Laser-induced Forward Transfer of Liquids for Miniaturized Biosensors Preparation

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Direct-writing techniques are adequate tools for rapid prototyping of diverse materials, since they avoid the usage of moulds or masks. Among them, laser-induced forward transfer (LIFT) has become a promising tool for rapid prototyping of microdevices due to the high focusing power of lasers, which provides a high resolution, and also to their non-contact and orifice-free nature, which avoids clogging and thus allows working with a wide range of materials. This makes LIFT an appropriate tool for biosensors preparation. In this article, immunoglobulin (IgG) microarrays were prepared through LIFT varying the laser pulse energy. It was found that there exists a minimum energy threshold, E_{min}, below which no material is deposited. Moreover, an analysis of the droplets volumes revealed a linear dependence of this parameter with the laser pulse energy, what allowed finding the existence of an energy density threshold, which is considered to be the threshold to generate an impulsion on the liquid film that only results in droplet ejection when the total energy overcomes E_{min}. Finally, the bioactivity of the transferred proteins was tested, showing no loss of their activity along the whole laser pulse energy range.

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1. Introduction

Biosensors are powerful devices for the detection and identification of different biological molecules, which offer multiple applications in important areas such as genomics, proteomics, and biomedical diagnostics. The preparation of such devices requires not only adequate techniques to deposit and immobilize biological molecules onto a substrate, but also to reach high levels of integration [1]. Direct-writing techniques meet these requirements, adding accuracy, low cost, and speed, and thus making them suitable for the rapid prototyping of miniaturized biosensors.

Although ink-jet printing [2] and dip-pen microspotting [3] are the most extended direct-writing techniques, they present some drawbacks, such as orifice clogging or contamination issues. Laser-based techniques overcome these problems as lasers are non-contact and orifice-free, and their high focusing power allows obtaining higher spatial resolution, what makes them interesting alternatives to the previous conventional techniques.

Laser-induced forward transfer (LIFT) is an additive direct-writing technique that uses laser pulses to transfer portions of material from a thin film previously deposited on a transparent holder to a substrate placed parallel and in close proximity to it. Although LIFT was initially carried out using solid films that were transferred in a process of vaporization and further recondensation, more recently it has been used to transfer pastes and liquids [4-6]. In such cases, the material is propelled by the laser pulse to the substrate without any phase change, which allows depositing complex materials without degrading their properties. This has been used to transfer biological solutions preserving their biological activity, such as DNA [7-9], peptides [10], proteins [11-13], living cells [14, 15] and micro-organisms [16], making LIFT an especially appropriate technique for biosensors preparation.

The study of the LIFT has not only been focused on testing the bioactivity of the transferred solutions, but some effort has also been addressed to the understanding of its transfer process [17-21]. In this work we carry out a study of the LIFT process of an immunoglobulin (IgG) protein solution through the characterization of the morphology of the droplets deposited at different laser pulse energies. In addition, the biological activity of the protein after transfer is also tested.

2. Experimental

A pulsed Nd:YAG laser (355 nm wavelength, 10 ns pulse duration, 1 Hz repetition rate) was used to print a protein-containing solution, which consisted of human IgG in a solution of PBS (0.01M, pH 7.4) with glycerol (40% v/v) at a concentration of 0.05 μg/μL. A volume of 20 μL of this solution was spread by means of a blade coater on top of a laser-transparent glass slide coated with a 50 nm thick titanium film, resulting in a 5 μm liquid film (estimated through its weight measurement). The use of a titanium coating is due to the transparency of both printed solution and glass slide to the laser radiation, which forces the presence of an absorbing layer that is supplied by the titanium film. Different materials such as titanium [1, 7-9, 12, 19, 21], gold [13, 14], or silver [16] have been used as absorbing layers to transfer biological solutions and no loss of bioactivity has been observed in any case.

The system formed by the liquid film and the glass slide coated with titanium, usually referred to as the ribbon, was then placed parallel to a receiving substrate at a separation distance of 25 μm, with the liquid facing the substrate. This distance was kept using spacers. The substrate con-

sisted in a commercially available poly-L-lysine coated glass, commonly used for biomolecules immobilization. The ribbon and the substrate were then placed in a computer-controlled XYZ translation stage. Using an in-house program, the ribbon-substrate system was displaced with respect to the laser beam and synchronized with laser firing so that every laser pulse produced a unique droplet, thereby obtaining droplets microarrays.

The laser beam presented an elliptical Gaussian intensity distribution, given by the expression:

$$F(x,y)=\frac{2E}{\pi\omega_x\omega_y}e^{-2\left(\frac{x^2}{\omega_x^2}+\frac{y^2}{\omega_y^2}\right)} \qquad (1)$$

where E is the laser pulse energy, and ω_x and ω_y the major and minor beam radii respectively, and it was focused on the titanium film using a 15x microscope objective optimized for the 355 nm radiation, with a numerical aperture of 0.32. In a first experiment a 16 columns per 5 rows microarray was prepared using a laser beam with ω_x = 27 μm and ω_y =19 μm, and varying the energy from row to row. In a second experiment, a 9 columns per 18 rows microarray was prepared using a beam of larger dimensions, with ω_x = 47 μm and ω_y =30 μm. The energy was also varied from one row to another.

The morphology of the deposited droplets was characterized using optical microscopy, and optical profilometry was used to obtain their three-dimensional profiles, which allowed calculating the droplets volume. The bioactivity of the transferred solution was evaluated by means of a fluorescence scanner operating at 635 nm exciting wavelength, as cyanine molecules (Cy5) were used as fluorescence tags.

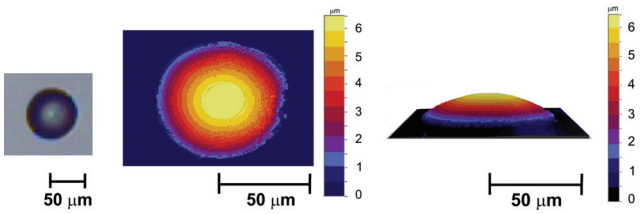

Fig. 2. Optical microscopy image of a droplet deposited at a laser pulse energy of 3 μJ and its pseudo-colour topographic image in two and three-dimensions obtained with the optical profilometer.

3. Results and discussion

An optical microscopy image of the first prepared microarray is presented in Figure 1. It is observed that all the droplets have a circular and well-defined contour. The three dimensional profile of one of these droplets, shown in Figure 2, reveals that they can be considered spherical caps. As it can be seen in Figure 1, the droplets diameters increase as the energy increases. However, there exists a minimum energy, E_{min}, below which no material is transferred. This explains the sporadic lack of droplets in the lower rows, since some laser shots do not reach E_{min} due to laser instabilities. The value of E_{min} sets the maximum resolution of the technique for fixed laser focusing conditions: the optimal printing results are obtained for energies slightly above this value.

In order to quantify these results, the volume of each droplet was calculated and plotted versus the laser pulse energy that caused its deposition, as shown in Figure 3. The appropriate parameter to characterize the transfer process is the droplets volume, owing to the dependence of the droplets contact angle, and thus its diameter, on the substrate nature. The points in Figure 3 present an upward

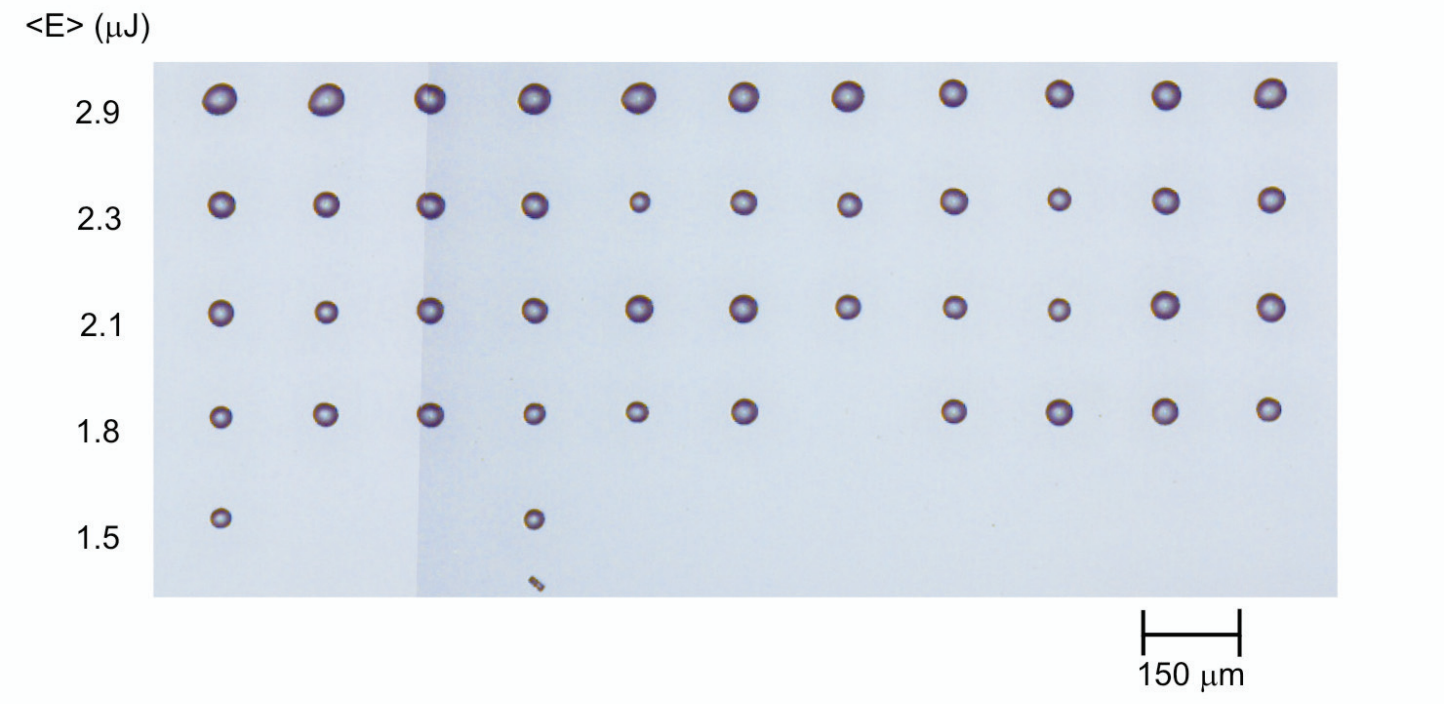

Fig. 1. Optical microscopy image of a portion of an array of droplets of the IgG solution obtained at different laser pulse energies at a fixed laser beam size of ω_x= 27 μm and ω_y=19 μm.

linear trend, a relationship between droplets volume and laser pulse energy which has also been found for two other different solutions [19, 21]. This strongly suggests that the linearity between the removed liquid volume and the laser pulse energy corresponds to a general behaviour, independent on the particular composition of the transferred solution.

The linear relationship found can be characterized fitting the experimental points to the expression:

$$V=K\,(E-E_0) \qquad (2)$$

where V is the droplet volume, E the laser pulse energy, and K and E_0 the fitting parameters. The parameter K, which has a value of 1.9 pL/μJ in the current experiment, is interpreted as the efficiency of the transfer process, whereas E_0, with a value of 1.3 μJ, is not a true energy threshold, as the fit may suggest. Actually, the true energy threshold is E_{min}, which has a value of 1.6 μJ. However, equation 1 allows calculating an energy density threshold using the following expression:

$$F_0 = \frac{2E_0}{\pi \omega_x \omega_y} \qquad (3)$$

as pointed out in Ref. 19. Such energy density threshold is considered to produce a perturbation in the liquid film that results in an impulsion of it. However, such impulsion is not sufficient to detach any material, unless the total energy of the laser pulse overcomes E_{min}. Then, the process results in a droplet deposition. According to equation 3, F_0 has a value of 0.16 J/cm^2, similar to that obtained with other liquid solutions [19].

An optical microscopy image of a portion of the second microarray is presented in Figure 4a. The features described in the preceding section are also observed in this second array, although the required energies to deposit droplets are higher due to the larger beam dimensions [19].

In order to test whether the deposited biomolecules preserve their bioactivity after transfer, a Cy5-conjugated rabbit anti-human IgG was applied to the microarray and it

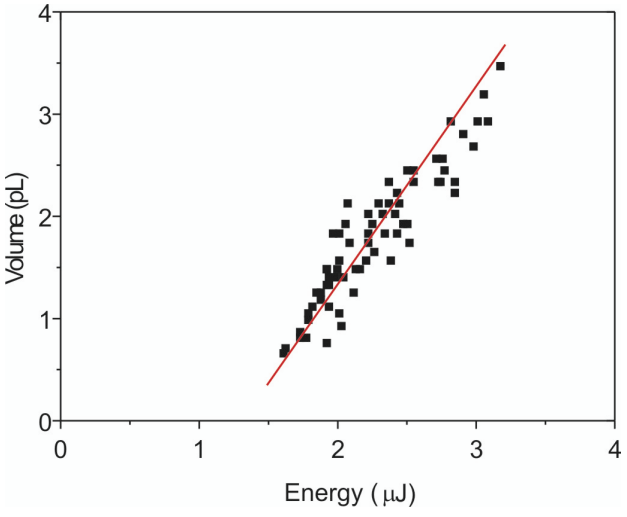

Fig. 3. Plot of the transferred droplets volumes vs the laser pulse energy.

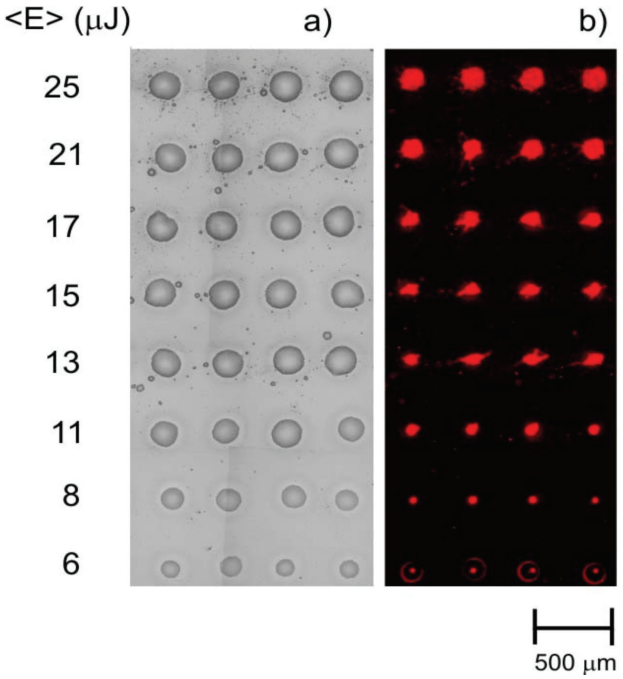

Fig. 4. a) Optical microscopy image of a microarray of the IgG solution obtained at different laser pulse energies. b) Fluorescence image of this array after fluorescence assay with Cy5-anti-IgG.

was subsequently submitted to a fluorescence assay. The fluorescence image obtained is presented in Figure 4b. It can be seen that there exists correspondence between the deposited droplets and the fluorescence red dots for the whole range of energies, which indicates that the transferred proteins have bond their complementary pair, and that, therefore, there is no loss of bioactivity in any case.

4. Conclusions

The printing of IgG-solution microarrays through LIFT has revealed the dependence of the droplets morphology on the laser pulse energy. All the transferred droplets present a circular and well-defined contour with a spherical cap shape, and a diameter which increases for increasing energies. However, there exists a minimum energy threshold, E_{min}, below which no transfer occurs.

The plot of the droplets volumes versus the laser pulse energy has demonstrated that there is a linear dependence between these parameters. This has allowed finding the existence of an energy density threshold considered the threshold to generate an impulsion in the liquid which only results in material release when the total energy overcomes E_{min}.

Finally, a fluorescence assay after having applied the IgG complementaries bond with fluorescence tags has proved that the biomolecules transferred through LIFT preserve their bioactivity, thereby making LIFT a promising technique for biosensors preparation.

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