A Biomaterials Approach for Programming Cell Fate

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Statement of Purpose: A cell's fate is tightly controlled by its microenvironment. Key factors contributing to this microenvironment include physical contacts with the extracellular matrix and neighboring cells, in addition to soluble factors produced locally or distally. Alterations to these cues can drive homeostatic processes, such as tissue regeneration/wound healing, or may lead to pathologic tissue dysfunction. *In vitro* models of cell and tissue microenvironments are desirable for enhanced understanding of the biology and ultimately for improved [illegible]. However, mechanisms to exert specific control over cellular micoenvironments remains a significant challenge. Herein we describe a novel strategy to engineer cells with an intracellular depot of phenotype altering agent/s that can be used for altering cell fate via both intracrine-, paracrine-, and endocrine-like mechanisms. Specifically, we show that human mesenchymal stem cells (MSCs) can be engineered with poly lactide-co-glycolic acid (PLGA) particles containing dexamethasone (DEX), which acts on cytoplasmic receptors. The controlled release properties of these particles allowed for sustained intracellular and extracellular delivery of agents to promote differentiation of particle carrying cells, as well as neighboring cells and distant cells that do not contain particles.

Methods: Rhodamine 6G dye (Sigma) or the osteogenic differentiation agent, DEX, were encapsulated in PLGA particles using a single emulsion encapsulation technique. To determine the encapsulation efficiency, 10 mg of DEX-PLGA particles were dissolved in anhydrous DMSO followed by quantification of DEX with a UV-Vis spectrophotometer. PLGA particle suspensions with concentrations of 0.1 mg/mL and 0.5 mg/mL in PBS were added to 90% confluent layers of MSCs for 10 min after which the PBS was removed and complete media was added. The MSCs were permitted to internalize particles for 24 hrs at 37 °C. To characterize particle internalization and stability of internalized particles, MSCs were loaded with DiO containing PLGA particles and characterized with confocal and transmission electron microscopy. Osteogenic differentiation of particle modified cells, neighboring cells, and distant cells, was examined by cell membrane associated alkaline phosphatase (ALP) activity after 21 days of culture.

Results: MSCs modified with DEX-PLGA microparticles exhibited sustained release of DEX (Fig. 1 A) and underwent osteogenic differentiation in the presence of ascorbic acid (A) and β-glycerol-phosphate (G)(Fig. 1 B). In addition, modified cells were able to induce differentiation of neighboring cells (without particles) as well as distant cells grown on a transwell membrane 2mm above the DEX-PLGA modified MSCs (Fig. 1 C-D).

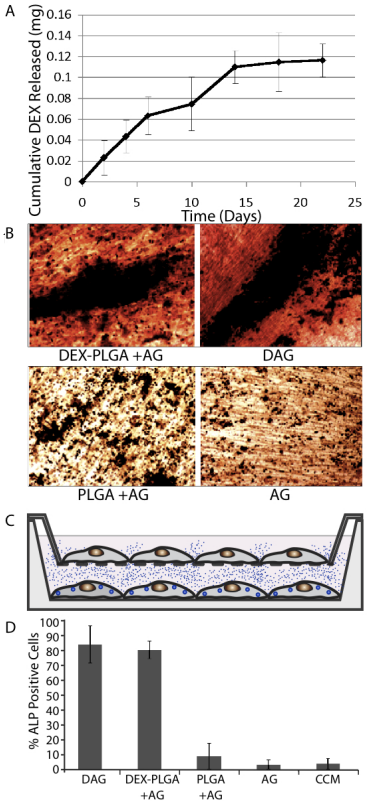

Figure 1. (A) Release kinetics of DEX from MSCs containing DEX-PLGA particles into media at 37 °C for 21 days (B) Bone nodules were identified via positive dual staining for Von Kossa and ALP in DEX and internalized DEX-PLGA particle containing cultures supplemented with A and G but not in the absence of DEX or DEX-PLGA particles (C) Illustration of DEX-PLGA modified MSCs controlling the fate of MSCs (without particles) separated by a transwell membrane. (D) Osteogenic differentiation of unmodified MSCs atop transwell membrane quantified through ALP staining. (CCM= expansion media, DAG= osteogenic media)

Conclusions: We have developed a strategy to engineer cells with an intracellular depot to impart intracellular and extracellular control of cell fate. In our pro of of concept studies we have shown that primary human mesenchymal stem cells (MSCs) can efficiently internalize 1-2μm biodegradable particles containing differentiation factors. Remarkably, differentiation factors released from the particles were shown to promote the differentiation of particle-carrying cells (intracrine-like signaling), neighboring cells (paracine-like signaling), and the differentiation of distant cells (endocrine-like signaling). In addition to use as an *in vitro* tool to create cell niches in culture where temporal and spatial control of cellular cues is critical, intracellular depots may permit exquisite control over transplanted cells and their microenvironment through impacting cellular phenotype and function. For example, agents could be used to impact cell survival, proliferation, differentiation, extracellular matrix production, cell death, or secretion of therapeutic peptides and proteins. We envision this intracellular drug depot will be useful for developing in vitro models to study cell-cell communication, and cell-based therapies for tissue regeneration, drug delivery and cancer therapeutics.