Adult Stem Cells from Adipose Tissue for Cardiac Repair

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Introduction: Heart failure remains a major public health problem in the western world and is the result of cardiomyocyte apoptosis and fibrous replacement. Any therapy aiming at increase of functional cardiomyocytes is expected to restore normal cardiac function. It is well known that adipose tissue contains not only the mature adipocytes, endothelial cells, smooth muscle cells, and circulating blood cells, but also multi-potent fibroblast-like stem cells. The present study was to develop methods for isolation, culture, identification, and labeling of the ADSCs for cardiac repair.

Methods: Subcutaneous adipose tissue from pigs and rats was rinsed with PBS and then digested with 2 mg/ml collagenase A for 45 min at 37°C. The digested mixture was filtered to eliminate un-digested tissue fragments. The filtered mixture was centrifuged and the cell pellets were collected and suspended in a culture medium. Suspended cells were then seeded in plastic plates at the initial density of 3-4 x 10^4 cells/cm^2 in a DMEM-F12 (1:1) medium. After 24-48 hours, non-adherent cells were removed and the isolated ADSCs reached confluence at approximately 5-6 days with a cell density of 3-5 x10^4 cells/cm^2. The ADSCs were then loaded with ferumoxide (50 μg/ml) for MRI study. The ADSCs were then infected with adenoviruses containing green-fluorescent-protein (GFP) gene for in vivo identifications. The ADSCs (2 x 10^6/ml) labeled with ferumoxide and GFP gene were embedded in a culture medium containing 0.1% agarose to assess the effects of ferumoxide on T_2, T_2*, and T_1 relaxation times of the ADSCs.

Results: With the above method we were able to obtain sufficient ADSCs from the adipose tissue (~10^5 ADSCs/10 g adipose tissue) before cell expansion. In contrast, only ~10^4 mesenchymal cells could be obtained from entire long-bone marrow of an adult rat. Moreover, the ADSCs isolated from the pig and rat exhibited common properties of the adult stem cells (data not presented). It was also found that the ADSCs from both animals could be readily loaded with ferumoxide (Fig. 1). The number of the ADSCs containing iron particles, however, decreased gradually as the cells proliferated (Table 1). Thus, signal reduction of T_2 images caused by ferumoxide was gradually attenuated as increase of cell pass (Fig. 2). Similar trends were found in T_2* and T_1 images of the ADSCs. Only 20% of the ADSCs at the pass 6 (6^{th} generation) were found to contain ferumoxide. Thus, the ADSCs region on T_2 images was not easily distinguishable as they were in earlier passes (Fig. 2). This was due to a gradual recovery of T_2, T_2*, and T_1 relaxation times of the ADSCs (Table 2). Finally, the ADSCs infected with adenovirus-GFP steadily produced the fluorescent protein (Fig. 3). Viability of the ADSCs seemed not affected significantly by iron loading and adenovirus infection (data not presented).

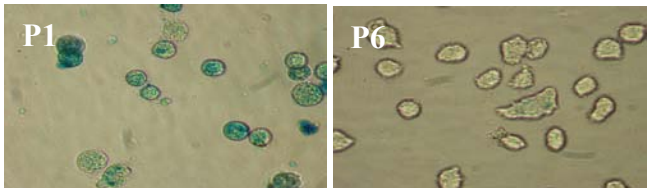

Fig. 1. Pig ADSCs labeled with ferumoxide that was stained green. P1, 1st pass of the ADSCs; P6, 6th pass of the cells. The pass-1 cells obviously contained more iron particles than the pass-6 cells.

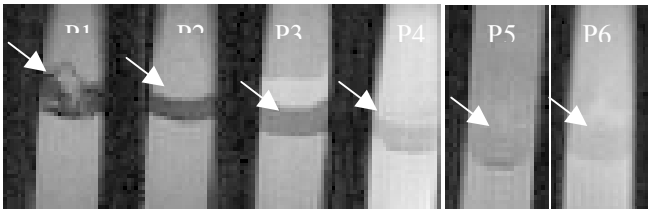

Fig. 2. T_2 images of the adipose-derived stem cells embedded in agarose-containing culture medium. Arrows point cell-embedded region.

Table 1. Relationship between cell pass and percentage of iron-containing ADSCs.

Passage	Cells/FOV	Labeled/Total (%)
P1	112	100
P2	107	100
P3	162	76
P4	140	51
P5	189	39
P6	144	20

Table 2. Relationship between the pass of ADSCs and their T_2, T_2*, and T_1 relaxation times following iron loading.

Cell Pass	1	2	3	4	5	6
T_2 (ms)	88	129	234	286	381	386
T_2^* (ms)	5.6	6.2	8.8	37		
T_1 (ms)	1051	1399	1855	1991	2175	

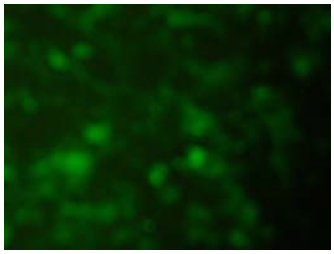

Fig. 3. Pig adipose stem cells labeled with GFP-containing adenoviruses. It is clear that most of the ADSCs displayed GFP.

Discussion and Conclusions: This study demonstrated that ADSCs could be obtained with a sufficient number. Therefore, in vitro cell expansion may not be necessary for cardiac repair. The ADSCs could be readily labeled with ferumoxide. Success of cell delivery and migration can be reliably monitored with MR imaging. However, the ADSCs could be observed with MRI for approximately two weeks only. Therapeutic benefits of the ADSCs on ischemic heart disease are currently under investigation in our laboratory.