±4% vs 31 ±5%), 18/22 (82%) had not an EF increase >5 % (p<0.0001 vs Group A), NYHA class (from 3.5 ±0.5 to 3.2 ±0.8) and exhibited ongoing remodelling (LVEDVI increased from 106 ±12 ml/m^2 to 111 ±8 ml/m^2, p=0.04)
The number of scarred segments was directly related (R^2 0.52, p<0.0001) while that of ischemic segments was inversely related to the change in EF (R^2 0.46, p<0.0001).
The degree of interventricular dyssynchrony (difference in LV and right ventricular (RV) mean phase angles) did not change from baseline to the final visit in Group B (11 ±17 vs 12 ±9), while it improved in Group A (13 ±17 vs 9 ±14; p<0.01). The degree of intraventricular dyssynchrony (standard deviation of the mean phase angle) significantly improved for LV in Group A (54 ±18 vs 51 ±19; p=0.04) but did not change in the LV (57 ±14 vs 56 ±19) in Group B.
Conclusion:
Treatment with BiV in patients with ischemic cardiomyopathy and a limited extension of scarred myocardium improves contractile synchrony, both inter- and LV intraventricular, EF, quality of life and determines reverse remodelling of the LV

1068-122 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 accessibility 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 1 after birth, digested with Collagenase (10mg/ml) and cultured for 1 day 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 plated under standard conditions and transfected with a lentivirus 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 co-culture 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/gram 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% +/- 9) 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. Immunohistochemistry 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 endothelial cell marker CD105 after 5 days in culture and are void of CD34,45 or CD117. Direct cell-cell contact through co-culture between SFT cells and rat CM leads to an early cardiomyocytic phenotype that expresses the sarcomeric protein Titin as shown by immunohistochemistry and RT-PCR.

1068-123 Akinetic Segments of Myocardial Infarction Contain Contracting Myocytes: A Finite Element Model Study

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Background:Infarcted segments of myocardium demonstrate a range of functional impairment. The properties that determine the severity of dysfunction are not fully understood. Using a finite element model, we sought to better define the relative contributions of passive 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 akinetic and not dyskinetic, it must contain contracting myocytes. Methods: Using two-dimensional echocardiographic images from a reperfused ovine anteroapical 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 myocardial material properties defined by isometric tension, T_{max}. A reduction in the ability of the infarcted region to develop active stress was accomplished by scaling the parameter T_{max} to represent the percentage 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). Akinesis was defined as an average RS between -0.01 and +0.01, where a more positive or negative value represents hypokinesis or dyskinesis, respectively. The diastolic and systolic properties necessary to produce akinesis were determined using an iterative process.We then modeled an infarct with no contracting myocytes and increased the diastolic stiffness required for akinesis until RS fell between -0.01 and +0.01. Results: As stiffness, *C*, was increased from normal (0.876 kPa) to ten times normal (10 kPa), the percentage of T_{max} necessary to achieve RS between -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 necessary to achieve akinesis. Conclusion: In this finite element model of left ventricular myocardial infarction, if wall stiffness is less than 250 times normal, the presence of contracting myocytes in the infarct zone is necessary to prevent dyskinetic wall motion.

1068-124 Nonviral FasL Protects Allogeneic Myoblasts Against Cell Death in Cardiomyogenesis

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Background: FasL, which promotes T-cell apoptosis through interaction with the death receptor Fas, has been proposed to immunoprotect allotransplanted cells. However, success with this strategy has been limited in part due to inflammatory side effects of viral vectors. We hypothesize that overexpression of FasL in myoblasts via a non-viral vector will provide protection of allotransplanted cells in heart.
Methods: C_2C_{12} myoblasts were stably transfected with FasL and co-cultured with activated T-cells to determine FasL activity. T-cell apoptosis was examined by annexin V expression. In vivo, control animals received untransfected DAPI-labelled myoblasts (2x10^6), while treated animals (FasL group) received transfected labeled myoblasts where 25% expressed FasL. Myoblasts were injected into cryoinjured myocardium, the kidney capsule or subcutaneously (n=12 each). We evaluated DAPI-positive cell survival and engraftment by histology at 1 hour and 3, 10 or 14 days post-injection.
Results: FasL transfected myoblasts induced a fourfold higher apoptosis rate in T-cells than untreated myoblasts in vitro (23.0±2.51% vs 7.05±3.93%, P<0.001). In vivo, FasL protected allogenic cells after injection into cryoinjured myocardium up to 14 days (21±8.1/HPF vs 8.9±9.1 control, P<0.05). Concurrently, the maximum infarct diameter was reduced (0.25±0.02 vs 0.29±0.03 mm; P<0.05) and infarct wall thickness was increased (0.11±0.03 vs 0.07±0.03 mm; P<0.05). Similarly, FasL cells protected allogenic cells at 1h, 3d and 10d after subcutaneous injection as compared to controls (251±6.0, 232±7.1 and 231±6.8 /HPF for FasL vs. 49±2.6, 0, 0 /HPF for controls, P<0.001). Surprisingly, FasL did not protected allogenic cells after injection under the kidney capsule.
Conclusion: Functional FasL, delivered non-virally, can more than double survival of allogenic cells after transplantation into injured myocardium or subcutaneously. FasL might be a useful tool in allogenic cell therapy.

1068-125 Cyclic Stretch of Adult Human Mesenchymal Stem Cells Induces Expression of Early Cardiac and Neuronal Genes

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Background: Human mesenchymal stem cells have shown to be plastic and amenable to transformation into cardiomyocytes. Myocardial regeneration using direct injection of stem cells into infarcted myocardium results in low yield of differentiated cardiomyocytes. Alternately, stem cells have been preprogrammed using chemicals, growth factors, and co-culture of cells into cardiomyocytes in vitro. We explored the role of cyclic stretch on human mesenchymal stem cells (hMSCs) as a stimulus to promote transdifferentiation.
Methods: hMSC were cultured in 6 well plates and subjected to programmable square cyclic stretch at 1 Hz using the Flexercell system with 4% and 8% elongation of cells alternating every 12 hours for 7 days in a humidified incubator with 5% CO_2 at 37^{o}C. Total RNA was isolated from the stretched hMSC and unstretched control experiments using microarray (Hu133A gene chips, Affymetrix Inc) and lineage specific gene expression was studied. Genes were profiled in triplicate for cardiogenic, adipogenic, neurogenic, osteogenic and skeletal lineage.
Results: The data was normalized and lineage specific genes were analyzed using Gene Spring V.5.0. Induction of synaptobrevin, macrotubule associated protein (MAP-2), and Galactocerebroside genes indicating early neurogenic lineage was observed, in addition there was also concurrent expression of Connexin-43 and BMP-2 indicative of early cardiogenic lineage. In contrast, there was no expression of adipogenic, osteogenic and skeletal genes.
Conclusions: These data suggest that adult human bone marrow stem cells are plastic and mechanical stretch bioengineers hMSC transdifferentiation to early cardiogenic and neurogenic lineage. This technique of preprogramming stem cells in vitro could potentially be used to increase high yield of lineage specific cells before cellular cardiomyoplasty.

1068-126 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 cardiomyopathy 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 stromal cells (ASCs) would retain this marker in culture and whether they could differentiate into a cardiomyocyte phenotype in vitro.
Methods: Subcutaneous adipose tissue biopsies or lipospirates were obtained from volunteers. The stromal fraction cells were cultured in EBM-2 or EGM-2 medium (Clonetics-Cambrex). The expression of CD34 was assessed on days 2, 4, 6 and 8 by flow cytometry. 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