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Non-invasive Quality Control for Production Processes of Artificial Skin Equivalents by Optical Coherence Tomography

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Abstract

The engineering of artificial tissue and organs is widely gaining relevance in biotechnological and biomedical research. Particularly by the law enforced reduction of animal testing, alternative methods need to be developed to simulate and test the effects of chemical substances on human organs. Regarding the branches for pharmaceuticals, chemicals and cosmetics most products need to be tested on penetration of the human skin tissue. As an alternative method to animal testing, skin equivalents (SEs) based on tissue engineering may be used to test the skin barrier function.
In the framework of a large scale research project, we built a fully automated production process based on the adaption of manual laboratory protocols to carry out the biological process steps for the manufacturing of SEs. In a first step, keratinocytes are mechanically and enzymatic isolated from human foreskin biopsies. In a second process, the cells are cultivated in MTP format cultivation dishes und undergo a series of passages. In a last process step, the keratinocytes are concentrated and dispensed in 24 well membrane based MTPs. Due to biological fluctuations in cell and tissue growth, the production process faces major challenges to control and process a vast variety in batch duration and timing. This results in a strong impact in the final tissue quality. For quality control in the manual process, histologies of the SEs are prepared followed by staining and microscopic imaging. To overcome this time intensive process, we developed a fully automated optical coherence tomography OCT system. OCT is a non-invasive and non-destructive imaging technique based on the interferometric detection of optical light feedback from the probe tissue in the near infrared light. OCT generates 2D and 3D tomographic images with a micron resolution and a tissue penetration up to 2 mm. We developed custom image processing algorithms, to detect the individual layer structure and corresponding layer thicknesses. The OCT results show a high degree of correlation with the histological findings regarding structure and layer thicknesses. Further, we show in a first study a method to use this OCT layer detection technique as a potential substitution measurement to the ET50 test used for analyzing the *in vitro* skin barrier function.

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Keywords: Optical Coherence Tomography; OCT; Tissue Engineering; Artificial Skin Equivalent; Quality Control.

1. Introduction

With the aim of placing greater responsibility on industries when it comes to chemical substances the European Commission released in 2006 the REACH (Registration, Evaluation, Authorization and Restriction) regulation to protect human health through the better and earlier identification of the intrinsic properties of chemical substances. In many cases public and regulatory requirements force producers to utilize alternative methods to animal testing for analyzing new substances or products regarding their safety. The increasing demand for substituting animal testing in consumer product development has lead to a growing market for *in vitro* test systems. Here, a significant

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number of product groups likely to get in contact with skin need to be tested regarding their effects on the human body. *In vitro* skin equivalents (SEs) may be used for irritation, penetration or toxicity tests (i.e. OECD guideline for the testing of chemicals 431: "In vitro Skin Corrosion: Human Skin Model Test") [1]. SEs are build up by keratinocytes undergoing a differentiation and proliferation process during the air-liquid-interface growth phase. On the basis of specialized growth culture condition, the SEs exhibit the four epidermal layers (fig. 1) achieving one part of the requirements for using it as alternative for skin irritation test.

Like most other tissue engineering processes, the built up of SEs based on primary human cells is a time consuming and complex manual process and results in a high demand for process automation. To face this challenge, we developed an automated production facility for SEs. The device is based on a 3 x 5m laminar flow unit with access through sterile benches. All process steps are automated starting from the isolation of cells from a biopsy to the air lift cultivation phase. The production unit allows to maintain very high standards in sterile production while the risk of contaminations are minimized. Another advantage in an automated production process, is the standardized protocol resulting in consistent growth conditions.

Due to biological fluctuations of the cell growth and a strong variance in different cell charges, each SEs shows slightly different properties in layer structure, layer thickness, morphology and skin barrier function. This leads to a high demand in quality control at the end of production. Therefore, we developed a fully automated Optical Coherence Tomography (OCT) device which allows to generate high resolution cross sectional images of the SEs [2]. OCT is a tomographic imaging technology based on the detection of the reflected light signal from a probe. Due to the low energy of light used, OCT is non ionizing and operates non invasive. This leads to a major advantage over histologies, since by applying the OCT measurement, the SEs do not need to be cut up for histological analysis. We developed a custom image processing software to detect the layer structures and thicknesses. Here, the major challenges are the automated measurement process, as well as the automated image processing and evaluation of the OCT B-scans (2D cross sectional scan). To realize an automated measurement, we developed a new Fourier Domain OCT device coupled to a three axis translational stage system. With an automatic focus algorithm, we are able to generate multiple B-scans of SEs in an MTP format dish.

2. Methods

2.1. Growth process of skin equivalents

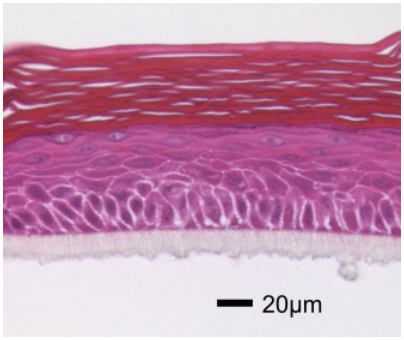

Fig. 1. Histology of an SE. The SE exhibits the typical morphology with basal, spinous, granular and cornified epidermal layers. In the *Stratum corneum* a cutaneous barrier is formed to provide the barrier exhibiting properties [3]

In order to provide the required cell material for cell proliferation and for the subsequent tissue structure build-up, skin cells are extracted from human foreskin tissue. First, the keratinocytes are isolated from the biopsy. A new automated process chain was developed comprising a combination of specific mechanical and enzymatic applications, focusing on high throughput and maximized efficiency while at the same time maintaining high cell vitality rates.

The process starts with the manual preparation of the biopsy to remove the fatty tissue and blood vessels. The applicable biopsy is inserted in the automated process through the lock and the automated process starts by gripping the biopsy out of a tube and disposing it on a petri dish. Next an automated cutting process of the epidermal layer of the biopsy with a scapel blade provides enzyme (Dispase) access to the basale membrane (*Stratum basale*). After enzymatic digestion (4 hours at 37 °C) of the biopsy, the epidermis separates in small pieces from the remaining dermis tissue by vortexing. Subsequently, the epidermis pieces are isolated from the biopsy by a filtration process. After a second enzymatic digestion (Trypsin, 5 min., 37 °C) the epidermal tissue is isolated to single cells, the keratinocytes. The major difference in the isolation of keratinocytes between the automated process and the manual laboratory protocol lies in the process of separating the two tissue layers (epidermis, dermis). In the manual laboratory process, the biopsy is cut up into small pieces for the over night enzymatic digestion at 4 °C. In a next step, the epidermal layer is separated from the dermis by two forceps after enzymatic digestion [4].

The primary keratinocytes are resuspended in specific cultivation media (Epilife, life Technologies, Germany) and transferred to the downstream process steps. The