Controlled Release of Stromal Cell Derived Factor-1 to Enhance Progenitor Cell Recruitment

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Statement of Purpose: Stromal cell derived factor-1 (SDF-1) is a chemokine that plays a role in the mobilization, trafficking, and homing of stem/progenitor cells by interacting through the exclusive membrane receptor CXCR4. Circulating progenitor cells (CPCs) are commonly positive for CXCR4, as well as the markers flk and c-kit. Following ischemic injury, SDF-1 is transiently over-expressed in the myocardium to provide a gradient with which progenitor cells can localize to the origin of SDF-1 production and release. This overexpression occurs for an acute period, indicating a limited time during which CPCs are recruited after insult. This study aimed to develop a biomaterial system for the controlled and prolonged release of SDF-1in order to enhance the mobilization and homing of CPCs to sites of interest, such as ischemic muscle.

Methods: SDF-1 containing microspheres were generated by forcing 5mL of 1.5% (w/v) sodium alginate containing 1µg murine SDF-1 through a Nisco J1 coaxial bead generator at 1mL/min with a nitrogen stream at 9.48L/min. Microspheres were collected and cross-linked for 20 minutes in 2% $CaCl_2$. CPCs were obtained by differential centrifugation in histopaque of freshly-isolated peripheral blood from healthy human donors and cultured for 4 days in endothelial basal medium on fibronectin-coated plates. Adherent cells were lifted and suspended in endothelial basal media (EBM) in the upper compartment of a modified Boyden chamber, and human SDF-1 containing microspheres were diluted to total SDF-1 concentrations of of 0, 10, and 25ng/mL and placed in the lower chamber. Migrated cells were collected after 24 hours and counted. Biomatrices consisting of 0.53% rat tail type I collagen and 2.1% chondroitin sulfate-c were created, and microspheres were incorporated during polymer cross-linking with N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide(EDC)/N-hydroxysuccinimide(NHS). Suspensions of microspheres in dPBS, with embedding within matrix and without, were prepared and suspension aliquots were taken over a 12-day period and analyzed for SDF-1 using an ELISA test kit. For evaluation *in vivo*, a bone marrow transplant mouse model was used with green fluorescent protein (GFP)+ donor cells. After 6 weeks of marrow reconstitution, hindlimb ischemia was induced by femoral artery ligation and animals received an intramuscular injection of SDF-1-microsphere-containing matrix or controls. On days 0, 1, 4, 7, and 14, circulating mononuclear cells were isolated and analyzed by flow cytometry for the cell surface markers c-kit, flk, and CXCR4.

Results: Microsphere populations were generated with a diameter of 39.8 +/- 11.4µm. Microspheres containing total SDF-1 concentrations of 10 and 25 ng/mL induced greater CPC migration (45.3+/-2.8% and 70.2+/-9.6%; respectively) than controls of 50ng/mL VEGF or PBS (28.4+/-6.0% and 24.2+/-5.3%; respectively; $p<.05$). The maximum concentration of SDF-1 released from microsphere impregnated collagen matrix was observed after 10.5 days, and 6 days from microspheres in media without matrix impregnation.

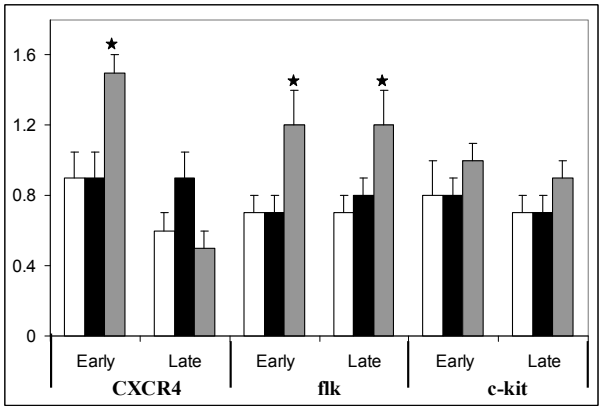

Figure 1. Relative changes, compared to pre-operative levels, in proportions of circulating mononuclear cells positive for CXCR4, flk, and c-kit early (1-4days) and late (7-14 days) stages post-op for PBS (white), sham (black), and SDF-1-releasing matrix (grey) treatments. Compared to controls, animals receiving SDF-1 treatment had greater flk expression at all stages post-treatment (by ≥47%;*$p<.05$), greater CXCR4 expression at the early stages (≥54%;*$p<.05$).

Conclusions: A dose-response relationship exists between CPCs and the chemotactic cytokine SDF-1. Release of SDF-1 from alginate microspheres can be prolonged up to 10.5 days by incorporation into collagen-based biomatrices, and treatment with SDF-1 microspheres impregnated in biomatrices can increase the levels of circulating progenitor cells in the blood. This system of SDF-1 control and release may be used to generate a gradient of released cytokines within the circulation for enhanced homing of stem/progenitor cells to the target tissue.