Polyurethane Membranes with Tunable Surface Properties for Biomedical Applications

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ABSTRACT: The use of surfaces with tunable properties triggered by external stimuli is effective in controlling the interactions between biomaterials and biological entities, such as proteins and cells. The goal of this work is to prove that the presence of poly-*n*-isopropylacrylamide (P-*N*-IPAAm) chains grafted onto polyurethane (PU) membranes and used for medical wound dressings can allow the behavior of the surfaces to be shifted from hydrophobic to hydrophilic by reducing the temperature to values lower than the low critical solution temperature (LCST) of the polymer, which is close to 32°C. The manipulation of this behavior can then be used to control cell and protein adhesion on the surface. Grafting of P-*N*-IPAAm was accomplished by treating the surface of polyurethane membranes with ultraviolet (UV) radiation, followed by polymerizing the isopropylacrylamide from the modified surfaces. The wettability of the surfaces was studied using contact angle measurements as a function of temperature. Infrared spectroscopy was also used to characterize these modified surfaces. The ability of the grafted surfaces to allow adsorption of proteins was evaluated as a function of temperature. The results showed that the amount of proteins adsorbed on the polyurethane membranes could be radically changed by altering the temperature above or below 32°C. *In vivo* biocompatibility tests were performed on P-*N*-IPPAm samples, and no indication of toxicity was noted after 7 days of implantation. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 121: 3501–3508, 2011

Key words: polyurethane; poly(*n*-isopropylacrylamide); wound dressing

INTRODUCTION

The modification of surfaces of polymers through the grafting of special macromolecules[1–3] that have properties that can be modulated through external stimuli is a very attractive approach to control the behavior of biomaterials in contact with proteins, cells, and tissues.[4,5] The ability of surfaces to be switched from hydrophobic to hydrophilic has been investigated recently to produce smart surfaces for DNA transfection,[6] nonfouling behavior,[7] cell culture[8,9] and biocatalysts.[10–12]

Poly(*n*-isopropylacrylamide) (P-*N*-IPAAm) has been particularly useful in creating smart biomaterials and surfaces because it displays a LCST (lower critical solution temperature) transition in water that is close to body temperature, which is usually reported to be 32°C.[10,13–15] During this transition, intermolecular forces between the water and polymer are disrupted, and nonpolar groups on the polymer are exposed, which leads to phase separation and conversion of the overall behavior of the material from hydrophilic to hydrophobic.[9,10,14] This change of behavior can then be used to control interactions between the polymer and biological entities. While hydrophobic, the polymer will favor the adhesion of proteins[15–17] and cells[8–13]; however, when hydrophilic (below the transition temperature), this behavior can force a loss of the adherence of the proteins and cells, a mechanism sometimes called "on-off."[8–10,18–20]

In this work, polyurethane (PU) membranes, which are often used as wound dressings and scaffolds for tissue engineering,[21,22] were modified by grafting P-*N*-IPAAm from their surfaces. These modified membranes were then able to generate novel biomaterials with an adhesion toward wounds or tissues controlled by external stimuli, such as temperature. Reducing the local temperature of the wound or tissue to values lower than the LCST of P-*N*-IPAAm allowed the biomaterial to be removed

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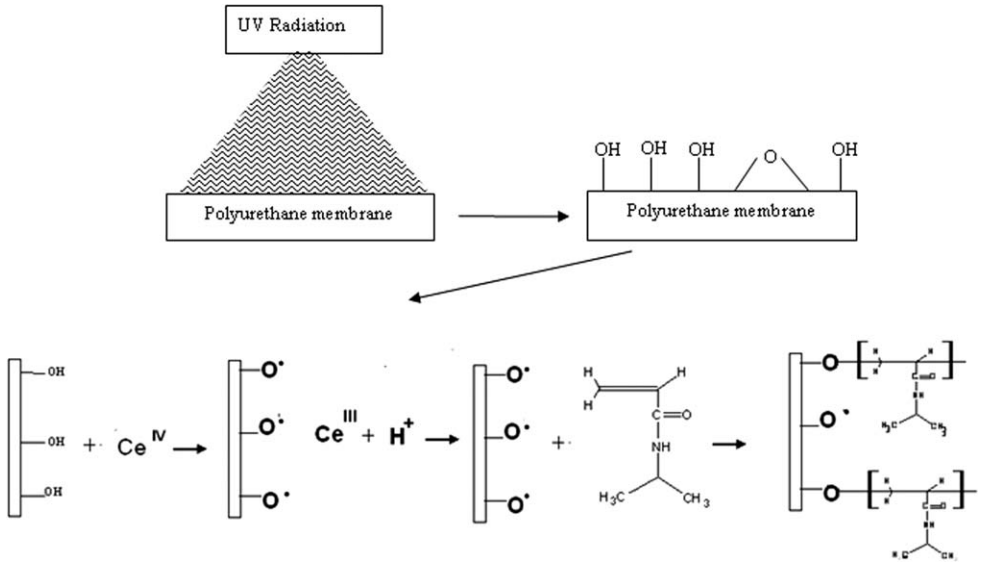


Figure 1 Representative outline of P-*N*-IPAAm grafting on the polyurethane surface.

without damaging the tissue undergoing repair. In this work, the hypothesis that PU membranes containing P-*N*-IPAAm grafts could have their wettability and ability to promote protein adsorption and desorption modified as a function of temperature was tested. The *in vivo* biocompatibility of P-*N*-IPAAm was also investigated to preliminarily investigate the possibility of using this type of material in biomedical devices.

EXPERIMENTAL

Preparation of polyurethane membranes

The thermoplastic polyurethane (PU) Elastollan ELA585A10 (Basf) (typically used in wound dressings), kindly provided by Polymeric Petropol, was dissolved in pyridine (20% w/v). Films were then obtained by casting the solution onto glass slides, followed by drying. Porous membranes were obtained by dispersing LiCl (10% w/w with respect to the polymer, with a 5-μm average particle size) into the polyurethane solution and washing the dried membrane at least five times with 500 mL of pure deionized water (DI) to remove the salt particles and any residual solvent.

UV treatment on polyurethane membranes

Ultraviolet radiation was used to oxidize the original surface of the polyurethane (PU) membranes to yield surfaces containing polar species and free radicals. PU membranes were placed 50 mm from a 30-W UV lamp with a wavelength range of 256–370 nm for 4 h. Figure 1 illustrates the procedure to modify PU films.

Grafting of *n*-isopropylacrylamide (*N*-IPAAm) from polyurethane

The grafting of *N*-IPAAm was accomplished by immersing the UV-treated PU membranes in an aqueous solution containing 5% w/v of the *N*-IPAAm monomer, 0.04*N* nitric acid, and 0.1% ceric ammonium nitrate. PU samples not exposed to the UV treatment were also used in the grafting process as a reference. Grafting of *N*-IPAAm was performed under a N_2 atmosphere at room temperature (20°C) for 12 h. Polyurethane membranes submitted to the grafting procedure were washed with pure water for 4 h to remove any nonreacted and nongrafted species. Films had their weight measured before and after the grafting procedure to provide information about the grafting yield. An average increase of 3 μg cm^{-2} was observed on the weight of films submitted to the grafting procedure. By using this information together with the initial content of monomer in the reactor, a grafting efficiency of 10% could be calculated, i.e., 10% of total monomer added in the reactor was actually grafted on the surface of PU films.

Characterization of PU modified films and membranes

The contact angle between deionized water and the surface of the films was measured both below and above 32°C. The hydrophilic and hydrophobic behaviors of the modified membranes were also monitored respectively, below and above the LCST by evaluating the degree of transparency of 0.5-mm thick PU membranes qualitatively. The modified membranes with P-*N*-IPAAm were immersed in DI water at different temperatures, and photos of a

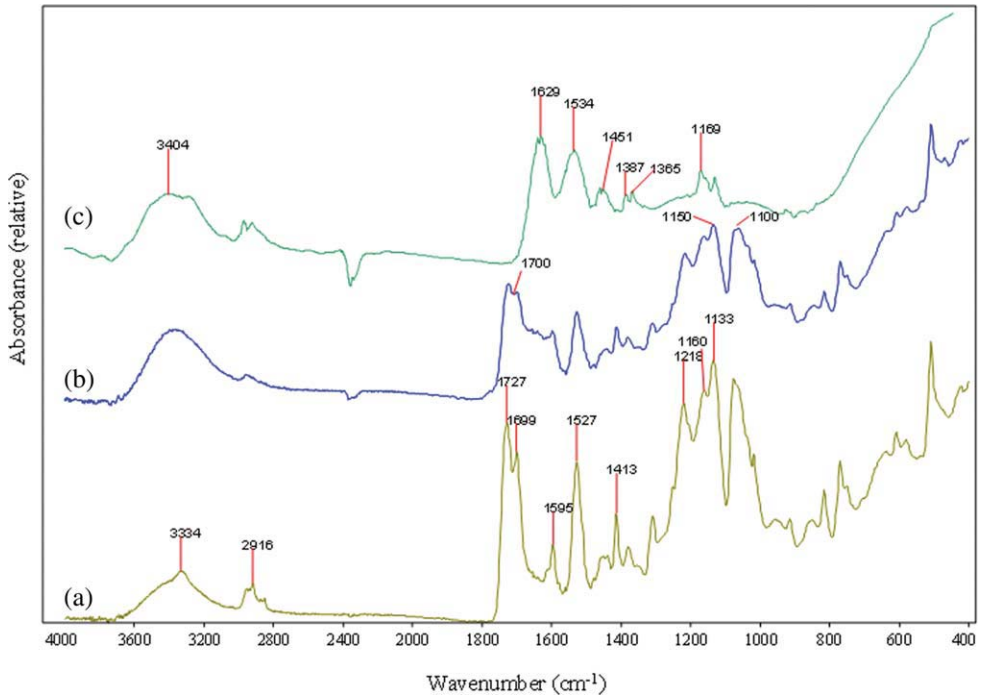


Figure 2 Infrared spectra (FTIR) of (a) pure polyurethane membrane, (b) polyurethane membrane after UV treatment and (c) polyurethane membrane with P-*N*-IPAAm grafts (c). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

barcode beneath the membranes were taken and compared.

Infrared spectra were collected in a Fourier transform infrared spectrophotometer (FTIR; Perkin–Elmer, model Spectrum 1000). Measurements were taken with the attenuated total reflectance (ATR) technique. Each spectrum was produced after 32 scans with a resolution of 4 cm^{-1}.

Protein adsorption and desorption on modified surfaces

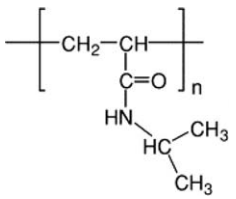
The ability to modulate protein adsorption and desorption (the on-off mechanism) was tested *in vitro* by dipping PU membranes with P-*N*-IPAAm grafts into an aqueous solution of bovine albumin (2 wt %) for 5 days both above and below 32°C. After the incubation period, the adsorbed albumin was extracted from the samples using DI water at different temperatures (again, above and below 32°C, which is equal to the LCST of P-*N*-IPAAm). Samples submitted to extraction were then dried at 105°C, and their weights were measured and compared with the initial ones. At least five samples were tested for each adsorption/desorption condition.

In vivo biocompatibility tests

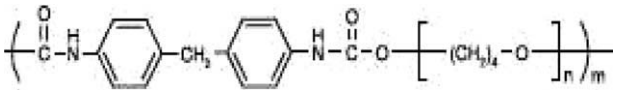
A proposal reporting the protocol associated with the *in vivo* tests was submitted and approved by the Committee of Ethics in Animal Experimentation of the Federal University of Minas Gerais (UFMG - CETEA protocol 069/09).

In vivo tests were performed to preliminarily check the biocompatibility of P-*N*-IPAAm-based materials. These tests were carried out using Swiss mice that were anesthetized and had their backs shaved. The animals were anesthetized with 80 mg kg^{-1} of ketamine and 10 mg kg^{-1} of xylazine. PU films (used as a control) and pure P-*N*-IPAAm-casted films, prepared using similar polymerization conditions as used for the grafting procedure, were tested *in vivo*. Prior to the *in vivo* test, samples were washed several times with DI water and sterilized in an autoclave at 130°C for an hour. Samples, which were an average of 0.5 mm in thickness, 5 mm in length, and 3 mm in width, were subcutaneously implanted in the dorsum of mice by producing a 10-mm long incision that was closed with Catgut suture afterward. Six mice (three mice for each type of sample) were used in the *in vivo* tests. After 7 days, the animals were euthanized in a gas chamber, and the samples together with the surround tissues were removed. The collected tissues and samples were processed by using traditional histotechniques, i.e., fixating them in formalin and embedding them in paraffin. The embedded tissues were then sectioned with a microtome and stained using hematoxylin and eosin (H and E). The thin samples were observed using an optical microscope (Zeiss Axiolab; Zeiss; Germany), and digitized images were analyzed with software (Kontron Eletronics, Carl Zeiss - KS300 v.2.0).

TABLE I
Typical FTIR Absorption Bands for Polyurethanes and P-N-IPAAm



Band (cm^{-1})	Assignment
P-N-IPAAm	
1653	C=O stretching/Amide I
1543	N—H stretching/Amide II
1386	CH_3 in isopropyl groups
1362	CH_3 in isopropyl groups



Typical aromatic polyurethane

3330	N—H stretching.
1720	Free C=O stretching
1700	Hydrogen-bonded C=O stretching
1540	N—H stretching
1150	C—O—C stretching

RESULTS AND DISCUSSION

The FTIR spectra of the PU membranes are shown in Figure 2. The ATR method used to acquire spectra allows the investigation of the surface of the samples in contact with the ATR crystal at a depth that is typically around 5 μm. This type of surface analysis then provided information about the chemical modifications that might have occurred on the surface of samples. Table I lists typical infrared absorptions bands expected in aromatic PUs and P-*N*-IPAAm.[23,24]

In the spectrum of the original pure PU [Fig. 2(a)], FTIR absorption bands due to carbonyl groups (1727 and 1699 cm^{-1}) in the urethane bonds can be observed. The 1699-cm^{-1} band is associated with hydrogen-bonded carbonyl groups, whereas the 1727-cm^{-1} band is related to nonhydrogen-bonded (free) carbonyl groups. Other FTIR bands typically seen in polyurethanes are noted in Figure 2(a): amine bonds at 3334 cm^{-1}, aromatic groups at 3100 cm^{-1}, aliphatic CH and CH_2 bonds at 2900 cm^{-1}, and NH bending vibrations at 1539 cm^{-1}. The spectrum of the UV-treated polyurethane is shown in Figure 2(b). In this spectrum, the presence of broader carbonyl (between 1750 and 1650 cm^{-1}) and C—O bands (close to 1100 cm^{-1}) can be seen when these bands are compared to the same bands in the spectrum of the original polyurethane. These results may indicate that the UV treatment led to the oxidation of the surface of the polyurethane.

The FTIR spectrum of the polyurethane after P-*N*-IPAAm grafting is shown in Figure 2(c) and reveals absorption bands typically assigned to chemical groups in P-*N*-IPAAm,[24] such as the carbonyl group in amide bonds (1639 cm^{-1}), the broad absorption band close to 3400 cm^{-1} due to water bonded to P-*N*-IPAAm (the spectrum was collected at temperatures below 32°C, and thus P-*N*-IPAAm displays a hydrophilic behavior that leads to adsorption of water molecules from the environment), *N*—H bonds at 1540 cm^{-1} and methyl groups (in isopropyl groups) at 1362 and 1386 cm^{-1}.

FTIR results proved that the procedure used to graft P-*N*-IPAAm from polyurethane, as outlined in Figure 1, was successful. Several types of grafting procedures for *N*-IPAAm on different materials have been employed, such as ATRP, plasma, UV, and gamma radiation, among others.[25–28] The energy associated with UV radiation is high enough to generate active groups, such as hydroperoxides, epoxide, carboxylic acids, and hydroxyl groups, on the surface of the polymers.[7] Wavelengths shorter than 400 nm correspond to energies higher than 3.1 eV.

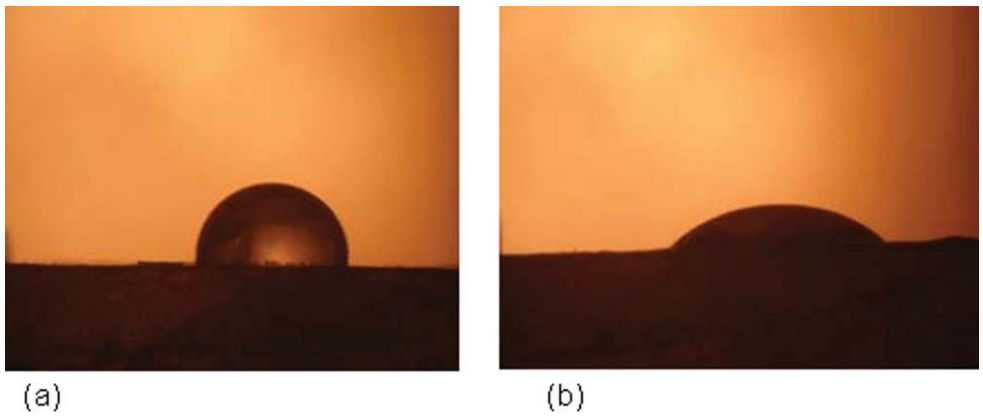


Figure 3 Contact angle of a drop of DI water on the surface of PU with P-*N*-IPAAm grafts at (a) 40°C and (b) 25°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE II
Contact Angle of Deionized Water and the Surface of Pure Polyurethane and PU with P-N-IPAAm Grafts at 25 and 40°C

Substrates	Contact angle
PU without treatment (pure) (25°C)	45° (±2°)
PU with P-N-IPAAm grafts (25°C)	28° (±2°)
PU with P-N-IPAAm grafts (40°C)	74° (±2°)

This level of energy is sufficient to break C—H bonds (with a bond energy close to 2.54 eV) and to generate active species, such as hydroxyl, carboxyl, and epoxide groups, on the surface of polymers. These species can reduce Ce^{4+} ions to Ce^{3+} ions and produce free radicals, as shown in the scheme of Figure 1.[25] In contact with oxygen or water, the active species can also form hydroperoxides that can further decompose and generate free radicals. Free radicals are able to initiate the chain growth polymerization of monomers containing C=C bonds, as in the case of *n*-isopropylacrylamide. Polymerization initiated by free radicals on the surface of polymers led to grafting of the macromolecule. Free radicals not attached to the surfaces initiate polymerization in bulk or solution and form free chains that can be extracted by using a good solvent, such as water at 25°C for P-*N*-IPAAm.

Contact angle measurements and optical properties

The data in Figure 3 and Table II were obtained by measuring the contact angle of a DI water drop on the surface of PU and modified PU materials. The measured contact angle of pure PU was close 45° at 25°C and shifted to 28° for PU with P-*N*-IPAAm at 25°C. This low contact angle indicates that the sample had a very hydrophilic surface at 25°C (i.e., below the LCST of P-*N*-IPAAm). At 40°C (above the LCST of P-*N*-IPAAm), the value of the contact angle changed to 75° for PU containing P-*N*-IPAAm grafts, showing that the surface had become much more hydrophobic. The photographs displayed in Figure 3 clearly show the changes in the shape of water drops deposited on the surface of PU with P-*N*-IPAAm grafts below and above the LCST of P-*N*-IPAAm (usually reported as 32°C).

The same type of result was obtained by qualitatively monitoring the transparency of PU membranes with P-*N*-IPAAm grafts. Photos in Figure 4 show a clear transition from a transparent material that is fully hydrated and swollen below 32°C (at 31°C) to a much more opaque material above 32°C (at 33°C) due to shrinkage and the water expelled from the grafts to the solution (increase in refractive index).

Verification of protein adhesion and the on–off mechanism *in vitro*

The possibility of tuning the ability of the surfaces for the adsorption of proteins was investigated *in vitro* by immersing PU membranes with P-*N*-IPAAm grafts into aqueous solutions containing bovine albumin. Albumin adsorption and desorption were monitored by measuring the weight of the dried samples after intense extraction using DI water at 25 and 40°C. In Figure 5, no adsorption of albumin after incubation at 25°C and extraction with water at 25°C was observed because no weight gain was measured for PU with P-*N*-IPAAm grafts. At this temperature, fully hydrated P-*N*-IPAAm chains should acquire a comb-like conformation that reduces the possibility of the high molar mass protein approaching and finding specific bond sites on the surface. Even when DI water at 40°C was used to extract albumin in samples incubated at 25°C, low levels of adsorption were again noted. For PU samples with P-*N*-IPAAm grafts incubated at 40°C in albumin solutions and extracted with water at 40°C, a much more pronounced increase in the weight of the samples could be measured, which indicates that the hydrophobic behavior of the surface at this temperature was able to promote adsorption of the protein. When cold water (at 25°C) was used to extract albumin, partial desorption of the protein could be

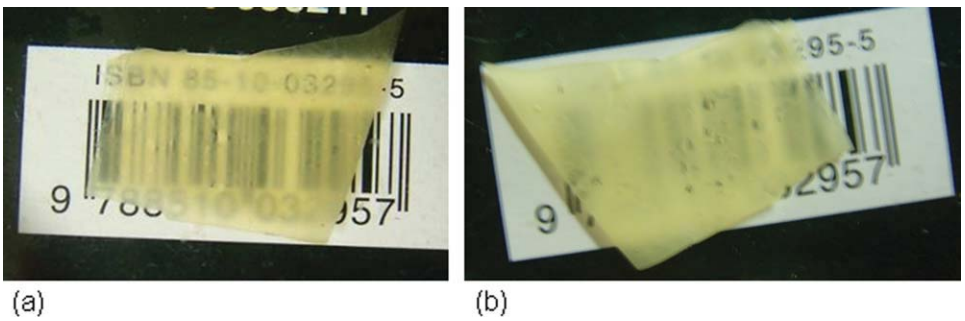


Figure 4 Polyurethane with P-*N*-IPAAm grafts at (a) 31°C and at (b) 33°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

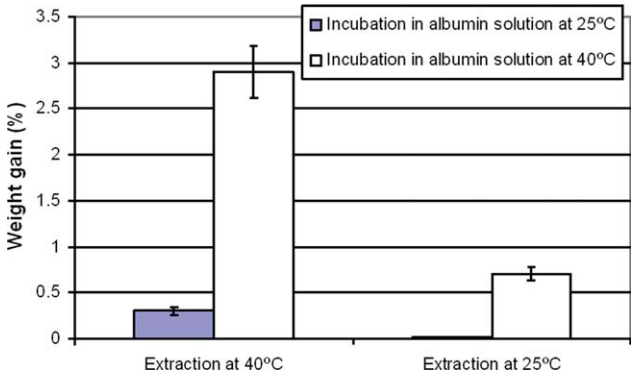


Figure 5 Adsorption and desorption of albumin of polyurethane membranes with P-*N*-IPAAm grafts. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

seen as a consequence of an increase in the hydration of the P-*N*-IPAAm grafts. Figure 6 outlines the changes in adsorption and polymer conformation during the *in vitro* tests.

These results prove that by changing the temperature across the LCST of P-*N*-IPAAm, it is possible to radically switch the behavior of the surfaces from "on," which allows adsorption of albumin, to "off," in which the surface neglects adsorption. Assuming that the behavior of albumin can be extended to other proteins, it is possible that scaffolds and wound dressings, for example, made of PU with P-*N*-IPAAm grafts could change their adhesion for tissues by changing the temperature. A reduction of the local temperature of the biomedical device to values lower than the LCST would allow them to be removed from the tissues without damage.

In vivo tests

In vivo tests were performed in PU and P-*N*-IPAAm-based materials to verify any possible toxic effect for biomedical applications. Figure 7 shows that after 7 days of implantation, pure polyurethane samples were encapsulated in a fibrous capsule composed of connective tissue rich in thin and low-packed fibers, as well as adipose tissue, inflammatory cells (macrophages and lymphocytes), and blood vessels. P-*N*-IPAAm samples were also encapsulated within a fibrous capsule as shown in Figure 8. The fibrous capsule rich in connective tissue had thicker and highly packed fibers, inflammatory cells and a larger number of new blood vessels.

The *in vivo* results suggest that neither material (PU or P-*N*-IPAAm) elicited a severe inflammatory

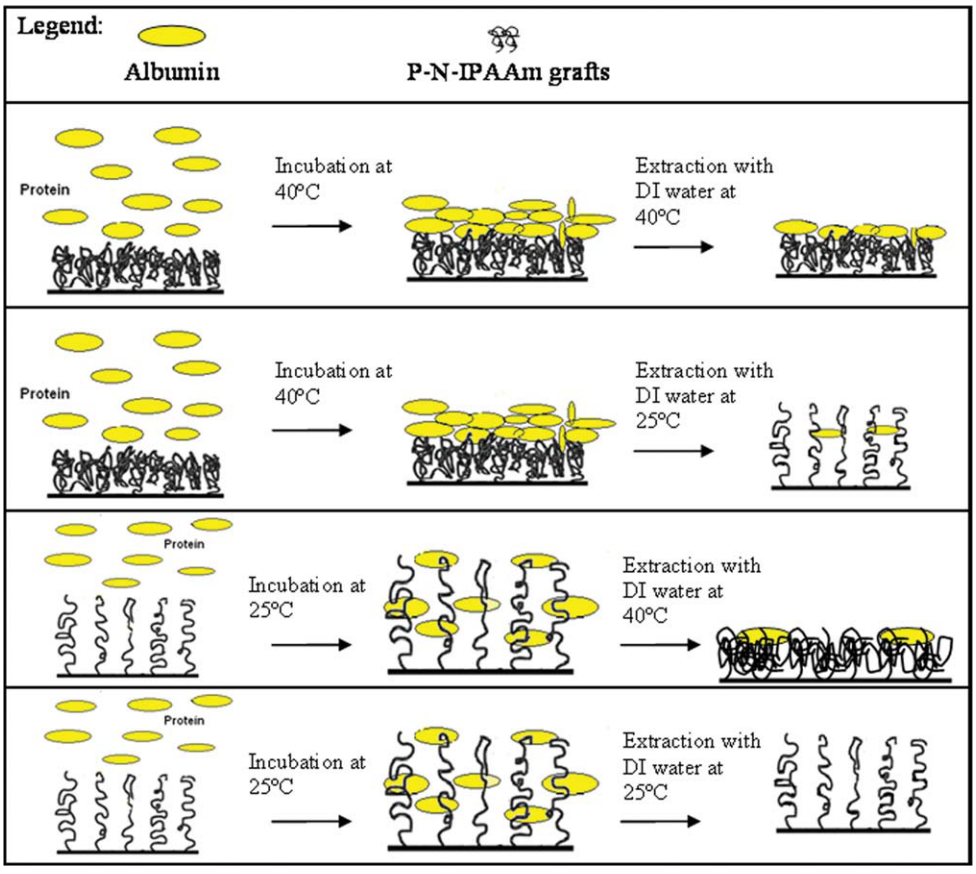


Figure 6 Representative outline of the adsorption and desorption of albumin from the surface of PU with P-*N*-IPAAm grafts. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

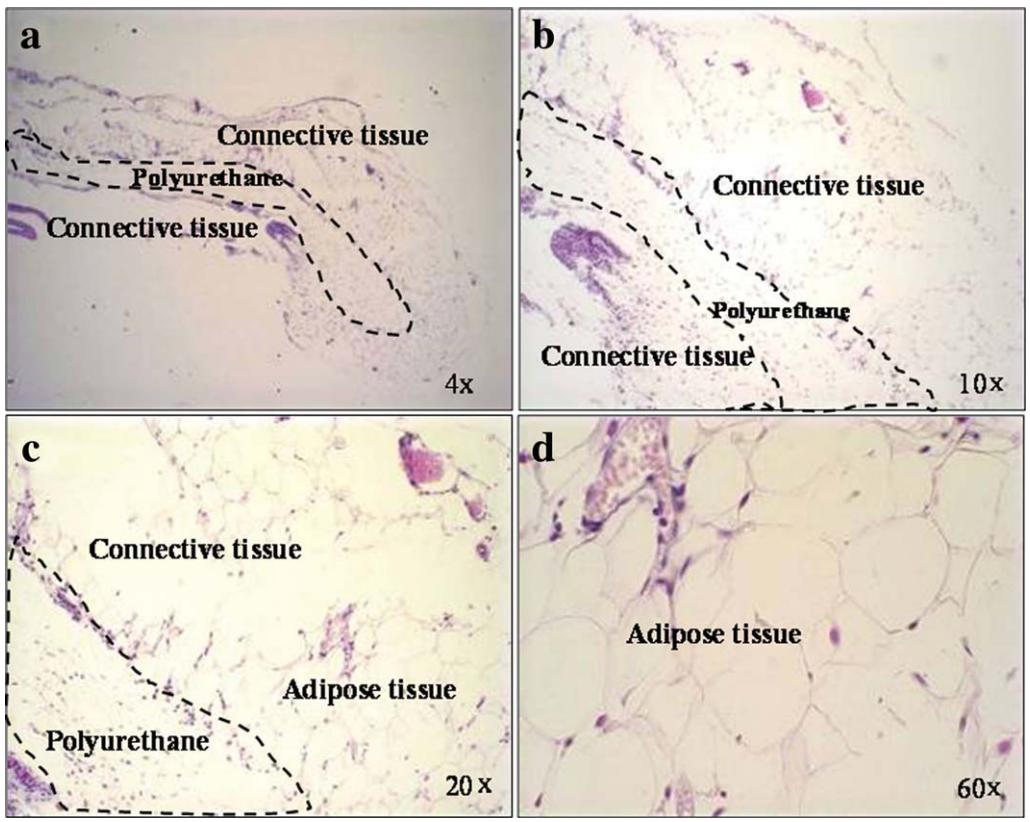


Figure 7 Histology of polyurethane samples after 7 days of implantation. (b, c, and d) are different amplifications of (a). H and E staining. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

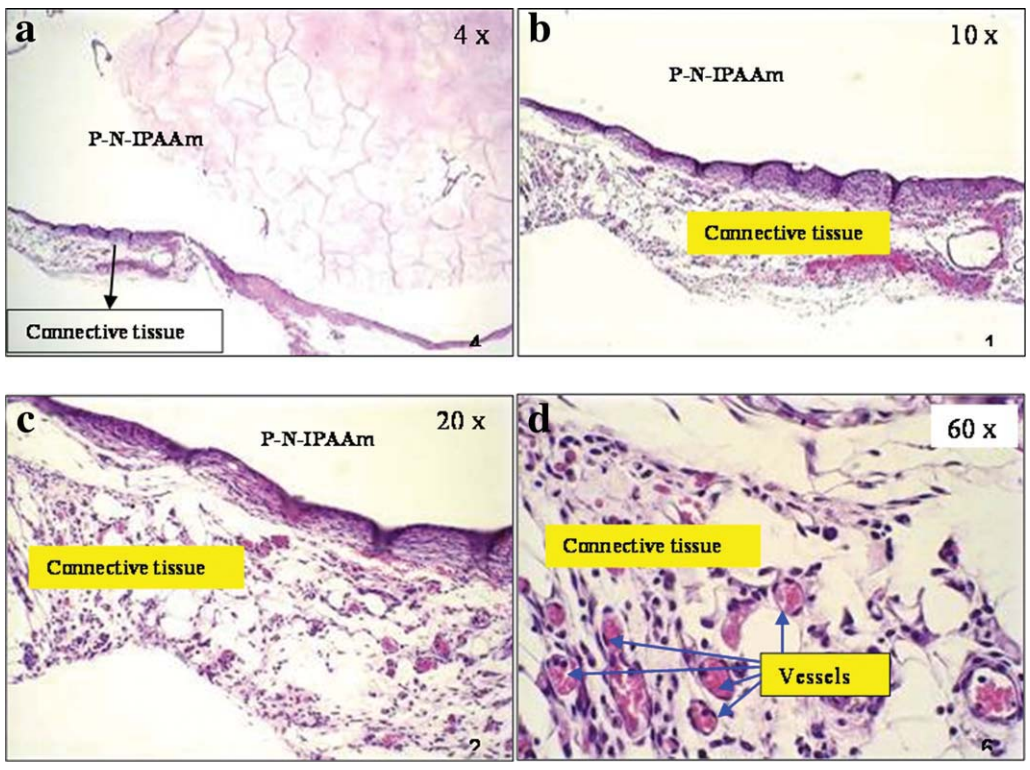


Figure 8 Histology of P-*N*-IPAAm samples after 7 days of implantation. (b, c, and d) are different amplification of (a). H and E staining. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

reaction, which indicates that they are nontoxic and can be considered for biomedical applications.

CONCLUSIONS

P-*N*-IPAAm was successfully grafted from UV-treated PU membranes as indicated by the FTIR-ATR results. The wettability of the surface of PU with P-*N*-IPAAm grafts could be switched from highly hydrophobic to hydrophilic by changing the temperature from 40 to 25°C (i.e., through the LCST transition of P-*N*-IPAAm). The change in temperature across the transition of P-*N*-IPAAm grafts was also able to tailor the adsorption of albumin toward the surface of PU with P-*N*-IPAAm grafts. The adsorption of albumin was practically eliminated when the temperature was reduced to values lower than the LCST of P-*N*-IPAAm. *In vivo* tests suggested that both PU and P-*N*-IPAAm were nontoxic. The overall results indicate that PU membranes with P-*N*-IPAAm grafts may be considered for biomedical applications, for example, for wound dressings in which the level of adhesion between the tissue and the surface of the biomaterial could be tuned by locally changing the temperature close to the biomedical device.

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