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Abstract (Doctor)

Title of Thesis	Controlled Cellular Perforation by Photoabsorber-Mediated Laser Irradiation: From Targeted Delivery to Selective Ablation
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Approx. 800 words

Controlled laser irradiation methods have emerged as a powerful tool in the field of modern biomedical engineering, offering precise administration of foreign molecules such as genetic material, drugs, and functional proteins in the intracellular microenvironment, changing the phenotype of cells, and enabling selective ablation of cancer cells to offer site-specific cancer therapy. Traditional methods cannot, however, target cells precisely at a site-specific location with high throughput. Photoabsorber-assisted laser irradiation methods overcome these drawbacks by uniquely combining the application of light-absorbing materials for converting light energy from laser to heat to create both temporary and permanent pores on the cell membrane to facilitate intracellular delivery and cell necrosis. This thesis discusses the development of site-specific cell perforation methods for the creation of transient and permanent pores on the cell membrane for the application in the field of intracellular delivery, photothermal therapy, and cell patterning.

Chapter 2 presents the development of a micropattern-assisted method to perform controlled intracellular delivery of Fluorescein Isothiocyanate (FITC) Dextran into cells. The system utilizes motorized stages to locate the target site of irradiation. The findings in the chapter highlight the importance of the size of the micropatterns and cell-specific properties in the determination of cellular response to laser-induced shockwave upon irradiation on a microstructure surface. This approach presents a robust distance-dependent method to systematically optimize the shockwave-mediated intracellular delivery, which balances delivery efficiency and cell viability.

Chapter 3 introduces a new approach to parallelize the method introduced in Chapter 2. We used a micropattern on the top setup, which facilitates the intracellular delivery of Fluorescein Isothiocyanate (FITC) Dextran in a massively parallel fashion. The shockwave induced from laser irradiation of the micropattern surface causes bio-effects on cells by creating transient and permanent pores, which results in intracellular delivery, cell peeling, and cell necrosis. Each of which contributes to either intracellular delivery or cell ablation. We have investigated the effect of varying laser fluence and micropattern pitch on the shockwave response of HeLa cells. This approach highlights the importance of processing parameters for desired outcomes on cells.

Chapter 4 shifts the focus of photoabsorber-assisted laser irradiation methods into the field of photothermal therapy. This chapter explores the combination of visible laser irradiation to plasmonic gold

nanoparticles, which facilitates the formation of vapor nanobubbles at the interface of nanoparticle and cell medium, whose collapse results in the formation of permanent pores on the cell membrane, resulting in cell death. The chapter further explores the applicability of the system for spot laser irradiation and scanning laser irradiation at specific sites to kill cells. Further, we explored the effect of varying concentrations of gold nanostars on the cell killing efficiency and applicability of the system for three cell types of HeLa, HEK, and SAOS-2.

Chapter 5 explores the applicability of Prussian blue nanoparticle clusters (PBNCCs) for application into intracellular delivery and photothermal ablation for patterning cells. First, we explored the effect of varying process parameters such as PBNCCs concentration and laser fluence for intracellular delivery of Fluorescein Isothiocyanate (FITC) Dextran into HeLa cells. Moreover, we check the applicability of the same setup for site-specific ablation of HeLa cells, which assists in the formation of precise cell patterns. We optimized similar process parameters for cell patterning. We further formed cell micropatterns of target size at the optimized parameters and analyzed the accuracy and precision of pattern formation. This chapter highlights that a similar photoabsorber setup can be used for creating both transient and permanent pores in cell membrane.

In summary, this thesis presents four different approaches for creating site-specific cell perforation in cell monolayers. The experiments conducted in this thesis highlight that careful selection of photoabsorber material and laser parameters facilitates obtaining a deterministic outcome of cell perforation in the form of transient pores to help in intracellular delivery and permanent pores for killing cells to apply for the application of photothermal therapy and cell patterning.