SHG IMAGING OF MATURE COLLAGEN FIBERS PRODUCED IN VITRO FOR TISSUE ENGINEERING APPLICATIONS

Jana Liskova, Daniel Hadraba, Zuzana Burdikova, Martin Capek, Lucie Bacakova

Institute of Physiology
Academy of Sciences of the Czech Republic
Videnska 1083, Prague 4, CZ-14220, Czech Republic
E-mail: jana.liskova@biomed.cas.cz

KEY WORDS: Second harmonic generation imaging, Collagen, Saos-2, VICs

Collagen is an important component of extracellular matrix (ECM) of connective tissue. Therefore, analysis of collagen deposition and remodeling is essential in tissue engineering. Second harmonic generation (SHG) imaging technique allows the imaging of non-centrosymmetric structures such as collagen. The primary pig valve interstitial cells (VICs), derived from the aortic heart valve, actively produce type I collagen and are therefore suitable for optimizing collagen visualization methods. The cells of human osteoblast cell line Saos-2 are able to grow extensively, and they can differentiate and produce ECM under specific conditions as well. They can be used for evaluation of biocompatibility of biomaterials for bone implants coatings, such as nanocrystalline diamond films (NCD) [1].

We analyzed the production of type I collagen by Saos-2 cells on nanocrystalline diamond films (NCD) and on glass coverslips after 2 weeks of cultivation in a medium with addition of 50 µg/ml ascorbic acid. The VICs were used as a positive control for the production and visualization of type I collagen and for the SHG signal measurements. The native type I collagen fibers were visualized by two-photon excitation microscopy and SHG imaging, together with immunofluorescence staining and fluorescence microscopy. For SHG imaging, the excitation wavelength 860 nm, detection filter 430 nm and the backward nondescanned mode were used. In order to recognize all collagen content and extracellular collagen by immunofluorescence we used permeabilization treatment. Additionally, the amount of collagen in ECM was measured by the Sircol assay in both VICs and Saos-2 cell layers.

The mature collagen fibers were detected in Saos-2 cells after a two-week culture in an osteogenic medium, on both glass and NCD films. In VICs, both the immunofluorescence staining and SHG imaging depicted collagen structures, although in permeabilized cells, also intracellular and immature collagen was observed. In non-permeabilized samples, only extracellular collagen was stained, which corresponded with the SHG signal of these samples. The image analysis and stereological analysis of SHG signal in the images and series of images are being performed. To be concluded, SHG imaging technique appears to be a promising tool for analysis of collagen I production associated with testing of material biocompatibility.

This work was supported within the project “The Centre of Biomedical Research” (CZ.1.07/2.3.00/30.0025). This project is co-funded by the European Social Fund and the state budget of the Czech Republic. Other supports were provided by the Grant Agency of the Czech Republic (grant No. P108/11/0794).