**Develop the co-culture models of human Uveal Melanoma and macrophages**

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**Background and aims.** Uveal melanoma (UM) is the primary intraocular cancer in adults with a 5-year survival rate of ~15% in metastatic UM patients. There are few therapeutics for UM, primarily due to a lack of physiologically relevant models to replicate the tumour microenvironment (TME). UM develops in an immune-privileged site without lymphatic drainage and resident macrophages to support its growth and immune evasion.

The traditional 2D cultures cannot accurately replicate the TME; however, 3D models better reflect tumour physiology. To mimic UM and its TME, this study aims to develop and characterise co-cultures of UM cells and macrophages in 2D and 3D format.

**Methods.** The typical UM cell line 92.1 were co-cultured with U937-derived macrophages on normal or transwell plates to obtain the 2D co-culture models, as well as bioprinted into 3D format with Rastrum bioprinter. The resultant UM-macrophage co-cultures were molecularly characterised for cell morphology with microscopy, cell growth using Incucyte imaging machine and metastatic potentials with cell migration assay. Subsequently, their response to drug treatment was assessed with a lead compound that has shown a high potency against UM.

**Results.** U937 cells were successfully induced to generate prototype (M0), pro-inflammatory (M1), and anti-inflammatory (M2) macrophage phenotypes. M0 and M2 macrophages increased the proliferation of 92.1 cells, while M1 macrophages suppressed this. M0 and M2 macrophages significantly induced 92.1 migration after 72 hours, but M1 macrophages have no impact on that. M1 macrophages enhances the sensitivity of 92.1 to drug treatment; M2 macrophages reduces its sensitivity and M0 macrophages have no impact.

**Conclusion/Discussion.** These findings suggest that UM cells behave differently when co-cultured with distinct macrophage phenotypes in 2D and 3D format. The use of 3D UM-macrophage co-culture systems are more predictive and physiologically relevant to study UM biology and develop new therapeutics.

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1. Liau, S. et al. (2023) Biochimie. 25;212:114-122.
2. Cha, YS. et al. (2025) Recent advances in 3D cell culture models in cancer drug development. J Pharm Investig. (Accepted)