

### 3D engineered human cardiac tissues as platforms for cardiotoxicity assessment

Pochettino E.<sup>1,2</sup>, Alliaud A.<sup>1</sup>, Gisone I.<sup>3</sup>, Fernandez-Arroyo S.<sup>4</sup>, Boffito M.<sup>1</sup>, Sartori S.<sup>1</sup>, Cecchetti A.<sup>5</sup>, Ceccherini E.<sup>3</sup>, Vozzi F.<sup>3</sup>, Ciardelli G.<sup>1</sup>

<sup>1</sup>Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy; <sup>2</sup>Department of medical and surgical sciences, Università di Torino, Turin, Italy; <sup>3</sup>Institute of Clinical Physiology IFC-CNR, Pisa, Italy; <sup>4</sup>Centre for Omic Sciences, Joint Unit Eurecat-Universitat Rovira i Virgili, Reus, Spain; <sup>5</sup>Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy

#### Introduction

Humans are constantly exposed to a wide range of environmental and pharmaceutical compounds, some of which may exert cardiotoxic effects. However, cardiotoxicity remains insufficiently investigated, particularly in relation to age-related differences in cardiac tissue response. Structural and functional changes occurring during myocardial aging can significantly influence cellular susceptibility to toxic agents. Therefore, reliable *in vitro* models capable of reproducing different stages of myocardial maturation are essential for more accurate evaluation of drug and chemical toxicity.

**Aim.** The aim of this work was to develop bioengineered 3D cardiac models that mimic different stages of myocardial aging as platforms for cardiotoxicity assessment.

#### Materials and Methods

The models consisted of fibronectin-functionalized scaffolds fabricated via melt-extrusion additive manufacturing using a custom-made poly(caprolactone)(PCL)-based polyurethane (PUR). These scaffolds were combined with photocurable gelatin methacryloyl (GelMA) hydrogels at 5% and 10% w/v concentration to reproduce the mechanical properties of young and mature myocardium, respectively. GelMA served as a carrier for a co-culture of human-induced pluripotent stem cell-derived cardiomyocytes and human coronary artery endothelial cells in an 80:20 ratio. The cellularized GelMA precursor was pipetted into the PUR scaffolds and subsequently photo-crosslinked (365 nm, 10 mW/cm<sup>2</sup>, 30 s) to obtain the final biphasic constructs. Multi-omics approaches consisted of transcriptomics (RNA-seq by Illumina platform), untargeted proteomics (by nLC-Orbitrap-MS), and targeted metabolomics and lipidomics (by LC-QqQ-MS).

#### Results

PUR synthesis was confirmed by infrared spectroscopy and size exclusion chromatography, while differential calorimetry and thermogravimetric analyses provided information on its thermal transitions and stability. Scaffolds with anisotropic architecture (filament diameter of ca. 0.41 mm) were produced and surface-functionalized with fibronectin to enhance biomimicry of cardiac extracellular matrix. Photo-rheological characterization of GelMA hydrogels revealed Young's modulus values of approximately 10 kPa and 55 kPa for 5% and 10% formulations, reproducing the mechanical properties of young and mature myocardium, respectively. Scanning electron microscopy of the assembled constructs revealed strong PUR-GelMA integration and an interconnected porous network that supports nutrient and oxygen diffusion. The cell-based models were cultured for up to 11 days, during which cell viability and oxidative stress were monitored. Multi-omics analyses (transcriptomics, proteomics, and metabolomics) revealed that myocardial aging was associated with mitochondrial dysfunction, loss of calcium homeostasis, enhanced protein degradation, impaired cell-cell adhesion, and alterations in amino acid and lipid metabolism, ultimately affecting contractile function and cellular maturation.

#### Conclusion

The developed 3D cardiac models successfully reproduced key features of young and mature myocardial tissue *in vitro*. These platforms represent promising tools for investigating age-related cardiac physiology and for improving the predictive assessment of chemical-induced cardiotoxicity.

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