

FEMTOSECOND LASER BASED TRANSFECTION OF MAMMALIAN CELLS

- A DOOR FOR NOVEL CELL THERAPEUTIC APPROACHES

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THE AIM

Transfection of macromolecules represents a key technique in molecular biology. The currently applied methods e.g. viral vectors, chemical carriers, lipofection and electroporation face several critical problems in terms of the achieved efficiency, toxicity, and reproducibility. Although for multiple applications the systems used are sufficient, several experimental approaches require more sophisticated methods. Parameters like a defined amount of induced molecule uptake e.g. DNA, reduced cell damage and high transfection efficiency represent critical parameters when working with cell type requiring extremely high complexity handling e.g. stem cells. "Opto-perforation" is an interesting alternative to conventional techniques for gene transfer into living cells. The cell membrane is perforated by femtosecond (fs) laser pulses, in order to induce an uptake of macromolecules. The laser nanodissection system (CellSurgeon, ROWIAK, Germany) used in this study allows a defined control of the described critical transfection parameters (Figure 1D).

The novel alternative method for cell membrane perforation using fs-laser pulses allows to avoid the described transfection-problematics. Whereas the whole membrane is perforated by electroporation, the fs-laser pulses are focused on a small region of the membrane less than one micrometer in diameter (Figure 1 A+B). Due to the shortness of the laser pulses, almost no heating of the irradiated volume occurs as the applied pulse duration is shorter than the thermal conduction time. The manipulation induced by these pulses is limited to the focal volume, because the effect is based on multiphoton absorption and therefore relies on very high photon densities. Thus, the perforation of the cell membrane by the fs-laser pulses does not damage the whole cell, affecting only a small volume of some femtoliters. The opto-perforation technique allows a "single cell targeting" and hence provides a key advantage in respect to selectivity, when compared to standard transfection (Figure 2 B).

EXPERIMENTAL DESIGN AND RESULTS

Laser parameter evaluation in terms of the viability of the cells and the efficiency

We evaluated the effects of fs-laser pulse energies from 0.7 to 1.1 nJ in combination with different irradiation times (40ms-60ms) and characterized the achieved effects on mammalian cells. Aim was to define parameters allowing a high transfection efficiency combined with a high viability of the transfected cells. For the following experiments we chose as transfection parameters an irradiation time of 40ms with an pulse energy of 0.9 nJ (Figure 2 A+C)

Transfection of canine MTH53a and ZMTH3 cells by fs-laser pulses with pEGFP-C1 and pEGFP-C1-HMGB1 vectors

The cells were settled in an experimental design according to "Figure 1C" and treated either in presence of 50 µg/ml non-recombinant pEGFP-C1 vector or recombinant pEGFP-C1-HMGB1 vector in the culture media. The transfection was performed as described above. The fluorescence was observed 24 and 48 h after treatment allowing an expression and processing of the respective recombinant proteins. The cells transfected with pEGFP-C1 vector showed a labeling of the complete cell (Figure 3 A+B) by the synthesized recombinant GFP proteins. The cells transfected with the pEGFP-C1-HMGB1 vector showed a specific labeling of the nucleus (Figure 3 C+D). These specific labeling shows that the cells are still able to synthesis the pEGFP-HMGB1 fusion protein and to transport the chromatin associated architectural transcription factor HMGB1 to its native cellular localization in the nucleus post transfection.

CONSEQUENCES

In this study, we successfully transfected two canine cell line (MTH53a and ZMTH3) with GFP vector or a vector coding for a GFP-HMGB1 fusion protein. The transfected cells were observed 48 hours after treatment and they were not showing any signs of apoptosis or necrosis. Based on measured membrane potential changes during the perforation, we were able to calculate and experimentally verify that the relative volume exchanged is 0.4 times the total cell volume (data not shown). Thus, for first time a quantitative predication of the amount of uptaken molecules and therefore a quantification of the transfection is possible. Consequently, this method offers new high efficient possibilities for critical transfection approaches involving high sophisticated cell types, e.g. primary and stem cells.

Acknowledgements

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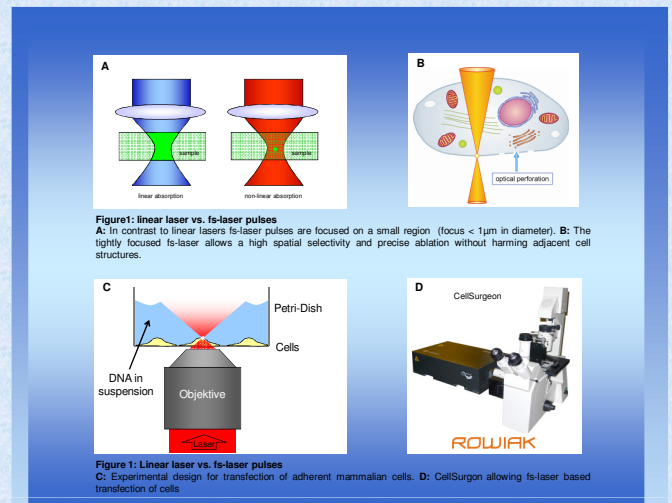


Figure 1: Linear laser vs. fs-laser pulses

A: In contrast to linear lasers fs-laser pulses are focused on a small region (focus < 1µm in diameter). B: The tightly focused fs-laser allows a high spatial selectivity and precise ablation without harming adjacent cell structures.

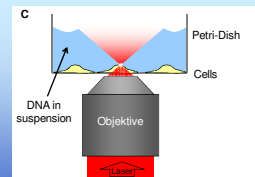


Figure 1: Linear laser vs. fs-laser pulses

C: Experimental design for transfection of adherent mammalian cells. D: CellSurgeon allowing fs-laser based transfection of cells

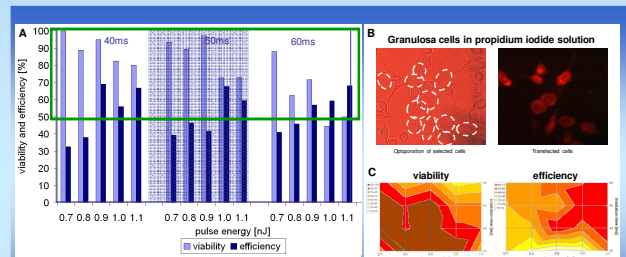


Figure 2: Viability of the optoperated cells linked to the achieved transfection efficiency

A+C: Cell viability and transfection efficiency depending on laser irradiation time and pulse energy. B: Granulosa cells showing the viability of propidium iodide containing media. The cells were observed for 90 minutes to exclude false positive viability results.

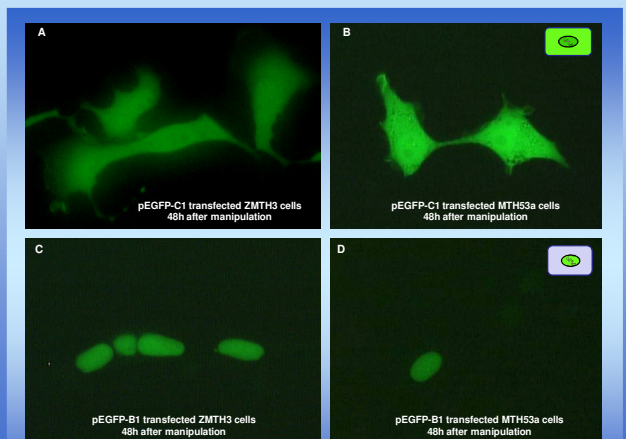


Figure 3: Optoperated transfected mammalian cells expressing GFP- and GFP-fusion proteins

A+B: Canine ZMTH3 and MTH53a cells transfected with an expression plasmid coding for EGFP showing fluorescence in the complete cell. C+D: Canine ZMTH3 and MTH53a cells transfected with a recombinant expression plasmid coding for a HMGB1-EGFP fusionprotein showing fluorescence in the nucleus. As HMGB1 is an architectural transcriptionfactor the fusionprotein is located in the nucleus.