Engineering 3D in vitro cardiac tissue models for cardiotoxicity assessment

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INTRODUCTION

Animal models represent the gold standard for toxicity assessment. However, due to anatomical and functional diversity, they are often unable to replicate the real human physio-pathological scenario with sufficient fidelity. Additionally, their use is currently strongly debated due to ethical issues. In this scenario, the development of in vitro bioengineered tissue/organ models could represent a significant step forward in establishing Novel Approach Methodologies, providing more reliable and robust methods for toxicity assessment [1]. In this contribution, we report the development of a new in vitro bioengineered model of cardiac tissue. The model results from the assembly of a polymeric 3D multi-layered structure obtained by melt extrusion additive manufacturing of a custom-made poly(ester urethane) (PU) with a cell-loaded (human induced pluripotent stem cell derived-cardiomyocytes hiPSC-CMs) methacryloyl gelatin bio-ink.

MATERIALS AND METHODS

The PU was synthesized using poly(ε-caprolactone) diol, 1,4-butane diisocyanate, and L-lysine ethyl ester [2] and characterized by Infrared (IR) spectroscopy, Size Exclusion Chromatography (SEC), tensile tests, Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA, in temperature ramp and isothermal conditions), and thermo-rheological tests (i.e., temperature ramp, time sweep, and frequency sweep tests). Then, the PU was microfabricated into multi-layered scaffolds with a 0°/90° lay-down pattern by melt-extrusion AM. In parallel, gelatin methacryloyl (GelMA) was synthesized by reacting gelatin (type A from porcine skin) with methacrylic anhydride (MA) (1 ml/g_gelatin). GelMA was characterized by IR and proton nuclear magnetic resonance (1H NMR) spectroscopies and the Ninhydrin colorimetric assay. GelMA aqueous solutions containing phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) as photo-initiator (0.05% w/V) were then characterized by photo-rheological (365 nm, 10 mW/cm2) tests to define a plethora of formulations for hiPSC-CM loading. hiPSCs were thawed and cultured in Essential 8™ Medium on Vitronectin VTN-N-coated 6-well plates. Subsequently, hiPSC cell differentiation was initiated using PSC Cardiomyocyte Differentiation Kits in accordance with the manufacturer's instructions. After 14 days, beating hiPSC-CMs were obtained, and cells were passaged onto VTN-N-coated 12-well plates for 2D culture or encapsulated into GelMA bio-ink, poured within the scaffolds and finally photo-crosslinked for 3D cultures. After further 14 days of culture, cell function (CellTiter-Blue) and viability (LDH) assays were performed. RealTime-PCR analyses of cardiac genes associated with the mature phenotype (TNNT2, BNP, CX43) were also performed.

RESULTS AND DISCUSSION

PU successful synthesis was assessed by IR spectroscopy and SEC. Tensile tests evidenced an elastomeric-like behavior, with Young's modulus and strain at a break of around 10MPa and 700%, respectively. DSC, TGA, and thermo-rheological tests provided information on PU behavior and stability at different temperatures, thus supporting the optimization of the printing protocol. Scaffolds with a 0°/90° lay-down pattern were successfully fabricated. The selected fiber size and spacing allowed the deposition of self-supporting molten filaments during the fabrication of highly porous multi-layered structures. The synthesis of GelMA with a 99% degree of methacryloylation was proved by spectroscopic analyses and the Ninhydrin assay. A plethora of GelMA formulations with tunable mechanical properties (storage modulus value of photo-cured gels within few and 15 kPa) was successfully designed by modulating GelMA content within 5 and 10% w/V concentration. Cell viability and function of hiPSC-CMs were increased in scaffolds with respect to 2D control as also the transcriptional expression of the analyzed genes.

CONCLUSIONS

Our results proved the potential of the developed structs as cardiac tissue models with tunable structural, mechanical, and biochemical features. Such models will allow the investigation of physio-pathological processes and cardiotoxicity testing.

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