Physiologically inspired Multi-Organ systems for Nanomedicine Investigations

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Nanomedicine is an evolving discipline that exploits the unique properties of nanoscale materials within biomedical and pharmaceutical sciences. Over the past decades, it has emerged as a field with strong potential to impact the diagnosis and treatment of human diseases [1]. However, despite its great potential, translation into clinical practice has been slow, with only a few nanoformulations approved by official health agencies, such as the FDA or the European Medicines Agency [2]. One of the main reasons for this slow-paced transition is the lack of robust preclinical models that can adequately replicate human physiology. In this context, microphysiological systems (MPS), also known as organs-on-chips, have emerged as more relevant platforms compared to traditional two-dimensional models and costly animal studies [3]. These advanced in vitro systems integrate engineered microtissues with microfluidic technology, enabling the replication of key biophysical cues naturally present *in vivo*.

While existing single-organ chips have shown great promise, they lack inter-organ communication, which is fundamental for studying systemic effects, nanoparticle biodistribution, and metabolism. Multi-organ-on-chip systems are therefore progressively being developed and represent a highly promising tool for nanomedicine investigations.

This work presents a multi-organ MPS designed to offer a physiologically relevant platform to investigate the biodistribution, efficacy, and metabolism of muscle-targeting nanoparticles. The system consists of two microfluidic devices: a muscle-on-a-chip, representing the target organ, and a liver-on-a-chip, mimicking metabolic processing (Fig.1). Three key aspects were considered when designing the system to increase its physiological relevance: organ proportions, endothelial barriers, and shear stress. The relative compartment sizes between the two organs were derived using a scaling method based on allometric scaling, a tool that is commonly used by drug developers to predict human pharmacokinetics from animal data [4]. Then, each device was provided with a perfusion channel to be lined with endothelial cells. The presence of this channel is crucial to mimic the natural vasculature present in vivo and allow for dynamic culture of the system. Its dimensions and flow conditions were tuned to match the physiological shear stress of liver sinusoids and skeletal muscle venules. Additionally, the liver device was provided with an additional channel to mimic the presence of a bile duct, representing the excretory system of the model.

The fabrication of the devices was carried out via traditional photolithography and soft-lithography techniques. Briefly, a master for each organ-chip was created by coating SU8-100 negative photoresist on silicon wafers and impressing the corresponding design via UV exposure of the resist through a photomask with the desired pattern. Then, PDMS was poured onto the wafers and cured to obtain a replica of the design, which was then bonded to a glass slide, constituting the final device.

At this early stage of system development, mouse cells were selected for easier comparison with *in vivo* data. C2C12 cells, a well-established muscle model, were seeded into the devices at a density of 30'000 cells/mL in a matrix composed of collagen and GFR Matrigel, to promote the formation of a 3D microtissue. Then they were cultured in low serum media conditions to induce differentiation. For the liver compartment, AML-12 hepatocytes were seeded in a collagen matrix at a lower density (10'000 cells/ml) to limit the collagen contraction phenomenon. For the endothelium, C166 cells were seeded in a medium-only suspension to promote monolayer formation around the walls of the channel.

Representative light microscopy images of the muscle compartment revealed progressive compaction of the muscle tissue into a fiber-like structure (Fig.2A), suggesting continuous remodeling of the muscle tissue, probably encouraged by the differentiation process. Confocal images of immunostained tissues confirm cytoskeletal alignment, as well as fusion of cells into multinucleated myofibers, indicating correct cell differentiation (Fig.2B).

In the liver compartment, cells display stable morphology, with the formation of aggregates in a well-connected cellular network. This aligns with findings in the literature of 3D liver constructs [5], confirming correct cell arrangement (Fig.3). Nevertheless, further studies will be conducted to assess the functionality of the tissue and its ability to metabolize compounds.

Finally, as shown by a z-stack reconstruction (Fig.4B), the endothelial cells were able to form a compact monolayer surrounding the walls of the perfusion channel, showing a thigh network (Fig.4A), ideal for permeability studies and to represent the *in vivo* vasculature.

Overall, these results show promising biological characteristics of the model, aligning with physiological organ and vasculature structure. The next steps will assess the ability of the system to model biodistribution, efficacy, and metabolism of known compounds and nanoparticles.

By connecting target efficacy with metabolic processing in the same system, this liver–muscle MPS represents a step toward "body-on-chip" technologies that could complement or replace animal models, in line with the FDA Modernization

Act (2022) guidelines. Ultimately, this platform could accelerate the design and preclinical validation of nanomedicines with improved predictive power and translational relevance.

References

[1] R. O. Rodrigues, P. C. Sousa, J. Gaspar, M. Bañobre-López, R. Lima, and G. Minas, *Small*, 16.59 (2020), p. 2003517

[2] Z. He, N. Ranganathan, and P. Li, *Nanotechnology*, 29.49 (2018), p. 492001

[3] N. Ashammakhi et al., *Small Methods*, 4.1 (2020), p. 1900589

[4] D. W. Lee, S. H. Lee, N. Choi, and J. H. Sung, *Biotechnol. Bioeng.*, 116.12 (2019), p. 3433

[5] J. Deng et al., *Micromachines (Basel)*, 10.10 (2019), p. 676

Figures

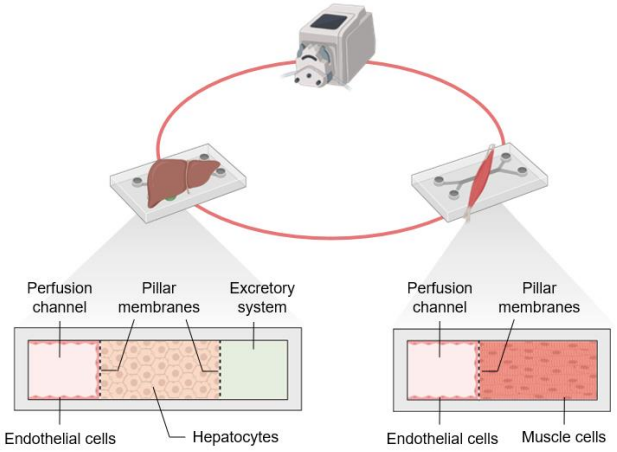

Figure 1. Multi-Organ system setup

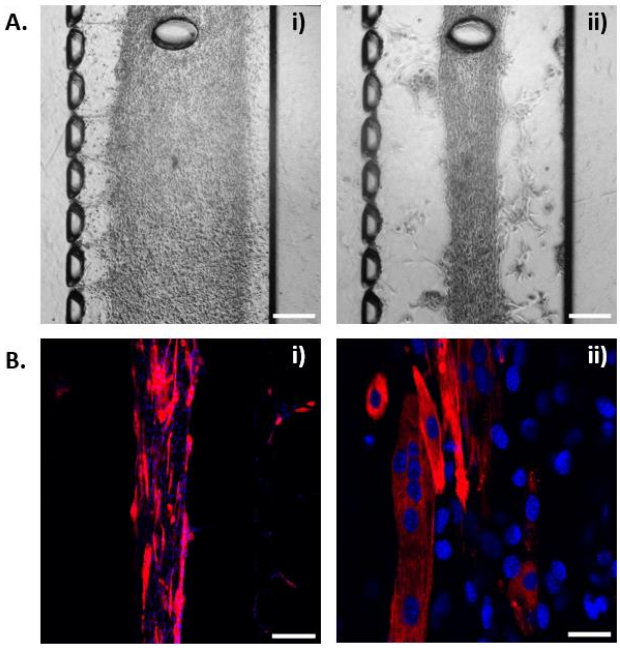

Figure 2. Muscle-on-a-chip: **A)** Brightfield images of muscle microtissue at (i) 24 hours and (ii) 9 days after seeding in the device. Scale bar 200µm. **B)** Confocal images of stained muscle microtissue (nuclei in blue, F-actin in red). Scale bars (i) 200µm and (ii) 30µm.

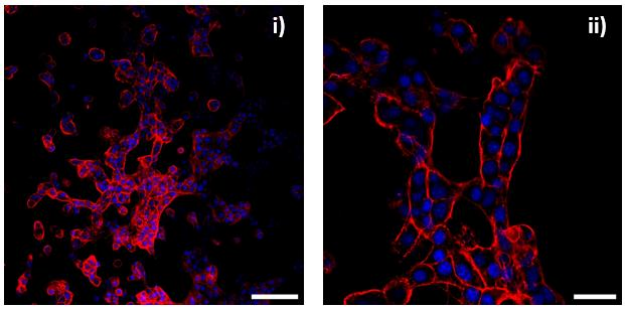

Figure 3. Liver-on-a-chip: Confocal images of stained liver microtissue (nuclei in blue, F-actin in red). Scale bars (i) 100µm and (ii) 30µm.

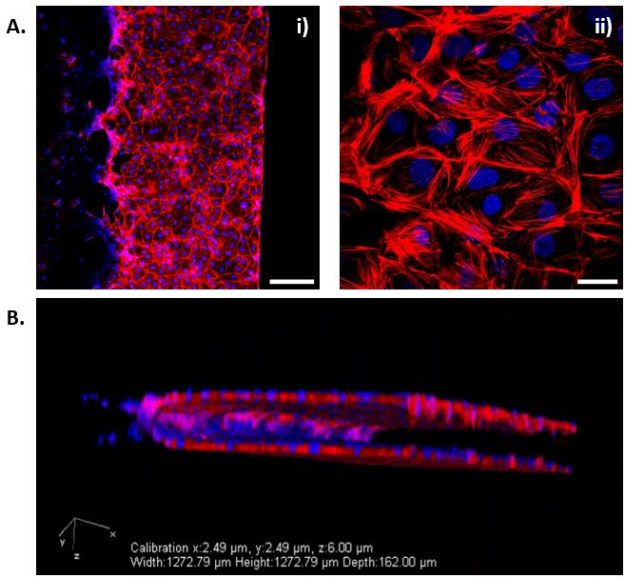

Figure 4. Endothelial compartment: **A)** Confocal images of endothelial monolayer (nuclei in blue, F-actin in red). Scale bars (i) 200µm and (ii) 30µm. **B)** 3D reconstruction of cells lining the wall of the channel.