Control of Mesenchymal Stem Cell Phenotype and Microenvironment through Intracellular Particles

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Statement of Purpose: Mesenchymal stem cells (MSCs) are of great interest for the treatment of a multitude of inflammatory conditions including graft versus host disease, Crohn's disease, and acute lung injury(*1*). While MSCs are being investigated in over 200 clinical trials for their immunomodulatory properties, MSCs fail to persist long term *in vivo* limiting their therapeutic effect. Therefore, there is a great need to augment MSC therapy. We address this problem by simultaneously controlling MSC phenotype and their local microenvironment. To achieve this, we harnessed a biomaterials approach by loading MSCs with glucocorticoid steroid doped particles. Steroids released from the intracellular particles can impact MSC phenotype and cross the cell membrane to impact the microenvironment. In addition to their potent anti-inflammatory effects on immune cells, steroids have also been shown to increase the expression of indoleamine 2,3-dioxygenase (IDO) in astrocytes(*2*). Since IDO has been implicated as a main contributor of MSC immunomodulatory properties, we investigated the effect of steroids on MSC IDO activity. By coupling the anti-inflammatory properties of glucocorticoid steroids with the immunomodulatory properties of MSCs, we sought to enhance the potency of MSC therapy by controlling the cell and its microenvironment. First, we examined the effect budesonide (BUD) has on MSC immunomodulatory properties to ensure it is not detrimental to MSC phenotype and function. We then formulated poly lactide-co-glycolic acid (PLGA) particles containing BUD and identified conditions that maximized particle uptake by the MSC. Intracellular release of BUD from particles enhances MSC phenotype and is transported across the cell membrane to impact the local microenvironment.

Methods: To investigate BUD's effect on MSC immunomodulatory properties we looked at the effect of BUD treatment on MSC IDO activity. After treating MSCs with 1 μM BUD for 24 hours, MSCs were additionally exposed to 100ng/ml IFN-γ for 48 hours. MSCs were collected, lysed to isolate IDO from the cells, and the quantity of L-kynurenine produced within 1 hour was measured with a colorimetric assay. BUD-PLGA particles were formed through a single emulsion encapsulation technique and the size was adjusted through alteration of the agitation speed. DLS and HPLC were used to measure the particles size and drug loading, respectively. MSCs were loaded with 0.1 mg/mL BUD-PLGA particle suspensions, with or without poly-l-lysine, in 1% serum media and incubated overnight. To characterize particle internalization, flow cytometry and confocal microscopy was performed.

Results: Treatment of MSCs with BUD resulted in significantly increased IDO activity (Figure 1A). MSCs preferentially internalized 1 μm BUD-PLGA particles compared to 1.4 μm particles, and internalized positively charged particles over negatively charged particles (Figure 1B). Particle loading of MSCs resulted in internalization of 15-20 particles/cell as shown by confocal analysis (Figure 1C).

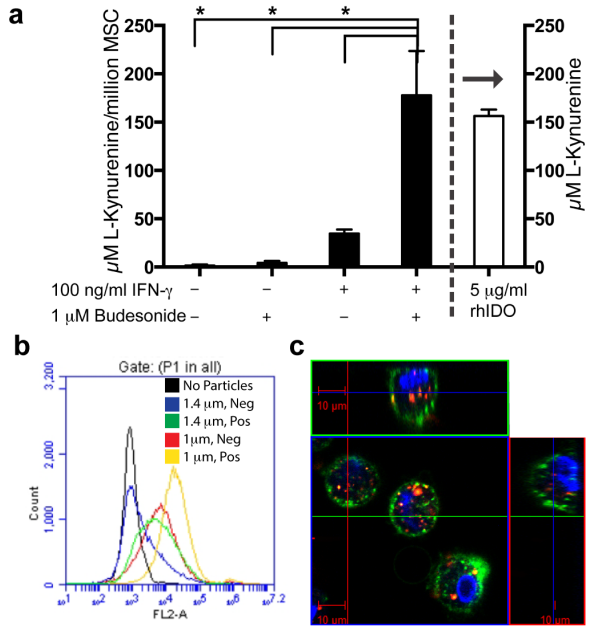

Figure 1. A. IDO activity measured from MSC exposed to 1uM budesonide and 100ng/ml IFN-gamma. (One-way ANOVA with Fishers LSD test, n=3, *p<0.0001). B. Flow cytometry of MSC loaded with DiI PLGA particles as metric of particle association with MSCs. C. Representative confocal image of MSCs modified with 1μm BUD-PLGA particles (Particles: red, Membrane: Green, Nucleus: Blue).

Conclusions: BUD treatment enhanced MSC immunomodulatory potential via a 4X increase in IDO activity. MSC readily internalized 1 μm BUD-PLGA particles and while the number of particles internalized per cell is heterogeneous, most cells in the population internalized particles. Thus, this platform should be useful to significantly improve control of the immunomodulatory properties of MSCs post transplantation.

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