



## Design of Smart Surface by Nanometric Grafting of NIPAAm with Benzophenone Initiator under UV Radiation

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### ABSTRACT

The UV radiation method is a suitable method for grafting processes. Poly-N-isopropylacrylamide was successfully grafted onto a polystyrene surface with benzophenone initiator under UV radiation. The ATR-FTIR analysis showed the existence of the graft poly-N-isopropylacrylamide (PNIPAAm) on the surface by this method. The scanning electron microscopy and atomic force microscopy (AFM) images clearly showed suitable grafting of the PNIPAAm on the PS surface. Surface topography and graft thickness in AFM images of the grafted samples showed that the thickness of these grafts was about 300-600 nm. The drop water contact angle of the grafted sample at 37°C and 4°C was 48° and 63° respectively, which showed the hydrophilicity and hydrophobicity of the grafted surfaces. Thermo-responsive polymers were grafted to dishes covalently, which allowed epithelial cells to attach and proliferate at 37°C; the cells were also detached spontaneously without using enzymes when the temperature dropped below 4°C. Also MTT analysis showed a good viability of cells on the grafted samples. Such a characteristic proved that this type of grafted material had the potentiality as a biomaterial for cell sheet engineering.

**Key words:** Nanometric Grafting, benzophenone initiator, PNIPAAm, Polystyrene Film, UV Radiation.

### INTRODUCTION

Poly-N-Isopropylacrylamide and its copolymers in due to its high-speed phase transition from liquid to solid and being critical dissolution temperature of 32 °C can be used in the field of medical science as well as drug delivery system and tissue engineering. At high temperatures of 32

°C, the material shows a solid and hydrophobic state; and at temperatures below 32 °C, the polymer shows fully hydrated and hydrophilic properties. PNIPAAm was synthesized in 1957 by Wooten.<sup>1</sup> With this smart polymer, surface modification of materials can be used in cell sheet engineering.<sup>2,5</sup> Different methods for surface modification of polymers like polyethylene, polypropylene,

polystyrene and polyethylene terephthalate with PNIPAAm grafting with chemical, physical methods such as gamma-ray exposure, plasma, ozone and ultraviolet and electron beam is used, each has advantages and disadvantages.<sup>6,9</sup> In all such methods, a radical is created on the surface; and during the collision with monomer, the polymerization process occurs.

Ultraviolet irradiation method in terms of simplicity and low cost as good as possibility of developing is a suitable method for biomaterial surface modification.<sup>10,11</sup> Factors such as the distance of radiation, absorption intensity, the wavelength used appropriately to the initiator, thickness and usual factors such as degassing, substrate and initiators and sensitizers contributed in the ultraviolet radiation.<sup>12</sup> Of course, this method is required for optical sensitizers or initiators such as anthraquinone<sup>13</sup> or benzophenone.<sup>14</sup> Selected solvents used in spectroscopy and polymerization with ultraviolet radiation are very important. In this study, benzophenone initiator was used for grafting PNIPAAm on the polystyrene surface by UV radiation. Finally, a nanometric uniform thickness was obtained. Also, the nanometric thickness of grafting was shown to have an important effect in the process of adhesion and separation of cells and cell sheets. In this study, technical knowledge was also provided to achieve intelligent surfaces with nanometric thickness by using this type of radiation.

## EXPERIMENTAL

### Materials

Polystyrene dishes (orange color) with the dimension of 1×1 cm<sup>2</sup> and 1 mm thickness, ethanol and methanol (Merck), NIPAAm (Aldrich), n-hexane (Merck), distilled water and tissue culture polystyrene (TCPS), and epithelial cells (pastor institute, Iran) were used in this study. Polystyrene dishes were put in the solution of ethanol-methanol with a 50/50 ratio for 24 hours to dissolve impurities and the oils existing on the surface of the dishes. After bringing out the dishes from the solution, they were washed by distilled water. For recrystallization of NIPAAm, 10.3 grams of NIPAAm (Aldrich Co) were dissolved in 125 ml n-hexane and the solution was inserted into a refrigerator to make the NIPAAm ready for grafting.

### Sample preparation

The NIPAAm monomers were dissolved in water solvent (30%W) with to constant ratio of benzophenone initiator (3% w/v) (Fluka co). The samples were degassed by nitrogen (2bar mass flow rate) for 30 minutes. This process was followed to increase the efficiency of the free radical polymerization (deoxygenating). Then, the solutions were poured in a plastic washer (diameter=1cm and height=3 mm) that was attached to the polystyrene substrate. The samples with solution were exposed by a UV radiation source (black light, 160 W, 365 nm: Philips) for 30 min. Irradiations were carried out in the air under ambient conditions; then, the samples were brought out and washed by distilled water and were put in distilled water for 72 hours and Soxhleted for removing the ungrafted monomer, then were taken out for analysis.

### Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR)

The samples were examined by ATR-FTIR (Thermo Nicolet, nexus 870 ft-sr-USA) before and after adjustment, and there were put under instrument to investigate.

### Surface Morphology Study

The surface characteristics of modified and unmodified films were studied with the help of SEM (Cambridge Stereo scan, model S-360) to analyze the changes in the surface morphology. The films were first coated with a gold layer (Joel fine coat, ion sputter for 2 hours) to provide surface conduction before their scanning. The surface topology characteristics and the thickness of modified as well as the unmodified films were studied with the help of AFM (TMX 2010 and the nano-surf easy scan 2 contact model) to analyze the changes in the surface topology.

### Contact Angle Analysis

Surface's static contact angle of the samples was investigated by the contact angle measuring device (Kruss G10) following the sessile drop method. The formed contact angle would be the angle between the solid/liquid and the liquid/steam joint surface. In order to review the sample surface's hydrophilic/hydrophobic behavior in high and low temperatures, a better sample was considered in two different temperatures of 4°C and

37°C and the contact angles were measured under these temperatures.

### Biocompatibility Study

Aliquots of cell suspension in the RPMI medium including 300,000 SW742 epithelial cells were seeded on a 6-multiwell cell culture plate (orange), which was pre-coated with samples. The plate was incubated in a incubator (37 °C, CO<sub>2</sub>) in 3 h for cell attachment, followed by rinsing off the loosely attached cells with the phosphate buffer solution, and by adding 2 ml of fresh medium because of the cell culture in an incubator (37 °C, CO<sub>2</sub>) for 7 days. The proliferation of cells was determined for measuring the viable cell number by MTT assay. The MTT tetrazolium compound was reduced by living cells into a colored formazan product that was soluble in a tissue culture medium. The quantity of formazan product was directly proportional to the number of viable cells in the culture. The assays were performed by adding 1 ml of MTT solution (Sigma) and 9 ml fresh medium to each well after aspirating the spent medium, and incubating at 37°C for 4 h with protection from light. The colorimetric measurement of formazan dyeing was performed at a wavelength of 570 nm using a microplate reader (RAYTO).

For cell detachment, SW742 cells were seeded onto the samples at the density of 1,000,000 cells, and were cultured at 37°C under a humidified atmosphere of 5% CO<sub>2</sub>. The cell detachment was evaluated by incubating the cultures at 4°C for up to 60 min. The culture medium that included the detached cells was transferred to a new well. The number of detached cells and the attached cells to the original well was determined by the MTT assay.

## RESULTS AND DISCUSSION

### ATR-FTIR Analysis

ATR-FTIR spectra results of the unmodified and the UV radiation modified polystyrene samples have been shown in Figure 1. The NIPAAm grafted by the UV radiated polystyrene ATR-FTIR spectrums have been shown below Figure 1B. The PNIPAAm picks characteristic includes 1601 cm<sup>-1</sup> which indicates –NH groups and 1730-1830 cm<sup>-1</sup> which indicates C=O groups and 3025 cm<sup>-1</sup> which indicates CH<sub>3</sub> groups and 3443

cm<sup>-1</sup> which indicates NH groups in PNIPAAm. All these picks are found in PNIPAAm grafted polystyrene samples. This conclusion shows grafting between the PNIPAAm with the polystyrene surface through UV radiation coating activation .

### Surface Morphology Study

The microscopic images for investigating the modified samples through UV radiation have been shown in figures 2 and 3. These images showed the PNIPAAm grafting on the polystyrene surface. Figure 2A is the obtained AFM image from the unmodified polystyrene samples. The surfaces topography and the created graft thickness on surface are shown in the AFM images. Figures 2B-D show the surface topography for the grafted sample in the different scales. The graft thickness average for the sample grafting was about 300-600 nm .

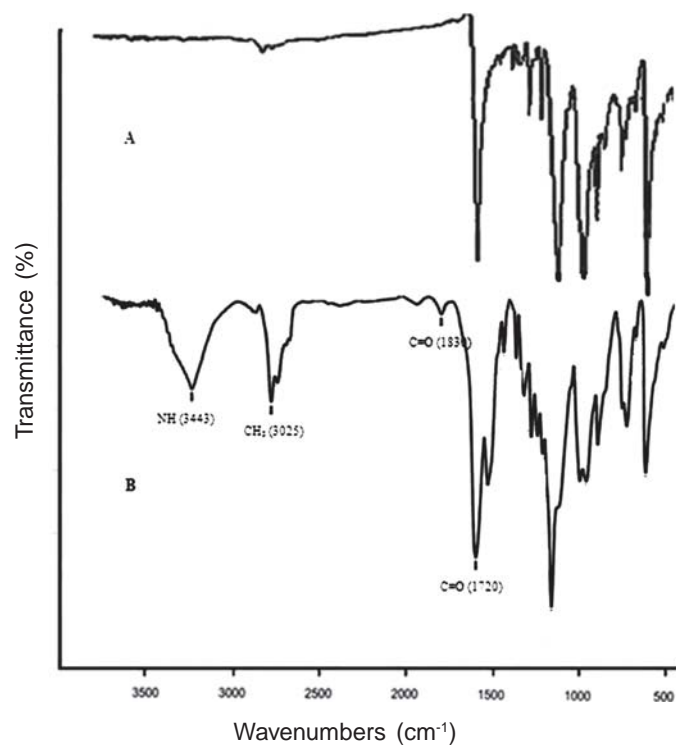
Figure 3 is the obtained SEM image from the unmodified and modified polystyrene samples. Figure 3A is the obtained SEM image from the unmodified polystyrene sample (1000 ×). Figures 3A and 3B are the obtained SEM images from the modified polystyrene samples at different magnification. The grafted surfaces with the PNIPAAm are shown in the SEM images

**Table 1: Contact angle for the unmodified and modified samples**

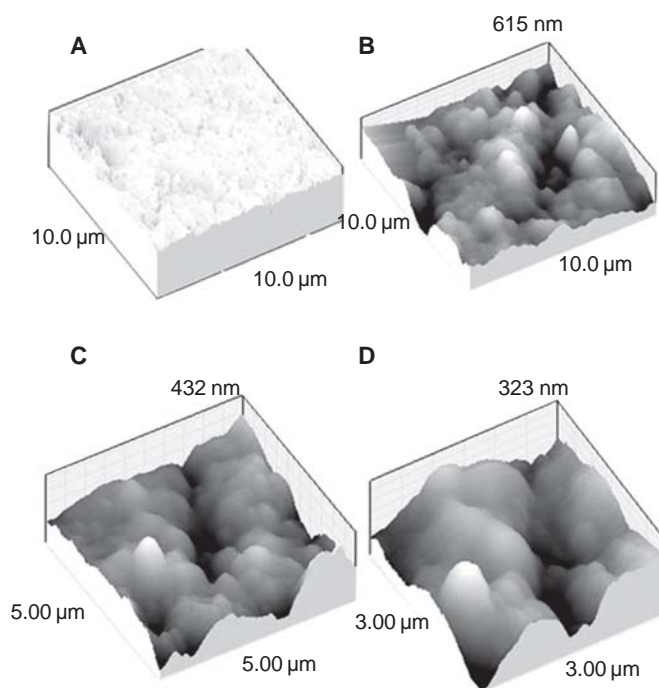
T(°C)	Normal $\theta_{H_2O}$	Grafted samples $\theta_{H_2O}$
25	90 ± 3.2°	60 ± 0.4°
37	90 ± 3.2°	63.2 ± 0.3°
4	90 ± 3.2°	48.5 ± 1.9°

**Table 2: MTT analysis of the samples**

Sample	Viability %(37°C)	Viability %(4°C)
TCPS	100	100
Grafted sample	80	68



**Fig.1.** ATR-FTIR spectra of the unmodified polystyrene (A).  
And spectra of the grafted polystyrene by UV ray (B)



**Fig.2.** Atomic force microscopy of grafted polystyrene under UV radiation .  
A. unmodified sample , B. grafted sample in the different scales:10×10, C:5×5, D:3×3μm

### Contact Angle Analysis

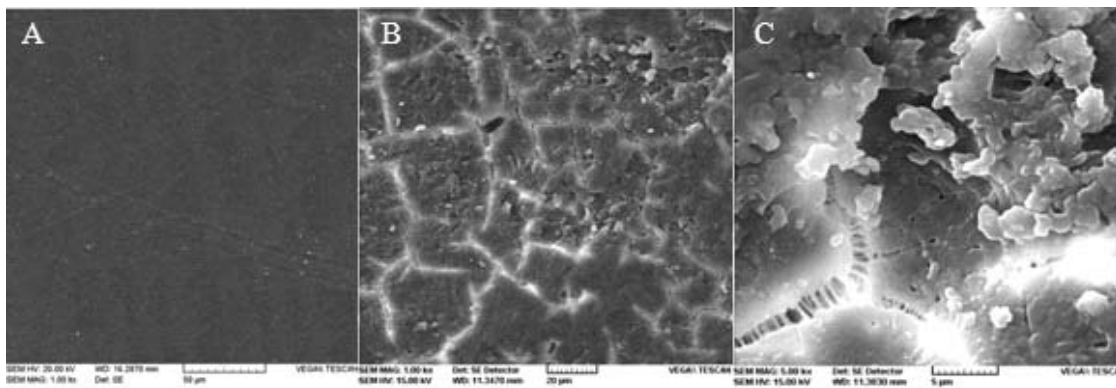
In the contact angle measurement process, the measured angle of normal polystyrene adjusted by UV radiated PNIPAAm surface samples in 4 °C and 37 °C temperatures has been shown in Table 1. The tabular data of the best grafted sample (with 40% of NIPAAm concentration resolved in the solvent of 9:1 (v/v) water/methanol ) indicate the fact that in 4°C and 37°C, the samples show different contact angles which is also another reason for the existence of PNIPAAm grafting onto polystyrene surfaces.

The contact angles average 48.5° and 63.2° have been calculated for 4°C and 37°C temperatures. The result indicated a contact angle decrease below the temperature of 32°C (4°C), and

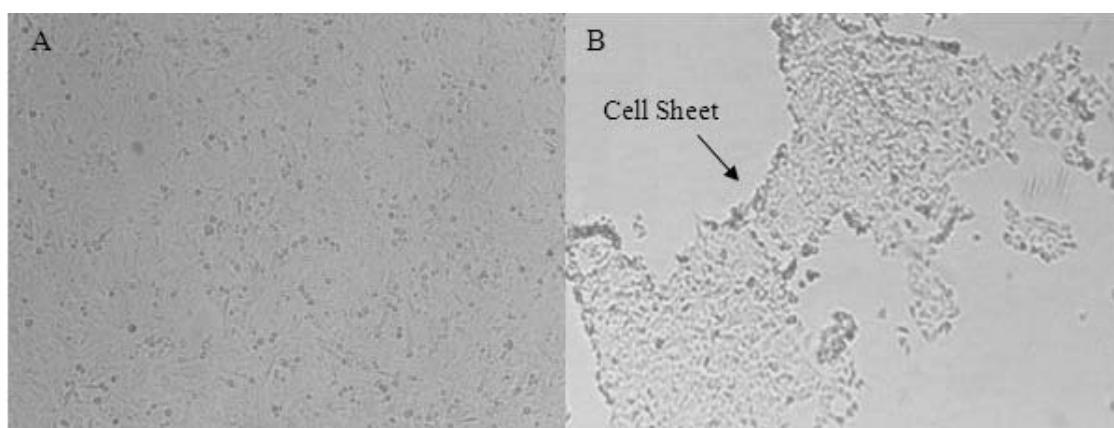
it showed the hydrophilic surface feature. The contact angle increased in above 32°C temperature (37°C) which also showed the hydrophobic surface feature.

### Biocompatibility Results

The biocompatibility data demonstrated that the grafted samples under UV radiation supported epithelial cell adhesion and proliferation; and the cells also maintained suitable viability (Table 2). After cultured for 7 days on grafted samples, many cells were alive, suggesting that the grafted samples were suitable for cell attachment and proliferation; and the viability was about 80%. When the cells were placed outside the incubator and the medium was cooled from 37°C to 4 °C, almost all of them were alive (the viability was about 70%) .



**Fig. 3: Scanning electron microscopy of grafted polystyrene under UV radiation. A. unmodified sample; B. grafted sample in the magnification 1000 x and C. 5000x**



**Fig. 4: The epithelial cells growth on the grafted Petri dish in 37°C (4A); and on fig.4B the Cells detached spontaneously when temperature decreased below 4°C on the grafted sample is showed**

Figure 4A shows a good cell growth on the grafted samples surface at the physiological temperature of 37°C. Figure 8b shows cells growth detached from the grafted samples surface spontaneously, in the absence of enzymes (trypsin/EDTA). Cell detachment efficiency from the grafted samples was high. In contrast, cells growth on the TCPS dishes did not show such temperature-dependent cell sheet detachment. After a longer period of cell cultivation for 7 days, confluent cells formed a continuous monolayer cell sheet on the surface of the grafted samples. The cell sheet was spontaneously detached from the surface of the thermo-reversible grafted samples when cooled to 4°C without treating by any enzymes. As shown (Figure 4B), the detachment of the cell monolayer created a cell monolayer. After 60 min incubation at 4°C, a monolayer cell sheet could be lifted up from the edge upon mild perturbation of the medium. A living cell sheet, detached from the culture surface, could be obtained within 60 min. Such results demonstrated that cold treatment effectively released the cell

sheet from the plate without considerable damage of the cell-cell connections

The polymer grafting on a polystyrene surface with chemical initiator under UV radiation was studied in this article. The ATR-FTIR spectrum showed the existence of the grafted polymer on polystyrene surfaces. The SEM images showed the grafted surfaces morphology in different magnifications; we could clearly observe and compare our graft increases. The topology of the surfaces shown in the AFM images also approved the claim. The graft thickness of the grafted samples in this study was about 300-600 nm. The contact angles 48° and 63° obtained in 4°C and 37°C temperatures. Thermo-responsive polymers were grafted to dishes covalently, which allowed the epithelial cells to attach and proliferate in 37°C. Also cells (cell sheet) were detached spontaneously when temperature decreased below 32°C, without using enzymes. Also MTT analysis showed good viability of grafted samples. This characteristic proved that such type of grafted materials had potential as biomaterials for cell sheet engineering.

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