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Studying rat retina anatomy and ca^{2+} signalling to optimize retinal implant design

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KEY WORDS: calcium signalling, retinal ganglion cells, two-photon microscopy.

Millions of people worldwide suffer from retinal degenerative diseases, such as retinitis pigmentosa. Common to all those diseases is the lost and malfunction of the photoreceptor cells. This abnormality prevents the phototransduction in the retina and incapacitates vision. To overcome retinopathies, several prosthesis are being designed for the partially restoration of sight in blind patients. These devices consist on a multielectrode array (MEA) which is implanted in the retina of the patient. The electrodes can be driven to produce electrical stimulation of the retinal ganglion cells (RGC) allowing the propagation of signal to the nerve fiber layer (NFL), and ultimately to the brain. The main goal of our project is to refine and improve the already existing MEAs using a graphene-based technology.

Calcium signalling plays an important role in neuronal synapsis. Calcium indicators are fluorescent molecules that respond to the binding of free $Ca2^{+}$ ions by changing their fluorescent properties. Therefore, they result an ideal readout for neuronal activity. To optimize the design of such implants, the calcium activity of the RGCs will be measured, upon stimulation.

In this work, we follow three main directions based on different staining procedures that serve us as a preliminary approach for the final optimization of such implants. First, we tested retinas' viability after dissection with a cell death marker. Secondly, to characterize the dimensions and potential heterogeneities of the retina, whole eye transversal sections were stained with hematoxylin and eosin (H&E). In addition, on excised and fixed retinas, we have fluorescently outlined the cytoskeleton and nuclei of all retina layers. Furthermore, we have specifically labelled the neurofilament H present in RGCs. Once the anatomy of our sample was better understood, we started a precursory approach on calcium imaging by labelling primary culture neurons with ACa1-AM, a two-photon fluorescent marker. In a next stage, we will test and compare the performance of different calcium indicators, as reporters of neuronal activity.

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The possibilities of optical non-invasive diagnostics for studying microcirculation disorders in tissues in patients with diabetes mellitus

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Disorders of microcirculation and metabolic processes in the diabetic foot have a key role in the development of ulcers and their subsequent insensitivity to treatment. Optical diagnostic methods have potential to find promising application in the diagnosing of diabetes disorders. Applying several diagnostic technologies simultaneously in one diagnostic volume is a promising way for a comprehensive diagnosis of the state of microcirculatory-tissue systems.

The aim of this study was to explore the possibility of joint application of laser Doppler flowmetry, fluorescence spectroscopy and diffuse reflectance spectroscopy methods to assess the severity of trophic disorders in diabetes mellitus.

The studies involved registration of changes in blood flow and the biological tissue coenzymes fluorescence during 4 stages. The first stage included registration of a basic test of LDF-record for a 4 min. The second stage included registration of a local cold test (t = 25 °C) for a 4 min. Third and fourth stages included registration of a local heating test (35 °C and 42 °C) for a 4 min each. Simultaneously with the registration of the perfusion at each stage a pair of fluorescence spectra were recorded at the probing wavelength of 365 nm and 450 nm. Registered perfusion parameters and the amplitude of the coenzymes fluorescence were evaluated simultaneously using a specially designed system in the same tissue volume (SPE "LAZMA" Ltd, Russia). The optical probe was installed on the dorsal surface of the foot on a point located on a plateau between the 1st and 2nd metatarsals. All studies were performed in the supine position. Before the beginning of each study at the specified point registration of the spectra of skin diffuse reflection was carried out by a compact spectrometer "FLAME" (Ocean Optics, USA). A total of 76 patients (including 14 ones with trophic disorders) and 46 controls took part in the study. Statistically significant differences in the registered and calculated parameters between the groups studied were obtained. As well, the possibilities of wavelet analysis of LDF signals were further investigated. The study of oscillating components shows a significant difference of the spectral properties even in the basal conditions. Local thermal tests induce variations both in the perfusion and its spectral characteristics, which are different in the groups under consideration.

Thus, skin fluorescence and the level of perfusion at a heating test may be markers of various degrees of complications, from the onset of diabetes to the formation of trophic ulcers. The results of joint non-invasive studies of the lower limbs by optical spectroscopy methods allows one to implement a comprehensive approach to the diagnosis of tissue metabolism and microcirculatory disorders in patients with diabetes.