

# 3D Biomimetic in Vitro Model of Self-induced Cardiac Ischemia Based on the Co-culture of Cardiomyocytes Derived from hiPSC and Primary Human Cardiac Fibroblast

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## Abstract

Large cardiac spheroids (i.e., > 500 µm diameter) that self-induce an ischemic core were generated by co-culture of cardiomyocytes derived from induced pluripotent cells (hiPSC-CMs) and primary cardiac fibroblast (hCF). Without modification of culture conditions, spheroids' large size allowed the recreation of a dead core, fibrotic remodelling and tissue stiffening.

## Introduction

Ischaemic heart diseases (IHD) are characterized by the imbalance between the myocardial blood demand and supply, usually caused by the occlusion of a coronary blood vessel. A gradient of nutrients and gases is then generated. Cardiomyocytes (CM) start developing the first signs of necrosis and slow altered contractil activity. The resulting injured microenvironment causes the recruitment of immune cells and the activation of cardiac fibroblast. The last ones increase their secretion of extracelular matrix, generating a stiff scar that further decreases the contractil capacity of the tissue (fibrosis) [1].

Modelling an ischemic myocardium *in vitro* calls for the recreation of its particular 3D architecture, closely related to the formation of nutrients and oxygen, along with the inclusion of the different cell types involved in ischemic events [2]. Spheroids and organoids represent the simplest approach to culture different cell types in 3D and generate gradients of cellular access to the media. However, the few reported models of cardiac ischemia worked with relatively small aggregates, requiring low oxygen incubation or deprived nutrient media to achieve an ischemic core [3, 4].

To better recapitulate the environment of cardiac ischemia, here, we co-culture hiPSC-CM and hCF

into large cardiac spheroids that self-induce the ischemic core. Then, we evaluate cellular viability and hints of fibrotic remodelling.

## Materials and methods

CMs were obtained by hiPSCs differentiation, following a biphasic Wnt modulation protocol with minor modifications, and cocultured with hCF (iCell) at a 70:30 (hiPSC-CM:hCF) ratio in low-adherence U-bottom wells to generate cardiac aggregates of 40.000 cells. Aggregates were cultured for up to 17 days and inspected at different time points.

Cellular viability within the whole spheroid was assessed by calcein/propidium iodide staining, following by a clearance procedure to visualize the inside of the spheroid by confocal microscopy. Cryosections of spheroids were used for immunostaining of caspase-3, vimentin and cardiac troponin T (cTnT). Nuclei were stained with Hoechst.

Spheroid stiffness was determined using a custom-made constriction methacrylate microfluidic device that consists of a single 400 µm tubular channel, which is reduced to 200 µm. Spheroids immersed in liquid were forced to pass through the constriction by increasing the pressure. Stiffness was calculated as a pressure/deformation relationship, where deformation was determined as the penetration length normalized by the spheroid initial diameter.

## Results

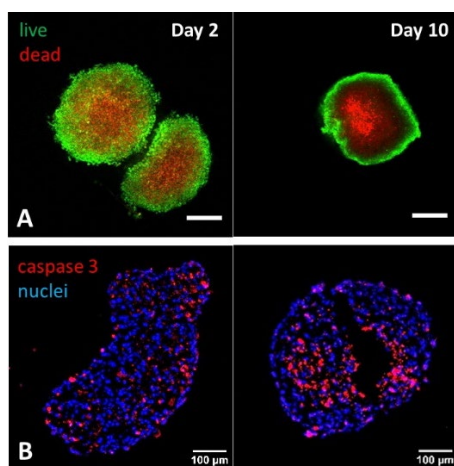
The culture of 40.000 cells per aggregate resulted in spheroids bigger than 500 µm of diameter, which should be enough to ensure the generation of a hypoxic core, since the range of oxygen diffusion in spheroids is around 200 µm. Indeed, a dead core could be already seen at day 2 since seeding (Figure

1A). Cell death did not correlate with presence of caspase-3 (Figure 1B) at day 2, suggesting that apoptosis is not the leading dead pathway at initial stages of our model. In fact, ischemia *in vivo* is known to cause CM death by different ways, with necrosis being the preponderant one. Interestingly, at day 10, the frontier between live and dead cells became sharper, with an increase in the apoptotic fraction at the center of the spheroid.

We next investigate the fibrotic tissue remodelling, another hint of ischemia. The initial random disposition of cardiac troponin T (cTnT, a CM marker) and vimentin (a CF marker) evolved to present a central aggrupation of cTnT-positive cells flanked by areas of vimentin-positive cells (Figure 2A). The fibrotic cellular reorganization seemed to lead to an increase of tissue stiffness (Figure 2B), thus recreating the *in vivo* formation of a stiff fibrotic scar.

## Conclusions

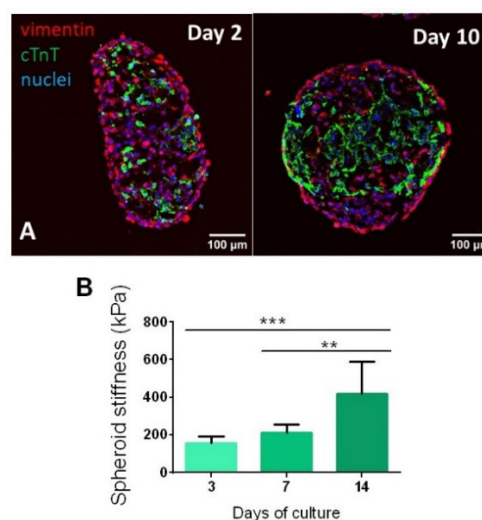
The large size of the spheroids resulted in the generation of a dead core without external inductors of ischemia, such as nutrient deprivation or incubation in hypoxic atmosphere. Cardiac spheroids also recreated hints of ischemic fibrosis, including tissue remodelling and stiffen. Data presented in this work entail the first steps to establish a complex biomimetic spatial recreation of human cardiac ischemia gradients.



**Fig 1. Dead core formation**, assessed by (A) live/dead staining with calcein (green) and propidium iodide (red) of whole spheroids and (B) caspase-3 (apoptotic marker) immunostaining in spheroid cryosections. Scale bar in A= 200  $\mu$ m. Scale bar in B = 100  $\mu$ m.

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**Fig 2. Cardiac spheroids recreate ischemic-related fibrosis.** A) Immunofluorescence of vimentin (CF marker, in red) and cardiac troponin T (cTnT, CM marker, in green) shows fibrotic tissue remodelling on spheroids cryosections. B) Spheroid stiffness, assessed by means of a constriction assay, increases overtime. Scale bar = 100  $\mu$ m.