Skin And Hair-On-A-Chip: Hair And Skin Assembly Versus Native Skin Maintenance In A Chip-Based Perfusion System

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Background and novelty: In recent decades, substantial progress to mimic structures and complex functions of human skin in the form of skin equivalents has been achieved. Different approaches to generate functional skin models were made possible by the use of improved bioreactor technologies and advanced tissue engineering. Although various forms of skin models are successfully being used in clinical applications, in basic research, current systems still lack essential physiological properties for toxicity testing and compound screening (such as for the REACH program) and are not suitable for high-throughput processes.
Experimental approach: In particular, further bioengineering is necessary for the implementation of adipose tissue, hair follicles and a functional vascular network into these models. In addition, miniaturization, nutrient and oxygen supply, and online monitoring systems have to be implemented in sophisticated culture systems. To become one step closer to the *in vivo* situation, we produced microfollicles as in vitro hair equivalents and integrated them into skin models. We also cultured *in vitro* skin tissues with and without subcutaneous tissue under static and dynamically perfused conditions and compared to *ex vivo* foreskin skin organ cultures.
Results and discussion: Data on the integration of hair follicle equivalents into skin models and their survival will be presented. Furthermore, the extension of culture periods of integrated skin explants in the perfused chip-based bioreactor platform will be demonstrated. The current status of the development of a blood-perfused in vitro skin model and remaining hurdles will be discussed.

Experimental Set up

Dermal Papilla Cells (DPCs), keratinocytes and melanocytes were isolated and expanded into multiple passages. Subsequently, DPCs have been kept under low adherent culture conditions resulting in the formation of dermal papilla-like aggregates. These spheroids underwent extra cellular matrix protein coating which mimics basal membrane compositions and thereby retained their inductive properties. Protein secretion and ultra-structural analysis of this neopapillae were performed. In following co-culture procedures keratinocyte and melanocyte attachment to the spheroids was forced allowing further follicular development (Fig. 1). We further introduced bioengineered microfollicles into skin equivalents which had been cultured under perfused conditions in a chip-based bioreactor for a step closer to build skin appendages (Fig. 2). Chip-based microfluidic system is called Multi-Organ-Chip (MOC) which has culture areas for standart 96-well Transwell insert (Fig. 3).
From another angle for skin model inprovements, we integrated subcutaneus tissue (SCT) collected from juvenile foreskin into *in vitro* skin model Mattek EpiDermFT. Combined tissues cultured under static and dynamic MOC conditions for 7 days (Fig. 4).

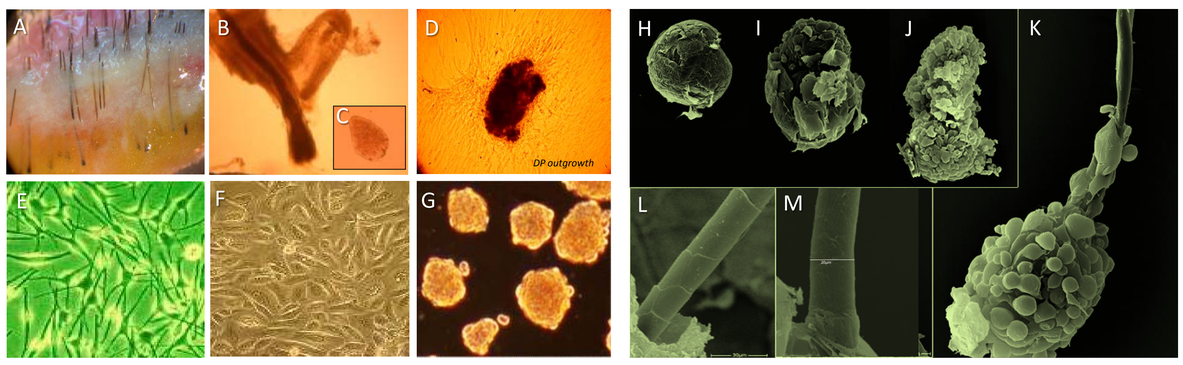

Fig. 1: From hair follicle isolation to Microfollicle formation. Dissection of hair follicle originated cells (A,B) isolated Dermal Papilla (C), cell culture of dermal papilla fibroblasts (D), melanocytes (E), keratinocytes (F). Neopapilla (DP condensate) formation after 48 hours (G) and 7 days plus additional ECM coating (H). Scanning electron microscopy (H-M). After adding keratinocytes and melanocytes microfollicle formation begins: polarization of the early aggregate (I). Assembly, orientation and sheath formation (J); Microfollicle with fiber production (K). (L) shows a cuticle sheathed hair from a human vellus hair follicle, whereas (M) represents a 1800× times magnified image of a microfollicle hair-like shaft.

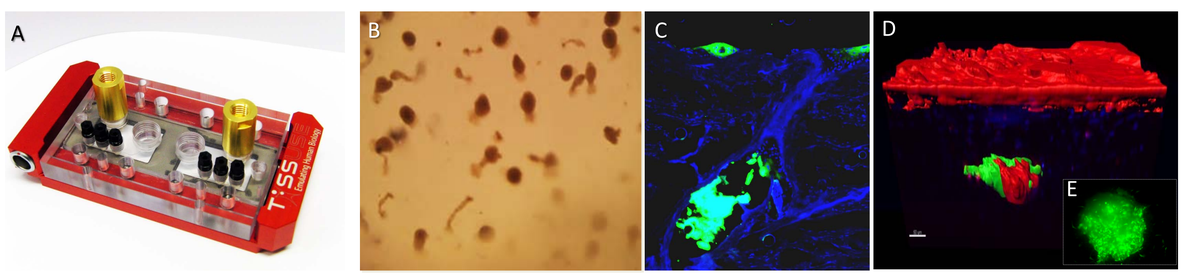

Fig. 2: Microfluidic device for perfused skin equivalent culture with integrated Microfollicle. Dynamic chip-based bioreactor for continuous perfusion culture of skin equivalents with integrated microfollicles (A). Evenly distributed microfollicles in a skin equivalent (B). PanCK immunoflourescent staining of a skin equivalent with an inserted microfollicle (C). 3D-rendering of a skin equivalent containing an early stage microfollicle (D). Here keratinocytes were labeled with live cell marker Celltracker (red), GFP labeled neopapilla (green), nuclei (blue). Fig. 2E shows an inserted GFP-labeled neopapilla taken by a fluorescence microscope (not-rendered, green).

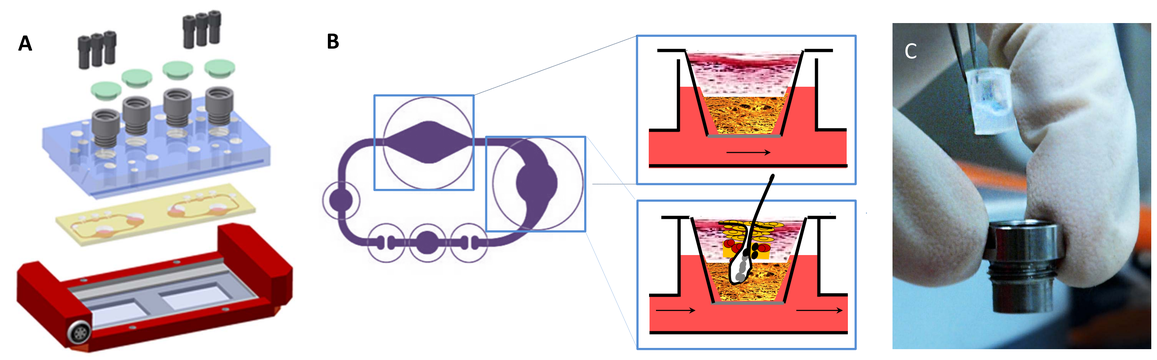

Fig. 3: Exploded view of the device comprising a polycarbonate cover-plate (blue), the PDMS-glass chip accommodating two microfluidic circuits (yellow; footprint: 76 mm x 25 mm; height: 3 mm) and a heatable MOC-holder (red) (A). Schematic drawing of one circuit and insert areas with a skin equivalent or skin equivalent with microfollicle (B). Standard 96well insert containing the skin equivalent being integrated into the chip (C).

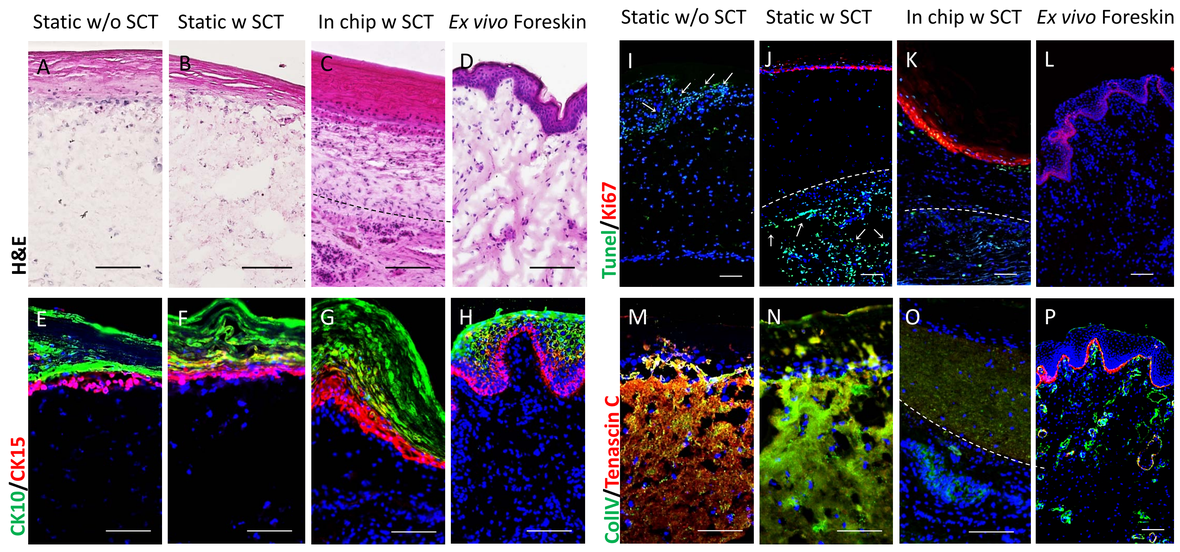

Fig. 4: 7D cultured EpiDermFT SE with subcutaneous tissue in MOC. *In vitro* skin equivalents (Mattek) cultured for 7 days in MOC or static conditions with and without subcutaneous tissue (SCT) and compared to *ex vivo* foreskin. H&E staining (A-D) and immunofluorescence staining for epidermal markers Cytokeratin 10 and 15 (E-H) and ColIV/TenascinC are applied (M-P). Ki67 and TUNEL assay for proliferation and apoptosis (I-L) Dashed lines mark the border between SE and SCT. Scale bars indicate 100µm.

Results

The formation of functional neopapillae needs more than 48 hours. After the addition of keratinocytes and melanocytes, the self-organizing microorganoids follow a stringent pattern of follicular-like formation by generating polarized segments, sheath formations and the production of a hair shaft–like fiber. Microfollicles in skin equivalents appeared viable more than 4 weeks in the perfused MOC-bioreactor. Commercially available skin equivalent EpiDermFT under MOC conditions for 7 days with SCT showed better viability and compareble histological results to native skin. Cellular and nutritional effect of SCT is visible even under static conditions. Presence of SCT decreased the expression of Tenascin C in dermis which is a marker for inflamation and fibrosis. Integritiy of the epidermis and proliferating cells in epidermis kept prominently in combined tissues. Perfusion of the combined tissue provides better integration and associated to viability of the SCT. In general, presence of SCT increased the longevity of the in vitro SE in both static and especially in MOC cultures with improved tissue architecture.

Summary and Outlook

We show that the *de novo* formation of human microfollicles *in vitro* is accompanied by basic hair follicle like characteristics. The microfollicles can be used to study mesenchymal-epithelial-neuroectodermal interactions and for the *in vitro* testing of hair growth-modulating substances and pigmentary effects. As the hair follicle is highly vascularized, it supports penetration of substances into the skin and further into the bloodstream. Testing of topically applied substances might therefore be performed with significantly enhanced validity by the incorporation of a microfollicle into a dynamic chip-based bioreactor containing a skin equivalent which mimics a physiological penetration route. An integrated SCT to in vitro skin equivalents can lead to more accurate evaluation of substance metabolism in skin in combination with a perfused microfluidic system which significantly improves the oxygen and nutrient supply. A skin equivalent with integrated microfollicles and SCT under dynamic perfusion will be the most suitable model for long-term cultivation and more efficient drug studies and one step closer to mimic skin.

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