Design of 3D bioengineered cardiac tissue models for the evaluation of chemical cardiotoxicity

Gianluca Ciardelli[1,*], Arianna Grivet-Brancot[1,2], Valeria Chiono[1], Susanna Sartori[1], Monica Boffito[1]

[1] Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Torino, Italy
[2] Department of Surgical Sciences, Università di Torino, Torino, Italy
[*] gianluca.ciardelli@polito.it

INTRODUCTION

Humans are continuously exposed to a huge amount and a variety of chemicals. Animal tests are the gold standard for toxicity testing. However, they often fail in finely replicating the real physio-pathological scenario and their use is associated with ethical issues. 3D *in vitro* tissue models more efficiently mimic the native human environment and bring clear ethical advantages.[1] In order to design a 3D bioengineered tissue model, the 3D matrix used to guide cell behavior, extracellular matrix (ECM) production and new tissue formation should replicate the architecture and composition of the native tissue. In this context, the proper selection of the biomaterial, the fabrication method and the functionalization protocol plays a pivotal role. In cardiac tissue engineering (TE), elastomeric polymers are required as constituents of porous structs replicating *in vitro* the myocardium architecture and mechanical properties. Moreover, surface functionalization with cardiac ECM proteins replicates *in vitro* the biochemical cues present in the native tissue. In this work the versatility of poly(urethane) (PU) chemistry was exploited to design a plethora of polymers with a wide range of physico-chemical properties. The most promising material for cardiac TE was then microfabricated through melt extrusion additive manufacturing (AM). Scaffolds were surface functionalized with cardiac ECM proteins (e.g., laminin, LN) and seeded with cardiac progenitor cells (CPCs) to establish cardiac tissue models.

EXPERIMENTAL METHODS

PUs were synthesized[2] using the same macrodiol (poly(ε-caprolactone) diol, 2000Da) and aliphatic diisocyanate, and different chain extenders (e.g., 1,4-butanediol, 1,8-octanediol, 1,12-dodecanediol, L-lysine ethyl ester, N-Boc serinol). As synthesized PUs were characterized by Infrared (IR) spectroscopy and Size Exclusion Chromatography (SEC). Thermal characterization was carried out through Thermogravimetric analysis (TGA), Differential Scanning Calorimetry (DSC) and rheology to assess polymer suitability for processing in the melt state. Finally, tensile tests were performed on PU dense films to evaluate their mechanical performances. The selected PU according to the measured mechanical properties was then microfabricated into scaffolds by melt-extrusion AM. The structs were surface plasma treated in the presence of acrylic acid vapor to expose -COOH groups and then grafted with ECM proteins through the carbodiimide chemistry.[3] Scanning electron microscopy, IR spectroscopy and X-ray photoelectron spectroscopy (XPS) were performed. CPC adhesion and proliferation were then assessed, while cardiac markers expression was tested by Real-Time Quantitative Reverse Transcription-Polymerase Chain Reaction.

RESULTS AND DISCUSSION

PU successful synthesis was assessed by IR spectroscopy and SEC. Aliphatic linear chain extenders gave stiff PUs (Young's Modulus (E) ≈ 350MPa) with elongation at break (ε%) ranging from few to tens %. In particular, 1,8-octanediol gave a PU with higher ε% (≈ 30-40%) compared to both 1,4-butanediol and 1,12-dodecanediol that resulted in highly brittle polymers. Conversely, N-Boc serinol gave a PU with around 150MPa Young's Modulus and ε% of approx. 150%. L-lysine ethyl ester instead provided the resulting PU with an elastomeric-like behavior (E and ε% of around 10MPa and 700%) that made it the optimal one for the fabrication of structs replicating the cardiac tissue. Rheological temperature ramp tests evidenced a solid-to-liquid transition temperature around 160°C, in agreement with DSC thermograms. Isothermal TGA analysis proved PU suitability for processing in the melt state. Scaffolds with a 0°/90° lay-down pattern were thus fabricated via melt-extrusion AM. Successful surface functionalization was proved by XPS and IR spectroscopy. LN functionalization promoted CPC proliferation and the expression of differentiation markers for endothelial and smooth muscle cells, and cardiomyocytes.

CONCLUSION

In this contribution we have demonstrated that PU versatile chemistry can be exploited to *ad-hoc* engineer a polymer best matching the mechanical requirements for cardiac TE. 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.

REFERENCES

1. Caddeo S. Front Bioeng Biotechnol. 2017;5:40.
2. Sartori S. React Funct Polym. 2013;73: 10.
3. Boffito M. PloS ONE 2018;13:7.

ACKNOWLEDGEMENTS

This work was supported by MIUR-FIRB 2010 Future in Research (grant # RBFR10L0GK), and the European Union's Horizon 2020 research and innovation program (grant # 101037090). The content of this abstract reflects only the author's view, and the Commission is not responsible for any use that may be made of the information it contains.