Beyond Barriers: Redefining Organ-on-Chip Architecture Through Emerging Designs

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Abstract

This study presents four barrier-free organ-on-chip devices using xurography, thermal bonding, and surface treatment for hydrogel confinement. Removing physical barriers enhanced cellular interaction. Devices were tailored for hypoxia, tumor invasion, immune response, and interstitial flow models, demonstrating a versatile, modular approach for physiologically relevant *in vitro* systems.

Introduction

Microfluidic organ-on-chip systems have become essential tools for replicating key aspects of tissue microenvironments, particularly in studying gradients, cell-cell interactions, and disease progression. However, existing platforms often employ physical barriers such as membranes, micropillars, or phaseguides to confine hydrogels, which introduce inert material between functional compartments [1,2]. These structures can hinder the formation of continuous chemical and cellular gradients, disrupt direct cell contact, and impair immune cell infiltration, limiting their ability to accurately mimic *in vivo* conditions. Alternative strategies, such as laminar flow patterning, eliminate physical barriers but are constrained by their inability to produce defined or compartmentalized geometries [3]. Photopatterning methods, while offering spatial control over hydrogel placement, may compromise the mechanical and biological properties of the hydrogel due to laser exposure, thereby affecting cell behavior and restricting natural interactions [4]. Overcoming these limitations requires fabrication strategies that ensure hydrogel localization while enabling seamless connectivity between compartments.

To address these challenges, this work explores the value of a microfluidic device fabrication approach known as the Surface Treatment Technique. By eliminating the need for physical barriers, this method enables the compartmentalization of co-cultures into distinct chambers while preserving direct interaction between cell populations. Its flexible design allows for straightforward geometric adjustments, supporting adaptation to a wide range of experimental conditions, including variations in reagent volumes, cell densities, and contact areas. The utility of the technique was evaluated through the development of four distinct organ-on-chip devices, each tailored to address a specific biological question.

Theory and experimental procedure

All devices were fabricated using this technique that combines xurography for planar patterning, selective surface modification to control hydrogel placement, and thermal bonding to assemble layers and integrate additional components. Originally developed to produce the Be-Gradient Barrier-Free device [5], it was subsequently adapted to create the four systems presented here. The use of oxygen-impermeable substrates and biocompatible materials ensured compatibility with long-term cell culture.

The simplest design, Twin-Stream, consisted of a single chamber and one channel per replica, allowing two replicas per chip (Figura 1A). Hydrogel boundaries were established via selective surface treatment without requiring additional structures. This configuration was ideal for studies requiring directional flow and well-defined gradient formation.

To support the inclusion and imaging of spheroids or organoids, the Open Port was created by increasing chamber height and area. A top opening and reservoir well were fabricated The well was created using stereolithography 3D printing, and it was bonded to the main structure using unpolymerized resin and UV treatment. This adaptation provided vertical access for spheroid loading and stable imaging, particularly suited for invasion and metastasis assays (Figura 1B).

The Multicompartment device included three interconnected central chambers and two lateral channels, all fabricated in a single layer (Figura 1C). Xurography patterns and surface treatment were adjusted to maintain hydrogel continuity across compartments, enabling direct interaction between multiple co-cultured cell types. This layout was optimized for studying immune infiltration, multicellular communication, and matrix effects.

The Trans-Mesh device was specifically designed to model interstitial fluid flow by incorporating a vertical configuration with two chambers separated by a nylon mesh membrane with regular 150 µm pores (Figura 1D). The membrane was chosen for its thermal resistance and was sealed into the device during thermal bonding, allowing the flow of medium while maintaining physical separation. This setup is applicable to studies involving nutrient exchange, barrier permeability, or drug transport.

Conclusions

New microfluidic platforms without pillars have been successfully developed to allow direct contact between cells, eliminate inert materials and recreate more biomimetic models. The results presented in this work demonstrates that this fabrication technique enables the recreation of different geometries that mimic tissue structures, as well as the generation of specific shear stress profiles, in order to obtain more accurate experimental models.

We demonstrated a versatile fabrication method for constructing barrier-free organ-on-chip platforms that maintain full hydrogel contact across compartments without relying on inert physical barriers. By applying this approach to four distinct chip designs, we showed how small but strategic adjustments, such as altering chamber dimensions, adding vertical access, or integrating permeable membranes, enable each platform to address specific experimental needs. The modularity and adaptability of this technique make it a powerful tool for developing physiologically relevant *in vitro* models, supporting a broad range of biological applications from cancer research to immune interactions and fluid dynamics studies.

Acknowledgments

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Figures

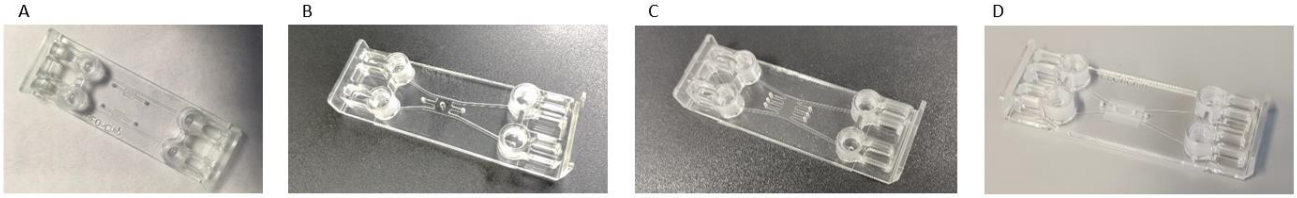

Figura 1: Images of the four organ-on-chip devices. (A) Twin-Stream, (B) Open Port, (C) Multicompartment and (D) Trans-Mesh. Scale bar: 5 mm.