LASER PROCESSING OF ORGANIC MATERIALS:
APPLICATIONS IN TISSUE ENGINEERING AND CHEMICAL SENSING

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Abstract. The production of micron-scale architectures is a major demand in several new fields in the biomedical and sensing area. In particular, engineering different materials can produce cell templates, allowing the analyses of cell adhesion and growth, cell-cell interactions, or cell clustering. In addition, the capability to spatially control the deposition of soft materials i.e. polymers onto different types of substrates is important for the development of sensors and biosensors. In this work, the application of laser methods i.e. laser direct-writing (LDW), matrix-assisted pulsed laser evaporation (MAPLE), and laser induced forward transfer (LIFT) for obtaining controlled sized designs or thin films from a variety of soft materials is shown. Organically modified silicates (ORMOSILS) have been investigated in order to obtain polymeric structures designed by LDW that would be further used as scaffolds for tissue engineering. Collagen has been deposited by MAPLE as a thin layer, proving the feasibility of MAPLE for the fabrication of implant materials or matrices. Finally, LIFT has been applied for the transfer of polymers aiming application in toxic gas sensors.

Key words: LDW, MAPLE, LIFT, SAW sensors, collagen, ORMOSILS.

1. INTRODUCTION

Direct laser writing (LDW) is a technique by which polymeric templates at micrometric and even nanometric scale can be realized [1, 2]. The polymerization process is initiated by multi photon absorption in the center of the focal volume. By moving the focused beam of an infrared laser with ultrashort pulses in the volume
of a photosensitive material, 3D structures can be fabricated in a single step process [3]. DLW can find applications in designing photonic crystals [4–7], for drug delivery and bio-medicine [8, 9] and tissue engineering [10].

Hybrid organic-inorganic materials are engineered materials that gather together the advantages of both organic (the possibility of being laser processed) and inorganic groups (thermal and mechanical stability). These materials span a large range of applications i.e. medicine [11], biosensors [12], coatings with tunable mechanical properties [13] and optical waveguides [14].

In this work, ORMOSILS (organically modified silicates), i.e. different laboratory synthesized hybrid methacrylates have been processed in order to obtain polymeric structures designed by DLW that would be further used as scaffolds for tissue engineering.

Furthermore, the application of laser methods for obtaining controlled sized patterns or thin films from a variety of synthetic and natural biomaterials widened the application areas from clinical and biomedical applications such as implant materials, matrices and scaffolds for guided tissue engineering to chemical application such as gas sensors.

Matrix-assisted pulsed laser evaporation (MAPLE) is a physical vapor deposition method, similar to the conventional pulsed laser deposition (PLD) that involves diluting the material of interest in a volatile, noninteracting matrix or solvent (0.1–5 wt%) freezing the mixture to create a solid target [15–18]. When the system is irradiated by laser beam, the solvent evaporates whereas the molecules of interest (polymer or biological compounds) are collected on a substrate. In the case of using MAPLE for coating various substrates with an active compound, such as collagen, the main advantages of using this approach are related to no structural damage to the protein, control over the thickness and surface morphology of the deposition by carefully choosing the processing parameters [19–21].

Laser-induced forward transfer (LIFT) is an advantageous method used for the deposition of a wide range of materials both in solid or liquid phase [22–25]. In LIFT, a laser beam is focused through a transparent support plate onto the backside of a metallic or polymer thin film coated with the material to be transferred (donor film). Each single laser pulse promotes the transfer of the thin film material (donor film) onto a receiver substrate that is usually placed parallel and facing the thin film at a short distance. The donor substrate can be previously coated with a polymeric layer (triazene polymer TP), which is called dynamic release layer (DRL) or sacrificial layer. This layer has the purpose to improve the process efficiency and to reduce the risk of damaging the layer to be transferred [26–28].

An important opportunity arises from the applicability of polymers to surface acoustic wave (SAW) devices for their broad range of applications aiming toxic gas detection [29, 30].
In this study, some results on the development of sensor arrays based on SAW resonators coated with chemoselective polymers shall be presented. These results prove the feasibility of LIFT as a deposition method of active material in sensors and sensor arrays.

2. EXPERIMENTAL

2.1. LASER DIRECT-WRITING

The laser direct-writing (LDW) workstation is coupled with an amplified femtosecond laser Clark CPA-2010 emitting at 775 nm, 200 fs pulse duration, and 2 kHz repetition rate. The sample is fixed and the laser beam is moved inside the photo sensible material by a system with galvanic mirrors and scanning speeds up to 10 mm/s, limited by the laser repetition rate. The laser beam is focused by a 100 mm focal length and 35 µm focal spot lens. The laser fluence is varied in the range 0.2 – 1 J/cm² and the scanning velocity of the laser beam with respect to the sample is in the range 1 – 3 mm/s. For the developing procedure after laser irradiation, the unirradiated monomer solution is gently washed out by isopropyl alcohol. The obtained structures are characterized by Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM).

2.2. MATRIX ASSISTED PULSED LASER EVAPORATION

A “Surelite II” pulsed Nd:YAG laser system (Continuum Company) working at 266 nm wavelength, 6 ns pulse duration, and 10 Hz repetition rate is used. The laser spot size measured on the surface of the frozen targets is 0.02 cm². The laser fluence applied is between 400 – 900 mJ/cm². A number of 25,000 pulses is used. Two thermocouples are placed at two different positions of the target holder for checking the temperature. Pfeiffer-Balzers TPU 170 turbomolecular pump is used for adjusting the background pressure (1 – 4×10⁻³ Pa).

The targets are solutions of collagen suspended in acetic acid (0.1 wt %), which are homogenized and flash frozen in a liquid nitrogen cooled copper container. The target is rotated with a motion feed through driven by a motor to avoid local overheating and drilling due to multiple pulses of laser irradiation.

The substrates are 1.3 cm² round glass coverslips. The substrates are carefully cleaned in an ultrasonic bath in acetone, ethyl alcohol, and deionized water and blow-dried with N₂ gas before use. During deposition the substrates are kept at ambient temperature and placed at a distance of 3 cm from the frozen target.

The surface of the thin collagen films deposited by MAPLE is investigated by AFM (XE 100 AFM setup from Park). AFM measurements in non contact mode are performed to analyze the surface roughness of the films.
In order to investigate the functionality of the thin collagen layers deposited, the films are submitted to a sterilization step followed by a cell assay. First, the films are placed on a 70% alcohol wet paper and kept for 60 min under a UV lamp. Then, the films are gently rinsed twice with sterile serum-free Hank’s balanced salt solution (SF-HBSS) in Petri dishes. “Cell Titer 96Aqueous One Solution Cell Proliferation Assay” Promega kit is used for the viability and proliferation testing of the cells. 2 ml of L929 cells suspension in Dulbecco’s Modified Eagle Medium DMEM (with phenol red, 10 % FCS and 0.1 % penicillin / streptomycin) are dropped onto the sterilized films surface. The films are then kept in a CO₂ incubator at a temperature of 37º C up to 48 hours.

A fluorescence microscope (CKX31) is used for studying the morphology of the previously colored (20 min with acridine orange stain) cells.

2.3. LASER-INDUCED FORWARD TRANSFER

The LIFT experimental setup has been described in detail elsewhere [30]. The laser pulses generated by a XeCl laser system (308 nm wavelength, 30 ns pulse duration) are imaged through a lens onto the backside of the donor film placed parallel to the receiving substrate on a motorized translation stage. The donor and the receiver substrates are placed in closed contact. [31] All transfer experiments are carried out in air, at ambient temperature.

The donor films consist of chemoselective polymers (polyepichlorohydrine (PECH), polyisobutylene (PIB), and polyethylenimine (PEI)) deposited by MAPLE onto the same substrate plate. [30] The aim is to create multi-ribbon targets, which allow single step transfer (by LIFT) of multiple polymer layers onto different sensors arranged in a matrix. Fused silica plates previously spin coated with a triazene polymer (TP) [32] as DRL are used as substrates for the MAPLE depositions. The TP film thickness is ~ 100 nm as determined by profilometer measurements (Veeco Dektak 8 Profilometer).

The receiving substrates are 2-port SAW resonators which consist in two inter-digital transducers (IDTs) arranged between two grating reflectors, all of them deposited on α-quartz substrates (ST-cut, x propagation). The metallic electrodes are made of Al (100 nm thickness). The Al films are grown by radio frequency magnetron sputtering from a 99.9 % pure Al target in Ar atmosphere. The patterns are transferred by photolithography using a poly(methyl-metacrylate) resist exposed by deep UV radiation, and lift-off procedure to remove the unexposed resist. Each IDT consists of 76 couples with a finger width of 2 µm and a metallization ratio of 0.5, resulting in a wavelength of 8 µm. The maximum finger overlap is 450 µm and IDTs are shaped with a Gaussian apodization in order to minimize spurious transverse modes. The number of reflecting fingers for each grating is 300 and the cavity length is 1278 µm. The operating frequency of the resonators results to be approximately 392 MHz.
The characterization of the SAW resonators is carried out with a Network Analyzer (HP8753A) as reported in a previous work. The SAW sensors are tested in a N₂ atmosphere upon exposure to different concentrations of dimethyl methylphosphonate (DMMP), dichloromethane (DCM), and ethylacetate (EtOAc). In order to obtain different concentrations of DMMP in N₂, the sensor array was exposed to a total flux of 1000 sccm, controlled by two flow meters: the main for the gas carrier (N₂) and the second for the analyte. The vapor concentrations are obtained by fluxing N₂ with a bubbler in the liquid analyte (i.e. DMMP, DCM, and EtOAc).

3. RESULTS

3.1. LDW

Grid-like structures of ormosils on large areas (up to 25 mm²) have been obtained by femtosecond laser pulses with different spacing between lines [33, 34]. In Fig. 1 a SEM images of such structures (3×3 mm²) having one polymer layer are shown. Different magnifications have been used in order to check for the absence of defects onto the structure as well as the geometrical characteristics of the scaffolds. The height of the lines is approximately 25 µm. AFM studies (Fig. 1.b) reveal that the surface of the polymer presents a low roughness, however at nanometric scale a surface structuring can be identified, and also the presence of a few pores. The chemical structure of the hybrid dimethacrylate is presented in Fig 1.c.

(a)
It has been shown before [35, 36] that this type of structures can accommodate different cells, like fibroblasts, melanocytes and keratinocytes, in simple cultures or co-cultures. In [36] we evaluated the interaction between a pseudo-dermis formed by primary human dermal fibroblasts grown on ormosils scaffolds and keratinocytes added on top and it has been seen that the geometry acts as organization guidance for the primary human dermal fibroblasts grown in contact with the polymer. The cells align with the parallel lines or anchor to the square corners of the grid structure [36]. Similar behavior has been noticed for human mesenchymal stem cells grown on different hybrid dimethacrylate polymeric structures (Fig. 2).

More studies are needed in order to make a step forward to in vivo tissue organization. There are many parameters influencing cell adhesion (surface wettability and polarity, micro and nano topography, functional groups at the surface etc. [37]). Todorka G. Vladkova in [37] points out the direction using different types of physical/chemical/biological stimuli. Temperature, pH variation, electrical stimulation or the presence of a specific chemical substrate are some of the solutions identified until now in the research community [37, 38].
3.2. MAPLE

3.2.1. Morphological study

The use of collagen layers deposited by MAPLE as substrates onto which fibroblast L929 cell populations can attach and proliferate as a function of surface topography characteristics is investigated. The 3D AFM images of the samples obtained by MAPLE are shown in Fig. 3 (a–e).

The surface of the samples does not exhibit any special topography features for the lowest fluence, except the presence of few grain-like structures. The grain-like structures have heights up to 50 nm and a density too low to influence the surface roughness. The average surface roughness measured over 5×5 µm area is 11 nm.
Fig. 3 – 3D AFM images of the samples obtained by MAPLE at (a) 400 mJ/cm², (b) 500 mJ/cm², (c) 600 mJ/cm², (d) 700 mJ/cm², and (e) 800 mJ/cm².

By increasing the laser fluence to 500 mJ/cm² the number of grain-like structures increases (Fig. 2b) the grains being spread all over the analyzed area, in contrast with the previous case where they were located as a drop like structure. The average surface roughness measured over 5×5 µm area is 17 nm. The roughness increases to 76 nm when the laser fluence increases to 600 mJ/cm²; however it decreased to less than 6 nm when a laser fluence of 800 mJ/cm² is used.

3.2.2. In vitro studies

The confirmation of the physiological functionality is given by the cell response to the collagen coatings. To monitor the L929 cell attachment the cells behavior on collagen thin films obtained by MAPLE (for the fluences ranging from 400 to 800 mJ/cm²) is compared to the control (bare glass) and to films obtained by drop casting.

In all cases of collagen films obtained by MAPLE, the proliferation rate assessed by MTS assay is higher than in the case of the standard tissue culture material (Fig. 4a).

Cells cultured on the collagen thin films obtained by MAPLE at 800 mJ/cm² the viability decreased only 10% as compared with the one from the films obtained with lower fluences.

From the labeling of cells with acridine orange, the cell morphology is analyzed 48 h after their seeding. Although the cells exhibit similar characteristic onto the tested surfaces, there are few differences observed for the samples with a higher roughness. Cells display a spindle like shape, induced by the surfaces aggregates formed in the MAPLE process on the surface of collagen thin films, as previously shown by AFM studies.
3.3. LIFT

As reported previously, the performances of the SAW sensors are mainly affected by the physical properties of the polymer coatings [39, 40]. In previous studies [39, 40] parameters such as (i) the laser fluence applied for the transfer, (ii) the thickness of the polymer layer, (iii) the laser wavelength, or (iv) transfer with DRL and without DRL have been investigated. However, in this study only the parameters which lead to a clean transfer of polymers onto SAW devices have been applied.

Ns-LIFT in the direct-writing of polymer patterns shows that:
- Better results in terms of polymer pixel morphology are obtained by using TP as a DRL compared to the LIFT process without a DRL.
- The thickness of the TP layer should be lower than 150 nm.
- The transfer with the 308 XeCl laser yields the best results i.e. clean cut pixels; TP has its maximum of absorption at 308 nm.
- The laser fluence applied for the transfer should be in the range 400–500 mJ/cm² as below 400 mJ/cm² no polymer is deposited onto the SAW device and above 500 mJ/cm² the SAW device is destroyed.

An optical image of PIB, PEI, and PECH pixels transferred at 450 mJ/cm² laser fluences onto the acoustic resonating cavity of SAW devices is shown in Fig. 5. It can be noticed that the transfer is “clean”, resulting in well defined pixels with regular edges.
The advantage of using SAW sensors for the detection of toxic gases is mainly related to their high sensitivity and selectivity. The mass sensitivity i.e. the signal increase in response to the increase in the mass per unit area on the SAW device surface results from the properties of the transferred polymer pixel. Selectivity refers to ability of the polymers chosen to be transferred by LIFT onto the SAW devices to specifically and selectively identify target gases. In addition, to analyze the sensing response of the LIFT printed SAWs to different toxic gases, principal component analysis (PCA) is used.

In Fig. 6 the projection of the data on the 1st and 2nd principal component (PC) is shown. From the plot in Fig. 6 it can be clearly seen that the sensor system can discriminate the three analytes i.e. DMMP, DCM, and EtOAc. In addition, the three analytes investigated are separated from each other, showing the capability of the PCA to discriminate between vapors and the discrimination increases at higher concentrations. The biplot of the loadings (blue insert) shows the contribution of each sensor to PC1 and PC2.
4. CONCLUSIONS

This study is focused on the use of laser based methods i.e. LDW, MAPLE, and LIFT to process sensitive materials such as polymers and proteins for biological and sensor application.

It has been shown that laser direct-writing is an advantageous method to write grid-like structures of ormosils on large areas. In addition, the surface of the polymer has low roughness and allows the accommodation of different cells, in this case of human mesenchymal stem cells.

MAPLE allows a facile control of film morphology via variation of the fluence, where the formation of nano-structured features on the deposited surfaces was strongly dependent on the fluence values. The topography of the surface induced few changes in the cell shape, from flat and spread type to spindle like shape. However, our results indicate that L929 fibroblast cells adhere and proliferate on thin films obtained by MAPLE within all the used fluence range (400–800 mJ/cm²).

LIFT is well suited for the transfer of chemoselective polymer pixels for the fabrication of surface acoustic wave sensors. Under appropriate experimental conditions the polymer pixels are functional as proven by their sensitivity and selectivity towards different analytes.
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