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 [1]. 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 [2] 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 [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 [2]. 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% CO_2, 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).

Fig. 1: Live/dead fluorescence assay; green = live, red = dead. AM-1 cells are viable in the construct.

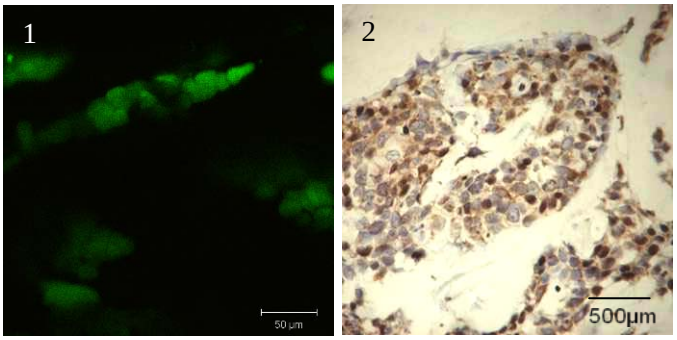

Fig. 2: IHC shows vimentin expression (brown) in AM-1 seeded bone construct.

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.

REFERENCES: [1] H. Harada, T. Mitsuyasu, N. Nakamura, Y. Higuchi, K. Toyoshima, A. Taniguchi (1998) *J. Oral Pathol. Med.* **27**: 207-212. [2] R.A. Brown, M. Wiseman, C. Chuo, U. Cheema, S.N. Nazhat (2005) *Adv. Funct. Mater.* **15**: 1762-1770.

ACKNOWLEDGEMENTS: This work was funded by an anonymous donor and the UCL Development Fund. Laboratory instruction was provided by Pauline Levey and Dr Nicky Mordan. The AM-1 human ameloblastoma cell line was a kind gift from Professor Harada, Iwate Medical University, Japan.