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Collagen gel as a 3D *in vitro* tissue model for ameloblastoma studies

C. Raison, S. Porter, S. Fedele, M. P. Lewis[1], V. Salih

Division of Biomaterials & Tissue Engineering, UCL Eastman Dental Institute and [1]University of Bedfordshire, 256 Gray's Inn Road, London, WC1X 8LD, UK

INTRODUCTION: Ameloblastoma is a rare locally invasive epithelial odontogenic tumour of the jaw which can cause significant and debilitating bone destruction. *In vitro* studies of ameloblastoma are sparse in the literature, and little is known regarding patterns of ameloblastoma cell growth and invasion, as well as relevant gene and protein expression. This study aims to (i) use plastic-compressed collagen gels as a robust and relevant biomimetic to culture ameloblastoma cells in a 3D *in vitro* tissue model [1] and (ii) perform histology, immunohistochemistry (IHC) and gene expression assays 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 cell line [2] and HOS and MG-63 osteoblast-like 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 [1]. Compressed gels were rolled into spirals to provide easy handling and provide a multi-layered 3D environment to observe tissue remodelling and cell distribution. Gels were incubated at 37°C with 5% CO_2, in the medium appropriate to the cell line (Gibco).

Gels were bathed in either mineralising or normal media, for up to 4 weeks in culture, to observe the extent of collagen remodelling and differences in gene expression at different time points. Each gel was halved at the end of its culture period. One half was processed for histology and the other used for TRIzol-based RNA extraction (Invitrogen) and subsequent reverse transcription and qPCR (Applied Biosystems). H&E staining was performed to histologically assess tissue remodelling and cell migration (histology was carried out at the Royal London Hospital Pathology laboratory). IHC will be performed to visualise the expression of bone- and cancer-associated proteins.

RESULTS: Expression of bone-associated proteins osteonectin and alkaline phosphatase, and ameloblastoma-associated proteins bcl-2 and MMP-2 will be detected by qPCR. Antibodies against bone-related and cancer-related proteins will be used in IHC visualisation. Pending results will help to characterise growth patterns across the different cell types, media types and culture times.

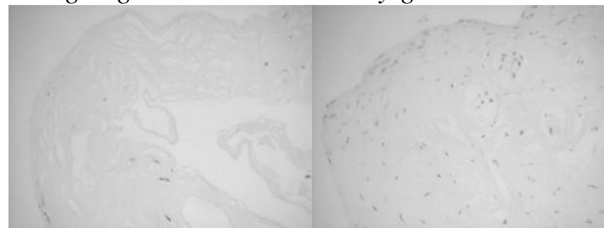

Fig. 1: Sample H&E-stained transverse sections of collagen gel rolls. These 20 day gels contain HOS cells and were cultured in normal (left) or mineralising (right) medium, producing different patterns of collagen disruption, cell viability and cell distribution.

DISCUSSION & CONCLUSIONS: Compressed collagen gel is an appropriate tissue model for research into ameloblastoma, due to its biological relevance; its ultrarapid and reproducible construction; tolerance of changing the experimental variables; and its support of cell viability and migration. Histology of processed gels and qPCR of extracted RNA will characterise cell growth and migration, collagen remodelling and gene expression in AM-1, HOS and MG-63 cell-seeded collagen gels under different culture conditions.

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