Skin Bioprinting: an innovative approach to produce standardized skin models on demand

**Introduction**

In the cosmetic industry the testing of cosmetic ingredients on animals is no longer tolerated as soon as appropriate in vitro skin test systems are available. Therefore artificial in vitro skin models are urgently needed. So far, skin model supplier use standard liquid handling robots to manufacture their product leading to very simple composed skin equivalents.

In a previous CTI project (CTI No.: 12148.2) we used the upcoming bioprinting technology to print a dermal equivalent in a layer by layer fashion. With alternating layers of Bioink (matrix) and fibroblasts in suspension the tissue was formed. This technique allows the creation of a biological composite system by controlling the exact deposition of cells, growth factors and extracellular matrix (ECM) molecules in a spatially-controlled manner. In the frame of the former CTIproject a bioink was developed, which is printable, cyto-compatible and photo-polymerizable serving as a matrix to build up the tissue. Furthermore, an in situ quality control was integrated using an OCT-system. Figure 1 shows the bioprinter (BioFactory) and the printing mode.

**Project Goals**

After the successful former CTI-project the aim of this project is developing the bioprinter further and providing a complete skin model print solution to the market. The following steps will lead to this goal:

1. A full-thickness skin model is printed including dermis and epidermis
2. Bioink for optimal epidermis growth is adapted from dermis Bioink (stiffness is adjusted)
3. Developed Bioink is tested on different cell types (Universal Bioink)
4. Optical coherence tomography (OCT)- system is further optimized for better image quality
5. Biological relevance of printed skin models is proven by comparing to state of the art skin models
6. Bioink for bioprinter is developed and ready for commercialization
7. SOPs and application notes are available to provide an all-in-one solution for printing of in vitro skin models.

**Key Findings**

First results of printing dermis containing fibroblasts and epidermis containing keratinocytes in a one step process were successful and are shown in Figure 2 and 3. In this protocol the epidermal Bioink composition was optimized for commercialization. Long time stability of the Bioink was analyzed with respect to logistics. Packaging of the Bioink product was developed and is in a syringe format ready to use with the bioprinting device. First contacts with a potential Bioink production partner was developed and is in a syringe format ready to use with the bioprinter. First tests were performed to verify the universal nature of the Bioink. Different cell types were encapsulated into the Bioink to analyze their viability over time. In Figure 4 an epithelial tumor cell line (BeWo b30) and primary osteoblasts were cultivated inside the polymerized Bioink for 5 days and remained viable shown with an MTT-assay. Different epithelial, endothelial cell lines and primary cells were also tested and viable after 5 days (data not shown).

Bionik composition was optimized for commercialization. Long time stability of the Bioink was analyzed with respect to logistics. Packaging of the Bioink product was developed and is in a syringe format ready to use with the bioprinting device. First contacts with a potential Bioink production partner was initiated and evaluated.

The OCT system is optimized (how?) to produce images of good quality.

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**Conclusion**

Fibroblasts and keratinocytes were successfully printed to build a skin model. The OCT-system is integrated and ready to monitor the differentiation process of the keratinocytes into a stratified epidermis. The here developed Bioink is compatible with a wide variety of cell types including cell lines and primary cells. In parallel to the skin model production a business plan to commercialize the Bioink has been initiated, which will give rise to an all-in-one product for in vitro skin production. Next steps include the differentiation of keratinocytes into the epidermis and proving of biological relevance of the printed tissue.