Collagen and Elastin Binding Polyphenols Protect Scaffolds and Stem Cells from Diabetes-Related Complications

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Statement of Purpose: The alarming prevalence of diabetes mellitus (DM) is of great concern due to its dual threat as a risk factor for cardiovascular disease (CVD). Elevated levels of inflammation and wound healing are hallmarks of DM contributing to cardiomyopathy, atherosclerosis, and valve disease. Though cardiovascular tissue engineered replacements are emerging as a revolutionary treatment option, there is still little insight as to how these constructs perform in patients with comorbidities (i.e. DM). The aims of this study are to identify the adverse effects of diabetic environments on both biologic scaffolds and stem cell differentiation; furthermore, we plan to mitigate these diabetic-related alterations using a non-toxic matrix-binding polyphenolic antioxidant, pentagalloyl glucose (PGG).

Methods: Porcine aortic valve leaflets and carotid arteries were decellularized and purged of cellular epitopes to achieve predominantly collagen scaffolds (yielded from leaflets) and elastin scaffolds (yielded from arteries). Half of these scaffolds were PGG-treated while the other half remained non-treated to serve as controls. Scaffolds were implanted subdermally in normal and streptozotocin-induced diabetic (STZ) adult male Sprague-Dawley rats and evaluated after 4 weeks by histological analysis, mechanical tests, and various assays for inflammation and remodeling.

To study the effects of diabetic environments on stem cells, human adipose stem cells (hASCs) were isolated from both healthy volunteers and diabetic patients from Greenville Hospital System using the Zuk procedure (Zuk PA. Tissue Eng. 2001;7:2110228). These cells were cultured 4 weeks in either control media (100mg glucose/dL) or diabetic media (250mg glucose/dL). Viability was analyzed via Live/Dead®. Cellular AGE products and oxidative stress were analyzed by immunofluorescence and glutathione detection assay, respectively. We also tested the effects of diabetes on human stem cell differentiation into endothelial cells and separately into smooth muscle cells using inductive factors (VEGF/IGF and heparin/TGF-β respectively. Differentiation markers were analyzed by immunofluorescence and RT-PCR.

Results*:* Histological evaluation confirmed complete cellular removal and good scaffold matrix preservation prior to implantation. Biaxial tensile tests revealed that scaffolds subjected to in vivo diabetic environments exhibited a significant increase in stiffness compared to the controls, suggestive of matrix crosslinking. This was further confirmed by differential scanning calorimetry (increase in Td). Using the same tests we noticed that PGG-treated scaffolds mitigate pathological stiffening and do not significantly alter physiological ranges of mechanical function. Immunohistochemistry for carboxymethyl lysine revealed accumulation of glycoxidation products, and pentosidine analysis by fluorescence revealed that non-treated scaffolds are

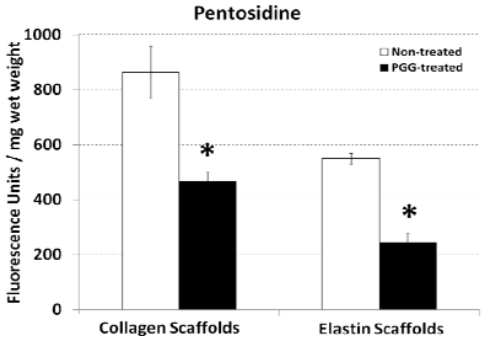

Figure 1: PGG-treatment lowers pentosidine content in both collagen and elastin scaffolds

susceptible to glycoxidation upon implantation in diabetic rats. Advanced glycation end products were significantly decreased in PGG-treated scaffolds implanted in diabetic rats. PGG also appeared to discourage inflammatory cell infiltration while allowing fibroblast proliferation. Inflammatory cytokine arrays confirm elevated inflammation in diabetes and pro-inflammatory attenuation in PGG-treated scaffolds. MMP activity was lowered in non-PGG treated scaffolds. PGG-treatment also inhibited *in vivo* calcification of elastin scaffolds.

The diabetic hASC cultures revealed greater amounts of general AGE products as well as increased oxidative stress. Diabetes did not appear to influence the ability of ASCs to differentiate into cardiovascular cell lineages such as endothelial cells and smooth muscle cells.

Conclusions: We have demonstrated adverse effects of diabetes on both collagen and elastin scaffolds including accumulation of glycoxidation products, scaffold stiffening, and pro-inflammation. Furthermore, we exhibit the remarkable ability of PGG to attenuate these diabetic-related complications while simultaneously allowing for cell infiltration and construct remodeling (Chow JP. Biomaterials. 2013;34:685-695). We also see similar hyperglycemic complications in static hASC cell culture; however, we are still able to encourage differentiation into target cardiovascular cells. The differentiation of diabetic cells is essential for an authentic diabetic patient-tailored tissue engineered approach. Differentiated diabetic stem cells combined with a diabetic-resistant scaffold would hold much potential for future tissue engineered treatment options, especially for high-CVD risk factor patients with DM.

References:

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