PRIMO-Powered 3D Structuration: Shaping Spheroids and Organoids using Photosensitive Hydrogels

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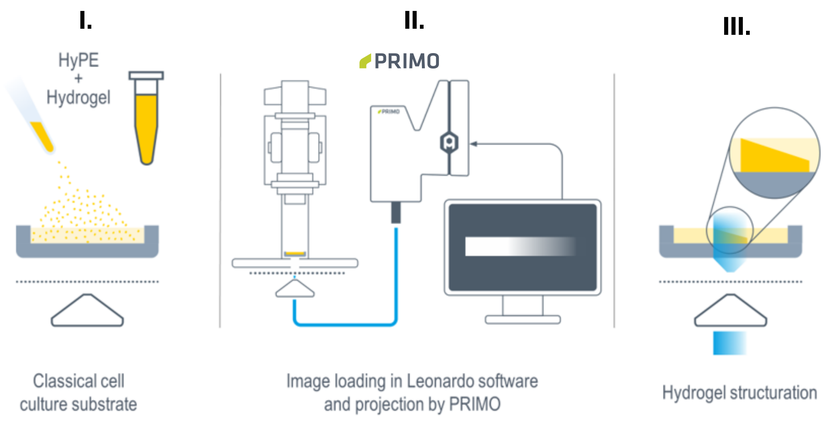
Introduction

Three-dimensional (3D) cell culture models, including spheroids and organoids, are significantly gaining importance in biology due to their enhanced physiological relevance compared to two-dimensional models. These 3D models provide the ability to create minimal biological systems within a controlled environment, allowing the study of organogenesis, disease modeling, drug testing, and regenerative medicine. In order to obtain this 3D cell culture, we need to engineer complex support structures. In this poster, we demonstrate the use of our UV-based device PRIMO, in combination with HyPE, a hydrogel photopolymerization solution. It allows precise and localized control of the height of PEG-based and biological hydrogels structures. We then show the wide range of applications of this technology, from the controlled and reproducible fabrication of spheroids and organoids to be further used for drug screening and personalized medicine, to the engineering of more complex 3D cellular models and organs on a chip for research.

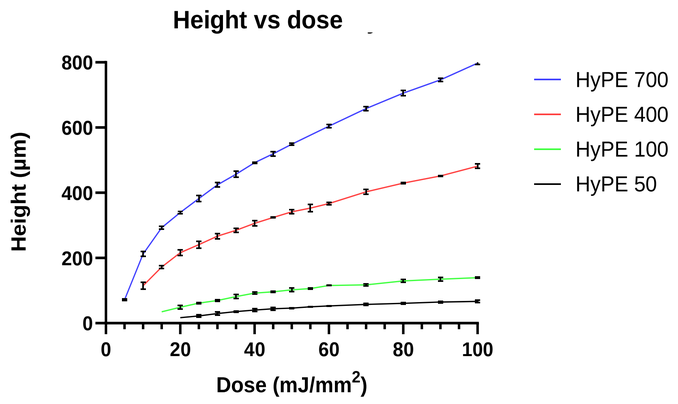
I. PRIMO-based hydrogel structuration with HyPE

Hydrogel photopolymerization process



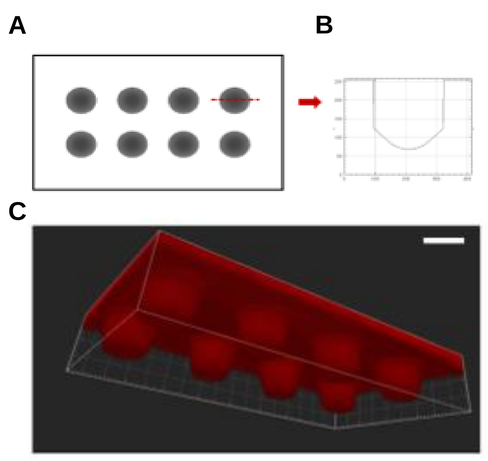
I. The **HyPE polymerization solution** is mixed with a hydrogel precursor. This mix is then poured into a petri dish. **II.** The sample is placed on a **PRIMO setup** and the pattern is loaded into the software. **III.** Illumination is spatially and temporally controlled by the software, allowing the creation of complex structure with define height.

Control of the height with the UV dose



The **height** of the structures is controlled by the **local UV dose** that is projected. We developed different HyPE solutions to obtain different heights ranging from 25 to 800 µm.
The data here represent 4-Arm-PEG-Acrylate hydrogels heights versus UV dose for different HyPE solutions. The range of tested UV doses goes from 5 to 100 mJ/mm².

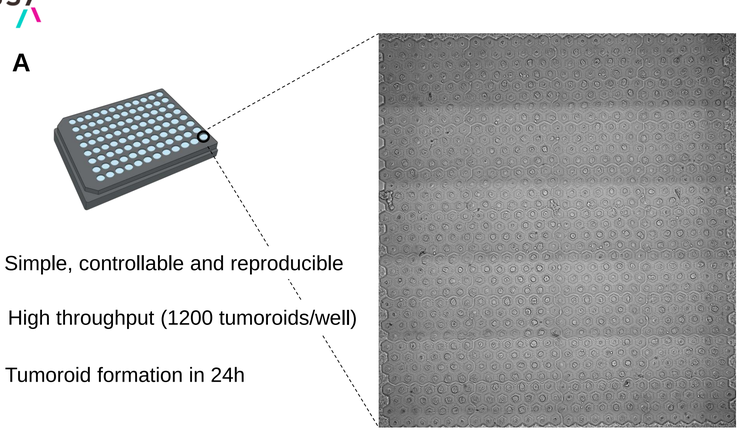
Control of the UV dose with the image gray levels



A) Image of a pattern loaded into the software with its corresponding **gray level** profile in **B)**. **C)** Inverted image of the obtained hydrogel using a confocal microscope. The surrounding medium is fluorescent and is represented in red.
Scale bar: 200 µm.

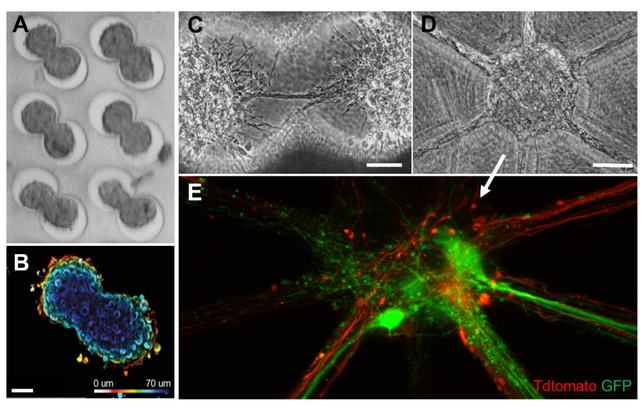
II. Applications

Patient-derived spheroids/organoids for high throughput drug screening and personnalized medicine[(1)]



Proof of concept of the utilization of hydrogel polymerization for high throughput drug screening and personalized medicine.
(A) Hundreds of non-adhesive microwells were produced in the wells of 96 (and 384) well-plates. Cancer cells isolated from patient tumors were then seeded in the wells and aggregated in the microwells to form tumoroids. After 24 hours of culture, a drug (Epirubicin) was added at different concentrations. **(B)** Pictures of tumoroids at T0 and after 24h (T24) for different concentrations of drugs. The figure show how the increasing concentration of drug perturbates the aggregation and proliferation of tumor cells. **(C)** Viability of cells at different time points from 0 to 24 hours after drug treatment measured by propidium iodide fluorescence assay.
This experiment shows that PRIMO allows, in combination with the HyPE photopolymerization solution, the culture of thousands of reproducible 3D cell aggregates in multiple well-plate for high throughput drug testing experiments.

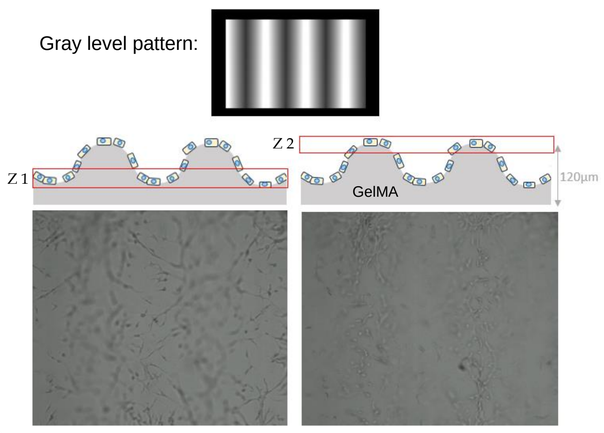
Customize the shape of 3D cell aggregates [(2)]



Owing to the versatility of PRIMO, it is possible to customize microwell shapes in order to orient spatial organization of organoids and other cell aggregates.

(A) brightfield image of non-spherical spheroids made with HEK cells using microwells of specific shapes. **(B)** Z-coded image of the same HEK spheroids imaged with a confocal microscope. **(C)** Brightfield image of two neurospheres in two communicating microwells and linked by an axonal bundle. **(D)** Brightfield and **(E)** Confocal images of a neurosphere from which have grown axonal bundles in a very controlled manner inside hydrogel channels. Scale bars = 50 µm.

Structuration of biological hydrogels: GelMA

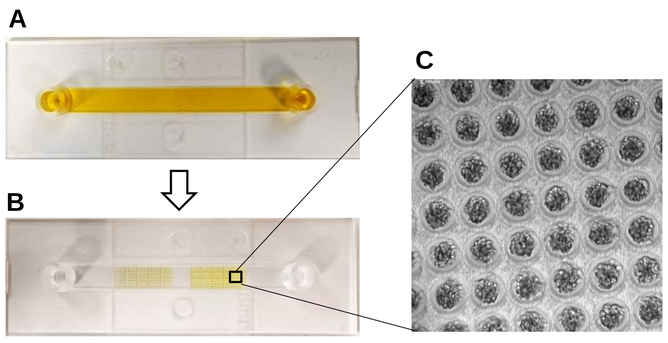


It is also possible to polymerize biological hydrogels (collagen, gelatin etc) that have been modified with methacrylate groups to make them UV-polymerizable.

In this example, some Gelatin-Methacrylate (GelMA) hydrogels have been structured with wave shapes before Cos-7 cells were seeded and cultured on their surface.
The two pictures have been taken at two different Z positions: Z1 and Z2.

In situ structuration in microfluidic channels

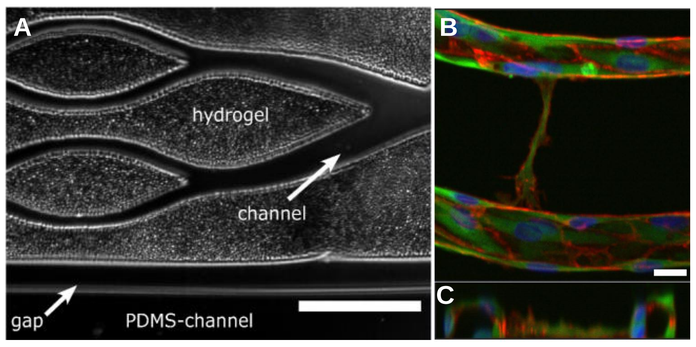
Spheroid formation in Ibidi channels



PRIMO being a contactless photopatterning device, it is possible to polymerize hydrogels *in situ* into microfluidic channels. In this example, we made hundreds of spheroid microwells into Ibidi Luer channels. This allow a gentle media change without shear stress, and to easily add then wash a drug solution or other reagents.

(A) HyPE+hydrogel mix into the channel. **(B)** Hydrogel microwells after polymerization and washing of the mix. **(C)** Spheroids formed and cultured in the channels.

Vascularized network of endothelial cells inside a PDMS chip[(3)]



In this example, a vascularized and injectable network was created with a specific shape 3D-Vessels-on-Chip to study effects of dynamic fluid flow on human induced pluripotent stem cell derived endothelial cells

(A) GelMA hydrogel was injected into a PDMS channel then polymerized with a specific shape thanks to the virtual mask. Scale bar = 200µm. **(B)** Human iPSCs-derived endothelial cells were seeded in the GelMA network and colonized it to create a 3D vessel-on-a-chip. Scale bar 10 µm. **(C)** Z section of the same image. 3D sprouts started to form between two branches.

(1) Collaboration with K. Schauer and E. Ouni – Institut Gustave Roussy, Cell Biology of Organelle Networks team, UMR1279 - Tumor Cell Dynamics Unit.
(2) Images from the “Cell Organ-Izers” joint lab IINS/Alvéole (A. Pasturel and S. Rahmati).
(3) Courstesy of Mees de Graaf, University of Leiden.

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