Specific analysis of osteoclast-mediated bone resorption by differentiation of primary human osteoclasts in 3D

Objectives. Osteoclasts are specialised bone-resorbing cells that drive the pathogenic osteolysis characteristic of varied conditions including rheumatoid arthritis (RA), osteosarcoma and multiple myeloma. The presence of osteoclasts within diseased tissue is commonly associated with aggressive disease and poor prognosis. *In vitro* investigation of factors affecting osteoclast activity is complicated by the inability to easily distinguish from effects on differentiation, which must be performed *in situ* on the resorption substrate. We have therefore developed an alternative 3D method of osteoclast differentiation, which allows specific investigation of the bone resorption capacity of mature osteoclasts when re-seeded in 2D culture.

Methods. CD14+ monocytes were selected from peripheral blood and seeded into 2 mg/ml collagen gels in 24 well plates at 1 x 10^6 CD14+ MON per gel. Osteoclastogenesis was initiated by application of M-CSF and RANKL for 8 days. Mature osteoclasts were released from the gel by digestion with collagenase I and re-seeded onto dentine discs for analysis of resorption (toluidine blue staining).

Results. Similar rates of osteoclast formation were observed in 2D and 3D culture by brightfield microscopy. Once released and re-seeded, the final size of the osteoclasts formed with respect to number of nuclei was also similar. Mature osteoclasts re-seeded onto dentine discs were able to resorb bone and responded to resorption stimuli such as hypoxia (2% O_2, 24 h, 2.6-fold induction, $p<0.05$) and HIF stabilisation (PHD enzyme inhibition, 25 μM FG4592, 2.1-fold induction, $p<0.05$).

Conclusion. Differentiation of osteoclasts in 3D collagen gels allows direct re-seeding of mature osteoclasts onto dentine discs for specific analysis of bone resorption activity.

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