Plasmonics on nanostructures for cell manipulation

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Abstract: A plasmon resonance based method for introducing foreign material into living cells is presented. By illuminating gold-coated or structured surfaces, near field enhancement is employed to selectively open the cell membrane using ultrashort laser pulses.

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1. Introduction

In the field of molecular biology the targeting of the cell membrane with short or ultrashort laser pulses allows the delivery of extracellular compounds to the target cell, as for example needed in gene therapy or high-throughput screening. Tightly focused laser pulses allow the precise and minimal invasive manipulation of cells and cell compartments with subcellular resolution. Several works have shown successful delivery with high cell viabilities in different model system over the past two decades [1]-[3]. However, due to their focusing conditions, these techniques are very limited with respect to cell throughput. To overcome these limitations, several approaches are possible, for example microfluidics [4] or special beam shapes [5] to mitigate this specific drawback. In contrast, other approaches employ micro- or nanoparticles [6]-[8], Wu et al. immobilized these particles on a surface [9]. Especially gold structures (among other noble metals) can confine the energy of a loosely focused laser beam into the near, due to the creation of localized surface plasmons. Here, we show an overview of these different approaches, especially gold-nanoparticles and nanostructured surfaces and presenting our work with different laser systems at 532nm and 800nm with pulse durations in the ps- and fs-regime.

2. Methods

Before experimental use, the different shapes of nanostructures on surfaces and sizes nanoparticles were modeled within COMSOL Physics (Multiphysics 4.2a) using a Finite Element Method (FEM). Different sizes of gold nanoparticles were simulated (between 20nm- and 200nm) and different surface structures, as for example nanoparticles attached to a surface or other structures. The irradiation wavelength was varied between 400nm-1100nm and the near-field enhancement was calculated ($\eta=|E|/|E_0|$). To study different wavelengths, an ultrashort tunable oscillator (COHERENT, Ultra II) was used, delivering 140fs pulses at 80MHz with a maximum output power of 4W and a wavelength range between 680-1080nm. Additionally, a microchip laser (HORUS LASER) at 532nm and 850 ps pulse duration at 20kHz with an output power of 100mW was used to excite at the plasmon resonance of gold. The structures and particles were incubated with a standard cell line (ZMTH3) and seeded 24 h before manipulation. As a standard molecule, lucifer yellow, a membrane impermeable dye was used to monitor the uptake of extracellular molecules upon laser irradiation.

3. Results

Using different substrates and shapes, successful cell manipulation (uptake of membrane impermeable extracellular molecules) could be demonstrated. Depending on illumination wavelength, the mechanism for optical perforation was found to be thermally induced or induced by nonlinear processes at the near-field of the particles, possibly due to photochemical and low-densities plasma effects. The found effects correlated well with the findings from the simulations.

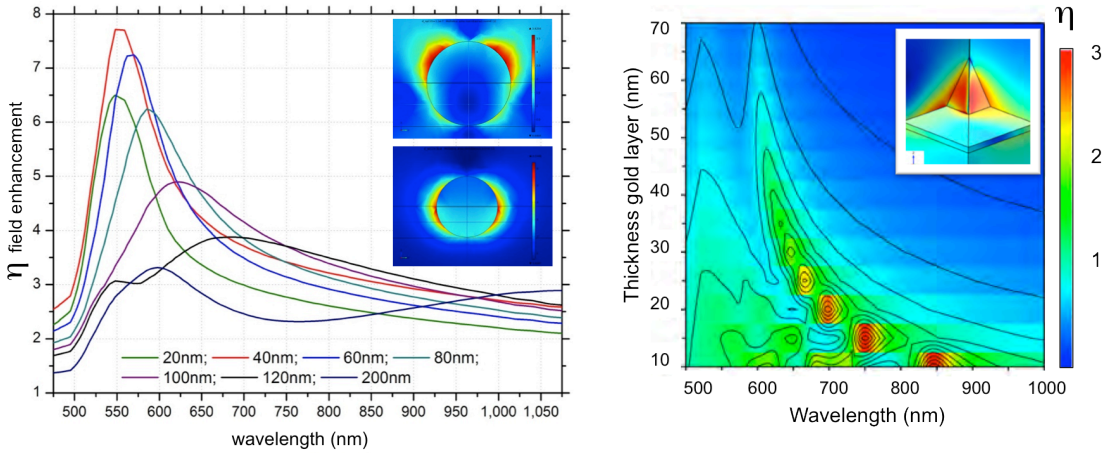

Figure 1: (Left) Near-field enhancement (η) for different gold nanoparticles sizes, particles are embedded in polymer (50%). (Right) Near-field enhancement (η) at a 3d surface structure (pyramidal shape) at different gold thicknesses.

4. Discussion

The plasmon-based cell perforation using different shapes and structures heavily depends on size, geometry and illumination parameters (pulse duration and wavelength) [10]. Different mechanisms can play a role, leading for example to a thermal fragmentation of the particles after exposition at 532nm in the ps regime, whereas nearly no thermal melting of structures or particles was found at NIR wavelengths in the fs regime. Furthermore, the relation between plasmon resonance and illuminating wavelength plays an important role as well. This has consequences for possible applications, as thermally denatured particles might act toxic in *in vivo* applications, whereas they might prove useful for screening applications [11].

5. Conclusion

Using gold-coated nanostructures and nanoparticles, the transient perforation of cell membranes by laser irradiation could be shown. Due to the moderate focusing conditions, high throughput using multi-well plates is enabled, allowing applications in siRNA-screening or gene knockdown. Depending on laser wavelength and pulse duration, different fundamental mechanisms for creating the membrane opening are found.

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