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

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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. 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.

Background

Nutrient supply in vitro is limited by the size of the organoid and ultimately affects 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 build up using 7mm punch biopsies of Matriderm® (Asclepios Medizintechnik). Human dermal fibroblasts (6·10⁴/cm²) were pipetted onto the top of the scaffolds and cultivated in 5% CO₂ at 37°C for 7 days. Keratinocytes (5·10⁴/cm²) 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.

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.