BIOPOLYMERIC SCAFFOLD FOR CELL VISUALISATION IN 3D ENVIRONMENT USING COHERENCE-CONTROLLED HOLOGRAPHIC MICROSCOPY

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[ENGINEERING OF BIOMATERIALS 153 (2019) 43]

Introduction
Coherence-controlled holographic microscopy (CCHM) is an emerging single-shot imaging technique used for fast processes visualisation [1]. Unlike fluorescence microscopy, which is currently one of the most used bioimaging technique, CCHM is a non-invasive, label-free technique with the ability to visualise cells in real-time. A challenging aspect of this technique is light scattering as the imaging in CCHM is based on the interference of the object and the reference light beams, which enables to detect the phase delay of light transmitted through the specimen.= [2]. Cells are overall weakly scattering absorbing specimens and highly scattering environment, such as polymeric substrates, can distort final images. Therefore till this day, CCHM has been successfully used mostly in visualising cells in a 2D environment even tough 3D visualisation represents physiological environment better [3,4].
Within the context, the presented work aimed to establish a microstructured scaffolding material for visualisation of cellular interaction in a 3D environment using CCHM.

Materials and Methods
The 3D microstructure of biopolymeric scaffolds was achieved using the process of freeze-drying. The stability upon disintegration in the environment of the culture medium was improved using chemical crosslinking reaction initiated by carbodiimides. Microstructure in dry state was visualised and evaluated using scanning electron microscopy (SEM). Optical transparency was evaluated using UV-VIS spectroscopy at 600 nm. Visualisation using CCHM was achieved at 37°C and 5% CO_2 atmosphere. The cell line of normal human dermal fibroblasts nHDF was maintained in Eagle's minimal essential medium supplemented with 10% fetal bovine serum.

Results and Discussion
The 3D microstructured biopolymeric scaffolds, fabricated in our study, have the advantage of high optical transparency resulting in minimal light scattering effect. Biopolymeric nature of the scaffolds simulates extracellular matrix by its chemical composition, therefore creates an environment that closely represents the physiological environment. Optimal concentration of biopolymeric substances was set to 0,2 % (w/w). At this concentration, the scaffolds were still easy to be manipulated with as well as had a minimal negative effect to light scattering. SEM visualisation revealed' microstructure of the scaffolds having high porosity and inhomogeneous pores. The cellular behaviour was investigated using CCHM in order to determine the effect of prepared biopolymeric scaffolds on cultured cells adhesion, alignment, orientation and morphology in comparison to glass microscope slides. The cultured cells were investigated at different time intervals to monitor their behaviour. Cells were able to attach and align to biopolymeric fibres within the microstructure of the scaffolds without forming a cluster or unaccustomed morphology. After addition of model presumably toxic substance, there were visible changes in cellular behaviour and morphology.

Conclusions
In presented work, we have achieved preparation and characterisation of microstructured biopolymeric scaffold with low optical density designed for the visualisation of cellular interactions in the 3D environment using CCHM.

Acknowledgments
This work was supported by the CEITEC 2020 (LQ1601) with financial support from the Ministry of Education, Youth and Sports of the Czech Republic under the National Sustainability Programme II and by Special Research project of Brno University of Technology under no. CEITEC VUT-J-19-6086.

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