

Integrating skin and vasculature in a Multi-Organ-Chip Platform

Katharina Schimek¹, Annina Markhoff¹, Frank Sonntag², Martin Blechert³, Roland Lauster¹, Uwe Marx^{1,4}, Gerd Lindner¹

¹TU Berlin, Institute for Biotechnology, Germany,

²Fraunhofer IWS, Dresden, Germany

³Fraunhofer IZM, Berlin, Germany

⁴TissUse GmbH, Spreehagen, Germany

Abstract

Tests for drug development require an almost perfect fit with the human (patho-) physiological microenvironment. The majority of skin equivalents currently commercially available are based on static culture systems emulating only human epidermis, or combining epidermis and dermis in so-called full thickness skin equivalents. None of the existing systems contain important elements, such as vasculature, skin appendices or an immune system. Therefore, current *in vitro* and animal tests are failing to accurately predict drug toxicity. Our Multi-Organ-Chip (MOC) platform is a micro scale bioreactor providing pulsatile dynamic perfusion for microscale organoids. Here, we combine skin equivalents with vasculature in our two-organ variant (2OC). This would be needed for physiological-like interactions, regulation and, eventually, homeostasis within the chip.

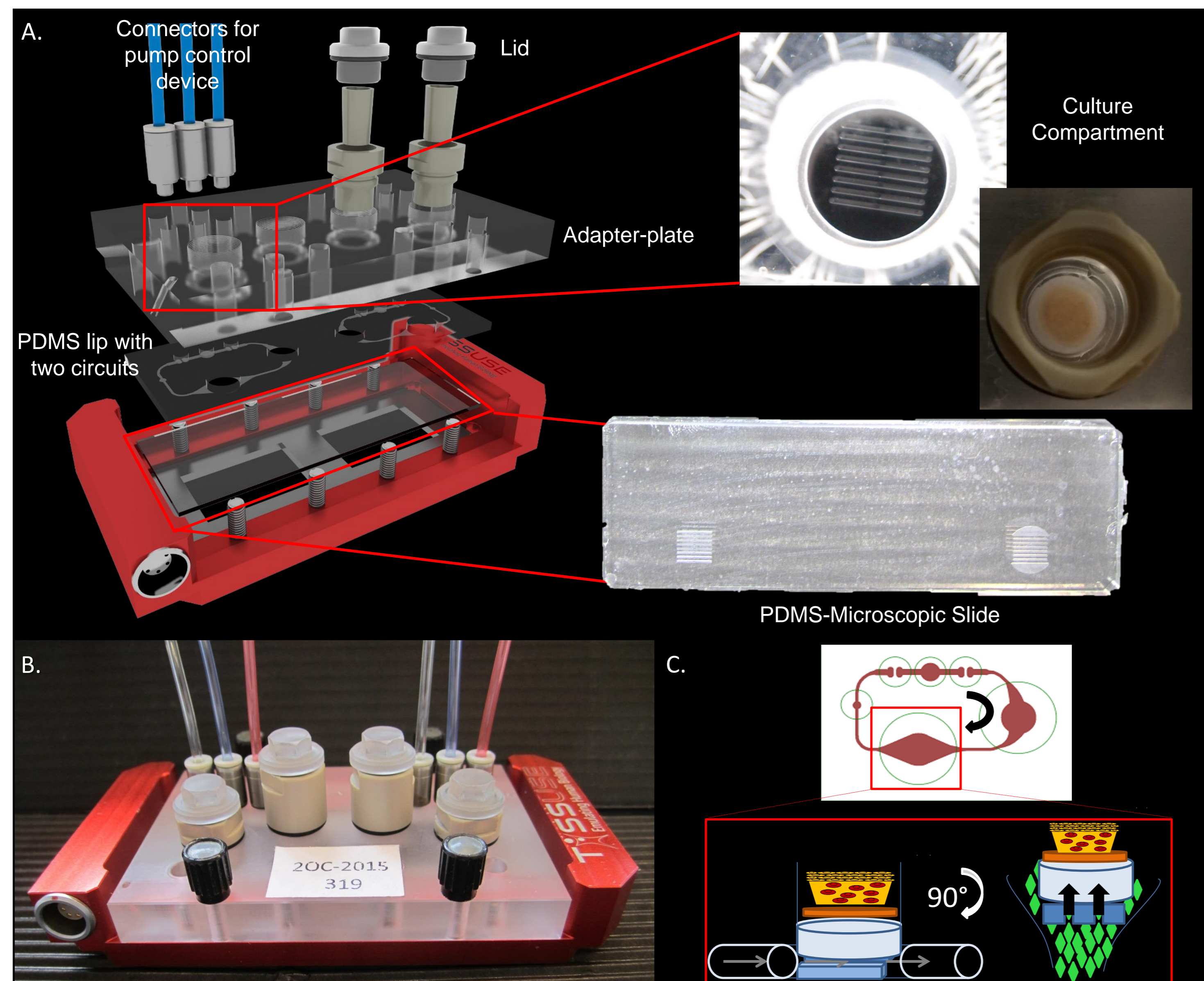


Figure 1: (A) Exploded view of the MOC platform comprising a polycarbonate adapter-plate and a PDMS chip accommodating two microfluidic circuits (footprint: 76 mm x 25 mm; height: 3 mm) each with one culture compartment and one Medium reservoir. (B) Photograph of a completely assembled MOC. (C) Footprint of the circuit in the PDMS layer. Direction of flow is indicated by the arrow. Tissue culture compartment is also indicated. Schematic sections through the tissue culture compartment which are used for culturing MatriDerm® full thickness skin equivalents in a pre-vascularized MOC. Barstructure of the PDMS bottom layer ensures Medium circulation in the culture compartment below the skin equivalent. Right insert area is used for medium exchange.

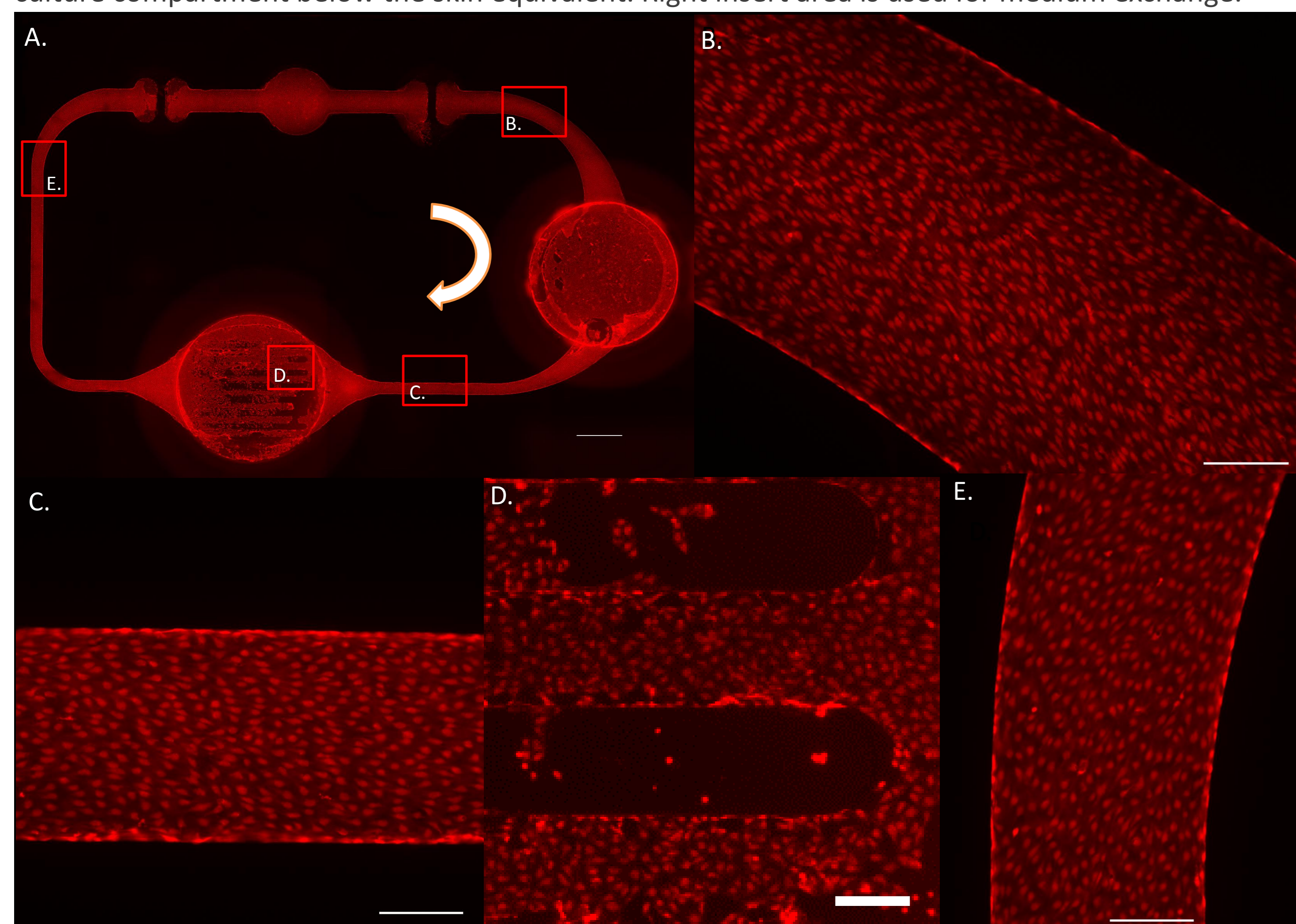


Figure 2: (A) Microvascular circuit in the MOC after 7d co-culture of skin equivalent and endothelial cells. Functionality of the microvascular vessel system demonstrated by live cell viability staining (CellTrace™ Calcein red-orange AM assay, red); endothelial cells maintain a confluent monolayer after 7 days of dynamic culture. Scale bar indicates 2mm. (B-E) Magnified sections of the circulation, as indicated in (A). HDMEC in the Channels are elongated and oriented into the direction of flow. Cells in the culture compartment showing a cobblestone-like morphology. Scale bars indicate 200µm.

Summary and Outlook

These results render the MOC used a useful tool for long-term co-culture of skin equivalents and endothelial cells, keeping most of their structures undamaged.

The on-chip micro-pump was able to provide a suitable shear stress environment for the endothelial cells. Dynamic perfusion of the MOC mediated the elongation with the direction of flow. HDMECs completely covered the channel walls of the entire circuit as presented by CalceinAM staining. Additionally, in comparison to cultures utilizing conventional static conditions, skin equivalents cultivated in our perfused MOC system together with the endothelial cells showed improved vitality and consistency of keratin 10/15, type 1 collagen, laminin 5 and vimentin expression.

In vitro testing of substances using the MOC, whether applied topically or into the medium, might be performed with significantly prolonged test periods, enhanced validity and online endpoint analysis compared to static cultures. Ultimately, combining different approaches, such as vascularization and integration of the MF model into skin equivalents, will provide the most predictive *in vitro* model of skin with hair follicles so far.

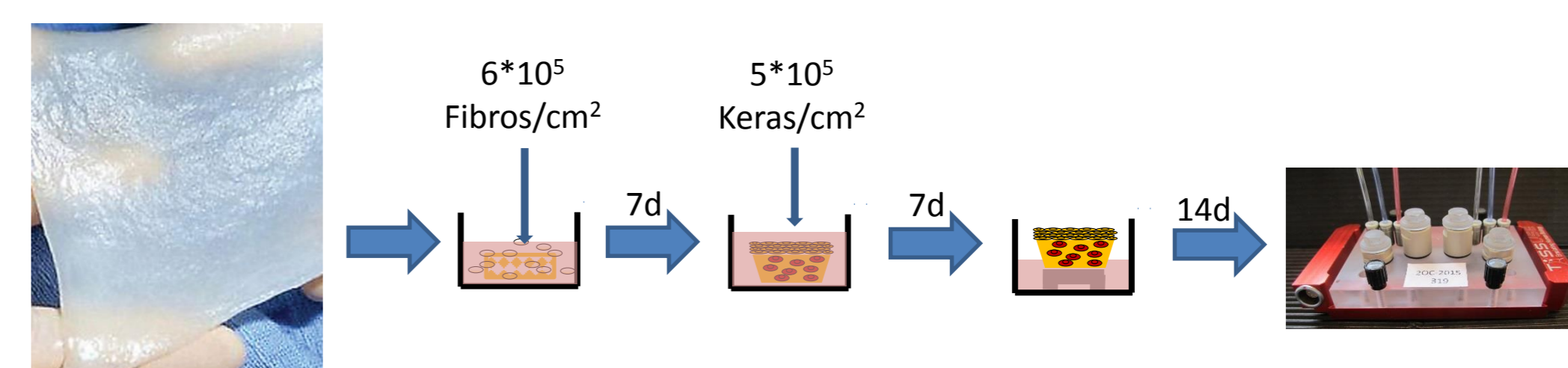
This project is funded by the German Research Foundation (DFG), LA 1028/7-1

Background

Nutrient supply *in vitro* is limited by the size of the organoid and ultimately effects differentiation efficiency of the tissues under static conditions. The multi-organ-chip (MOC) platform is a micro scale bioreactor providing pulsatile dynamic perfusion for microscale organoids.

Experimental Set Up

Skin equivalents were built up using 7mm punch biopsies of MatriDerm® (Asclepius Medizintechnik). Human dermal fibroblasts ($6 \times 10^5/\text{cm}^2$) were pipetted onto the top of the scaffolds and cultivated in 5% CO₂ at 37°C for 7 days. Keratinocytes ($5 \times 10^5/\text{cm}^2$) were then added as a drop onto the matrix. After 1h incubation for attachment of the cells, skin equivalents were cultivated submerged for another 7 days. Then, skin equivalents were lifted to the air-liquid interface and cultivated for further 14 days. After 28 days of static culture, the skin equivalents were added to the MOC (day zero of MOC culture).



The MOC platform provides a constant pulsatile flow of medium via its built-in micropump and ensures oxygen and nutrient supply. The system is filled with up to 600 µL of medium. No external reservoirs need to be attached that would otherwise dilute the enriched medium.

Human dermal microvascular endothelial cells (HDMECs), isolated from human foreskin, were seeded into the microfluidic channel system using a syringe. After even cell infusion inside the circuit, the device was incubated in 5% CO₂ at 37°C under static conditions for 3 h to allow the cells to attach to the channel walls. A frequency of 2 Hz was applied for continuous dynamic operation and, after 3 days of monoculture, the skin equivalent was added for co-cultivation for another 7 days.

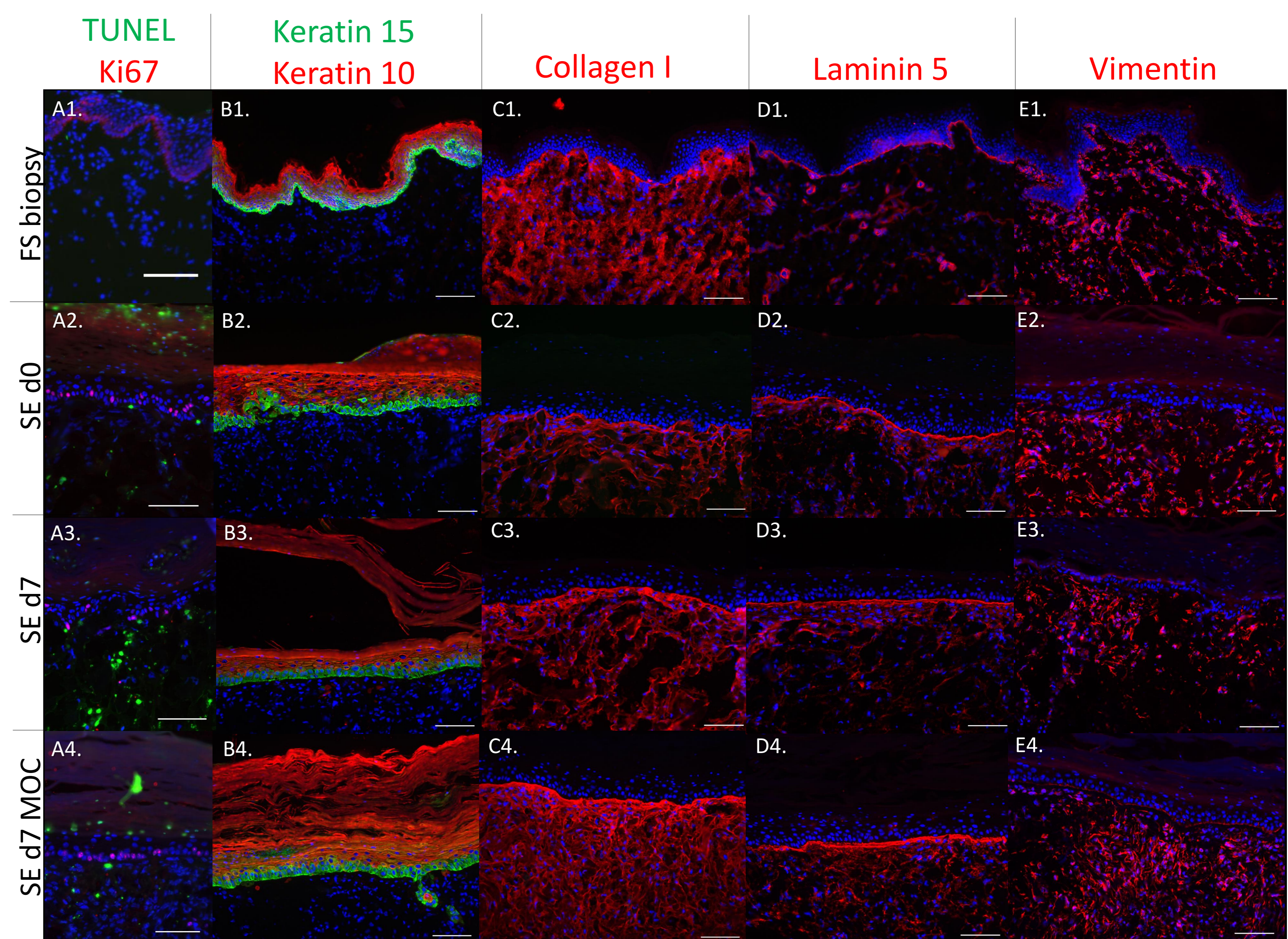


Figure 3: Characterization of skin tissues. A1-E1 native foreskin (FS) biopsy. A2-E2 skin equivalent (SE) at d0 of MOC culture. A3-E3 SE cultured statically outside the MOC for 7 days. A4-E4 SE cultured in the MOC platform for 7 days. (A) Indirect immunofluorescence for Ki67 (red) in indicated skin tissues marks proliferating cells. Click-iT® TUNEL Alexa 488® (green) staining indicates apoptotic cells. There were more viable and proliferating cells in the SEs cultured in the MOC compared to the SEs with static culture conditions. (B) Indirect immunofluorescence for keratin 10 (red) and keratin 15 (green) shows all non-basal and basal cells in the epidermis, respectively. Both were similarly expressed in all cultures. (C) Type I collagen (red) predominates in the dermis of both native skin and SEs. (D) Laminin (red) accumulates along the dermal-epidermal junction in all shown skin tissues. (E) Vimentin (red) was used to show mesenchymal cells in the dermis. Scale bars indicate 100 µm.

