Isolation and Endothelial Differentiation of Adipose-Derived Stem Cells from Diabetic Patients

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INTRODUCTION: Diabetes is a significant risk factor for coronary and peripheral arterial disease, as well as end stage renal disease. Surgical bypass and dialysis access creation are common procedures performed in the diabetic population. Unfortunately only 45% of diabetic patients have autologous vascular tissue available for use as a conduit for these procedures, and alternative conduits demonstrate decreased patency and increased infection rates. Our laboratory has developed an alternative conduit composed of a vascular scaffold seeded with autologous adipose-derived stem cells (ASC) differentiated into endothelial cells to create a non-thrombogenic graft lumen. Herein, we test the hypotheses that ASC from diabetic patients (DM) are similar to those from non-diabetics (NDM) in terms of isolation efficiency, proliferation, commitment towards endothelial lineage, and seeding onto a vascular scaffold.

METHODS: ASC were isolated from adipose tissue harvested from patients undergoing elective vascular procedures via peri-umbilical liposuction (DM, n=53; NDM, n=145) by collagenase dispersion. Isolation efficiency was defined by the number of cells isolated within the stromal vascular pellet normalized to gram of adipose tissue. Proliferation was assessed by constructing growth curves over 14 days. ASC were differentiated in endothelial growth medium (EGM2) up to 3 weeks. Endothelial differentiation was determined by measuring endothelial cell-specific gene expression (CD31, vWF) using quantitative PCR. Cord formation by ASC seeded on Matrigel was assessed and quantitated by Wimasis image analysis software. Lastly, ASC were seeded onto decellularized saphenous vein scaffolds and flow conditioned from 0 to 9 dynes over 5 days. Confocal microscopy was used to evaluate retention of cells onto the scaffold at physiological levels of shear stress.

RESULTS: Isolation efficiency of stem cells did not differ significantly between DM and NDM patients (224,028+/-147284 cells/gm vs. 259,345+/-185,290 cells/gm, respectively; P=0.21). The growth curves for DM (n=4) and NDM (n=5) ASC also appeared similar, with no significant differences observed in cells counts between days 1-14. Both cell populations achieved maximum number by day 10, at which point the curves leveled suggesting contact inhibition. After 3 weeks in culture, no significant difference in CD31 or vWF expression between DM (n=5) and NDM (n=5) cells was observed. Preliminary data also show that ASC from DM form capillary-like cords when seeded on Matrigel, as previously demonstrated for ASC from NDM. Finally, retention of ASC to vascular scaffolds under physiological shear stress was similar between the two groups.

CONCLUSIONS: This study demonstrates that ASC are isolated from diabetic patients in quantities similar to those observed in non-diabetic patients. These cells grow at rates similar to non-diabetic ASC, exhibit differentiation into an endothelial phenotype, and are capable of lining the luminal side of a tissue engineered vascular graft. These results suggest that adipose tissue may prove to be a more reliable source of autologous stem cells than blood or bone marrow, where the presence of diabetes and other co-morbidities significantly decrease the availability of stem cells for use in regenerative therapies.