Enhancing the Osteogenic Potential of Bioabsorbable Implants through Control of Surface Charge

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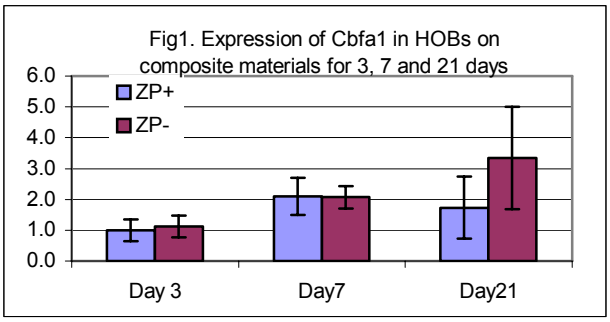
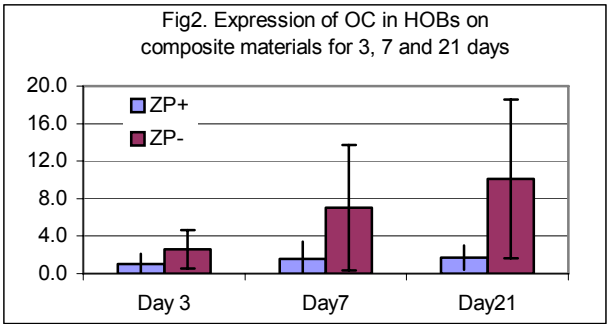
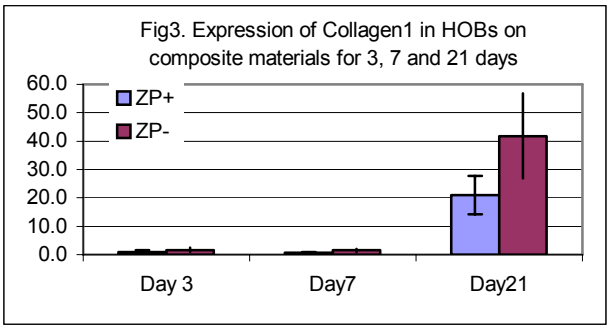
Statement of Purpose: Cells and proteins are colloidal particles. In aqueous suspensions, *in vitro* or *in vivo,* their interactions are influenced by the relative sign and magnitude of the acquired surface charge (zeta potential - ZP). Any implant material will also display a surface charge and it is therefore reasonable to assume that the charge exhibited by an implanted material will influence its biological response. Eriksson[1, 2] reported on the relationship of surface energies and charge effects on the bone induction principle and showed, from ZP measurements and data from Urists seminal study[3], that the materials having the highest osteoinductive potential had the greatest negative surface electric charge. Krukowski in a series of papers[4, 5, 6] demonstrated a significant *in vivo* response of both hard and soft-tissue to charged resin beads. The objective of the present study was to assess the osteogenic response of human osteoblasts (HOBs) to calcium phosphate (TCP) bone graft particles having positive and negative ZP values.

Methods: Both positive and negatively charged TCP powders were moulded into a poly-L-lactic acid matrix to provide a convenient, 7mm X 5mm flat surface 'carrier' for standardised cell seeding conditions. Ten samples were used per material per time point. The studies were carried out using primary human osteoblasts (HOBs) in the first passage, grown in Dulbecco's modified eagles medium (DMEM) containing 10% fetal calf serum (FCS, Sigma, Poole, UK), 50µg/ml ascorbic acid and 10nM dexamethasone in an incubator at 37°C and humidified atmosphere of 95% air and 5% CO_2. Biomaterial contact was performed with cells expanded to the third passage. Suspensions of 50µl (5×10^4 cells per one sample) were seeded onto the test materials in 24 well plates. The HOBs were cultured in direct contact with both materials for 3, 7 and 21 days. Quantified evaluations of changes in gene expression were performed by real time RT-PCR for the osteogenic markers (i.e. proteins involved in the bone-healing cascade): Alkaline Phosphatase (ALP), CBFA-1, Collagen 1 (Coll 1), Osteocalcin (OC), Osteonectin (ON) and Osteopontin (OP).

Results/Discussion: Total RNA concentration, indicative of cell proliferation, and ALP, an osteogenic marker, were similar for both materials at all time points indicative of the favourable response of HOBs to both materials. However, CBFA-1 (Fig 1), OC (Fig 2), Coll I (Fig 3), ON and OP all showed enhanced up-regulation on the negatively charged TCP compared to the positively charged material, indicating an enhanced osteogenic response on this negatively charged substrate.







Conclusions: The importance of ZP to HOBs activity *in vitro* is confirmed. A negatively charged substrate presents a more favourable surface for osteogenic activity. Future studies will establish whether the *in vitro* response translates to *in vivo* performance.

References:

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