

Short-term responses of stem cells and osteoblasts to a novel mechanical force application technique.

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INTRODUCTION: This study describes short-term changes in the membrane potentials of both MG63 human osteoblast-like and human mesenchymal stem cells (hMSCs). Magnetic particles were attached to individual cells and an oscillating magnetic field was applied, allowing force application to specific regions of the cell membrane at controlled frequencies. The degree of force was theoretically [2,3] calculated to be in the order of in the order of a few piconewtons.

METHODS: Ferromagnetic microparticles (4.0-4.5µm diameter) were coated with Arg-Gly-Asp (RGD). Time-varying magnetic fields were applied using a computer controlled drive system (Fig1). Magnetic source used in all experiments was a rare earth NdFeB magnet. Commercially purchased (Cambrex) human mesenchymal stem cells (hMSCs) and the osteosarcoma cell line MG63 were tested. The stretch activated ion channel (SAC) antagonist Gadolinium (Gd) was used for both MSCs and MG63. Large conductance calcium-activated potassium channel (BK ion channel) antagonists Tetraethylammonium chloride (TEA) and 4-Aminopyridine (4-Ap) were only used with MG63 cells [1]. All electrophysiological recording were taken by impaling cells with a glass electrode, as previously described [4]

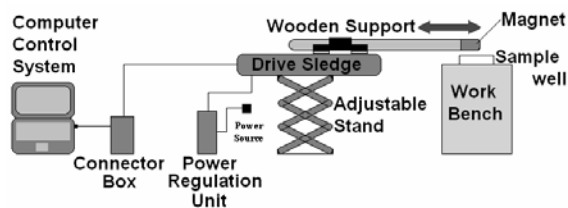


Fig. 1: A schematic representation of the computer controlled magnetic field delivery system.

RESULTS:

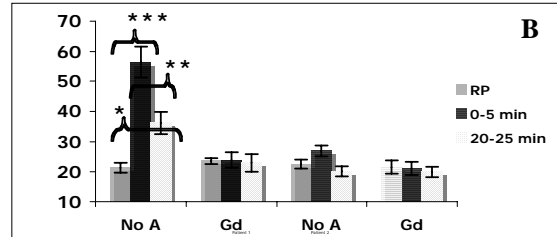
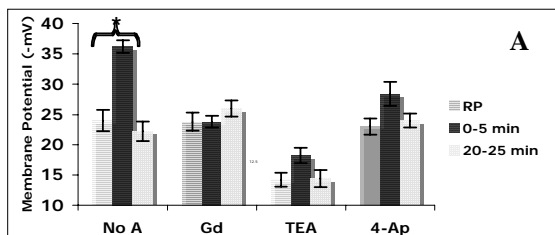


Fig 2: A summary of the effects of magnetic particle based force application with and without antagonists on: **A** MG63 cells * $p < 0.01$, **B** hMSCs * $p < 0.01$ ** < 0.01 *** 0.01 . No A = No Antagonist, Gd = 10µm Gadolinium, TEA = 20mM TEA, 4-Ap = 3mM 4-Ap

DISCUSSION & CONCLUSIONS: Both patient 1 hMSCs and MG63 cells showed a significant increase in membrane hyper-polarisation in the experimental group. The presence of gadolinium completely abrogated the hyper-polarisation response in both hMSCs and MG63 cells, indicating the change in membrane potential may be due to stretch activated ion channels (SAC), possibly resulting in calcium flux⁵. The presence of TEA and 4-Aminopyridin significantly reduced the response in MG63 cells, indicating that BK ion channels may play a role in their hyper-polarisation. The exact mechanisms regulating BK channel induction remains unclear but may be due to the activation of specific cell signaling cascades mediated by the opening of SAC.

REFERENCES: ¹M. Wright, R. Stockwell, G. Nuki. (1992). *Connect Tissue Res* **28**: 49-70. ²J. Dobson, A. Keramane, A. A. El Haj. (2002) *European Cells and Materials* **4**: 42-44. ³Q. Pankhurst, J. Connolly, S. Jones, J. Dobson. (2003) *Journal of Physics D: Applied Physics* **36**: R167-R181. ⁴Salter.D.M. et al (2000) *Journal of Bone and Mineral Research* **15**:9: 1746-1755. ⁵Hughes. S et al (2003) *European Cells and Materials* **6**: 2:43.

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