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MECHANICAL CONDITIONING OF BONE CELLS *IN VITRO* USING MAGNETIC MICROPARTICLE TECHNOLOGY

S.H.Cartmell, J.Dobson, S.Verschueren, S.Hughes & A.J.El Haj

Centre for Science and Technology in Medicine, Keele University, England, UK

INTRODUCTION: Application of mechanical strains directly to cells has been performed using magnetic fields applied to magnetic particles that have been coated in a variety of proteins such as collagen type1[1], RGD[2], fibronectin[3], bovine serum albumin[4] and poly-l-lysine[5]. Translational stretches and torques have been applied to individual cells in this manner and an upregulation of Ca2+ influx into cells and significant alterations in the cytoskeletal network such as actin filament stiffening has been reported as a result of such strains. However, application of mechanical strain to cells has been predominantly focused on short-term single cell interactions. In this report we go further to investigate the use of magnetic microparticle technology on multiple cells grown in culture long-term. We have studied the effects of applying a cyclical mechanical strain directly to primary human osteoblasts for a 21-day period using magnetic particle technology.

METHODS: Ferromagnetic (CrO_2) particles (diameter ~4.5 microns, Spherotech) were coated with RGD (50mg/ml PBS) and adhered to primary human osteoblasts at a concentration of approximately two particles per cell. Approximately 100,000 cells (plus adhered particles) were placed into wells of a 6 well plate and cultured in the presence of culture medium (alpha-MEM), antibiotics, 10% fetal calf serum and osteogenic supplements (dexamethasone, â-glycerophosphate ascorbic acid) for 21 days. A 1 Hz/60 milliTesla, (max.) magnetic field generated by an oscillating NdFeB magnet array was applied to the cells plus adhered particles each day for 30 minutes (fig.1).

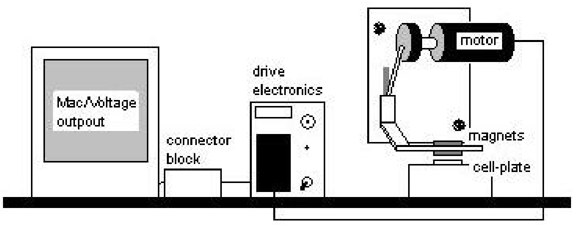

Fig.1: *Sketch of set-up used to apply cyclical magnetic field to cell/magnetic particle samples.*

Six sample groups were analysed (table 1). At 21 days, live/dead fluorescent staining (propridium iodide/syto9, Molecular Probes) and SEM analysis were performed to evaluate cell viability and morphology. Also at 21 days, a von kossa stain for phosphate deposits (mineralized bone matrix) was performed on all six samples. At this same time point, real-time RT-PCR was performed to quantify bone-related gene production of type I collagen, osteocalcin and osteocalcin normalised to GAPDH. Prostaglandin E_2 present at day three and osteocalcin present at day 21 in the culture media were quantified by ELISA. Laser scanning confocal microscopy (LSCM) was performed at week 1 on sample group (3) to visualize actin filaments and cytoskeletal arrangement.

Table 1. Sample groups and description.

(1)	Cells plus particles, with applied magnetic field, with 30 minutes at room temperature (as magnet was applied at room temperature for 30 minutes).
(2)	Cells with no particles, with applied magnetic field, with 30 minutes at room temperature.
(3)	Cells with particles, with 30 minutes at room temperature, with no magnetic field applied.
(4)	Cells with no particles, with 30 minutes at room temperature, with no magnetic field applied.
(5)	Cells with no particles, maintained in a CO2 incubator with no magnetic field applied.
(6)	No Cells, no magnetic field, maintained in a CO2 incubator.

RESULTS: Flourescent imaging showed for sample groups (1) – (5) a viable, confluent monolayer of cells covering the surface of the wells in the tissue culture plates. SEM imaging showed confluent cells in monolayer spread out and attached to the surface of the tissue culture well in samples (1) - (5). Von Kossa staining of the wells in sample groups (2) – (6) showed no phosphate deposition. However, sample group (1) (the experimental group) showed a small amount of mineralization. Real-time RT-PCR showed an upregulation of osteopontin in sample group (1) (the experimental group) in comparison to the control sample group (3) (sample group consisting of cells with particles but no magnetic field) (figure 3).

Fig. 2: Flourescent microscope image of phalloidin stained actin filaments of 3 week primary human osteoblasts with internalised microparticles (left image) DAPI stain of nucleus (light microscope) of same cell (right image)

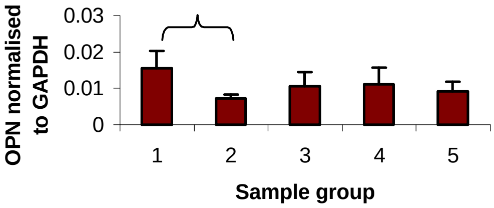

Fig. 3: Real-time RT-PCR data showing osteopontin (OPN) levels normalised to GAPDH. $p<0.05$.

There was no significant difference in the mRNA levels of osteopontin expressed between the other sample control groups. There was also no significant difference in the amount of osteocalcin or type 1 collagen mRNA produced at 21 days between sample groups (1) – (5).

The ELISA's performed showed a statistically significant ($p<0.05$) upregulation of prostaglandin E_2 from group (1) (the experimental group) in comparison to groups (2), (4), (5) and (6) (fig. 4). There was no significant difference in prostaglandin E_2 production between group (1) and group (3). The osteocalcin ELISA performed showed no significant difference between the sample groups at day 21.

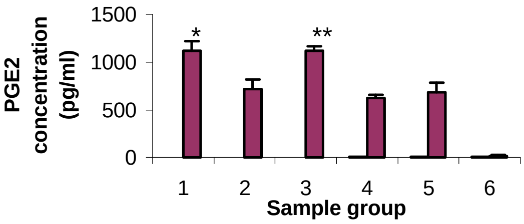

Fig. 4: *Prostaglandin E_2 ELISA data from three day culture media. * Group (1) is significantly different from groups (2), (4) – (6) ($p<0.05$). ** Group (3) is statistically significantly different from groups (2), (4) – (6) ($p<0.05$).*

The LSCM performed showed no significant alteration in the actin filament structure in the osteoblasts. It was clear that the majority of the particles had been internalized by the cells and that many of these particles appeared to be located close to the nucleus (figure 2).

DISCUSSION & CONCLUSIONS: The live/dead stain performed showed that the presence of the magnetic particles was not causing cell necrosis and particles were still present at 21 days. The small amount of mineralisation (as seen by the von kossa stain) in the sample group that had adhered particles plus magnetic field applied may be due to an upregulation in bone matrix production by the cells in response to mechanical stimulation from the particles in the applied magnetic field. Also, real-time RT-PCR results show an upregulation of osteopontin in experimental sample group (1) in comparison to group (2) which may also correlate to the same mechanical stimulation response. The increase in prostaglandin production in groups (1) and (3) in comparison to the other groups may be due to the internalization of the particles, which may also result in prostaglandin release. A comparison of the relationship between internalization effects and mechanical stimulation is currently being investigated which includes understanding how these particles are internalized.

Initial results indicate that adherence of RGD-coated, 4.5 ìm ferromagnetic particles to primary human osteoblasts does not initiate cell necrosis up to 21 days *in vitro*. Also, though these results are preliminary, mechanical stimulation of primary human osteoblasts by magnetic particle technology appears to have an influence on osteoblastic activity.

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