Platelet-Derived Growth Factor Stimulated Migration of Bone Marrow Mesenchymal Stem Cells into an Injectable Gelatin-Hydroxyphenylpropionic Acid Hydrogel

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Statement of Purpose: For certain regenerative medicine applications, the reparative potential of endogenous mesenchymal stem cells (MSCs) maybe tapped by recruitment of the cells into the injury site. In the case of injury defects, this will require a defect-filling matrix that incorporates MSC chemoattractants and is permissive of MSC migration. Gelatin-hydroxyphenylpropionic acid (Gtn-HPA),[1] an injectable natural biopolymer hydrogel that is covalently cross-linkable *in vivo*, has been shown to accommodate neural stem cell migration[2] and is thus commended for MSC recruitment into defects. Bone marrow-derived MSCs (bMSCs) express tyrosine kinase and macrophage-derived chemokine receptors, including platelet-derived growth factor (PDGF) and stromal cell-derived factor (SDF)-1α CXCR-4 receptors, providing the rationale for employing PDGF-BB and SDF-1α as chemokines and mitogens for inducing bMSC migration and proliferation. Polyelectrolyte complex nanoparticles (PCNs) prepared from dextran sulfate (DS) and chitosan (CS) can serve as controlled release vehicles for growth factors[2] and may enhance the thermal stability of the proteins.[3] The aim of our study was to evaluate the effects of PDGF-BB and SDF-1α, incorporated directly into the Gtn-HPA or into PCNs, on bMSC migration and proliferation in Gtn-HPA.

Methods: Gtn-HPA was synthesized as previously described.[1] The protein-loaded PCNs were prepared by mixing recombinant rat SDF-1α or human PDGF-BB with DS (500kDa) and CS (15kDa, ~84% deacetylation) at a ratio of 0.2:1:0.33.[3] The 3D-migration assay comprised 10^5cells/ml bMSCs in a 2mg/ml type I collagen annular, tissue-simulating gel (containing 10ng/ml FGF-2), surrounding a 2% Gtn-HPA core gel containing: blank PCNs; 2μg/ml soluble proteins; or PCN-encapsulated proteins (Fig. 1A). Inverted microscope images were taken on days 4 and 7 to quantify the number of cells in the core gel. A proliferation assay was performed with a bilayer hydrogel construct with Gtn-HPA gel at the bottom layer and a collagen gel seeded with 10^5 cells/ml bMSCs on top (Fig. 2A). Proliferation was measured on days 4 and 7 using PicoGreen DNA quantification assay.

Results and Discussion: The Gtn-HPA gel was highly permissive of bMSC migration (Fig. 1B). The bMSCs showed a significantly enhanced migration into gels containing soluble PDGF-BB and PCN-encapsulated PDGF-BB compared to the blank gel and gel with blank PCNs (Fig. 1C). PDGF-BB also strongly stimulated bMSC proliferation (Fig. 2B). On day 7, while there was a 2-fold increase in the number of the cells that had migrated into the PDGF-containing gels relative to controls, there was only ~25% increase in bMSC proliferation, reflecting the active recruitment of cells from the annular tissue-simulating gel into the core gel. Also of interest was the longer distance into the gel traveled by the bMSCs under the influence of PDGF-BB (Fig. 1D). SDF-1α attracted fewer cells into the Gtn-HPA core gel and stimulated less proliferation compared to PDGF-BB. On day 7, blank PCNs modestly enhanced migration compared to the blank gel control (Fig. 1C), but had no effect on proliferation (Fig. 2B).

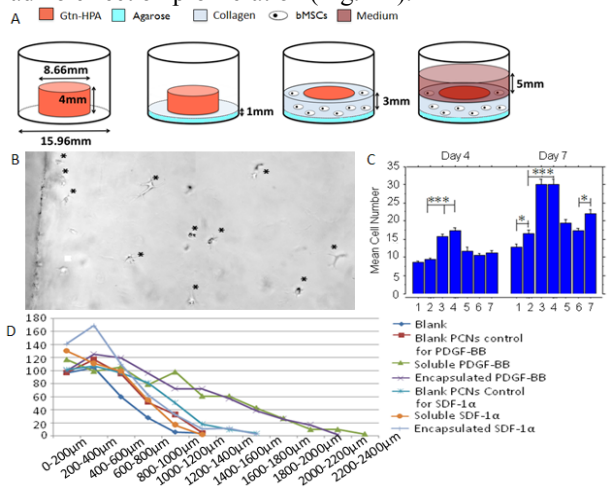

Fig. 1: (A) Annulus-core migration model. (B) A typical migration micrograph on day 4; asterisks show bMSCs. (C) Migrated cell numbers/mm of interface in each group. (D) Migration distance on day 7(* P<0.05;*** P<0.001).

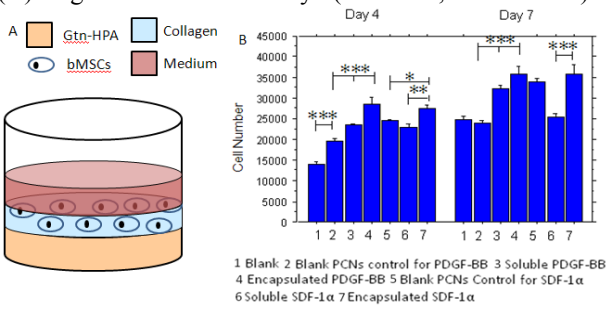

Fig.2: (A) Bilayer proliferation model. (B) Cell numbers on days 4 and 7. (*P<0.05; **P<0.01; ***P<0.001).

Conclusions: Gtn-HPA is permissive of bMSC migration, and PDGF-BB incorporated directly into the gel or into PCNs greatly stimulates the number of migrating bMSCs and the distance that they travel in the gel. PDGF-BB released from Gtn-HPA and from PCNs incorporated into the gel also stimulates proliferation of bMSCs in an adjacent tissue-simulating (collagen) gel.

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References: (1) Wang LS. Biomaterials 2010;31:8608-8616.(2) Lim TC. Biomaterials 2012;33:3446-3455.(3) Huang M. Biomacromolecules 2007:1607-1614