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Metabolic and morphological evaluation of an artificial full-thickness skin model using a multimodal photonic system

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ABSTRACT

The fabrication of artificial tissue and organ models is one of the important directions of the development of modern biomedicine. Assessment of the morphology, maturation, and viability is an important part of such developments. Here, we report on the validation of our custom-build fluorescence spectroscopy (FS) system with optical coherence tomography (OCT) for assessing the metabolism and morphology of the full-thickness skin equivalence (FSE) model. FS along with OCT has been used for the metabolic activity evaluation of the developed FSE model and 3D imaging of its structure. Thus, we have developed a multimodal optical system that can be used in the future for a full-profile assessment of the maturation and viability of 3D-printed models of biological tissues in time-course development.

Keywords: keratinocytes, fibroblasts, artificial full-thickness skin model, scaffolds, fluorescence spectroscopy, optical coherence tomography.

1. INTRODUCTION

The regeneration of damaged tissues in the human body is limited, and the development of new technologies for the creation of 3D artificial biological tissues and organs is one of the key directions of modern medical and biological practice.[1] For the generation of artificial tissues model, 3D cell culturing is gaining popularity.[2] Human skin is the largest organ of the body, and artificial skin models create a platform for studying the effects and testing the safety of new drugs, as well as open up wide opportunities in transplantology.[3,4] At the same time, the development of this industry is inextricably linked to the development of methods for controlling the growth of models.

In this work, we validate our custom-built multimodal optical system consisting of fluorescence spectroscopy (FS) and optical coherence tomography (OCT) channels for the evaluation of the morphology and viability of a full-thickness skin equivalence (FSE) model grown by us on special 3D scaffolds.

2. MATERIALS AND METHODS

A spectroscopy system with a fibre optical probe was used to make fluorescence intensity measurements at 365 and 450 nm. The choice of these wavelengths was due to the requirement to excite NADH and FAD fluorescence, as these coenzymes are known to be key elements in cellular energy metabolism.[5] The output power of both excitation sources did not exceed 3 mW. Fluorescence in the range of 400–900 nm was analysed using a Flame spectrometer (Ocean Insight, USA). Longpass filters (Edmund Optics, USA) were used to attenuate the backscattered radiation of the excitation sources. This is a widely used approach when building such systems.[6–8] Considering the compact form factor, affordability and excellent performance, the Lumedica OCT imaging system was chosen to evaluate the morphological features of FSE.

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Fluorescent measurements were carried out on the suspensions of two types of cells. We cultured immortalised HaCaT keratinocytes (StratiCELL, Belgium) and primary fibroblast cells (StratiCELL, Belgium) to healthy confluent layers. The FSE model was prepared using keratinocytes and primary fibroblasts according to the methodology developed. Next, we evaluated the possibilities of OCT to control the growth of this FSE model.

3. RESULTS AND DISCUSSION

First, to validate the FS channel of our optical system and assess the sensitivity limit and signal-to-noise ratio (SNR), we studied the concentration dependence of the fluorescence intensity of the NADH and FAD solutions. PBS was used as a buffer. It was found that the developed system is able to distinguish between concentrations of NADH and FAD in a solution below 10 μM, which corresponds to real concentration levels in biological tissues.

The performance of our system using primary fibroblasts and immortalised HaCaT cell lines was then evaluated. Fig. 1 presents the statistical analysis of HaCaT and fibroblast cells suspension for two different excitation light wavelengths. Histograms represent the statistical analysis of the fluorescence intensity of HaCaT and fibroblast cell suspensions that was performed for three different concentrations. With increasing the concentration of cells, the value of intensity also increases.

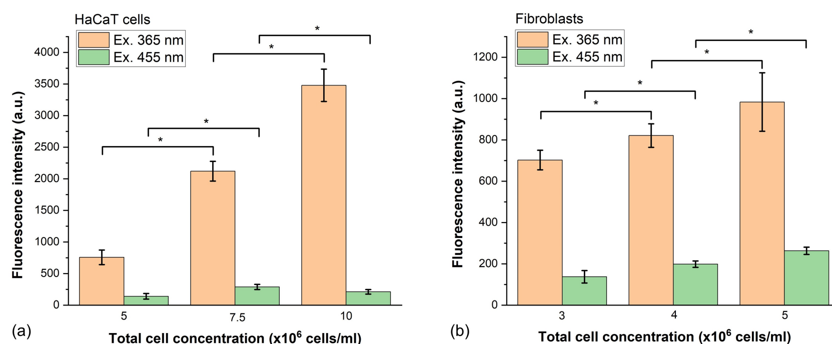

Figure 1. Fluorescence intensity histograms for (a) HaCaT cells and (b) fibroblast suspensions at three different concentrations for 365 and 455 nm excitation light (n=3). *Confirmed statistically significant difference between different concentrations of cell suspension (p<0.05).

Fig. 2 represents the detected fluorescence intensity of metabolic coenzymes in the suspension of HaCaT and fibroblast cells associated with different cell ages. The decreasing trend represents the change in the intensity of the fluorescence of metabolic coenzymes with ageing of cells.

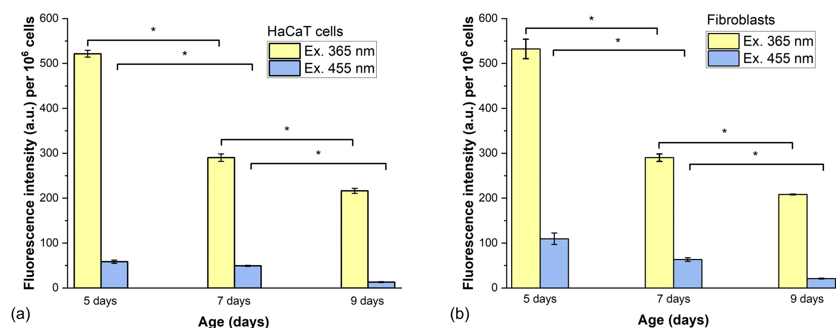

Figure 2. Fluorescence intensity histograms for (a) HaCaT cells and (b) fibroblast suspensions at different ages. *Confirmed statistically significant difference between different ages (p<0.05).

Fig. 3a,b presents OCT images of a scaffold used for growing the FSE model and a two-layers FSE model, accordingly. The skin model has been developed using fibroblast and HaCaT cells, and OCT images provide information about the morphological structure of this artificial skin. These figures show a layer of HaCaT cells over the fibroblast cells in fibrinogen.

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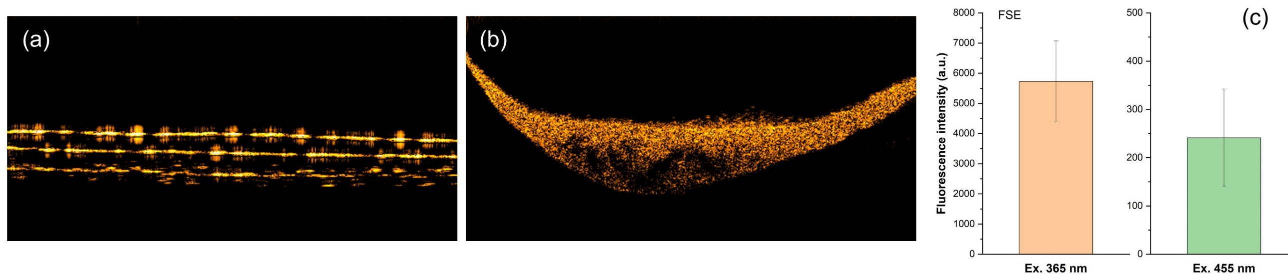

Figure 3. OCT images of (a) scaffold and (b) full-thickness skin equivalence model.

The fluorescence measurements shown in Fig. 3c demonstrate the possibility of measuring the metabolic activity of FSE models when the fluorescence of NADH and FAD is excited. The change in the fluorescence level can serve as a reliable parameter to evaluate the effectiveness of growth and ageing of such tissue models.

4. CONCLUSION

Here, we demonstrated our custom-build multimodal optical system for assessment of the morphology, maturation, and viability of human skin cells in suspension and full-thickness skin equivalence model. NADH and FAD are important participants in cell energy metabolism, and monitoring their fluorescence can be a reliable marker of cell growth. At the same time, an inexpensive OCT system can provide high-quality information on the structure of artificial tissue models. Thus, we have developed an optical system that can be used in the future for a full-profile assessment of the maturation and viability of 3D-printed models of biological tissues.

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