Building a Heart-on-Chip: First Steps Towards a Vascularized Cardiac In Vitro Model

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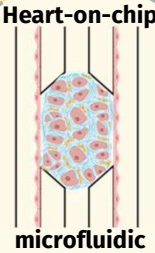
Background

Animal models — Complexity / Result extrapolation / Cost — **2D cellular cultures**

Lack of predictive cardiac models

→ **Clinical trial failures** of new therapies

Heart-on-chip



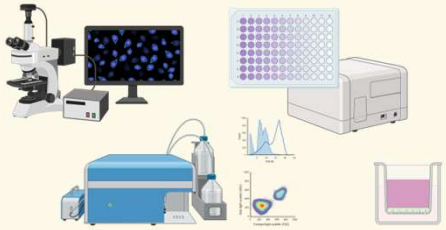
microfluidic device

Goals

1. **Characterization** of the 3 main cardiac populations: induced cardiomyocytes (**iCM**), cardiac fibroblast (**hCF**) and endothelial cells (**hCAEC**)
2. Definition of a **co-culture medium**
3. Establishment of the **endothelial barrier**

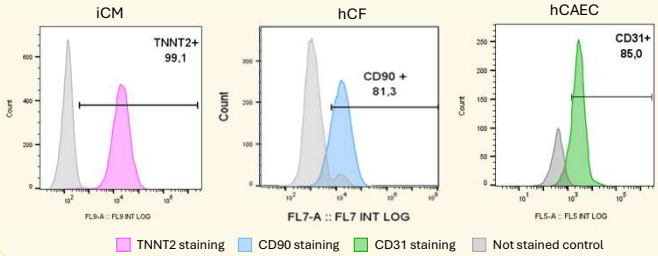
Materials & Methods

- **Immunophenotyping** with lineage-specific antibodies by **flow cytometry**
- **Metabolic activity** by **resazurin assay** of the 3 cellular population with 4 co-culture media candidates
- Characterization of the **endothelial barrier** in transwells (**2D**) by **permeability assay** with dextrans of different molecular sizes (40 kDa and 70 kDa) and **immunofluorescence** for the intercellular junctions.
- **Endothelization** of the **lateral channels** of the **chip platform** and **permeabilization assay with** 70 kDa dextran within the chip.



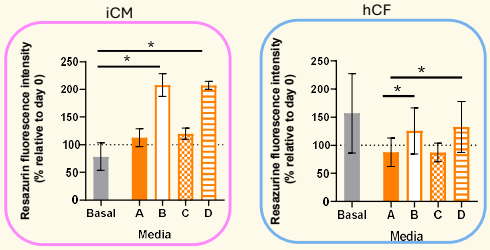
Results

Immunophenotyping of cardiac cell lineage



The different cell types express lineage-specific markers, namely **TNNT2** for **CMs**, **CD90** for **hCFs** and **CD31** for **hCAEC**.

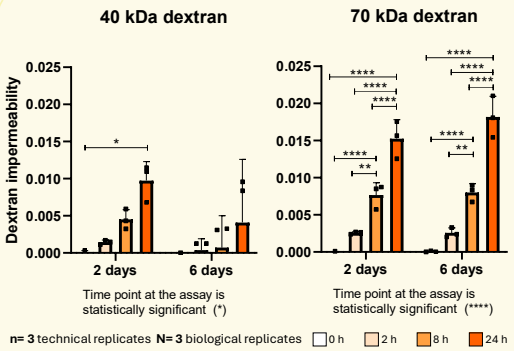
Co-culture medium determination



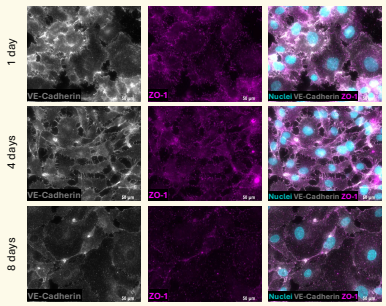
n= 5 technical replicates
N= 3 biological replicates

Media C supported the viable cultures of iCM and hCF, which exhibited similar metabolic activity compared to their basal media after 7 days in culture, while for hCAEC it supported their non-proliferative culture.

Endothelial barrier characterization

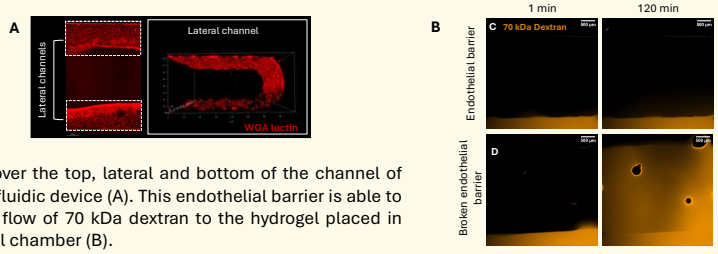


We have successfully established an **endothelial barrier** in transwells cultured with the **co-culture medium C** that was able to significantly decrease the permeability of 40kDa and 70 kDa dextrans at different time points (2 and 6 days of culture).



The **cellular junctions** (adherent and tight junctions) between hCAEC, typically established when forming an endothelial barrier, are present at different time points while culturing them in the co-culture medium.

Endothelization of the lateral channels



hCAEC cover the top, lateral and bottom of the channel of the microfluidic device (A). This endothelial barrier is able to block the flow of 70 kDa dextran to the hydrogel placed in the central chamber (B).

Conclusions

- We have confirmed the lineage-specific **phenotype** of the three human cellular cardiac populations.
- We have established a **co-culture medium** that supports the endothelial barrier viability in static conditions.
- We have set a procedure for **endothelializing the lateral channels** of the microfluidic device.
- This study provides ground evidence of the capacity to create a heart-on-chip with a functional vascular barrier.

References

[1]Paz-Artigas, L., Montero-Calle, P., Iglesias-García, O., Mazo, M. M., Ochoa, I. & Ciriza, J. Current approaches for the recreation of cardiac ischaemic environment in vitro. *Int. J. Pharm.* **632**, 122589 (2023). [2]Rexius-Hall, M. L., Escopete, S. S., Li, X., Hu, J., Yuan, H. *et al.* A myocardial infarct border-zone-on-a-chip demonstrates distinct regulation of cardiac tissue function by an oxygen gradient. *Sci. Adv.* **8**, eadd4909 (2022). [3]Lee, J., Mehrotra, S., Zare-Eelanjegheh, E., Rodrigues, R. O., Akbarinejad, A., Ge, D. *et al.* A Heart–Breast Cancer–on–a–Chip Platform for Disease Modeling and Monitoring of Cardiotoxicity Induced by Cancer Chemotherapy. *Small* **17**, 2100282 (2021)

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