Understanding the Immunomodulatory Effects of MSCs in Hydrogels on Macrophages and the Foreign Body Reaction

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Statement of Purpose: Nearly all non-biological materials elicit a foreign body reaction (FBR) when implanted *in vivo* [1]. Our group has previously reported the negative effects of macrophages and the FBR on dermal fibroblasts when encapsulated in poly(ethylene glycol) (PEG) hydrogels, suggesting that the FBR may have a negative impact in tissue engineering [2]. With the promise of mesenchymal stem cells (MSCs) in tissue engineering and recent reports on their secretion of trophic factors with anti-inflammatory properties [3, 4], we hypothesized that MSCs encapsulated within a PEG hydrogel attenuate the FBR, but lose this ability upon differentiation. To test this hypothesis, PEG based hydrogels with encapsulated MSCs or MSCs osteogenically differentiating were employed. Studies were also performed to elucidate the trophic factors secreted by encapsulated MSCs, which attenuate macrophage activation.

Methods: Hydrogels were formed by photo-polymerization from PEG-diacrylate (10 wt %) and 2.5 mM acrylate-PEG-YRGDS in the presence of I2959, a photoinitiator. Murine (C57BL/6) bone marrow derived MSCs were differentiated in bone differentiation medium for 4, 10, or 21 days in 2D. MSCs or differentiating MSCs were encapsulated in hydrogels at 10^7 cells/ml. Murine (C57BL/6) macrophages derived from bone marrow monocytes were seeded on acellular and MSC-laden hydrogels at 2.6×10^3 cells/mm^2. Hydrogels were treated with lipopolysaccharide (1µg/ml) to simulate an inflammatory environment. Macrophages were assessed via gene expression (qRT-PCR) and protein secretion (ELISAs). Acellular and MSC-laden hydrogels were implanted subcutaneously into C57BL/6 mice for 28 days following an IACUC approved protocol. Data are reported as mean with standard deviation as error bars.

Results: *In vitro* experiments demonstrated that macrophages seeded on synthetic hydrogels with encapsulated MSCs had a reduced response to LPS after 24 hours (Fig 1a) compared to acellular hydrogels. The presence of MSCs led to decreased macrophage gene expression of proinflammatory cytokines (IL-1β by 77% ($p<0.05$), IL-6 by 88% ($p<0.05$), TNF-α by 99% ($p<0.05$)), but increased gene expression of the wound-healing molecule arginase by 8-fold ($p<0.05$) compared to acellular hydrogels.

To identify the factors that led to MSC attenuation of macrophage activation by LPS, macrophages were treated with medium conditioned (CM) by encapsulated MSCs. In the presence of LPS, a dose-dependent decrease in macrophage secretion of TNF-α was observed with CM (Fig 1b). The addition of a COX2 inhibitor during medium conditioning reduced prostaglandin E2 (PGE2) secretion by MSCs, which partially recovered LPS-induced macrophage secretion of TNF-α ($p<0.01$ for 50% CM and $p<0.001$ for 75% CM, Fig 1b). No TNF-α was detected in MSC CM. Exogenous PGE2 confirmed a significant reduction in macrophage secretion of TNF-α in a dose-dependent manner.

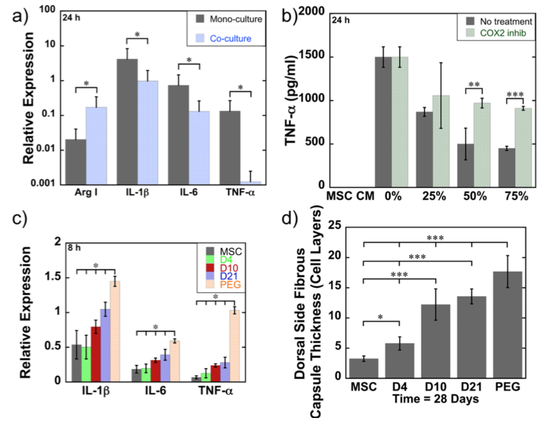

Fig. 1. a) Macrophage gene expression of wound healing (Arginase) and proinflammatory (IL-1β, IL-6, and TNF-α) genes in the absence (mono-culture) and presence (co-culture) of encapsulated MSCs. b) Macrophage secretion of TNF-α in the presence of MSC conditioned medium with LPS and with or without a COX2 inhibitor, which inhibited PGE2 synthesis. c) Macrophage gene expression in the presence of encapsulated MSCs or differentiating MSCs (D4, D10, D21). d) Dorsal fibrous capsule thickness as measured in cell layers following a 28 implantation of MSCs, differentiating MSCS, or acellular PEG hydrogels. *<0.05, **< 0.01, and ***<0.001.

As MSCs differentiated down the osteogenic pathway, their ability to attenuate LPS-activation of macrophages diminished (Fig. 1c). Because the *in vivo* FBR is more complex, cell-laden and acellular PEG hydrogels were subcutaneously implanted into immuno-competent mice for 28 days. The presence of MSCs reduced the fibrous capsule thickness, measured by the number of cell layers in the fibrous capsule (Figure 1d, $p<0.001$) compared to cell-laden hydrogels containing differentiating MSCs and acellular PEG hydrogels. With MSC differentiation, fibrous capsule thickness increased ($p<0.001$), and was highest for acellular PEG hydrogels.

Conclusions: Our results show that MSCs are able to reduce macrophage activation *in vitro* and the FBR *in vivo*, likely through the secretion of PGE2. However, as MSCs differentiate (at least down an osteogenic lineage) they begin to lose their immunomodulatory properties and the ability to mitigate the FBR.

References: [1] Anderson JM. Semin Immunol. 2008;20:86-100. [2] Swartzlander MD. Biomaterials. 2013;34:952-64. [3] Nemeth K. Nat Med. 2009;15:42-9. [4] Choi H. Blood. 2011;118:330-8.

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