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Endothelialization of Perfused Vascular Models Using Tunable Gelatin-Based Biomaterials for Advanced Drug Screening

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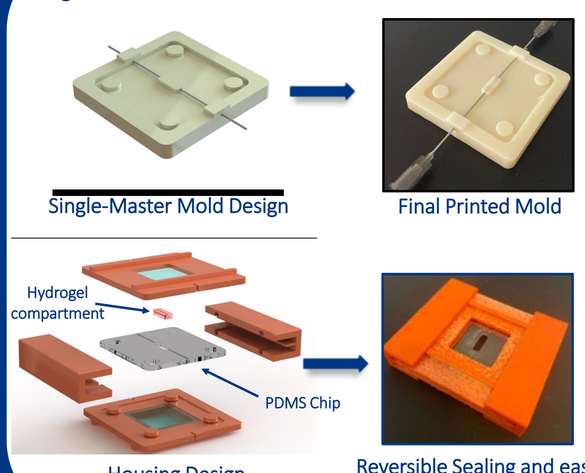
1. Introduction

The integration of a **vascular compartment** has become crucial in tissue engineering and regenerative medicine, as it enhances **the physiological relevance of *in vitro* tissue models**. Perfused vasculature **significantly improve drug screening**, allowing **direct administration of drugs, carriers, or small molecules,** thereby reflecting real-world clinical scenarios.

In this study, we present a **versatile millifluidic bioreactor** designed to engineer **functional, perfusable blood vessels**. The platform has been used for a systematic **optimization of endothelialization** by investigating multiple parameters affecting endothelial cells (**ECs**) monolayer formation. Following the formation of a stable EC monolayer, the role of **human Mesenchymal Stem Cells** (**hMSCs**) as supportive cells was evaluated. Moreover, the platform can be adapted for **spatial organization** of **multiple cell populations** exploiting **bioprinting** and integration of **multiple channels,** providing a flexible tool that paves the way for more predictive models for **disease modelling** and **therapeutic testing**.

2. Device Design

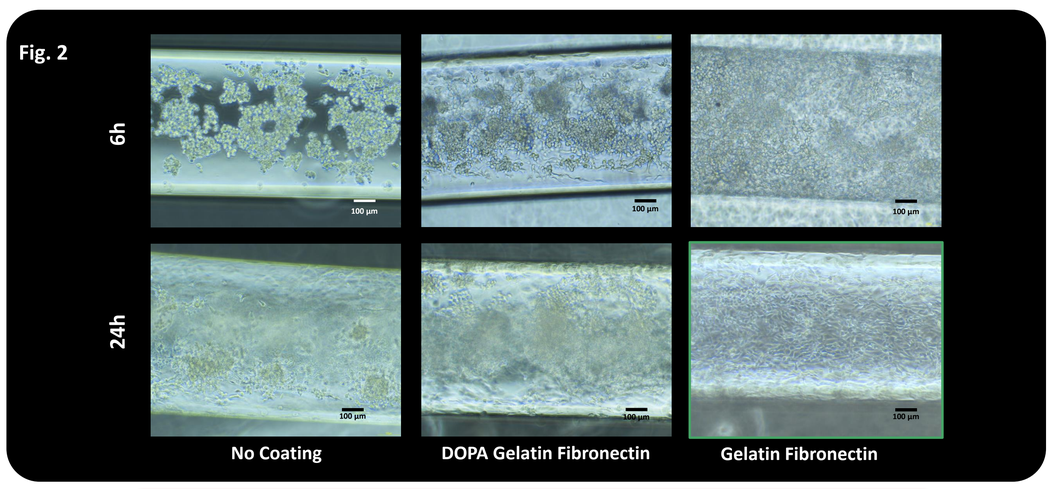
Fig. 1



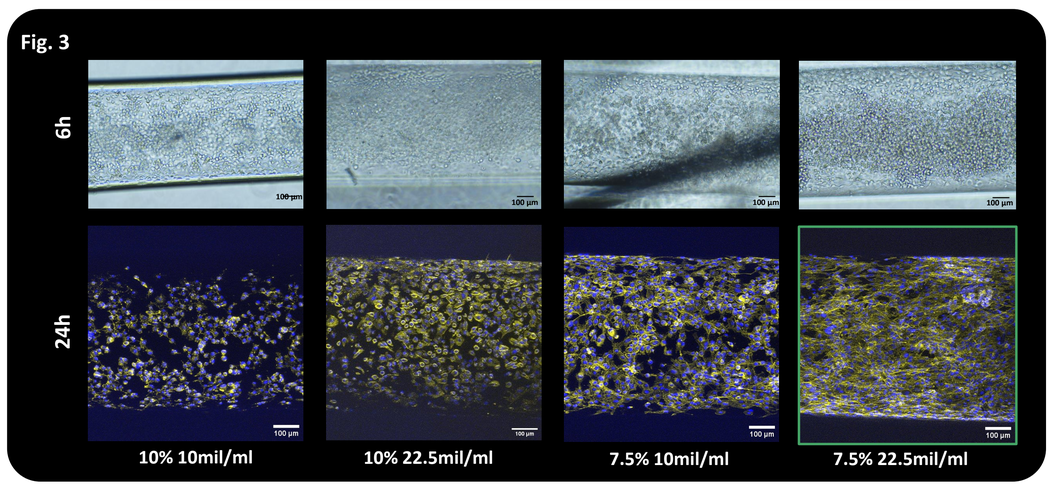
A **single-piece PDMS chip** was obtained, with **suspended channel horizontal access** for needle insertion and tubing connection to an external **perfusion** system. The device is designed to host a **hydrogel compartment**, which contains both the **perfusable channel** and cells embedded within the hydrogel matrix. Consequently, a housing system was designed to ensure **tubing stabilization** and allowing **reversible sealing** (*Fig. 1*).

3. Optimization of endothelialization parameters

Gelatin methacrylate (GelMA) was used due to its tunable mechanical properties and exceptional biological performances. **Human Umbelical Vein Endothelial cells** (**HUVECs**) were used as they are one of the most represented lines in the field. Firstly, two different channel coatings were tested, showing good initial adhesion and cell spreading after 24h when using **gelatin** and **fibronectin** (*Fig. 2)*. Following that, **7.5% w/v GelMA from Type A Gelatin** and **22.5 millions of cells/ml** were identified as the optimal condition, as confirmed **by DAPI (blue)** and **phalloidin (yellow)** staining observed by confocal microscopy (*Fig. 3)*.



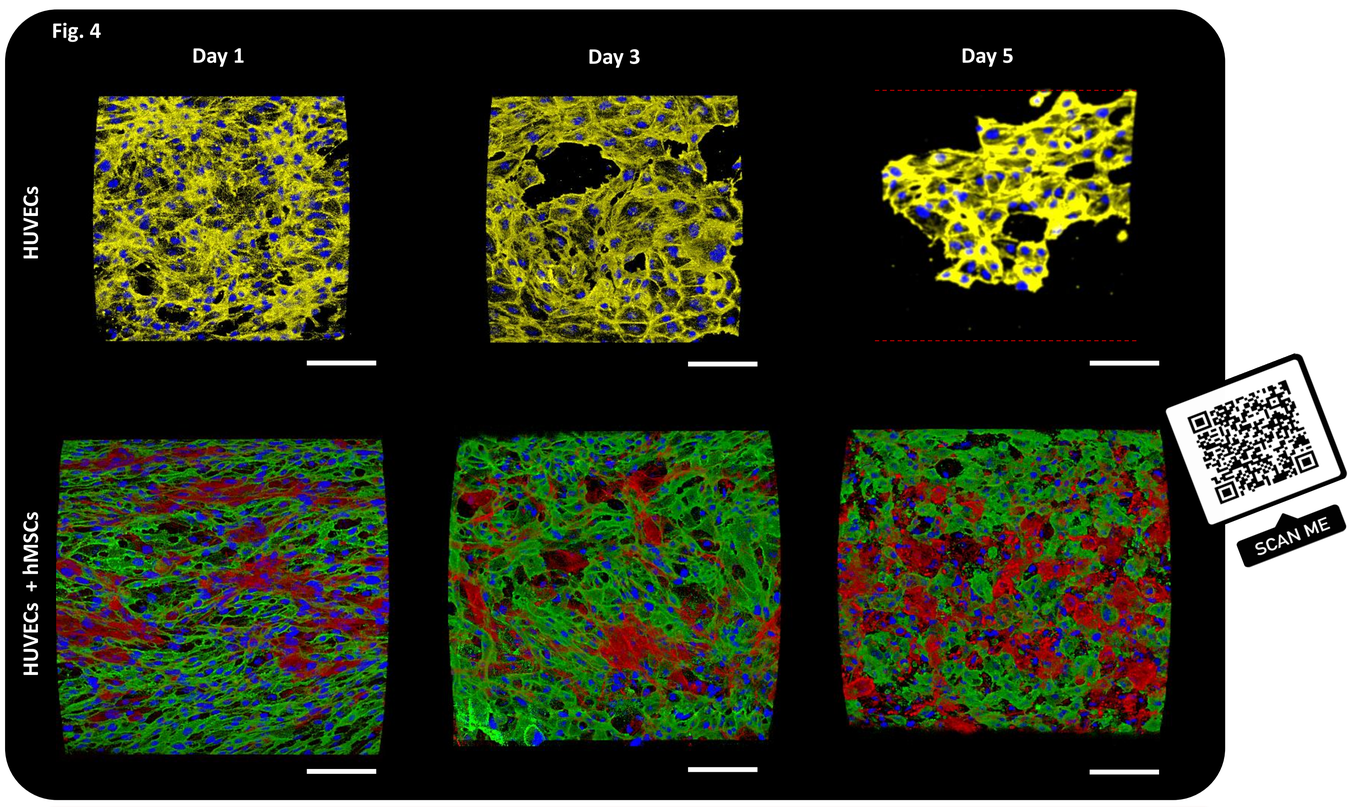
✓ Coating improves homogeneous coverage



✓ Optimal GelMA concentration and seeding density identified

4. Effect of supporting cells in static cultures

As **HUVECs** alone struggled to mantain a **confluent monolayer** over an extended period of time, **bone marrow-derived hMSCs** were used as support cells to help mantaining coverage over time as suggested in literature**.** HUVECs and hMSCs were **co-seeded** at a **3:1 ratio**. The single culture was stained with **DAPI** (**blue**) and **Phalloidin** (**yellow**). To distinguish the two cell lines **CD31 (green)** and **Vimentin (red)** were used as markers for **HUVECs** and **hMSCs**, respectively, as shown in *Fig. 3 (Scale bar = 150um)*.



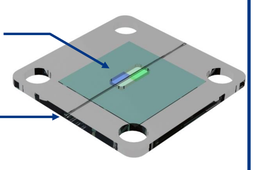
✓ hMSCs introduction greatly aids in mantaining good channel coverage

5. One Chip – Multiple Possibilities

In-Chip Bioprinting of Spatially Organized Cell Populations

Assembled Device

- Open-top chip allowing printing
- Needle can be inserted mid-printing and removed after



Bioprinted multicellular architecture

- Up to two different cell population can be printed via cell-laden hydrogels
- After needle removal endothelial cells can be seeded to obtain a vascular compartment

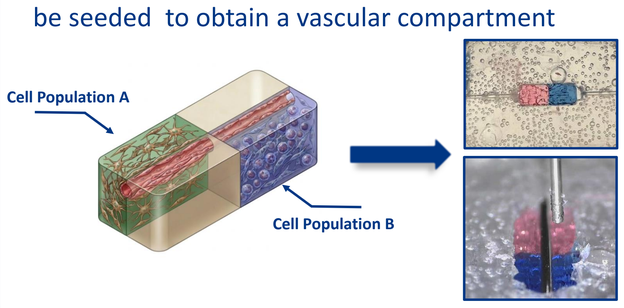


Fig.5 Combination of in-chip multimaterial printing and sacrificial templating.

Introduction Of Secondary Compartment

Gel + Spheroids/Organoids Seeding at controlled distance

Recirculating Molecules/Carriers Compartment

Lymphatic System Parallel Channel

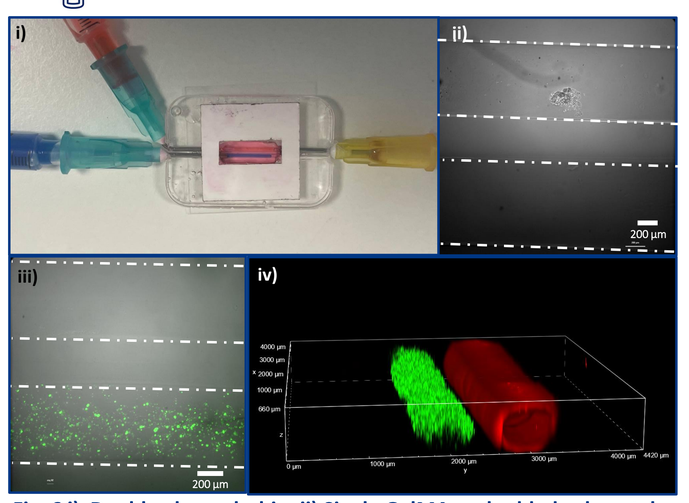


Fig. 6 i) Double-channel chip; ii) Single GelMA-embedded colorectal cancer patient derived organoid; iii-iv) A549-GFP⁺ cells – laden GelMA

6. Conclusions and future perspectives

- **Channel seeding** and **endothelialization optimizaton**
- **Monolayer stabilization** in static condition using a **co-culture** approach
- Proof of concept of **in-chip multimaterial 3D printing**
- Auxiliary channel manufacturing
- **Dynamic culture** characterization
- Introduction of **diseased compartment**
  - **In-chip bioprinting of diseased cell lines**
  - **Introduction of 3D cell structure through the auxiliary channels**
- **Drug testing**