LASER BIO-PRINTING OF CONIDIA AND LIVING CELLS USING ABSORBING FILM ASSISTED LASER INDUCED FORWARD TRANSFER

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Abstract

Absorbing film assisted laser induced forward transfer arrangement was developed and applied for the controlled transfer of living biological samples, fungus conidia, living rat Schwann and astroglial cells and pig lens epithelial cells. A KrF excimer laser beam (λ=248 nm, FWHM=30 ns) was directed through a fused silica plate and focused onto its silver-coated surface onto which the biological samples were spread as a thin film. The applied laser fluence was 355 mJ/cm^2 and each laser pulse transferred a pixel of target material. We found that the transferred conidia pixels were germinated and all three cell types survived, proliferated and differentiated under cell culture conditions and regained their original phenotype few days after cell transfer. Our time-resolved studies proved that the ejection velocities of the investigated conidia and astroglial cells from the donor surface during the transfer procedure were 1140 and 120 m/s, respectively. On the basis of the recorded snapshots the estimated average accelerations of the emitted conidia and cells were approximately 10^9 and 10^7×g, respectively. Our results proved that the living biological samples tolerated the extreme high acceleration and the deceleration during the impact to the acceptor plate without significant damages.

1. Introduction

Laser processing is increasingly finding applications in the transfer of biological materials and organisms because its capabilities surpass what can be done by conventional techniques. A number of methods have been reported for the controlled movement of different living biological samples.

MAPLE direct write (MAPLE DW) is a new laser-based direct-write technique which combines the basic approach employed in laser-induced forward transfer (LIFT) with the unique advantages of matrix-assisted pulsed-laser evaporation (MAPLE). MAPLE DW utilizes an optically transparent substrate coated on one side with a matrix consisting of the material to be transferred mixed with a polymer or organic binder. As in LIFT, the laser is focused through the transparent substrate onto the matrix. When a laser pulse strikes the matrix, the binder decomposes and aids the transfer of the material of interest to an acceptor substrate placed parallel to the matrix surface [1]. Ringeisen and co-workers reported the successful deposition of active protein thin films using MAPLE technology [2]. Biomaterials ranging from polyethylene glycol to eukaryotic cells, i.e. Chinese hamster ovaries, were also deposited with no measurable damage to their structures or genotype. Deposits of immobilized horseradish peroxidase, an enzyme, in the form of a polymer composite with a protective coating (polyurethane) retained their enzymatic functions. A dopamine electrochemical sensor was fabricated by MAPLE DW using a natural graphite composite [3].

Barron and co-workers have developed a laser-based printing technique, called biological laser printing, which is a non-contact, orifice-free technique that deposits fL to nL scale volumes of biological material with spatial accuracy better than 5 μm [4]. This technique was applied to print single-layer patterns of pluripotent murine embryonal carcinoma cells. It was found that when cells are printed onto model tissue scaffolding such as a layer of hydrogel, more than 95% of the cells survive the transfer process and remain viable [5]. The report of Barron et al. describes the use of this laser-based printing method to transfer genetically-modified bacteria capable of responding to various chemical stressors onto agar-coated slides and into microtiter plates. This technology enabled smaller spot sizes, increased resolution, and improved reproducibility compared to related technologies [6]. Fernández-Pradas et al. focused a Nd:YAG laser beam (355 nm wavelength) through a transparent glass plate on a titanium film that coated its rear side and acted as radiation absorber. The

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titanium film was, in turn, coated by a liquid solution of salmon sperm DNA. In this way they deposited microarrays of micrometric droplets of this solution [7]. Zergioti et al. presented the direct micro-printing of biomaterials such as enzyme patterns by laser-induced forward transfer method using 500 fs laser pulses emitted at 248 nm. The dynamics of the process was investigated by stroboscopic Schlieren imaging for time delays up to 3 ms following the laser irradiation pulse [8].

Here we report our results obtained with a modified laser induced forward transfer (LIFT) arrangement developed for controlled transfer of living materials.

2. Materials and Methods

Absorbing Film-Assisted Laser-Induced Forward Transfer (AFA-LIFT) was developed and applied for the controlled transfer of *Trichoderma longibrachiatum* conidia, rat Schwann and astroglial cells and pig lens epithelial cells. A thin Ag film deposited onto a transparent (Suprasil2) holder carried the actual biological sample to be transferred. A glass plate coated with a thin culture medium (in the case of conidia) or wet gelatin layer (for the cell experiments) faced the donor plate with a 0.6 mm gap between them. The silver film was irradiated by single pulses of a KrF excimer laser (λ=248 nm, FWHM=30 ns) through the quartz plate (Fig. 1).

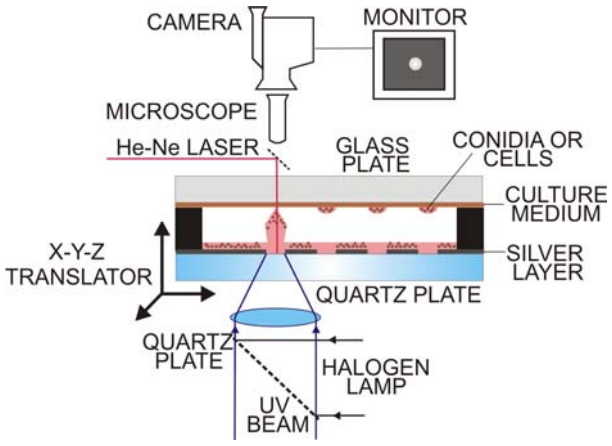

Figure 1: Experimental arrangement

In all experiments reported here the average area of the illuminated spots was 0.075 mm^2 and the average applied fluence was 355 mJ/cm^2. The sample was translated shot by shot and each laser pulse irradiated a virgin target surface area. According to this a matrix of droplets containing the transferred conidia or cells appeared on the culture medium/gelatin layer corresponding to the spatial positions of the illuminated donor spots.

3. Results

It was found that the transferred conidia pixels were germinated on the culture medium surface (Fig. 2) and all three cell types survived, proliferated and differentiated under cell culture conditions and regained their original phenotype few days after cell transfer (Fig. 3).

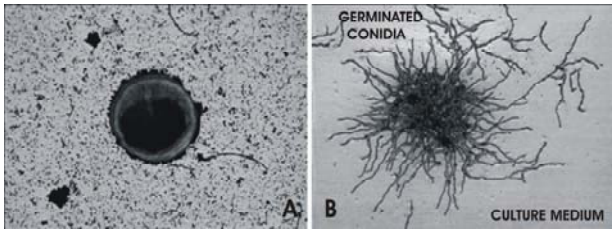

Figure 2: Optical microscopic pictures of the irradiated donor surface (A) and the transferred and germinated conidia (B) after 20 hours incubation time.

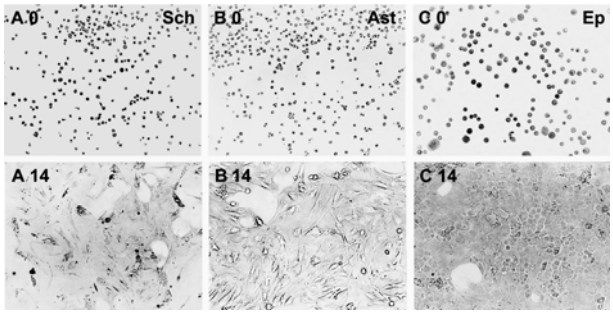

Figure 3: Optical microscopic pictures of the transferred Schwann (A), astroglial (B) and lens epithelial (C) cells right after the procedure and after 14 days.

For the detailed description of the forward transfer process time-resolved investigations were carried out. The donor plate was irradiated by the excimer laser beam from below and its surface was illuminated by an electronically delayed nitrogen laser pumped Coumarin 153 dye laser beam (λ=543 nm, FWHM=1 ns) (Fig. 4).

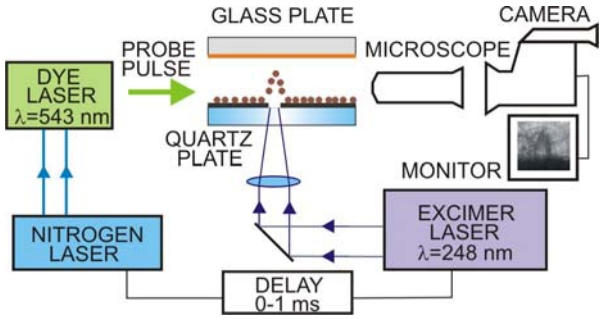

Figure 4: Photographic arrangement for the time-resolved investigation of the transfer processes

The time delay between the processing and the probe pulses could be varied in the range of 0-1 ms. The processes taking place on and above the target surface during AFA-LIFT were observed and recorded by a microscope-camera system. We realised single shot measurements in this case, too: after each UV pulse the sample was moved to a new place with a precision x-y translator. Fig. 5 shows the snapshots of the material ejection process observed and recorded by a

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microscope-video camera-monitor system during AFA-LIFT of *Trichoderma* conidia (a) and astroglial cells (b) at different delays.

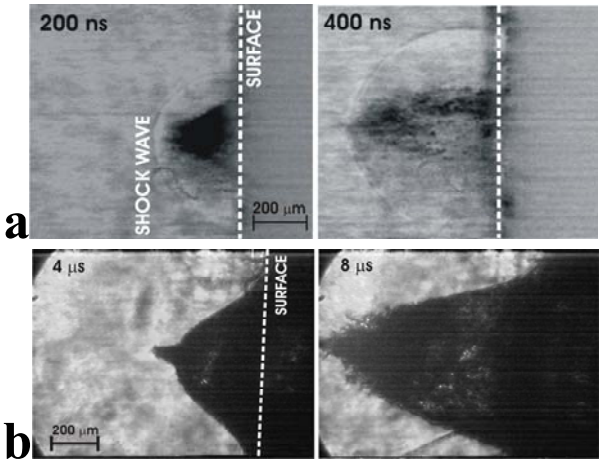

Figure 5: Pictures of the ejected conidia cloud (a) and liquid jet containing living astroglia cells (b) during the AFA-LIFT procedure at different delay times

Using the recorded pictures we could measure the ejection distances at given delays for both conidia and cells (Fig. 6).

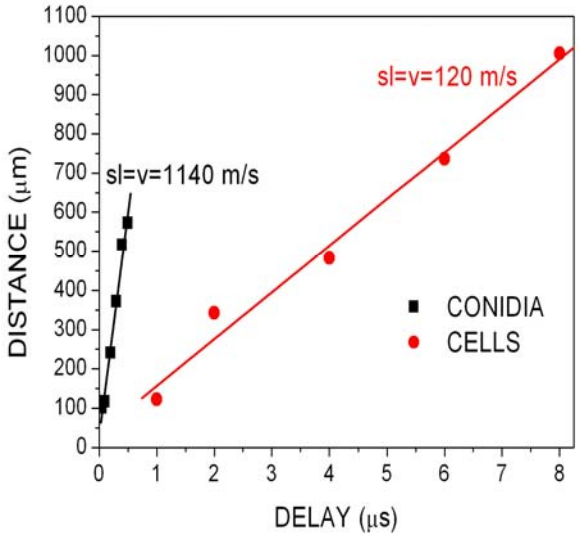

Figure 6: Traveled distances of the ejected conidia cloud and liquid jet containing living cells during the transfer process as a function of delay time.

The slopes of the lines fitted to the measured data correspond to the average velocities of the ejected conidia and cells. According to these values the minimal average accelerations of the biological samples could be estimated. The calculated velocities were divided by the smallest delays, where material ejection could be detected. The estimated accelerations were approximately 10^9 and $10^7 \times g$ for conidia and cells, respectively. These values could be even higher, since the real acceleration periods less than the delays used in our calculations, were not known.

The results suggest that the majority of the studied conidia and cells tolerate extremely high acceleration at the beginning of the ejection and the deceleration during the impact against the acceptor plate without significant damage to the original phenotype.

4. Conclusion

For the interpretation of the results using the one-dimensional heat-flow equation we calculated the temperature of the irradiated silver thin films in the case of conidia transfer process. During our calculations the measured reflection from quartz-silver interface (36%), the phase transformations and the temperature dependences of the specific heat capacity and heat conduction for silver, fused silica and air were taken into consideration. The absorption coefficient of silver was measured to be 71 1/µm, which means approximately 14 nm penetration depth. Fig. 7 shows the calculated temperature of the irradiated silver layer on the fused silica plate.

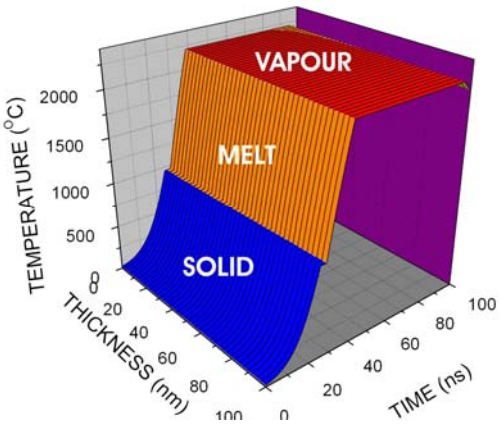

Figure 7: Calculated temperature of the excimer laser irradiated silver layer covered by conidia

It can be seen that with the exception of an approximately 8 nm thick layer cooled by the contacting fused silica plate the whole silver layer was evaporated 40 ns after the onset of the UV pulse.

On the basis of our results and calculations the process of the conidia transfer described as follows: The UV pulse energy is absorbed in a thin layer (few tens of nanometers) of the absorbing metal film (Fig. 8a). In consequence of this the irradiated silver volume is already evaporated during the laser pulse (Fig. 8b). The very quickly expanding metal vapour sweeps along the conidia giving them a drastic push resulting in an extremely high acceleration (Fig. 8c).

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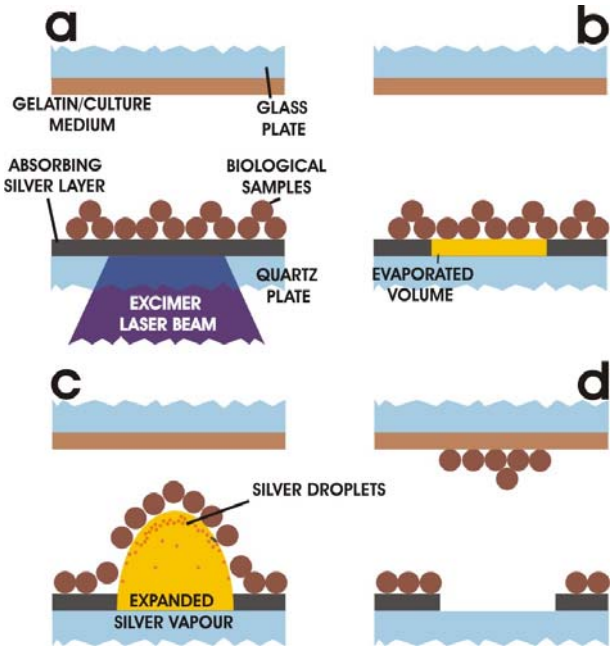

Figure 8: The mechanism of the absorbing film assisted laser induced forward transfer procedure

In our following calculation the silver vapour in the initial stage was used as an ideal gas. Solving the ideal gas equation the evaporation pressure was calculated.

$$pV = nRT\,, \qquad (1)$$

where V is the evaporated silver volume (~irradiated area × film thickness), n is the number of moles, R is the universal gas constant and T is the evaporation temperature (2483 K). It was found that the initial vapour pressure is 20000 atm. The average cross-section of conidia (A) was measured by an optical microscope. Multiplying this value with the pressure we could determine the force (F) influencing a given conidium.

$$F = A \times p = 7.7 \times 10^{-12}\, m^2 \times 2 \times 10^9 \frac{N}{m^2} = \\ = 0.0154\, N. \qquad (2)$$

Applying the second law of motion the maximum acceleration of a conidium (a) could be estimated:

$$F = m \times a \Rightarrow a = \frac{F}{m} = \frac{0.0154 N}{1.4 \times 10^{-14} kg} = \\ = 1.1 \times 10^{12} \frac{m}{s} \approx 10^{11} \times g, \qquad (3)$$

where m is the average mass of the conidia. This value is in relatively good agreement with the results of our time-resolved measurements. The difference can be due to the unknown acceleration duration. Comparing the temperature calculation with the photographic results it is probable that the acceleration begins not earlier than 40 ns after the excimer pulse (when the silver layer is evaporated) and it is finished till 50 ns (when the velocity of the ejected conidia cloud becomes constant). The other reason of the difference can be that we could determine only an average value of the acceleration using the recorded snapshots, while its momentary value can exceed this at the very beginning.

As summary, our results suggest that the AFA-LIFT technique appears to be suitable for several potential applications in tissue engineering and medical tissue repair technologies.

5. Acknowledgements

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