Morphological Assessment of HepG2 3D Spheroid Culture

Morphological assessment of HepG2 spheroids by brightfield microscopy (Zeiss Axiovert 25, Germany) revealed clear differences between static and perfusion culture conditions over the 15-day period (Figure. 8). In the static condition (top row), spheroids progressively increased in size and adopted irregular, lobulated morphologies with increasingly darkened and opaque cores. This pattern is consistent with the development of central hypoxia and the onset of necrotic core formation, a well-documented hallmark of avascular 3D tumor models under diffusion-limited, static conditions [5, 12]. The progressive spreading and irregular peripheral margins observed at later time points further reflect the unrestricted outgrowth characteristic of static 3D cultures lacking mechanical or fluidic constraints.

In contrast, spheroids maintained under continuous perfusion (bottom row) retained a more compact, rounded architecture with smoother boundaries throughout the culture period. The cores remained more translucent and morphologically uniform compared to their static counterparts, suggesting more homogeneous oxygenation across the spheroid volume, an observation directly supported by the stable pO_2 readings ($\sim 86\%$ air saturation) recorded in the perfusion chamber throughout the culture period (Figure. 7). The absence of progressive core darkening in perfused spheroids indicates that continuous media exchange was sufficient to prevent the oxygen depletion and associated cellular deterioration observed in the static condition.

Together, these morphological findings corroborate the oxygen data and reinforce the conclusion that the perfusion platform sustains a physiologically more controlled tumor microenvironment over extended culture periods, one that more faithfully reflects the constrained, flow-regulated conditions experienced by tumor nodules in vivo, compared to the unrestricted and increasingly hypoxic conditions that develop in static culture.

Platform Accessibility and Broader Applicability

A key outcome of this work is the demonstrated accessibility of the platform. By leveraging SLA 3D printing with a commercially available biocompatible resin, fabrication is achievable within hours and at a fraction of the cost of photolithographic alternatives. The modular, screw-top design not only enables intermediate culture access for direct sampling, microscopy, or sensor spot replacement, but also allows the chamber body to be reused after cleaning and re-sterilisation, reducing per-experiment consumable costs. Compatibility with standard borosilicate glass coverslips ensures seamless integration with conventional inverted fluorescence microscopes, enabling high-resolution live-cell imaging without platform modification.

The same architecture is directly transferable to other 3D tumor models embedded in BME, broadening the platform's applicability across oncology research. Future developments will focus on parallelisation of chambers for throughput enhancement, integration of additional electrochemical sensors (pH, glucose), and incorporation of co-culture chambers.

Conclusion

We have presented a modular, reusable OoC platform fabricated entirely by accessible 3D printing technologies, SLA for the primary chamber body and FDM for auxiliary components and validated for long-term 3D tumor culture perfusion culture with integrated optical oxygen sensing. The platform eliminates cleanroom requirements, reduces fabrication costs, and enables intermediate culture access while maintaining a controlled, physiologically relevant microenvironment. Experimental validation with HepG2 monolayers and spheroids over up to 15 days demonstrated robust oxygen tracking across perfused and static conditions, revealing key differences in metabolic dynamics that static systems cannot resolve. These results position the platform as a practical and scientifically rigorous tool for advanced in vitro oncology research, drug screening, and translational studies in standard biological laboratories.

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