



Effect of Plasma Radiation on Intelligent Surface Grafted to NIPAAm with Chemical 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. In this study, grafted polystyrene was exposed to microwave plasma treatment with oxygen gas. The ATR-FTIR analysis showed the existence of the graft poly-N-isopropylacrylamide (PNIPAAm) on the surface by this method also attenuated total reflection Fourier transform infrared spectra investigations of irradiated sample indicated clearly the presence of functional groups. Atomic force microscopic images of samples irradiated with active gas and grafted sample indicated nanometric surface topography. Sample irradiated with oxygen plasma showed more roughness compared with those unmodified samples. Surface roughness increased with increasing duration of exposure, which could be due to reduction of the contact angle of samples irradiated with oxygen plasma. Contact angle analysis showed reduction in samples irradiated with plasma. Samples irradiated with oxygen plasma showed a lower contact angle compared with those unmodified samples. 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 especially for modified sample with plasma. Such a characteristic proved that this type of grafted material had the potentiality as a biomaterial for cell sheet engineering.

Key words: Grafting, Surface modification, PNIPAAm, UV Radiation, Oxygen plasma.

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.¹

With this smart polymer, surface modification of materials can be used in cell sheet engineering.^{2,5} 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.^{6,9} 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^{10,11}. 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 radiation¹². Of course, this method is required for optical sensitizers or initiators such as anthraquinone¹³ or benzophenone.¹⁴ Selected solvents used in spectroscopy and polymerization with ultraviolet radiation are very import. Control of surface properties is very important for good adhesion. Biomaterial wettability is an important factor in the surface modification of materials. Surface modification of hydrophobic polymer surfaces can be achieved by wet (acid, alkali), dry (plasma), and radiation treatments (ultraviolet radiation and laser).¹⁵⁻¹⁷

Physical surface modification and its effects on wettability are an interesting field for surface engineers. It should be noted that there has been a lot of scientific work done on molecularly smooth or modeled 'simply rough' surfaces,¹⁸ but little work has been done on wettability and the spreading phenomena of real engineering surfaces.¹⁹

In this study, benzophenone initiator was used for grafting PNIPAAm on the polystyrene surface by UV radiation then this grafted polystyrene was exposed to microwave plasma treatment with oxygen gas. The samples analyzed and investigated with to cellular study.

EXPERIMENTAL

Materials

Polystyrene dishes (orange co) with the dimension of 1×1 cm² and 1 mm thickness, ethanol and methanol (Merck co), NIPAAm (Aldrich co), n-hexane (Merck co), 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.

Surface modification

We used a microwave plasma source to modify the surfaces of the grafted samples. Microwave sources can be operated at low pressures of 10⁻³ to 10⁻¹ millibars, which reduce the risk of gas phase contamination during processing.

Moreover, plasma properties can be controlled conveniently by adjusting the microwave power. We demonstrate the effect of microwave plasma on the surface property of polystyrene with oxygen (active) at 1 minute. Samples were placed

in a plasma chamber and exposed to oxygen gas for 60 second. Samples irradiated for these times were investigated by structural analysis and microscopy. Plasma surface treatment was achieved in microwave-induced plasma, with surface waves at a power level of 100 W. The experimental apparatus is shown in Figure 1

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 Topography Study

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..

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, CO₂) 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, CO₂) 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% CO₂. 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 grafted and the modified samples have been shown in Figure 2. ATR-FTIR spectra of the normal polystyrene have been shown in Figure 2A . The NIPAAm grafted by the UV radiated polystyrene ATR-FTIR spectrums have been shown below Figure 2B. The PNIPAAm picks characteristic includes 1601 cm⁻¹ which indicates -NH groups and 1730-1830 cm⁻¹ which indicates C=O groups and 3025 cm⁻¹ which indicates CH₃ groups and 3443 cm⁻¹ 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 .

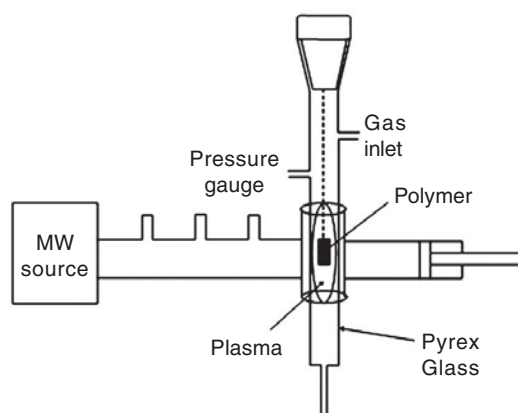


Fig. 1: Experimental setup of microwave plasma

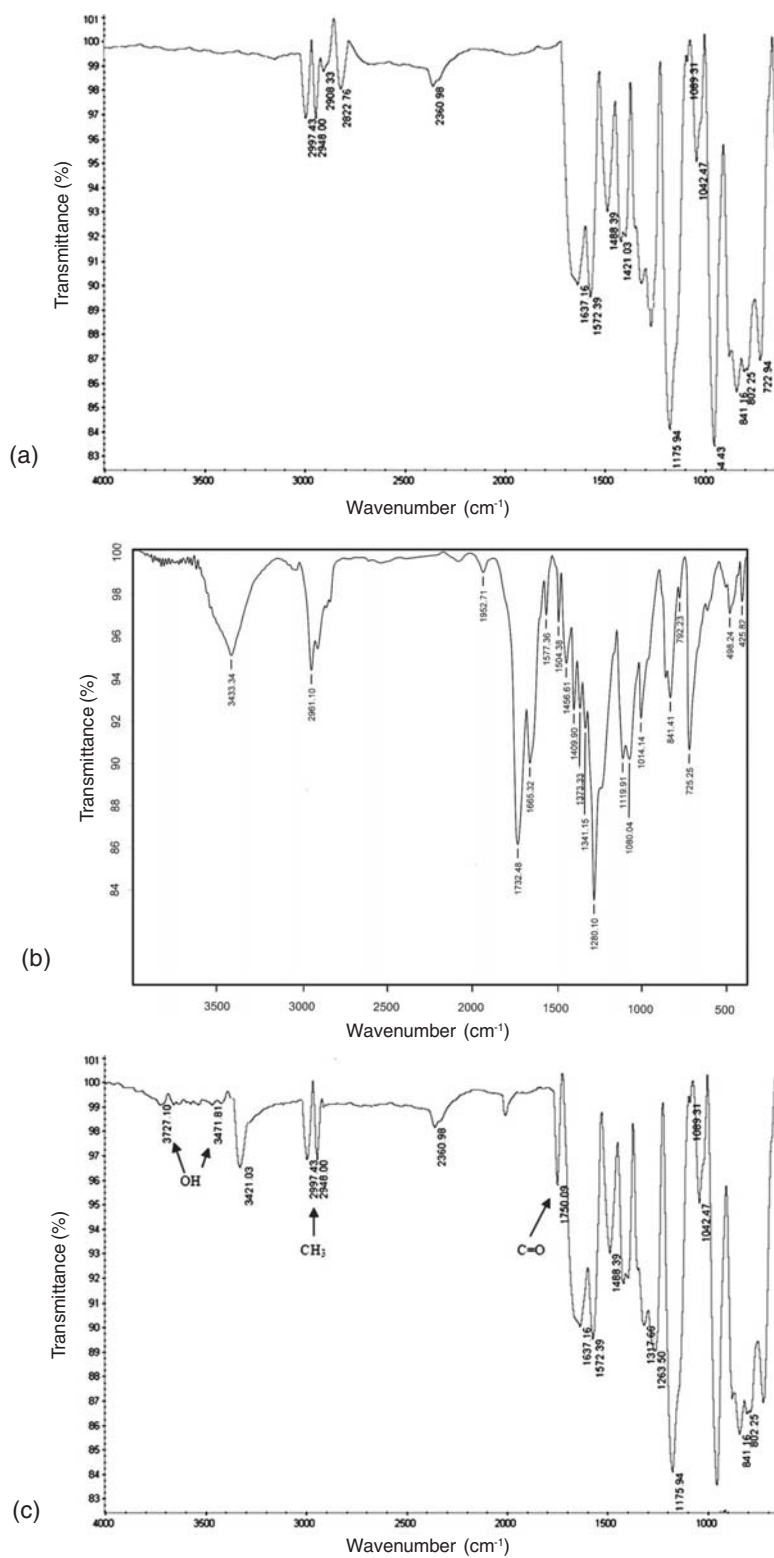


Fig. 2: ATR-FTIR spectra of the unmodified polystyrene (A)
The grafted polystyrene by UV ray (B) And spectra of the grafted and modified sample (C)

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

T (°C)	Normal θ_{H_2O}	Grafted samples θ_{H_2O}	Modified samples θ_{H_2O}
25	$90 \pm 3.2^\circ$	$60 \pm 0.4^\circ$	$43 \pm 0.1^\circ$

The ATR-FTIR spectra of the grafted and modified sample with oxygen plasma is shown in Figure 2C. The spectrum observed at 1750 cm^{-1} could be related to the C=O group, and showed successful surface modification with oxygen plasma. The tension peak at $1000\text{--}1300\text{ cm}^{-1}$ could be related to the C=O group and a peak at 3000 cm^{-1} could be related to the $-\text{CH}_3$ group. Moreover, the tension peak observed at $3400\text{--}3700\text{ cm}^{-1}$ could be

related to an OH group that demonstrated the effect of surface treatment on the samples.

Surface Topography Study

Figure 3A is the obtained AFM image from the grafted polystyrene sample. The surfaces topography and the created graft thickness on surface are shown in the AFM images. Figure 3B show the surface topography

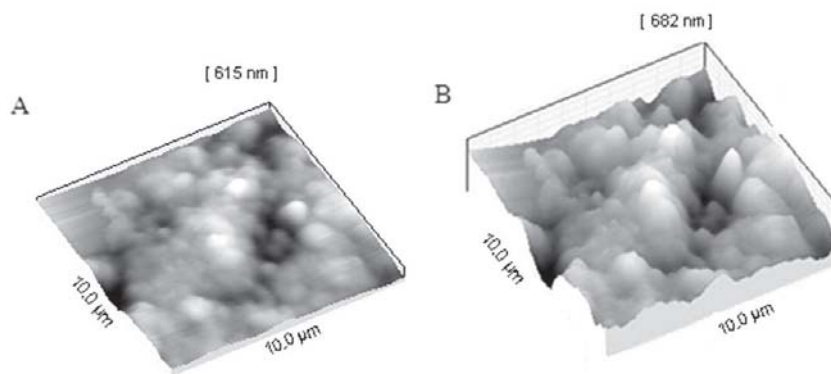


Fig. 3: Atomic force microscopy of grafted and modified samples .
A) unmodified grafted sample, B) grafted modified sample in the scale: $10 \times 10\ \mu\text{m}$.

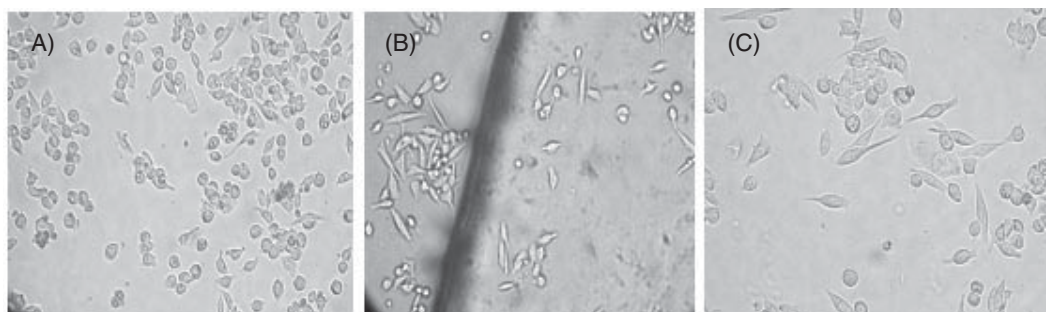


Fig. 4: The epithelial cells growth on the samples. (A) Control (TCPS), (B) the grafted sample, (C) the modified sample with oxygen plasma

for the grafted and modified sample with to oxygen plasma. The graft thickness average for the grafted and modified samples were about 600 and 700 nm separately.

The results show that surface roughness increased with increasing duration of exposure. A lot of difference was observed in surface topology with increasing duration of exposure. The surface roughness of the grafted samples and samples irradiated with oxygen plasma are shown in figure 3. The results show that the roughness of the samples modified with oxygen plasma was higher in comparison with unmodified samples.

Contact Angle Analysis

In the contact angle measurement process, the measured angle of normal polystyrene adjusted by UV radiated PNIPAAm surface samples and modified with to plasma in 25°C temperature has been shown in Table 1.

The contact angles average 90° and 60° and 43° have been calculated for normal polystyrene adjusted by UV radiated PNIPAAm surface samples and modified with to plasma in 25°C temperature. The results showed that samples modified using either gas reduced the contact angle.

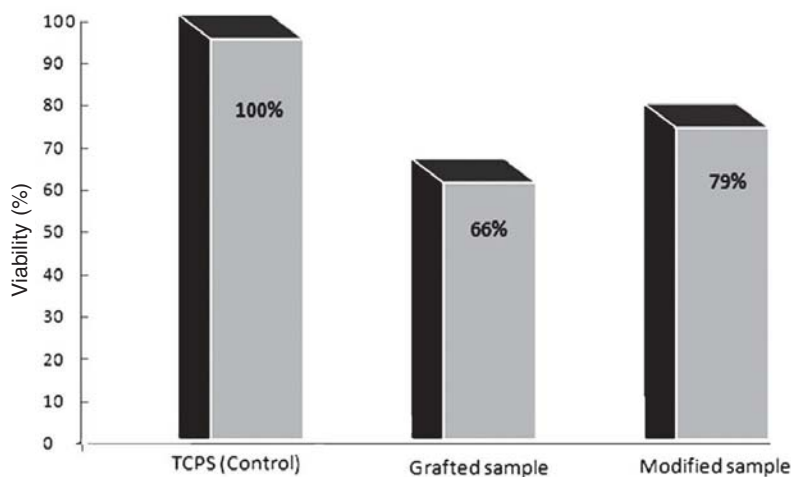


Fig. 5: MTT analysis of the samples

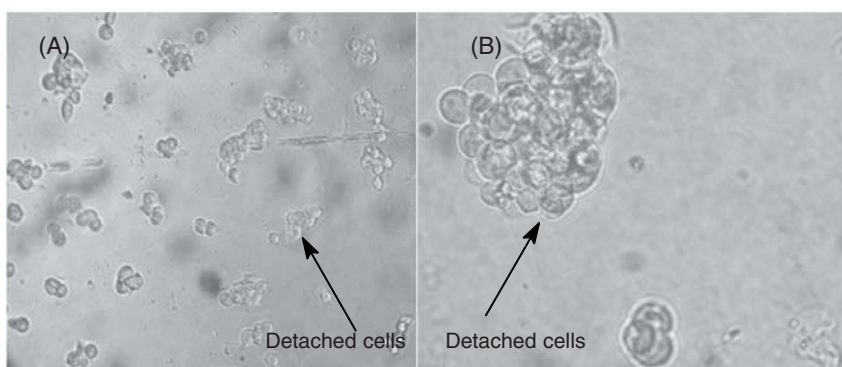


Fig. 6: The epithelial cells detached spontaneously when temperature decreased below 4°C on the grafted sample (6A); and on the modified sample (6B)

Samples modified with oxygen plasma showed a higher level of contact angle reduction in comparison with the unmodified sample, indicating that the sample modified with oxygen plasma was more hydrophilic than the others.

Biocompatibility Results

The biocompatibility data demonstrated that the grafted and modified samples under radiation supported epithelial cell adhesion and proliferation; and the cells also maintained suitable viability (figure 4). After cultured for 7 days on grafted samples, many cells were alive, suggesting that the two samples were suitable for cell attachment and proliferation; and the viability was about 70% (figure 5).

Figures 4B and 4C show a good cell growth on the grafted and modified samples surface at the physiological temperature of 37°C. Figures 6B and C show cells growth detached from the 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 6), 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 obtained and effect of plasma radiation on the grafted sample studied in this article. The ATR-FTIR spectrum showed the existence of the grafted polymer on polystyrene surfaces and also functional groups produced by oxygen plasma. The topology of the surfaces shown in the AFM images also approved the claim. The graft thickness of the grafted and modified samples in this study was about 600 and 700 nm separately. The contact angles 60° and 43° obtained for the grafted and modified samples. 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 samples. This characteristic proved that such type of grafted materials had potential as biomaterials for cell sheet engineering.

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