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

Carolina Gómez-Moreno[1,2], Laura Paz-Artigas[1,3], Hazel Santander-Badules[2,4], Laura Ordovás[2,4,5,6], Jesús Ciriza[1,3,7].

[1] Grupo TME Lab, Instituto de Investigación en Ingeniería de Aragón (I3A), Universidad de Zaragoza, 50009 Zaragoza, Spain.
[2] Grupo BSICoS, Instituto de Investigación en Ingeniería de Aragón (I3A), Instituto de Investigación Sanitaria Aragón (IISA), 50009 Zaragoza, Spain.
[3] Grupo TME Lab, Instituto de Investigación en Ingeniería de Aragón (I3A), Instituto de Investigación Sanitaria Aragón (IISA), 50009 Zaragoza, Spain.
[4] Grupo BSICoS, Instituto de Investigación en Ingeniería de Aragón (I3A), Universidad de Zaragoza, 50009 Zaragoza, Spain.
[5] Fundación Agencia Aragonesa para la Investigación y el Desarrollo (ARAID), 50018 Zaragoza, Spain.
[6] Grupo BSICoS, CIBER-BBN, 50018 Zaragoza, Spain.
[7] Grupo TME Lab, CIBER-BBN, 50018 Zaragoza, Spain.
Tel. +34-976762707 e-mail: carolina.gomez@unizar.es

Abstract

Cancer and cardiovascular diseases are the leading causes of mortality worldwide. However, the development of new advanced treatments is limited by the lack of predictive human cardiac models. This study lays the groundwork for developing a human *heart-on-chip* that incorporates the interactions between the three main cardiac cell types.

Background

Cancer and cardiovascular disease are the world's leading causes of death, highlighting the need to develop new advanced treatments. However, this is currently limited by the lack of predictive humanized cardiac models, resulting in a high rate of failed clinical trials.

Currently, two-dimensional human cellular cultures and animal models are primarily used, but both have drawbacks and fail to accurately model the pathophysiology of the human heart. Consequently, research is focused on developing new heart models. [1]. *In vitro* modelling of the human heart has grown significantly in recent decades, thanks to the advavnces in the development of stem cell technologies for obtaining heart-derived cells. However, robust cardiac models with a functional endothelial barrier that mimics the natural impediment to the distribution of therapies are still lacking [2–3].

The development of microfluidic and microfabrication technologies, in combination with the differentiation of pluripotent stem cells, opens the door to unprecedented opportunities for the biomimetic recapitulation of human tissue characteristics *in vitro,* including vascularized cardiac tissues, through the creation of organs-on-chip.

Therefore, we aim to give the first steps in the establishment of a vascularized heart-on-chip (HoC) that is designed to model human cardiac processes such as chemotherapy-induced cardiotoxicity or isquemia.

Materials and Methods

Cardiomyocytes derived from human induced pluripotent stem cells, human cardiac fibroblasts and human endothelial cells have been characterized by flow cytometry.

As a critical step in developing the HoC, we have defined a co-culture medium that supports the viability of all the human cell types involved determined by metabolic activity assay with resazurine. Then, we have determined the capacity of this co-culture media to support the creation of a functional cardiac endothelial barrier in static models by permeabilization assays (Multimode Microplate Reader, BioTek Synergy H1) and immunofluorescence (MICA WideFocal, Leica). Furthermore, we have determined conditions for the endothelialization of the lateral channels of a microfluidic device by confocal and time lapse microscopy (Eclipse TE2000-S confocal microscope, Nikon).

Results

More than 99% of the human iPSC-derived progeny was identified as cardiomyocytes (iCMs) due to their expression of TNNT2. Human cardiac fibroblasts (hCF) and human coronary artery endothelial cells (hCAEC) expressed lineage-specific markers, namely, CD90 and CD31, respectively.

We tested several different coculture media and one of the candidates (media C) supported the viable culture of iCM and hCF in terms of similar metabolic activity compared to their basal media after 7 days in culture, while hCAEC maintained their metabolic activity as compared to de initial timepoint.

In static models, we have successfully established an endothelial barrier cultured with the co-culture medium that was able to significantly decrease the dextran permeability at different time points (Fig. 1A). Furthermoner, we endothelialiazed the lateral channels of the microfluidic deviced and assessed its permeability with a 120 minute lapse (Fig. 1B)

Conclusions

We have characterized the phenotype of the three cellular populations and established a co-culture medium that supports the endothelial barrier viability in static conditions. Furthermore, we have set a procedure for endothelializing the lateral channels of the microfluidic device, providing ground evidence of the capacity to create a heart-on-chip with a functional vascular barrier.

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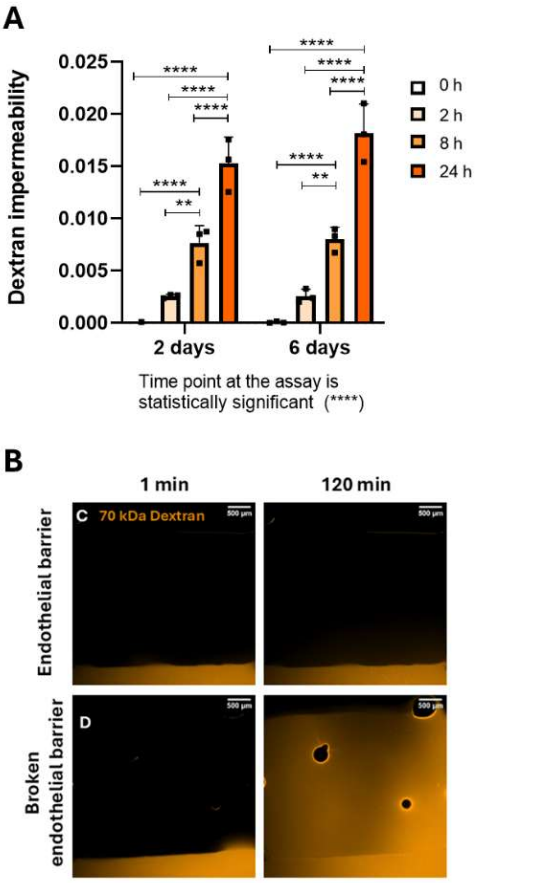

Figure. 1. Dextran permeabilization assays with hCAEC. **(A)** Characterization of the hCAEC barrier static model in media C. represented as the degree of impermetability of the barrier to the 70 kDa fluorescently labelled dextran, at different timepoints after 2 or 6 days of culture. **(B)** Representative images of dextran diffusion at 1 and 120 minutes in the presence of a functional (top panels) or disrupted endothelial barrier (bottom panels).