In vitro 3D tissue modelling: insights into ameloblastoma pathogenesis

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INTRODUCTION: Ameloblastoma is a rare, benign oral tumour. Tumours develop within the jaw bone and are highly destructive and invasive, with cells migrating into the jaw and surrounding soft tissue. This is a little-understood disease which if left untreated causes dramatic bone destruction and maxillofacial disfigurement. Current treatment is radical surgery, often resulting in extensive loss of function and tissue. An ameloblastoma-derived cell line, AM-1, has been established. Cells were isolated from a human tumour and immortalised by the addition of HPV-16 DNA. This study aims to (i) make a 3D in vitro ameloblastoma disease model, using plastic-compressed collagen gel seeded with AM-1 cells, and (ii) use this bone construct to characterise tissue remodelling, cell growth and invasiveness.

METHODS: Collagen type I, isolated from rat tails (First Link UK), was used to make hydrated gels suitable for seeding cells. Ameloblastoma AM-1 cells were added to the gel. Plastic compression was then used to expel the water content, rapidly increasing the gel’s mechanical strength without compromising cell viability. Compressed gels were rolled into spirals to provide easy handling and provide a biomimetic 3D environment to observe cell viability and behaviour. Gels were incubated at 37°C with 5% CO₂, in keratinocyte-serum-free medium (Gibco) supplemented with fibroblast growth factor (Peprotech UK).

Constructs were cultured for up to 4 weeks to observe the extent of collagen remodelling and differences in cell viability at different time points. Immunohistochemistry was performed to visualise the expression of bone- and cancer-associated proteins (Abcam, Vector Labs). The alamarBlue cell growth assay (AbD Serotec) and live/dead fluorescence assay (Invitrogen) were carried out to assess AM-1 cell growth and viability in bone construct culture. IHC was carried out at the Royal London Hospital Pathology laboratory.

RESULTS: AM-1 cells are viable in this bone construct, demonstrated by the alamarBlue growth curve and live/dead fluorescence assay (see Fig. 1). Expression of epithelial marker vimentin, early bone marker alkaline phosphatase and matrix metalloproteinase-2 (MMP-2) was detected in AM-1 seeded bone constructs (see Fig. 2).

DISCUSSION & CONCLUSIONS: Compressed collagen gel is an appropriate tissue model for research into ameloblastoma, as it is a native material which provides good mimicry of bone tissue; its construction is ultrarapid and reproducible; and it allows cell growth and migration. AM-1 cells proliferate in the bone construct, as shown by the viability assays. Expression of vimentin, alkaline phosphatase and MMP-2 show that this disease model retains in vivo behaviours of ameloblastoma, therefore we have made progress towards a representative disease model.


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