Chitosan Particles Induce Human U937 Macrophages to Release Anti-Inflammatory Factors and Mesenchymal Stem Cell Chemokines Through Pathways Involving STAT-1

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Statement of Purpose: Marrow stimulation is a cartilage repair procedure where micro-perforations are surgically created in the subchondral bone to elicit a repair tissue from the bone marrow into the cartilage lesion. It was previously shown that chitosan, a biodegradable polysaccharide of glucosamine and ~20% N-acetyl glucosamine (GlcNA), can be used during marrow stimulation procedures in order to improve cartilage repair, through increased attraction of bone marrow mesenchymal stem cells (BMSCs) [1,2]. Analysis of the repair response revealed that chitosan attracts more arginase-1+ macrophages to the bone defect granulation tissue than surgery-alone [3]. Macrophages play a central in wound healing, notably through the release of mesenchymal stem cell chemotactic factors [4]. In this study, we tested the hypothesis that chitosan potentiates the release of stem cell chemotactic factors from macrophages that are primed towards distinct states of differentiation, in a structure-specific manner.

Methods: Human macrophage-like cells were obtained through chemical differentiation of U937 monocytes with phorbol myristate acetate (PMA). PMA-differentiated macrophages (D-U937) were then primed in serum-containing media pH 7.4 for 24 hours with or without biodegradable chitosan microparticles (80M: 80% degree of deacetylation (DDA), 240 kDa) and factors known to elicit different polarization states: IFN-γ/LPS for M1 inflammatory (M1 D-U937), IL-4 for alternatively activated M2a (M2a D-U937) and IL-10 for deactivated M2c (IL-10 D-U937) phenotypes. Latex beads were used as a control for non-specific phagocytosis. Low serum media was applied to polarized, chitosan-stimulated cells and cell-conditioned media (CM) was collected and analyzed for pro-inflammatory (IL-6, CXCL8/IL-8, CXCL10/IP-10, CCL2/MCP-1) and anti-inflammatory (IL-1ra, IL-10) cytokines. The ability of CM from macrophages (stimulated or not with chitosan) to attract primary human BMSCs was determined in a transwell migration assay (n=4 donors, Inst. Regen. Med., Texas A&M). Cells were also analyzed for viability and chitosan uptake using rhodamine-labeled chitosan. Cell lysates were analyzed by Western blot for time-dependent activation of STAT-1 and STAT-6 after stimulation by IFN-γ, IL-4, or particles of 80M chitosan, or non-biodegradable 98M chitosan (98% DDA, 150 kDa).

Results: Macrophages were successfully polarized towards M1 and M2a phenotypes with no evidence of M2c polarization. Macrophages internalized both chitosan particles and latex beads while retaining high viability, although IFN-γ/LPS treatment was slightly cytotoxic, with and without chitosan. Chitosan specifically stimulated the release of chemokines CXCL10/IP-10, CCL2/MCP-1 and anti-inflammatory factors IL-1ra and IL-10, without influencing CXCL8/IL-8 or IL-6 release. Chitosan stimulated CXCL10/IP-10 and IL-1ra release to CM, irrespective of macrophage polarization state. BMSCs migrated to CM from D-U937, M2a- and IL-10-treated D-U937, but not M1-polarized D-U937. Chitosan partly restored stem cell chemotactic activity in M1 D-U937 cells, with no added stem cell chemotactic effect for other polarization states (Figure 1).

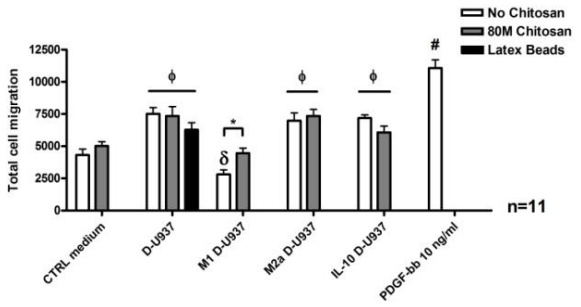

Figure 1: Human BMSC migration to macrophage-conditioned medium. Migration assays were performed 2 to 3 times with 4 different human donors. * $p<0.05$; ϕ $p<0.01$ vs. CTRL medium; δ $p<0.01$ vs. D-U937 no chitosan, $p<0.01$ vs. CTRL medium; # $p<0.001$ vs. all conditions. Mean±S.E.

We investigated the molecular mechanism behind the chitosan-induced release of CXCL10/IP-10 and IL-1ra, soluble mediators normally induced by IFNγ/STAT-1, and IL-4/STAT-6, respectively. Chitosan induced a delayed activation of STAT-1, and no activation of STAT-6 during 24 hours of D-U937 stimulation. We further show that the activation of STAT-1 and the release of CXCL10/IP-10 induced by chitosan are positively influenced by increasing polysaccharide GlcNA content.

Conclusions: Our novel findings show that chitosan can guide macrophages to release therapeutic chemotactic factors under different polarization states. We report for the first time that chitosan can be used to activate STAT-1 in a delayed time course compared to IFN-γ, to selectively improve stem cell attraction and to potentiate the release of anti-inflammatory factors that can be beneficial for joint health. The DDA-dependent STAT-1 activation and CXCL10/IP-10 release show that the biomaterial chitosan can be optimized based on GlcNA content to control the release of chemokines and cytokines by macrophages.

References: [1] Hoemann CD. J Bone Joint Surg Am. 2005;87(12):2671-86, [2] Chevrier A., Osteoarthritis & Cartilage. 2007;15(3):316-27, [3] Hoemann C.D., Am J Sports Med. 2010;38(9):1845-56, [4] Anton K., PLoS One. 2012;7(4):e35036.