Optical transfection with a microfluidic cell system using sub-15 femtosecond lasers

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Through cell reprogramming somatic cells can be turned into induced pluripotent stem cells (iPS). The latter are similar in every way to embryonic stem cells (ESC), and as such can differentiate into every somatic cell type of the human body. The conventional iPS technologies require the introduction of exogenous genetic material into the donor cell (transfection). Gene delivery can be achieved through optoporation, *i.e.*, the creation of transient holes in the cell membranes using femtosecond (fs) laser pulses.[1] This method, unlike conventional ones, is contact-free, non-toxic, reproducible, virus-free, and easy to perform.
In this work, we demonstrate the use of fs pulses for laser-assisted transfection and reprogramming of suspension cells in a microfluidic system. For optical transfection, the cells were flowing in a microfluidic cell system with an inner diameter of about 100 μm. A pump system was used maintain a continuous flow of cells. Transient permeabilization of the cell membranes was achieved using a Ti:sapphire laser generating sub-15 fs pulses at a repetition rate of 85 MHz. A quasi-Bessel beam was generated using an axicon lens to generate an axially elongated focal region with a length in excess of 100 μm. The focused laser light was then scanned in a direction perpendicular to the cell flow across the microfluidic cell system so as to generate a kind of scanned light sheet through which the cells passed. The average interaction time between the cells and laser light was about 1 ms. The mean laser power used was 135 mW. Using this transfection method, a very large number of cells could be addressed. Survival rates between 96% and 100% were obtained. The transfection efficiency was confirmed through the expression of green fluorescence protein (GFP).[2]–[4] GFP expression was observed about 48 to 72 hours after optoporation and remained for several weeks. Introduction of transcription factors Oct4, Klf4, Sox2, and c-Myc linked to GFP resulted in the formation of spherical cell clusters between day 4 and 6 following transfection. Live cell staining revealed the presence of a pluripotency marker, showing therefore successful cell reprograming.[3]
Using a microfluidic cell platform coupled with an ultrashort laser source, different cell lines can be transfected and reprogrammed. Moreover, this system allows to directly expand optoporated cells on feeder cells.

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