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Development of multicellular organoids for skin injury and disease modeling

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Skin condition, skin related diseases and injuries remain a major problem for women's health. To provide proper development of new skin care, healing, and therapeutic agents it requires new functional in vitro models. Cell spheroids and organoids are widely used as a useful tool for drug testing and disease pathology in 3D microenvironment. In this study we show that the novel normal cell skin organoids (SO) can be utilized as a universal platform for different applications, including skin-like physiology modeling, in vitro chemical skin irritation testing, radiation and disease modeling in immersive conditions.

Key human skin cells were induced to form SOs and then organoids were characterized at 7, 14, and 21 days of culture. SO physiology was tested with application of retinol, Lucifer Yellow and pigmentation analysis. For radiation modeling SOs were exposed to UVB (150mj/cm^2). For chemical irritation testing, SOs were incubated with 1% Triton solution for 6h, Isopropanol, Hexyl Salicylate, 5% KOH for 15min on day 7. For disease modeling, two types of cancer cells have been tested in SO – SK-MEL-28 melanoma cells and cancer-associated fibroblasts (CAF) were added on days 0 and 7. Methods of analysis included Live/Dead assay, histology, IHC, Photometry, Cell Titer Glo assay, and RT-PCR. Statistical analysis was performed using Graphpad Software Inc.

Up to day 21, SOs demonstrated skin-like layered microstructure, with the surface zone formed by epidermis cells and the central core formed by dermal and hypodermal cells. SO showed skin-like functionality with epidermal barrier formation, vasculogenesis, and active melanogenesis. The generated SO were capable of physiologically relevant response to retinol by utilizing it into retinoic acid.

After UVB radiation SOs developed the ER-stress and apoptosis that could be blocked. In the chemical testing SOs responded to skin-irritating chemicals accordingly to their irritation index.

Modeling of melanoma in the SO revealed inhibition of CAF proliferation within an organoid. Outside of the organoid, CAF formed rapidly growing tumor spheroid. SK-MEL-28 cells had an unrestricted proliferation in and out of the SO with metastasis formation.

Therefore, this study shows that novel multicellular SO are capable of recapitulating skin structure functionality and of high-throughput analysis under immersive conditions. Ultimately, this technique provides an *in-vitro* skin model and could be used as a platform for dermatopathology and cancer biology research.