Elevated Ca\textsuperscript{2+} greatly increases human adipose-derived stem cell mineralization in the absence and presence of osteogenic supplements

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INTRODUCTION:
Extracellular calcium (Ca\textsuperscript{2+}) is a potent regulator of stem cell behavior, biomineralization, and signaling within bone (1-2). \textit{In vivo}, Ca\textsuperscript{2+} concentrations range from 8-40 mM during osteoclastic resorption, and this enriched ionic environment stimulates the proliferation, matrix maturation, and eventual mineralization of osteoblasts and osteoblast progenitors (1-2). Previous research not only indicates a correlation between elevated Ca\textsuperscript{2+} levels and mineral formation, but also upregulation of osteogenic genes and proteins including cyclooxygenase-2, bone morphogenetic protein-2, and osteocalcin for a variety of bone and bone progenitor cells (2). Human adipose-derived stem cells (hASCs) are a multipotent stem cell line capable of undergoing osteogenic differentiation and provide an abundant and proven source of cells for bone tissue engineering; to date, no research has assessed the effect of enriched calcium levels on the mineralization of hASCs. Thus, the objective of this research was to evaluate the effect of varying ionic Ca\textsuperscript{2+} levels on the viability, proliferation, and osteogenic differentiation potential of hASCs seeded on electrospun PLA scaffolds under both growth and osteogenic differentiating medium conditions.

METHODS: Scaffold Fabrication
Scaffolds were prepared using the electrospinning technique. Commerical PLA with an M\textsubscript{n} of 70,000 Da was solubilized in chloroform and dimethyl formamide at a ratio of 4:1 to yield a 12 wt% solution. The solubilized polymer was electrospun at a flow rate of 50 μL/min, working distance of 15 cm, apiled voltage of 15 kV, and a deposition time of 1.5 hrs (Figure 1).

After electrospinning, scaffolds were punched into 1.27 cm discs with a thickness of ~150 μm and sterilized with 70% ethanol.

Cell Culture
Human ASCs from 2 donors (49 y.o. females) were cultured on electrospun scaffolds at an initial seeding density of 20,000 cells/cm\textsuperscript{2}. Two days after seeding, media was changed to either complete growth medium (CGM), osteogenic differentiating medium (ODM) at normal Ca\textsuperscript{2+} level (1.8 mM), or either medium enriched with CaCl\textsubscript{2} at either 8 or 16 mM Ca\textsuperscript{2+} and hASCs cultured for up to 14 days. Cell viability was determined by quantifying DNA on days 1, 7, and 14. Proliferation was determined by quantifying DNA on days 1, 7, and 14. Dystrophic and cell-mediated calcium content were evaluated on days 7 and 14 using the Ca\textsuperscript{2+} Liquicolor Assay. Data is presented as average ± standard error mean. Statistical analysis was performed using ANOVA and Tukey-Kramer HSD tests (significance at p-value < 0.05).

RESULTS:
Human ASCs were able to adhere and remain viable on electrospun PLA scaffolds kept in elevated Ca\textsuperscript{2+} media throughout the duration of the experiment (Figure 2). Human ASC osteogenic differentiation and mineral production was significantly increased in the presence of elevated Ca\textsuperscript{2+} levels with 8 mM causing the highest Ca\textsuperscript{2+} deposition (Figures 3 and 5). Elevated Ca\textsuperscript{2+} in CGM increased the amount of mineral laid down (Figure 3). DNA analysis indicated that cell proliferation was significantly hindered for the 16 mM CGM group, while other groups were not significantly different at day 14 (Figure 4).

DISCUSSION:
Our findings indicate that elevated Ca\textsuperscript{2+} levels of 8 mM can significantly increase the mineralization rate of hASCs with or without osteogenic inductive factors of dexamethasone, ascorbic acid, and β-glycerol phosphate. Culturing hASCs in 16 mM Ca\textsuperscript{2+} CGM proved to be inhibitory as determined by both viability and proliferation data. Overall, culturing hASCs in elevated Ca\textsuperscript{2+} in ODM can substantially increase the amount of mineral deposited. This is the first study to evaluate the effect of ionic Ca\textsuperscript{2+} on hASCs and indicates that in vitro expansion of hASCs in 8 mM Ca\textsuperscript{2+} could greatly increase local Ca\textsuperscript{2+} levels to maximize mineral deposition prior to implantation.

REFERENCES:
1: Biomaterials 26 (2005) 4847–4855

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