

## Mechanical Stimulation of Calcium Signaling Pathways in Human Bone Cells Using Ferromagnetic Micro-particles: Implications for Tissue Engineering

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**INTRODUCTION:** Mechanical forces are known to influence bone cell behaviour *in vivo* and *in vitro*, leading to a range of cellular responses that ultimately influence bone matrix production. Techniques have previously been described that allow mechanical forces to be applied to specific cell surface receptors, such as integrins, via the use of micron-scale magnetic particles coated in specific ligands or antibodies <sup>(1, 2)</sup>. We are investigating the potential for using magnetic particle based techniques to mechanically condition cells via the activation of specific mechanotransduction pathways. Here we provide evidence that both primary human osteoblasts and bone marrow stromal cells respond to magnetic particle mediated mechanical stimulation via the generation of intracellular calcium transients.

**METHODS:** 4.5µm ferromagnetic particles were attached to integrin receptors on primary human osteoblasts and bone marrow stromal cells via an RGD peptide coating. Typically 4-6 particles were attached to each cell. Cells were then exposed to static magnetic fields (600G) using permanent rare Earth magnets. The forces acting on the membrane bound particles are estimated to be in the range of 10-30pN. Fluo-3 was used in conjunction with laser scanning confocal microscopy and time-lapse software to monitor intracellular calcium levels of populations of cells in real time. Cells were monitored over a period of 5-15 minutes and the activity of stimulated cells was compared to normal cells without particles, and also to cells with particles but no applied magnetic field. The effect of magnetic field alone was also investigated. The number of cells responding, magnitude, time to onset and duration of calcium responses was evaluated.

**RESULTS:** Magnet field alone had no effect on either cell type. The presence of magnetic particles in the absence of an applied magnetic field also had little effect on calcium signalling, although there was a slight increase in the percentage of cells exhibiting Ca<sup>2+</sup> transients compared to normal cells. Both osteoblasts and bone marrow stromal cells exhibited increased levels of calcium activity in response to magnetic loading. There were however significant differences in both the

background levels of spontaneous calcium activity and the characteristics of magnetically stimulated calcium responses between the different cell types. Bone marrow stromal cells typically responded with a single Ca<sup>2+</sup> transient whereas responsive osteoblast cells demonstrated both single and oscillating Ca<sup>2+</sup> transients. Time to onset of response was also found to vary between cell types.

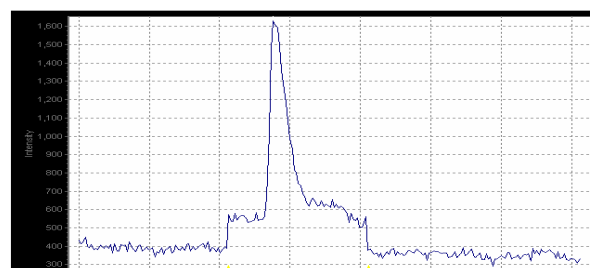


Fig 1. Ca<sup>2+</sup> trace from hBMSc exposed to magnetic loading. Magnet applied between yellow arrows. Image every 2 sec.

**DISCUSSION & CONCLUSIONS:** Magnetic particles offer a valuable tool for mechanically stimulating cells *in-vitro*, and possibly *in-vivo*. The ability to apply mechanical forces directly to cell surface receptors has several advantages over more traditional approaches where compressive or tensile forces are applied to cell seeded scaffolds. It is clear that the different cell types investigated herein displayed differential responses to similar modes of stimulation. These differences may be of importance when deciding on the pattern of loading and optimal cell source to be used for the generation of tissue engineered bone constructs *in-vitro*. Future directions include the development of strategies that will allow the magnetic localisation and differentiation of human mesenchymal stem cells *in vivo* for the repair of damaged or diseased tissue.

**REFERENCES:** 1) Wang N. et al, *Science*, 260, 1124-1127, 1993. 2) Pommerenke H. et al, *J. Bone Mineral Res*, 17, 603-611, 2002.

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