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Presentation Title: Impact of Calcium Concentration on Cell Viability, Transfection Efficiency, and Osteogenic Differentiation in Bone Marrow-derived Stem Cells

Abstract:

Statement of Purpose: With the rising use of bone marrow stem cells (BMSCs) embedded in calcium-coated scaffolds for bone tissue regeneration, understanding the role extracellular calcium plays at the cellular level is paramount for the continued optimization of BMSC-seeded scaffolds. Recent publications have demonstrated that the dissolution of the calcium coating is composition¬¬-dependent1, and calcium concentration affects both cell viability and differentiation2. Here we present the effects that varying extracellular calcium concentrations, over a broader range than previously tested, have on BMSCs with respect to their cell viability, their susceptibility to transfection, and their capacity to differentiate.

Methods: BMSCs were purchased from ATCC, and the passage number remained below ten for all experiments. Cell viability was determined using an MTS assay [Promega] at 24, 48, and 72 hours post transfection Nanoplexes made from polyethyleneimine (PEI) [Sigma] complexed with enhanced green fluorescent protein (eGFP) plasmid were used to transfect the BMSCs, and expression of eGFP was quantified using flow cytometry [Becton Dickinson FACScan]. Osteogenic differentiation was qualitatively assessed after 14 days using alizarin red staining [Sigma-Aldrich], and the presence of osteogenic markers was quantified using RT-PCR for osteocalcin and alkaline phosphatase on day three and seven. As a proof-of-concept study, supernatants from cultures of BMSCs transfected with PEI nanoplexes containing plasmid encoding bone morphogenetic protein (pBMP-2) PEI were analyzed with an ELISA kit for BMP-2 content [Thermofisher].

Results: The variation of calcium concentrations resulted in increased protection against the cytotoxic effects of the PEI, whilst not significantly impacting the transfection efficiency of the complexes. In addition, the levels of calcium increased the expression of osteocalcin, but alkaline phosphatase was not significantly altered. The BMSCs transfected with the pBMP-2/PEI nanoplexes produced significantly more BMP-2 at the higher concentrations of calcium media as compared to controls.

Conclusions: Our results indicate BMSCs are heavily influenced by the presence of extracellular calcium ions. We have successfully characterized the role calcium plays in cell viability, transfection, and differentiation. Also, the wide range of calcium concentrations analyzed provides a more complete view of the effects calcium has on BMSCs than previously recognized. This work provides a strong foundation for optimizing calcium coatings for bone tissue regeneration. Future work will focus on testing a variety of calcium carbonate and hydroxyapatite coatings for the concentration of calcium ions present in the scaffold microenvironment and the effects on the aforementioned cellular properties.

References:

1. (Lee JS. Adv Mater. 2011; 23: 4279-4284)

2. (Cheng S. Hum. Cell. 2013; 26: 114-121)