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

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Main Industrial Partner: regenHU Ltd., Marc Thurner, Michael Kuster, Andreas Scheidegger
Start: 01.06.2012, **Duration:** 15 months

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 CTI-project 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.

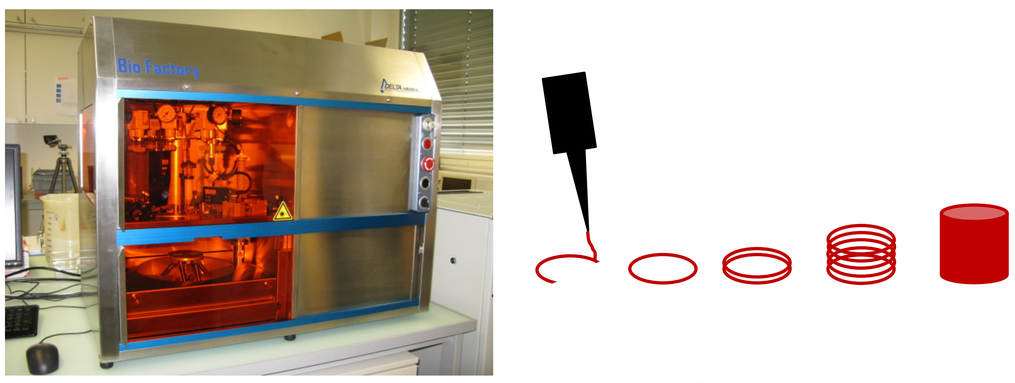

Figure 1: Bioprinting instrument on the left. The Biofactory creates 3D composite tissue models by combining up to 8 different media into a biological system. The printing mode (layer by layer) to from tissues is shown on the right side.

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 adapted. Figure 2 shows an HE-staining of a skin model with a thick dermal layer and a single cell layer of keratinocytes on top. This figure displays the layer by layer deposition process of the Bioink and cells.

Top

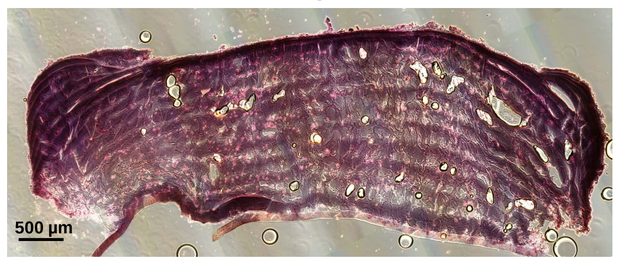

Figure 2: HE-staining of a printed skin model. A dermis equivalent containing fibroblasts was printed in a layer by layer mode. On top of the dermis a thin layer of keratinocytes was printed. The skin equivalent was cultivated for 48 hours.

In Figure 3 a fluorescent image of a printed skin model is outlined. Printed fibroblasts are labeled with DAPI to visualize cell nuclei (blue) and keratinocytes were labeled green-fluorescent prior printing and they're deposited as a thin layer on top of the dermis.

Top

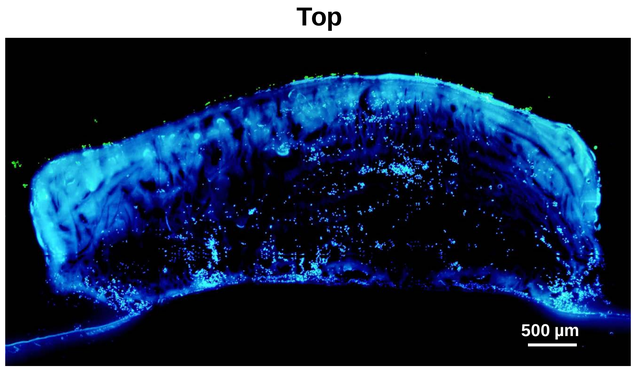

Figure 3: Fluorescence-staining of a printed skin model. A dermis equivalent with fibroblasts was printed in a layer by layer mode. On top of the dermis green-fluorescent keratinocytes were printed in thin layer. Cell nuclei are stained with DAPI (blue spots). The skin equivalent was cultivated for 48 hours.

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).

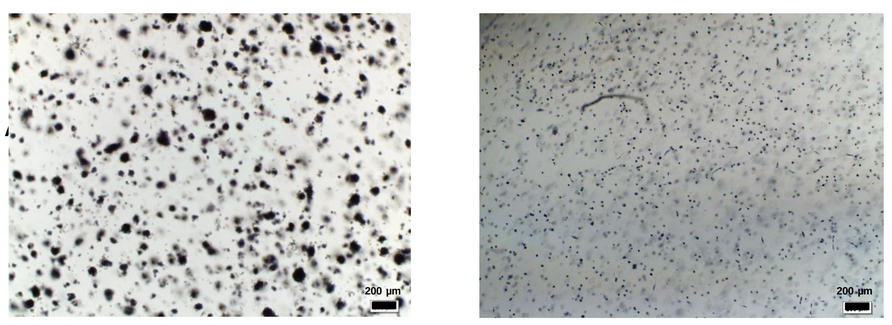

Figure 4: Cell type compatibility of Bioink. In A) an epithelial cell line (BeWo b30) was cultivated for 5 days in Bioink. In B) primary osteoblasts wer cultivated for 5 days in Bioink. The cells were stained with the viability-staining MTT and the viable cells are shown as dark spots.

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

Acknowledgements

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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.