Calcium and Gap Junction Intercellular Communication Enhance Differentiation of Bone Marrow Stromal Cells

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Statement of Purpose:

Bone engineering seeks to regenerate tissue through the full volume of a fracture or defect. However, in cell-based tissue engineering, transport limitations prevent regeneration in the core of a scaffold. Calcium-phosphate (Ca/P) templates may be physiologically favorable because they mimic the extracellular environment and release ions that aid in the regeneration of bone. In addition to the Ca/P surface, soluble calcium ions may also regulate cell differentiation. The abundance of these ions throughout the construct may help overcome these transport limitations. Another way to overcome this limitation is by micromass cell seeding (MM), which increases cell capacity, nutrient transport and cell-cell communication (Rossello, Trans. Soc. Biomat. 2005, 30:734). Cell-cell communication could also be augmented by overexpressing Connexin 43 (Cx43). Cx43 is a gap junction protein that plays an important role in the coordination of bone modeling and remodeling (Stains, BioChim. BioPhys. 2005, 1719, 69-81,), and may enhance the propagation of signals to enhance differentiation. This study examined: (1) the effects of extracellular calcium (Ca^{2+}_e) on intracellular calcium (Ca^{2+}_i) and gap junction intercellular communication (GJIC) in BMSCs transfected with Cx43 or cultured in micromasses, and (2) the differentiation of these cell groups under normal and increased calcium environment.

Methods:

BMSCs from the femoral and tibial cavities of five-week old male C57BL/6 mice were cultured in α-MEM supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 µg/ml penicillin G-streptomycin) at 37°C in 5% CO_2/95% air. MM culturing was performed by pipetting dense cell clusters (10^5cells/cm^2) into the cell culture surface. Cx43 infection via lentivirus LV-GFP-Cx43, was performed by culturing cells with 8 ml of filtered virus-containing media for 12 h (infection), followed by replacing with fresh media for 12 h.

Ca^{2+}_i levels were quantified using imaging techniques with Ca^{2+} sensitive dye Fura-2. Prior to Ca^{2+} exposure, cells were incubated with Fura-2AM in media and washed with PBS. BMSCs, MM seeding or cells transfected with Cx43 were measured for their Ca^{2+}_i in a control environment and their response after increasing the concentration by 1.5mM. Following alteration of Ca^{2+}_e Ca^{2+}_i was monitored over 24 hrs. GJIC was assessed by lucifer yellow transfer to adjacent cells after 10minutes.

Real-Time PCR was used to detect effects of Cx43 over-expression, MM culturing, and extracellular calcium on ALP expression at 2, 8 and 16 days; normalized to 18s expression).

Results/Discussion:

Ca^{2+}_i was altered when the Ca^{2+}_e was increased by 1.5mM (fig. 1). In all cases the Ca^{2+}_i level spiked up 4-6 fold after exposure to higher calcium in the media. MM cultured cells showed a significantly higher initial Ca^{2+}_i level (190+/-23.2nM, $p<0.001$) than all other groups (BMSC-Cx43:113+/-19.2nM, BMSCs: 105+/-12.4nM) before being exposed to 1.5mM increase of Ca^{2+}_e. All profiles decreased with time and leveled at their base level, except for the Cx43-cells which was significantly higher (193 +/-18.3nM) than at the beginning ($p<0.001$) suggesting more Ca^{2+}_i retention. Dye transfer showed that increasing Ca^{2+}_e did not affect the GJIC. However, Cx43 transfected cells had significantly higher GJIC (43.2+/-5.1 cells) than MM (33.1+/-7.3), and BMSCs (26.1+/-3.9).

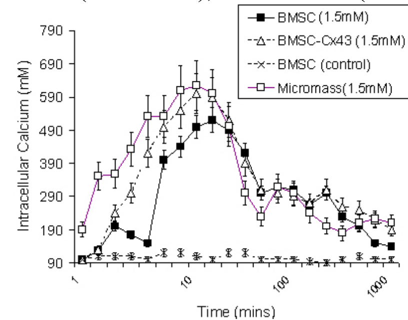

Figure 1: Intracellular calcium profile.

ALP expression was significantly enhanced by MM seeding and Cx43 overexpression at 2 and 8 days (figure 2). The effect of the extracellular calcium is observed in the 8th day, with significantly more ALP activity in the higher calcium environment ($p<0.001$, both day 8 and 16) than BMSCs in the control environment.

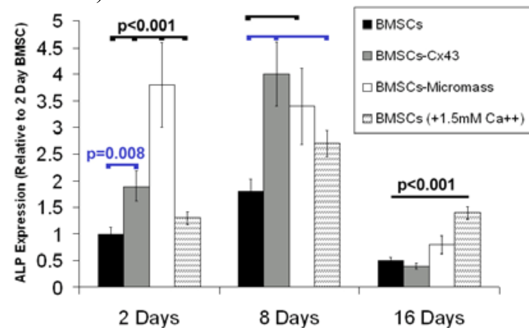

Figure 2 : ALP expression is enhanced early by MM seeding and Cx43 overexpression, and late with increased Ca^{2+}_e environment (bars indicate significant differences).

Conclusions:

Higher Ca^{2+}_e, such as in the environment created during dissolution of Ca/P scaffolds, increases Ca^{2+}_i in cells, and enhances differentiation. This conclusion supports using a mineralized template rich in soluble calcium to enhance bone formation. The overexpression of Cx43 also enhanced differentiation. In MM cultures, GJIC was greater than BMSCs only; suggesting that this increase is one reason for the success of the MM seeding technique. However, the effects cannot be solely attributed to the increase in GJIC, as differentiation was higher than or equal to cells expressing more Cx43.

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