Omentum-derived stromal cells improve myocardial regeneration in pig post-infarcted heart through a potent paracrine mechanism

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Introduction: Myocardial Infarction (MI) represents one of principal causes of human death. Cell-based therapy could be a valid new approach for MI. Different kind of cells, including embryonic stem cells as well as adult/progenitor stem cells, have been proposed as candidates for therapeutic purposes. However, many aspects, ranging from ethical questions to functional efficacy, still remain to be clarified. Among adult/progenitor stem cells, Adipose-derived stromal cells (ADSC) seem to have some advantages, mainly because of their easy tissue accessibility and iv tro an adequate rate of growth.

Aim of the study: To investigate the capacity of transplanted ADSCs, through functional, haemodynamic and histopathological assessment, to improve myocardial infarction and regeneration of experimental heart ischemia induced by permanent IVA-ligation in pigs.

Methods: ADSCs isolated from human adipose tissue (omentum fat) were cultured, expanded, and phenotipically characterized. Furthermore, *in vitro* pro-angiogenic, anti-inflammatory and anti-apoptotic properties were analyzed. 50x10^6 cells/pig were transplanted by intramyocardial injection in acute infarcted hearts (treated-group, n=12 cell-injected pigs). Two months after MI induction echocardiographyc and haemodinamic follow-up was performed. In addition, hystopathological examination was conducted.

Results: As shown in Figure 1, i*n vitro* ADSCs secreted high levels of pro-angiogenic, anti-inflammatory and immunomodulatory cytokines (VEGF, HGF and IL-6). Furthermore, they prevented monocytes activation as well as cardiomyocytes apoptosis (Figure 2). Finally, *in vitro* but not *in vivo*, ADSCs were able to trans-differentiate into cardiomyocyte-like cells (Figure 3). *In vivo*, ADSCs injection along the border of the ischemic area (Figure 4), reduced post-infarct pigs mortality, produced a significant ameliorative effect on heart haemodinamycs parameters and slightly improved echocardiographyc profile. Histological and immunohistochemycal examination demonstrated some cardio-regenerative capacities of ADSCs, showing an increase of vascular and cardiomyocyte markers only in animals treated with ADSCs (Figures 5 and 6).

Conclusions: Implanted ADSCs derived from omentum could improve myocardial function and regeneration through the concomitant capacity to release molecules, restore angiogenesis, reduce inflammation and prevent cardiomyocytes apoptosis. Since adipose tissue is one of the body's richest known sources of regenerative cells, ADSCs could play a critical role in limiting or reversing heart damage caused by a heart attack.

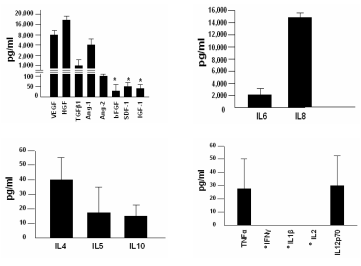


Figure 1. *Release of growth factors and cytokines by ADSCs in culture.* All cell supernatants were collected between 5-7 passages and just before cell detachment for injection. The values in the figure represent the mean ± SD of growth factors and cytokines released by 10^6 cells during the last 72 hrs of culture.

* Indicates that the corresponding factor was detected in around 50% of ADSCs cultures.

° undetectable; below the level of kit sensitivity.

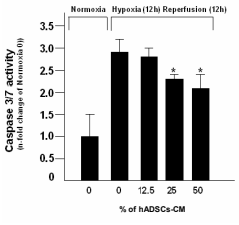


Figure 2. *In vitro differentiation of ADSCs.* Control cells cultured under specific cell lineage culture condition differentiated into cardiomyogenic cell lineages.

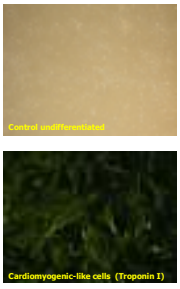


Figure 3. *In vitro differentiation of ADSCs.* Control cells cultured under specific cell lineage culture condition differentiated into cardiomyogenic cell lineages.

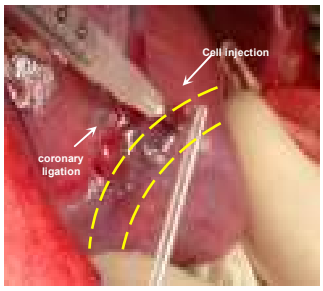
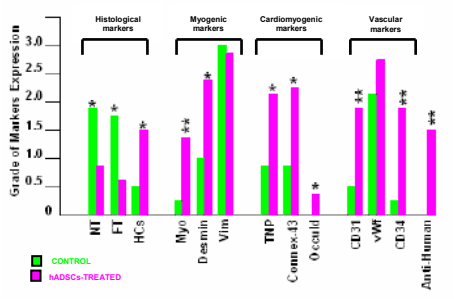
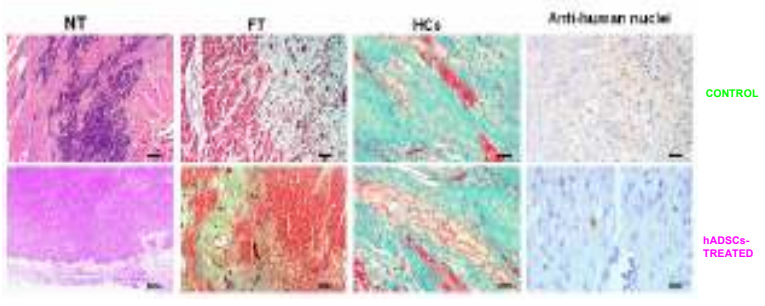


Figure 4. *Intramyocardial injection of ADSCs cells.* Cells between 5 and 7 passages were detached, resuspended in Tissu-coll® solution and administered by 4 to 6 injections all around the border ischaemic zone. 50x10^6 cells/pig were injected 45' after IVA ligation.





Figures 5 and 6. *Hystology and immunohystochemistry of ADSCs and control hearts.* At 2 months, post-infarct animals were sacrificed and hearts were analysed by hystology for the presence of necrotic calcified tissue (NT), fibrotic tissue (FT) and presence of Hypertrophic cardiomyocytes (HCs) by hematossilin-eosin and tricromic Masson staining; the expression of myogenic, cardiomyogenic and vascular markers was investigated by immunohystochemistry. On the ordinate is indicated Grade of markers expression. Note that NT and FT is significantly reduced the presence of HCs as well as myogenic, cardiomyogenic and vascular markers was increased in treated-pigs respect to control-pigs.*p<0.05; **p<0.01 versus controls.