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DIRECT OBSERVATION OF ATHERMAL PHOTOFLUIDISATION IN AZO-POLYMER FILMS

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(*Note – the article is dedicated to Almeria Natansohn*)

Abstract

Surface relief gratings (SRGs) can be generated when azo-polymer films are exposed to laser beams interference as result of mass migration. Despite of considerable research effort over the past two decades, this complex phenomenon remains incompletely understood. Here we show, in premiere, the athermal photofluidisation of azo-polysiloxane films exposed to 488 nm light, directly monitored by optical microscopy. A new approach of SRG formation is proposed, based on three different processes: (1) the polymer photo-fluidization in the illuminated regions (2) the mass displacement from illuminated to the dark regions and (3) the inverse mass displacement, from dark to the illuminated regions. The mechanical properties of the films during UV light irradiation were also investigated by classical rheology and, in premiere, by using amplitude modulation-frequency modulation atomic force microscopy (AM-FM AFM). Applications of the azo-polysiloxanes films as cell culture substrates are also reported.

Almost 20 years ago, A. Natansohn *et al.* and J. Kumar *et al.*[1, 2] reported independently one of the most exciting behaviours of azo-polymers: the capacity to generate sinusoidal surfaces as a consequence of light irradiation through an interference pattern. Surface relief gratings (SRG) are the result of the *trans-cis* isomerisation process of the azobenzene groups, either linked to a polymeric chain[1-7] or as part of azo dye doped polymers (guest-host systems)[8, 9]. Despite the large number of studies attempting to characterize this process, the mechanism underlying the SRG formation is still unclear. Several models have been proposed so far, such as: the isomerisation pressure[10], the gradient electric force[11], the permittivity gradient[12], the asymmetric diffusion (migration)[13,14], the mean-field model[15], the theory of light-induced deformation[16], the cage-breaking model[17] or the statistical model based on fundamental molecular dipole[18] - none of them succeeding to comprehensively explain the surface relief formation. In the case of continuous laser irradiation, it is clear that the nanostructuration mechanism involves the directional displacement of the material from the exposed to the masked regions[4, 19-20].

One of the most recent applications of the azo-polymers surfaces is their use as support for cell cultures. Understanding the ability of cells to process and respond to different signals received from the extracellular matrix (ECM) was the objective of many research studies reported in recent years[21-25]. The ECM geometry and rigidity can influence cell adhesion, with direct consequences on cell morphology and migration capacity, tissue architecture and regeneration[21, 26]. Moreover, stem cells fate/differentiation can be induced by the flexibility and geometry of the ECM[27]. This tight relationship led to the development of the so called “third-generation” of biomaterials, capable to influence cell behaviour at nanometre scale in a well-defined manner[28, 29]. Among the variety of materials used as 3D-ECM, the azo-polymers distinguish themselves by certain advantages, especially the one-step procedure for surface nano-structuration[30-32].

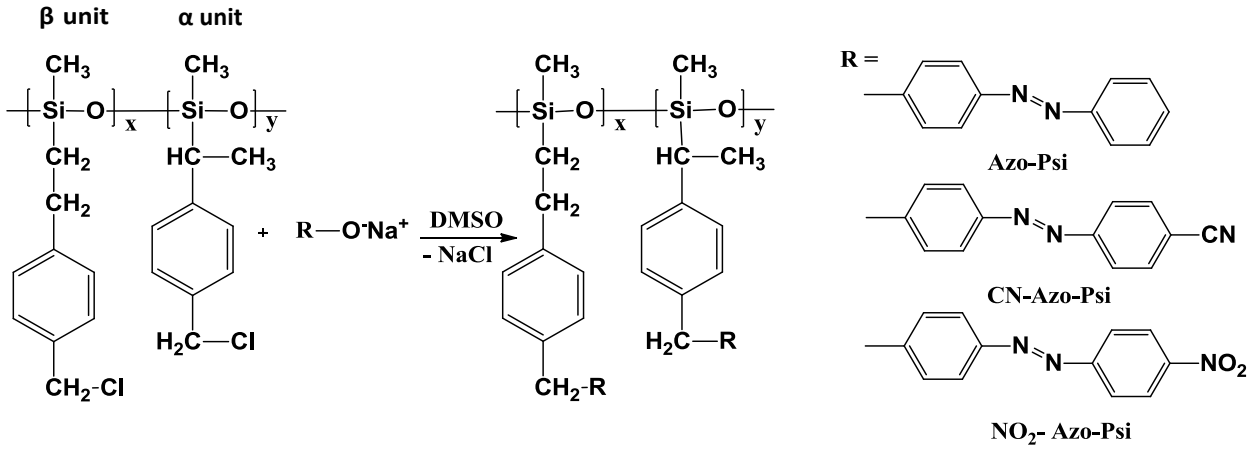
Here, we report the athermal photofluidisation resulting from the interaction between light and an azo-polysiloxanic film using optical microscopy. The photofluidisation and the directional mass displacement processes occurred independently, demonstrating the co-existence of multiple processes during nanostructuration. A new approach of SRG formation is proposed, based on three processes: (1) the polymer photo-fluidization in illuminated regions, (2) the mass displacement from illuminated to dark regions and (3) the inverse mass displacement from dark to illuminated regions. The mechanical properties of the films during UV light irradiation were also investigated by classical rheology and by using amplitude modulation/ frequency modulation atomic force microscopy (AM-FM AFM). Potential applications of the azo-polysiloxanes films as cell culture substrates are also reported.

Results

The azo-polymers investigated in this study were obtained from a polysiloxane containing chlorobenzyl groups in the side chain, modified by a substitution reaction with different azo-phenols. Three types of azo-groups were connected to the polysiloxanic chain: 4-phenylazophenol [Azo-Psi], 4-((4'-nitrophenyl)azo)phenol [NO_2-Azo-Psi] and 4-((4'-hydroxiphenyl)azo)benzonitrile [CN-Azo-Psi]. The chemical structure and synthesis route of these azo-polysiloxanes are shown in Scheme 1. Details of polymer synthesis and characterization were reported previously [33]. The UV-VIS absorption spectra, corresponding to the synthesized polymers are presented in Fig. 1S.

These azo-polymers can be used as support for cell cultures, a property investigated by our laboratory using plane and nano-structured surfaces [32, 34]. Our study has shown that the chemical structure of the azo-polymer has an important impact on cell adhesion and proliferation, independent of surface geometry. This observation prompted us to evaluate further the influence of both, plane film thickness and chemical composition on cell behaviour. The azo-polysiloxanic films were prepared by spin-coating onto silanised glass or poly (methyl methacrylate) supports. The film thickness was calibrated using

trichloromethyl-ethane solutions of various polymer concentrations. Details of the main polymers and films characteristics are presented in Table 1.



Scheme 1. Synthesis route of the azo-polysiloxanes. The starting polysiloxane contains two types of structural units, named **α** and **β**, with the x/y ratio = 3/1. The polysiloxane was modified using a SN_2 reaction in DMSO as solvent.

Table 1. The main characteristics of the synthesized polymers and films.

Sample code	Azo-group substituent	Substitution degree (%)	Mn	Tg* (°C)	Film support	Film thickness (nm)
1	4-phenylazophenol	85	7,300	36	glass	380
2	(**Azo-PSi**)				glass	970
3					PMMA	700
4	4-((4'-nitrophenyl)	82	7,750	48	glass	500
5	azo)phenol				glass	1150
6	(**NO_2-Azo-PSi**)				PMMA	500
7					PMMA	1150
8	4-((4'-hydroxiphenyl)	80	8,200	65	glass	300
9	azo)benzonitrile				glass	1300
10	(**CN-Azo-PSi**)				PMMA	300
11					PMMA	1300
12					PMMA	3700

*DSC method

In order to evaluate the adhesion properties of cells grown on the azo-polysiloxanic films, the architecture of the cytoskeleton structural elements was analysed by fluorescence microscopy, following a standard fixation step in 4% p-formaldehyde for 20 minutes at room temperature (22 °C) and incubation with the actin stain Alexa Fluor488-Phalloidin. The human hepatoma-derived HepaRG cells were chosen as a model in these experiments due to their unique ability to differentiate towards hepatocyte, as well as biliary-like phenotypes under specific culture conditions[35].

During this preliminary analysis a very interesting behaviour of the NO_2-Azo-PSi film was evidenced, when the 488 nm light (filtered HBO lamp light) was used to visualize the actin filaments. After a few seconds of microscopic visualization of samples at room temperature, the azo-polysiloxanic film began to fluidize (Video 1). The polymer «melting» is the result of the athermal photo-fluidization process induced by the trans-cis azo-groups isomerisation. To our knowledge, this is the first report showing that a fluid state induced by light can be directly observed by optical microscopy (Figure 1 and Video 1).

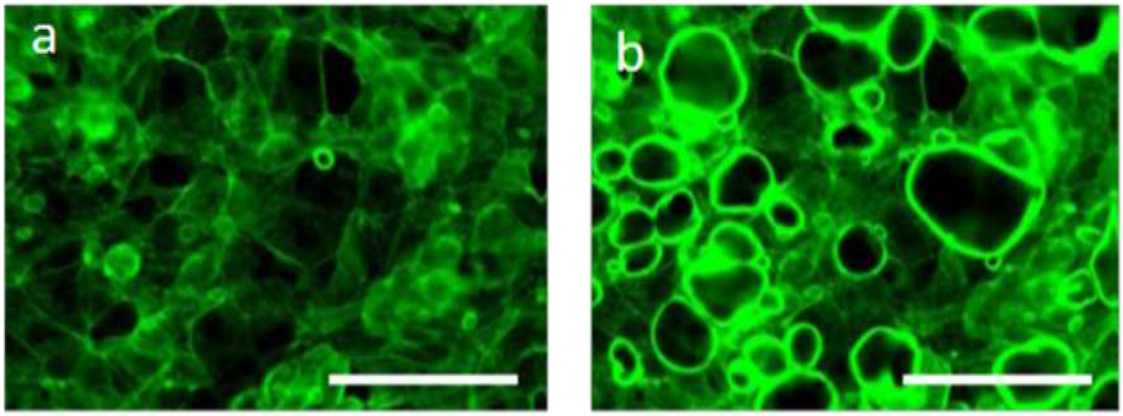


Figure 1. Athermal photofluidisation of the azo-polysiloxanic film (NO_2-Azo-PSi) during exposure to 488 nm light. Actin filaments in HepaRG cells seeded on the polymer film were stained with Alexa Fluor 488-labeled Phalloidin and visualized for 2 **(a)** or 8 **(b)** seconds. Scale bar is 100 µm.

Video 1. Athermal photo-fluidization of the azo-polysiloxanic film corresponding to NO_2-Azo-Psi. The video shows the polymer film fluidization as a result of its interaction with the 488 nm light (microscope HBO lamp source). The displacement of cells during the film fluidization can be observed.

To determine whether the intriguing behaviour of the NO_2-Azo-PSi film when exposed to 488 nm light is an intrinsic property of the polymer or is induced by the growth of the cell monolayer, the experiment was repeated using a similar plane film, in the absence of cells. To enable monitoring the process at the same wavelength, the polymer was first incubated with Alexa Fluor 488-labeled goat antibody. As shown in Video 2, the athermal photofluidisation was clearly detected demonstrating that it occurs independently of film-cell interactions.

Video 2. Athermal photo-fluidization of a 1 µm thick NO_2-Azo-PSi film stained with Alexa Fluor 488-labeled goat antibody.

Interestingly, none of the other two types of azo-polysiloxanes responded to 488 nm light. This observation supports the idea that the *cis-trans* equilibrium value, characteristic to each azo-group, plays a very important role in fluidization.

To gain more insights into the athermal photo-fluidization process, a series of complex, complementary studies were performed using rheological methods, AFM, UV-VIS spectroscopy and laser irradiation in different operational conditions.

The analysis of the rheological properties of the polymers in the presence and absence of light is expected to provide very important mechanistic information concerning the photo-fluidization process. Based on the results obtained by optical microscopy, the rheological studies in the presence of light should ideally employ irradiation of samples at the same wavelengths.

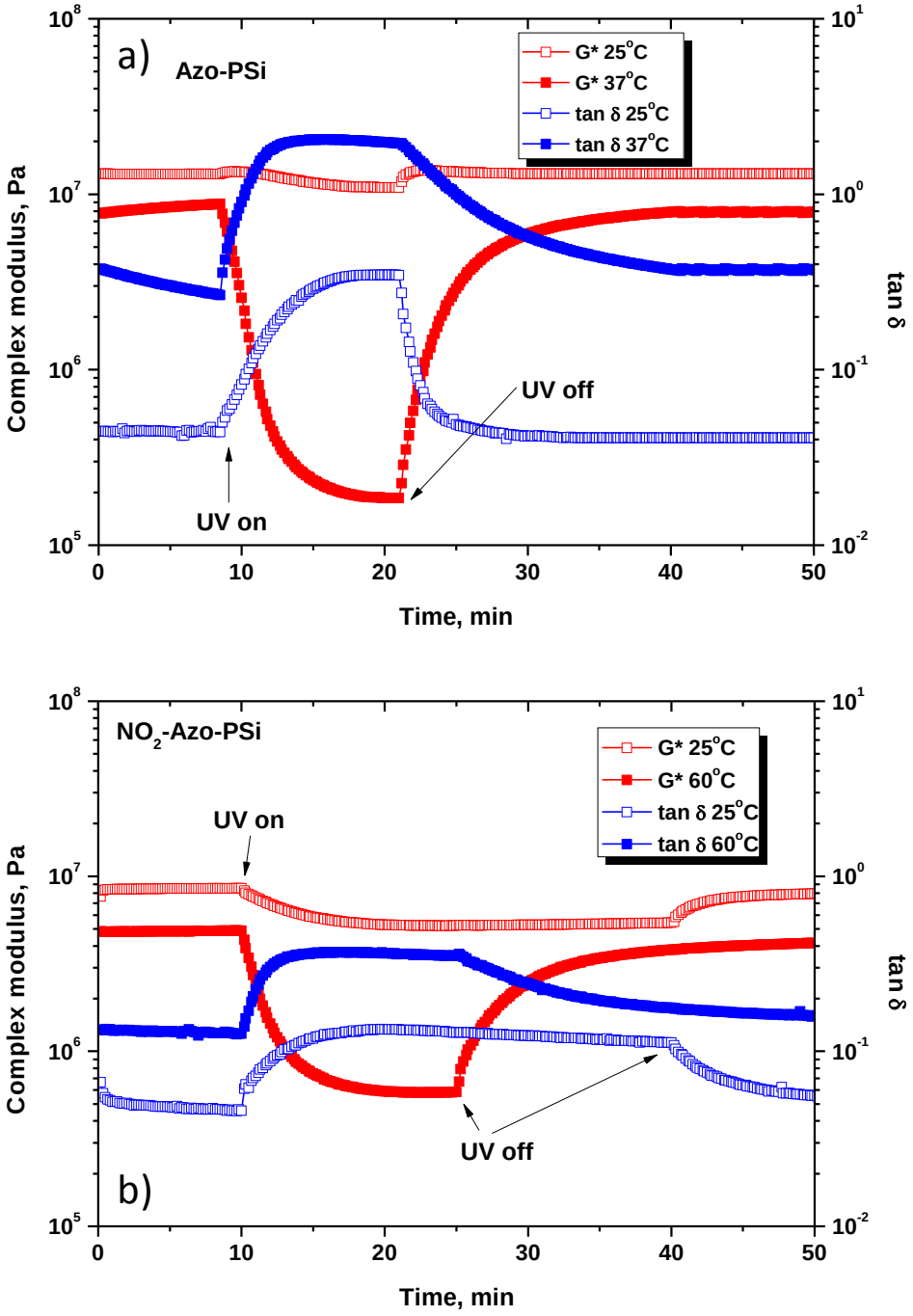


Figure 2. Rheological behaviour of the Azo-Psi **(a)** and NO_2-Azo-Psi **(b)** samples during UV irradiation (365 nm) at different temperatures. Sample film thickness was 400 microns. After 10 minutes from the beginning the rheological test the UV light source was switched on and the film was irradiated at constant temperature until the stabilization of the complex module value.

As the rheometer is not equipped with light sources in the visible range, the studies were conducted using the closest wavelength of 365 nm. In addition, it should be noted that the minimum thickness of the film used in rheological tests was around 400 µm. The complex modulus (G*) values corresponding to samples Azo-Psi and NO_2-Azo-Psi samples are in the range of 8.5 - 10.5 MPa, evidencing a behaviour situated between soft (G* ≤ 1 MPa) and rigid materials (G* ≥ 1 GPa)[39].

When the Azo-Psi sample is UV irradiated at 25 °C (Fig. 2a) a slow diminution of the complex modulus (G*) is observed accompanied by an increase of the tan δ value; however the system is not in a real fluid state yet, since the tan δ value is not situated above 1. When the temperature is increased to 37 °C an important decrease of the G* value is evidenced during the UV exposure. Accordingly, the tan δ value increases above 1, reflecting the fluid state of the film. This suggests that the UV light induces *trans-cis-trans* isomerisation cycles of the azo-groups, thus increasing the movement of the polymer chain segments. This rheological behaviour of the Azo-Psi sample confirms the concept of *conformational instability* which we have proposed previously[33].

A similar variation of the G* and tan δ values was observed during the UV irradiation of the NO_2-Azo-Psi sample (Tg = 48 °C, Table 1). However, a real fluid state was not obtained in this case, despite sample heating above the Tg value, the tan δ value remaining below 1. This intriguing rheological behaviour can be explained by the differences of the photoisomerization rates and the trans-cis equilibrium values corresponding to unsubstituted and para NO_2 – substituted azo groups.

We next performed viscoelastic mapping Amplitude Modulated/ Frequency Modulated Atomic Force Microscopy (AM FM AFM) before, during and after light irradiation. This method not only offers calibrated quantitative evaluation of sample viscoelasticity (as it provides data on loss tangent) but it also allows the possibility to monitor the mechanical properties with time and at the same location precisely. Scanning the film surface in the fluid state was practically impossible, due to capillary forces trapping the probe into the polymer mass. Therefore, we applied irradiation conditions that only

diminish the modulus value, without a real fluidization of the sample. In order to obtain comparable results with the rheology study, the azo-polysiloxane films were irradiated using an UV lamp (365 nm). A first general observation regarded the mechanical properties measured by AFM, which were different for very thin films (500-2000 nm), compared to the thick films (above 400 µm) tested by rheometry. Rheometry's shearing elasticity moduli were in the MPa range while the AM-FM AFM compressive moduli were in the GPa domain.

Figure 3 shows general trends in Moduli and topography of the Azo-Psi, NO_2-Azo-Psi and CN-Azo-PSi samples, before, during and after irradiation with 365 nm light. For the first two samples, the first pass (scan 1) of the scanning cantilever induced an increase in the modulus of approximately 1GPa, which is considered to be an effect of sample compression upon contact with the tip. The CN-Azo-PSi polymer seemed to maintain its mechanical properties after the first pass. Both, Azo-PSi and CN-Azo-PSi polymers reacted instantaneously to irradiation, the modulus dropping by approximately 1.5 and 0.8 GPa respectively (scan 2). The polymer containing nitro substituents reacted significantly more slowly, the reduction of modulus appearing only at the end of Scan 2, after 200 seconds from the start of irradiation. Overall, the Azo-PSi and CN-Azo-Psi polymers have similar behaviours, partially recovering after irradiation. In striking contrast, the NO_2-Azo-Psi polymer softens in scan 3 and hardens in the subsequent pass, suggesting plastic deformation. After the UV light is turned off, the polymer softens again. This opposite behaviour of polymers with substituted azobenzene in their structure suggests that the photofluidisation mechanism depends on the nature of the substituents and probably, on the percentage of *cis* and *trans* isomers.

It is worth noting that the topography of the sample changes in terms of RMS roughness upon irradiation and relaxation, dropping from 1.0 to 0.6 nm for Azo-PSi, 11.5 to 9.3 nm for NO_2-Azo-PSi and 3.1 to 1.7 nm for CN-Azo-PSi polymers (Table 2 S)

The evolution of the mechanical properties under UV irradiation and the relaxation in the dark are critical to understand the photofluidisation mechanisms. To our knowledge, the recordings of Moduli evolution upon UV excitation, employing AM-FM viscoelastic mapping are a premiere. The AFM micrographs corresponding to the height and Young's modulus of the synthesized polymer films are presented in Figures 2S and 3S respectively.

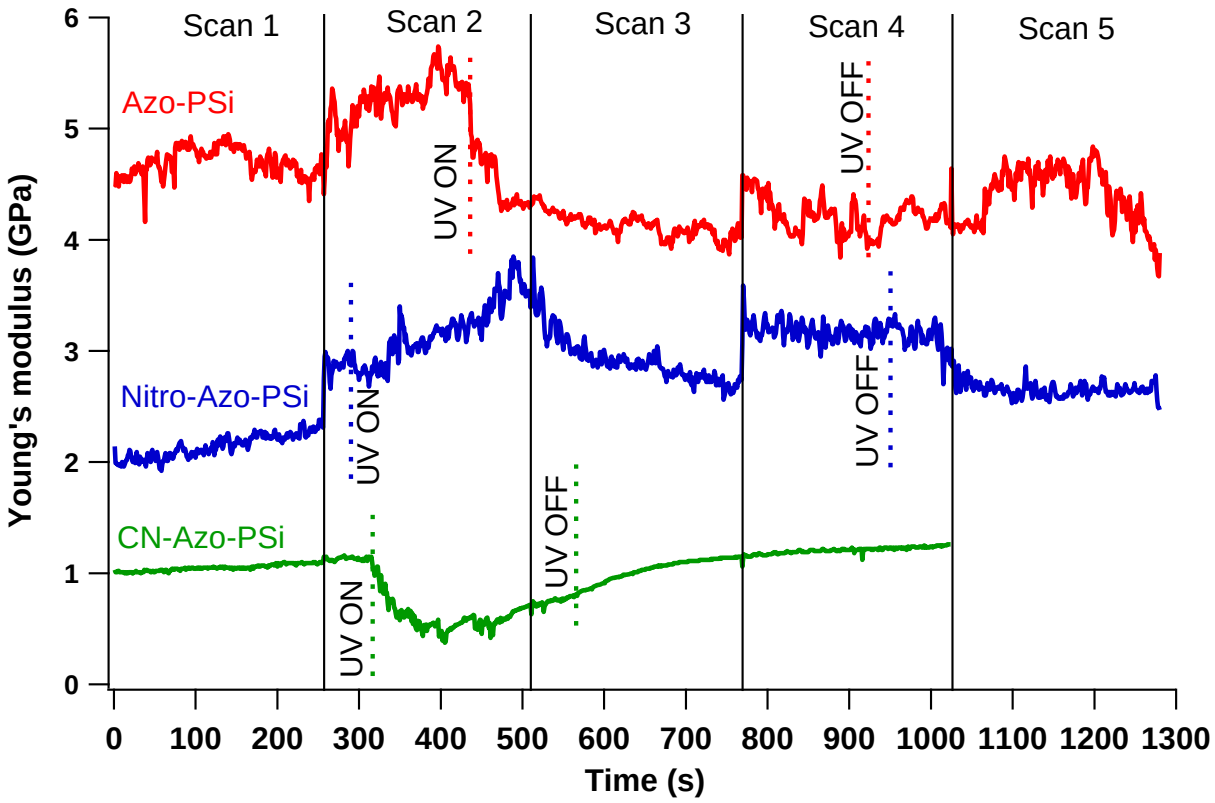


Figure 3. Young's modulus variation over time during five scans of Azo-Psi, NO_2-Azo-PSi and CN-Azo-Psi thin films at the same location

Another surprising behaviour of azo-polysiloxanes was observed during the laser nanostructuration of the Azo-Psi polymer film consisting in a parallel erasure process leading to the reduction of the relief amplitude. The surface relief was generated using a 488 nm laser polarized horizontally (180 mW/cm^2).

As shown in Fig. 4, the diffraction intensity decreases after 50 - 60 min of irradiation, indicating the surface erasure. The experiments were performed using 650 nm -thick films and different illumination times in order to correlate the diffraction curves with the evolution of the surface amplitude. The process was further confirmed by AFM, a reduction of the relief amplitude from 239 to 114 nm being observed. These amplitude modulations were detected when maximum and minimum diffraction intensities were reached (239 nm after irradiation for 1 hour and 114 nm after irradiation for 2 hours). In this case, the erasure process stops after 2 hours of irradiation and the imprinting restarts. As a consequence, after 3 hours of irradiation the relief amplitude increases to 180 nm. Fig. 4 (a, b) shows typical profiles of the surface modulation measured by AFM.

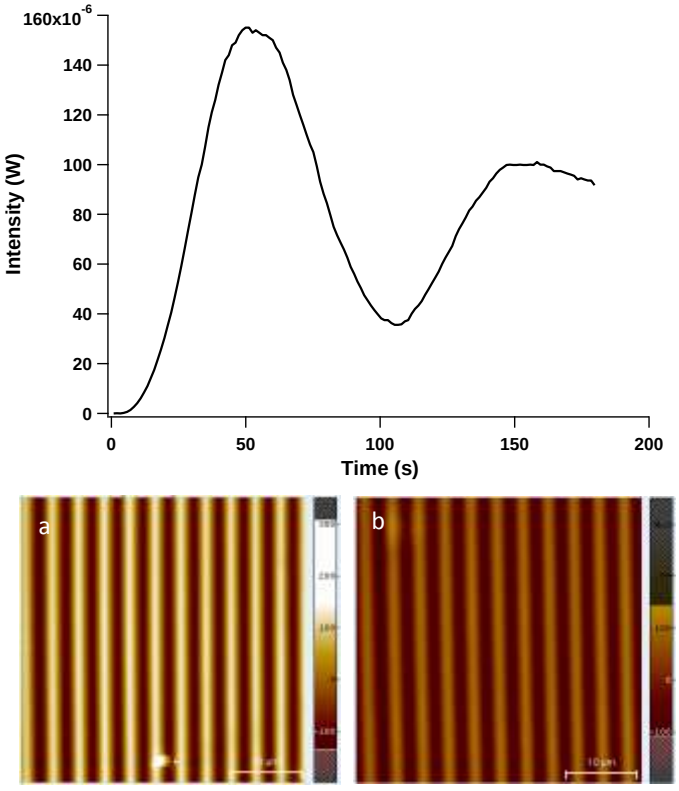


Figure 4. Diffraction curve reflecting the formation and erasure of the surface relief of a film corresponding to Azo-Psi polymer after 3hours of irradiation. AFM micrograph of the surface relief obtained when illumination is stopped after 1 hour (maximum of diffraction efficiency) **(a)** or after 2 hours (minimum of diffraction efficiency during erasing) **(b)**.

This unexpected behaviour suggests that an inverse mass displacement process from dark to illuminated regions takes place. Very recently, Accary and Teboul[36] proposed a similar mechanism, using molecular modelling simulations. The authors attributed the phenomenon to a local material softening occurring around isomerising azobenzene molecules. The process was shown to increase with the illumination intensity, i.e. with the azobenzene isomerisation rate, favouring local molecular reorganisations against a long range material migration. In our case, the reverse migration occurs after a certain time while the illumination intensity is maintained constant, evidencing a competition between the two processes. Their balance may be influenced by the evolution of the mechanical properties of the film during illumination. In this respect, the low Tg of the material investigated is a critical parameter in revealing the occurrence of the two the different processes. As well, the reverse migration is not always present if the film is thermally pre-treated before irradiation (50 °C, 1h). In addition, it must be noted that higher values of the relief profiles are obtained in the case of thermally -treated compared to untreated films. This suggests that the initial chain conformation (that is, before UV/VIS irradiation) is essential for the erasing process.

The next step of our investigation was to test the possibility to use azo-polysiloxanes as ECM in cell culture. A typical cell response to each azopolymer type is shown in Fig. 5, evidencing the better cell adhesion properties of NO_2-Azo-PSi and CN-Azo-Psi polymers. The cells were well-adhered, as judged by the pattern and intracellular distribution of the actin filaments and microtubules. In contrast, the PMMA support (sample 3 – Table 1) was less compatible with cellular proliferation, a lower number of cells being detected on the film surface, compared to control. The cells appear to grow better on NO_2- Azo-PSi polymers than on control surface or Azo-PSi films, as a higher number was detected on both, glass and PMMA supports (samples 4-7, Table 1). These results confirm our previous studies showing that of the series tested, this type of polymer is most suitable for cell culture applications[32]. A better adhesion is

observed when the cells are seeded on films layered on glass substrates, which also appear to induce cell clustering (more evident in the case of sample 4).

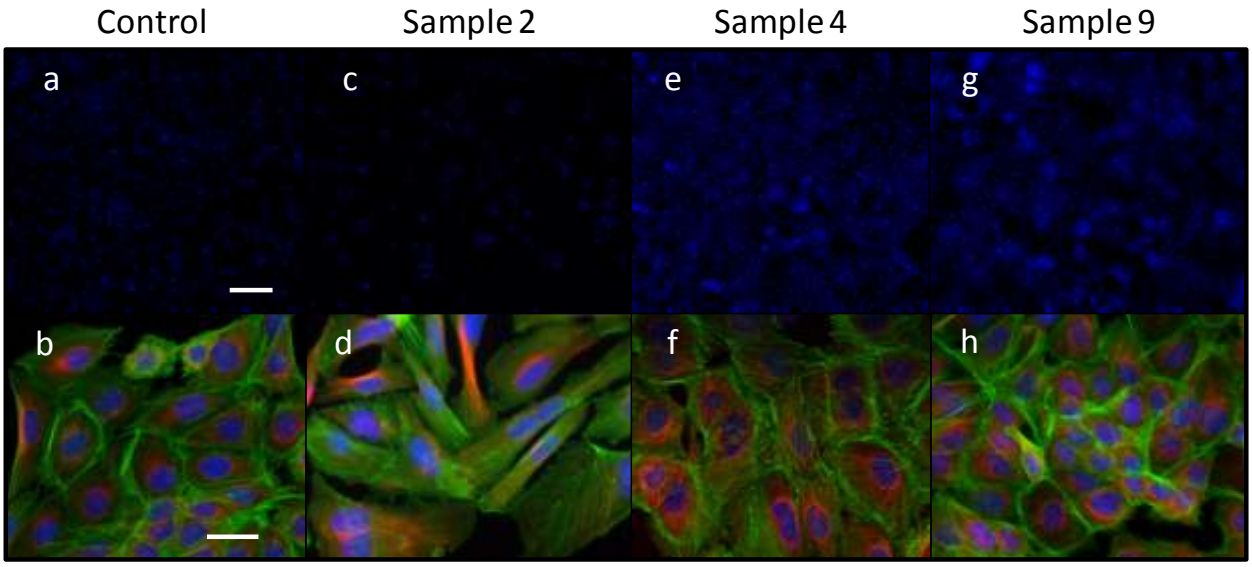


Figure 5. Immunofluorescence microscopy images of HepaRG cells grown on glass (Control: a and b) or polymer films corresponding to Samples 2 (c, d), 4(e, f) and 9 (g, h) (Table 1) showing the cell nuclei (a, c, e, g; scale-bar 100 µm) and the organization of cytoskeleton elements (b, d, f, h; scale-bar 30 µm) ; actin and microtubules are shown in green and red, respectively.

This observation could be relevant for the differentiation of HepaRG cells, a long process requiring especial culture conditions, and will be investigated in detail in a future study. A similar behaviour was observed for the cells grown on CN-Azo-PSi polymers (samples 8-12, Table 1). In this case, the cell division and adhesion are favoured by the film thickness, regardless the substrate used to deposit the polymer. This polymer also appears to promote cell clustering (samples 9 and 12, Table 1).

Considering the potential development of these materials as cell culture substrates, it was of immediate interest to determine their stability in aqueous milieus. Interestingly, the AFM investigations showed that following 3 hours of contact with water the Azo-PSi and CN-Azo-PSi films adopt an «island»-like surface relief (Fig. 6). This observation opens up new directions of research in the field of biomaterials, as this surface instability is expected to influence the cell adhesion properties. It would be very

interesting to exploit this controllable feature of the Azo-PSi and CN-Azo-PSi films, in future studies addressing the influence of surface geometry on cell signalling and development. Unlike the other two materials, the NO_2-Azo-PSi surface remains stable in contact with water, which is in agreement with the viscoelastic mapping showing a better stability of this material.

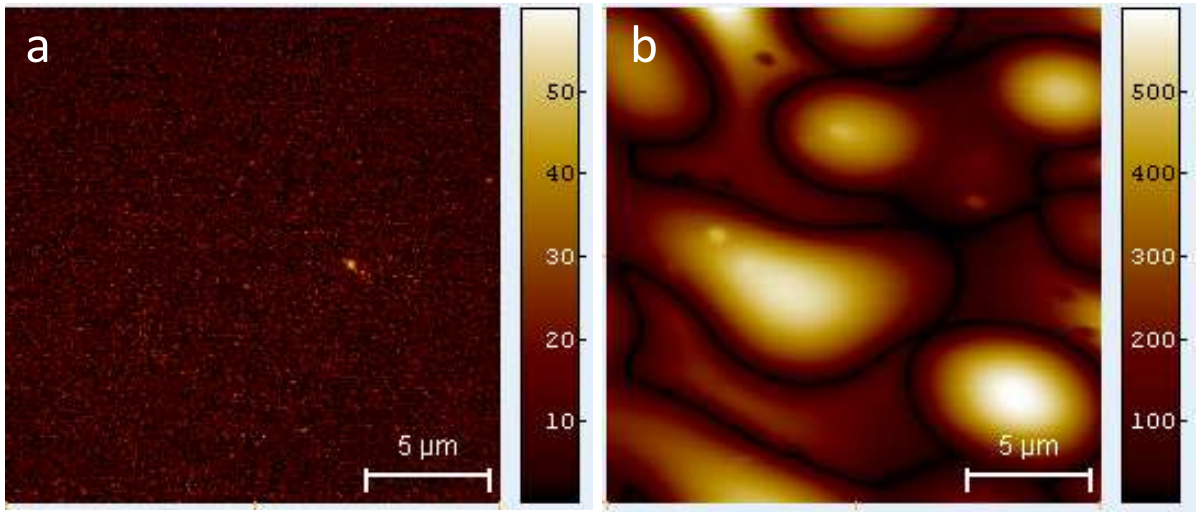


Figure 6. AFM image of an island-like relief formation (Azo-Psi film) as a result of the contact between the surface film and water: (a) before the contact with water; (a) after 3 hours in contact with water.

Discussion

In this study we have confirmed the existence of photo-fluidity and demonstrated that the material viscosity upon interaction with UV/VIS light is strongly influenced by the polymer chemical structure. The presence of the photo-fluidization process during the light irradiation of azo-polymers was a subject of major debate between different research groups. The neglect of this process was, in our opinion, one of the reasons why the mass transport mechanism has not been completely elucidated until now. Recently however, an important number of research studies began to acknowledge the existence of photo-fluidity[4, 14, 36-38]. The softening degree of the azo-polymers is expected to be different according to

their Tg values, ranging from plastic to fluid state. Softening/fluidization is the first stage of the irradiated material, preceding probably the mass displacement process. The low Tg values of the azo-polysiloxanes investigated in this work allowed us to separate the parallel processes that most likely overlap during the surface nano-structuration.

Based on these results, a new approach of SRG formation is proposed, involving at least three processes: (1) the polymer photo-fluidization in the illuminated regions (2) the mass displacement from illuminated to the dark regions and (3) the inverse mass displacement from dark to the illuminated regions (Fig. 4S). These processes take place at the same time, one or the other being dominant, depending on the polymer chemical structure and operational conditions. It must be highlighted that the inverse migration from dark to illuminated regions occurs only if the laser source is on. The erasing process stops once the laser is switched off.

A parallel study of the mechanical properties of the films, based on classical rheology and AFM, was also conducted in this investigation. The results evidenced important differences in the magnitude of elastic moduli as a function of film thickness. The polymer has an elastic behaviour when layered on thick films of approximately 400 microns, while acting as rigid bodies in very thin films (500-2000 nm). This difference can be a consequence of the interactions with the glass substrate, which becomes more prominent in the case of thin films.

The athermal photo-fluidization process has potential in biological applications, considering that the cells grown onto an azo-polysiloxanic film can be easily detached from the support, using light. This property may be particularly relevant for manipulation of certain cell types for which more destructive methods, such as mechanical scrapping or enzymatic trypsinisation are not recommended. The results from the tissue culture experiments show that the polymer chemical structure, the film thickness and its support influence greatly the cell adhesion and morphology. The best results regarding the cellular behaviour on the plane azo-polysiloxane films were obtained for NO_2-Azo-Psi, the only stable material

upon contact with water. Together, the results obtained by our group recommend the azo-polysiloxanes as a very promising class of materials which can find interesting applications in biomedical research. Under certain light irradiation wavelengths and power, azo-polysiloxanes films are able to display particular mechanical properties, which can be tailored by changing their chemical structure. This unique feature could be used to drive specific responses, such as directed differentiation of pluripotent stem cells towards certain lineages. Moreover, the characteristics of the relief geometry obtained by laser irradiation cannot be reached using classical lithography methods[40].

Methods

Azo-polysiloxanes synthesis

The polysiloxane containing chlorobenzyl groups in the side-chain was obtained by a two-step reaction, starting from dichloro(4-chloromethylphenylethyl)methylsilane. A hydrolysis reaction takes place in the first step, resulting a mixture of linear and cyclic oligomers.. The second step ensures a cationic equilibration in the presence of triflic acid and 1,3-divinyl-1,1,3,3-tetramethyldisiloxane as chain blocker resulting in formation of linear polymers (Mn around 5,000). The polysiloxanes are then modified with different azophenols using a SN_2 reaction, in DMSO. Details concerning polymers synthesis and characterization were previously reported[33].

Rheological measurements

The rheological measurements were performed on a Physica MCR 501 rheometer (Anton Paar, Austria) equipped with electronically commutated synchronous motor, allowing rheological measurements in controlled-stress and controlled-strain modes and a Peltier device for the temperature control. The temperature can be adjusted between -40 and 200 °C. For regular tests parallel plate geometry with serrated plates was used to avoid slippage of the sample. The upper plate made of stainless steel has 50 mm in diameter. Simultaneous UV irradiation and rheological studies could be performed using an advanced UV system, under a Peltier controlled P-PTD-UV chamber on Physica MCR 501 rheometer. As measuring system a 43 mm parallel glass plate fixture was used. The UV source was an Omnicure Series

1000 manufactured by EXFO (Vanier, QC, Canada), using a 100 W lamp able to deliver UV light of 365 nm using a Mercury bulb.

Viscoelasticity mapping during UV irradiation in dark

All experiments have been done using a Cypher S AFM (Asylum Research/ Oxford Instruments, Santa Barbara, CA) in AM-FM (Amplitude Modulation – Frequency Modulation) viscoelastic mapping mode employing Multi75-G (Budget Sensors, Bulgaria) probes with nominal resonance frequency f_N = 75 kHz and nominal spring constant k_N = 3 N/m. All probes have been mounted in a high frequency/ low noise AMFM cantilever holder. For the AM pass probes were actuated to free air amplitude of 1.5 V, at the nominal resonant frequency and engaged at 800 mV setpoint. The second pass was done actuating for the second harmonic at approximately 1.2 MHz for free air amplitude of 100mV, while maintaining phase to 90 degrees. Sample engagement was done so that probe tapping was in repulsive mode at a phase less than 50 degrees for the AM pass. Irradiation was done employing a Maxima-ML-3500/FA (Spectroline, NY) lamp (λ = 365 nm) calibrated at 75 ± 10 mW/cm^2 for a 15 cm working distance. Before sample analysis, the cantilever's conversion constant was determined fitting the Young's modulus (E = 1.526 GPa) of a known sample (UHMWPE-8456 standard reference material from National Institute of Standards and Technology, USA) at 22 ± 0.2°C. A typical experiment involves the simultaneous acquisition of topography data and the modulus mapping before, during and after irradiation of the sample with 365 nm light. The evolution of modulus with time graphs are generated by extracting the layer of 256x256 pixels and averaging by line for a scan rate of 1Hz.

Polymer film surface optical modulation

The polymer films are illuminated with an interference pattern produced by the superposition of two coherent beams incident symmetrically onto the film with respect to the film surface normal direction (Fig. 5S). The beam delivered by a 488nm wavelength laser diode is incident onto a beam splitter and the resulting beams are superposed onto the film after reflection onto two mirrors. The resulting interference pattern presents a sinusoidal modulation of the intensity along the polymer surface. The intensity in both beams is adjusted with optical densities to be equal. An average intensity of 180mW·cm^{-2} was used for the experiments and the beam polarization was set on the incidence plane,

i.e. perpendicular to the interference pattern fringes. A 633 nm wavelength Helium-Neon laser is employed to record the evolution of the 1rst order diffraction efficiency during the film illumination.

Fluorescence microscopy

Cell adhesion and growth were monitored by Immunofluorescence microscopy, at 24 hours post-seeding on different substrates, using a Zeiss Axio Microscope. Actin filaments and microtubules were visualized with the 60X objective, using Alexa Fluor 488-labelled Phalloidin and mouse anti-human tubulin antibodies, followed by Alexa Fluor 594-labelled goat anti-mouse antibodies, respectively. Cell nuclei were stained with DAPI and visualized with the 20X objective. Images were taken using the AxioVision Rel4.8 software.

Topography measurements by AFM

Topographic measurements evidencing the laser induced modulations and the influence of water on the polymer films were performed with a PicoLE 5100 AFM supplied by Agilent Technologies. The AFM was driven in the acoustically driven mode (tapping) in order to minimize the interaction of the tip with the surface during the scan. Arrow NCR Silicon probes from Nanoworld with nominal resonance frequency f_N = 285 kHz and nominal spring constant k_N = 42 N/m have been used. Experiments were performed within a liquid cell enabling direct measurement of the topography evolution of the films while exposed to water. 512x512 pixels images were generated with a scan rate frequency of 0.2 to 0.4 Hz.

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Acknowledgements

The Romanian authors thank to ANCS for financial support of this research (Grant CEA - C1- 01/2010).

NN and AM were supported by the Institute of Biochemistry through the Project 3 of the Romanian Academy. This work was performed in part at the Queensland node of the Australian National Fabrication Facility, a company established under the National Collaborative Research Infrastructure Strategy to provide nano and micro-fabrication facilities for Australia's researchers.