Stem Cell Reactivity to Cardiovascular-Specific Differentiation Cues is Altered in Diabetic Conditions

Zhang, H.S., Maivelett, J., Stowers R., Mary E. Tedder, Simionescu, D., Simionescu, A.
Biocompatibility and Tissue Regeneration Laboratory, Department of Bioengineering, Clemson University, Clemson, SC

Statement of Purpose: Tissue engineered constructs based on scaffolds and autologous stem cells are currently being developed for treatment of cardiovascular diseases, but very little information exists regarding the fate of tissue engineered devices in the compromised patient, and more specifically in diabetic environments. Diabetes is characterized by elevated levels of blood glucose and formation of advanced glycosylation end products (AGE) which induce endothelial dysfunction, accelerated atherosclerosis, activation of inflammation, fibrosis and impaired healing, which are not conducive to the desired integration and remodeling of tissue engineered constructs. Moreover, the outcome of reparative surgery and organ transplantation is very complex in diabetic patients. Our **hypothesis** states that high glucose environment alters stem cell differentiation abilities and that diabetes-induced complications could affect the outcome of current tissue regeneration efforts.

Methods: Rat adipose-derived stem cells (AdSCs) were obtained by lipectomy and isolated by collagenase digestion from diabetic rats (STZ-treated rats, glycemia of between 450-550 mg/dl) and normal age matched controls. Diabetic cells were maintained and propagated in diabetic culture medium (DMEM, 10% FBS, 550 mg/dl glucose) while normal AdSCs were maintained at physiologic glucose levels (100 mg/dl).

Study 1: To test effect of high glucose on stem cells normal AdSCs were cultured in DMEM containing normal (100 mg/dl), high (550 mg/dl) and extremely high (900 mg/dl) glucose levels. After 4 days, cells were stained by immunofluorescence (IF) for Prolyl-Hydroxylase and α-smooth muscle cell actin.

Study 2: To test effect of biochemical cues on differentiation, cells were cultured for 2 weeks in static conditions in media formulated to induce stem cell differentiation into endothelial cells (ECs) and separately into myofibroblasts/valvular interstitial cells (VICs). To evaluate differentiation, IF staining for CD31, eNOS, alpha-smooth muscle cell actin, and N-(carboxymethyl)lysine (CML, early marker of protein glycoxidation) was performed.

Study 3: To test effect of dynamic mechanical cues, normal and diabetic stem cells were seeded onto collagen-coated membranes and subjected to equibiaxial 14% stretch loads at 1 Hz for 3-4 days in a Flexercell system and properties compared to static controls. In addition to IF staining for actin, vimentin, CML and collagen type III, gene expression was assessed by RT-PCR and matrix metalloproteinase (MMP) activity measured by gelatin zymography followed by densitometry.

Results and Discussions: Cell morphology, distribution of collagen synthesis enzymes and of smooth muscle cell markers changed as a response to increasing glucose levels indicating that exposure to abnormal glucose levels may influence stem cell reactivity and differentiation. When exposed to differentiation media, diabetic AdSCs expressed less CD31 and eNOS as compared to normal stem cells, suggesting that endothelial regeneration may be challenging in diabetic subjects. Conversely, diabetes stimulated formation of actin-positive cells similar to activated VICs, indicating that valvular tissue engineering may be feasible in diabetes. Diabetic AdSCs expressed very high levels of (CML)-positive proteins indicating that cells maintained high levels of protein glycosylation and AGE products during differentiation (**Figure 1**).

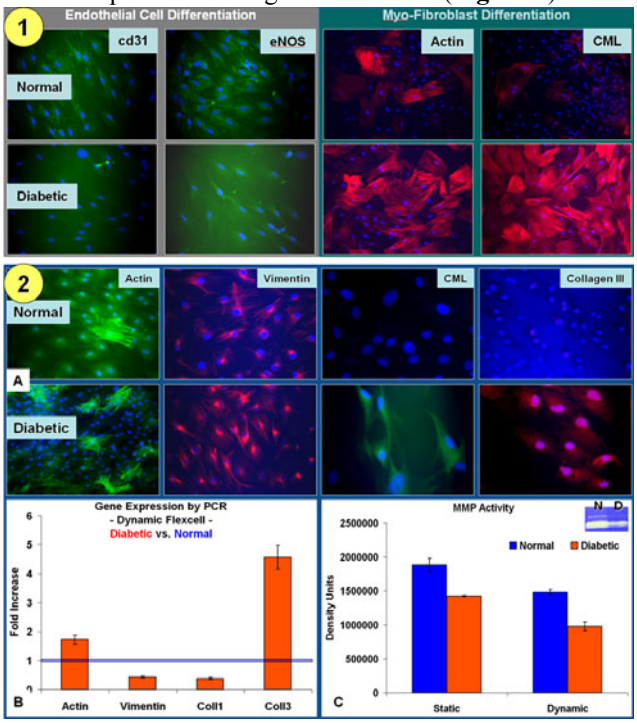

Figure 1. In vitro differentiation of AdSCs in normal and diabetic conditions into endothelial cells. Immunofluorescence for CD31 and eNOS (green) and actin and CML (red); DAPI stain (blue).

Figure 2. Effect of dynamic loads on diabetic and normal stem cells. Immunofluorescence stained for actin (green), vimentin (red), CML (green) and collagen type III (red). B) Gene expression by PCR in diabetic cells vs normal cells. C) MMP activity by zymography.

Study 3 showed that as a response to loads that mimic those present in heart valve cusps, diabetic AdSCs up-regulated actin and collagen III synthesis but not vimentin when compared to normal cells (**Figure 2**). IF data was confirmed by PCR. Diabetic cells expressed lower levels of MMP suggesting lower capacity for matrix remodeling.

Conclusions: Biochemical and mechanical cues successfully induced differentiation of normal AdSCs into EC and VIC-like cells capable of matrix turnover. Diabetic stem cells displayed altered in vitro reactivity to biochemical and mechanical stimuli, reduced matrix remodeling activity and thus may exhibit different propensity to differentiate into cardiovascular cells for use in tissue regeneration studies.

Acknowledgements: Funding for ZHS was provided by NSF grant EEC 0609035.