Two-photon microscopy with time resolution to assess the metabolic status of cells grown on porous ceramics.

T. B. Lepekhina[1], V. V. Nikolaev[1], H. Zuhayri[1], M. S. Snegerev[1], A. S. Lozhkomoev[2], E. I. Senkina[1,2]

[1]Tomsk State University, Lenin Ave. 36, 634050, Tomsk, Russia;
[2]Institute of Strength Physics and Materials Science of the Siberian Branch of the Russian Academy of Sciences (ISPMS SB RAS), 634021 Tomsk, Russia

Nowadays new materials are being actively developed as osteoimplants. However, the survival process can be disrupted due to incorrectly selected material. Two-photon microscopy offers prospects for assessing cell survival on osteoimplants. After analyzing the fluorescence lifetime of endogenous NADH (nicotinamide adenine dinucleotide) and FAD (flavin adenine dinucleotide) fluorophores by two-photon tomography, we can conclude about the metabolic activity and cell survival on the materials used as the manufacture of osteoimplants. The aim of this study was to develop an approach to assess the metabolic state of 3T3 mouse fibroblasts near the surface of osteoimplants,

Using a two-photon tomograph (MPTflex, Germany, Jena), measurements were made of 3T3 mouse fibroblasts incubated on 10 samples of cover glass (control group), 5 samples of stabilized zirconium ceramics (Y-TZP), 5 samples of yttrium-stabilized zirconium ceramics hardened with aluminum oxide (ATZ).

The analysis of the intensity and of fluorescence lifetime of endogenous NADH and FAD fluorophores was carried out using a phasor plot to FLIM data processing. Such a graphical representation of the results made it possible to distinguish between the free and bound shapes of NADH and FAD of the cells used, based on data on the intensity and fluorescence lifetime of these coenzymes and, accordingly, to obtain information about the metabolic state of the cells. This approach can be used as an additional analysis in assessing the biocompatibility of materials used for the manufacture of osteoimplants.

The research was carried out with the support of a grant under the Decree of the Government of the Russian Federation No. 220 of 09 April 2010 (Agreement No. 075-15-2021-615 of 04 June 2021).