Integration of Cysteine-rich angiogenic inducer 61 (CYR61) into collagen biomaterial promotes the therapeutic potential of circulating angiogenic cells

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Statement of Purpose:
Myocardial infarction (MI) is a leading cause of death in the world. Cell therapies are a promising approach to treat MI by promoting revascularization and regeneration. For revascularization, circulating angiogenic cells (CACs) are a good candidate cell source as they directly contribute to the generation of new blood vessels and secrete proangiogenic cytokines. Animal models[1] and clinical trials[2] have highlighted the potential for these cells to treat MI; however the benefits associated with this type of therapy remain modest due to low cellular retention and engraftment. To overcome this hurdle, a collagen-based biomaterial has been developed to deliver and promote the therapeutic potential of the CACs;[3] however improvements are still needed. Therefore, this study aimed to modify our collagen-based biomaterial to improve the function and therapeutic efficacy of CACs.

Methods:
Matrix preparation: Collagen I and chondroitin sulfate-C were blended on ice, cross-linked by glutaraldehyde and quenched using glycine. CYR61 was immobilized to the biomaterial using EDC/NHS crosslinking. Cell Isolation: Mononuclear cells from human peripheral blood were isolated using Histopaque 1077 density centrifugation, and CACs were enriched during a 4-day fibronectin culture. CACs were lifted and re-plated on fibronectin or on a collagen type I based biomaterial. RT-qPCR: mRNA expression of 18 α- and 8 β- integrins were analyzed from the highly potent pro-angiogenic $CD34^+$ subpopulation of CACs purified by fluorescence-activated cell sorting. Functional assays: CACs treated with and without CYR61 were assayed for adhesion, migration, proliferation and angiogenic potential. Hindlimb ischemia model: The left proximal femoral artery was ligated under 3% isoflurane. Ligation and subsequent recovery was assessed using laser Doppler perfusion imaging.

Results:
mRNA expression of integrins α5, α7, αM, αV and β3 were significantly up-regulated by 56±5.5, 60±6.4, 15±4.2, 55±4 and 67±7.5 fold, respectively, in $CD34^+$ cells cultured on collagen vs. fibronectin while integrin α3 and β7 were down-regulated by 30±4.5 and 58±6.8 fold, respectively (all $p<0.05$). Since αV, β3 and αM interact with CYR61, the functional response of collagen cultured CACs to CYR61 was examined. Adhesion of CACs to collagen matrix containing CYR61 was increased by 2.2±1.0 fold ($p=0.03$) over matrix lacking CYR61 and 4.8±2.4 fold ($p=0.02$) over fibronectin-cultured cells. Using CYR61 as a chemoattractant, CAC migration was increased 5.0±2.1 fold ($p=0.04$) compared to serum free control media. CACs pretreated with CYR61 for 1h prior to an angiogenesis assay increased the incorporation of CACs into tube-like structures by 4.1±1.6 fold ($p=0.03$) over CACs from collagen and 7.3±1.4 fold ($p=0.02$) over CACs from fibronectin. *In vivo*, CACs pre-treated with CYR61 resulted in a greater perfusion recovery in a hindlimb ischemia mouse model over both PBS ($p=0.0005$) and collagen-cultured CAC ($p=0.03$) injections (Fig. 1).

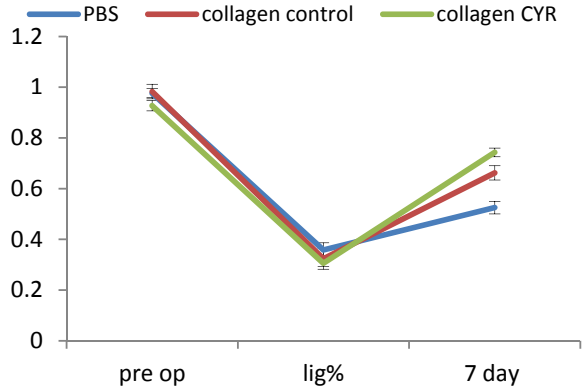

Fig. 1. Blood flow (ischemic/non-ischemic ratio)

Conclusions:
We demonstrate that the expression of integrins is significantly altered when culturing CACs on a collagen matrix. The discovery of which specific integrins are expressed under these conditions helped identify CYR61 as a potential protein to improve the matrix. CYR61 added to the matrix enhanced CAC migration and adhesion, and promoted vascularization and perfusion of ischemic tissue. These findings demonstrate a novel mechanism which may be used to better restore perfusion and function of ischemic tissue in cell-matrix therapy.

References:
1. Doyle, B., *et al.* Progenitor cell therapy in a porcine acute myocardial infarction model induces cardiac hypertrophy, mediated by paracrine secretion of cardiotrophic factors including TGFbeta1. *Stem Cells Dev* 2008;**17**, 941-951.
2. Beeres, S.L., *et al.* Intramyocardial injection of autologous bone marrow mononuclear cells in patients with chronic myocardial infarction and severe left ventricular dysfunction. *Am J Cardiol* 2007;**100**, 1094-1098.
3. Kuraitis, D., *et al.* Ex vivo generation of a highly potent population of circulating angiogenic cells using a collagen matrix. *J Mol Cell Cardiol* 2011;**51**:187-197.