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# Direct laser interference patterning of polystyrene films doped with azo dyes, using 355 nm laser light

M.F. Broglia[a,b], S. Suarez[b], F. Soldera[b], F. Mücklich[b], C.A. Barbero[a], R. Bellingeri[a], F. Alustiza[a], D. Acevedo[a,*]

[a] Universidad Nacional de Río Cuarto, Departamento de Química, Ruta 36 km 601, Río Cuarto, Córdoba 5800, Argentina
[b] Saarland University, Department of Materials Science, Campus, D-66123 Saarbrücken, Germany

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ABSTRACT

The generation of line-like periodic patterns by direct laser interference patterning (DLIP) of polystyrene films (PS) at a wavelength of 355 nm has been investigated. No structuration is achieved in plain PS due to the weak absorption of the polymer at 355 nm. On the other hand, patterning is achieved on films doped (PSd) with an azo dye (2-anisidine → 2-anisidine) which is incorporated in the polymer solution used for film preparation. Periodic micro-structures are generated. DLIP on PSd results in the swelling of the surface at low fluences, while at high laser intensities it causes the ablation of the regions at the interference maxima positions. The results contrast with the usual process of DLIP on PS (at shorter wavelengths, like 266 nm) where only ablation is detected. The results suggest that decomposition of the azo dye is the driving force of the patterning which therefore differ from the patterning obtained when plain PS is irradiated with laser light able to be absorbed by the aromatic ring in PS (e.g. 266 nm). The biocompatibility of these materials and adhesion of cells was tested, the data from in vitro assays shows that fibroblast cells are attached and proliferate extensively on the PSd films.



## 1. Introduction

Polymeric materials are widely due to their rapid and easy preparation, high resistance to corrosion and chemicals, good electrical properties and excellent surface characteristics [1–3]. In the recent years, the fabrication of polymer functionalized surfaces has received considerable attention in virtue of its possible use in chemistry, biology, physics, and material science [4–6]. Polymer ablation using direct laser interference patterning technique [7] has been extensively applied to polymers such as, polyaniline [8], poly(styrene-co-methyl methacrylate) [9], polyimide [10], polyacrylamide gels [11] and others [12]. Ablation of the pure polystyrene [13,14] as well as PS doped with different substances such as dyes has been studied [15,16]. However, in the best of our knowledge there are not studies dealing with structuration of PS using near UV (355 nm) light. At 355 nm, PS has low absorption, making necessary to dope the polymer with light absorbing substances (dyes, polymers, etc.) to allow PS microstructuring by pulsed ultraviolet laser light. The dopants reduce the ablation threshold and increase the quality of ablated features [17]. There are many organic compounds such as dyes and pigments that can be used as light sensitizers. Due to their photosensitive properties and ability to absorb light by high absorption coefficients in the spectral range between 300 and 400 nm, compounds containing the azo group (–N=N–) have been used as photosensitive materials in photolithography [18] and laser ablation [19]. Moreover, the photochemistry and the microstructuring properties of several classes of azo compounds have been investigated in earlier studies [20,21].

On the other hand, methods for micro and nano-patterning of polymeric materials are commonly referred as lithography, which involves a flow of information that typically begins with the design of a pattern in the form of a dataset and ends as a patterned array of features on the surface of a substrate [22]. Various techniques have been used in the past to produce such modulated surfaces with controlled dimensions (e.g. optical lithography [23], electron-beam, ion-beam and laser writing [24,25]. In this study, we used a simple method for developing microstructures on doped PS, direct Laser Interference Patterning (DLIP). DLIP permits the fabrication of repetitive 1D and 2D patterns and microstructures by direct irradiation of the sample surface with coherent beams of light [26,27]. Furthermore, we show that it is possible to produce microstructures with DLIP lines-type by illumination of a

* Corresponding author. Tel.: +54 0358 4676157.
*E-mail addresses:* dacevedo@exa.unrc.edu.ar, dacevedo@ing.unrc.edu.ar (D. Acevedo).

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single pulse suitably doped PS with at wavelength of 355 nm. The micro-modification of the surface topography of PS could enhance the properties of this material (e.g. changing the contact angle, modified its wetting properties). In a previous manuscript we [28] reported the fabrication of line-like sub and micro structures with periods from 500 nm to 10 μm on polyimide surfaces using the method of DLIP. The range of laser fluence necessary to produce sub and microstructures for this material ranging 0.2–1.0 J cm$^{-2}$. The results of these studies indicate that to obtain sub-μm spatial periods (500 nm) is necessary laser fluences of ca. 0.3 J cm$^{-2}$ in order to preserve the morphology. Therefore to generate sub-μm range structures is necessary to produce ablation with low values of fluence. For the system present here (PSd) to generate of sub-μm periods we should use low laser energy, however using low energies values is impossible to produce ablation on PSd and in such conditions if the ablation is produce the material swell, this behavior inhibits the generation of a sub-micrometric periodic and regular structure. Also it will be possible to develop new interesting properties allowing to extend the PS uses to areas such as biology, material science, and medicine or to improve the performance of this material in an existing application.

The structured surfaces can be used for cell and tissue growth. Therefore, we evaluate the material cytotoxicity using an in vitro assay. Additionally, fibroblast cells are shown to attach and proliferate successfully on the PSd films.

## 2. Experiments

### 2.1. Synthesis of polymer

Polymer of styrene was synthesized in bulk as follows. The polymerization of pure liquid styrene (Merck) was initiated with benzoyl peroxide (BDH Chemicals, Ltd.) as initiator. Dissolved oxygen was removed from the reaction solution by nitrogen purging for 30 min. Then, the test tube (1 cm diameter) containing the polymerization mixture was immersed in a water bath at 60 °C. The polymerization process was executed for 4 h. Styrene (Merck), and benzoyl peroxide (BDH Chemicals, Ltd.). All the solvents used were reagent grade.

### 2.2. Synthesis of azo compounds

2-Anisidine solution was diazotized with sodium nitrite and concentrated HCl in an ice bath [29,30]. Then, the diazonium salt is mixed with equimolar amount of 2-anisidine suspended in TRIS buffer (pH = 8) in an ice bath. After 15 min, the solid dye was filtered under vacuum and washed first with distilled water. The product was filtered out of the mixture under vacuum and dried (dynamic vacuum for 48 h).

### 2.3. Laser interference experiments

A high-power pulsed Nd:YAG laser (Quanta-Ray PRO 290, Spectra Physics) was used for the laser interference experiments. The pulse duration was 10 ns and only 1 laser pulse was used in each experiment. To obtain the line-like periodic patterns, the fundamental laser beam was split into two sub-beams and guided by mirrors to interfere on the sample surface. For two-laser beam configuration, the period line-like pattern is given by:

$$P = \frac{\lambda}{2\sin\alpha} \quad (1)$$

where $2\alpha$ is the angle between the laser beams, and the period can be controlled by changing the angle between the laser beams and the wavelength ($\lambda$). More details about the experimental setup have been published elsewhere [31]. The laser fluence (energy per unit area) was varied from 0 to 1200 mJ/cm$^2$. All experiments were conducted in air at normal conditions of pressure and temperature.

### 2.4. Sample preparation

For preparation of the films, solutions of the PS and of the dopant were dissolved in chloroform. The dopant concentration was 0.3% by weight. Fifteen milliliters of the solution were poured into a glass dish of 5 cm diameter, with a bottom plate of good planarity. The solvent was allowed to evaporate for 24 h. The film was then cut out of the dish and the final thickness of the substrate was between 100 and 400 μm.

### 2.5. Surface characterization

All samples were imaged with a high-resolution scanning electron microscope (SEM) equipped with a field emission gun (FEI Strata DB 235) at 5 kV acceleration voltage. The depth and period of the micropatterns were characterized using a white light interferometer (WLI) and a New View 2003-D imaging surface structure analyzer (Zygo), with a vertical and lateral resolution of 0.3 and 360 nm, respectively.

### 2.6. UV–vis spectroscopy

UV–vis absorption spectra were obtained using a Hewlett-Packard 8453 diode array spectrophotometer.

### 2.7. Adhesion assay

Fetal bovine fibroblasts were seeded onto modified polystyrene at a cell density of $5 \times 10^4$ cell/mL. Cultures were incubated for 24 h at 37 °C with 5% $CO_2$ in air, in a humidified incubator. To determine cellular morphology on the surfaces, cells were observed under inverted microscopy. Images were captured on a Nikon microscope equipped with a Nikon digital camera (DS-5M).

### 2.8. In vitro cytotoxicity test

Polystyrene samples were cut into squares (10 mm edge length and 0.25 mm height). These samples were prepared following the recommendations of ISO 10993-12 at a ratio of 117.8 mm$^2$ of sample surface area/mL of cell culture medium at 37 °C and 5% $CO_2$ [32]. Fetal bovine fibroblasts were routinely cultivated in DMEM supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 mg/mL) at 37 °C and 5% $CO_2$. Into each well of a 24-well plate, $8 \times 10^4$ cells were seeded and incubate for 24 h at 37 °C. After overnight cultivation, the culture medium was replaced with fresh medium and test material was added. Non-treated cell culture was used as positive control. After 24 and 48 h, the exposure medium was discarded. Cell viability after exposure was determined using the MTT assay, which is a colorimetric test that measures the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium to a purple formazan product [33]. The absorbance of viable cells was immediate determined at 570 nm using a UV–visible single beam spectrophotometer (Jasco, V-630 Bio). The absorbance of viable cells was converted into a percentage, assuming that cell control absorbance was 100% viability.

### 2.9. Statistical analyses

Statistical analyses were performed using Infostat software (GrupoInfostat/FCA, 1998). For in vitro cytotoxicity assay 3 replicates of each concentration were performed for each test; the tests were repeated 3 times to ensure reproducibility. The significance of differences between the groups was statistically analyzed by

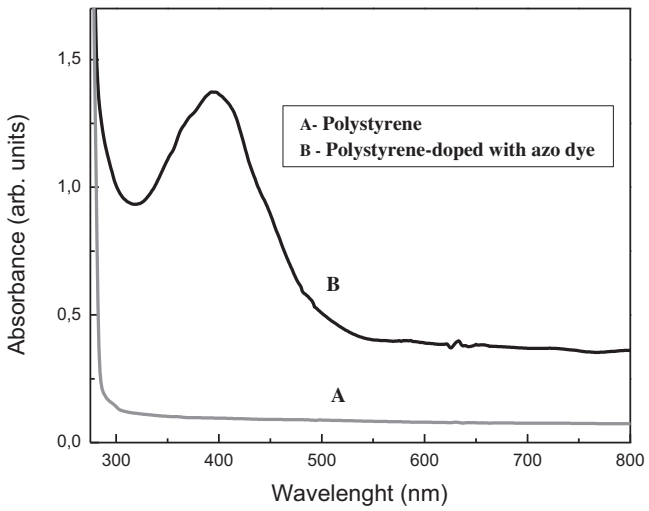


**Fig. 1.** UV–vis absorption spectra of PS films, without (A) and with (B) dopant.

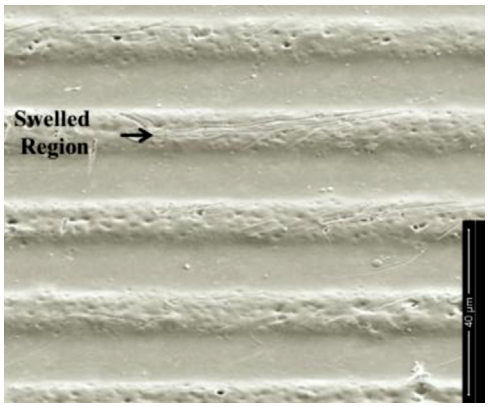


**Fig. 2.** Line-like patterns on a PSd film with laser fluence: 90 mJ/cm$^2$. The periodic is of 20 μm. Beam tilt = 45°.

one-way variance. Repeated measures of ANOVA were followed by Bonferroni's multiple comparison post hoc test, and a $P$ value less than 0.05 was considered statistically significant.

## 3. Results and discussion

The formation of different periodic microstructures in PSd films with azo compound (2-anisidine → 2-anisidine) obtained by using DLIP was investigated as a function of laser fluence. A two-laser beam configuration was used to obtain line-like interference patterns. In all cases, the utilized wavelength was 355 nm, which is commonly available in Nd-YAG tripled lasers. This dopant dye was selected because it has a high absorption coefficient in that region, which assures that the light energy will be effectively coupled to the polymeric matrix. Two process could occur: (i) the azo group absorbs light and breaks down with release of nitrogen which help to remove the solid material from the ablated region; (ii) the heat absorbed by the dye is transferred to the polymer producing sublimation and/or breakage of the polymer chain.

Fig. 1 shows the visible spectra of the PS films without and with dopant. As can be seen, 2-anisidine → 2-anisidine displays mainly one band. This band is located between 300 and 450 nm and is assigned to π–π* transition in the azo groups which is red shifted by conjugation with aromatic rings [34]. A broad UV absorption band, probably due to the aromatic ring transition in PS and azo is exhibited at around 290 nm [35]. When the irradiation light is of 355 nm, only the dye absorbs light and induces the patterning of PS. We found that doped PS can be micro-structured in the line-like patterns by DLIP at 355 nm in one single laser pulse, due to local and periodic heating/reaction of the materials at the interference maximum position.

Fig. 2 shows the scanning electron microscopic (SEM) of the irradiated PSd films. As can be observed, at low laser fluences (ca. 90 mJ/cm$^2$), the swelling of the surface takes place only at the maximum of interference. A different behavior is observed at higher laser fluences.

Fig. 3 shows SEM images indicating the evolution of the surface topography for PSd irradiated at different laser fluence values (525, 750, and 1125 J cm$^{-2}$). As shown in Fig. 3, at higher laser fluences the swelled structure is broken, pores open and are arranged periodically at the interference maximum positions.

The results also indicate that variations of the laser beam intensity produces a change of the line width and depth of the structure generated. It was also possible to fabricate very uniform structures at high laser intensities. However, the samples irradiated with low laser fluences show less regularity (Fig. 3(a)–(c)). This means that the material response to laser interference patterns is strongly dependent on laser intensity. The pores observed at interference maximum position resulted from the gas produced during the laser irradiation process. Furthermore, we consider that the pores observed in the samples irradiated may have been produced by gas ejection from the irradiated area. Previous work has shown that ablation of undoped polystyrene occurs without generation of gaseous products [9]. Therefore, the particular behavior in PSd is probably due to the presence of gaseous products generated by azo compound decomposition. Since only the dopant absorbs at 355 nm, their photosensitivity and the superior structuring properties are mainly due to the liability of the (–N=N–) group. During irradiation, the dye heats up and nitrogen as well as other

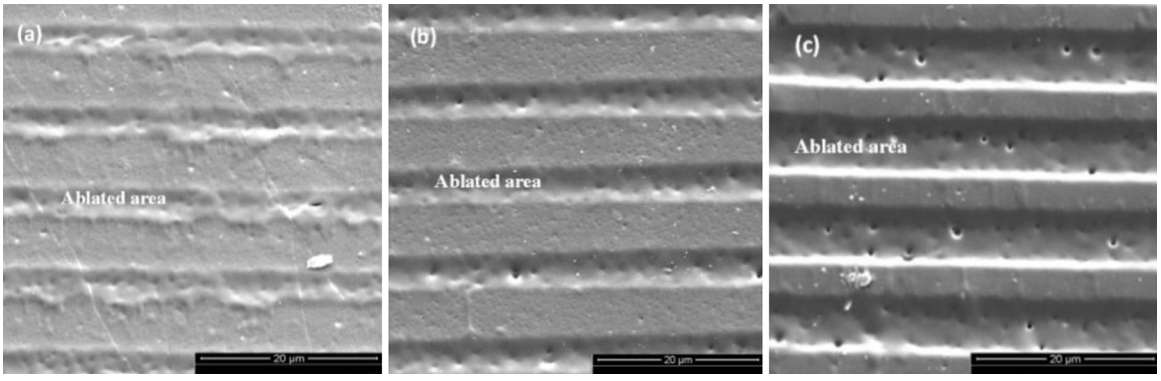


**Fig. 3.** Line-like patterns on a doped PS film with laser fluence: (a) 525 mJ/cm$^2$, (b) 750 mJ/cm$^2$, (c) 1125 mJ/cm$^2$. The period is of 10 μm. Beam tilt = 30°.

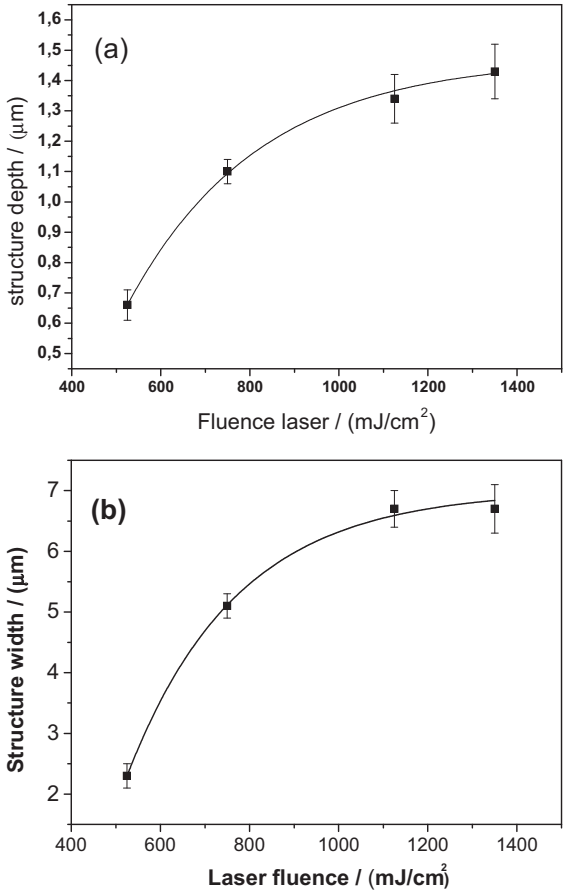


**Fig. 4.** (a) Structure depth and width (b) as function of laser fluence for doped PS irradiated at a wavelength of 355 nm.

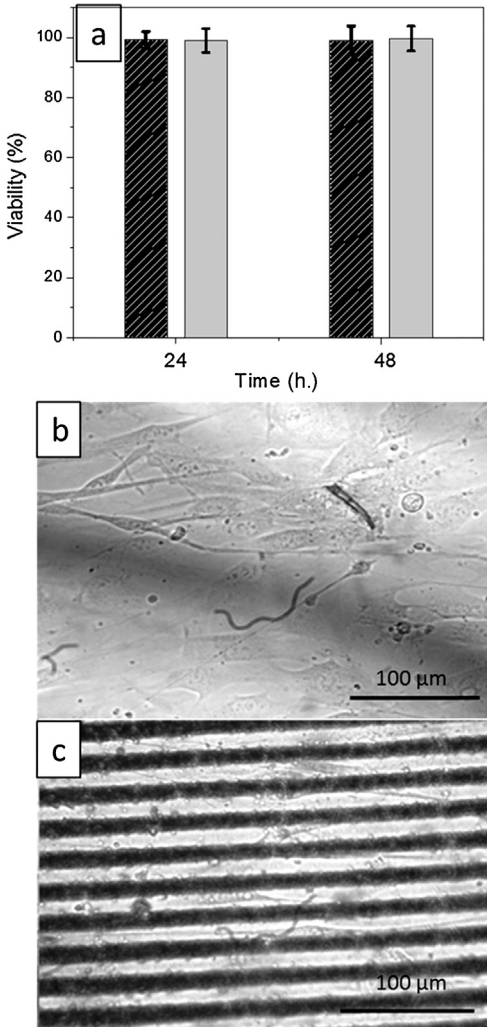


**Fig. 5.** (a) MTT proliferation assay of fibroblastic cells cultured in the presence (grey bar) and absence (black bar) of doped PS for 24 and 48 h. Similar results were obtained in three independent experiments. Error bars indicate standard deviations of the triplicate experiments. Phase contrast microscopic images of fibroblastic cells 24 h. after seeding on (b) PSd and (c) PSd structured (scale bar = 100 μm).

small organic fragments are released without leaving any residuals on the surface [19]. Nitrogen which is released in the course of the azo compound photochemical decomposition is thought to act as a driving gas for the ejection of material from the irradiated area [36]. In Fig. 3, it is possible to observe that the amount of bubbles increases with the laser intensity. This phenomenon is explained by the fact that a few nanoseconds after the laser irradiation the photolabile group (–N=N–) contained in the polymeric matrix decomposes creating gaseous products. Then, the material is ejected from the surface at supersonic velocity, producing a microstructured regular pattern [37,38]. Therefore, the DLIP process is controlled by the chemistry of the dopant and is different from that occurring when plain PS is irradiated with laser light at wavelengths where PS has significant absorption (e.g. 266 nm).

For PSd films, the laser fluence variation can be used to control the pattern depth and width. Fig. 4(b) shows the relationship between laser fluence and the width of the pattern. It is possible to observe that for all the laser fluences tested, when the laser fluence increases the pattern width increases. For fluence values up to 1200 mJ/cm$^2$ the width of the pattern increases proportional to fluence, while above this value, the width of the structure remains almost constant. This behavior is quite usual because, when the surfaces are ablated; there is the decrease of laser action resulting from the effective protection of fragments released by the plasma produced during the ablation process [39].

Also, DLIP can be used to vary the period of the structure of PSd films by controlling the angle between the laser beams, as in (1) [31]. Figs. 2 and 3(a)–(c) show different periodic arrays on doped PS films with 20 μm and 10 μm respectively.

To be used in biological applications, the biocompatibility and adhesion of cells have to be tested. Viability (%) of the cells exposed to the polymeric material after 24 and 48 h are shown in Fig. 5(a). No significant differences between the viability of cells exposed to doped PS and cell controls were observed ($P > 0.05$), with viability ratio above 95%. The data from in vitro assays shows that fibroblast cells are attached and proliferate extensively on the PSd films. The morphology of adhered cells, as observed in an inverted microscope, shows a normal appearance (Fig. 5(b) and (c)).

## 4. Conclusions

In summary, we have shown the possibility of fabricating different periodic arrays in PS films applying two-dimensional light interference patterns at 355 nm. We have demonstrated that doped PS with 2-anisidine → 2-anisidine dye is actually ablated when irradiated with pulsed laser light at 355 nm. We have also shown the possibility of fabricating regular microstructure line-like arrays with different periods and width on PSd, by varying the laser fluence could also be used to change the topography type of the periodic structure from swelled to ablated-like structure. The ablation of doped PS seems to be due to the absorption of laser light by

the dopant contained in the polymeric matrix, which decomposes to give gaseous products. The released nitrogen acts as driving gas and facilitates the removal of fragments from the surface. Finally the use the molecular dopant may be effective to improve the laser ablation of polymers. These micropatterns seem to be adequate for use as substrate for cell cultures [10], as it shows negligible cytotoxicity. Moreover, biological cells are shown to attach and proliferate on the surfaces. The structuring could be used to alter the wetting properties of the surface [40], and/or to fabricate microfluidic devices.

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