Human Fat Tissue-Derived Stem Cells Show Cardiomyocytic Differentiation After Coculture

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Introduction: Cells isolated from subcutaneous fat tissue (SFT) can give rise to cells from all three germ layers. So far differentiation into a cardiomyocyte (CM) lineage by means of co-culturing has not been shown for cells isolated from human SFT. The acces-

sibility and obtainable cell numbers would make SFT an excellent source of cells for tissue repair after myocardial infarction. Methods: CM from neonatal rat hearts were isolated on day 8 of age, digested with Collagenase (100mg/ml) and cultured under standard conditions. SFT from patients undergoing liposuction was collected and digested with Collagenase for 90 minutes to obtain a single cell suspension. Cells were cultured under standard conditions and transfected with a lentivector encoding eGFP. SFT cells were then co-cultured with the neonatal rat CM in a 1:1 ratio. FACS analysis of adherent SFT cells was done at day 1 and 5 prior to coculture for CD34, CD45, CD105, and CD117. Immunohistochemistry of cardiac specific Titin, Troponin T and eGFP of co-cultured cells was done after 14 days or total RNA was harvested and an RT-PCR was performed. Results: More than 1.3x10^6 cells/cm^2 could be isolated routinely from SFT and 30% did adhere to tissue culture plastic. A FACS analysis of the adhering SFT cells showed an expression of CD 34 (48% +/- 8) and CD45 (6% +/- 2) after 1 day in culture which dropped to undetectable levels after 5 days. CD105 expression was 50% (+/-8, day 1) and increased to 97% (+/-2, day 5). CD117 could not be detected at any time. The co-
cultures were dispersed after 14 days and re-plated. Immunohistocompatibility staining showed dual positivity of SFT for eGFP and Titin but not for Troponin T. This was confirmed in the RT-PCR reaction with human specific Primers for Titin that do not cross react with the rat CM. The RNA expression level in the CM and SFT coculture was 11 fold above control (corrected for GAPDH). Conclusion: SFT cells show the predominantly osteogenic and skeletal lineage. The expression of the stem/progenitor cell marker CD34 on human ASCs was assessed on days 2, 4 and 8 by flow cytometry. The expression of CD34 was assessed on days 2, 4, 6 and 8 by flow cytometry. The expression of CD34 was assessed on days 2, 4, 6 and 8 by flow cytometry. The expression of CD34 was assessed on days 2, 4, 6 and 8 by flow cytometry.

Akinetic Segments of Myocardial Infarction Contain Contracting Myocytes: A Finite Element Model Study

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Background:The extent that the jeopardy of dysfunction are not fully understood. Using a finite element model, we sought to better define the relative contributions of pas-
sive material properties (stiffness) versus active properties (contracting myocytes) in determining regional wall motion. We tested the hypothesis that in order for a segment to be aki-
netic and not dyskinetic, it must contain contracting myocytes. Methods: Using two-
dimensional echocardiographic images from a perfused ovine anterolateral infarct, we developed a three-dimensional finite element mesh of the left ventricle. The model describes both diastolic material properties defined by a constant, C, and systolic myo-
cardial material properties defined by isometric tension, T_{iso}. A reduction in the ability of the infarcted region to develop active stress was accomplished by scaling the parameter T_{iso} to represent the contribution of contracting myocytes between 0% and 100%. The simulated change in wall thickness between end-diastole and end-systole was measured using the fractional change of average radial strain (RS). Akihness was defined as an average RS between 30% and 0, where a more positive or negative value repre-
sents hypokinesis or dyskinesis, respectively. The diastolic and systolic properties neces-

sary to produce akinhness were determined using an iterative process. We then modeled an infarct with no contracting myocytes and increased the diastolic stiffness required for akihness until RS fell below -0.01 and -0.01 increased from 20-50%. When the percentage of contracting myocytes was assumed to be zero, a stiffness of C=250 kPa was neces-
sary to achieve akinhness. In this finite element model of left ventricular myo-
cardial infarction, as wall stiffness is less than 250 times normal, the presence of

Cultured Adipose Tissue-Derived Stromal Cells Express the Stem Cell Marker CD34 and Show Evidence of Differentiation Into a Cardiomyocyte Phenotype

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Background: Autologous cell therapy using pluripotent cells for cardiac repair and regeneration in patients with cardiac dysfunction is limited by the fact that the required cell numbers often exceed the number of available pluripotent cells. We have recently shown that a substantial proportion of non-adipocyte stromal cells in adipose tissue express the stem/progenitor cell marker CD34. We therefore examined whether adipose stem cells (ASCs) could retain this marker in culture and whether they could differentiate into a card-

iomyocyte phenotype in vitro.

Methods: Subcutaneous adipose tissue biopsies or liposates were obtained from vol-
unteer. The stromal fraction cells were cultured in EBM-2 or EGM-2 medium (Clonetics). Cardiogenic expression of CD34 was assessed on days 2, 4, 6 and 8 by flow cyto-
metry. To assess differentiation of ASCs, they were labeled with the red fluorescent dye Dii (Molecular Probes) and added to culture wells containing neonatal rat cardiomyocytes (CMCs). The co-culture was observed for four days to identify spontaneously beating cells. At the end of the co-culture period, cells were fixed and stained for cardiac-specific alpha-actinin and the nuclear stain DAPI.

Results: The expression of the stem/progenitor cell marker CD34 on human ASCs was present at consistently high levels during the first week of culture (Day 2: 95%, Day 4: 91%, Day 6: 89%, Day 8: 77%). Multiple ASCs showed evidence of spontaneous beating.