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Morphological and morphometric analyses of Human Amniotic Fluid Stem Cells (AFSC) induced to differentiate into endothelial-like and cardiomyogenic phenotypes

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Preclinical studies performed in cell cultures and animal models have shown the outstanding ability of embryonic and induced pluripotent stem cells to repair ischemic heart by promoting the formation of new blood vessels and new myocytes. Although stem cells *in vivo* administration to patients affected with myocardial ischemia have revealed only modest results, adult cells remain a primary option for clinicians interested in therapeutic cardiovascular repair. Human amniotic fluid stem cells (AFSC) are adult stem cells, recently identified and characterized as pluripotent and non tumorigenic in animal models, that could represent a safer and alternative source of cells to deliver for regenerative purposes(1). The present study was done on AFSC expanded *in vitro* up to the 4th passage in proliferation medium containing DMEM, 20% FBS, 1% P/S and 5 ng/ml basic Fibroblast Growth Factor (FGF2). AFSC were induced to differentiation into cardiomyocytes with 5-aza-2'–deoxycytidine (5-AZA-dC) or to endothelial-like cells through VEGF supplementation. Control or induced AFSC at day 22 of culture were fixed with 70% ethanol, absolute methanol or 2% paraphormaldehide in PBS, stained with conventional or myofibrils-specific staining mixtures (haematoxylin eosin solution, phosphotungstic acid haematoxylin) and observed on a ZEISS Axioskop microscope equipped with a CoolSnap videocamera. Metamorph analysis software was used to acquire computerized images and to perform morphometric analyses. Parallel immunofluorescence analyses were carried out to evaluate the presence of cardiomyogenic markers in differentiated cells. Our results demonstrate that almost the 35% of human AFSC, unselected for c-kit (CD117), display shape and dimensions typical of a cardiomyogenic phenotype and a positive reaction for late markers of differentiation like α actinin or myofibrils, although they do not appear to be functional. This preliminary *in vitro* investigation on total human AFSC confirms the already demonstrated potential of differentiation into cardiomyocytes and endothelial-like cells of c-kit positive human AFSC sub-population(1). Our cell model could represent a cheaper and alternative approach to obtain *in vitro* human differentiated cardiomyocytes to use for regenerative medicine procedures instead of undifferentiated AFSC which spontaneously differentiate into a chondro-osteogenic mass when transplanted into animal models(2).

1. De Coppi P *et al*. Nat Biotechnol 2007; 25(1): 100-6.
2. Dai & Kloner, J Mol Cell Cardiol 2007; 42: 730-732.

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