Institut für Biotechnologie
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TISSUSE Emulating Human Biology

Long-term Culture of Dermal Units in Multi-Organ-Chips

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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.
We are aiming here to model a microvasculature-driven cutaneous homeostasis of skin and hair follicle biopsies, as well as their bioengineered equivalents in our perfused, self-contained and endothelialized multi-organ-chip (MOC) system. Our MOC platform uses a miniaturized circulatory network with an integrated micropump to provide pulsatile circulation of a microliter-volume of medium to support milligrams of human tissue constructs.
In comparison to cultures utilizing conventional static conditions, dermal units cultivated in our perfused MOC system showed remarkable consistency of cutaneous structure and vitality, rendering the MOC system a useful tool for long-term culture.

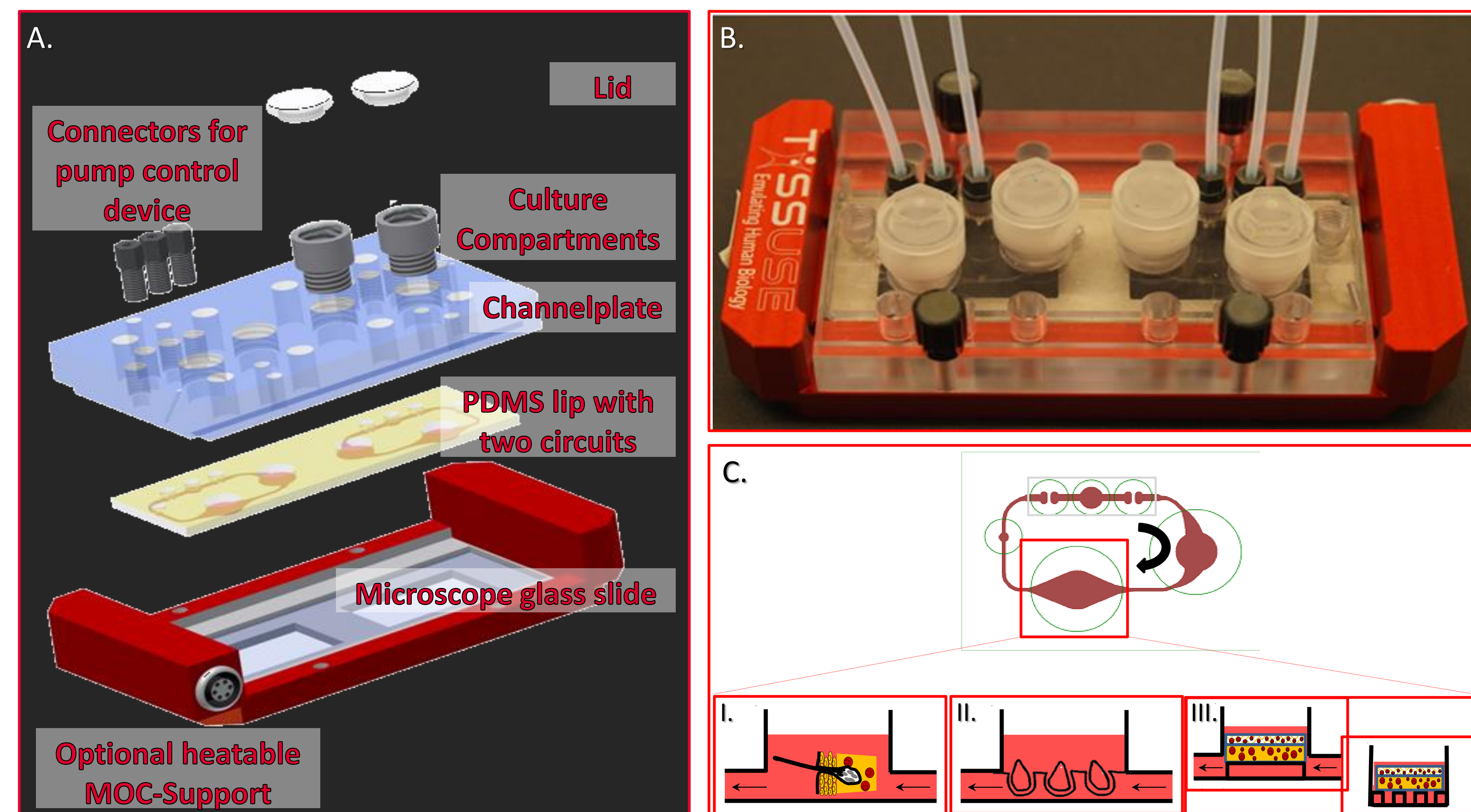

Figure 1: (A) Exploded view of the MOC platform comprising a polycarbonate cover-plate (blue), a PDMS-glass chip accommodating two microfluidic circuits (yellow; footprint: 76 mm x 25 mm; height:3 mm) and an optional heatable MOC-support. (B) Photograph of a completely assembled MOC. (C) Footprint of 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 in different approaches for culturing (I) *ex-vivo* follicular units (FUEs) directly in the stream, (II) MFs submerged in a PC-based cell culture scaffold, and (III) dermal equivalents composed of cellularized fibringel (yellow) and MatriDerm® in a pre-vascularized MOC. Right insert area is used for medium exchange.

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

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.
Approach (I): Complete philosebaceous units freshly taken during a hair transplantation procedure utilizing standardized follicular unit extraction (FUE) were cultured for 14 days in the MOC (Fig. 2). Immunohistology was performed to reveal *ex vivo* spatiotemporal development and to characterize viability and maintenance of the hair follicle structures after long-term culture.
Approach (II): Polycarbonate (PC)-based microthermoformed 3D cell culture material was used to transfer *in vitro* microfollicle (MF) culture to the MOC platform (Fig. 3). This PC-based material is formed as microcavities with latent tracks providing perfusion from each dimension. MFs were formed under static conditions, transferred onto the scaffold and dynamically cultured for 14 days in the MOC. Immunohistology was performed as an end point analysis to characterize different morphological changes. The model for MF formation is composed of cells from additional germ layers including keratinocytes, melanocytes and endothelial cells.
Approach (III): 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% CO_2 at 37°C under static conditions for 3 h to allow the cells to attach to the channel walls. A frequency of 0.476 Hz was applied for continuous dynamic operation and, after 6 days of monoculture, a dermal equivalent was added for cocultivation for another 14 days. The dermal equivalent was built up by adding a fibrin gel, and on top, a 5mm MatriDerm® punch both cellularized with HDMECs and/or adipocyte stromal cells (ASCs) (Fig. 4).

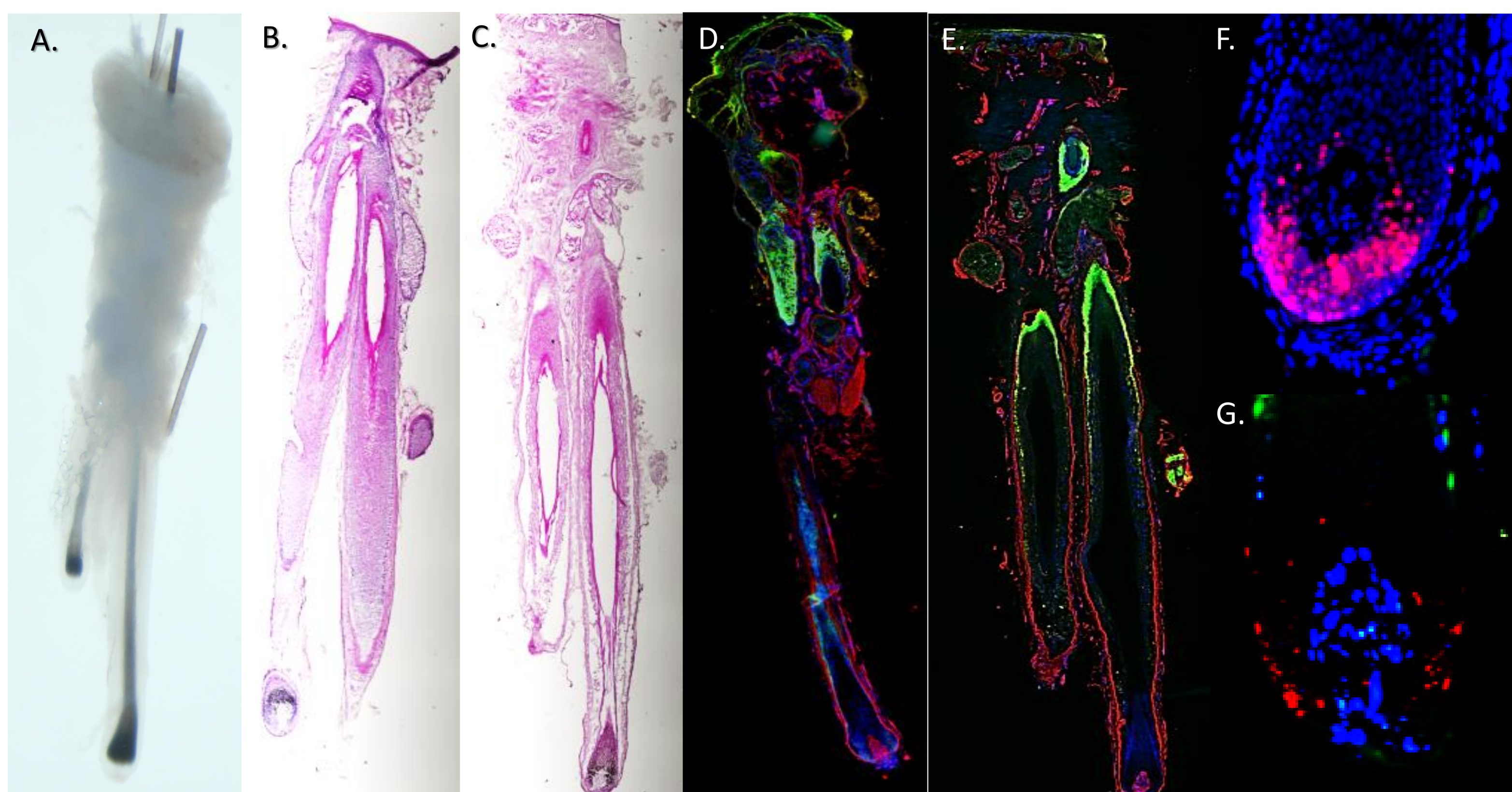

Figure 2: Characterization of FUEs cultured in the MOC device for 14 days. (A) Light microscopy image of a hair follicle. (B, C) H&E staining for day 0 (B) and day 14 (C) sample. (D, E) Immunofluorescence staining for collective cytokeratins (PCK) and collagen IV for day 0 (D) and day 14 (E) samples. (F, G) TUNEL assay and Ki67 staining (red) shown for the bulb region of the hair for day 0 (F) and day 14 (G) sample, respectively (arrows indicate TUNEL positive cells in the DP).

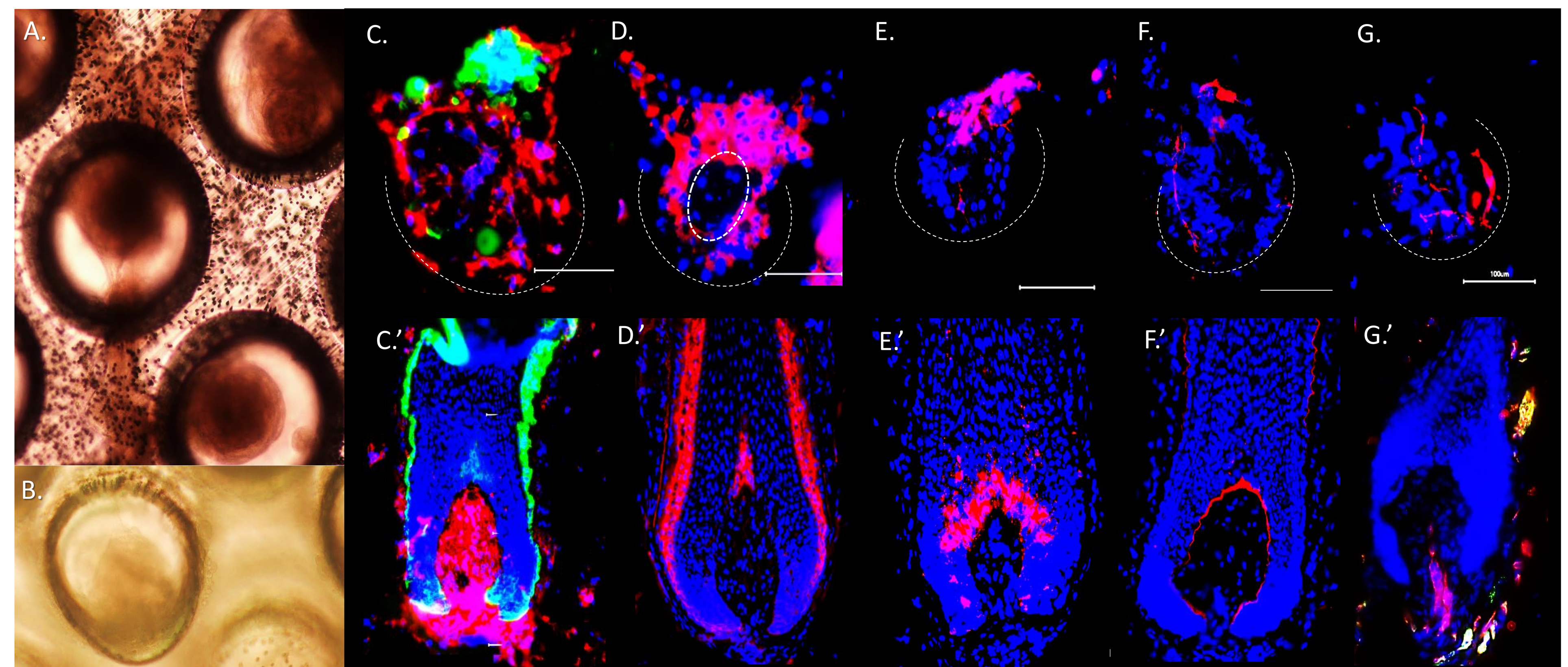

Figure 3: The cultured MFs showed polarization, keratinocyte differentiation and a hair follicle (HF)-related expression pattern. We observed polarization of MFs from day 7 onward (A, B), while end point analysis was carried out at day 14. Vimentin (red) was used to stain for mesenchymal distinction of the MFs (F-H) and CK15 (green) as a hair stem cell and ORS marker (C, C'). Keratin 25 is expressed in Henle and Huxley layers and the IRS cuticle of the HF, as we also observed in MFs (D, D'). Pmel17 is a premelanosomal protein that we used to detect the melanogenic melanocytes in the MF (E, E'). We labeled MFs with laminin to determine the basement membrane deposition (F, F'). CD31 (red) is used to label the ECs (G, G').

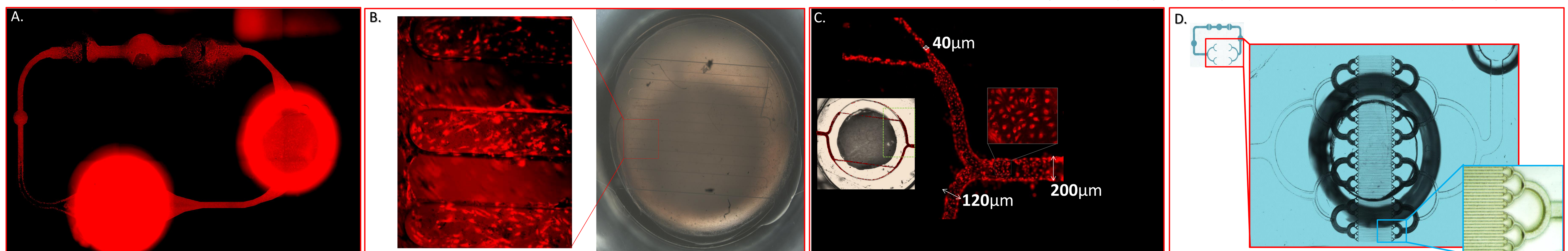

Figure 4: Coculture of dermal equivalent and endothelial cells: (A) Microvascular circuits in the MOC. Functionality of the microvascular vessel system demonstrated by live cell viability staining (Calcein red-orange AM assay, red); endothelial cells maintain a full monolayer after 14 days of dynamic culture. (B-D) Magnified sections of the culture compartment showing different designs of microchannels. Microchannels were seeded with endothelial cells (red) and dermal equivalents were cultured on top of the channels (B).

Summary and Outlook

These results render the MOC used a useful tool for long-term culturing of dermal units, namely hair follicle biopsies, *in vitro*-generated microfollicles, and dermal equivalents, keeping most of their structures undamaged.
The PC film with its perforated micro cavity structure is a suitable material for MF cultures. It provides an adjusted geometry for adequate 3D tissue formation based on optimized polarization and differentiation processes as well as permanent nutrient supplementation through medium perfusion. Under dynamic MOC culture conditions within the PC cavities, signs of polarization and differentiation of MFs are observed between days 7 and 14.

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.

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