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# SCIENTIFIC **Reports**

### Tissue-engineered 3D melanoma model with blood and lymphatic capillaries for drug development

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## Background and introduction

#### PROTOCOL

### Tissue-engineered 3D human lymphatic microvascular network for *in vitro* studies of lymphangiogenesis

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- One of the central issues with cancer therapy development is the dismal low rate of reproducibility between results observed in animal models and in the patients: less than 10% of data obtained from animal models can be translated to humans.
- To improve preclinical screening and reduce the use of animals, more physiological human models are needed.
- Tissue-engineered skin allows the production of 3D tissues partially reproducing either the native or the tumor microenvironment (TME).

# Method of production of the 3D microvascularized skin melanoma model.



### Assembly of HMVEC into blood and lymphatic capillaries in the 3D melanoma model



Representative flow cytometry analysis of the cultured HMVEC population indicating the presence of two distinct populations (BEC and LEC) before their incorporation into the reconstructed skin. (**B**) Lumens (arrows) were observed after hematoxylin and eosin staining of HMVEC-enriched skin sections, but not in (**C**) non-endothelialized skin (scale bars: 50 µm). *s: stratum corneum*, *e*: epidermis, *d*: dermis.



(**D**–**I**) Immunolabelings revealed the presence of two networks after staining for (**D**,**G**) podoplanin (PDPN, red signal) and (**E**,**H**) CD31 (green signal), **F** and **I** represent merge signals (scale bars: **D**–**F**: 50 μm, **G**–**I**: 150 μm).

Confirming metabolic activity of capillary networks: HMVEC specifically secrete (J) angiopoietin-2 (ANG-2) in the reconstructed skin. \*\*p-value  $\leq 0.01$ , Statistical analysis: Student t-tests.



Formation of melanoma spheroids using the hanging drop method.



Spheroids formed by (**A**) Malme 3 M, (**B**) A375, (**C**) RPMI 7951, (**D**) SK-MEL 28, (**E**) WM983A, (**F**) WM983B cell lines after 14 days of culture in GravityTRAP<sup>TM</sup> plates. (Scale bars: 100  $\mu$ m). (**G**) Hematoxylin and eosin staining of paraffin embedded spheroid section showing heterogenous composition and formation of compact spheroids by WM983A cells (Scale bar: 100  $\mu$ m). (**H**) Tumor spheroids incorporated to skin tissues can easily be located (red arrows, Malme 3 M cells). Scale bar: 1 cm.



Tumor growth in a tissue-engineered microvascularized environment. (A-C) Immunofluorescence of Malme-3M melanoma cells (red/pink signal) on (A) 8 µm and (B,C) 70 µm transverse cryosections of the complete melanoma model. Podoplanin staining (green signal) revealed lymphatic capillaries in the dermis. The dermo-epidermal junction is indicated by a white dotted line. Nuclei were counterstained with Hoechst (blue). A375 and RPMI 7951 melanoma cells secreted significant amount of (D) pro-lymphangiogenic factor VEGF-C and (E) VEGF-A as measured by ELISA in media conditioned by spheroids only. (N = 2donors, performed in triplicate). \*\*\*pvalue  $\leq$  0.001. Statistical analysis: one-way ANOVA with Bonferroni post-test. Scale bars: (A) 50 µm, (**B**,**C**) 100 µm.



Melanoma spheroids integration into the human reconstructed cutaneous microenvironment. (A–D) Immunostainings of selected cutaneous proteins (green signal): (A) fibronectin staining of the dermis, (B) laminin 332 staining indicating the presence of a basement membrane, (C) filaggrin and (D) involucrin stainings (scale bar: 100 µm). Melanoma cells (SK-MEL 28) are labeled in red following premelanosome protein (PMEL) staining while nuclei are stained with Hoechst (blue signal). (A'-D') Magnified views of the boxed areas in (A-**D**) respectively. (**E**) Hematoxylin and eosin staining of the melanoma model (SK-MEL 28), with (E') boxed area (Scale bars: 200 µm, boxed areas: 50 µm). Results are representative of two independent experiments, performed in triplicate.

# Change in melanoma tumor morphology by vemurafenib treatment.



(A) Hematoxylin and eosin staining of WM983A melanoma model revealed a proliferative nodule morphology whereas (B) the WM983B model showed a more invasive morphology. (C) Macroscopic aspect of non-treated WM983B model with dense tumors and (D) corresponding histological appearance after hematoxylin and eosin staining. (E) WM983B model treated with 5  $\mu$ M vemurafenib for 12 days showed tumor pigmentation, which is also visible on (F) hematoxylin and eosin stained tissue sections of the same model. Results are representative of two independent experiments, performed in triplicate. Scale bars: (A,B) 100  $\mu$ m, (C,E) 2 mm, (D,F) 20  $\mu$ m.

### Melanoma cell response to vemurafenib treatment in the 3D microenvironment



(A–D) Evaluation of melanoma cell proliferation in the model after 12 days of treatment with vemurafenib. Ki67 immunostaining (green signal) is indicative of cell proliferation in the WM983B melanoma tumor (B,D, red signal, PMEL). (E) Proliferative index of WM983A and WM983B cells in the 3D model in response to increasing vemurafenib concentrations.



(F–I) Co-labeling of apoptotic cells after staining for cleaved caspase 3 and tumor marker NG2. (F,H) Immunostaining for cleaved caspase 3 (red signal) in the melanoma model with (G,I) immunostaining of melanoma cells (neural/glial antigen 2 (NG-2), green signal and Hoechst, blue signal). (J) Quantification of cleaved caspase-3 expressing cells per total number of tumor cells as a function of vemurafenib concentration. (\*p ≤ 0.05 as determined by ANOVA followed by a multiple comparison). Results are representative of two independent experiments, performed in triplicate.