Advanced *in vitro* platforms for investigating melanoma-skin interactions

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Introduction

Malignant melanoma is the most aggressive form of skin cancer, and despite recent therapeutic advances remains a major clinical challenge due to frequent therapy resistance and metastatic potential. Although significant progress has been made in targeted therapies, effective preclinical translation remains limited, as conventional 2D culture systems and animal models only partially recapitulate the complex architecture of human skin and its tumour microenvironment. To address these limitations, we have developed physiologically relevant human-based *in vitro* models of melanoma-skin interaction[1,2]. These include skin organoids (SO) derived from human induced pluripotent stem cells[3] and full-thickness skin equivalents[4] (FTSE) generated from primary skin cells, which provide a promising *in vitro* platforms to investigate melanoma progression and tumour-stroma interactions under more physiologically relevant conditions.

Organotypic Full-Thickness Model of Malignant Melanoma

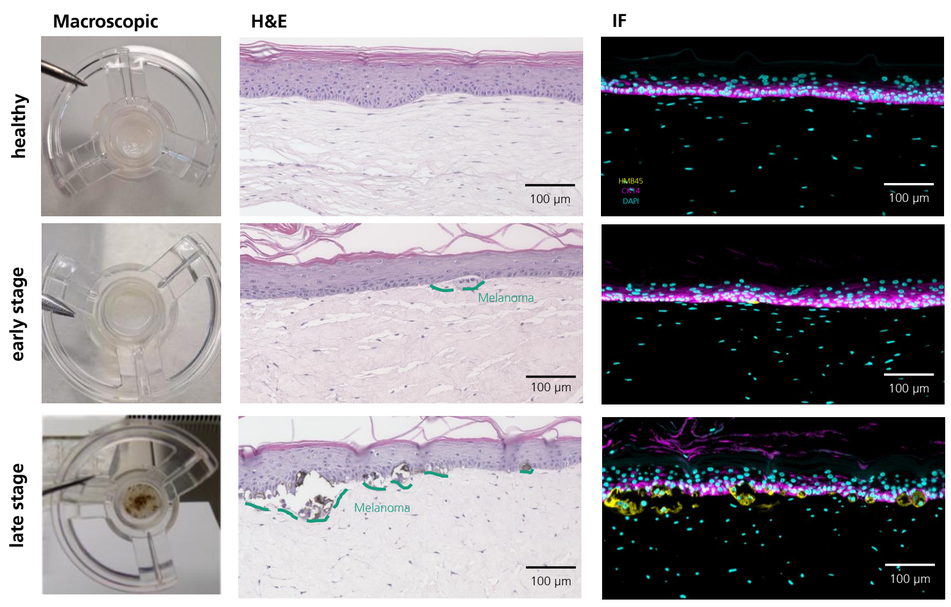


Fig. 1: Generation and histological characterisation of dermal-epidermal melanoma model representing early and late tumour stages

Macroscopic images, hematoxylin and eosin (H&E) staining, and immunofluorescence (IF) analysis of melanoma models showing stage-dependent tumour development. Microtumours were visible macroscopically as dark spots. H&E staining revealed clearly demarcated melanoma clusters. Late-stage models exhibited more numerous and larger **tumour nests** than early-stage models, **primarily located at the epidermal-dermal junction** and surrounded by healthy tissue, reflecting their physiological localization in native skin. IF staining for HMB45 and CK14 further confirmed increased tumour nest formation in late-stage skin equivalents.

Integration and Evaluation of the Influence of Cancer Associated Fibroblasts

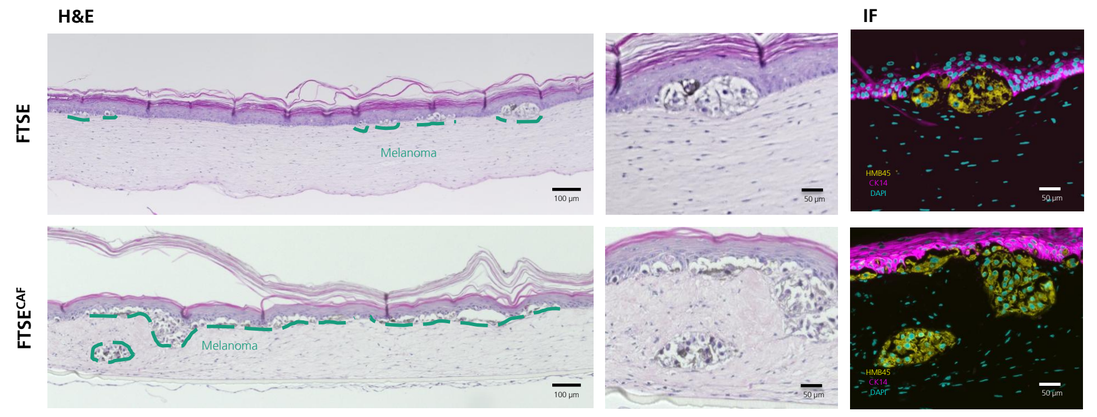


Fig. 2: Histological analysis of dermal-epidermal melanoma models with cancer-associated fibroblasts (CAFs)

Melanoma full-thickness skin equivalents (FTSEs) were generated with different fibroblast populations to assess the impact of cancer-associated fibroblasts (CAFs) on tumour progression. Human dermal fibroblasts (hdFs) were either used in their unactivated state or CAF-like phenotype prior to incorporation into the dermal compartment of the melanoma models. Models containing activated CAF-like hdFs exhibited partially thinner epidermal strata and developed a greater number of larger and more irregular melanoma nests. Immunofluorescence revealed CK14-positive basal layers in all models, while HMB45-positive melanoma cells were distributed throughout the tumour nests and infiltrated the dermal compartment more extensively in CAF-containing models. By contrast, models with unactivated fibroblasts displayed smaller, more confined tumour clusters with limited invasion, indicating that **CAF-like fibroblasts promote melanoma progression, enhanced tumour invasiveness, and deeper dermal infiltration within the *in vitro* skin equivalent**.

Establishment of a Melanoma Skin Organoid

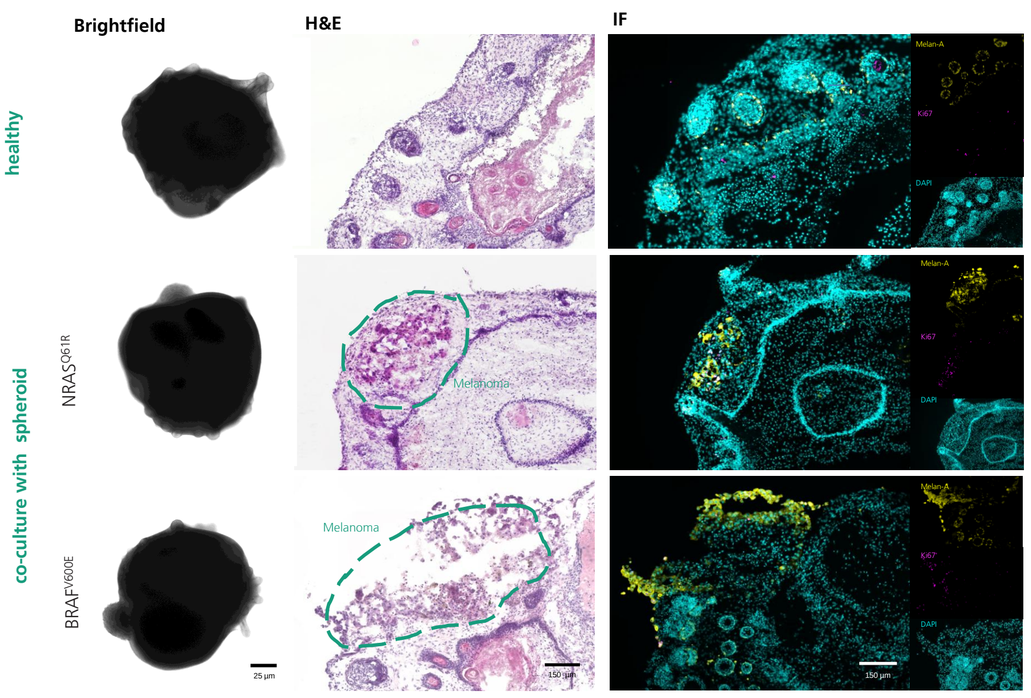


Fig. 3: Melanoma infiltration and interactions in human skin organoids

Melanoma spheroids with distinct BRAF and NRAS mutations were co-cultured with human skin organoids. Brightfield imaging shows spheroids adhered to the organoid surface, indicating successful establishment of the co-culture. H&E staining confirms **visible tumour attachment, integration, and initial structural changes at the interface with the organoid tissue**. Areas of melanoma invasion associated with altered extracellular matrix structure are outlined in green, illustrating the onset of tumour-induced stromal modulation. Immunofluorescence analysis for Melan-A and Ki67 showed that, while Melan-A-positive cells were confined to hair roots in control organoids, in co-cultured organoids **melanoma cells infiltrated deep into the organoid**, highlighting early invasion into the dermal compartment. PCR analysis reveals significantly elevated MMP3 expression and decreased MMP2 levels across genotypes, consistent with shown early ECM remodelling and limited basement membrane penetration.

Summary

These advanced *in vitro* models provide **human-specific tools** for studying melanoma-skin interactions. Hair-bearing skin organoids have been shown to replicate the structural complexity of human skin, and full-thickness skin equivalents offer a precise experimental set-up. Together, these features overcome the limitations of conventional models, thereby enhancing the value of preclinical research and drug testing. Collectively, they **support the development of more efficacious melanoma therapies.**

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