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Development of a macrophage-containing in-vitro 3D model of oral cancer

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Objectives: Tumour-associated macrophages (TAM) represent a prominent component of the leukocytic infiltrate of human tumours and their accumulation in oral squamous cell carcinoma (OSCC) has been shown to be a predictor of poor prognosis. Evidence suggests that the tumour microenvironment drives TAM into a pro-tumour phenotype that exacerbates tumour growth but evidence for this in OSCC is lacking. In this study, we further develop a 3D *in vitro* model of OSCC to incorporate human macrophages to be used as a tool to examine the role of TAM in OSCC.

Methods: Human monocyte-derived macrophages were cultured with human oral fibroblasts within a type 1 collagen hydrogel with either human normal oral keratinocytes or OSCC cell lines seeded on top and cultured at an air-to-liquid interface. After 14 days, key features of the tumour microenvironment were measured using ELISA, immunohistochemistry and rheology. Macrophages were retrieved from the connective tissue by collagenase digestion and analysed using qPCR and 8-colour flow cytometry.

Results: Macrophages were viable and functional after 14-day 3D culture, with IL-6 and CXCL8 release observed upon LPS stimulation. Comparison of a malignancy-free environment to an induced tumour microenvironment showed a positive correlation between OSCC cell invasion and matrix stiffness that was enhanced by the presence of macrophages. In addition, flow cytometric analysis showed a marked increase in the number of macrophages expressing key markers, such as CD163 in an induced cancer compared to normal environment.

Conclusions: Macrophages remain viable and responsive to exogenous stimuli even when cultured within a 3D *in vitro* model for prolonged periods. These immuno-responsive 3D models provide a new, reproducible and adaptable tool that are able to mimic the tumour microenvironment during OSCC and therefore will be of great use in gaining a deeper understanding of the role of TAM and the tumour stroma in OSCC progression.