Poly-lactic acid and fibrinoin scaffolds as three-dimensional device to differentiate cardiac stem cells: *in vitro* and *in vivo* studies

Valentina Di Felice[1], Angela De Luca[1], Antonella Marino Gammazza[1], Claudia Serradifalco[1], Patrizia Catanese[1], Luigi Rizzuto[1], Rosario Barone[1], Filippo Macaluso[1], Patrizia Di Marco[2], Giovanni Cassata[2], Roberto Puleio[2], Lucia Verin[3], Antonella Motta[3], Annalisa Guercio[2], Giovanni Zummo[1]

[1] Department of Experimental Biomedicine and Clinical Neurosciences, Section of Human Anatomy, University of Palermo, Italy
[2] Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italy
[3] BIOtech Laboratories, University of Trento, Italy

Introduction The rapid translation of preclinical cell-based therapy to restore damaged myocardium has raised questions concerning the best cell type as well as the best delivery route, and the best time of cell injection into the myocardium. Intramyocardial injection of stem cells is by far the most-used delivery technique in preclinical studies. We have recently demonstrated that c-Kit+ cardiac progenitor cells are able to organize themselves into a tissue-like cell mass in three-dimensional cultures, and with the help of an OPLA scaffold, many cells can create an organized elementary myocardium.

Hypothesis We designed random porosity and oriented poly-lactic acid and fibrinoin scaffolds to be used as three-dimensional systems to favour differentiation of cardiac progenitor cells. We tested them in vitro and in vivo to study their potentiality as surgical devices in cardiology.

Methods For the synthesis of PDLLA scaffolds, the Poly (D,L lactic acid) (RESOMER® 207, MW = 252 kDa) polimer were used (6.7%) in Dicloromethane/Dimetilformamide (DCM/DMF) 70/30 (v/v). The three-dimensional structure was obtained by salt-leaching, using NaCl crystals as porosity agent (NaCl < 224 μm and <150 μm). For the synthesis of fibrinoin scaffolds, degummed silk fibres were dried and dissolved into 9.3 m LiBr water solution (20% w/v) at 65°C for 3h. Scaffolds with different porosities, pore size, and properties were made by freeze-drying and salt-leaching. Scaffolds embedded with collagen I and cardiac progenitor cells were implanted in the subcutaneous dorsal region of athymic *Nude-Foxn1^{nu}* mice.

Results Cardiac progenitor cells differentiated into cardiomyocytes in vitro into PDLLA scaffolds in a M-199 medium supplemented with 20% FBS within 21 days. A less extent of differentiation has been obtained in the dorsal subcutaneous region, and a foreign body reaction has been recorded.

Key words
Cardiac stem cells, cardiac surgery, scaffolds, tissue engineering