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Biosensors and Bioelectronics xxx (2004) xxx–xxx
BIOSENSORS & BIOELECTRONICS
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Short communication

DNA deposition through laser induced forward transfer

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Received 21 May 2004; received in revised form 28 July 2004; accepted 4 August 2004

Abstract

Laser induced forward transfer (LIFT) is a laser direct write technique that appears to be specially adequate for the production of biosensors, since it permits to deposit patterns of biomolecules with high spatial resolution. In the LIFT technique, a laser pulse is focused on a thin film of the material to be transferred through a transparent support, and under the action of the laser pulse, a small fraction of the film is transferred to a receptor substrate that is placed parallel to the film-support system. In the case of biomolecules transfer, the thin film consists in a liquid solution containing the biomolecules. In this work, microarrays of two different cDNAs have been both spotted by LIFT and pin microspotting onto a poly-L-lysine treated glass slide. Once transferred, all the microarrays have been submitted to hybridization with the complementary strands of the spotted cDNAs, each one tagged with a different fluorochrome. Comparative fluorescence scanner analyses have revealed that the microarrays transferred through LIFT are equivalent to those transferred through pin microspotting in terms of signal intensity and gene discrimination capacity, and that the action of the laser pulse does not result in significant damage of the transferred DNA.

Keywords: Laser direct writing; LIFT; DNA; Microarrays

1. Introduction

The interest in direct write techniques for the production of biosensors is growing fast, since they represent an interesting alternative to photolithographic-based techniques in terms of versatility, speed and cost. In particular, and due to the high focusing power of lasers, the sub-set of laser direct write techniques appears to be an interesting choice in applications requiring a high degree of spatial resolution. Among these techniques laser induced forward transfer (LIFT) has been revealed as a promising option, since it allows to "write" patterns at a microscopic scale by deposition of either inorganic or organic materials.

The method of operation of LIFT is represented in Fig. 1. A tiny amount of the material to be deposited is removed from a thin film by the action of a laser pulse, and deposited onto a receptor substrate placed parallel to the film. It is a requirement of the technique that the thin film support be transparent to the laser radiation, since the laser beam is focused on the film interface through the support. The short separation distance between the substrate and the film, and the reduced dimensions of the focused laser beam provide the process with the high spatial resolution desired in most of the applications.

Although LIFT was initially developed to transfer metals (Bohandy et al., 1986), its use was quickly extended to oxides (Fogarassy et al., 1989) and other inorganic materials (Zergioti et al., 2002). In all these cases, the thin film was solid and the action of the laser pulse resulted in the vaporization of a small amount of material that recondensed in the substrate after transfer. However, this scenario is clearly not possible when working with biological materials, since vaporization would lead to the irreversible decomposition of the molecules. The discovery of the possibility of transferring liquids with LIFT (Young et al., 2001; Fitz-Gerald et al.,

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doi:10.1016/j.bios.2004.08.047

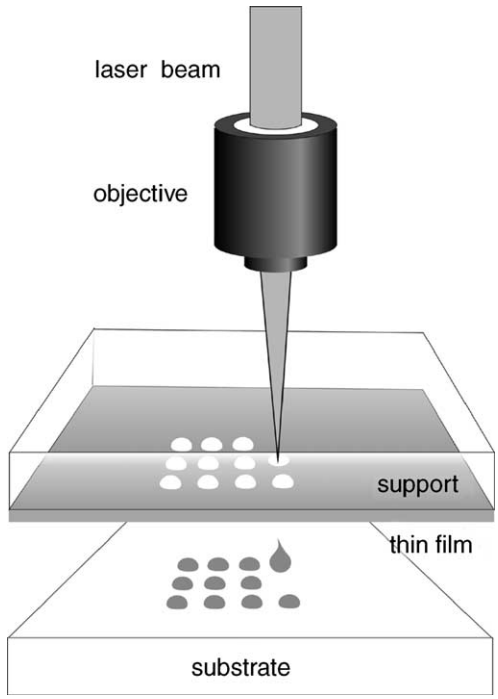

Fig. 1. Laser induced forward transfer scheme.

2002) allowed to overcome this problem. Biological materials in solution could be used to prepare a liquid film for LIFT. In this case, the action of the laser pulse results in the expulsion of a micrometric droplet that is deposited onto the substrate, and the solvent, that acts as transport vector, prevents the biomolecules of decomposition, since there is no longer need for direct coupling between the laser radiation and the fragile molecules. In this way, different biological materials, like proteins (Chrisey et al., 2003; Serra et al., 2004) and cells (Ringeisen et al., 2002), have been successfully transferred through LIFT.

Once demonstrated that LIFT could be used to transfer droplets of a solution containing biomolecules without alteration of their biological activity, it appears interesting to apply this technique for the production of DNA microarrays. The high spatial resolution of LIFT makes it competitive in front of more conventional techniques like pin deposition or ink-jet printing (Heller, 2002; Campàs and Katakis, 2004). The first step in that sense were given by Karaiskou et al. (2003), who demonstrated that undamaged DNA could be transferred through LIFT. However, they carried out the transfer directly from a solid DNA film by means of a sub-ps laser. They claimed that the fragile DNA molecule was not damaged by the laser beam due to the extraordinarily short pulse duration that did not allow for sample heating. In any case, the transfer in solution seems to be a better approach for DNA microarrays production, since more affordable ns lasers could then be used, and the sticking of the molecules to the treated glass substrates should be, in principle, much better. In a previous work (Fernández-Pradas et al., 2004), we demonstrated that good adhesion of DNA transferred through LIFT from a liquid film onto a treated slide could be achieved by means of an ns laser. However, none of these works allowed to grant that the amount of undamaged DNA transferred per spot was enough to be detected through a conventional scanner in a hybridization assay.

The aim of the present paper is to demonstrate that DNA microarrays with good detection level and selective enough to allow gene discrimination can be prepared through LIFT. In addition, we try to determine whether the LIFT process results in a significant amount of DNA damaged by the action of the laser pulse. To carry out this study, we have used LIFT and pin microspotting to prepare similar microarrays containing two different cDNAs, and submitted them to a hybridization assay. The comparison between the two techniques should allow to achieve the pursued objective of the work.

2. Experimental

Pin microspotting and LIFT were used to print three different solutions on the same substrate. Every solution contained a common buffer solvent consisting of glycerol (50% in volume), Tris-EDTA (25% in volume) and dimethyl sulfoxide (25% in volume). Though glycerol is not a usual component of the buffer solvents used with conventional spotters, it was added in this case to optimize the rheological properties of the solution for LIFT spotting. In addition, two of the spotted solutions contained each one a different human cDNA clone insert, amplified by PCR, at a concentration of 250 ng/μl. The cDNAs corresponded to the mitogen-activated protein kinase 3 gene (MAPK3, 525 base pairs long, from the IMAGE clone 809939) and the v-ets avian erythroblastosis virus E26 oncogene homolog 2 (ETS2, 2205 base pairs long, from the IMAGE clone 260303). The third solution contained the buffer solvent alone, and was used as a negative control test.

Blade coating was used to spread 20 μl of each solution in a different titanium-coated microscope slide in order to prepare the films for LIFT. This resulted in liquid films 10 μm thick. The titanium coatings had been previously deposited by evaporation and their thicknesses were about 50 nm. The system formed by the titanium-coated slide and the solution thin film has been named ribbon (Wu et al., 2001). Commercially available glass treated with poly-L-lysine was used as substrate. The ribbon was placed parallel to the receptor substrate, with the liquid film facing the poly-L-lysine treated surface at a distance of 100 μm kept through the intercalation of two small kapton pieces in the slides extremes.

A pulsed Nd:YAG laser tripled in frequency (355 nm wavelength, 10 ns pulse duration) was used to transfer by LIFT microarrays with each one of the prepared ribbons. The laser beam was focused on the titanium coating through the glass slide by means of a 15× microscope objective, resulting in a beam diameter of about 80 μm. Although the laser beam can be focused at much smaller diameters, the present focusing conditions were set in order to obtain droplets with a

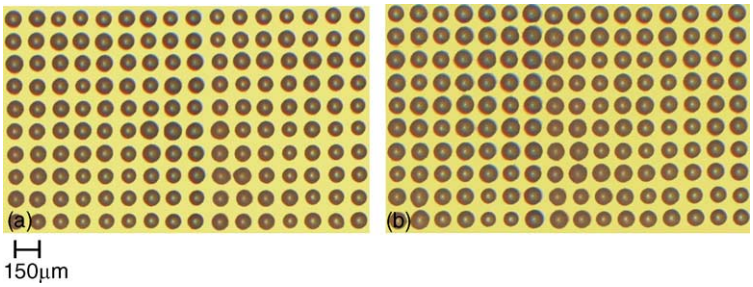

Fig. 2. Optical microscopy images of the microarrays of LIFT spotted droplets containing the cDNAs corresponding to the (a) *MAPK3* and the (b) *ETS2* human genes.

similar size to those normally obtained with pin spotters. The average energy of the laser pulse during transfers was 20 μJ. The system ribbon-substrate laid on a xyz translation stage, and the transfer was carried out in such a way that each droplet of the microarray was propelled by a single laser pulse. Once transferred the droplet, the stage was displaced a distance of 150 μm and a new droplet was transferred at a new substrate position. This procedure was repeated up to completing the microarrays, each one with 10 rows and 16 columns.

The process to print the microarrays was carried out as follows: first, three microarrays, each one containing a different solution, were spotted through LIFT. Once the three microarrays had been transferred, they were submitted to a heating process in an oven at 80 °C for 3 h to evaporate the solvent. Subsequently, a Genetix Qarray pin arrayer was used to print three microarrays identical to the LIFT printed ones in another location of the substrate. Finally, the substrate was submitted to another heating process at 80 °C for 3 additional hours. Once the last printed droplets were evaporated, the post printing processing of the poly-L-lysine protocol was carried out (Massimi et al., 2002). This protocol includes: microarrays rehydration, fixation of DNA to the substrate by UV cross-linking at 130 mJ, blocking of the DNA-free sites with succinic anhydride and prehybridization (Ideker et al., 2002). The complementary strands of the transferred cDNA (20 ng each) were tagged with different fluorochromes: Cy3 for MAPK3 and Cy5 for ETS2 using the Rediprime™ II Ramdom Prime Labelling System kit from Amersham Biosciences. Labeled DNAs were combined with hybridization buffer (50% formamide, 2.5× Denhardts, 6× SSPE and 0.5% SDS) and hybridized to the microarrays for 16 h at 42 °C. Slides were washed at room temperature for 10 min with solution A (0.1× SSC, 0.1% SDS) and twice 10 min with solution B (0.1× SSC). Finally, the microarrays were analyzed through a fluorescence scanner operating at 635 nm and 543 nm to excite Cy5 and Cy3, respectively, (GenePix 4000 from Axon Instruments).

3. Results and discussion

Microarrays spotted by LIFT were evaluated by means of optical microscopy in order to check the size and the morphology of the transferred droplets before drying. Fig. 2 shows optical microscopy images of the MAPK3 and ETS2 microarrays. It can be seen that the transferred droplets are well aligned, round shaped, with an average diameter of about 90 μm, and with absence of satellites or spallation. These good morphological characteristics can be attributed to transfer mechanisms where droplets remained intact during the flight from the film to the substrate. Additionally, a small dispersion in the diameter of the droplets is observed. In situ monitoring of the laser pulse energy during the transfer allowed to attribute this dispersion to energetic fluctuations of the laser pulse, caused by the typical instabilities of the laser systems.

The fluorescence images of the same microarrays obtained after the hybridization process are presented in Fig. 3a and b. The microarray containing the MAPK3 cDNA, whose complementary strand was tagged with Cy3, presents green spots and the ones corresponding to the microarray with the ETS2 cDNA, whose complementary strand was tagged with Cy5, are red. Taking into account that the fluorescence intensity

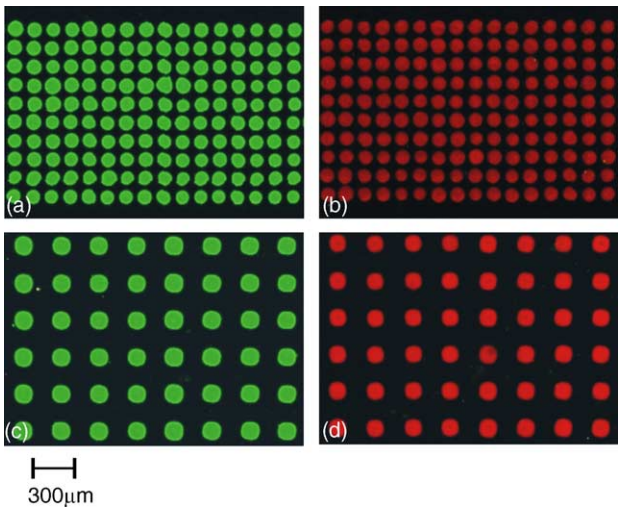

Fig. 3. Fluorescence scanner images obtained after hybridization of the microarrays spotted through (a, b) LIFT and (c, d) pin microspotting. The green colored spots in (a) and (c) contain the *MAPK3* human gene cDNA, and the red colored spots in (b) and (d) contain the *ETS2* human gene cDNA. In the hybridization target solution the MAPK3 cDNA is tagged with Cy3 (green emission) and the ETS2 cDNA with Cy5 (red emission).

Table 1
Average spot fluorescence intensity per unit droplet volume (arbitrary units) in the microarrays spotted by LIFT and pin microspotting

	$(F_{532})_g$	$(F_{635})_r$	$(F_{532})_r$	$(F_{635})_g$	$(F_{532})_g/(F_{532})_r$	$(F_{635})_r/(F_{635})_g$
LIFT	29.5	9.5	0.22	0.23	134	41
Pin spotting	24.5	12.1	0.18	0.25	136	48

The notation $(F_{\text{wavelength}})_{\text{color}}$ has been used to indicate the fluorescence intensity recorded at 532 nm or 635 nm in the green or red spots.

is proportional to the hybridization level with the correct complementary strand, this result indicates that each cDNA hybridized mainly with its perfect complementary and that, therefore, the prepared microarrays are selective in gene discrimination. In the slide zone where the buffer microarray had been spotted (not presented in the figures), no fluorescence intensity was detected. Additionally, it has to be mentioned that after the hybridization process the spots present a round shape, a diameter of about 110 μm, and a similar fluorescence level between spots in the same array. Moreover, the fluorescence intensity distribution within the whole spot area is always uniform. The comparison between the as-transferred droplets and the fluorescence spots, gives evidence of a remarkable correspondence in distribution, shape and diameter dispersion. However, there is a significant difference in their diameters, being the spots surprisingly larger than the droplets. This could be attributed to the increase of the surface tension with temperature during the drying process prior to hybridization that would make the droplets to spread away their initial borders before complete evaporation. Fig. 3c and d show the fluorescence images of the microarrays printed by pin microspotting. Both microarrays responded correctly to the hybridization process, leading to uniform, round shaped, green and red spots for the MAPK3 and ETS2 cDNAs, respectively, with average diameters of 140 μm. It is important to point out the similarity at least from a qualitative point of view between the fluorescence scanner results corresponding to the LIFT printed spots and those corresponding to the spotted ones. This similarity already allows to state that LIFT is a viable technique for microarray preparation, since it grants that enough DNA is transferred to allow selective gene detection, in a similar way to other conventional techniques, like pin microspotting. However, in order to get a deeper knowledge on the LIFT process and to reveal whether a significant amount of DNA is damaged during the transference, quantitative analysis of the fluorescence results are required.

The results of the quantitative analysis carried out to all printed microarrays are presented in Table 1. The average of the spot total fluorescence intensity per unit droplet volume (F) was calculated for every microarray and emission wavelength. This value is proportional to the DNA concentration of the transferred droplets and, therefore, its analysis should allow to determine whether there has been significant DNA damage during LIFT. The fluorescence intensity was directly provided by the scanner and the volume of the droplets was obtained through calculation of the volume of the observed spherical cap, which projected radius, and contact angle were measured through optical microscopy. The F values do not contain the fluorescence background intensity that was previously subtracted. The notation $(F_{\text{wavelength}})_{\text{color}}$ has been used to indicate the fluorescence intensity recorded at 532 nm or 635 nm either in the green or red spots. Thus, the values where the wavelength and the color of the spot coincide (for example 635 nm and red) correspond to specific hybridization, and those with different wavelength and color (for example 532 nm and red) correspond to unspecific hybridization. The first important characteristic to remark in the table is that the intensities corresponding to the LIFT microarrays are similar to those corresponding to the pin spotted ones and that the small differences between them are not biased in favor of any of the techniques. This result confirms that the printed microarrays responded to the hybridization process in a similar way, independently on the employed printing technique, which indicates that the amount of biologically active DNA spotted through both techniques is similar and that, therefore, there is no significant amount of DNA damaged by the action of the laser pulse during LIFT deposition. It can be also observed in the table that the fluorescence intensity of the green spots is higher than that in the red ones in all the cases. This fact is due to differences in the DNA tagging efficiency between Cy3 and Cy5 (DeRisi, 2002). Although the phenomenon could be also attributed to differences in the hybridization efficiencies of DNA strands of different length, this possibility has been discarded through experiments where the strands were tagged in the reversal way (MAPK3 with Cy5 and ETS2 with Cy3); these experiments also showed higher fluorescence intensities for the green spots. In order to quantify the ratio of specific hybridization respect to the unspecific one, the ratios $(F_{532})_g/(F_{532})_r$ and $(F_{635})_r/(F_{635})_g$ have been calculated. This values, not affected by the tagging efficiency of the fluorochromes, represent the amount of DNA strands hybridized to their perfect complementary respect to the ones attached to the DNA strands corresponding to a different gene. In all the cases these values are high and, of course, similar for the LIFT spotted microarrays and the pin spotted ones. The observed difference between $(F_{532})_g/(F_{532})_r$ and $(F_{635})_r/(F_{635})_g$ is attributed to the difference in length between the MAPK3 and the ETS2 cDNAs that would result in a lower specific hybridization ratio for the longer strand. Summarizing, this comparative study not only confirms the viability of the LIFT technique for DNA microarray production already pointed out in the previous paragraph, but also allows to state that there is no significant amount of damaged DNA during the LIFT process.

4. Conclusions

The optical microscopy analyses of the material transferred through LIFT have shown that this technique allows to transfer arrays of regularly arranged, uniform, round-shaped droplets of a cDNA containing solution onto a poly-L-lysine glass treated substrate. The diameter of the deposited droplets is about 90 μm, but it has to be pointed out that this value has not been optimized and that, by varying the transfer technological parameters, smaller droplets can be obtained.

The results of the hybridization process carried out with two different cDNAs corresponding to different human genes have revealed that the LIFT spotted DNA microarrays are perfectly functional, with fluorescence intensity levels detectable by conventional fluorescence scanners, and specific in gene discrimination.

The comparative study between the LIFT spotted microarrays and microarrays obtained through conventional pin spotting with similar morphological characteristics have demonstrated that no significant amount of DNA is damaged during LIFT deposition.

Summarizing, it can be stated that LIFT allows to prepare DNA microarrays with functional characteristics identical to the ones obtained through more conventional techniques, and with the potential advantage of allowing for a higher degree of integration. Therefore, LIFT constitutes a viable alternative DNA microspotting technique.

Acknowledgments

The authors would like to thank D. Fernández for his technical support in the pin microspotting process. This work is a part of a research program financed by MCYT of the Spanish Government (Project MAT2001-3757), Fondo Europeo de Desarrollo Regional (FEDER) and DURSI of the Catalan Government (2001 SGR00080).

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