# Non-genetic, Transient Engineering of Mesenchymal Stem Cell Secretome via Intracellular Controlled Drug Delivery

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**Statement of Purpose:** Mesenchymal stromal cells (MSCs) are promising therapeutic candidates given their potent immunomodulatory and anti-inflammatory secretome. However, controlling the MSC secretome post-transplantation is considered a major challenge that hinders their clinical efficacy. Genetically engineered hMSCs pose challenging long-term regulatory hurdles, and while preconditioning hMSCs with cytokines/small molecules may be safer, the phenotype-altering effects are transient. We hypothesized that engineering MSCs to induce a specific secretome profile under a simulated host microenvironment may maximize their therapeutic utility. In this study, using TNF-α stimulated NF-κB activation as a model of inflammation, we show that microparticle-mediated continuous intracellular delivery of a small molecule inhibitor (TPCA-1) of NF-κB can provide sustained inhibition of the hMSC pro-inflammatory secretome and thereby inhibit monocyte migration, cardiac myofibroblast (CMF) differentiation, and collagen deposition (Ranganath et al, 2016).

**Methods:** hMSCs were treated for 48 h with 10 ng/ml TNF-α, or 10 mM TPCA-1 and stimulating with 10 ng/ml TNF-α, followed by conditioned media (CM) collection. Quantitative measurement of 250 human cytokines in the stimulated hMSC CM was performed using Quantibody Human Cytokine Array 5000. TPCA-1 was encapsulated in PLGA microparticles (μPs) using single-emulsion solvent evaporation technique. To enhance μP uptake and loading in hMSCs, the surface of μP was modified using poly-L-lysine. Analysis of the composition and kinetics of preconditioned and μP-engineered hMSC secretome was done by incubating hMSCs with 200 μg/ml of TPCAμPs or no μPs with/without TNF-α. Concentrations of IL-6, MCP-1 and RANTES in CM were quantified using ELISA kits. Quantification of human monocyte migration in response to hMSC CM in transwell plates was performed with optical microscope. The effect of μP-engineered hMSC CM was analyzed on human and murine CMF through α-SMA immunostaining and collagen deposition assays.

**Results:** Sustained TPCA-1 treatment in TNF-α-stimulated hMSCs significantly reduced the secreted levels of pro-inflammatory mediators (Fig. 1). Flow cytometry analysis and confocal imaging confirmed that PLL-coated μPs had greater interaction with hMSCs and revealed internalization. The μPs released TPCA-1 completely within 20 days of incubation in PBS at 37 ºC with a near zero-order release kinetics. Also, μPs loaded with TPCA-1 when delivered to hMSCs attenuated secretion of pro-inflammatory factors for at least 6 days in vitro. Intracellular TPCA released from μPs inhibited NF-κB nuclear translocation in TNF-α-stimulated hMSCs. Conditioned medium (CM) derived from TPCA-μP-loaded hMSCs also showed reduced ability to attract human monocytes and prevented differentiation of human cardiac fibroblasts to myofibroblasts, compared with CM from untreated or TPCA-1-preconditioned hMSCs .

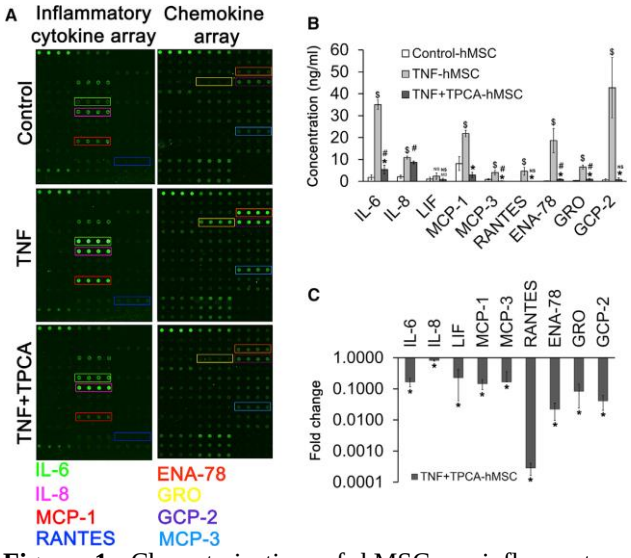


**Figure 1.** Characterization of hMSC proinflammatory secretome upon TNF-a stimulation and TPCA-1 treatment

**Conclusions:** Here we have demonstrated that the pro-inflammatory hMSC secretome could be inhibited using a microparticle engineering approach delivering an intracellular NF-κB inhibitor, TPCA-1. The approach of microparticle engineering of an exogenous cell population by modulating a central regulatory pathway, may find application in other cell types and pathways and could provide an attractive strategy for harnessing any cell secretome for therapy.

**Reference:** Ranganath SH, et al. Stem Cell Rep. 2016;6:926-939.