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# Organoids-on-chip models to mimic liver physiology

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## 1. Introduction

The development of relevant *in vitro* models requires properties and functionalities closely mimicking human physiology. Classic 2D cultures fail to mimic physiological functions, while *in vivo* models, widely used in research and preclinical testing, have a low translatability to humans. In the last years, the MicroPhysiological Systems (MPS) technology, including Organ-on-Chips (OoC) and organoids, has emerged as a promising tool for building more relevant *in vitro* models. Using these MPS, mechanical stimuli can be applied and tuned by controlling various parameters (flow rate, use of hydrosccaffold, microstructures). They offer better conditions for cells/tissues development, with enhanced longevity and activities. These models allow complex 3D tissue engineering and can also recapitulate cell-cell and cell-matrix interactions, zonation, physiological flows and architectures of organs (Lee et al., 2023).

Here, we propose two models of ‘organoids-on-chip’ using Primary Human Hepatocytes (PHHs). We first used biochips integrating hydrosccaffold to improve cell adhesion and 3D organization. (Messelmani et al., 2022). In parallel, biochips with a microwells design were used to directly engineer liver spheroids. Dynamic cultures were run for several weeks, and metabolic analyses were carried out to compare activities of both models. We discuss the important biological and mechanical parameters for the development of each model.

## 2. Methods

### 2.1 Biochips manufacturing

The biochips consisted of two polydimethylsiloxane layers (PDMS, Sylgard 184, Dow Corning) manufactured by replica molding process using SU-8 mold fabricated by photolithography process. The

layers were sealed together after surfaces activation with reactive air plasma (1 min; Harrick, USA). Two biochip designs were used (Figure 1a). The top layer (depth of 100  $\mu\text{m}$ ) was the same for both. It included an inlet and outlet microfluidic network for homogenous culture medium distribution.

The bottom layer of the first design (hydrosccaffold-biochip) is composed of a series of microchambers and microchannels (depth of 100  $\mu\text{m}$ , Jellali et al., 2016). A hyaluronic acid (HA)-based hydrosccaffold (BIOMIMESYS® Liver; HCS Pharma, France) was integrated into the biochip and crosslinked *in situ* (Messelmani et al., 2022). It was composed of RGDS-grafted HA (sites for cell adhesion), galactosamine-grafted HA and extracellular matrix (ECM) proteins (collagen type I and collagen type IV). The elastic modulus and porosity of the hydrosccaffold were of 0.6 kPa and  $120 \pm 20 \mu\text{m}$ , respectively.

For the second design (microwells-biochip), the bottom layer, used to trap cells/form spheroids, was composed of 600 microwells measuring 400  $\mu\text{m}$  in diameter (depth of 300  $\mu\text{m}$ ), and spaced by 50  $\mu\text{m}$  (Essaouiba et al., 2020).

### 2.2 Cell culture

The cells (PHHs; Cytes biotechnology, Spain) were thawed using PHHs thawing medium (manufacturer recommendations). Then, the cells were suspended in PHHs plating medium and  $5 \times 10^5$  cells were seeded in each biochip. After 24h in static condition, the biochips were connected to the IDCCM box (Integrated Dynamic Cell Cultures in Microsystems, Jellali et al., 2016) and the perfusion was started using a peristaltic pump (25  $\mu\text{L}/\text{min}$ ). For the dynamic step, HCM medium (Lonza, 4 mL for each biochip) was used and renewed every two days. During the experiment, the medium was sampled for albumin (Elisa assays) and urea (QuantiChrom assay kit) quantification.

## 3. Results and discussion

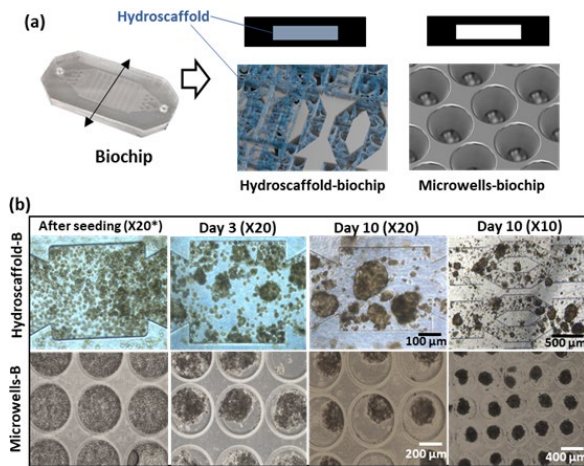
### 3.1 Engineering of 3D organoid-on-chip

In the first strategy (hydrosccaffold-biochip), the HA pre-polymer (HA and crosslinker: adipic acid dihydrazide) was easily integrated in the biochip and crosslinked *in situ* forming HA-hydrosccaffold. The microscopy observation showed the homogeneous distribution of the hydrosccaffold in the whole device. Furthermore, the addition of hydrosccaffold don't lead to significant differences in the flowrate.

A total of  $5 \times 10^5$  PHHs were seeded in the hydrosccaffold-biochips. The microscopic observations (after seeding) showed homogenous distribution of the cells inside the biochip (Figure 1b). After 24h, the cells were embedded in/adhered to the hydrosccaffold and started to aggregate. The spheroid shape formation was observed from day 3 and the final spheroid size was of  $165 \pm 40 \mu\text{m}$  (day 10). However, non-spherical shapes

and several small aggregates (10-50  $\mu\text{m}$ ) were also observed (Figure 1b).

Regarding the microwells-biochips, an anti-adherent coating (pluronic acid) was used to prevent cells attachment and improve spheroids formation. After seeding, PHHs showed a homogeneous distribution, most of the cells dropping and filling every well (similar cell density in the wells). After 24h, agglomeration of PHHs started and the dynamic perfusion was launched. The spheroids formation was complete at day 3, with spherical shape and a final size of  $200 \pm 20 \mu\text{m}$  (day 10). This morphology was maintained during the cultures and was not affected by dynamic perfusion (Figure 1b).



**Figure 1.** (a) biochips design (b) microscopy images of spheroids obtained in hydro scaffold- and microwells-biochips (\* magnification X20).

### 3.2 Functionality of organoids-on-chip

The assessment of PHHs functionality was performed by measuring albumin and urea production (Table 1).

**Table 1.** Albumin and urea secreted by PHHs organoids in hydro scaffold- and microwells-biochips.

		Hydro scaffold-biochip	Microwells-biochip
Alb*	D3	$320 \pm 40^{\pi}$	$466 \pm 30^{\pi}$
	D7	$500 \pm 35^{\S}$	$600 \pm 21^{\S}$
	D10	$460 \pm 15^{\#}$	$660 \pm 55^{\#}$
Urea**	D3	$280 \pm 40$	$330 \pm 35$
	D7	$250 \pm 25$	$195 \pm 10$
	D10	$180 \pm 15$	$200 \pm 20$

(\* ng/h/ $10^6$  seeded cells; \*\*  $\mu\text{g/d}/10^6$  seeded cells; ( $\pi$ , #,  $\S$ ) data statistically different between two culture modes,  $P < 0,05$ , t-test).

In both culture modes (hydro scaffold- and microwells-biochips), a continuous and relatively constant secretions of albumin and urea were found. Albumin secretion was higher in microwells-biochip ( $320 \pm 40$  to  $500 \pm 35 \text{ ng/h}/10^6$  cells), than in hydro scaffold-

biochip ( $466 \pm 30$  to  $660 \pm 55 \text{ ng/h}/10^6$  cells). This difference can be attributed to the removing of small aggregates/isolated cells by perfusion in the hydro scaffold-biochip (decrease of cells number compared to microwells-biochip). Regarding urea secretion, no significant difference was observed between the two types of biochips.

## 4. Conclusions

Here, two different models of organoids-on-chip were presented. They have shown similar results with long viable culture and metabolic activities. However, the microwells-biochip strategy enhances cell organization, the control of spheroid shape and size, as well as their distribution in the device. Organoids in microwells-biochip are trapped in the bottom of the 300  $\mu\text{m}$ -deep wells, in a zone of low shear stress, while organoids embedded in the hydro scaffold are exposed to larger shear rates and faster nutrient exchanges. Further simulations and experiments will be performed to guide users towards the most relevant model according to the clinical situation of interest.

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## Conflict of Interest Statement

BIOMIMESYS® hydro scaffold was provided by HCS Pharma, Loos (France).

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