Signaling Molecules to Recruit Endogenous Stem Cells for an Engineered Cartilage Healing Response

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Statement of Purpose: Articular cartilage does not mount an effective healing response to injury, resulting in progressive joint degeneration. Current clinical treatments do not regenerate durable hyaline cartilage. We are pursuing a novel strategy to engineer a cartilage healing response that uses biomaterials and signaling molecules to induce the healing stages that occur in other tissues, i.e., chemotaxis, proliferation, and differentiation of endogenous mesenchymal stem cells (MSC). Essential for a successful healing response is the formation of a dense cell condensation in the wound defect by MSC chemotaxis and proliferation. The synovial membrane has been shown to be a rich source of local endogenous MSC (sMSC) with superior potential for cartilage repair [1,2] (Fig. 1). Our objective was to screen candidate signaling molecules for those that induce robust sMSC chemotaxis and proliferation.

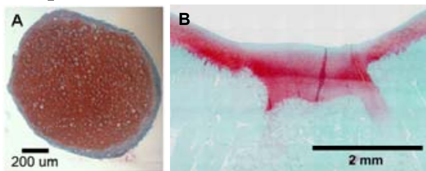

Figure 1. A) Chondrogenic differentiation of synovial cells in pellet culture, B) Regeneration in a rabbit osteo-chondral defect using our cell-free scaffold and multiple growth factor delivery (both Safranin O fast green stain).

Methods: Synovial membrane cells were isolated from 3 month-old New Zealand White rabbits by enzymatic digestion, expanded to passage 2, and cryopreserved. Stocks included all plastic-adherent cells. Signaling molecules that induce chemotaxis or proliferation in bone marrow MSC were selected from the literature [3]. Concentrations that induced the strongest response were selected for sMSC testing: HB-EGF, TGF-α, HGF at 5 μg/ml; PDGF-BB, FGF-2, TGF-β1 at 20 μg/ml; PDGF-AB at 50 μg/ml; IGF-1 at 100 μg/ml.

Chemotaxis was assayed using a dual-compartment chamber consisting of a FluoroBlok Transwell insert (8 μm pore) in a 24-well plate (BD Biosciences, San Jose, California). Cultures were maintained in serum-free medium for 24 hr prior to the experiment. Immediately before the experiment, cells were labeled with CellTracker™ Green dye (Molecular Probes, Eugene, Oregon). The upper surface of the insert was seeded with 40,000 cells. Medium containing the signaling molecule was placed in the lower chamber. Plates were incubated for 6 hr at 37°C, 5% CO_2. Fluorescence intensity of the cells that migrated to the undersurface of the inserts was measured using a plate reader in bottom-read mode. Samples were tested in quintuplicate. Wells containing medium only were used as controls for chemokinesis.

For the proliferation assay, 96-well plates were seeded at 6,000 cells/cm^2 and cultured in low serum medium (1%). After 48 hr incubation, the medium was exchanged and signaling molecules were added. The plates were incubated for an additional 48 hr at 37°C, 5% CO_2. The cells were then labeled with CyQuant® fluorescent dye (Molecular Probes) and fluorescence intensity was measured. Samples were tested in octuplicate. Control wells contained medium only.

Responses were expressed as a multiple of the control. Differences between treatment and control groups were tested using a 2-tailed students t-test.

Results: Chemotaxis was induced by the addition of TGF-α to the culture medium resulting in migration of 1.9 times more cells to the lower chamber than in chemokinesis controls (p=0.004) (Fig. 2A). Significant migration was not induced by the other signaling molecules. Greater proliferation was induced by the addition of PDGF-AB, PDGF-BB, HB-EGF, TGF-α, FGF-2, IGF-1, and HGF to the culture medium (Fig. 2B). The greatest response was induced by FGF-2 with 1.6 times more cells than controls after 48 hr (p<0.001).

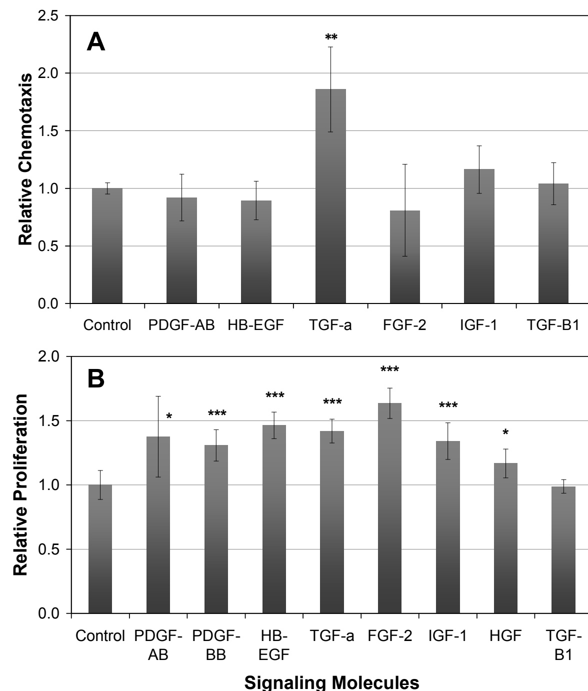

Figure 2. A) Chemotaxis and B) proliferation for selected signaling molecules expressed relative to controls (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).

Conclusions: Our approach to cartilage injury is novel because it focuses on engineering a healing response. Selection of signaling molecules that induce robust sMSC chemotaxis and proliferation is an essential step in orchestrating healing. This research lays the groundwork for programming a clinically effective healing response without the need for cell or tissue transplantation.

References: 1) Sakaguchi Y. Arthritis Rheum. 2005;52:2521-2529. 2) Vanden Berg-Foels WS. BMES Oct. 2009;OP9-3-13D. 3) Ozaki Y. Stem Cells Dev. 2007;16:119-129.