Delivery of Platelet-derived Growth Factor as a Chemotactic Factor for Mesenchymal Stem Cells

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Statement of Purpose: Bone marrow-derived multipotent mesenchymal stem cells (MSCs) have shown great potential in cell-based therapies for the regeneration of mesenchymal tissues, including bone. Despite their great potential a significant barrier to the effective implementation or recruitment of MSCs for bone regeneration is the inability to target these cells with high efficiency [1]. Few studies have provided insights to mechanisms of MSC mobilization. However, there is increasing body of evidence to show that both human and animal MSCs, like immune cells, exhibit chemotaxic response to selected chemokines and growth factors. It appears that among them, platelet-derived growth factor (PDGF) may be a potent chemotactic factor for MSCs. Our laboratory has developed a bone-like three-dimensional electrospun fibrous scaffold composed of polycaprolactone (PCL), collagen I (Col) and hydroxyapatite (HA). These scaffolds provide functional matrices for MSC adhesion, and also serve as growth factor/chemokine delivery vehicles. In this study we have investigated the potential of these fibrous scaffolds as a tool for delivery of PDGF as a chemotactic factor for GFP-expressing MSCs.

Methods: Fibrous triphasic scaffolds were electrospun from a mixture of PCL, type I Col, and HA nanoparticles with dry weight ratio of 50:30:20 in hexafluoroisopropanol. Purified PDGF-BB was passively absorbed to PCL/Col/HA or PCL scaffolds in PBS at 4°C. A specific ELISA was used to determine PDGF-BB release at 37°C over an 8-week period. Chemotaxis was assayed in Boyden chamber units with transwell inserts. Transmigration of GFP-MSCs was visualized by a stereomicroscope. A modified 2-D cell migration was developed; In 8-well rectangular culture plates PDGF-coated scaffolds was placed in tissue culture inserts 1.5 cm horizontally away from the edge of the GFP-MSC monolayers. After 72 h co-culture cell migration was examined and imaged microscopically.

Results: As a first step toward identifying chemotactic factors for MSCs, PDGF, BMP2 or a chemokine mixture containing SDF-1α, CXCL16, MIP-1α, MIP-1β and RANTES was tested in Boyden chamber assays to compare the chemoattractive ability of these molecules for GFP-expressing human MSCs. Our results clearly demonstrated that PDGF-BB is the most potent chemotactic factor for MSCs as compared to BMP2 and the group of chemokines (not shown). We then evaluated the capacity of PCL/Col/HA scaffolds to adsorb and release PDGF-BB. We found that significantly more PDGF-BB was adsorbed (91% vs. 55% of 1.5 μg) to, and subsequently released from, PCL/Col/HA than PCL alone scaffolds over an 8-week period (Fig 1). We also demonstrated that the released PDGF (6.0 ng/mL, as measured by ELISA) was chemotactically active in standard Boyden chamber assays (not shown). The degree of transmigration induced by released PDGF was equivalent to that elicited by a comparable dose of purified PDGF (not shown). Furthermore, we developed a modified cell migration assay, in which the distance between the PDGF-coated scaffolds and the cell front of MSC monolayers could be manipulated. We found that even as far as 1.5 cm away from the PDGF origin, MSCs showed substantial migration toward to the scaffolds loaded with chemotactic factor (Fig 2).

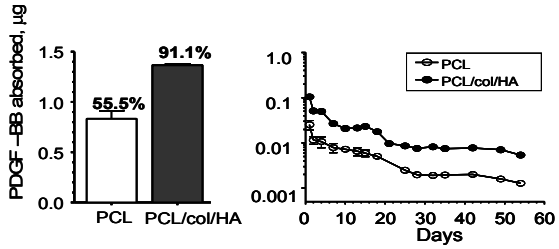

Figure 1 Scaffolds were incubated with PDGF-BB in PBS at 4°C for 42h, and then washed in PBS. ELISA assays were used to measure residual (unbound) PDGF-BB in the supernatants and wash solutions. Absorption of PDGF-BB to the scaffolds was determined by subtracting the unbound from 1.5 μg of PDGF-BB initially added. Data are from three independent experiments.

These results indicate that the amount of PDGF released is sufficient to promote MSC transmigration, and also that adsorption to scaffolds and its subsequent release does not alter bioactivity of the PDGF. Our data strongly suggest that PDGF-BB is a potential candidate of biological agents in the recruitment of MSCs for bone regeneration, and PCL/Col/HA composite scaffolds could serve as a delivery vehicle.

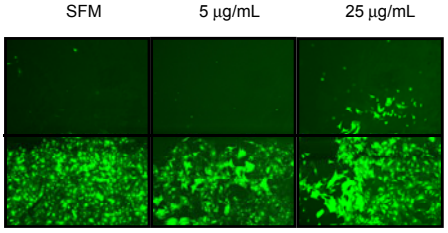

Figure 2. *Rat PDGF-BB induced 2-D migration of rat MSCs.* rat GFP-MSCs were pre-seeded in 8-well rectangular plates . On the next day, the monolayers of the cells were completely removed from the top half of the well by scraping along a pre-drawn central line. Subsequently duplicates of PDGF-BB-absorbed PCL/col/HA/ scaffolds held in 3 mm-inserts were placed 1.5 cm distant from the central lines. After a 72 hr-incubation, migration of GFP-MSCs was visualized by fluorescence microscopy .

Conclusions: PDGF-BB is a potential candidate of biological agents in the recruitment of MSCs for bone regeneration, and PCL/collagen/HA composite scaffolds could serve as a delivery vehicle in MSC-based therapies.

References:

[1] Karp, M. Jeffrey and G. Sock, and L. Teo *Cell Stem Cells.* 2009; 4:206