

## Establishing An Osteoblast:Osteoclast Co-culture System For Use In Bone Tissue Engineering

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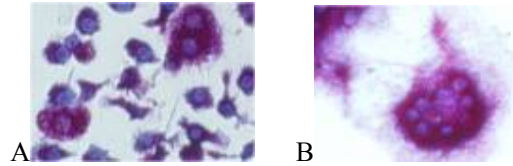
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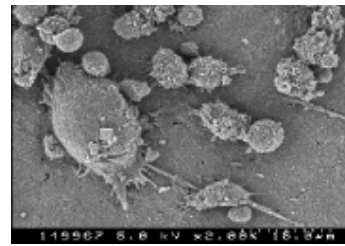
**INTRODUCTION:** Current bone tissue engineering strategies often focus on creating constructs consisting of a degradable scaffold and a single cell type, usually osteoblasts, the mineralised matrix producing cell of bone tissue. We hypothesise that by co-culturing osteoclasts on the same construct with osteoblasts, that the cell signalling pathways that are inherent in the physiological body, will, at a basic level, be replicated in the in-vitro 3D environment. This cell-cell communication has the potential to actively assist in the creation of a functional tissue engineered bone construct. The resorbing of the calcium based scaffold by the osteoclasts on which the co-culture is presiding may in turn promote the osteoblasts to produce an increased amount of mineralised matrix in comparison to a scaffold seeded with just osteoblasts alone. In this way we anticipate that this dynamic co-culture system will have synergistic effects on mineralised matrix deposition thus creating a functional tissue engineered construct in a shorter preparation time in-vitro whilst maintaining the potential of an increased acceptance rate into the body once implanted into a fracture site in-vivo. In this study, we describe our initial experiments in setting up this co-culture where we have analysed osteoclast activity on a 2D dentine slice using traditional analysis of SEM and histology as well microCT imaging.

**METHODS:** Monocytes were isolated from murine bone marrow, seeded onto dentine slices (5mm diameter, 3µm thick, IDS) at a density of  $1 \times 10^6$  cells per slice. The monocytes were then differentiated towards osteoclasts using macrophage colony stimulating factor (MCSF) and receptor activator of NFκB ligand (RANKL)<sup>1</sup>. Osteoclasts were visualised using Tartrate Resistant Acid Phosphatase (TRAP) positivity in multinucleate cells after 4 and 7 days culture in conjunction with microCT and SEM analysis for resorption pit determination.

**RESULTS:** *Figure 1*  
*MicroCT image of dentine slice prior to culture*



*Figure 2 A. TRAP positive cells B. TRAP positive multinuclear osteoclast cell (x40)*



*Figure 3 SEM image of cells adhered to a dentine slice after 4 days of culture. (x2000)*

**DISCUSSION & CONCLUSIONS:** We have established a murine system of differentiating osteoclasts from bone marrow derived monocytes that is consistent with the published literature. MicroCT analysis of the calcium phosphate matrices used in this project is proving an effective high resolution tool to locate and quantify mineralised matrix turnover. Our future studies now will look into the cell seeding ratio of osteoblast (derived from both murine bone, mesenchymal progenitors in the bone marrow) versus osteoclasts (derived from haemopoietic progenitors in the murine bone marrow) on dentine slices using these same techniques. Once we have established this information we will begin to quantitate the efficacy of this technique on novel scaffolds for bone tissue engineering including collagen/hydroxyapatite scaffolds in collaboration with Dr Jan Czernaska, Oxford University and Dr Antonella Motta, University of Trento.

**REFERENCES:** 1. Dotard et al. 2005. *Clinica Chimica Acta.* 356(1-2): 154-63

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