ImmunoTools *special* Award 2014

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Quick production of spherical aggregates of human cardiac progenitor cells for scaffold-less tissue engineering by means of a novel methylcellulose hydrogel-based system

Cardiovascular diseases are accompanied by the progressive loss of myocytes due to necrosis and apoptosis phenomena promoted by the oxidative stress associated with the reperfusion that follows the ischemic phase. The loss of cardiomyocytes is at first balanced by hypertrophy of the remaining contractile cells and then it evolves in ventricular dilatation/remodelling and chronic heart failure (CHF). Indeed the replacement of the lost cardiomyocytes is highly inefficient and relies on the few cardiac progenitor cells. These cells, however, can sustain only organ homeostasis in physiological situations, and, moreover, decline numerically with age. Pharmacological or device-assisted treatments are only palliative and thus heart transplantation is still the only possible treatment in severe cases. Major problems for this practice are the insufficient organ availability, complex surgical procedures, costs and long-term immunosuppression treatments. For these reasons, in recent years alternative therapeutic strategies have been suggested, including cellular therapies, mainly based on adult stem cells. However, very few (<1%) stem cells directly injected in the heart or in the blood stream can survive; for this reason other strategies, such as implantation of cell aggregates maintaining their extracellular matrix (ECM) appears to partially solve this problem. It is well reported in literature that a cell culture system employing methylcellulose (MC) hydrogel wells allows the production of implantable stem cell spherical aggregates.

Thus, during my PhD, we decided to generate 3D multicellular aggregates (named hCPC spheroids) composed of human cardiac progenitor cells for scaffold-less cardiac tissue engineering.

After the formation of hCPCs spheroids, the spherical clusters were analyzed for the expression of stemness, cardiac and ECM markers by immunofluorescence and for cell survival by Live/dead assay. The spheres expressed many

stemness/mesenchymal (CD90, CD44 and vimentin) and ECM (collagen I, fibronectin and laminin) markers. Moreover they retained the expression of some early cardiac markers (connexin 43, GATA-4 and MEF2C), although at a low level.

We have also analyzed the ability of re-plated hCPC spheroids to migrate: cells from spheroids migrated with more efficiency than cells maintained in 2D conventional culture systems in a wound healing assay in response to an agonist monoclonal antibody directed against the Hepatocyte Growth Factor Receptor.

We have started also *in vivo* experiment: spheroids were injected into the mouse heart wall and we found that some human cells survived, migrated and engrafted into the host tissue, at least for 1 week after transplantation.

I would like to take into considerations two other aspects of this project. The first one would be related to the factors involved in hCPCs migration which I will test *in vitro* as I already did in the case of the HGF-R mediated response. This would give me the information that could be translated for *in vivo* experiments. Moreover, the treatment of spheroids with growth/mobility factors could also help in increasing the viability of cells, which will undergo *in vivo* transplantation.

The second aspect that I want to investigate is relative to the possible inflammatory reactions induced by the transplanted spheroids. For this purpose I intend to use antibodies against different cells involved in this events such as macrophages, NK cells, neutrophils and dendritic cells. Since we will immunosuppress the animals with cyclosporine A, I would also like to test the involvement of cells of immune response such as CD4, CD8 positive cells.