Supplemental Magnesium Ions Altered Human Embryonic Stem Cell Morphology while Retaining Pluripotency

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Statement of Purpose: The purpose of this manuscript is to determine magnesium's (Mg's) potential as a scaffold material for stem cell therapies by focusing on human embryonic stem cell (hESC) responses to high Mg^{2+} concentrations (0.4-40 mM). Another motivation is to explore the possible embryotoxicity of these magnesium ion concentrations, since magnesium sulfate is clinically used to treat pregnant women with eclampsia and its safety and mechanism are still unknown (Euser AG, Stroke, 2009; 40:1169-1175).

Mg is an attractive biomedical implant and scaffold material because it has desirable biodegradable and mechanical properties. Mg degrades with water in the following overall reaction:

$$2Mg + 2H^+ + 2H_2O \rightarrow 2Mg^{2+} + 2OH^- + 2H_2 \quad (1)$$

As an implant, the control of Mg's degradation is necessary to moderate the release of potentially harmful hydroxide ions (which causes an increase in pH) and hydrogen gas. In developing Mg based implant materials, it is important to understand the effects of each degradation product on the surrounding cells or tissues. The goal of this study is to focus on the effects of high Mg ion (Mg^{2+}) concentrations from Mg degradation.

Methods: The H9 hESCs (WiCell, Madison, WI) were stably transfected with an octamer-binding transcription factor-green fluorescent protein (OCT4-GFP) reporter plasmid as previously described (Chatterjee P, J Vis Exp, 2011; 56: 3110). These H9-OCT4 hESCs were then cultured in serum-free, defined media mTeSR®1 (STEMCELL Technologies, Vancouver BC (V5Z 1B3), Canada) supplemented with different dosages of Mg Chloride (yielding media with Mg^{2+} concentrations ranging between 0.4-40 mM). A cell culture observation system (Nikon BioStation CT, Melville, NY) was used to record time-lapse phase contrast images and fluorescence images every six hours for 72 hours at two randomly selected points in each well, while incubating the cells under standard cell culture conditions. The post-culture media was collected and replenished with corresponding fresh Mg^{2+} supplemented mTeSR®1 media every 24 hours. At the endpoint of the experiment at 72 hours, immunocytochemistry was used on washed and fixed cells to label different pluripotency antigens expressed by the H9-OCT hESCs. Antibodies used for this purpose included anti-Sox2 (1:200, R&D systems), anti-SSEA3 (1:40, Millipore), and anti-OCT4 (1:200, Santa Cruz biotechnology). Fluorescence images were obtained using an inverted fluorescence microscope (Nikon Eclipse Ti, Melville, NY). Endpoint cell pellets from different Mg^{2+} test concentrations were also obtained to derive expression levels of pluripotency markers using RT-PCR and qPCR.

Results: All cultures of H9-OCT4 hESCs supplemented with Mg^{2+} dosages grew to confluency with no observable cell death. Time-lapse phase contrast images revealed that the hESCs cultured in media supplemented with 10-40 mM Mg^{2+} underwent a morphology change in which the cells became more dispersed with greater cellular extensions and reduced cell-cell adhesion (Fig. 1). Immunocytochemistry stains of all hESCs cultures supplemented with Mg^{2+} dosages were positive for OCT4, SSEA3, and SOX2 pluripotency markers. OCT4 and SOX2 were markers of the hESC nucleus while SSEA3 was a marker of cell surface. Since DAPI stains the cellular DNA, the images of the OCT4, SSEA3, and DAPI stains were merged to give a qualitative idea of the amount of cells that were not expressing the pluripotency markers. As seen in merged images in Fig. 1, most of the cells presented a teal color (a merged color of blue from the DAPI and green from the OCT4), indicating that most hESCs expressed the pluripotency marker OCT4. Furthermore, quantitative qPCR results show no statistically significant difference between the expression levels of the pluripotency markers of all hESC cultures supplemented with Mg^{2+} dosages (data not shown).

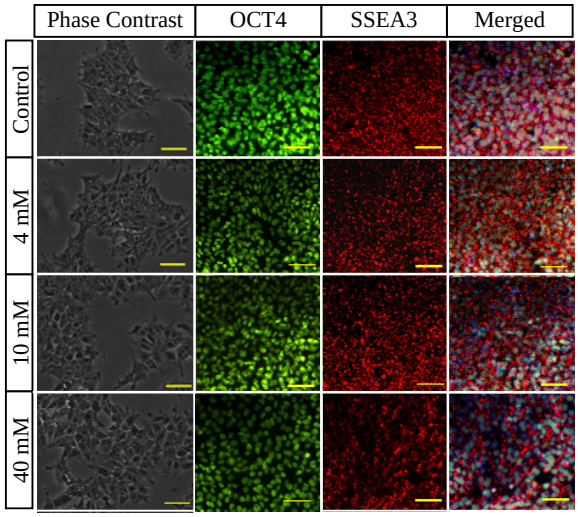

Fig. 1: Phase contrast (left) and immunostained (middle /right) images of hESCs cultured in media supplemented with additional Mg^{2+}. Scale bar = 100 µm.

Conclusions: Since hESCs grew to confluency while retaining pluripotency (indicated by the immunocyto-chemistry and qPCR results), high levels of Mg^{2+} concentrations (up to 40 mM) do not adversely affect pluripotency. Further studies are needed to explore stem cell growth or inhibition in the presence of supplemental Mg^{2+} dosages and possibly correlate this with changes in stem cell morphology. The maximum extracellular Mg^{2+} concentration that does not impose adverse effects on cellular functions can be used as a design guideline for future magnesium based scaffolds and implants.