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Chapter 10

Smart Thermoresponsive Surfaces Based on pNIPAm Coatings and Laser Method for Biological Applications

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Abstract

Various applications within last decades such as bacterially resistant surfaces, soft robotics, drug delivery systems, sensors and tissue engineering are poised to feature the importance of the ability to control bio-interfacial interactions. An enhanced attention is dedicated to designing smart stimuli-responsive interfaces for DNA, drug delivery, protein and cell based applications. Within this context, the thermoresponsive materials, especially poly(N-isopropylacrylamide) (pNIPAm) have been intensively used in tissue engineering applications for a controlled detachment of proteins and cells with a minimum of invasive effect on protein and cell structural conformation. The properties of smart bio-interfaces can be controlled by its composition and polymer architecture. Therefore, appropriate methods for obtaining controlled coatings are necessary. Laser methods were successfully used in the last decades for obtaining controlled organic and inorganic coatings for various types of applications, from electronics to tissue engineering. Among these, Matrix-Assisted Pulsed Laser Evaporation (MAPLE) technique bring us a step forward to other laser methods by avoiding damage and photochemical decomposition of materials. In this chapter we describe materials and approaches used for design of smart bio-interfaces aimed at controlling protein and cells behavior *in vitro*, focusing MAPLE method for tuning coatings characteristics in relation with biological response.

Keywords: bio-smart interfaces, temperature responsive, laser processing, mammalian cells

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1. Introduction to poly(N-isopropylacrylamide) (pNIPAm) characteristics, its thermoresponsive mechanism and its applicability in bioengineering

The design of smart bio-interfaces for a wide variety of applications is based nowadays on stimuli-responsive surfaces. Depending on the type of bioengineering applications implying bio-adhesion, manipulation of microorganisms or mammalian cell, bio-adsorption of proteins, various surfaces that can respond to different stimulus (e.g., temperature, pH, light and magnetic field) [1–3] could be obtained by tuning not only the nature of material or its characteristics and chemistry but also the obtaining method. Among these types of smart- or stimuli-responsive materials, thermoresponsive group of smart polymers consists of the polymers which exhibit rapid, reversible phase transition/phase separation phenomena in response to changes in temperature. The thermosensitive behavior is based on the reversible solubility of the smart polymers caused by increasing temperature above a critical transition temperature (lower critical solution temperature (LCST) or "cloud point") [1–5].

There are several groups of thermosensitive polymers with different transition temperatures such as poly(ethylene glycol) with a transition temperature of 100°C, poly(N-alkyl-substituted acrylamides) and poly(N-vinylalkylamides) with a transition temperature ranging from 32 to 39°C depending on polymer molecular weight as well as other polymers such as poly (N-vinyl piperidine) with low-transition temperature of 4–5°C.

Particularly, poly(N-isopropylacrylamide) (pNIPAm) coatings are of great promise in both basic developmental biology studies and regenerative medicine, as thermoresponsive smart bio-interfaces responding within the physiological temperature range [1–9]. Its chemical formula is $(C6H11NO)_n$, having a white solid form. Starting from 1968, from the first report on the phase-transition temperature as a function of pNIPAm concentration, there are various studies implying the use of reversible behavior phase transition of swelling/shrinking cycles that can be performed by a gel at around 32°C, with no sign of material fatigue [9]. For example, one of the first studies on the deswelling mechanism [6] demonstrated that bound water molecules are freed at LCST (38.5°C) which lead to a collapse phenomenon, followed by trapping water with a layer/skin. Moreover, depending on how dense the polymeric layer is, this process could have biphasic kinetics (slow for dense-packed surfaces, fast for less dense-packed surfaces) [6].

The abovementioned process implies a phase separation by changing the hydrophobic-hydrophilic moieties balance of the uncharged polymer. Solubility in water is based on the hydrogen bonding with water molecules which, however, is dependent/reduced with temperature increase, leading eventually to separation phase. Therefore, there are two phases formed: an aqueous phase containing practically no polymer and a polymer-enriched phase that could be easily separated by centrifugation, decanting, or even filtration. Moreover, the temperature of phase transitions also depends on the polymer concentration and its molecular weight and it can be further modified by incorporating various hydrophilic co-monomers or coupling other active compounds. For example, as phase transition at increased temperature of thermosensitive polymers is the result of hydrophobic interactions between polymer molecules, by increasing salt concentrations, a shift of cloud

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point to lower temperatures can be achieved. The opposite effect can be obtained by deteriorating the hydrophobic interactions by the simple addition of organic solvents, detergents and other active agents [7–10].

Besides modifying or adjusting the cloud point of pNIPAm with salts or surfactants, interesting results were achieved by copolymerization with other hydrophilic or hydrophobic co-monomers, nanoparticles, bioactive agents such as peptides and enzymes [10–15]. The resulted block copolymers based on the pNIPAm thermosensitive part and on hydrophilic monomers conducted to an elevating LCST while those based on the hydrophobic monomers lead to a decrease of the LCST. Additionally, these modifications imply also the stimuli response, as being able to respond not only to temperature variations but also to pH variations. For example, studies have been reported on the increase in the LCST of pNIPAm from 32 to 38°C, as well as adding a pH response after carrying out its copolymerization with hydrophilic methacrylic acid and PEG (polyethylene glycol). Depending on the followed application, it is essential to control the characteristics of the smart polymeric systems, especially for applications envisaging bioengineering area.

As previously mentioned, due to its response within the physiological temperature range, pNIPAm coatings are of great promise not only in basic developmental biology studies but also in drug delivery and regenerative medicine. There are several directions in which pNIPAm in the form of coatings could be applied within these areas [16–22].

For example, by obtaining a responsive polymer surface consisting of a densely packed monolayer of copolymer molecules onto a solid substrate, the switching of the surface properties upon changing solvent selectivity or pH of the aqueous environment facilitated the patterned cell seeding and coculture [18, 19]. Polymer brushes, which consist of an assembly of polymer chains that are attached by one end to the surface, were obtained for tuning physicochemical surface characteristics such as wettability, surface charge and morphology, for further even more complex environments such as for fabrication and manipulation of tissue-like architectures with multiple cell types [19, 20]. Another important direction is the use of the smart polymer networks in the delivery of therapeutic drugs at body temperatures as well as structures for tissue engineering [21, 22].

2. Approaches for obtaining pNIPAm surfaces

Nevertheless, nowadays, there are various approaches for obtaining pNIPAm surfaces, in different forms such as membranes, thin films and nanoparticles. Within the context of cell culture substrates or drug-delivery platforms, the ideal use of pNIPAm bio-interfaces is as controlled coating. Based on the fact that the coating response toward and within the biological medium is closely related to its structural and architectural characteristics, a variety of methods are envisaged and developed to comply the specific requirements. However, there are cases when some surfaces based on pNIPAm do not support cell adhesion even above the LCST, making them unsuitable as culture substrates. That is why the choice of surface fabrication methods is directly correlated to the ability to use the smart coating within the desired

application. Some of the most used techniques [5, 16–33] for obtaining smart bio-interfaces based on stimuli-responsive polymers within a large area of applications are listed below:

- Radiation cross-linking. By using this method, the polymer molecular mass is increased by linking the polymer chains under irradiation initialization. The irradiation produces free radicals which recombine forming the cross-links (depending on the polymer type and radiation dose). Graft polymerization can be initiated by high-energy irradiation such as γ-ray, swift heavy ions and electron beam (e-beam) [23].
- Atom transfer radical polymerization (ATRP) is another simple method to obtain well-defined and high functionalized (co)polymers. It allows a good control of polymers functionalities, topologies and compositions. pNIPAm surfaces obtained using surface-initiated atom transfer radical polymerization (ATRP) technique show strong adhesive properties and can be used for cell sheets for tissue engineering applications [24]. By combining the self-assembled monolayer of initiator and atom transfer radical polymerization (ATRP), relatively homogeneous polymer brush can be obtained [25].
- Electron-beam (e-beam) processing of polymers is also widely used for cross-linking of polymer chains in order to improve chemical properties [26–31]. The method implies two steps: first uniformly coating a surface with NIPAm monomer solution and irradiating it using 0.3-MGy EB [30] and second washing the non-grafted NIPAm monomers with deionized (DI) water. It was observed that cell adhesion depends on the grafting density of the pNIPAm layers obtained by e-beam (cell attachment/detachment significantly decreased for high grafting densities). Surfaces with optimized thicknesses of around 15–20 nm were used for temperature-controlled adhesion and detachment studies implying various cell lines. Meanwhile, it was observed that if the thickness is increase around 30 nm, grafted pNIPAm surfaces do not support cell adhesion at any temperature anymore.
- Plasma polymerization or plasma-deposited pNIPAm coatings provide a thermoresponsive surface that is covalently attached to a solid substrate with a good retention of the monomer integrity [30–32]. This method is based on gradually decreasing the plasma glow discharge for the deposition of the pNIPAm coating in the outer surface. The polymer retained its monomeric structure, with the preservation of the phase transition. Fourier transform infrared spectroscopy (FTIR) measurements revealed that the functional groups remained the same after plasma discharge. Cell culture studies as well as thermal detachment of the cell performed on pNIPAm surfaces proved successful, but no influence of the thickness of the films on cell was observed. The atmospheric plasma treatment followed by free radical graft copolymerization was used also for obtaining pNIPAm surfaces onto nylon. The atmospheric plasma exhibits the activation capability to initiate graft copolymerization [30–32].
- Other methods for the modification of large surface area with thin polymer films include solvent casting, dip, spin, or spray coating [30, 33, 34]. These methods have been employed to create bulk pNIPAm films which are not grafted to a substrate, but conjugated with some bioactive agents and deposited on a solid support such as tissue culture-grade polystyrene (TCPS) or glass.

- Another method of producing thermoresponsive surfaces involves using UV light irradiation to produce cross-linked surface layer. Moreover, this method can be used for engineering micropatterned surfaces with thermoresponsive regions. The first step involves using photolithography followed by the solvent washing from the shadowed regions [30, 35–37]. By using this approach, cells can be selectively detached from the grafted regions providing a good spatial control over detachment of adhered cells and therefore with high potential in the spatial distribution of different cell types in coculture systems.

2.1. The limitation of the current approaches and laser method perspectives

Most of the methods previously described use the hydrophobic-to-hydrophilic switch to recover cell sheets, instead of disintegration of the coating, present just in the case of solvent casting or spin coating. Although most of the methods ensure that the coating is not harvested with the cell sheet, keeping the cell construct free of unwanted soluble polymer after detachment, there are still several disadvantages such as high cost, the lack of flexibility in controlling the surface density, morphology or thickness of the films, adhesion onto substrate and even the abnormal cellular activity due to physiological alteration within cell microenvironment. Especially for applications such as cell sheets engineering or drug delivery, the efficiency of attachment/detachment of certain cell types or drugs is varied to the thickness and density of grafted pNIPAm. Thus, developing of simple strategy, allowing a rapid recovery of cell sheet transfer with the cell-extracellular matrix (ECM) intact but also which would allow a controlled release of active compounds, is desirable.

Nevertheless, laser methods proved to be viable solutions in the last decades, for processing various and multiple material surfaces. Due to the laser unique ability for surface heating, providing that the applied energy to be placed precisely on the needed area, laser can be used as a unique tool for surface engineering not only in research but also in industry and medicine [38, 39]. The distinct advantages compared to alternative methods of processing the materials can be summarized as follows:

- Versatility, as it can be applied to a wide range of organic and inorganic materials/biomaterials.
- Controlled thermal penetration allowing features high-resolution characteristics, up to tens of nanometers in size, speed, adaptability and scalability through parallel processing.
- Controlled thermal profile, shape and location of heat, therefore providing flexibility in making topographical features defined on a wide variety of biomaterials with no affected region in a noncontact way.
- Flexibility in the selection of parameters that can allow handling sensitive biological and living cells without losing their activity.

The most commonly used techniques for thin films or coatings at this moment are the laser-based methods of laser-assisted chemical vapor deposition (LCVD) and pulsed-laser deposition (PLD) [39]. LCVD is characterized by localized heating produced by a laser beam on a substrate. This technique is traditionally used to directly deposit complex geometries of

different materials, including metals and ceramics. PLD has been applied to a wide range of materials such as semiconductors, metals and alloys, presenting numerous advantages: the ability to control the thickness of the monolayer, good adhesion of film-substrate material consumption low and low temperature substrate.

These methods, however, are not suitable for the deposition of large and sensitive molecules, such as polymers and biomaterials as molecular bonds in the polymers can be destroyed by the laser energy pulse. There are few exceptions, the polymer, such as Teflon (PTFE), polymethylmethacrylate (PMMA), polypernaphthalene (PPN), or polyisobutylene (PIB) which were successfully deposited as coatings by using PLD [40, 41]. However, as most of the organic materials are damaged by the direct interaction of the laser beam, that is why, in order to avoid damage and photochemical decomposition of materials caused by PLD method, a new technique based on laser evaporation (matrix-assisted pulsed-laser evaporation—MAPLE) was introduced in the end of the 1990s at the Naval Research Laboratory. It provides multiple advantages for the area of biosensors, active multifunctional bio-coatings and multilayered coatings where the organic and sensitive materials are required to maintain their structure and functionality. By providing a much "softer" transfer by pulsed-laser evaporation, the degradation or the thermal decomposition of the materials is avoided.

An extension of MAPLE is resonant infrared matrix-assisted pulsed-laser evaporation (RIR-MAPLE) using free electron lasers or Er:YAG lasers, aiming to control and reduce the degradation of the materials during deposition process. The advantage of RIR-MAPLE method is based on using infrared radiation in order to excite specific molecular vibrational bond stretches in the host material.

MAPLE technique [41–52] brings us a step forward to laser method by avoiding damage and photochemical decomposition of materials and it is now used for obtaining different types of thin films such as follows:

- Fibronectin thin films were transferred stable and functional onto silica substrate for studies on human osteoprogenitor cells [49];
- Complex polymer or organic molecules (functionalized polysiloxanes and carbohydrates) were deposited on different substrates chemical sensor applications [40, 42];
- Light proteins thin films (such as lysozyme and myoglobin) with possible applications in drugs industry [50];
- Collagen thin films deposited on Si substrates with high structural fidelity as well as good uniformity and surface smoothness [51];
- Biodegradable hybrid polymeric thin films without or with embedded antitumoral agents (lactoferrin, cisplatin, or their combination with a biodegradable polycaprolactone) which can act as a hybrid platform with increased antitumor efficiency [43, 44];
- Polymer-inorganic nano-composite films with fluorescence properties with a potential for applications such as light emitters and chemical sensors [45];

- Composite thin films of calcium alendronate monohydrate, octacalcium phosphate as well as CaAl-H_2O/OCP on different titanium substrates with applications in implant coatings [46];
- Lipase enzyme for applications in enzymatic production and biosensors [47].

3. MAPLE techniques for smart pNIPAm thin-film engineering

Within the above discussed context, as most of the implied or required applications within bioengineering field require controlled bio-coating and sterile condition, laser-based techniques (i.e., matrix-assisted pulsed-laser evaporation—MAPLE) are contact-free technique and also easy to be integrated with required sterile processes.

Moreover, by controlling laser and target parameters in MAPLE process, an overall control over the thickness, porosity and architecture of mono- or multilayer can be easily achieved with no limitations in the use of materials to be deposited or structured, the ability to deposit multilayers without interlayer blending, as well as flexibility in engineering a wide range of simple or hybrid materials (nanoparticles, polymers, ceramics and biological compounds).

Although MAPLE technique was successfully used for a variety of biomaterials, there are only two groups that used recently the laser evaporation for engineering either simple pNIPAm coatings for mammalian cells attachment and detachment studies [5] or a mixed antimicrobial oligo (p-phenylene-ethynylene) (OPE)-PNIPAAm) coatings with biocidal activity, which provides also on-demand bacteria-releasing functionality [39]. In the last example, the multifunctional films of OPE-PNIPAAm with tailored chemical composition were deposited on substrates by using a sequential co-deposition mode, namely resonant infrared matrix-assisted pulsed laser evaporation (RIR-MAPLE) [39].

3.1. MAPLE system description for pNIPAm coatings deposition

The MAPLE process takes place in a vacuum chamber and it implies the use of an external laser source (UV, excimer, IR), a solid cryogenic target and a receiving substrate. In an ideal system for the MAPLE process, the material to be deposited (guest material) is mixed or suspended into a solvent (host matrix), rapidly frozen in a copper holder using liquid nitrogen and placed inside the vacuum chamber. During the deposition procedure, in order to avoid target melting problem during laser irradiation, the copper holder has to be continuously kept at low temperature by using a cooling system specifically designed for the transfer and circulation of liquid nitrogen inside the deposition chamber. Another step taken in order to avoid the melting was the rotation of the target holder, which avoids local overheating of the target as well as drilling of the copper holder due to multiple laser pulses. The material is ejected from the solid cryogenic target due to the laser energy absorbed into the target, followed by the evaporation of the host matrix solvent which transport and deposit the guest material on the substrate positioned above the target. The substrates are placed at a fixed distance of 3–4 cm from the target matrix. The background pressure ($1–4 \times 10^{-3}$ Pa) in the vacuum chamber

is maintained by using a turbomolecular pump (i.e., Pfeiffer-Balzers TPU 170). A simplified MAPLE setup is presented in **Figure 1**.

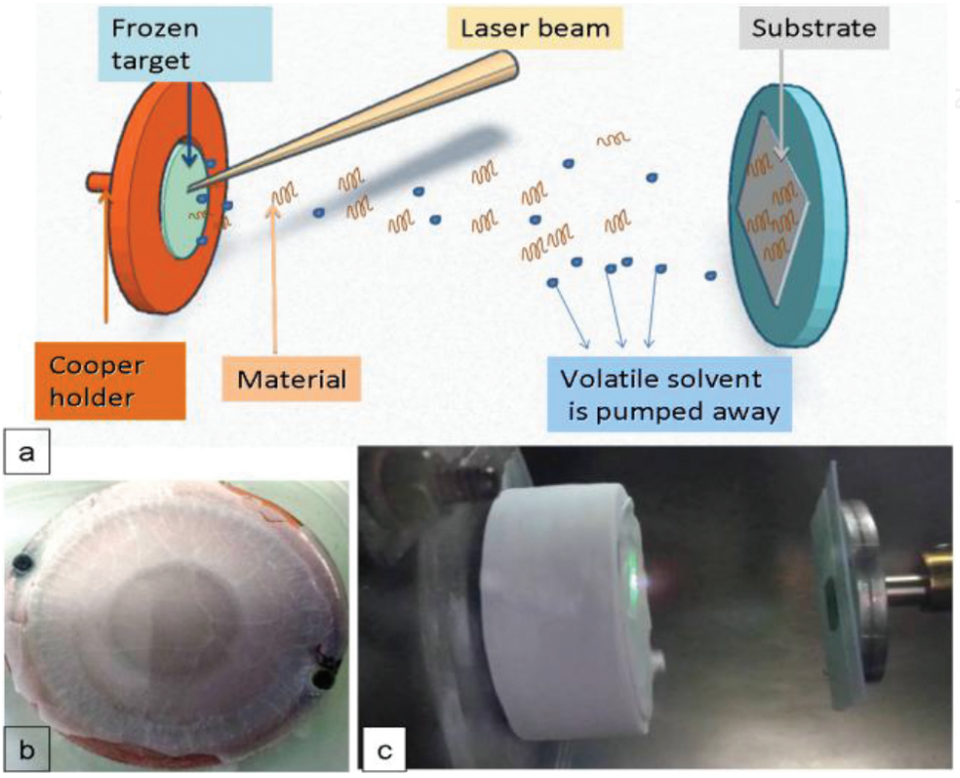


Figure 1. Schematic representation of MAPLE setup (a), image of the target system containing the frozen pNIPAm solutions and (b) inside chamber view during the deposition process (c).

3.2. Structural and morphological characteristics of the smart bio-interfaces engineered by laser methods

3.2.1. The structural characteristics of the pNIPAm coatings deposited by MAPLE

The characteristic vibrations of functional groups in the deposited pNIPAm thin films were subsequently analyzed and compared with a reference, that is, those obtained by drop-cast method by using Fourier transform infrared spectroscopy (FTIR). Fourier transform infrared spectroscopy is a technique for nondestructive chemical analysis used for characterizing thin films, allowing the identification of organic and inorganic chemical compounds. This technique is based on the chemical properties of the different groups to have specific resonance frequencies in the IR range of the electromagnetic spectrum (absorption bands presented/transmission). By comparing the film spectrum subjected to the action of the laser radiation to the spectrum of the control film, obtained by evaporation in air of a drop of solution from which was made the target, it can be determined whether the transfer was carried out with keeping its chemical composition.

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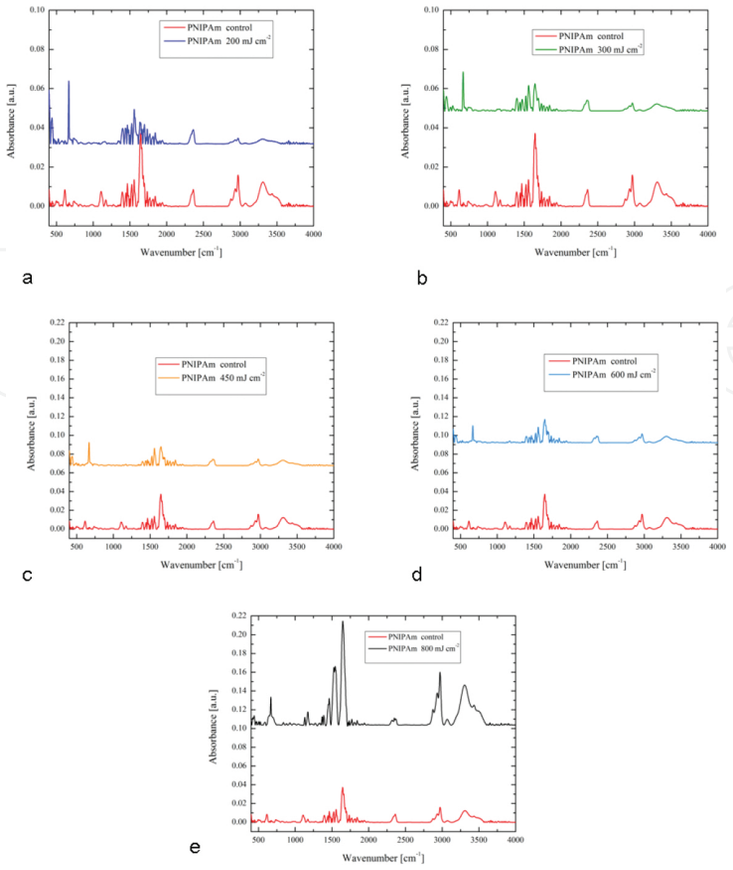


Figure 2. FTIR spectra for the coatings obtained with the following laser fluences: (a) 200, (b) 300, (c) 450, (d) 600 and (e) 800 mJcm^{-2}. A Jasco FT/IR-6300 spectrometer was used in transmission mode by accumulation of 1024 scans in the spectral range from 400 to 4000 cm^{-1}, with a resolution of 4 cm^{-1} [5]. The characteristic vibrations of functional groups in the deposited pNIPAm thin films obtained at different laser fluences were subsequently analyzed and compared with control thin films obtained by drop cast revealing the structural integrity of our samples.

As seen in **Figure 2a–e**, FTIR spectra confirmed the functionality of the polymer thin films obtained by MAPLE. At all laser fluences used, specific bonds corresponding to secondary amide C=O stretching of amide I bond at 1650 cm^{-1} and, respectively, to N–H stretching of amide II bond at 1550 cm^{-1} were observed on the thin films when compared with the control films [5]. The differences in FTIR spectral between these two bonds are correlated with the laser fluence used during MAPLE experiments. The absorption bands, which can be observed at 1368 and 1388cm^{-1}, correspond to the presence of isopropyl methyl (–CH(CH_3)$_2$)) deformation bands in the thin films. It can be observed also, in the high wave-number region, the presence of secondary amide N–H stretching around 3308 and 3437 cm^{-1} which are associated to free N–H stretching [5]. Specific vibrations of CH_2 and CH_3 radicals in the polymeric thin films are evidenced through the superposition of CH_2-scissoring and CH_3-rocking vibrations around 1465 cm^{-1}, as well as the symmetric- and asymmetric-stretching modes of CH_3 at 2873 and 2971 cm^{-1}, respectively and to the asymmetric-stretching vibration of CH_2 at 2934 cm^{-1} [5]. The appearance of a sharp positioned at 3067 cm^{-1}, especially at 600 and 800 mJ cm^{-2}, was observed, which corresponds to the solvent chemical composition. A possible explanation is that the pNIPAm thin films obtained by MAPLE contain chloroform due to the fact that the

solvent could not be totally evaporated and absorbed outside the vacuum chamber because of the rapid transfer of the target material to the substrates. For possible biomedical applications, the presence of the chloroform could impede the cell development/adhesion.

3.2.2. The morphologic characteristics of the MAPLE-deposited coatings

The coating characteristics in the case of MAPLE are related to wavelength, pulse duration, repetition rate, solvent absorption, target composition and percentage (preferably under 5–10% in mass). For example, using same number of pulses (Nd: YAG, 266 nm, 72-k pulses) but different laser fluences, pNIPAm coating with different arrangement of pNIPAm nanoparticles within the deposited coatings were obtained: uniform and low roughness (below 20 nm) coatings obtained for fluence value of 250 mJcm^{-2}, (**Figure 3a**), and highly rough coatings obtained for fluence value of 600 mJ cm^{-2} (**Figure 3b**). The obtained features and the film's surface roughness were determined by atomic force microscopy (AFM) measurements performed in air in noncontact mode.

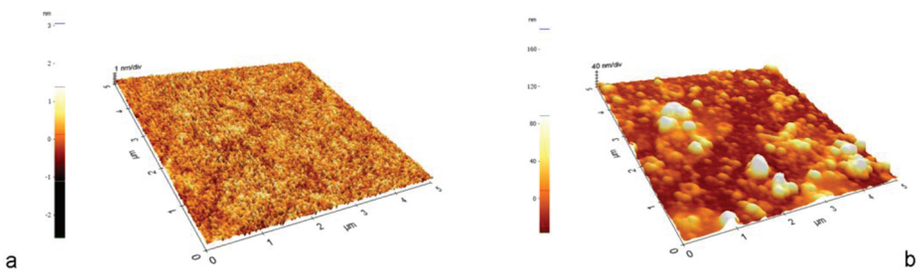


Figure 3. AFM images of pNIPAm thin films morphological characteristics obtained with 250 and 600 mJcm^{-2}. Scanning of the sample was carried out with a sharp tip with a diameter of about 100 Å and a length of the order of microns, positioned at the end of a cantilever having a length of 200 μm. The device used was the type XE100 AFM Park Systems, with maximum vertical movement of 8 μm, lateral resolution of tens of nanometers and optical resolution of 1 μm.

The surface of the samples can be tuned to exhibit special topography features on the surface from smooth, with roughness below 25 nm, to grain-like structures or porous surfaces, with roughness in the range of 110–150 nm [5] by changing either the fluences used or the target composition. It was shown that the surface roughness can impact both protein adsorption and desorption but also cell detachment process and time [5].

3.2.3. Thermoresponsive character of pNIPAm coatings obtained by MAPLE

In order to assess the behavior of the deposited materials upon hydration and heating, the step height was observed at 37°C water and in room temperature (RT) water (**Figure 4**). The decrease in temperature in water leads to an increase in coating thickness of 28 nm. The change in step height confirmed the stimuli-responsive character of the MAPLE-deposited pNIPAm coatings due to the change in temperature.

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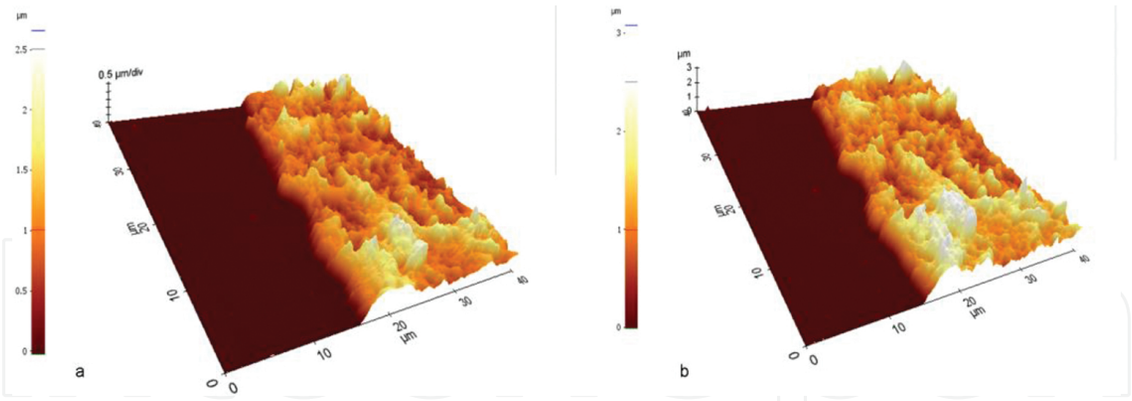


Figure 4. AFM images of pNIPAm samples measured in liquid at room temperature (RT) (a) and 37°C (b). Each sample was hydrated for ~60 min before measuring the step-height variation due to temperature change.

A change in the contact angle value from 47° (measured at 37°C) to 30° (measured at 21°C) by applying temperature as external stimuli confirmed the thermoresponsive character of the pNIPAm coatings (obtained with Nd:YAG laser, 266 nm, 72-k pulses, 10 Hz, 250 mJcm^{-2}).

An explanation for the above observations could be given by the fact that in a dehydrated state, the hydrophobic groups from the polymer surface are oriented outward to maximize hydrogen bonding underneath the surface [53], while in the aqueous environment at room temperature, the hydrophobic isopropyl groups appear to bend inward. In this way, the hydrogen bonding of the polar amide groups with water is facilitated to lower surface energy. Moreover, increasing temperature, there takes place a rearrangement of the surface based on tuning the intramolecular hydrogen bonds under the surface and freeing the bound water molecules [53].

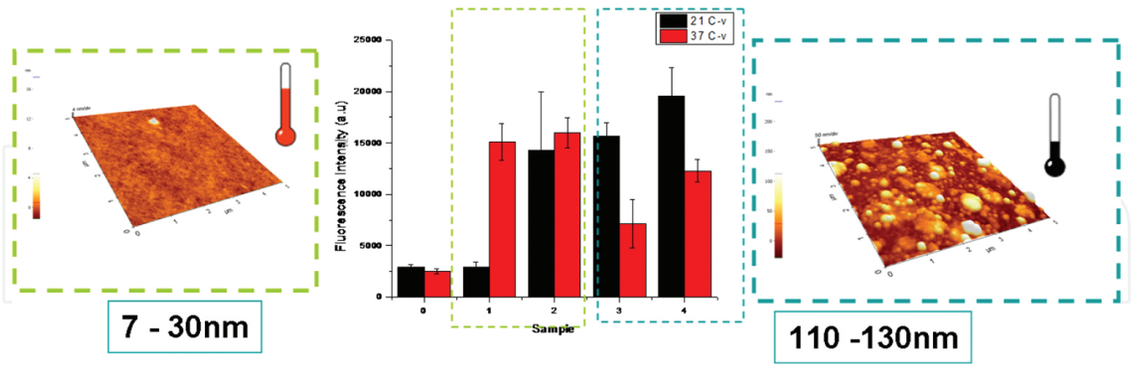


Figure 5. Protein adsorption studies on the coatings obtained by MAPLE using fluorescein isothiocyanate (FITC)-labeled BSA in PBS. The total fluorescence was determined using the built-in FITC filter of the microscope, with a constant exposure time, magnification and image area for all the surfaces. Duplicate images of each coating before and after incubation with BSA were recorded using a CCD camera (ANDOR iXon DU897 E-CSO-UVB) and Olympus IX71 microscope. The total fluorescence from BSA was calculated by extracting the background fluorescence for each coating type (zero, one and two samples with roughness in the range of 7–30 nm and three and four samples with roughness in the range of 110–130 nm). The left side of the image represents an example of AFM image of low roughness surface (7–30 nm) while the right side presents an example of AFM image of pNIPAm coatings with higher roughness (110–130 nm).

However, both protein adsorption and cell reversible attachment are correlated to pNIPAm density, grafting, or conformation onto a substrate [54]. In the case of pNIPAm coatings obtained by MAPLE, a model protein (bovine serum albumin (BSA)) was used to probe the changes in the interfacial properties coatings and two trends depending on roughness surfaces were observed. Negligible protein adsorption at 21°C was noticed for low roughness surfaces (samples 0–7 nm, 1–20 nm and 2 with roughness up to 30 nm) and increase of protein on denser and rougher pNIPAm coatings (samples 2–110 nm and 3 with roughness up to 130 nm) (**Figure 5**).

However, the changes in the pNIPAm surface properties (roughness) affected protein adsorption, with lower protein adsorption on dense and rough coatings at 37°C and higher adsorption on low roughness surfaces, which is in contradiction with the measurements reported on pNIPAm obtained by other methods [54]. This discrepancy could be explained by the swelling ratio which decreases with increasing material density, as the chains in more densely packed coatings swell less upon full hydration. When compared to the modification of the heights of the coatings under temperature influence (about 24 nm) to the protein dimensions (length 14 nm, height and width 4 nm), it can be assumed that the lowest density is insufficient for exceeding primary adsorption and the proteins are able to penetrate the rough coatings and they would adsorb to the underlying surface. Nevertheless, the thin-film irregularities or roughnesses were shown to be important factors in influencing the mobility of adsorbed proteins [5].

4. Cell interaction and answer to pNIPAm-based interfaces obtained by MAPLE

Cell interaction and answer to pNIPAm substrates/surfaces are determined, besides the chemical characteristics, also by the thickness and surface morphology. The porosity and uniformity of the thin films as well as low roughness plays an important role in the cell adhesion/detachment behavior. The thickness of the films has an important role in the temperature-induced alterations of the films (changing from hydrophilic to hydrophobic properties [5–7, 28–34]. The cell culture-based studies (phase-contrast microscopy, fluorescence microscopy and MTT assays) have proved a good biocompatibility and a cell behavior correlated with the chemical composition of biomaterial substrates in the case of pNIPAm coatings obtained by MAPLE.

4.1. Mammalian cells adhesion and morphology on pNIPAm coatings obtained by MAPLE

A first observation of the effect of pNIPAm coatings onto the behavior of L929 and human mesenchymal stem cells (hMSC) is given in **Figure 6**, where cells adhesion and morphology were analyzed by phase-contrast microscopy, showing that cells spread on the coatings surfaces without shape alteration.

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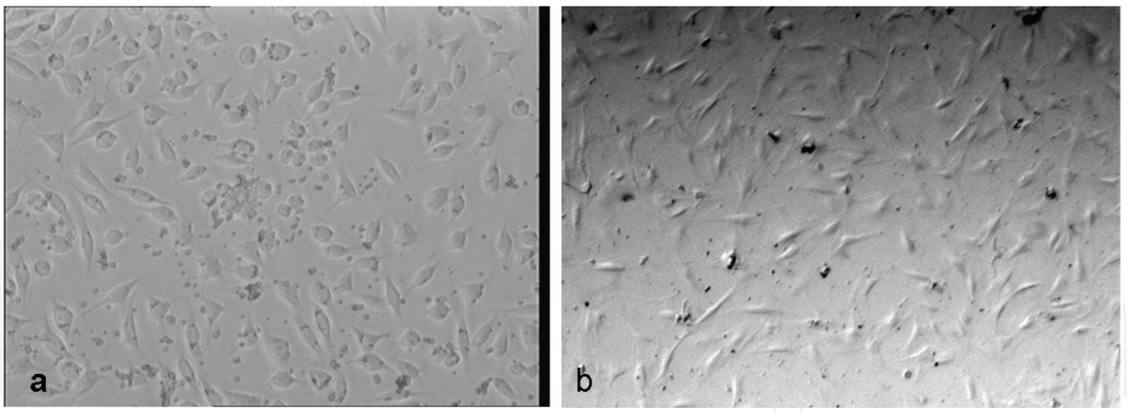


Figure 6. The attachment and morphology of the L929 cells (a) and hMSC cells (b) on pNIPAm coatings observed after 72 h of culture (using SteREO DiscoveryV20 microscope, with PlanApo S 1.0× objective, 32.5× magnification with an AxioCamICm1 camera attached to the microscope setup). L929 (mouse fibroblast) cells and human mesenchymal stem cells (hMSC) were cultured in Dulbecco's minimal essential medium (DMEM) containing 4-mM L-glutamine (Gibco) and supplemented with 10% fatal calf serum (FBS) and 50-U/ml penicillin/streptomycin (50 mg/ml) and maintained at 37°C with 5% CO_2.

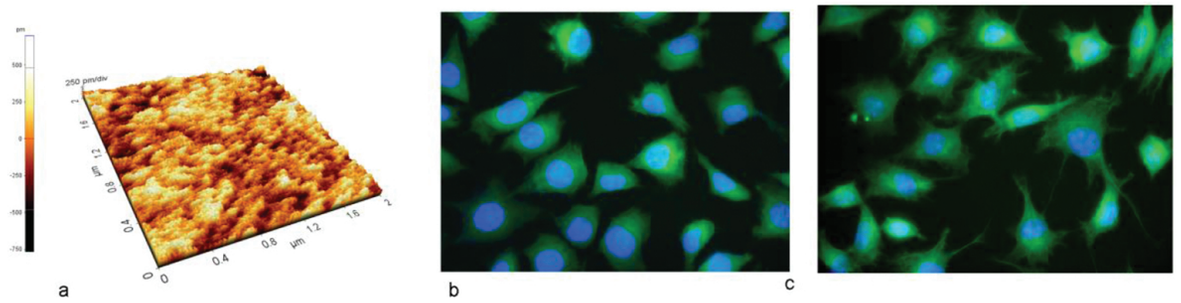


Figure 7. AFM image of pNIPAm coating obtained for a fluence of 400 $mJcm^{-2}$ (a) and (b) fluorescence microscopy image of L929 cells stained with Hoechst (Sigma) nucleus—blue and fluorescein phalloidin (ThermoFisher) green-actin fibers on pNIPAm samples and on controls (c).

The above observations were confirmed by the fluorescence microscopy analysis. The initial cell attachment, spreading and morphological features of fibroblasts cells were assessed and correlated to surface characteristics.

As fibroblasts are ubiquitous in the body and are the first to populate prostheses surface (implants), the morphology of the cells (seeded at a density of 5000 cells/100 μl) for 24 h can give a first information on how and if pNIPAm coating obtained by MAPLE can support cell adherence and if the confluence on highly dense coatings is possible.

As shown by florescence images in **Figure 7**, L929 cells adhere and spread uniformly onto the laser-engineered pNIPAm surfaces and start projecting long dendrites along the surface area. These results are confirmed by scanning electron microscopy (SEM) analysis of cells cultured onto the pNIPAm coatings for 24 h (**Figure 8**).

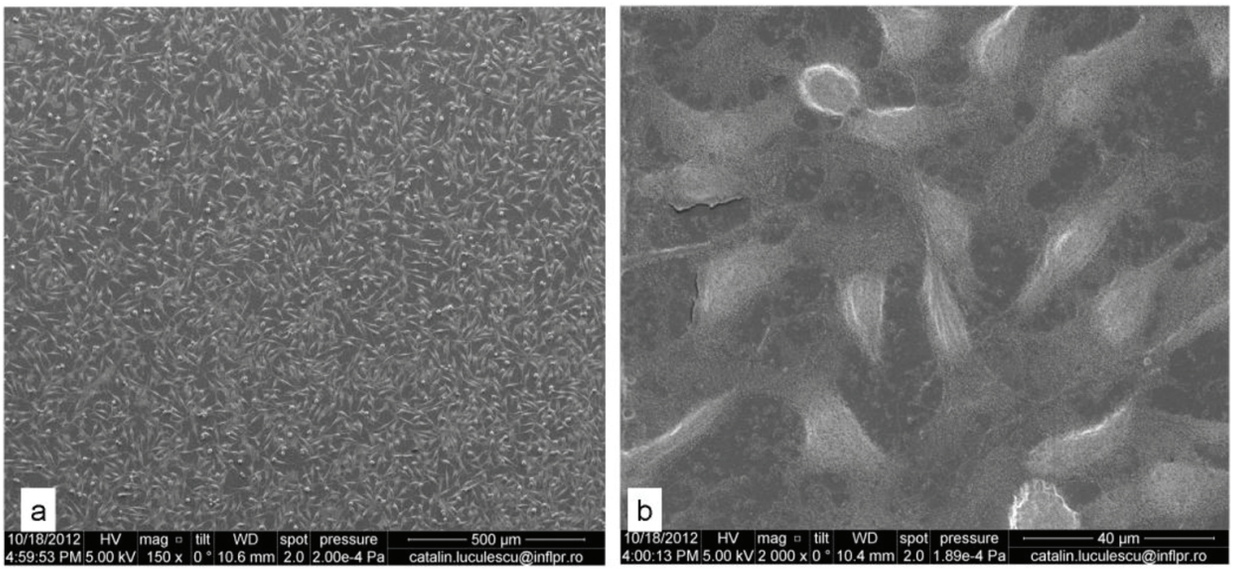


Figure 8. SEM images of the cells seeded on pNIPAm materials for 24 h: overview on the cells distributions over large area (a) and a close-up on the shape of the adhered L929 cells (b). For preparation of the samples for SEM analysis, the samples were washed with PBS and fixed with 2.5% glutaraldehyde in PBS for 20 min and subjected for ethanol gradient dehydration.

Nevertheless, the spreading of the cells and adherence can be inhibited by either surface chemistry or surface morphology [5]. The viability of the fibroblast cells after 3 days of culture on the pNIPAM coatings was calculated showing similar proliferation rate for the coatings obtained in the range of 200–600 mJcm^{-2}. A cytotoxic effect and a decrease in proliferation rate in the case of the coatings obtained for higher fluences [5] were observed.

4.2. Cell harvesting based on temperature change

A recent study by Rusen et al. [5] on the cell detachment upon temperature reduction showed that the roughness of the thin films represents a significant parameter in tuning the detachment time. The quantitative analysis on the fibroblast cell harvesting upon temperature reduction showed that if cells detached from rougher films within 10–20 min, the detachment time on smooth surfaces increased with more than 25%.

The sequence of images presenting an example of the cell detachment from a pNIPAm thin film obtained by MAPLE is shown in **Figure 9**. The cells presented flat, spread, normal morphology at the beginning of the observations (**Figure 9a**), when the temperature was 37°C. However, by lowering the temperature the cells morphology started to change, becoming less spread and rounder (**Figure 9b–d**). When compared with previously reported data on cell detachment from pNIPAm coatings obtained by MAPLE [5], the detachment time is increased due to the low roughness (20 nm) pNIPAm surfaces used in this study. Moreover, the cells collected from pNIPAm coating obtained by MAPLE did not change their shape or viability. This represent a strong indication that a nondestructive reversible cell detachment takes place when using pNIPAm bio-interfaces obtained by MAPLE [5].

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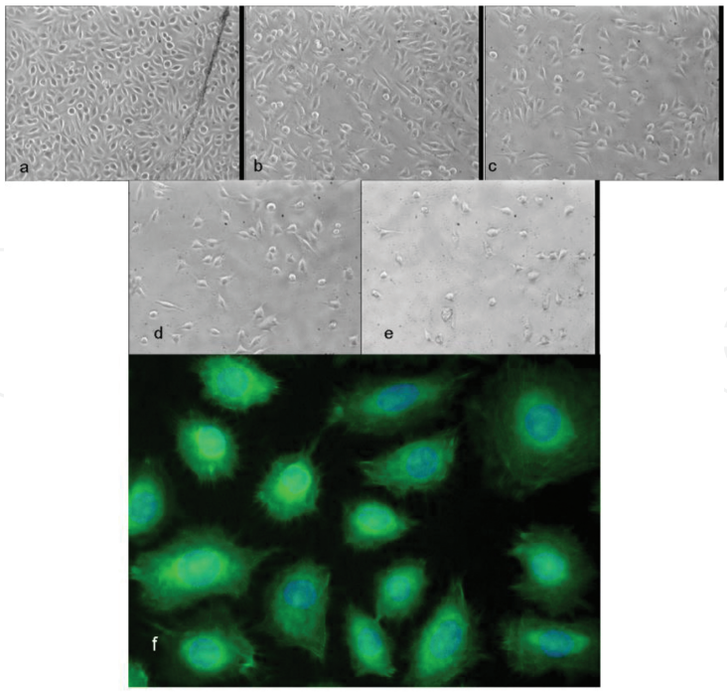


Figure 9. Cell detachment sequence images at 0 min (a), 5 min (b), 10 min (c), 15 min (d), 20 min (e) from pNIPAm coatings obtained by MAPLE at 250 mJ cm^{-2}. (f) Fluorescence microscopy image of L929 cells grew on other pNIPAm surfaces, indicating no damage to either the structural or functional aspects of the cells recovered from pNIPAm samples.

4.3. Multifunctional pNIPAm-based surfaces with biocidal and releasing properties obtained by MAPLE

Based on the same principle, resonant infrared matrix-assisted pulsed-laser evaporation (RIR-MAPLE) was used to co-deposit two components OPE/pNIPAm films with precise ratio control [39]. The sequential co-deposition mode allows depositions of a multifunctional coating comprising OPE and PNIPAm, used to kill bacteria and then release the accumulated dead bacteria from the film surface [39]. If laser fluence was previously used to change surface morphology [5], in this case, both surface morphology and wettability were adjusted by changing the OPE/PNIPAAm volume ratio in emulsion target used in the sequential deposition mode in RIR-MAPLE.

The biocidal activity and bacterial-releasing ability of the multifunctional films implied the use of both Gram-negative and Gram-positive bacteria strains such as *Escherichia coli* K12, respectively, *Staphylococcus epidermidis*. An inverse relationship between the biocidal activity and capability for bacterial release based on OPE content was observed. In this way, by increasing the quantity of OPE within the coatings, the resultant films showed increased biocidal activity but lower bacterial release capability. By optimizing the OPE/PNIPAAm ratio, coatings with enhanced biocidal activity against attached bacteria as well as good-removal capacity for the debris and bacteria when rinsing the films at a temperature below the LCST [39] were observed.

5. Conclusions and future prospects

In this chapter, the new and rapidly emerging importance of smart-coating engineering was introduced, with focused attention on smart thermoresponsive pNIPAm interfaces obtained by matrix-assisted laser evaporation-based method. In this chapter, the thermoresponsive-coatings characteristics obtained by MAPLE-based method were emphasized along with some of the deposition parameters and used for biological assays *in vitro* implying BSA model proteins, mammalian cells and microorganisms.

MAPLE as technique for obtaining smart polymeric coatings with specific characteristics envisaging biological application provides the advantage of tailoring not only the thickness of the pNIPAm layer, which is an important parameter in the cell attachment, but also the morphology of the deposited thin films for influencing protein and cells detachment and its increased stability in the fluid medium. Although significant progress has been achieved in the field of smart coatings based on stimuli-responsive materials, the materials and methods discussed within this chapter still have limitations in practical applications that need to be overcome in the future.

Although the majority of the previous works in this field have used insoluble pNIPAm-based coatings, future research should be more directed toward biomimetic bio-interfaces, with integrated analysis platforms able to address the complexity of bio-environments accordingly to the desired application.

Considering the abovementioned advantages of the MAPLE method on tuning not only the surface characteristics and properties but also the chemical composition and film functionality, this approach could provide a new strategy to engineer multifunctional films for biological studies, regenerative medicine, tissue engineering and industrial applications.

Conflict of interest

The authors declare no competing interests.

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