Development of a 3-D melanoma model for anti-cancer therapy assessment

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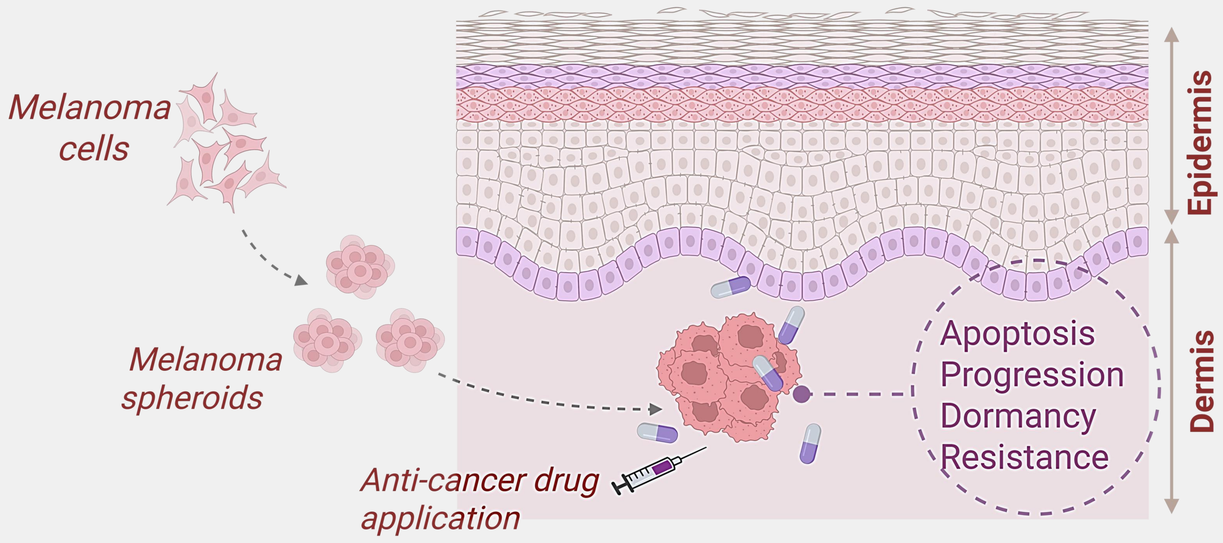
Empa Materials Science and Technology

TOXBOX

Background & Objective

- **Melanoma** is an aggressive form of skin cancer with metastatic potential.
- Challenges such as therapy resistance and non-responsiveness limit therapy options for patients.
- Clinically-relevant *in-vitro* 3-D models offer a platform to investigate melanoma progression and evaluate novel therapy.

Aim: This study aims to develop a **scaffold-free 3-D melanoma model** that recapitulates the tumour microenvironment and cellular interactions. The models is used to evaluate the response of melanoma tumour to Vemurafenib, a targeted BRAF inhibitor.



Summary

- **2-D culture:** A375 melanoma cells were susceptible to Vemurafenib-induced cytotoxicity compared to normal skin cells.
- **3-D spheroids:** Vemurafenib suppressed melanoma spheroid growth and reduced spheroid viability.
- **Melanoma skin models:** Vemurafenib decreased melanoma spheroid size and induced caspase-3 activation. Presence of melanoma spheroids within the skin models led to extracellular matrix (ECM) modulation.
- Future studies will investigate mechanisms of **drug resistance** after prolonged Vemurafenib exposure.

Approach

- Melanoma spheroids were generated by self-assembly of BRAF+ A375 melanoma cells in low-adhesion plate.
- Fibroblasts were coated alternatingly with fibronectin and gelatin and assembled with the melanoma spheroids.
- Keratinocytes were added on the top of the dermis and exposed to air to initiate epidermal differentiation

Key Findings

Vemurafenib selectively inhibits melanoma cells

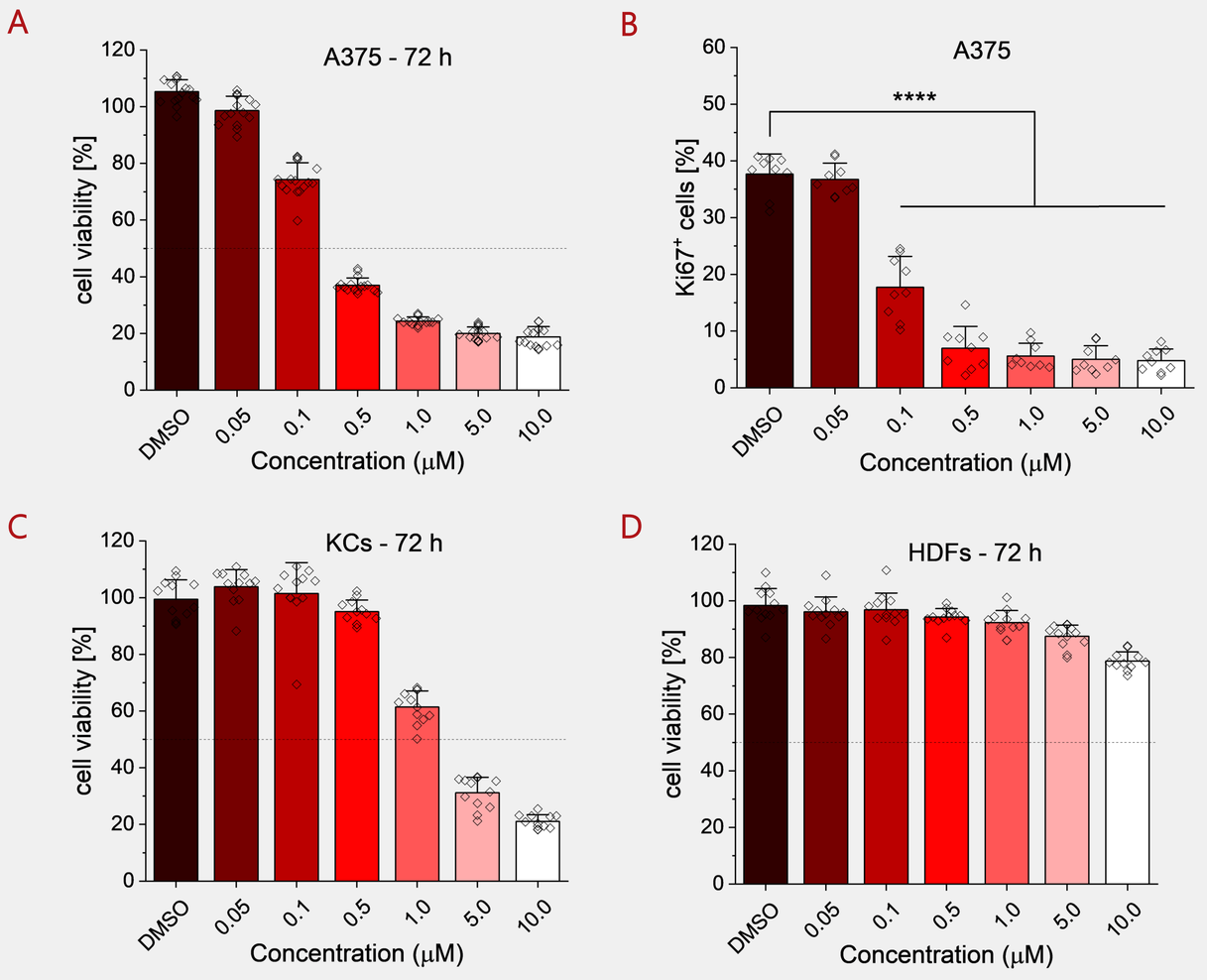


*Figure 1: A) Vemurafenib reduce 2-D melanoma cell viability (n=3) B) Vemurafenib suppress melanoma proliferation, as shown by lower ki67 labelling (n=3, **** p<0.0001 C,D) Keratinocytes and fibroblasts remain viable following exposure to 0.5 μM vemurafenib (n=3, dotted line= 50% viability).*

Vemurafenib triggers melanoma apoptosis in 3-D models

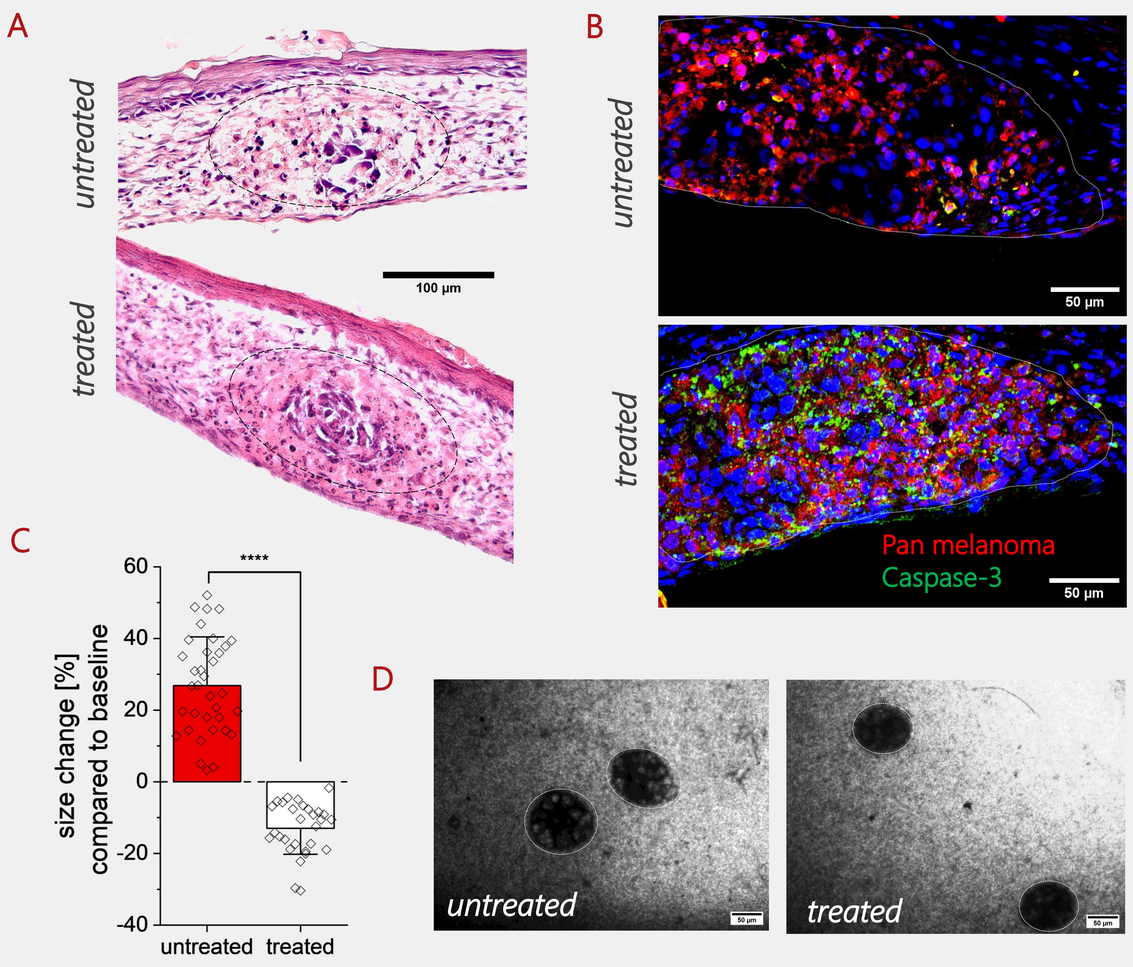


*Figure 3: A) H&E staining of the untreated and vemurafenib-treated 3-D melanoma models, B) immunofluorescence of pan-melanoma markers and caspase-3 C,D) Quantification of the spheroid size show significant reduction post-treatment (n=4, **** p<0.0001)*

Vemurafenib reduces melanoma spheroid size and viability

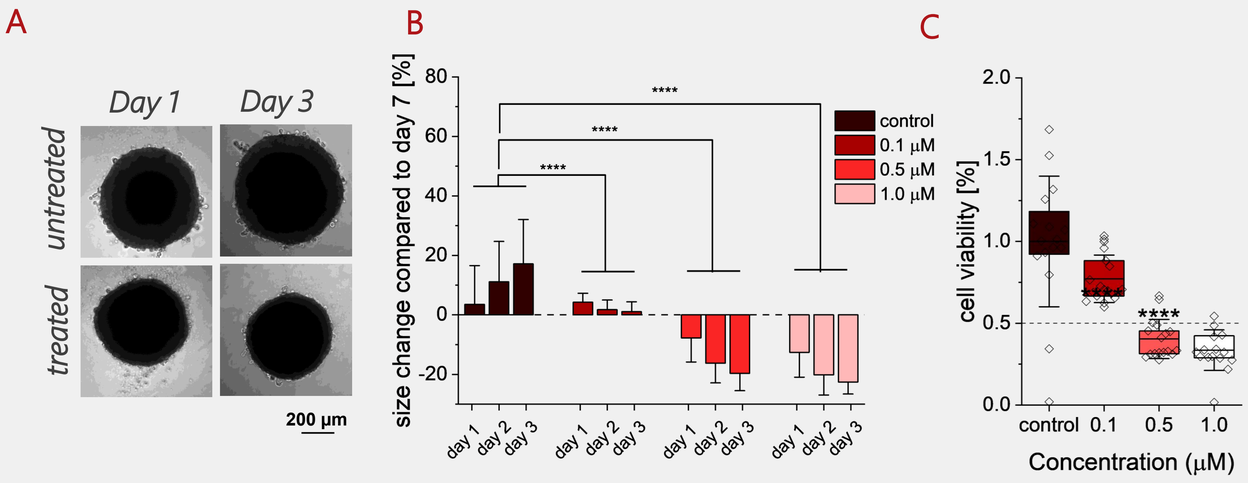


Figure 2: A, B) Quantification of dose-dependent effect of vemurafenib on spheroid size (n=3) C) Spheroid viability decrease with increasing concentration of vemurafenib (n=3, dotted line=50% viability)

Melanoma induces ECM remodelling in 3-D skin models

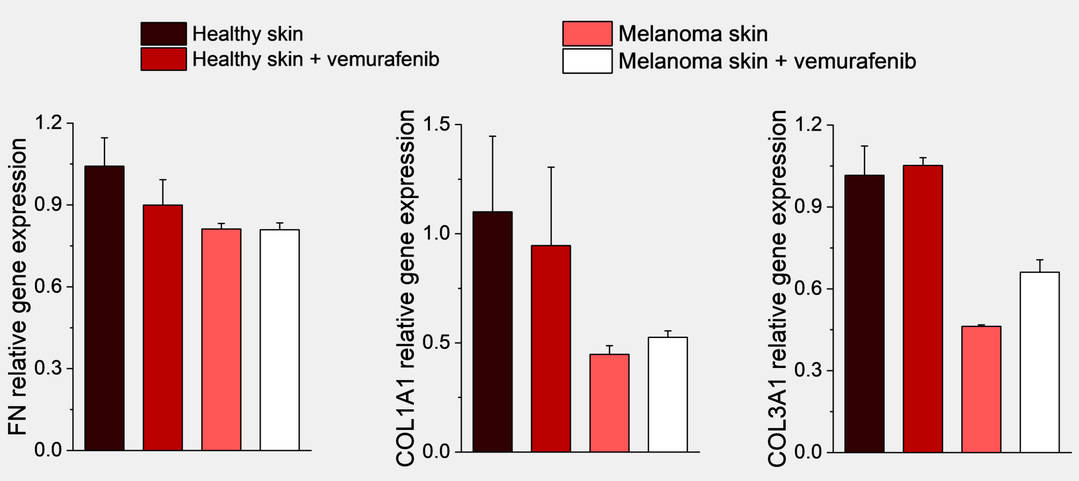


Figure 4: qPCR analysis of FN, COL1A1, and COL3A1. Presence of melanoma spheroid reduce COL1A1 and COL3A1 expression, while FN shows only a slight decrease (n=2 biological replicates, 2 technical replicates)