OPTICAL REPROGRAMMING WITH ULTRASHORT FEMTOSECOND LASER PULSES

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We report on the use of femtosecond-laser pulses for laser-assisted non-viral reprogramming of somatic cells into induced pluripotent stem (iPS) cells. Reprogramming of a cell can be evoked through the ectopic expression of defined transcription factors. Conventional approaches utilize retro/lenti-viruses to deliver genes and transcription factors as well as to facilitate the integration of these factors into the host genome. However, the viruses may lead to insertional mutations due to a random integration of genes. This significantly limits the potential for future clinical use of iPS cells which are generated in this way because of the high risk of subsequent cancer formation. Femtosecond-laser assisted transfection provides an alternative reprogramming method which is completely virus free. Femtosecond-laser pulses which are focused onto a cell can induce transient cell-membrane permeabilization, so-called optoporation. We have used this method to introduce transcription factors into human fibroblast to generate iPS cells [1-2]. The experiments were performed with an experimental platform comprising of a femtosecond oscillator, a laser-scanning microscope with beam shaping optics as well as home-made software control to automate the laser illumination for adherent cells. In a second setup, flowing cells inside a micro flow tube were transfected by optoporation [2]. Details of the experimental setup as well as the transfection results will be reported.