Journal of Photochemistry and Photobiology C: Photochemistry Reviews 28 (2016) 1–28

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology C: Photochemistry Reviews

journal homepage: www.elsevier.com/locate/jphotochemrev

Review

In situ patterning and controlling living cells by utilizing femtosecond laser

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ARTICLE INFO

Article history:
Received 9 March 2016
Accepted 4 July 2016
Available online 9 July 2016

Keywords:
Femtosecond laser cavitation
Femtosecond laser ablation
Living cell manipulation
Living cell patterning
Cell-cell interaction
Cell migration

ABSTRACT

Photo-induced processes have high potential in *in situ* patterning and controlling living cells, whose developments are introduced and recent progresses by utilizing femtosecond laser are described. Photochemical and photothermal surface modification performed by conventional light and nanosecond laser irradiation is summarized and their applicability is considered. Femtosecond laser ablation has superior features due to its photomechanical mechanism, which is confirmed by ultrafast spectroscopy and imaging of a model film under laser ablation. Femtosecond laser ablation of physiological solutions generates shockwave and cavitation bubbles, which is employed for patterning and manipulating living cells. Femtosecond laser ablation fabricating cytophobic and cytophilic domains enable us to form living cell patterns and to study cell migration and cell–cell interaction. Finally summary and perspective are presented.

Contents

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http://dx.doi.org/10.1016/j.jphotochemrev.2016.07.001
1389-5567/

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1. Introduction

Lasers were introduced to study molecular spectroscopy and photochemistry very early in 1960's and they have been used as light sources for spectroscopic measurements as well as for inducing photochemical reactions. Chemists started to use pulsed lasers for time-resolved spectroscopy, and then they soon became aware that laser irradiation induced vaporization, decomposition, and fragmentation of materials and found that the irradiated surface was etched, which was later called laser ablation by Srinivasan [1]. Its systematic application was quickly developed to fabricate microstructures of various materials by electronics engineers and to microsurgery by medical doctors. Laser ablation is one of the representative nonlinear photochemical phenomena and has a threshold with respect to laser fluence. Initially most of laser ablation studies were carried out for metals, semiconductors, ceramics, and glasses [2], while organic materials [3–6], cells [7,8], tissues [9,10], and organs [11] have recently been receiving more and more attention.

In general ablation mechanism of organic and biomaterials was discussed from photochemical and photothermal viewpoints. The former is based on the understanding that observed photon excitation energy is sometimes higher than ionization potential and chemical bond energy, leading to ion formation and bond cleavage, respectively. The molecules in the irradiated volume may decompose typically to small gas phase molecules and the volume should be etched. This interpretation is due to thermodynamic consideration, and the time scale of ~10 ps in which the decomposition occurs is not examined. When the relaxation from the higher excited states to the first excited state is fast, the excess energy is converted to heat before ionization and decomposition takes place from the higher excited states. The irradiated area is instantaneously heated to high temperature and shows explosive melting, leading to fragmentation and ejection. This decomposition behavior is interpreted as photothermal ablation, indicating that higher excited status formed by multiphoton absorption does not always undergo photochemical decomposition. At the early stage of ablation studies of molecular materials such as molecular assembly, supramolecules, molecular crystals, polymers, membranes, liposomes, living cells, and tissues, such mechanistic considerations seemed reasonable and were presented in many papers [1–6,10,12]. From theoretical viewpoints some photothermal models were presented for polymer ablation [13,14], while a simple sublimation model was proposed assuming the similarity between polymers and inorganic materials [15,16], which was later developed to discriminate various models [17].

Considering characteristic high potential in laser ablation, one of the promising applications is to make various patterns on glass, semiconductor, metal, and polymers substrates. Its future has extended from micro- to nano-electronics and from micro surgery to living cell manipulation. Noncontact and spatio-temporally controllable patterning has been requested for long time to develop new methods for studying cell functions, controlling cell behaviors, and utilizing them toward bio-mimic devices, and now being achieved by dynamic patterning using femtosecond laser ablation. Here first we shortly summarize the patterning methods applied for the purposes.

The patterning was started by introducing photolithography which needs photomasks to fabricate fine structures on a substrate. The resolution below 100 nm is made possible with ultraviolet irradiation of 157 nm [18]. DNAs, proteins and living cells could be patterned on substrates [19–24]. However, the photomask projection lithography has been time-consuming and laborious because so many steps of resist processing are necessary and indispensable. The conventional photolithography was developed to soft lithography and it has been widely applied for patterning proteins and living cells [25–29]. This is well known