Developing Three-dimensional In-vitro Culture Models of Skin

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Developing novel therapeutics for fibrosis remains bottlenecked at the translation between pre-clinical to clinical stages due to the lack of physiological relevance of commonly used tools. Traditional monolayers of skin cells often paint an altered representation of cell behaviour, while animal studies are expensive, slow and can lack biological significance due to interspecies differences. Three-dimensional (3D) culture systems can potentially be designed to recapitulate the 3D biophysical and biochemical cues to provide a more physiologically relevant alternative to monolayer and animal models, as well as integrate into a scalable methodology for high-throughput data generation, suitable, for example, to drug development. However, these systems must be characterised fully for their successful application. Current commercially available full-thickness skin equivalents (FTSE) mimic the 3D organisation of the dermal and epidermal layers and are frequently used for screening purposes. However, the long maturation period of skin construct fabrication (~2-3 weeks) limits data throughput in the study of skin biology. Therefore, there is a need to 1) develop a scalable platform for FTSEs, and 2) improve the speed and ease of fabricating a robust FTSE.

Here, work has been carried out to develop and characterise a layered coculture organoid of human dermal fibroblasts (nF) and keratinocytes (nK) from healthy donor skin. Organoids were achieved within six days by sequential self-assembly of nF followed by nK in low-adhesion 96-well plates. Immunocytochemistry results show that nF and nK in organoids exhibits spatially dependent expression of target proteins for keratinocyte maturation (cytokeratin-10) and the dermal epidermal junction (collagen-iv, collagen-vii), found towards the organoid periphery and the nF-nK interface respectively, reminiscent of the expression patterns observed in skin tissue. However, proteins expressed during the later stages of keratinocyte cornification (filaggrin, involucrin) were not observed, likely due to the lack of an air-liquid interface. Nevertheless, these results demonstrate that skin organoids capture 3D aspects of native skin, and due to its self-assembling nature, the integration into automated systems (such as bioprinting) as well as scaling to a 384-well format can be further explored, potentially enabling high-throughput screening of skin biology.