Imaging microviscosity in mesenchymal stem cells and their differentiated counterparts using a viscosity-sensitive molecular nanorotor and FLIM

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Changes in microscopic viscosity (microviscosity) signal about the ongoing intracellular alterations or the onset of various diseases, e.g. diabetes or atherosclerosis [1]. Imaging microviscosity before and after differentiation would enable a greater understanding of the differentiation mechanism and a more accurate characterization of stem cells, e.g. mesenchymal stem cells (MSC), which are able to differentiate into fat, bone or other connective tissue. Microviscosity bioimaging can be achieved by utilising molecular nanorotors with fluorescence lifetime imaging microscopy (FLIM) [1,3]. In this work, we utilise BDP-H molecular nanorotor (Figure 1). In low-viscosity environments, intramolecular rotation of BDP-H meso-phenyl ring is unhindered, and the relaxation from the excited state primarily occurs through a non-radiative energy transition leading to a shorter fluorescence (FL) lifetime. In more viscous environments, the rotation is restricted and energy is released through a radiative decay, thus resulting in longer FL lifetime [1,2]. The aim of our study was to determine intracellular localization of BDP-H and to apply the molecular nanorotor for microviscosity determination in live human skin MSC and their differentiated cultures – adipocytes and osteoblasts. Live cells were imaged with a confocal laser scanning microscope. FL lifetimes of BDP-H in live MSC were determined using time-correlated single photon counting based FLIM. Localisation experiments showed that BDP-H accumulates in lipid droplets (LD) and cytosol of live MSC (Figure 1A). In addition, BDP-H intensely accumulates in enlarged LD of adipocytes, while in osteoblasts, cytosolic staining is more pronounced. Finally, by using a calibration curve obtained in toluene-castor oil mixtures of known bulk viscosities, and the τ_1 of BDP-H decays obtained with FLIM (Figure 1B), we were able to assign microviscosities values of ca. 120 cP and 104 cP to LD of undifferentiated MSC and mature adipocytes, respectively.

References

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Figures

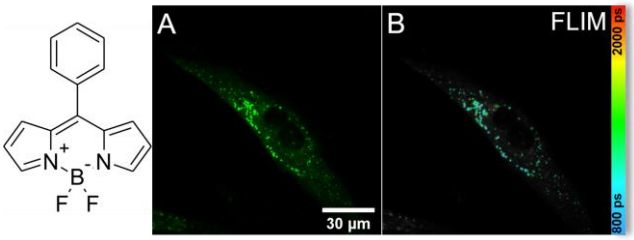

Figure 1: Structure of the BDP-H molecular nanorotor and its accumulation in lipid droplets of MSC (A). B – FLIM image obtained by applying a biexponential fit (τ_1 = 871±3 ps, mean lifetime – 1215±4 ps) (λ_{ex} = 490±5 nm).

Acknowledgements: supported by the Research Council of Lithuania, Grant No. S-MIP-19-6.