ABSTRACT

Wang, Xiaoling. Responsive Biomaterial Surfaces (under the direction of Dr. Marian McCord and Dr. Sam Hudson).

Responsive biomaterial surfaces were fabricated by grafting stimuli-responsive polymers onto polymer material surfaces via a novel Atmospheric Plasma Treatment (APT). They have potential uses in cell adhesion/detachment control, tissue engineering, medical device, drug delivery, bioreactor, bioseparation, and responsive clothing.

Temperature sensitive poly(N-isopropylacrylamide) (PNIPAM) was grafted onto various substrates via two novel methods using APT, i.e., atmospheric plasma treatment followed by free radical graft copolymerization (two- step method), and atmospheric plasma treatment of a NIPAM monomer coated surface (coating method). The substrates included nylon film, non-tissue culture treated polystyrene (PS) plates, and cotton fabrics. FTIR confirms the grafting of PNIPAM. The addition of Mohr's salt in the two-step method suppresses homopolymerizaiton and enhances graft yields. The contact angle of PNIPAM-grafted polymer surfaces increases dramatically at *ca.* 32^{o}C, indicating the temperature sensitivity of the grafted surface, i.e., the change of surfaces from hydrophilic to hydrophobic as temperature increases. Atomic Force Microscope (AFM) shows different topography of original, plasma treated, and PNIPAM grafted surfaces. For the first time, AFM was employed to characterize the grafted surface topography upon changes from dry to wet conditions and from below to above the LCST of PNIPAM.

The grafted surface is rough when dry at 22^{o}C, and smooth when wet at 22^{o}C. However, the surface becomes rough again in water at 40^{o}C in response to conformation changes in the PNIPAM hydrogel. Human epithelial cell line HEPG2 cells adhere and proliferate on PNIPAM grafted PS plates at 37^{o}C as on tissue culture plates. However, they detach from the surface automatically at 0^{o}C because of the phase change of PNIPAM. The detachment of HEPG2 cells upon cooling down can be used to recover continuous sheets of tissues from a bioreactor. The tensile property of PNIPAM grafted cotton fabrics was studied. The grafting of PNIPAM still have good tensile property. Comfort test shows thermal sensitivity of the PNIPAM grafted cotton. At 10^{o}C in wet conditions (sweating), less heat transfers from the skin model through the grafted cotton than through the original cotton (control); however at 35^{o}C, more heat transfers from the skin model through the fabric than control.

pH responsive Poly(acrylic acid) (PAA) was also grafted on the nylon surface via atmospheric plasma treatment two- step method. The FTIR and water contact angle confirmed the grafting.

Compared with conventional vacuum grafting methods, the APT method has several advantages, including no vacuum requirement, low cost, availability to be integrated into a continuous process, and no effect no bulk properties. The APT coating method is especially suitable for industry continuous process.

RESPONSIVE BIOMATERIAL SURFACES

by
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DEDICATION

This thesis is dedicated to my husband Xiangwu Zhang, for his devoted love and support. I am very grateful the life journey we have together.

BIOGRAPHY

Xiaoling Wang was born on April 4th, 1979 in Qingyuan, Zhejiang, China as a daughter of Yanping Wang and Jinyue Huang. She has two sisters, Xiaoqiong Wang and Xiaoqing Wang. She graduated from Qingyuan High School in July 1996 and then she obtained a B.S. degree in Polymer Materials and Engineering at Zhejiang University, Hangzhou, China in July 2000. During the undergraduate study, she began to have interests in Polymeric Biomaterials. She also met Xiangwu Zhang, later becoming her husband, in the same department of the University. After getting married in 2002, Xiaoling Wang moved to U.S. with her husband. In August 2003, she began to pursue her master degrees in Textile Engineering and Biomedical Engineering both focused on biomaterials field at North Carolina State University.

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Chapter One: Literature Review

1. Stimulus Responsive Polymers

1.1 Introduction

Functional polymers, which can react, adjust or modulate their physicochemical character, i.e., in most cases their water-solubility, in response to an external stimulus, are generally referred as stimulus responsive polymers (SRP), or "smart materials". The environment stimuli can be pH, temperature, ions, solvents, electrical field, magnetic field, light, pressure, chemical and biochemical compounds (e.g. glucose and others). The physical basis of this "smart" behavior is fast and reversible change of the polymer microstructure from a hydrophilic to a more hydrophobic one, triggered by small changes in the environment. These microscopic changes result at the macroscopic level, for example, in the formation of a precipitate or in a change in the wettability of a surface to which the smart polymer is grafted. The changes are usually reversible and the system returns to its initial state when the stimulus is removed [1].

Among the various stimulus responsive polymers, the most commonly used ones are temperature- and pH-responsive polymers, since the temperature and pH are relatively easy to change. These two kinds of stimulus responsive polymers will be discussed in detail. Some of other stimulus-responsive polymers will be discussed briefly as well.

1.2 pH-responsive polymers

In the case of pH-responsive polymers, the driving force behind the transitions is usually a neutralization of charged groups in the polymer by a pH shift. As the environmental pH changes, the degree of ionization in a polymer bearing weakly ionizable groups is dramatically altered at a specific pH which is called pKa. This rapid change in net charge of pendant groups causes an alternation of the hydrodynamic volume of the polymer chains. As shown in Figure 1, hydrogel volume (hydration) increases abruptly at a pH region above its

PKa for acidic polymer, below its PKa for basic polymers, and either side of bioelectric point(IEP) for amphoteric polymers[2]. The transition from collapsed state to expanded state is explained by the osmotic pressure exerted by mobile counterions [3]. The chain repulsion due to charge on the chain is the other reason for the formation of gel.

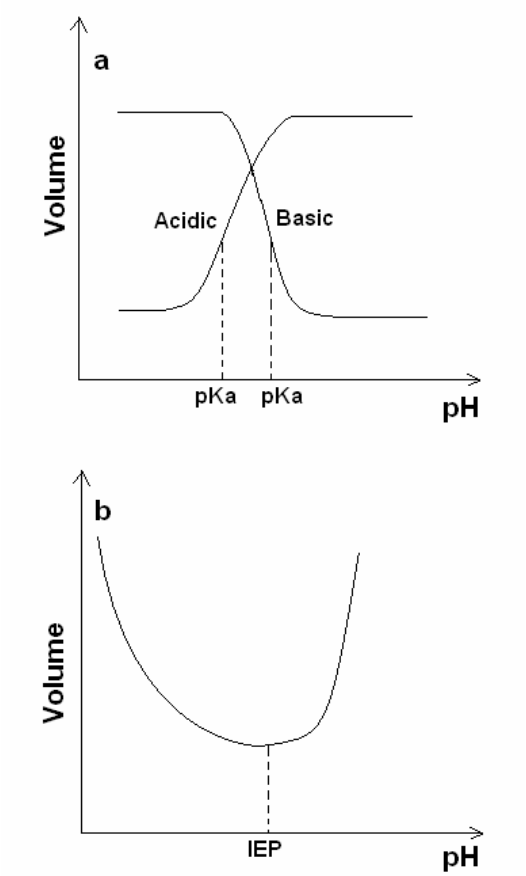
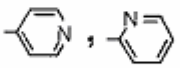


Figure 1: Schematic pH dependent swelling of pH responsive polymers in water. a) Acidic and basic polymers; b) amphoteric polymers (redrawn from [2]).

Some examples of pH sensitive polymers and their PKa and IEP are presented in Table 1 [2]. For the desired pH responsiveness, one may choose a polymer containing either acidic or basic groups, or both. It is also shown in Table 1 that pH-responsive polymers can be prepared from both natural and synthetic sources. Natural polymers such as chitosan and collagen have weakly ionizable groups, such as $–NH_2$, -COOH. Synthetic pH-responsive polymers include weak polyacids, weak polybases, and amphoteric polymers. For example, Weak polyacids such as poly(acrylic acid) (PAA) accept protons at low pH and release

protons at neutral and high pH. The leave of proton causes the anions in the polymer chain, which repulse each other. On the other hand, polybases like poly(4-vinylpyridine) is protonated at high pH and positively ionized at neutral and low pH.

Table 1. Example of pH sensitive of polymers [2].

Ionic groups	Polymer name	pH sensitive group	pK_a or IEP
Weak acid	Poly(acrylic acid)	–COOH	4.2–5.5
	Poly(methacrylic acid) (PMAA)		5.6–7.0
	Poly(carboxyacrylanilide–MMA)		5–7
	Cellulose acetate phthalate		?
	Inulin acetate succinate		6.5
Strong acid	Poly(sulfoxyethyl methacrylate)	$-SO_3H$	1.5
Weak base	Poly(aminoethyl methacrylate)	$-NH_2$	
	Poly[(N,N-dimethylamino)ethyl methacrylate]	$-N(CH_3)_2$	5.5–6.5
	Chitosan	$-NH_2$	6.4
	Polyvinylpyridine		3.9, 3.5 (2.8-5.0)
Ammo-nium	Poly[(vinylbenzyl)trimethylam-monium chloride]	$-N(CH_3)_3^+Cl^-$	1.2
Ampho-teric	Poly(acrylic acid– methacrylamidopro-pyltrimethylammonium chloride)	–COOH / $-N(CH_3)_3^+Cl^-$	
	Poly[sodium 2-acrylamido-2-methylpropylsulfonate–N-3-(dimethylamino)propylacrylamide]	$-SO_3Na$ and $-N(CH_3)_2C_3H_7$	
	Collagen	$-NH_2$ and –COOH	5-6

1.3 Temperature responsive polymers

Temperature is the most widely used stimulus in environmentally responsive polymer systems. The change of temperature is not only relatively easy to control, but also easily applicable both *in vitro* and *in vivo* [4]. One of the unique properties of temperature-responsive polymers is the presence of a critical solution temperature. Critical solution temperature is the temperature at which the phase of polymer and solution (or the other polymer) is discontinuously changed according to their composition. If the polymer solution has one phase below a specific temperature, which depends on the polymer concentration, and is phase-separated above this temperature, these polymers generally have a lower critical solution temperature (LCST), the lowest temperature of the phase separation curve on concentration–temperature diagram [4].

Many polymers composed of both hydrophobic and hydrophilic residues are characterized by LCST. Example of these polymers include poly(N-alkylacrylamide), poly(ethylene oxide)-poly(propylene oxide) (PEO-PPO) block copolymers, pyrrolidone containing polymers, and cellulose ethers[2]. These polymers dissolve in aqueous media at about room temperature, and precipitate from solution at certain higher temperatures.

Among various LCST polymers, poly(N-isopropylacrylamide)(PNIPAM) and its copolymer are the most extensively studied , because of their relatively sharp transition (narrow transition temperature region). PNIPAM exhibits a lower critical solution temperature (LCST) of 32°C. Above 32°C, the polymer is in a solid state and is hydrophobic. Below 32°C, the polymer is fully–hydrated, and exhibits a hydrophilic property. PNIPAM is particularly advantageous for potential medical applications because the LCST falls between body temperature and room temperature. Heskins and Guillet first reported in 1968 that LCST of PNIPAM solution decreases with increasing polymer concentration, and then increases after reaching a minimum [5]. Synthesis of PNIPAM had been reported by Wooten as early as in 1957[6].

It is widely believed [1] that the thermosensitivity (phase transition) of PNIPAM is due to the change of hydrogen bonding of groups in its segments with the water molecules at different temperatures. The efficiency of hydrogen bonding decreases in general with increasing

temperature. Consequently, at lower temperature, PNIPAM has sufficient hydrogen bonding with water molecules and is soluble in water. However, above a critical temperature, 32^{o}C, the efficiency of hydrogen bonding becomes insufficient to maintain the macromolecule in solution (segment/segment contact preferred over segment/water contact), the macromolecule collapse and aggregation/precipitation usually take place. Therefore, PNIPAM undergoes phase transition because it becomes progressively more hydrophobic with increase in temperature.

However, Wu et al [2] think that both hydrophobic interactions of PNIPAM segments and polymer-water hydrogen bonding are involved in the phase transition of PNIPAM at ca. 32^{o}C. They think that hydrogen bonding on its own is unlikely to be significant, because polyacrylamide(PAAm), that has no hydrophobic N-alkyl groups, is soluble in water at all temperatures. Furthermore, poly-N-alkylacrylamides with more hydrophobic N-alkyl groups exhibit lower LCSTs, again suggesting that polymer-water H-bonding may not be the only factors. So it appears that both hydrophobic interactions of PNIPAM segments and polymer-water hydrogen bonding play roles in the phase change. As temperature decreases, at some point the hydrophobic interactions between polymer molecules become more favorable than polymer-water interactions and the polymer molecules collapse.

There are other temperature-responsive polymers with similar structure to PNIPAM. For example, poly(N-vinylisobutyramide) with a transition temperature of 39^{o}C, or poly(N-vinyl caprolactam) with a transition temperature of 32-33^{o}C[1]. The critical temperature of a given thermosensitive (co)polymer can often be fine-tuned by adding more hydrophilic or more hydrophobic co-monomers to the structure. The former increases the transition temperature of the polymer while hydrophobic co-monomers have the opposite effect [7].

Beside the synthetic polymer, some biopolymers exhibit temperature responsive behavior too. One representative is Gelatin. It is a protein that is obtained by breaking the triple-helix structure of collagen into single-strand molecules. Gelatin forms gels in aqueous solution by cooling the temperature as the chains transform their conformation from random coil to

triple-helix, during which physical junctions are promoted and gel networks occur. At high temperature, the network breaks down [8].

1.4 Glucose responsive polymer or polymer complex

Polymers with glucose responsive phase transition have received extensive attention in recent years because of their potential application in self regulated insulin delivery for treatment of insulin-dependent diabetes. Various mechanisms have been employed to induce glucose responsive insulin release, which can be classified into direct and indirect glucose triggers, both of which undergo changes in swelling in response to glucose. The direct system is based on competitive substrate binding to glucose and hydrogel, and the indirect system involved pH sensitive hydrogels [2].

Polymer complexes that are directly sensitive to glucose are based on polymer-polymer or glucose-protein binding. One example involves competitive binding of glucose and PVA to PVP-4-aminophenylboronic acid [12]. Hydroxyl groups of PVA form boronate ester with boronic acid polymer, and lead to the formation of a hydrogel network. In presence of glucose, the gel reverts to sol due to competitive binding of glucose with borate groups.

pH sensitive hydrogels containing weakly basic or acidic groups in combination with glucose oxidase provide indirect sensitivity to glucose. Glucose is oxidized by glucose oxidase to gluconic acid, which reduces the pH of the medium, leading to increased hydrogel swelling (for weak base) or shrinking (for weak acid). Polymer carrying amino groups, are used as the pH sensitive component. pH decreases with increasing glucose concentration, leading to increased protonation of the amino groups on the polymer, increased hydrogel swelling, and higher solute permeability through the gel matrix [13]. Thus, amino polymers are potentially suitable for preparation of glucose sensitive hydrogels for controlled insulin delivery.

The direct glucose triggered systems have somewhat faster response than indirect systems, but binding of glucose to OH groups in boronic acid is not specific. The indirect glucose sensitive systems rely on enzyme oxidation of glucose, and hence are highly specific. But

they are more complex than nonenzymeic direct systems, and a particular challenge is to maintain the activity of the immobilized enzyme.

1.5 Other stimuli responsive polymers

In addition to response to pH, polymers with ionic groups are sensitive to electric field owing to their ionic charge, and hence their phase transition behavior is altered by electric field. This property of such polymers has been applied for bio-related applications such as drug delivery systems, artificial muscle, or biomimetic actuators [9]. It is reported that hydration of crosslinked hyaluronic acid hydrogels decreases as the gel is exposed to a DC voltage of 5v/cm or 10v/cm, and increases again when the current is switched off [10].

Light-responsive polymers can change their structures upon UV or visible light irradiation. It originates from their chromophores, i.e., residues with double or triple bonds or other conjugated systems [2]. The mechanism of light sensitivity including cis-trans isomerization, ionization, and ring opening that increase polymer hydrophilicity, and hence lead to irradiation stimulated phase transition (increased hydration). Typical examples of polymers carrying light sensitive residues including poly (N, N-dimethylacrylamide-4-phenylazophenyl acrylate), partially esterified poly(NIPAM-hydroxyethylacrylamide), and poly(NIPAM-triphenylmethane leuconitrile)[2]. Light-responsive polymers can be used in optical switches, displays, drug delivery.

An antigen responsive system was reported recently by Miyata et al. [11], consisting of poly(AAM-vinyl-GAR IgG) and poly(AAM-vinyl rabbit IgG). The reaction of immobilized antibody (GAR IggG) and antigen (rabbit IfG) on the two polymers forms a crosslinked gel. As free antigen is added to the system, it replaced the immobilized antigen, resulting in reduced crosslinkng density, and thus increased swelling. The authors report that the process is reversible and permeation of a model protein (hemoglobin) through the gel is antigen responsive. The antigen responsive property could thus be specifically utilized in a drug delivery system.

2. Stimuli-responsive polymer grafted surface and its application in biomedical fields

2.1 The advantages of stimuli-responsive polymers grafted surface

The stimuli-responsive polymers (SRP) can change their properties dramatically upon environment stimuli. Several applications have been proposed in a variety of area including sensors, drug delivery, and chromatography. However most SRP like PNIPAM and PAA form hydrogels with water and are crosslinked. There are two problems in the applications, the crosslinked hydrogels respond slowly to environment stimuli, and the mechanical property of hydrogels are poor.

In order to overcome these problems, SRP have been grafted or covalently coupled to solid surfaces (polymer or textile surface). Although SRP chains are covalently coupled to the surfaces, they still can respond to environment stimuli. So the graft of SRP endows the material surfaces with a new property, i.e., stimuli-responsive ability. The advantage of SRP-grafted surface is that the material substrate acts as a dimensionally stable matrix for mechanical support, whereas the conformation change of grafted SRP on the surface induced by environment stimuli results a stimuli-response surface. In addition, the stimulus response of the grafted SRP may be faster than that of crosslinked SRP hydrogel. Because the grafted chain has a freely mobile end, distinct from the typical crosslinked network structure with a relatively immobile chain end, a more rapid conformation change is expected.

SRP grafted surfaces may revolutionize certain areas of biotechnology, medicine, and smart textile due to the ensuing ability for self–recovery, self-adjustment or control, self-diagnosis, stand-by capability for detecting nonlinear onset, but also improved mechanical strength and quick stimulus response. The possible application of stimulus responsive polymers including responsive clothing, separation membranes, controlled drug release, site-selected drug delivery, cell adhesion and detachment, tissue engineering, bioreactor, biosensors, etc. Some types of application of SRP grafted surfaces will be described in detail in the rest of this section.

2.2 Cell detachment from PNIPAM grafted surface

The change in surface properties of the polymer from hydrophobic above the critical temperature to hydrophilic below it has been used successfully to detach mammalian cells

from their substrate. Mammalian cells are cultivated on a hydrophobic solid substrate and are usually detached from it by protease treatment, which often damages them. At a temperature of 37°C, a substrate surface grafted with PNIPAM is hydrophobic because this temperature is above the critical temperature of the polymer and the cells grow well. However, decreasing the temperature to 25°C or below results in the surface becoming hydrophilic, and the cells can then be easily detached without any damage.

Okano and his coworkers have successfully used the PNIPAM grafted Tissue Culture Polystyrene (TCPS) dishes to achieve temperature-modulated cell adhesion detachment control [14-18]. Active cell adhesion and proliferation on the PNIPAM-grafted surfaces are observed on surfaces modified with PNIPAM amounts ranging from 1.5 to 2.0 $\mu g/cm^2$ [14, 15]. They found that on PNIPAM grafted surface with less than 1.5 $\mu g/cm^2$, cells adhere and proliferate appropriately, but these cells never detach from the surfaces by lowering the temperature below 32 ^oC unless a digestive enzyme (trypsin) is used. Likewise, cells do not adhere to surfaces with more than 2.0 $\mu g/ cm^2$ graft yield or with a graft thickness of more than 30 nm. This cell behavior can be correlated with similar surface presence of the cell-adhesive protein, fibronectin (FN) on the PNIPAM-grafted surfaces with different PNIPAM graft amounts.

Various cells, including bovine hepatocytes[17], endothelia cells[14,15], and human keratin cells[18], have been grown on the PNIPAM grafted surfaces and has been detached by reducing temperature. For example, hepatocytes were cultured on PNIPAM grafted surfaces. After decreasing the temperature to 4^oC, nearly 100% of the hepatocytes were detached from the surfaces without loss of intercellular junctions [17]. On the contrary, in the case of trypsin treatment, these hepatocytes lose their intercellular junctions resulting in single cells. Therefore, confluent cells on PNIPAM-grafted surfaces can be recovered as contiguous intact cell monolayers by lowering the culture temperature, which avoids use of digestive enzymes and chelating agents. This process has been defined as cell sheet engineering recently [16] and Figure 2 shows the schematic and pictures of cell sheets release from the PNIPAM grafted surface. Use of PNIPAM allows temperature reduction harvest, retaining cell–cell junctions and permitting cell confluency maintenance post-harvest in large area sheets.

Furthermore, these monolayers of cells maintain basal surface extracellular matrix proteins after detachment, which can be used in readhesion of the released cell sheet on other surface. To prevent cell sheet shrinking, rigid support hydrophilic poly(-vinylidene difluoride) (PVDF) membranes [19, 20] are used to peel off the cell sheet from the PNPAM grafted surface.

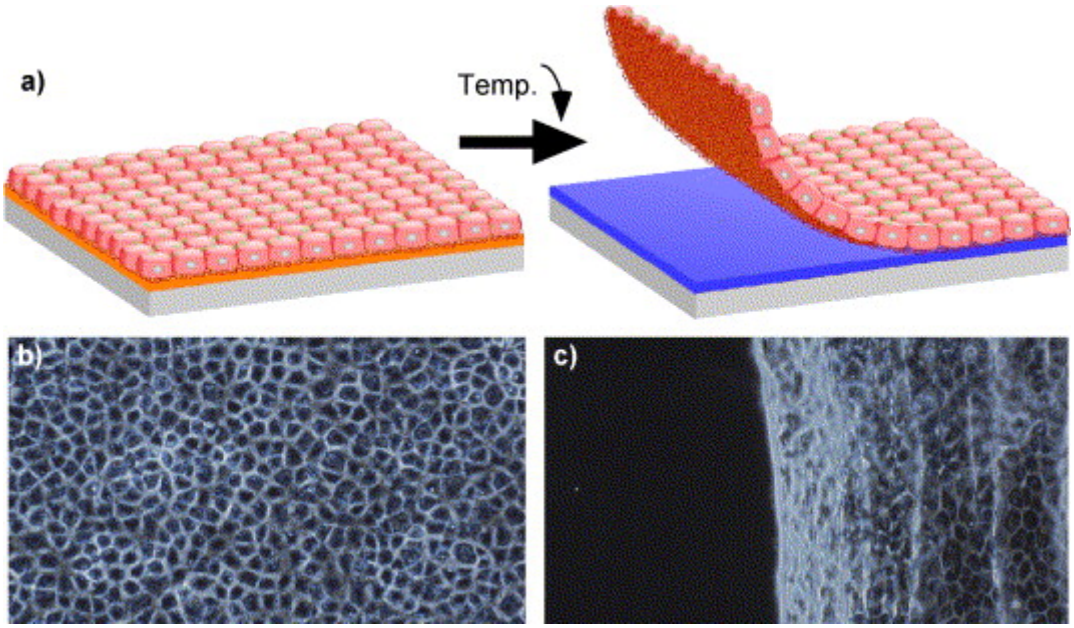


Figure 2. Cell sheet engineering using PNIPAM grafted surfaces. (a) Schematic illustration for temperature-induced recovery of intact monolayer cultures. (b) Confluent culture of endothelial cells on PNIPAM-grafted dishes at 37 °C. (c) Detaching endothelial cell sheet by lowering culture temperature to 20 °C [16].

Cell sheet engineering can be used not only as automatic cell release devices, but also in many other applications. For example, keratinocyte sheets from thermoresponsive dishes[18] are currently used clinically to treat burns and bruises. A corneal epithelium sheet transplantation is an effective treatment for patients suffering from alkali burns, or from the Stevens–Johnson syndrome[21]. Thermoresponsive culture dishes are also be utilized to prepare multi-layer tissue constructs with both homotypic [22] and heterotypic cells[23].

Ratner *et al*[24], Werner *et al*[25], and Cassinelli *et al* [26] also found cell detachment behavior on PNIPAM grafted surface after reducing the temperature. A photolithographically fabricated microheater array is used to create a cell adhesive and non-

adhesive patterned surface, which is used to direct site-specific cell attachment. Patterned adhesion of two types of cells has been visualized on the array through fluorescent markers [24].

2.3 Temperatures-Responsive Chromatography

The idea to change the structure and adsorptivity of polymer-grafted surfaces by a slight change of the temperature or the pH is very attractive, because a dramatic change in the interaction (for example adsorptivity) of this surface with a given solute may thus be achieved. One area, which depends strongly on the controlled interaction of solutes with adsorptive surfaces, is chromatography. For example, the chromatographic separation of some steroids and drugs, using HPLC-columns packed with PNIPAM grafted beads, was strongly dependent on the temperature with a steep increase in both retention and resolution when the temperature was increased from 5^{o}C to 50^{o}C.[27, 28] Especially the retention times of the more hydrophobic steroids were significantly longer at higher temperature. The retention times observed for the reference column packed with nongrafted beads, on the other hands were much shorter and decreased in face with increasing temperature. At low temperatures, retention was preferably through hydrogen bond acceptors, while hydrophobic interactions dominated the retention of the solutes at higher temperatures.

On the other hand, a combination of temperature-responsive polymeric grafts with biorecognition elements, such as affinity ligands proved to be a successful strategy for temperature controlled protein chromatography. PNIPAM interacts efficiently with Cibacron Blue (a triazine dye), which is widely used as a ligand in the dye-affinity chromatography of various nucleotide-dependent enzymes (e.g. lactate dehydrogenase[29]). The polymer binds strongly by multiple interactions with the dye ligands. At high temperatures, the polymer molecules are in a compact globular conformation capable of binding to only a few ligands on the matrix. Lactate dehydrogenase from porcine muscle has good access to the ligands and binds to the column. On lowering the temperature, the polymer molecules undergo a transition to a more expanded coil conformation; the polymer molecules now interact with more ligands and begin to compete with the bound enzyme for the ligands, thereby

displacing the enzyme. Lactate dehydrogenase has been purified using only the temperature change as the eluting factor [30]. This approach seems quite promising because it eliminates the step of separating the target protein from the eluent, which (for dye-affinity chromatography) usually involves adding a competing nucleotide or increasing the salt concentration.

2.4 **Stimuli-responsive membrane**s

One type of stimuli-responsive membranes is prepared by grafting stimuli-responsive polymers possessing functional groups onto porous membrane substrates [31-33]. The porous substrate provides mechanical strength and dimensional stability, while the conformational changes of the grafted functional polymers result in environment-responsive characteristics. These environmental responsive membranes may find applications ranging from controlled drug delivery, to chemical separation, to water treatment, to bioseparation, to chemical sensor, to tissue engineering, etc. There is an increasing interest in these intelligent membranes.

Chu and his coworkers have prepared a series of thermoresponsive gating membranes[32], with a wide range of grafting yields, by grafting PNIPAM onto porous poly(vinylidene fluoride) (PVDF) membrane substrates with a plasma-induced pore-filling polymerization method. Diffusional permeation experiments showed that two distinct types of temperature responses were observed, depending on the grafting yield. The diffusional coefficient of a solute across membranes with low grafting yields increased with temperature, while that across membranes with high grafting yields decreased with temperature. A schematic illustration of the diffusional permeability through PNIPAM-grafted membranes with different grafting yields is shown in Figure 3. When the grafting yield was low, the diffusional coefficient of the solute across the membrane was higher at temperatures above the LCST than that below the LCST, owing to the pores of the membrane being controlled open/closed by the shrinking/swelling mechanism of the grafted PNIPAM gates, while when the grafting yield was high, the diffusional coefficient was lower at temperatures above the LCST than that below the LCST, owing to the hydrophilic/hydrophobic phase transition of the grafted PNIPAM gates.

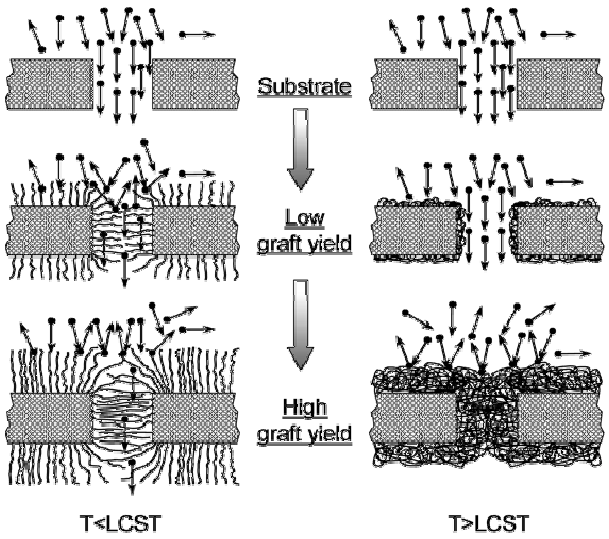


Figure 3. Schematic illustration of thermoresponsive diffusional permeability through PNIPAM-grafted membranes with different grafting yields [32].

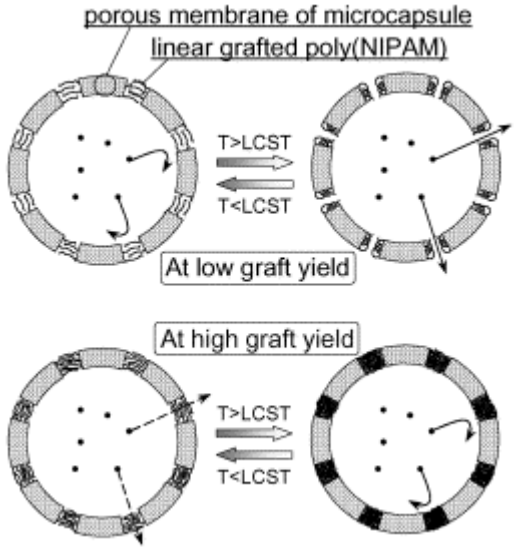


Figure 4. A schematic representation of the thermo-responsive release principle of core-shell microcapsules with a porous membrane and thermo-responsive polymeric gates [33].

Chu and his coworkers have extended this system to a thermo-responsive core-shell microcapsule with a porous membrane and poly(*N*-isopropylacrylamide) (PNIPAM) gates(Figure 4)[33]. It was prepared using interfacial polymerization to prepare polyamide core-shell microcapsules, and plasma-graft pore-filling polymerization to graft PNIPAM into

the pores in the microcapsule wall. The proposed thermo-responsive microcapsule could be a positive thermo-response controlled-release one or a negative thermo-response one by changing the PNIPAM graft yield.

2.5 Controlled drug release

Controlled drug release is a system in which a drug is liberated in response to a chemical signal (e.g. insulin release in response to rising glucose concentration). Then this drug can be controlled released or site-selected released. The swelling or shrinking of SRP-grafted surface in response to small changes in pH or temperature can be used successfully to control drug release, because the diffusion of the drug out of the surfaces depends on the gel state. When a smart polymer is integrated into a microcapsule wall, the conformational transition of the polymer affects the integrity of the porous film or microcapsule and allows the regulated release of the drugs loaded into the microcapsule or liposome.

The development of a glucose-sensitive insulin-releasing system for diabetes therapy is a long-standing challenge for biomedical engineers. Unsurprisingly, therefore, it became a popular model for the systems using smart polymers. An insulin-loaded matrix has been produced by mechanical mixing and compression a dry pH-responsive polymer, poly[(*N*,*N*-dimethylamino)ethyl methacrylate-*co*-ethylacrylamide], glucose oxidase, bovine serum albumin and insulin. Exposure to glucose resulted in the oxidation of glucose to gluconic acid and thus a decrease in the pH, protonation and swelling of the polymer, accompanied by insulin release. The insulin release stopped within 10 min of glucose removal and could be restimulated by glucose addition [34]. A variety of designs for an insulin-delivery system that responds to glucose were also developed by Klumb and Hotter using glucose-sensitive membranes containing glucose oxidase [35].

The specific release of insulin in response to glucose could also be designed in the form of a ‘chemical valve’ (Figure 5) [36]. Glucose oxidase can be immobilized on a pH-responsive poly(acrylic acid) layer grafted onto a porous polycarbonate membrane. Under neutral conditions, polymer chains are densely charged and have an extended conformation, preventing insulin transport through the membrane by blocking the pores. Upon exposure to

glucose, the pH drops and the polymer chains become protonated and adopt a more compact conformation. The blocking of pores is reduced and insulin is transported through the membrane [37].

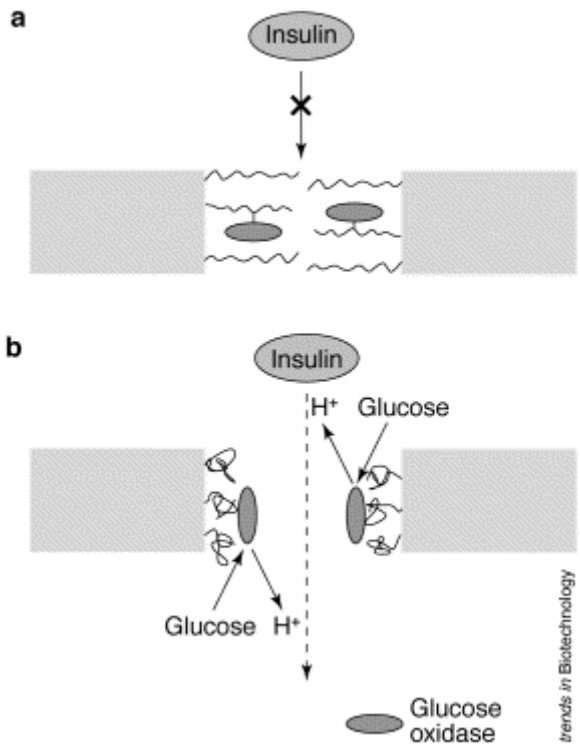


Figure 5. Schematic presentation of a 'chemical valve'[36]. Glucose oxidase is immobilized on pH-responsive polyacrylic acid, which is grafted onto a porous polycarbonate membrane. (a) Poly(acrylic acid) is in an expanded conformation blocking insulin transport. (b) The oxidation of glucose is accompanied by a decrease in pH and the transition of poly(acrylic acid) into a compact conformation, resulting in the opening of the pores and the transport of insulin[36].

3. Grafting of SPR on polymer surface

3.1 Introduction

Grafting SRP onto the surfaces endows the surface with considerable stimuli-responsive properties. The SRP-grafted polymer surfaces have many advantages compared to pure SRP.

They may revolutionize certain areas of biotechnology, medicine, and smart textiles. Unsurprisingly, therefore, grafting of SRP on polymer surface has become a popular research topic recently. Therefore, various grafting methods of SRP will be reviewed in this section.

There are in principle two methods for producing grafted surfaces, as schematically illustrated in Figure 6: direct coupling reaction of existing polymer molecules to the surface and graft polymerization of monomers to the surfaces[38].

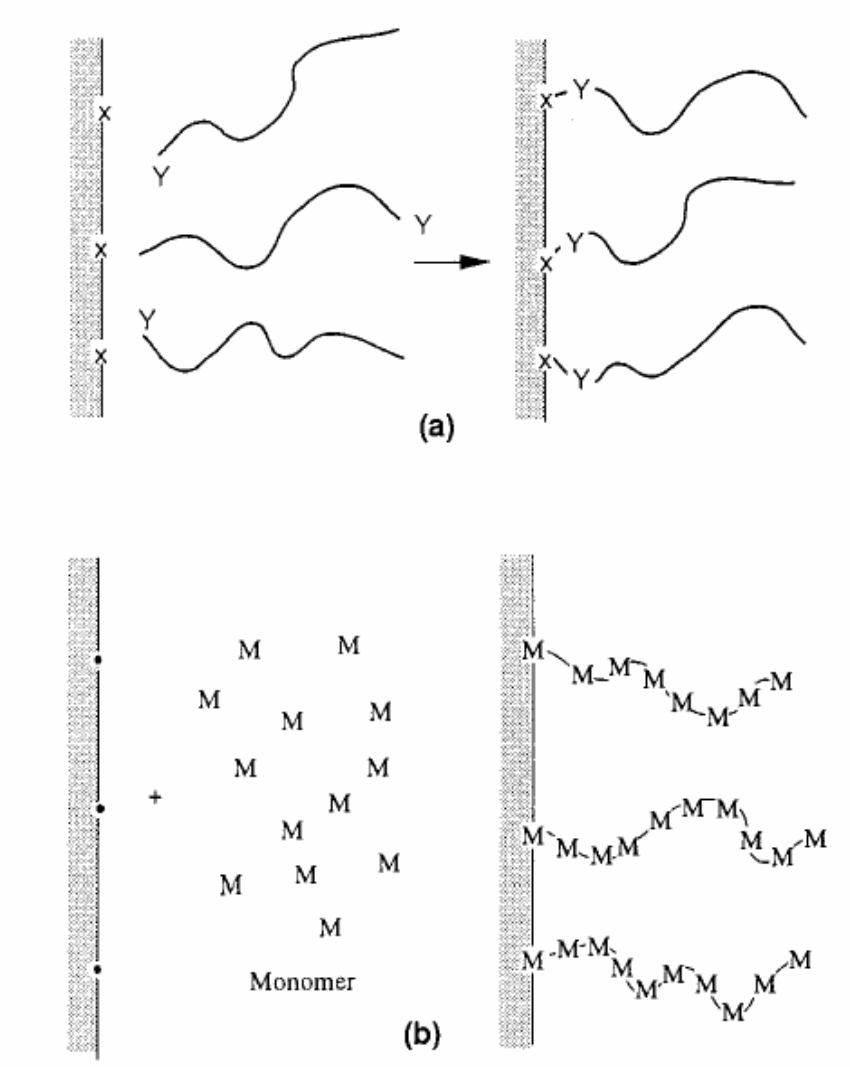


Figure 6. Creation of grafted surfaces by: a direct polymer coupling reaction; b graft polymerization.

If the polymer surface to be modified possesses reactive groups capable of reactive with SRP, surface modification can be readily conducted by chemical coupling reaction (Figure 8a). For example, PAA has been grafted onto Nylon surface using the reaction of the carboxyl groups with amide end groups on Nylon [39]. The direct polymer coupling reaction will not be discussed further in this review. Because normally this procedure needs two special groups, it is not straightforward enough for industry applications, although the precise control of chain structure.

Most material and monomer do not have paired coupling bonds, so the grafting can not happen in a direct polymer coupling reaction. However, the second grafting method, i.e., graft copolymerization (Figure 8), can be used. The material surface can be activated by chemical reagent, or high energy irradiation. Activation creates active species, including free radicals, peroxide groups, and ions formed on the surface. The active species can initiate graft polymerization when they are in contact with monomer. Monomers usually have double bonds or cyclical bonds, which can be broken to initiate and propagate the polymerization.

A variety of methods for graft polymerization onto different substrate surfaces from recent reports will be described below. Plasma induced graft copolymerization is very versatile and convenient, hence, it will be described in the next section in detail.

3.2 Chemical reagent grafting

Many chemical reagents have been used to activate material surfaces and initiate graft copolymerization. The activation is usually through a redox reaction between tertian chemical groups and chemical reagents. Free radicals are formed on the surface as a result of the reaction [40]. For example, Cerium in its tetravalent state (Ce^{4+}) is a versatile oxidizing agent that through various redox reactions with many different organic substrates can create free radicals capable of initiating graft copolymerization [41]. Therefore, one application of ceric ion initiation is the graft copolymerization of cellulose with vinyl monomers. For example, PNIPAM has been grafted onto cellulose substrate by ceric ions[42, 43]. Polyacrylic Acid (PAA) has been grafted onto rayon filament by ceric ions and Co-60 high

energy source [44]. It was found that ceric ion initiation produced more degradation than the Co-60 initiation. Beside ceric ions, other chemical reagents are also used in grafting, e.g., Poly(Methyl Methanacrylic acid) has been grafted onto chitosan with Fenton's reagent (Fe^{2+}-H_2O_2) as a redox initiatiator[45]. Thermoresponsive Hyaluronic Acid (HAs) were prepared by graft polymerization of NIPAM on HA using dithiocarbamate which is a kind of iniferte(initiator, transferator, and terminator)[46].

3.3 UV grafting

UV energy has been extensively applied for surface graft polymerization of polymers with the aid of a photo initiator or photo sensitizer, such as benzophenone (BP). Earlier reports were concerned with UV irradiation at the vapor phase of monomer and sensitizer under a reduced pressure or in the presence of inert gas. The surface of polyethylene (PE) and Polystyrene (PS) was modified by grafting with Acrylic Acid (AA)[47]. BP and AA in the vapor phase were UV-irradiated in the presence of a polymer substrate. Grafting was affected by the solvent/carrier used and the type of polymer to be grafted. PS was easier to graft, presumably because it contains easily abstractable, tertiary hydrogens. PNIPAM was also grafted onto PS and Polyethylene terephthalate (PET) surface by immersing substrates into monomer solution under N2 and exposing them to UV radiation [48]. A novel thermo-sensitive switching membrane regulated by pore-covering polymer brushes was prepared by photografting NIPAM onto the PET track membranes with BP as initiator[49].The photografting was carried out as presented in Figure 7 and 8.

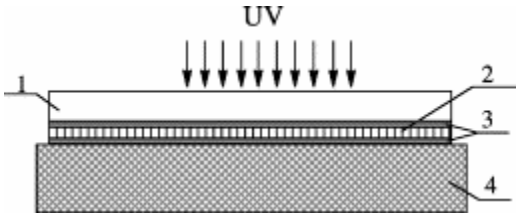


Figure 7. Schematics of photografting: (1) quartz glass; (2) PET track membrane; (3) reaction solution; (4) temperature controllable plate [49].

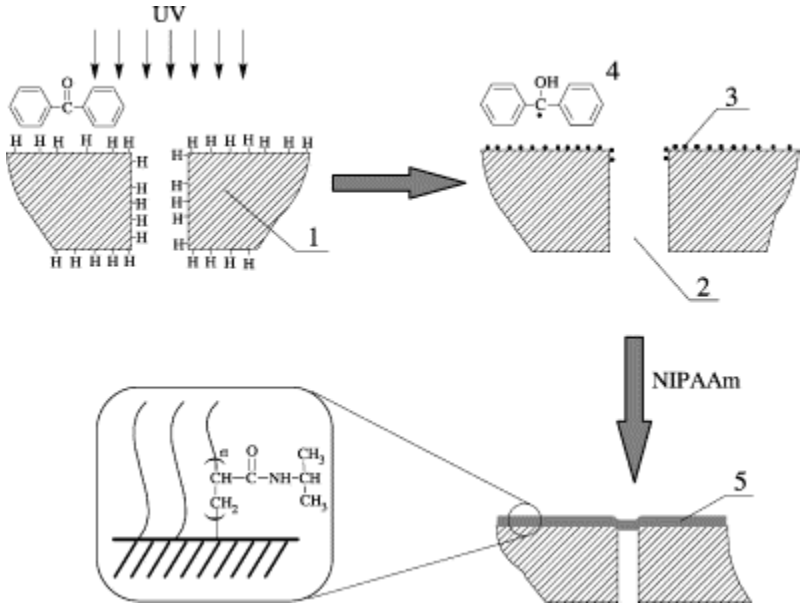


Figure 8. Chemistry of BP initiated photografting polymerization: (1) PET nucleopore membrane; (2) membrane pore; (3) surface free radical; (4) semipinacol radical; (5) graft layer of PNIPAM [49].

3.4 Ozone treatment grafting

Exposure of isotactic Polypropylene (PP) to ozone resulted in surface oxidation and formation of peroxides and hydrogen peroxides . 2-hydroxyethyl methacrylate was grafted to the ozonated samples, and the graft copolymer acted as a continuous matrix consequently linked to and reinforced by the PP crystals[50]. Cellulose fiber-supported hydrogels sensitive to pH were prepared by ozone-induced graft polymerization of AAc using cotton linters and wood pulp fiber substrates [51]. Immediately after ozone treatment, the substrates were placed in the monomer solution prepared by diluting monomer in deionized water containing ammonium sulfate hexahydrate salt (Mohr's salt), which forms a redox initiator. Polyacrylamide was also grafted on poly(ether urethane) by ozone pretreatment[52].

3.5 γ- irradiation grafting

PNIPAM and PAAc have been grafted on various substrates, including PTFE, PVDF, and cotton, by means of γ- irradiation [53-55]. Typically, substrates are exposed to γ- irradiation

to form free radicals and peroxide groups in the surface. Then the preirradiated substrate was immersed in grafting monomer solution at desired temperature with bubbling N2 for various a certain period of time. Beside γ-induced graft copolymerization can also be carried out by simultaneous irradiation and grafting through in situ forming free radicals. Jan et al. [56] modified a porous poly(vinylidene fluoride) membrane to introduce positive charge by γ irradiation in the presence of vinyl-triphenyl-phosphonium bromide monomer.

3.6 Electron Beam (EB) grafting

Various vinyl polymers were grafted onto an ultrahigh molecular weight polyethylene (UHMWPE) fiber surface after pretreatment with electron beam irradiation[57]. Acrylamide was also grafted onto PCL using EB preirradiation followed by graft copolymerization [58]. Okano and his coworkers have grafted PNIPAM onto Tissue culture grade polystyrene(TCPS) dishes via EB irradiation and used the grafted TCPS as cell release device and cell sheet engineering[15]. Briefly, 30~100 μl amounts of PNIPAM 2-propanol solution is coated uniformly over 6 well TCPS plate surfaces, followed by electron beam irradiation with a dose of 0.3MGy at 150kv. During this procedure, the NIPAM monomer is grafted on to the TCPS surfaces. And then it is rinsed by water. Various types of cultured cells adhere and proliferate on PNIPAM-grafted culture dish surfaces under normal culture conditions at 37 ^{o}C with 5% CO2. These adhesion–proliferation processes are comparable to that on conventional TCPS dishes.

4. Grafting of SRP by vacuum plasma treatment

4.1 Introduction of vacuum plasma

Traditionally plasma is a vacuum system. Plasma is an ionized gas containing both charged and neutral species, including free electrons, positive and/or negative ions, atoms, and molecules. Ions and free radicals are formed from ion and electron collisions. It is typically obtained when gases are excited into energetic states by radio frequency (rf), microwave, or electrons from a hot filament discharge. Plasma is a highly unusual and reactive chemical

environment in which many plasma-surface reactions occur. Low-pressure (vacuum) plasma has been used extensively to modify the surface properties of materials, including wettability, finishing, dyability, functionalization, sterilization, and cross link. The plasma is effective at near-ambient temperature and can modify almost any kinds of geometry. Furthermore, plasma treatment modifies only the near surface of treated substrates and does not change the bulk material properties [59].

4.2 Vacuum plasma grafting

It should be stressed that polymer surfaces can also be modified by graft copolymerization utilizing free radical or peroxides generated by the plasma treatment, similar to the other irradiation systems. When polymeric materials are exposed to plasma, radicals are created in the polymer chains. These radicals can and do initiate grafting copolymerization reactions when put in contact with monomers in the liquid or gas phase. Since the plasma produces radicals only close to the surface of the polymers, plasma-grafting copolymerization is restricted to the near surface [60].

In the literature, only conventional vacuum plasma is used to graft vinyl polymer on material surfaces. There are mainly three different methods to graft monomers onto polymer surface using plasma, i.e., i) grafting in monomer solution after plasma treatment, ii) grafting in monomer vapor after plasma treatment, iii) simultaneous plasma treatment and grafting in vapor phase.

4.2.1 Grafting in monomer solution after plasma treatment.

Grafting in monomer solution after plasma treatment is the most commonly used method. Briefly, the substrates are pretreated by plasma for certain time and then immersed into monomer solution under N_2 (or other inert gas) at a specific temperature for specific time. For example, a PTFE surface was modified by the graft polymerization of sodium vinylsulfnate [61]. The PTFE film surface was irradiated by Argon plasma for 10-300 seconds to form carbon radicals on the PTFE surface. It was subsequently exposed to air for

5min to modify carbon radicals to peroxide groups, which are able to initiate graft copolymerization. The PTFE film was then immersed into an aqueous solution of sodium vinylsulfonate at 65~80^{o}C for 1-72 hour under N_2. Similarly, PNIPAM was grafted onto porous PE membranes [62-64] and poly(vinylidenefluoride) (PVDF) [32] membrane by a plasma induced graft copolymerization. Poly(acrylic acid) was grafted using this methods to various substrates, including silicone rubber[65], PVDF[66], polyamide[67], poly[1-(trimethylsilyl)-1-propyne] [68], and PU[69].

Besides grafted on the surface, PNIPAM has also been grafted onto the pores of Polyamide microcapsules by a pore filling polymerization. Interfacial polymerization is used to prepare polyamide core-shell microcapsules, and plasma-graft pore-filling polymerization to graft PNIPAM into the pores in the microcapsule wall [33].

4.2.2 Grafting in vapor monomer solution after plasma treatment.

After plasma treatment, the plasma grafting copolymerization can also occur in the vapor phase beside liquid phase. The polymer materials are put into plasma chamber. After the plasma treatment is completed and the plasma gas is cut off, the monomer vapor will be introduced into the chamber, and graft copolymerization occurs. For example, polar monomers have been grafted onto polyolefin surfaces with the aid of inert gas plasma [4]. In the first stage, inert gas plasma (argon plasma) was used to generate free radicals on the polyolefin surface. In the second stage, the plasma generator was turned off and a vinyl monomer was introduced as a vapor. Polymer was surface grafted by free radical polymerization of the monomer vapor.

4.2.3 Simultaneous plasma treatment and grafting in monomer vapor phase.

Above methods contains two steps, plasma pretreatment and graft polymerization. SRP can be grafted onto material surface via one step methods, too, i.e., simultaneous plasma treatment and grafting in monomer vapor phase. Ratner et al [70] has grafted PNIPAM onto various substrates by this method. Figure 9 shows this method. Briefly, the monomer vapor is

introduced to the plasma chamber and the chamber pressure is controlled independently by a throttle valve and, in this case, maintained to 100mT. When the substrate is irradiated by plasma, the monomer grafting occurs simultaneously. Gancarz et al. [71] modified polysulfone membranes with acrylic acid (AA) using plasma-initiated graft polymerization via three methods, i.e., (1) grafting in solution; the plasma-treated polymer membrane was exposed to air for 5 min and dipped into deaerated aqueous solution of monomer; (2) grafting in vapor phase; when Ar plasma treatment on polymers was completed, the argon flow was cutoff and monomer vapor was introduced into the chamber; (3) simultaneous plasma treatment and grafting: a polymer membrane was placed in a plasma reactor; the reactor was evacuated and Ar was introduced and vapors of monomer were introduced to give a desired pressure. Comparing the above three methods, the polysulfone membrane with AA plasma-initiated grafting in the vapor phase of the monomer seemed to be the most promising from the point of view of filtration properties.

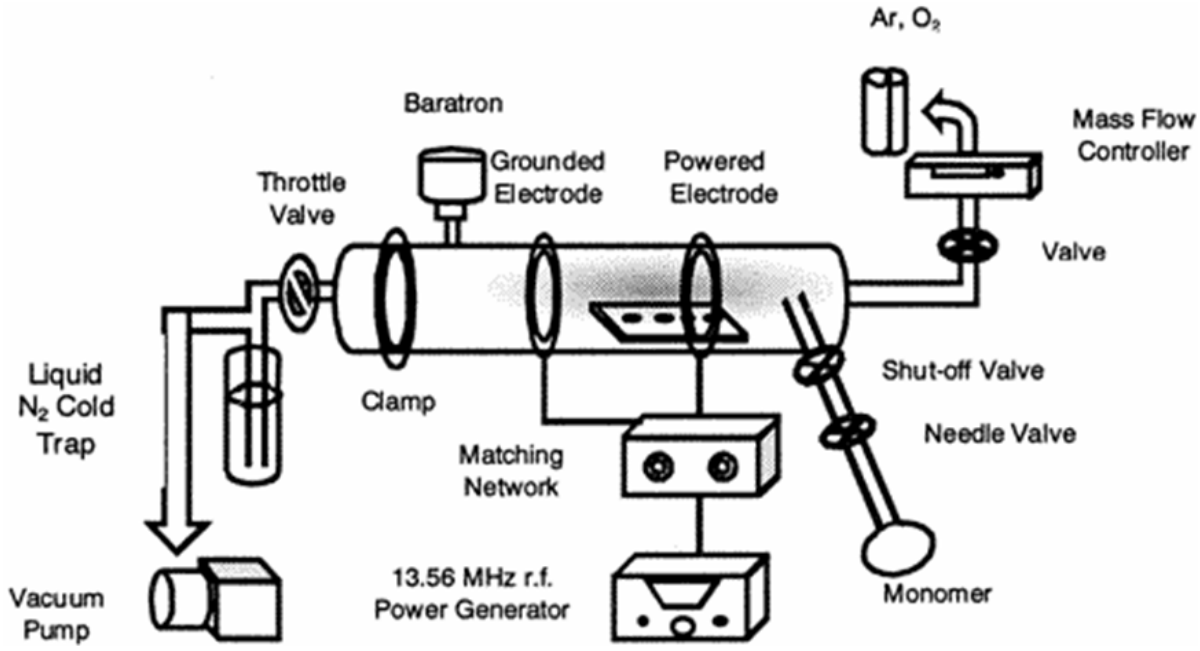


Figure 9. Schematic diagram of radio frequency plasma polymerization reactor utilized for the deposition of smart PNIPAM coating [70].

4.3 Disadvantages of vacuum plasma grafting and atmospheric plasma system

Over all, vacuum plasma is widely used to graft polymers on material surface. Compared to other irradiation grafting methods, vacuum plasma offers flexibility, effectiveness, safety, and environmental friendliness [59].Although vacuum plasma process has so many advantages over other methods, it require low-pressure conditions. Therefore, there is still need a high cost and limitation of processing of large surface area and continuous production lines.

In light of the disadvantages of low-pressure plasma systems, atmospheric pressure plasma treatment has recently emerged as a novel technique. By using an atmospheric plasma treatment device, the machine can operate in a pre-existing continuous processing line. Atmospheric pressure can also save the cost of vacuum environment. It has been successfully used to change material surface adhesion, wettability, and finishing processing [72-74].

There is no research about grafting of vinyl monomers using atmospheric plasma treatment in literature yet. Therefore, atmospheric plasma treatment will be used to graft various substrates in this thesis. pH sensitive polyacrylic acid and thermoresponsive PNIPAM will be grafted on nylon and PS surfaces. Cell adhesion/detachment will be studied on the PNIPAM grafted PS cell culture plates. Responsive textile will be made by grafting PNIPAM on cotton fabrics and then heat diffusion at different temperature will be studied

5. References:

1. I.Y. Galaev and B. Mattiasson, Stimulus Responsive Surfaces: Possible Implication for Biochromatography, Cell Detachment and Drug Delivery. *In*: R. Freitag (ed) *Synthetic Polymers for Biotechnology and Medicine(p 116~129).* Georgetown, Texas: Landes Bioscience 2003.
2. X.Y. Wu, Q. Zhang, and R. Arshady, Stimuli Sensitive Hydrogels. In: R. Arshady (ed) *Introduction to Polymer Biomaterials*. London, UK: Citus, 2003.
3. A.S. Lee, V. Butun, M. Vamvakaki, S.P. Armes, J.A. Pople and A.P. Gast, Structure of pH-dependent block copolymer micelles: charge and ionic strength dependence. *Macromolecules*, 35: 8540–8551, 2002.
4. E.S. Gil and S. M. Hudson, Stimuli-responsive polymers and their bioconjugates. *Progress in Polymer Science*, 29: 1173-1222, 2004.
5. M. Heskins and J.E. Guillet, Solution properties of poly(N-isopropylacrylamide). *J. Macromol. Sci. Chem.* 2:1441-1455, 1968.

6. W.C. Wooten, R.B. Blanton, and H.W. Coover, Effect of pH on homopolymerization of N-isopropylacrylamide. *J. Polym. Sci.* 25:403-412, 1957.
7. H.G. Schild, Poly(N-isopropylacrylamide): Experiment, theory and application. *Prog Polym Sci*, 17:163-249, 1992.
8. A.J. Kuijpers, G.H.M. Engbers, J. Feijen, S.C. De Smedt, T.K.L. Meyvis, J. Demeester, J. Krijgsveld, S.A.J. Zaat and J. Dankert, Characterization of the network structure of carbodiimide cross-linked gelatin gels. *Macromolecules,* 32: 3325–3333, 1999.
9. T. Shiga, Deformation and viscoelastic behavior of polymer gels in electric fields. *Adv. Polym. Sci.i* 134: 131–163, 1997.
10. R. Tomer, D. dimitrijevic, A.T. Florence, Electrically controlled-release of macromolecules from cross-linked hyaluronic-acid hydrogel. *J. Control . Release*, 33: 405-413, 1995.
11. T. Miyata, N. Asami, and T. Uragami, A reversibly antigen-responsive hydrogel. *Nature*, 399: 766-769, 1999.
12. S. Kitano, I. Hisamitsu, Y. Koyama, K. Kataoka, T. Okano, and Y. Sakurai, Effect of the incorporation of amino groups in a glucose-responsive polymer complex having phenylboronic acid moieties. *Polym. Adv. Tech.*, 2: 261-264, 1991.
13. K. Podual, F. J. Doyle III, and N. A. Peppas, Glucose-Sensitivity of glucose oxidaase-containing cationic copolymer hydrogels having poly(ethylene glycol) grafts. *J. Control. Rel.,* 67: 9-17, 2007.
14. Y. Akiyama , A. Kikuchi , M. Yamato , and T. Okano T, Ultrathin poly(N-isopropylacrylamide) grafted layer on polystyrene surfaces for cell adhesion/detachment control. *Langmuir*, 20 (13): 5506-5511, 2004.
15. Y. Tsuda, A. Kikuchi, M. Yamato, S. Yasuhisa, and U. Mitsuo, Control of cell adhesion and detachment using temperature and thermoresponsive copolymer grafted culture surfaces. *J. Biomed. Mater. Res.,* 69A:70-78, 2004.
16. A. Kikuchi and T. Okano, Nanostructured designs of biomedical materials: Applications of cell sheet engineering to functional regenerative tissues and organs. *Journal of Controlled Release*, 101 (1-3): 69-84, 2005.
17. F. A. Tobiesen and S. michielsen, Method for grafting poly(acrylic acid) onto nylon 6,6 using amine end groups on nylon surface. *J. Polym. Sci. Part A: Polym. Chem.*, 40: 719-728, 2002.
18. M. Yamato, M. Utsumi, A. Kushida, C. Konno, A. Kikuchi, and T. Okano, Thermo-responsive culture dishes allow the intact harvest of multilayered keratinocyte sheets without dispase by reducing temperature. *Tissue Engineering*, 7: 473-480, 2001.
19. A. Kushida, M. Yamato, C. Konno, A. Kikuchi, Y. Sakurai and T. Okano, Decrease in culture temperature releases monolayer endothelial cell sheets together with deposited fibronectin matrix from temperature-responsive culture surfaces, *J. Biomed. Mater. Res.*, 45: 355-362, 1999.
20. M. Hirose, O.H. Kwon, M. Yamato, A. Kikuchi and T. Okano, Creation of designed shape cell sheets that are noninvasively harvested and moved onto another surface. *Biomacromolecules*, 1(3): 377–381, 2000.
21. K. Nishida, M. Yamato, Y. Hayashida, K. Watanabe, N. Maeda, H. Watanabe, K. Yamamoto, S. Nagai, A. Kikuchi, Y. Tano and T. Okano, Functional bioengineering

corneal epithelial sheet grafts from corneal stem cells expanded ex vivo on a temperature-responsive cell culture surface. *Transplant*, 77(3): 379–385, 2004.
22. T. Shimizu, M. Yamato, Y. Isoi, T. Akutsu, T. Setomaru, K. Abe, A. Kikuchi, M. Umezu and T. Okano, Fabrication of pulsatile cardiac tissue grafts using a novel 3-dimensional cell sheet manipulation technique and temperature-responsive cell culture surfaces. *Circ. Res.,* 90: e40–e48, 2002.
23. M. Harimoto, M. Yamato, M. Hirose, C. Takahashi, Y. Isoi, A. Kikuchi, and T. Okano, Novel approach for achieving double layered cell sheets co-culture: overlayering endothelial cell onto monolayer hepatocytes utilizing temperature-responsive culture dishes. *J. Biomed. Mater. Res.*, 62(3): 464–470, 2002.
24. X. Cheng, Y. Wang, Y. Hanein, K.F. Böhringer, and B.D. Ratner, Novel cell patterning using microheater-controlled thermoresponsive plasma films. *Journal of Biomedical Material Research Part A*, 70A (2): 159-168, 2004.
25. D. Schmaljohann, J. Oswald, B. Jorgensen, M. Nitschke, D. Beyerlein, and C. Werner, Thermo-responsive PNiAAm-g-PEG films for controlled cell detachment. *Biomacromolecules*, 4 (6): 1733-1739, 2003.
26. M. Morra and C. Cassinelli, Thermal recovery of cells cultured on poly(N-isopropylacrylamide) surface-grafted polystyrene dishes. In B. D. Ratner and D. G. Castner (Ed) *Surface Modification of Polymeric Biomaterials*(175-181), New York and London: Plenum Press 1997.
27. H. Kanazawa, Y. Kashiwase, K. Yamamoto et al, Temperature-responsive liquid chromatography. 2. Effects of hydrophobic groups in N-isopropylacrylamide copolymer-modified silica. *Anal. Chem.*, 69:823-830, 1997.
28. K. Hosoya, K. Kimata, T. Araki et al, Temperature-controlled high-performance liquid chromatography using a uniformly sized temperature-responsive polymer-based packing material. *Anal. Chem.*, 67:1807-1911, 1995.
29. B. Mattiasson, I.Y. Galaev and N. Garg., Polymer-shielded dye-affinity chromatography. *J. Mol. Recog.*, 9: 509–514, 1996.
30. I.Y. Galaev, C. Warrol and B. Mattiasson., Temperature-induced displacement of proteins from dye-affinity columns using an immobilized polymeric displacer. *J. Chromatogr. A*, 684: 37–43, 1994.
31. W. C. Wang, G. T. Ong, S. L. Lim, R. H. Vora, E. T. Kang and K. G. Neoh, Synthesis and characterization of fluorinated polyimide with grafted poly (N-isopropylacrylamide) side chains and the temperature-sensitive microfiltration membranes. *Ind. Eng. Chem. Res.*, 42: 3740-3749, 2003.
32. Y. Li, L. Y. Chu, J. H. Zhu, H. D. Wang, S. L. Xia, and W. M. Chen, Thermoresponsive gating characteristics of poly(N-isopropylacrylamide) –grafted porous poly(vinylidenefluoride) Membranes. *Ind. Eng. Chem. Res.*, 43: 2643-2648, 2004.
33. L. Y. Chu, S. H. Park, T. Yamaguchi, S. I. Nakao, Preparation of thermo-responsive core-shell microcapsules with a porous membrane and poly(N-isopropylacrylamide) gates. *J. Memb. Sci.*, 192:27-39, 2001.
34. S.H. Yuk, S.H. Cho and S.H. Lee, pH/Temperature-responsive polymer composed of poly((N,N-dimethylamino)ethyl methacrylate-co-ethylacrylamide). *Macromolecules*, 30**:** 6856–6859,1997.

35. L.A. Klumb and T.A. Horbett, Design of insulin delivery devices based on glucose sensitive membranes. *J. Control. Release*, 18**:** 59–80, 1992.
36. I. Y. Galaev and B. Mattiasson, 'Smart' polymers and what they could do in biotechnology and medicine. Trends in Biotechnology, 17 (8): 335-340, 1999.
37. Y. Imanishi and Y. Ito, Glucose-sensitive insulin-releasing molecular systems. *Pure Appl. Chem.,* 6*7* (1995), pp. 2015–2021.
38. Yoshikimi Uyama1, Koichi Kato2, and Yoshito Ikada1, Surface Modification of Polymers by Grafting. Advances in Polymer Science, 147:1-39, 1998.
39. F. A. Tobiesen and S. Michielsen, Method for grafting poly(acrylic acid) onto nylon 6,6 using amine end groups on nylon surface. *J. Polym. Sci. Part A- Polym. Chem.*, 40 (5): 719-728, 2002.
40. D.W. Jenkins, S. M. Hudson, Review of vinyl graft copolymerization featuring recent advances toward controlled radical-based reactions and illustrated with chitin/chitosan trunk polymers. *Chemical Reviews*, 101 (11): 3245-3273, 2001.
41. S. Nagarajan, S. Sudhakar, K. S. V. Srinivasan, Block copolymerization initiated by Mn(III)-poly(ethylene glycol) redox system- general features and kinetics. *Colloid and Polymer Science*, 272 (7): 777-783, 1994.
42. J. Xie, and Y. L. Hsieh, Thermosensitive poly(N-isopropylacrylamide) hydrogels bonded on cellulose supports. *J. Appl. Polym. Sci.*, 89:999-1006, 2003.
43. K. C. Gupta and K. Khandekar, Temperature-responsive cellulose by Ceric(IV) ion-initiated graft copolymerization of N-isopropylacrylamide. *Biomacromolecules*, 4:758-765, 2003.
44. D. J. McDowall, B. S. Gupta, and V. T. Stannett, A comparison of the properties of ceric ion and preirradiated acrylic acid grafts to rayon fibers. *Polymer Journal*, 19(5): 643-648, 1987.
45. A. Lagos and J. Reyes, Grafting onto chitosan. I. graft copolymerization of methyl methacrylate onto chitosan of methyl methacrylate onto chitosan with Fenton's reagent (Fe2+-H2O2) as a redox initiator. *J. Polym. Sci Part A*, 26:985-991, 1988.
46. S. Ohya, Y. Nakayama, and T. Matsuda, Thermoresponsive artificial extracellular matrix for tissue engineering: hyaluronic acid bioconjugated with poly(N-isopropylacrylamide) grafts. *Biomacromolecules*, 2: 856-863, 2001.
47. K. Allmer, A. Hult and B. Rånby , Surface modification of polymers. I. Vapor phase photografting with acrylic acid. J*. Polym. Sci.*, Polym Chem, 26: 2099–2111, 1988.
48. P. S. Curti, M. R. De Moura, E. Radovanovic, A. F. Rubira, and E. C. Muniz, Surface modification of polystyrene and poly(ethylene terephthalate) by grafting poly(N-isopropylacrylamide). *Journal of Material Science*: Materials in Medicine, 13: 1175-1180, 2002.
49. B. Yang, W. T. Yang, Thermo-sensitive switching membranes regulated by pore-covering polymer brushes. *Journal of Membrane Science*, 218 (1-2): 247-255, 2003.
50. P. Gatenholm, T. Ashida, and A.S. Hoffman , Hybrid biomaterials prepared by ozone-induced polymerization. 1. Ozonation of microporous polypropylene. *J. Polym. Sci.*, 35(8): 1461–1467, 1997.
51. J.O. Karlsson and P. Gatenholm , Cellulose fiber-supported pH-sensitive hydrogels. *Polymer*, 40 (1999), pp. 379–387.
52. K. *Fujimotok*, Y. Takebayashi, H. Inoue, and Y. Ikada, Ozone-induced graft polymerization onto polymer surface. *J. Polym. Sci. Part A*, 31:1035-1043, 1993.

53. J. Liu, M. Zhai, and H. Ha, Pre-irradiation grafting of temperature sensitive hydrogel on cotton cellulose fabric. *Radiation Physics and Chemistry*, 55: 55-59, 1998.
54. R. Mazzei, E. Smolko, D. Tadey, and L. Gizzi, Radiation grafting of NIPAAm on PVDF nuclear track membranes. *Nuclear Instrumentas & Methods in Physics Research B- Bam Interacions with Materials & Atoms*, 170: 419-426, 2000.
55. O. Palacios, R. Aliev, and G. Burillo, Radiation graft copolymerization of acrylic acid and N-isopropylacrylamide from binary mixtures onto polytetrafluoroethylene. *Polymer Bulletin*, 51: 191-197, 2003.
56. D. Jan, J. S. Jeon, and S. Raghavan. *J. Adhesion Sci. Technol.*, 8:1157–1168, 1995.
57. L. Zhang, Z. Liu, N. Yang, A. Zhang. Zhongguo *Fangzhi Daxue Xuebao*, 21: 88–93, 1995.
58. M. Ohrlander, T. Lindberg, A. Wirsën, and A. C. Alebertsson, the grafting of acrylamide onto poly(ε-caprolactone) and poly(1, 5- dioxepan-2-one) using electron beam preirradiation. II. An in vitro degradation study on grafted poly(ε-caprolactone). *J. Polym. Sci. Part A*, 37: 1651-1657, 1999.
59. I. H. Loh, Plasma surface modification in biomedical application. *AST Technical Journal*, 2004 (www.astp.com).
60. P.K. Chu, J.Y. Chen, L.P. Wang, and N. Huang, plasma-surface modification of biomaterials", *Material Science and Engineering R.*, 36:143-206, 2002.
61. N. Inagaki, S. Tasaka, Y. Goto, Surface modification of poly(tetrafluoroethylene) film by plasma graft polymerization of sodium vinylsulfonate. J. *Appl. Polym. Sci.*, 66:77-84, 1997.
62. J. Huang, X. Wang, X. Chen, and X. Yu, Temperature-sensitive membranes prepared by the plasma-induced graft polymerization of N-isopropylacrylamide into porous polyethylene membrane. *J. Appl. Polym. Sci.*, 89:3180-3187, 2003.
63. X. Wang, J. Huang, X. Chen, and X. Yu, Graft polymerization of N-isopropylamide into a microporous polyethylene membrane by the plasma method: technique and morphology. *Desalination*, 146: 337-343, 2002.
64. J. Huang, X. Wang, W. Qi, and X. Yu, Temperature sensitivity and electrokinetic behavior of a N-isopropylacrylamide grafted microporous polyethylene membrane. *Desalination*, 146:345-351, 2002.
65. S. Lee, G. Hsiure, P. C. Chang, and C. Kao, Plasma-induced grafted polymerization of acrylic acid and subsequent grafting of collagen onto polymer film as biomaterials. *Biomaterials*, 17:1599-1608, 1996.
66. D. Klee, Z. Ademovic, A. Bosserhoff, H. Hoecker, G. Maziolis, and H. Erli, Surface modification of poly(vinylidenefluoride) to improve the osteoblast adhesion. *Biomaterials*, 24:3663-3670, 2003.
67. Y. M. Lee, S. Y. Ihm, J. K. Shim, and J. H. Kim, Preparation of surface-modified stimuli-responsive polymeric membranes by plasma and ultraviolet grafting methods and their riboflavin permeation. *Polymer*, 36:81-85, 1995.
68. T. Masuda, M. Kotoura, K. Tsuchihara, and T. Higashimura, Glow-discharge-induced graft polymerization of acrylic acid onto poly[1-(trimethylsilyl)-1-propyne] film. *J. Appl. Polym. Sci.*, 43:423-428, 1991.
69. H. Choi, Y. Kim, Y. Zhang, S. Tang, S. Myung, and B. Shin, Plasma-induced graft co-polymerization of acrylic acid onto the polyurethane surface. *Surface and coating technology*, 182:55-64, 2004.

70. Y. V. Pan, R. A. Wesley, R. Luginbuhl, D. D. Denton, and B. D. Ratner, Plasma polymerized N-isopropylacrylamide: Synthesis and characterization of a smart thermally responsive coating. *Biomacromolecules*, 2:32-36, 2001.
71. P. Auroy, L. Auvray, and L. Leger, Building of a graft layer. 1. Role of the concentration of free polymer in the reaction bath. *Macromolecules* , 24:5158–5166, 1991
72. M. G. McCord, Y. J. Hwang, P. J. Hauser, Y. Qiu, J. J. Cuomo, O. E. Hankins, and M. A. Bourham, Modifying nylon and polypropylene fabrics with atmospheric pressure plasmas. *Textile. Res. J.*, 72(6):491-498, 2002.
73. Y. Qiu, C. Zhang, Y. J. Hwang, B. L. Bures, and M. McCord, The effect of atmospheric pressure helium plasma treatment on the surface and mechanical properties of ultrahigh-modulus polyethylene fibers. *J. Adhesion Sci. Technol.*, 16(1):99-107, 2002.
74. Y. J. Hwang, S. Matthews, M. McCord, and M. Bourham, Surface modification of organic polymer films treated in atmospheric plasmas. *Journal of the Electrochemical Society*, 151(7):C495-C501, 2004.

Chapter Two: Grafting of PNIPAM onto Nylon and Polystyrene Surfaces by Atmospheric Plasma Treatment Followed with Free Radical Graft Copolymerization

ABSTRACT

Temperature-responsive polymer materials have potential uses in drug delivery, tissue engineering, bioreactors, and cell-surface adhesion control. Temperature-responsive surfaces were fabricated by grafting poly(N-isopropylacrylamide) (PNIPAM) onto nylon and polystyrene (PS) surfaces via a new procedure, i.e., He atmospheric plasma treatment followed with free radical graft copolymerization. The atmospheric plasma exhibits the activation capability to initiate graft copolymerization. The procedure is suitable for integration into a continuous manufacturing process. To reduce homopolymerization and enhance graft yield, Mohr's salt was added. The graft of PNIPAM was confirmed by Fourier transform infrared spectroscopy (FTIR) and Atomic Force Microscopy (AFM). Dramatic water contact angle increase was found for PNIPAM-grafted polymers at *ca.* 32^{o}C, indicating the temperature sensitivity of the grafted surface, i.e., the change of surface from hydrophilic to hydrophobic when temperature increases. The addition of Mohr's salt enhances the grafting reaction and the magnitude of temperature sensitivity.

1. Introduction

Polymer materials have been widely used in the tissue engineering and biomaterial fields because of their good resilience, low density, and low cost. However, due to problems including nonselective protein adsorption and nonselective cell adhesion, polymer biomaterials are limited in their applications. Hence, surface modification of polymers to improve their biocompatibility has been a significant issue in this field [1]. It is desirable for biomaterials to direct or participate in specific biomaterial/biological tissue interfacial responses. Biomaterials with responsive surfaces can meet this requirement since it can exhibit markable property changes in respond to stimuli such as temperature, pH, liquid

composition, photo, electric stimulation, etc. This response can be used to regulate the activity of biological tissues, for example, the automatic cell detachment from the substrate.

A responsive biomaterial surface can be made by grafting a stimuli-responsive polymers (SRP) onto another polymer surface. SRPs are defined as polymers which can react, adjust or modulate their physicochemical characters, i.e., in most cases, their water-solubility, in response to an external stimulus. The environment stimuli can be pH, temperature, ions, solvents, electrical field, magnetic field, light, pressure, and chemical/biochemical compounds [2]. Poly(N-isopropylacrylamide) (PNIPAM) is one kind of thermo responsive polymers among SRPs. It shows remarkable changes in aqueous swelling with a change in temperature. The thermoresponsive polymer shows fully hydrated and extended conformation below 32^{o}C. Over 32^{o}C, however, it extensively dehydrates and exhibits a compact chain conformation [3]. Grafting thermoresponsive polymers, such as PNIPAM, onto a surface endows the surface with considerable thermoresponsive properties – hydrophilic below the LCST and hydrophobic above it [4]. The PNIPAM grafted surfaces offer possibilities for a number of novel applications, which include smart and thermally responsive coating as cell culture substrate to control the attachment and detachment of cells, the recovery of cultured cells, a biofouling releasing coating, temperature responsive membranes, controlled release of drugs and growth factor, and temperature responsive chromatography. Thus, a PNIPAM grafted surface is an enabling technology that can facilitate experiments and applications which were previously difficult or impossible [5].

Because of wide biomedical application of PNIPAM grafted surface, grafting of PNIPAM on polymer biomaterial surfaces has been a hot topic recently. Different methods about grafting PNIPAM on surface have been reported, some example are ultraviolet (UV) irradiation [6, 7, 8], vacuum plasma treatment [5, 8, 9, 10], ozone treatment [11, 12], electron-beam [3, 13, 14] and γ irradiation [15], and chemical treatment [16], etc. Among them, irradiation is a convenient way to graft PNIPAM, including UV, vacuum plasma, ozone, electron-beam and γ irradiation. Irradiation results in the formation of active species on the surface, which are capable of initiating the copolymerization of PNIPAM monomer. For example, PNIPAM was grafted on polypropylene membrane surface using vacuum plasma technique to activate

the surface, which provides the polypropylene membrane a thermo responsive permeability [10]. Polyamide has also been activated by ozone treatment and then grafted by PNIPAM [12].

Among many irradiation methods, the vacuum plasma treatment technique has been widely used due to its commercial advantages. It offers flexibility, effectiveness, safety and environmental friendliness. The vacuum plasma is effective at near-ambient temperature without damage for most heat-sensitive biomaterials. Vacuum plasma treatment modifies only the near surface of treated substrates and does not change the bulk material properties. It can be used to modify any kind of substrate geometry. However, it has some disadvantages, including the need for a vacuum environment, relatively high cost, and being limited to continuous process [17].

In contrast, atmospheric plasma operates at atmospheric pressure while maintaining the positive effects. Compared with conventional vacuum plasma treatment, atmospheric plasma method has several advantages, including no vacuum requirement, and therefore lower cost, and application in continuous processing [18]. In this investigation, atmospheric plasma treatment with subsequent graft copolymerization was used to graft PNIPAM onto Nylon and PS surfaces. The PNIPAM grafted surfaces were investigated by FTIR, contact angle, and AFM.

2. Experiments

2.1 Materials

Nylon 6,6 (McMaster-Carr) film and 60mm non-tissue culture treated polystyrene (PS) plates (Corning) were used as the substrates for graft polymerization. Nylon and PS were cut into 3 $\times$ 3 cm^2 samples, which were washed with acetone, dried in an oven at 60^oC, and weighed. N-isopropylacrylamide (NIPAM) was generously provided by Kohjin Co, Tokyo, Japan. It was recrystallized using hexane prior to grafting. Mohr's salt ($Fe(NH_4)_2(SO_4)_2.6H_2O$, EM

Science) and hexane (95%, Acros Organic) were used without further purification after purchase.

2.2 Atmospheric Plasma Treatment

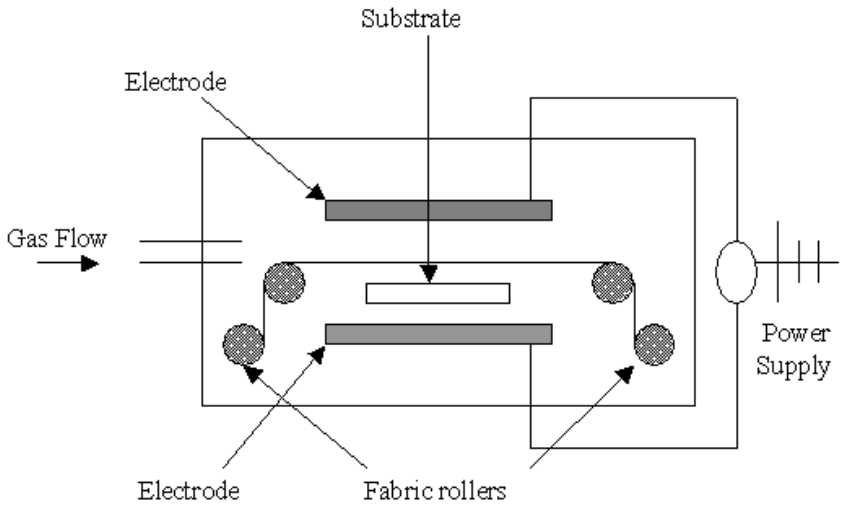


Figure 1. A Schematic of the Atmospheric Pressure Plasma System.

The atmospheric pressure plasma treatment system set up in the College of Textiles at North Carolina State University was used. It is an atmospheric pressure glow discharge (APGD) device. Figure 1 shows a schematic drawing of the experimental facility. It is a capacitively coupled chamber and contains two horizontal parallel electrodes. The radio frequency power is coupled to plasma via electrodes through an oscillating electric field. Each electrode is covered with a dielectric material to limit the current in the discharge and force the charge to spread out over a large area instead of constricting to an arc. The dielectric barrier prevented the transition of the discharge to an arc when acoustic frequency voltage signal was applied. The device has two chambers. An inner plasma chamber is for batch treatment. The outer chamber is equipped with a fabric rolling system for continuous fabric modification treatments. Therefore, the device is capable of batch treatment of fabric/film pieces using a test cell, as well as continuous operation using the roller feed system for large fabric rolls or

continuous filaments and yarns. Since the chamber is not pumped down, it operates at atmospheric pressure.

In this investigation, PS and Nylon were treated in the batch chamber with atmospheric plasma generated from 100% He. The power level used was 4.8 kW, the frequency was 5 kHz. The flow rate of He was 10.18 L/min. All nylon and PS samples were treated for 1 min.

2.3 Graft Polymerization of NIPAM onto Nylon and PS

After Nylon and PS were treated by He plasma for 1 min, they were immersed immediately into a NIPAM aqueous solution (5%wt) in a reaction kettle. The monomer solution was degassed with N_2 for 30 min to remove the existing O_2. The kettle was then sealed under N_2 and placed in a 60 ^oC water shaking bath to begin the graft copolymerization. After 24 hours reaction time, the PNIPAM grafted films were washed by agitating in ultrapure water at room temperature for 24 h to remove unreacted monomers and ungrafted homopolymers. Then the samples were dried at 60^oC for 3 hours and weighted. Mohr's salt was added in the monomer solution at a concentration of 0.345mM in half of the reactions to adjust the degree of grafting.

2.4 Graft Yield

The graft polymerization of PNIPAM was evaluated by weighing the nylon and PS samples before and after the graft polymerization. The amount of PNIPAM grafted on any PS surface was calculated using

Graft Yield (mg/cm^2) = $(W_1-W_0)/A$,

where W_0 is the weight of untreated film, W_1 is the weight of grafted film, and A is the film surface area.

2.5 FTIR Measurement

Fourier transform infrared spectroscopy (Nicolet 510P FTIR spectrometer) was used to examine the surface chemistry of the grafted nylon and PS. The spectra were collected at 4 cm^{-1} resolution with an FTIR microscopic spectrometer over 32 scans. The sampling area was coupled with an attenuated total reflection accessory and a 45° KRS-5 crystal.

2.6 AFM

Surface topography of original, plasma treated, and PNPAM-grafted nylon and PS was examined using Atomic Force Microscopy (JEOL JSPM-5200) under Tapping Mode in air. Measurements were carried out with a silicon probe (Olympus AC-160) at a scan area of $5\mu m^2$.

2.7 Water Contact Angle Measurement

The water contact angle of PNIPAM grafted nylon and PS was measured in air at 20, 25, 30, 32, 37, and 42 ^oC using the sessile method with a goniometer (Model A-100, Ramé-Hart, Inc.). The temperature of the test cell of the goniometer was controlled by a circulated water bath. ultrapure water (12 μl) was placed on the sample surfaces using a syringe. The contact angle was read after 1 min. The contact angle reported was an average of eight readings at different places on the same sample.

3. Result and Discussion

Nylon has been widely used in artificial joints, blood vessels, and kidney dialysis. PS are commonly used as various tissue cell culture plates for a long time. PNIPAM alone undergoes a reversible phase transition in response to temperature; hence, graft copolymerization of PNIPAM makes nylon and PS surfaces smart and temperature sensitive. The PNIPAM grafted nylon and PS will have intelligence applications in bioreactors, drug delivery, tissue scaffolds, and cell detachment control.

The process for grafting PNIPAM onto nylon and PS surface consists of a He atmospheric plasma treatment of nylon and PS and subsequent graft copolymerization in NIPAM monomer solution. A schematic presentation of the grafting method is depicted in Figure 2. The atmospheric plasma treatment activates the substrate surface and forms free radicals on the substrate surface. Since the plasma chamber is not sealed, there is always oxygen in the system, and the free radical react with oxygen to form hydrogen peroxide groups either inside the chamber or outside the system while being taken out. The hydrogen peroxide groups are thermally labile in nature and initiate the graft copolymerization of NIPAM to introduce graft brush layers on the surface [1].

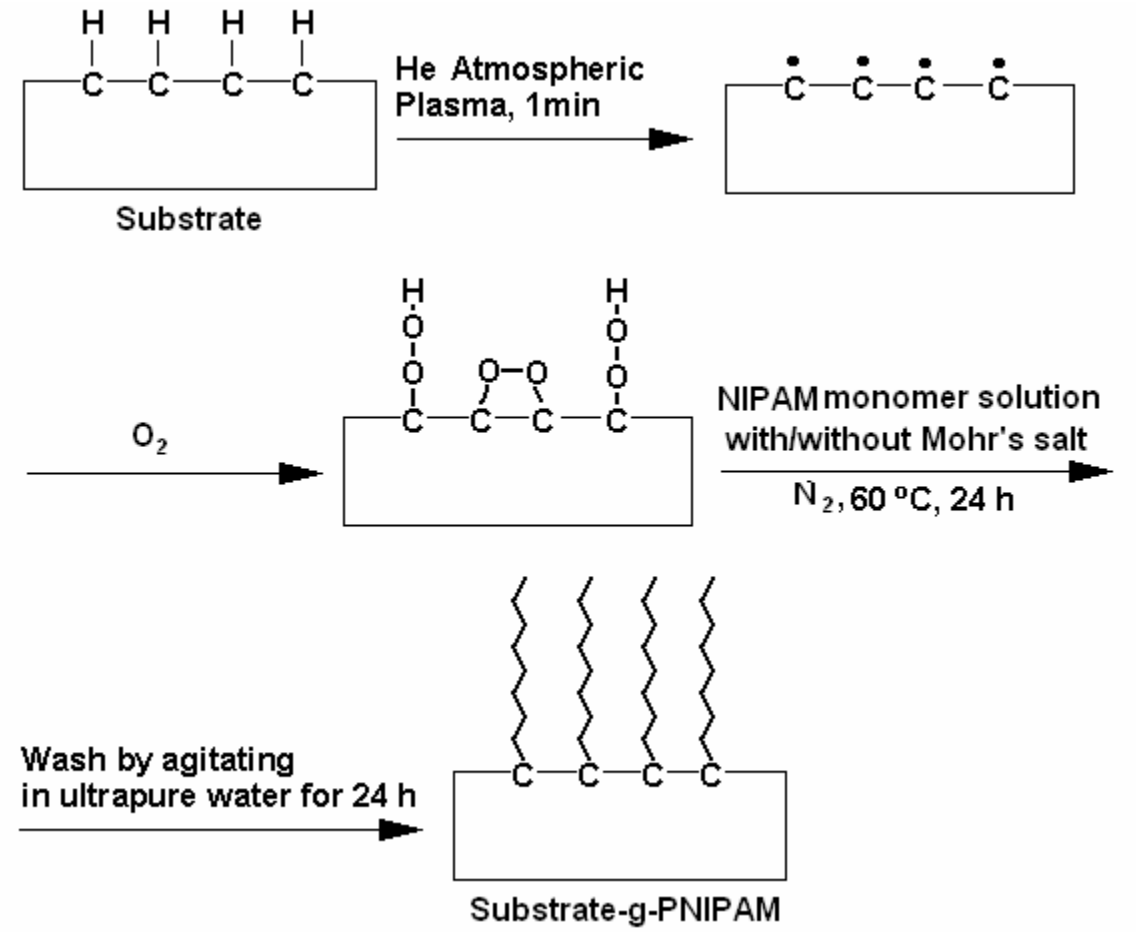


Figure 2. The schematic of graft copolymerization of NIPAM on PS and nylon surfaces.

3.1 FTIR

FTIR spectra of original PS and PNIPAM-grafted PS are shown in Figure 3. Absorption bands of 1540, 1650, and 3350 cm^{-1}, attributed to the secondary amide C=O stretching and secondary amide N-H stretching of PNIPAM chains [9], respectively, are found in both PNIPAM-grafted PS samples, indicating that PNIPAM has been successfully grafted onto the PS surface. The addition of Mohr's salt increases the intensity of the absorption bands at 1650 and 3350 cm^{-1} of PNIPAM grafted PS, indicating that the Mohr's salt enhances the graft copolymerization and increases the PNIPAM chain density on the PS surface.

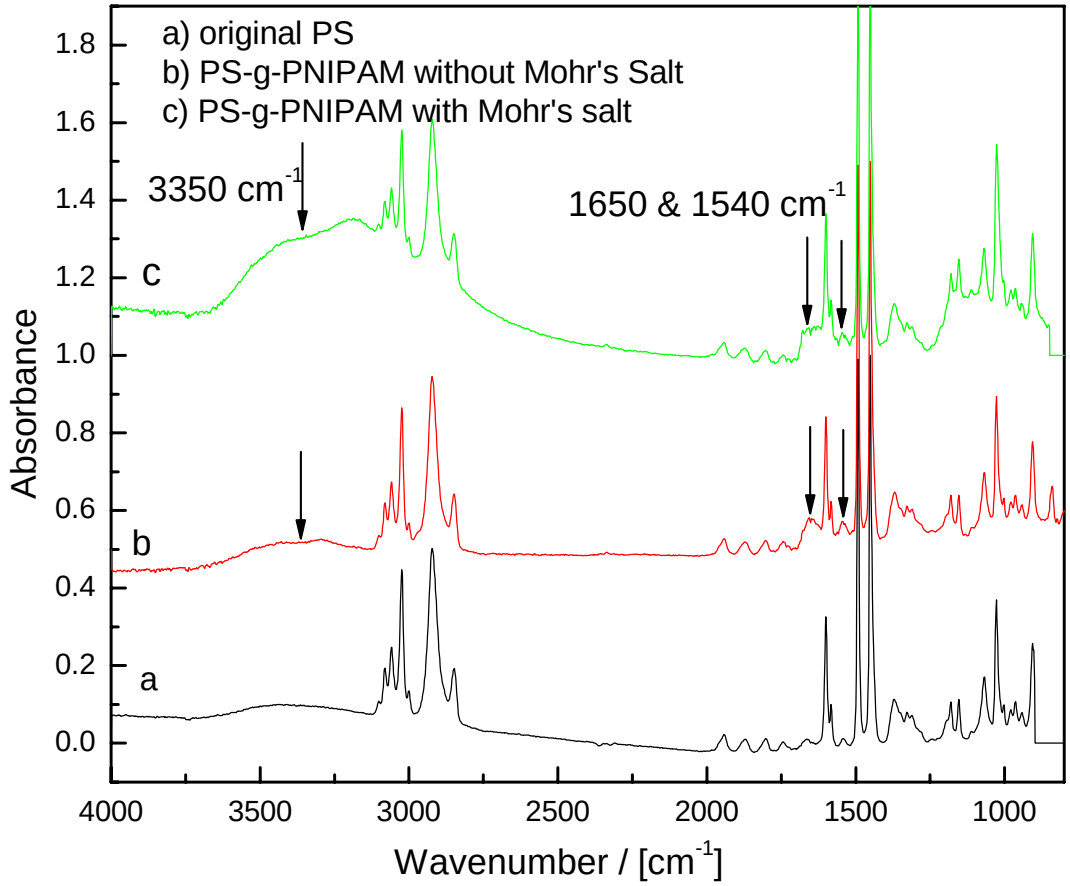


Figure 3. FTIR spectra of original and PNIPAM-grafted PS.

Figure 4 shows FTIR spectra of original nylon and PNIPAM-grafted nylon. Since there is a –NHCO- group in both nylon and PNIPAM structure, the absorption bands of 1540, 1650, and 3350 cm^{-1} cannot be used to detect PNIPAM chains on the nylon surface. Therefore, the adsorption band of 2970cm^{-1}, attributed to the $–CH_3$ asymmetric stretching of PNIPAM chains, is used to monitor the grafting of PNIPAM on nylon. The 2970 cm^{-1} peak is present in both PNIPAM grafted nylon surfaces with or without Mohr's salt, which confirms the

grafting of PNIPAM on the nylon surfaces. The intensity of this adsorption band increases with the addition of Mohr's salt, indicating that Mohr's salt can enhance graft copolymerization of PNIPAM on nylon.

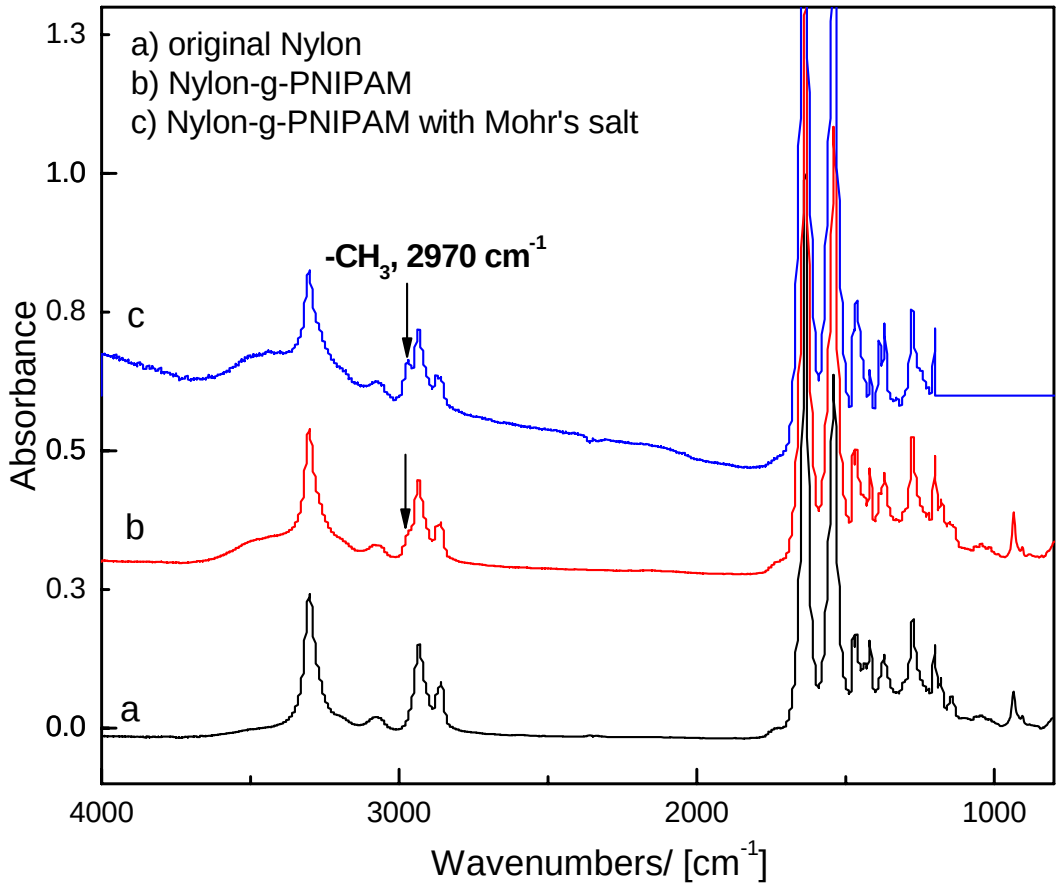


Figure 4. FTIR spectra of untreated and PNIPAM-grafted nylon.

In the atmospheric plasma system at NC State University, each electrode of the atmospheric plasma is covered with a dielectric material to limit the current in the discharge and force the charge to spread out over a large area instead of constricting to an arc. The dielectric barrier prevented the transition of the discharge to an arc when acoustic frequency voltage signal was applied. The resulting discharge is a nonequilibrium ionized gas that has many of the beneficial characteristics of low-pressure plasma treatment at increased pressure without the potentially damaging thermal energy [19]. Hence, the atmospheric plasma rather than vacuum plasma may be used to initiate graft copolymerization as vacuum plasma. In

atmospheric plasma, the surface is activated by forming free radicals, which initiates the graft copolymerization.

3.2 Influence of Mohr's salt on the grafting of PNIPAM

Graft yields of PNIPAM grafted nylon and PS were measured and shown in Table 1. The PNIPAM grafted PS and nylon by He atmospheric plasma induced graft copolymerization have graft yields of 0.056 and 1.67 mg/ cm^2, respectively. Generally the grafting copolymerization is competing with homopolymerization. If the surfaces were not atmospheric plasma pretreated, grafting copolymerization hardly did occur and the reaction was only a homopolymerization. However, as in studies using vacuum plasma, atmospheric plasma produced free radicals on the surface, which induced graft copolymerization. The graft yield for nylon is 20 times higher than that for PS. This is due to their different chemical structures. Nylon has many N-H and C=O bonds, which are more susceptible to chain scissor and free radicals formation under atmospheric plasma

Although atmospheric plasma is used to activate the surface, the grafting of NIPAM onto nylon and PS surfaces is still generally accompanied by the formation of PNIPAM homopolymers. This leads to large-scale monomer waste and may adversely affect the kinetics of the grafting process. As anticipated, the addition of Mohr's salt ($Fe(NH_4)_2(SO_4)_2.6H_2O$) suppressed the homopolymerization and enhanced graft copolymerization. It was found (Table 1) that the addition of Mohr's salt increased the graft yield of PS from 0.056 to 0.087 mg/cm^2, and graft yield of nylon from 1.67 to 1.93 mg/cm^2. Hsiue and Wang (1993) also found that the presence of Mohr's salt in the plasma grafting of vinyl monomers increased the graft yields. This was further supported by Hirotsu's results in the plasma grafting of different monomers onto polypropylene films.

The mechanism can be explained as follows. Mohr's salt, by virtue of its reducing nature, modifies the usual thermal decomposition of hydrogen peroxide and suppresses the formation of hydroxyl radicals. Hydroxyl radicals (eq. 1) are partly responsible for homopolymerization during the grafting reaction. In the presence of Mohr's salt, the

hydrogen peroxide group is transformed to hydroxyl ion, and the primary radical PO·, which initiates the grafting reaction (see eq.2)[1]:

$$POOH=PO\cdot+OH\cdot \quad (1)$$

$$POOH+Fe^{2+}= PO\cdot+OH^{-}+Fe^{3+} \quad (2)$$

Therefore, Mohr's salt can suppress homopolymerization [eq.(1)] and results in a higher degree of grafting.

Table 1: Graft yields of PNIPAM grafted nylon and PS by atmospheric plasma induced graft copolymerization with or without addition of Mohr's salt.

Samples	Graft yield (mg/cm^2)
PS-g-PNIPAM	0.056
PS-g-PNIPAM with Mohr's salt	0.087
Nylon-g-PNIAPM	1.67
Nylon-g-PNIPAM with Mohr's salt	1.93

3.3 AFM images of PNIPAM grafted surface

Figure 5 shows AFM images of untreated, plasma-treated and PNIPAM-grafted PS with Mohr's salt. Ungrafted PS has a flat surface, while the topography changes after the surface treatment. The plasma-treated PS becomes less flat and has many isolated rough spots due to the irradiation. Hwang *et al* [20] also found that atmospheric plasma treatment changes the morphology and roughness of polyester film and it was due to the etching and redeposition effects of plasma. PNIPAM-grafted PS in the presence of Mohr's salt contains a large amount of projections (ca. 0.2 μm), which were also found by Curti *et al* [21] on a PNIPAM-grafted polyester surface. The remarkable topography change is attributed to the grafting of PNIPAM chains on the PS surface.

AFM images of untreated, plasma-treated and PNIPAM-grafted nylon with Mohr's salt are shown in Figure 6. Like PS, untreated nylon also has flat surface, which becomes rougher

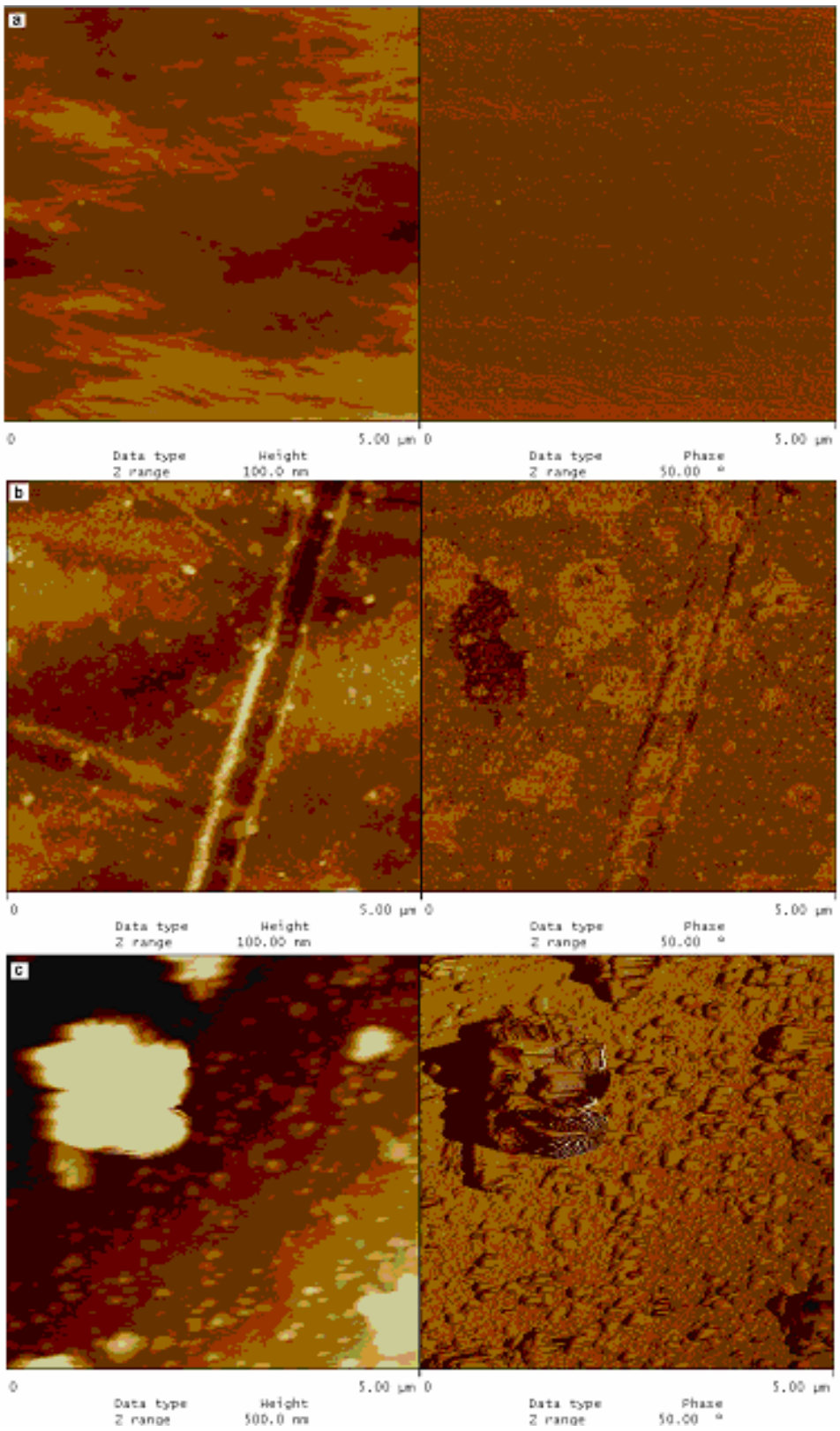


Figure 5. AFM images of PS (left images are topography while the right ones are phase): a) untreated PS; b) plasma-treated PS; c) PNIPAM-grafted PS.

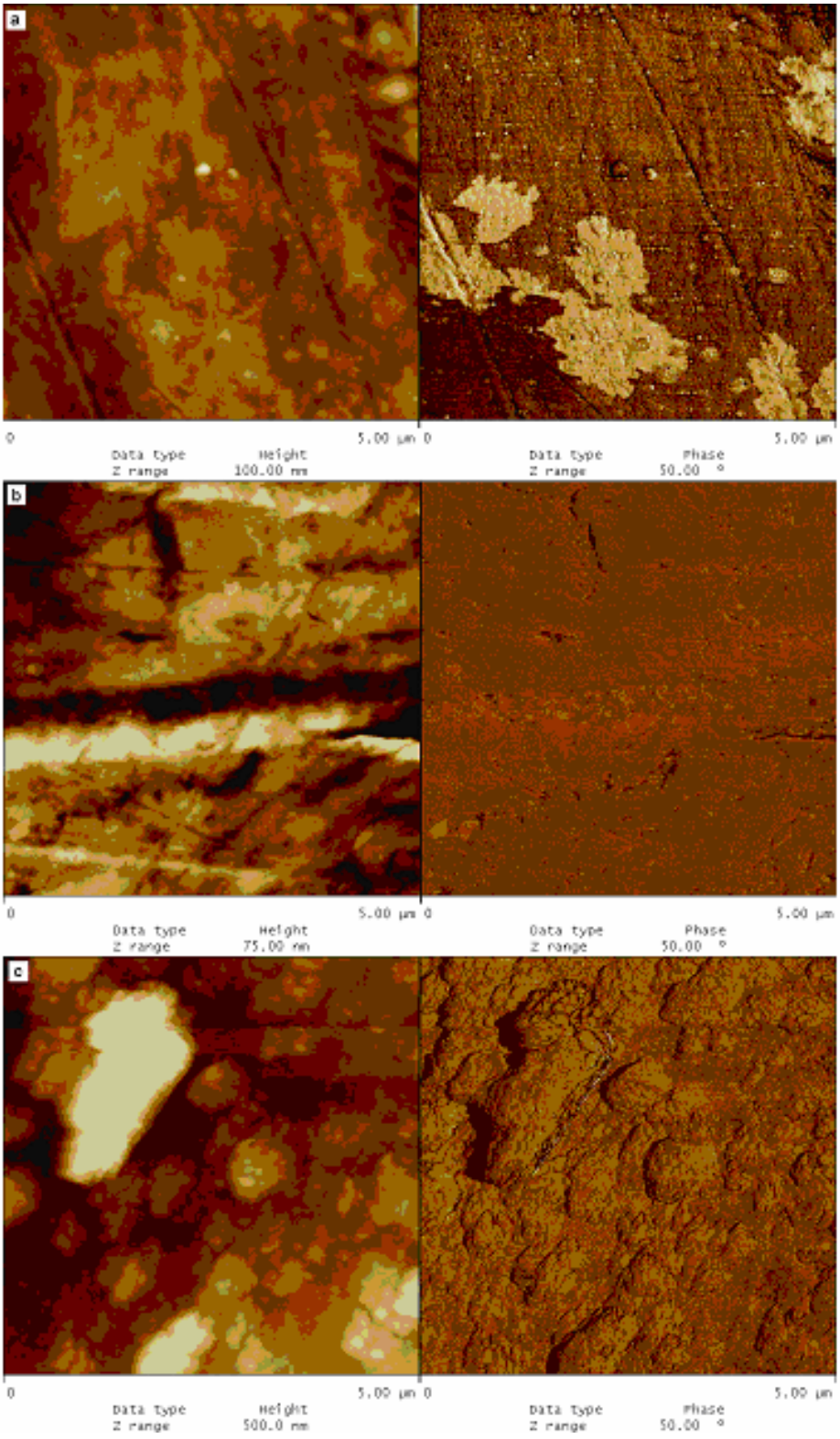


Figure 6. AFM images of nylon(left images are topography while the right ones are phase): a) untreated nylon; b) plasma-treated nylon; c) PNIPAM-grafted nylon with Mohr's salt.

after the plasma treatment. PNIPAM-grafted nylon in the presence of Mohr's salt shows many isolated rough spots (*ca.* 0.6 μm), which are even larger than those found on PNIPAM-grafted PS. This corresponds with the higher (about 20 times) graft yield of nylon than the one of PS.

3.4 Temperature sensitivity

PNIPAM alone undergoes reversible phase transition in response to temperature; hence, graft copolymerization of PNIPAM makes a surface smart and temperature sensitive. There are several methods to test the temperature sensitivity of PNIPAM grafted surface, including swelling, permeability, and contact angle evaluation at low and high temperatures.

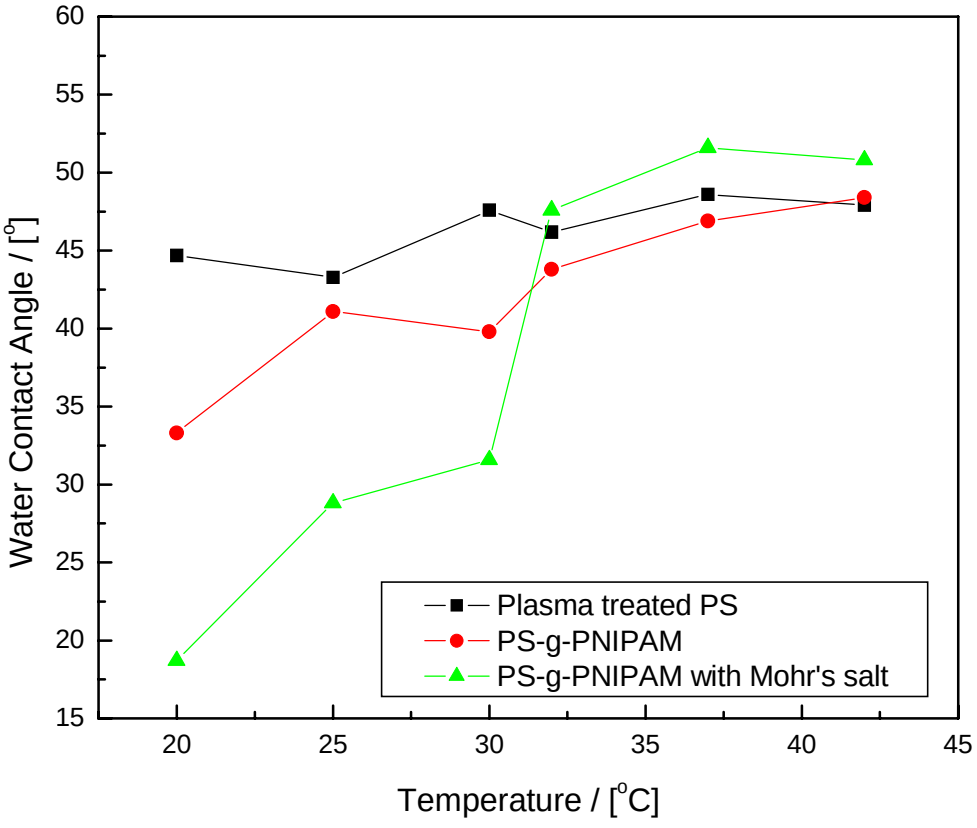


Figure 7. Contact angles of PNIPAM-grafted PS.

Here, the temperature sensitivity of PNIPAM-grafted PS and nylon was investigated using water contact angle measurement at various temperatures and the results are shown in Figure 7 and 8, respectively. Contact angles of plasma-treated PS and nylon are about 46 and 47^{o}, respectively. The contact angle for the plasma treated samples remains almost constant between 20 and 42 ^{o}C. However, all the PNIPAM-grafted PS and nylon surfaces show a temperature responsive behavior, i.e., the contact angle increases dramatically at a temperature around 32 ^{o}C, which is the Lower Critical Solution Temperature (LCST) of PNIPAM. This indicates that PNIPAM undergoes a phase change on the nylon and PS surfaces after being grafted. The grafted surfaces are hydrophilic at low temperatures and become relatively hydrophobic at temperatures higher than 32 ^{o}C. In addition, the magnitude of contact angle increase is affected by the grafting method. The contact angle increases are in the range of 10 to 40^{o}. Samples grafted in the presence of Mohr's salt show a larger difference in contact angle between the tested range of temperature than the samples grafted without Mohr's salt.

Temperature-response of the PNIPAM-grafted surface is partly attributed to conformation changes of the grafted PNIPAM chains. The temperature-responsive polymer chains have fully-hydrated and extended conformation at low temperatures. However, the PNIPAM polymer is essentially dehydrated and has compact chain conformation at temperatures higher than 32 ^{o}C. The temperature response is believed to be due to the change of hydrogen bonding between polymer segments and water molecules [2]. In general, the efficiency of hydrogen bonding decreases with the increasing temperature. As a result, PNIPAM can form sufficient hydrogen bonding with water molecules at lower temperatures. However, at temperatures higher than 32 ^{o}C, the efficiency of hydrogen bonding becomes insufficient to maintain the solubility of PNIPAM polymer in water, and the polymer chains collapse and form an aggregation/precipitation.

However, Wu et al [22] believe that both hydrophobic interactions of PNIPAM segments and polymer-water hydrogen bonding are involved in the phase transition of PNIPAM at ca. 32^{o}C. They claimed that hydrogen bonding on its own is unlikely to be significant, because polyacrylamide(PAAm), that has no hydrophobic N-alkyl groups, is soluble in water at all

temperatures. Furthermore, poly-N-alkylacrylamides with more hydrophobic N-alkyl groups exhibit lower LCSTs, again suggesting that polymer-water H-bonding may not be the only factor. It appears that both hydrophobic interactions of PNIPAM segments and polymer-water hydrogen bonding play roles in the phase change. At some point the hydrophobic interactions between polymer molecules become more favorable than polymer-water interactions and the polymer molecules collapse.

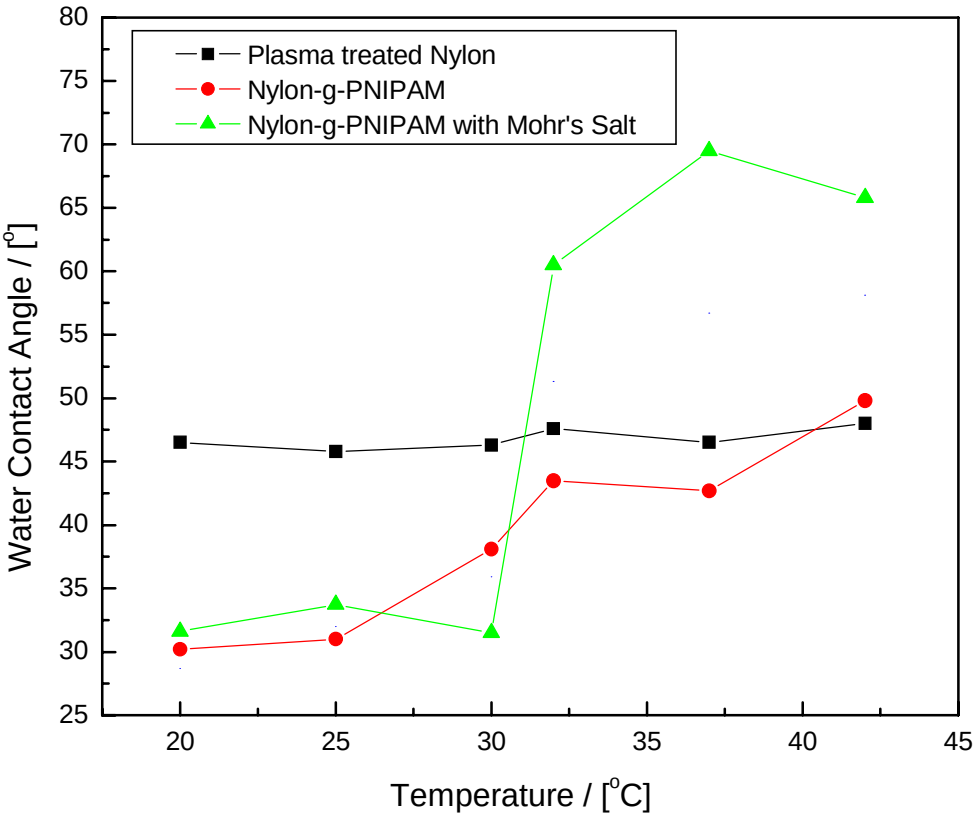


Figure 8. Contact angles of PNIPAM-grafted nylon.

4. Conclusions

Atmospheric plasma treatment is capable of activation of polymer surfaces and induction of graft copolymerization as conventional vacuum plasma. PNIPAM was successfully grafted onto nylon and PS surfaces by atmospheric plasma induced grafting copolymerization. The grafting of PNIPAM was confirmed by FTIR and Atomic Force Microscopy. The

temperature sensitivity of the PNIPAM grafted surface was demonstrated by water contact angle measurement at different temperatures. Addition of Mohr's salt in the graft copolymerization of NIPAM suppressed the homopolymerization of NIPAM and enhanced the graft copolymerization. It also increased the magnitude of water contact angle changes of PNIPAM grafted surface around 32^{o}C. PNIPAM grafted surfaces have many potential uses in biomedical field, such as drug delivery, bioreactors, tissue engineering, and cell detachment control.

5. References:

1. B. Gupta, J. G. Hilborn, I. Bisson, and P. Frey, Plasma-induced graft polymerization of acrylic acid onto poly(ethylene terephthalate) films. *J. Appl. Polym. Sci.*, 81: 2993-3001, 2001.
2. I.Y. Galaev and B. Mattiasson, Stimulus Responsive Surfaces: Possible Implication for Biochromatography, Cell Detachment and Drug Delivery. *In*: R. Freitag (ed) *Synthetic Polymers for Biotechnology and Medicine(p 116~129).* Georgetown, Texas: Landes Bioscience 2003.
3. N. Yamada, T. Okano, H. Sakai, F. Karikusa, Y. Sawasaki, and Y. Sakurai, Thermoresponsive polymer surfaces – control of attachment and detachment of cultured-cells. *Macromol. Chem. Rapid Commun.*, 11(11): 571-576, 1990.
4. I. Y. Galaev and B. Mattiasson, 'Smart' polymers and what they could do in biotechnology and medicine. *Trends in Biotechnology*, 17(8): 335-340, 1999.
5. D. Schmaljohann, J. Oswald, B. Jorgensen, M. Nitschke, D. Beyerlein, and C. Werner, Thermo-responsive PNiAAm-g-PEG films for controlled cell detachment. *Biomacromolecules*, 4 (6): 1733-1739, 2003.
6. S. Chen, J. Tsai, C. Chou, M. Yang, and J. Yang, Effects of additives on the photo-induced grafting polymerization of N-isopropylacrylamide gel onto PET film and PP nonwoven fabric surface. *Material Science and Engineering C*, 20: 203-208, 2002.
7. B. Yang and W. Yang, Thermo-sensitive switching membranes regulated by pore-covering polymer brushes. *Journal of Membrane Science*, 218: 247-255, 2003.
8. Y. M. Lee, S. Y. Ihm, J. K. Shim, and J. H. Kim, Preparation of surface-modified stimuli-responsive polymeric membranes by plasma and ultraviolet grafting methods and their riboflavin permeation. *Polymer*, 36: 81-85, 1995.
9. Y. V. Pan, R. A. Wesley, R. Luginbuhl, D. D. Denton, and B. D. Ratner, Plasma polymerized N-isopropylacrylamide: Synthesis and characterization of a smart thermally responsive coating. *Biomacromolecules*, 2: 32-36, 2001.
10. S. Y. Kim, T. Kanamori, and T. Shinbo, Preparation of thermal-responsive poly(propylene) membranes grafted with N-isopropylacrylamide by plasma-induced polymerization and their permeation. *Journal of Applied Polymer Science*, 84: 1168-1177, 2002.
11. J. O. Karlsson and P. Gatenholm , Cellulose fiber-supported pH-sensitive hydrogels. *Polymer*, 40: 379–387, 1999.

12. W. C. Wang, G. T. Ong, S. L. Lim, R. H. Vora, E. T. Kang and K. G. Neoh, Synthesis and characterization of fluorinated polyimide with grafted poly(N-isopropylacrylamide) side chains and the temperature-sensitive microfiltration membranes. *Ind. Eng. Chem. Res.*, 42: 3740-3749, 2003.
13. T. Okano, N. Yamada, M. Okuhara, H. Sakai, and Y. Sakurai, Mechanism of cell detachment from temperature-modulated, hydrophilic-hydrophobic polymer surfaces. *Biomaterials*, 16: 297-303, 1995.
14. M. Yamato, M. Utsumi, A. Kushida, C. Konno, A. Kikuchi, and T. Okano, Thermo-responsive culture dishes allow the intact harvest of multilayered keratinocyte sheets without dispase by reducing temperature. *Tissue Engineering*, 7: 473-480, 2001.
15. R. Mazzei, E. Smolko, D. Tadey, and L. Gizzi, Radiation grafting of NIPAAm on PVDF nuclear track membranes. *Nuclear Instruments & Methods in Physics Research B- Bam Interactions with Materials & Atoms*, 170: 419-426, 2000.
16. J. Xie, and Y. L. Hsieh, Thermosensitive poly(N-isopropylacrylamide) hydrogels bonded on cellulose supports. *J. Appl. Polym. Sci.*, 89:999-1006, 2003.
17. I. H. Loh, Plasma surface modification in biomedical application. *AST Technical Journal*, 2004 (www.astp.com).
18. Y.J. Hwang, thesis: Characterization of atmospheric pressure plasma interactions with textile/polymer substrates. *North Carolina State University*, 2004.
19. B. L. BURES, thesis: Rapid mortality of pest arthropods by direct exposure to a dielectric barrier discharge", *North Carolina State University*, 2004.
20. Y.J. Hwang, S.Matthews , M. McCord, and M. Burnham, Surface modification of organic polymer films treated in atmospheric plasmas, *Journal of the Electrochemical Society*, 151 (7): C495-C501, 2004.
21. P.S. Curti, M.R. DE Moura, E. Radovanovic, A.F. Rubira E.C. Muniz, and R.A. Moliterno, Surface Modification of Polystryrene and Poly(ethylene terephtalate) by Grafting Poly(N-isopropylacrylamide). *Journal of Materials Science*: Materials in Medicine, 13: 1175-1180, 2002.
22. X.Y. Wu, Q. Zhang, and R. Arshady, Stimuli Sensitive Hydrogels. In: R. Arshady (ed) *Introduction to Polymer Biomaterials*. London, UK: Citus, 2003.

Chapter Three: Thermoresponsive Polymeric Surfaces Prepared by Atmospheric Plasma Coating for Controlled Cell Detachment

Abstract

Temperature sensitive poly(N-isopropylacrylamide)(PNIPAM) was grafted on nylon film and tissue culture polystyrene(PS) dish surfaces by a novel method, i.e., atmospheric plasma treatment of NIPAM monomer solution coated substrates. The FTIR confirms the grafting of PNIPAM. Water contact angle confirms the surface changes from hydrophilic to hydrophobic around 32^{o}C when temperature increases. Novel Atomic Force Microscopy (AFM) techniques were employed to characterize the grafted surface topography changes upon the transition from dry to wet conditions and from low to high temperatures. The grafted surface consists of rolling hills and valleys in dry condition at 22^{o}C and smooth in water phase at 22^{o}C. The surface becomes tortuous again in the water phase at 40^{o}C because the PNIPAM hydrogel compacts at high temperature. The human epithelial cell line HEPG2 cells adhere and proliferate on PNIPAM grafted PS plates at 37^{o}C. However, they detach without agitation from the surface automatically at temperature below 32^{o}C. This detachment of HEPG2 and other types of cells can be used in recovery of continuous sheets of tissue from a bioreactor.

1. Introduction

Poly(N-isopropylacrylamide) (PNIPAM), a thermo-responsive polymer, shows remarkable changes in aqueous swelling with changes of temperature. The thermo-responsive polymer shows full-hydrated and extended conformation below its Lower Critical Solution Temperature (LCST) of 32 ^{o}C. Over 32 ^{o}C, however, it extensively dehydrates and changes to a compact chain conformations. When PNIPAM is grafted on another surface, the modified surface is relatively more hydrophobic in nature at temperatures higher than 32 ^{o}C, and becomes relatively more hydrophilic as the temperature falls below the LCST. Because PNIPAM changes its property dramatically and its LCST (32 ^{o}C) is near human body

temperature, PNIPAM grafted surfaces have many potential uses in the biomedical field, such as drug delivery, bioreactors, bioseparation, and responsive tissue scaffolds.

The change in surface properties of the PNIPAM grafted surface from hydrophobic above LCST to hydrophilic below it can be used to detach mammalian cells from their substrate. Okano and coworkers have successfully used a PNIPAM grafted surface to achieve temperature-modulated cell adhesion/detachment control [1-6]. PNIPAM was grafted onto Tissue Culture Polystyrene (TCPS) plates by electron beam irradiation. TCPS grafted with PNIPAM allows cell to adhere and proliferate above the LCST. Decreasing the temperature to below the LCST results in detachment of cells. The detached cells include endothelial cells [1, 2], bovine hepatocytes [4], human keratinacytes [5], etc. Further investigation into the mechanism of cell attachment revealed that strong interactions between hydrophobic PNIPAM and fibronectin, an extra-cellular matrix (ECM) protein, mediate cell attachment above LCST. Decreasing the temperature below the LCST makes the surface hydrophilic and results in detachment of ECM along with the cultured cells [6].

Ratner et al[7] have also reported that Bovine Aortic Endothelial Cells (BAEC's) detaches from the PNIPAM grafted surfaces when temperature decreases below the LCST. Werner et al[8] found that Mouse Fibroblast Cells(MFC's) adhere, spread, and proliferate on a PNIPAM and poly(ethylene glycol) (PEG) immobilized surface at 37 ^{o}C and become completely detached after reducing the temperature by 3 ^{o}C.

The temperature triggered cell detachment from PNIPAM grafted surfaces provides a gentler alternative to harvesting cells as compared to trypsin and ethylenediaminetetraacetic acid (EDTA). Enzymatic treatment results almost complete dissociation of cells from one another, thus interrupting simple paracrin cell to cell signaling. Furthermore, cells harvested by lowering the temperature may be recovered as a single sheet with intact cell-to-cell junctions [9] and maintain their differentiated function [10]. These sheets of cells can be recultured and be fabricated into new multi-layer tissues [3].

In this study, PNIPAM was grafted onto nylon film and non-tissue culture treated Polystyrene(PS) plates surfaces by a novel method, atmospheric plasma treatment of a PNIPAM coated surface. Compared to other irradiation methods, the plasma treatment technique has commercial advantages. It offers flexibility, effectiveness, safety and environmental friendliness [11]. Furthermore, the atmospheric plasma treatment, not conventional vacuum plasma, does not require a vacuum environment, which lowers cost and makes continuous processing possible [12].

Human HEPG2 cells were grown on the PNIPAM grafted PS for the first time and their detachment from the surface while reducing the temperature was studied. The detachment of HEPG2 cells upon cooling shows that recovery from various culturing systems can be achieved without chemical treatment. The recovered HEPG2 cells have the potential to be used as joined cell-to-cell precursors for future studies

2. Experiments and methods

2.1. Materials

Nylon 6,6 film (McMaster-Carr), 60mm non-tissue culture treated polystyrene (PS) plates (Corning) (for FTIR, water contact angle, and AFM), and 6-well non-tissue culture treated polystyrene(PS) plates (Falcon) (for cell culture), were used as the substrates for graft polymerization. N-isopropylacrylamide (NIPAM) was generously provided by Kohjin (Tokyo, Japan). It was recrystallized using hexane prior to grafting. Hexane (95%, Acros Organic) and 2-propanol (Aldrich) were used without further purification after purchase.

2.2. Atmospheric Plasma Treatment

The atmospheric pressure plasma treatment system set up in the College of Textiles at North Carolina State University is an atmospheric pressure glow discharge (APGD) device. Figure 1 shows a schematic drawing of the experimental facility. It is a capacitively coupled chamber and contains two horizontal parallel electrodes. The radio frequency power coupled

to plasma via electrode through an oscillating electro filed. Each electrode is covered with a dielectric material to limit the current in the discharge and force the charge to spread out over a large area instead of constricting to an arc. The dielectric barrier prevented the transition of the discharge to an arc when acoustic frequency voltage signal was applied. The device has two chambers. An inner plasma chamber is for batch treatment. The outer chamber is equipped with a fabric rolling system for continuous fabric modification treatments. Therefore, the device is capable of batch treatment of fabric pieces using a test cell, as well as continuous operation using the roller feed system for large fabric rolls or continuous filaments and yarns. Since the chamber is not pumped down, so it operates at atmospheric pressure.

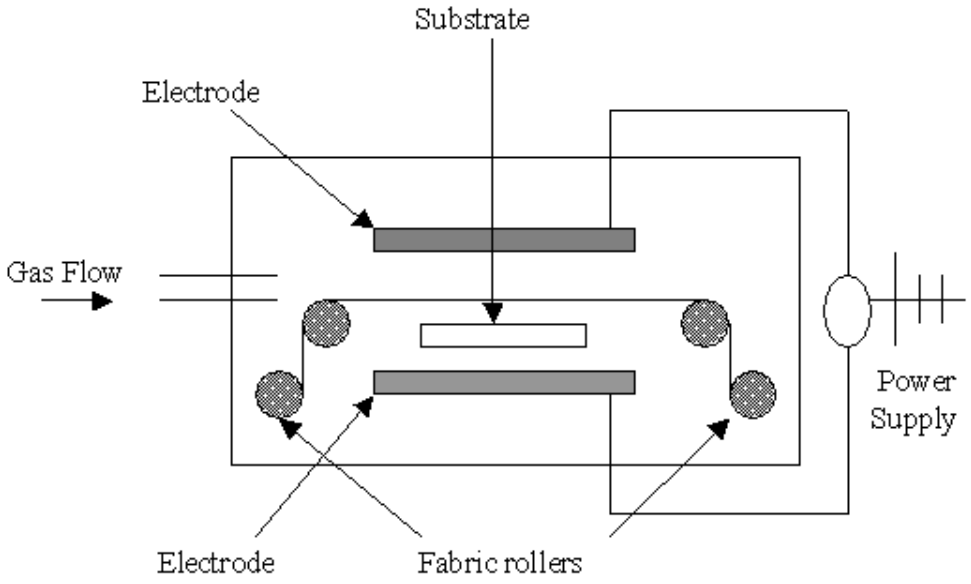


Figure 1. A Schematic of the Atmospheric Pressure Plasma System.

In this investigation, PS and nylon were treated in the batch chamber with atmospheric plasma generated from 100% He gas. The power level used was 4.8 kW, and the frequency was 5 kHz. The flow rate of He was approximately 10.18 L/min.

2.3 Grafting Copolymerization

The detailed process of grafting copolymerization was shown in Figure 2. The nylon film and PS plates were exposed to He pretreatment for 1, 2, or 3min to activate the surface. Certain amount of 55% NIPAM 2-propanol solution was added into PS plates and spread evenly. The nylon was dipped in 55% NIPAM 2-propanol solution and removed. After these coating procedures, the film and plates were immediately exposed to He plasma for 1, 2, 3, 5, 7, and 10 min post treatment. The treated sample were rinsed with water rigorously and dried.

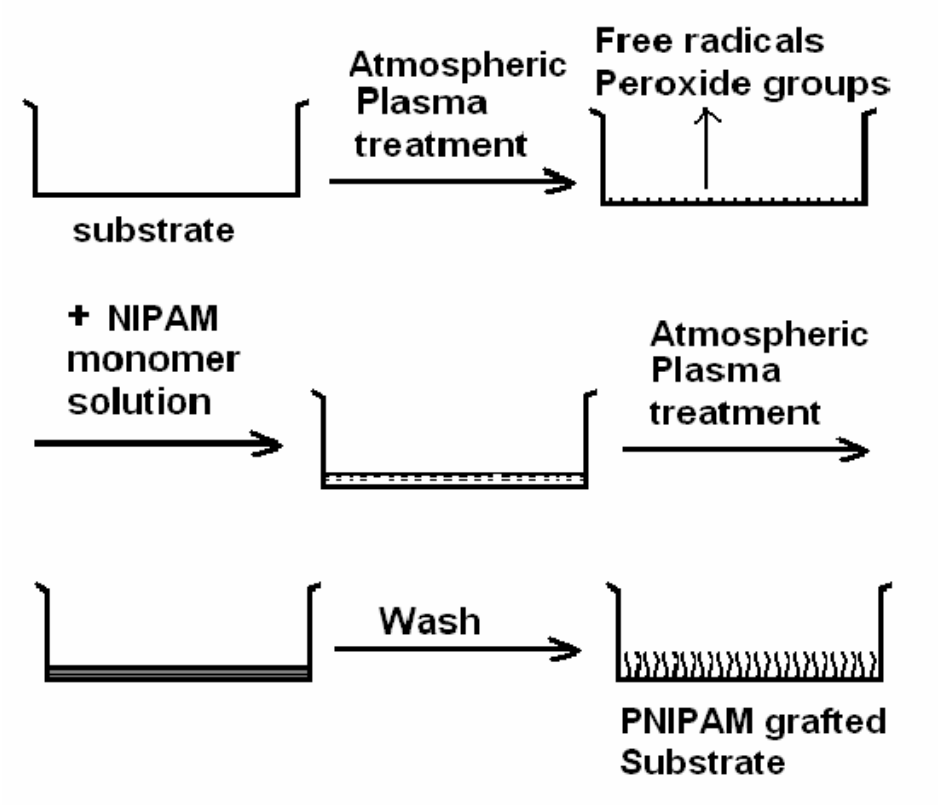


Figure 2. The schematic of graft polymerization of NIPAM on PS plates.

2. 4 FTIR measurement

Fourier transform infrared spectroscopy (Nicolet 510P FTIR spectrometer) was used to examine the surface chemistry of the grafted nylon and PS. The spectra were collected at 4 cm^{-1} resolution with an FTIR microscopic spectrometer over 32 scans. The sampling area was coupled with an attenuated total reflection accessory and a 45° KRS-5 crystal.

2.5 water contact angle measurement

The water contact angle of PNIPAM grafted nylon and PS was measured in air at 20, 25, 30, 32, 37, and 42 ^{o}C using the sessile method with a goniometer (Model A-100, Ramé-Hart, Inc.). The temperature of the test cell of the goniometer was controlled by a circulated water bath. Purified water (12 μl) was placed on the sample surfaces using a syringe. The contact angle was then read after 1 min. The contact angle reported was an average of eight reading at different places for each sample.

2.6 Surface topography

Surface topography of PNIPAM-grafted PS was examined using atomic force microscopy (JEOL JSPM-5200) under Tapping Mode in dry (vacuum) and in wet phase (water). Measurements were carried out with a silicon probe (Olympus AC-160) on a scan area of 5μm^2. In the water phase measurement, the temperature was controlled at room temperature (22.2 °C) and 40 °C by a microheater.

2.7 Cell culture

PNIPAM grafted PS plates were sterilized with gamma irradiation using a Cesium source at a dose of 40 Grey over 13 minutes. The plates were then placed in an incubator at 37 ^{o}C in preparation for cell plating.

The cells used in the experiments were human epithelial cell line HEPG2 cells. They were obtained from the Lineberger Cancer Center at University of North Carolina at Chapel Hill. The culture medium contains 500 ml EMEM (Gibco-Invitrogen, Carlsbad CA), 5ml AAS (Gibxo-Invitrogen, Carlsbad CA), 5ml L-Glutamine (Gibco-Invitrogen, Carlsbad CA), 50ml FBS (Atlanta Biological, Lawrenceville GA). HEPG2 cultures at confluence in a T-75 flask (Corning, Corning NY) were washed free of culture media, and treated with 5ml of 5x trypsin (Gibco-Invitrogen, Carlsbad CA). After lifting from the culture plate bottom, cells were treated with 5ml of FBS to stop the trypsin effects. Cells were centrifuged at 1400 rpm

and the supernate removed. Cells were resuspended in HEPG2 growth media, counted with a Coulter counter, and plated on PNIPAM grafted PS 6-well plates at a density of 12865 cells/plate. Media changes took place on day 4 of incubation and detachment was studied on day 10 after seeding.

2.8 Cell detachment by lowering temperature

Cells were dyed with a Mitotracker Red dye which infiltrates the mitochondrial walls of living cells (Molecular Probes, Eugene OR). Addition of the vital dyes was for a 30 minute incubation time period in normal EMEM supplemented media. Mitotracker Red was used at a concentration of 250 nM.

Cell culture plates were kept warm using an incubator until time for imaging. Images were taken on a Nikon Olympus microscope Olympus with a preheated stage using a Deltaphase Isothermal Pad for 37 °C temperature stabilization (Braintree Scientific, Braintree MA). Cultures were warmed while on stage using a set thermometer for the stage and a radiant heat lamp. After initial photographs were taken, cells were then cooled for 5 minutes in an ice bath. Secondary pictures were made after removal of the cell plates from the bath. Thirdly pictures were taken after the media is removed.

3. Results and Discussion

3.1 FTIR

FTIR spectra of untreated PS and PNIPAM-grafted PS (by plasma coating) are shown in Figure 3. The PNIPAM grafted PS plates was prepared by 1 min plasma pretreatment, 100 ul NIPAM solution coating, and 1 min post plasma treatment on the 60mm non-tissue culture treated polystyrene (PS) plates. Characteristic peaks of PNIPAM [13] including 1540 cm^{-1} (secondary amide N-H stretching), 1650 cm^{-1} (secondary amide C=O stretching), 2970 cm^{-1} (-CH_3 asymmetric stretching), and 3301 cm^{-1} (secondary amide N-H stretching), are all found

in PNIPAM-grafted PS samples. This confirms that PNIPAM was successfully grafted onto the PS surface by atmospheric plasma treatment of NIPAM monomer coated plates.

Figure 4 shows FTIR spectra of original nylon and PNIPAM-grafted nylon. Since there is a –NHCO- group in both nylon and PNIPAM structure, the absorption bands of 1540, 1650, and 3350 cm^{-1} cannot be used to detect PNIPAM chains on the nylon surface. Therefore, the adsorption band of 2970 cm^{-1}, attributed to the $–CH_3$ asymmetric stretching of PNIPAM chains, is used to monitor the grafting of PNIPAM on nylon. The 2970 cm^{-1} peak are present in PNIPAM grafted nylon, which indicates the grafting of PNIPAM on nylon surface.

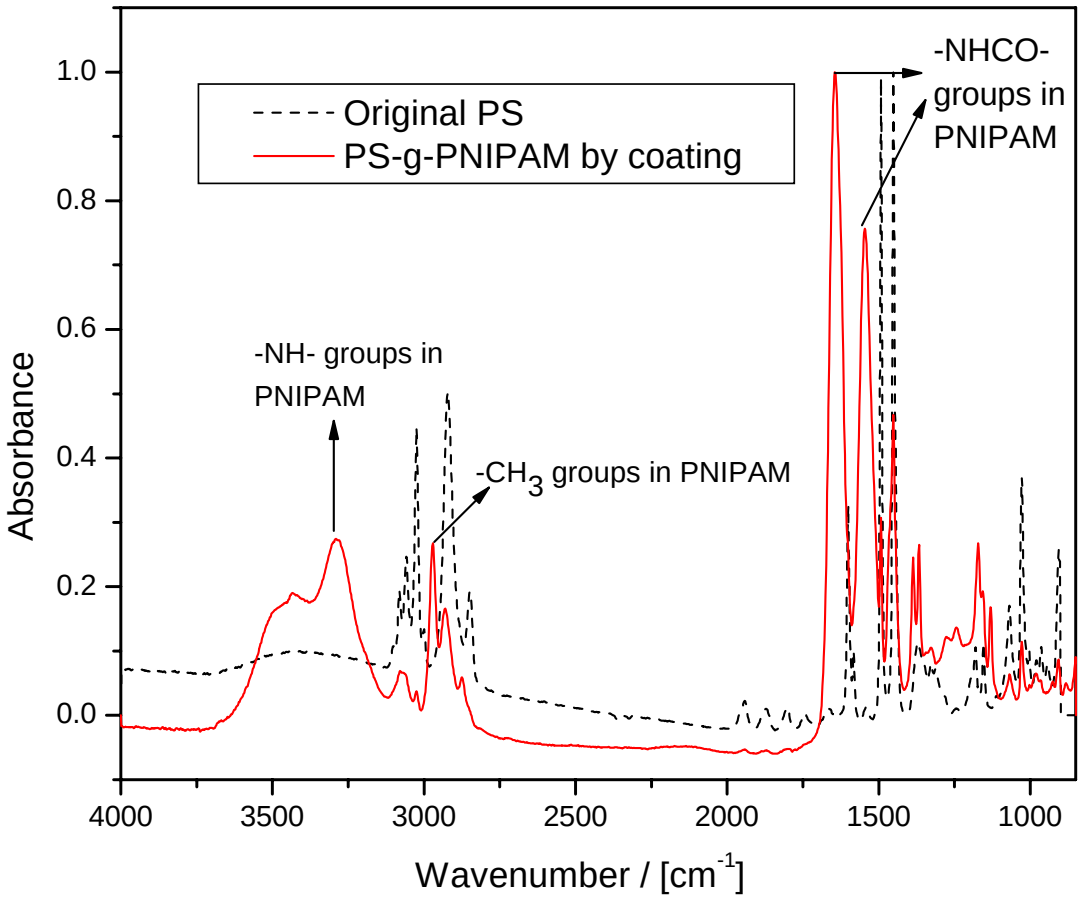


Figure 3. FTIR of original and PNIPAM grafted PS.

PNIPAM is grafted on both nylon and PS surface based on the FTIR results. This indicates that atmospheric treatment of NIPAM monomer coated surface is an effective way to graft

PNIPAM onto various material surfaces. Atmospheric plasma can work at room temperature and require no vacuum environment. It provides a very affordable and convenient way to fabricate PNIPAM grafted surface. Atmospheric plasma treatment of NIPAM monomer coated plates can be integrated into a continuous manufacturing process suitable for industrial application.

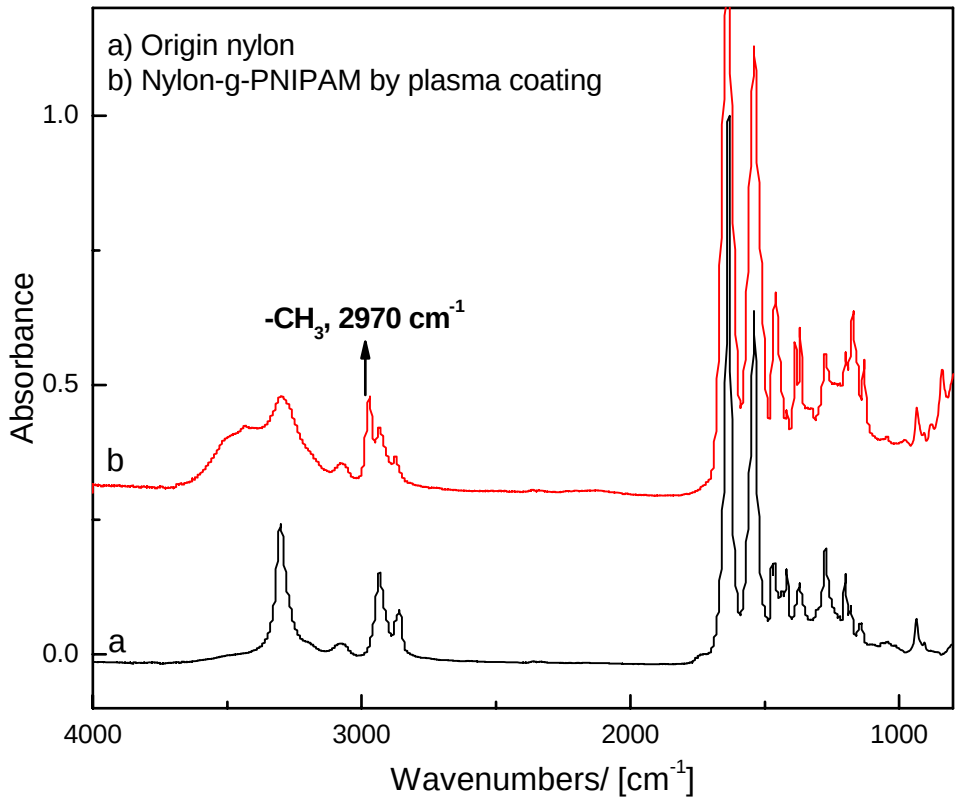


Figure 4. FTIR of original and PNIPAM grafted nylon.

3.2 Surface wettability changes with temperature

Surface wettability is often related to cell adhesive behavior on surfaces, showing optimal values [14]. PNIPAM alone undergoes reversible phase transition in response to temperature; hence, graft copolymerization of PNIPAM makes a surface smart and temperature sensitive, i.e., the surface changes from hydrophilic to hydrophobic as temperature increases. Static

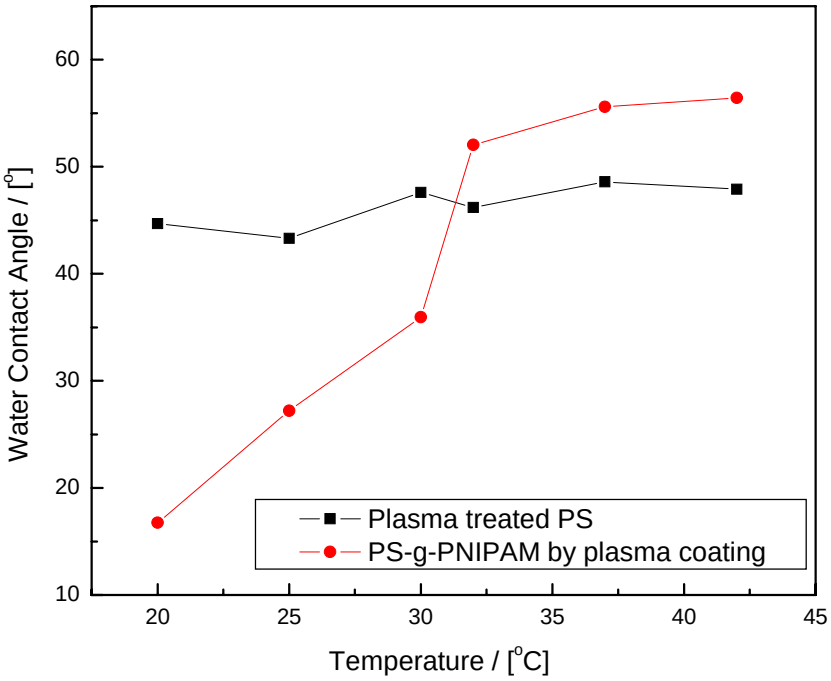


Figure 5. Contact angles for PNIPAM-grafted PS.

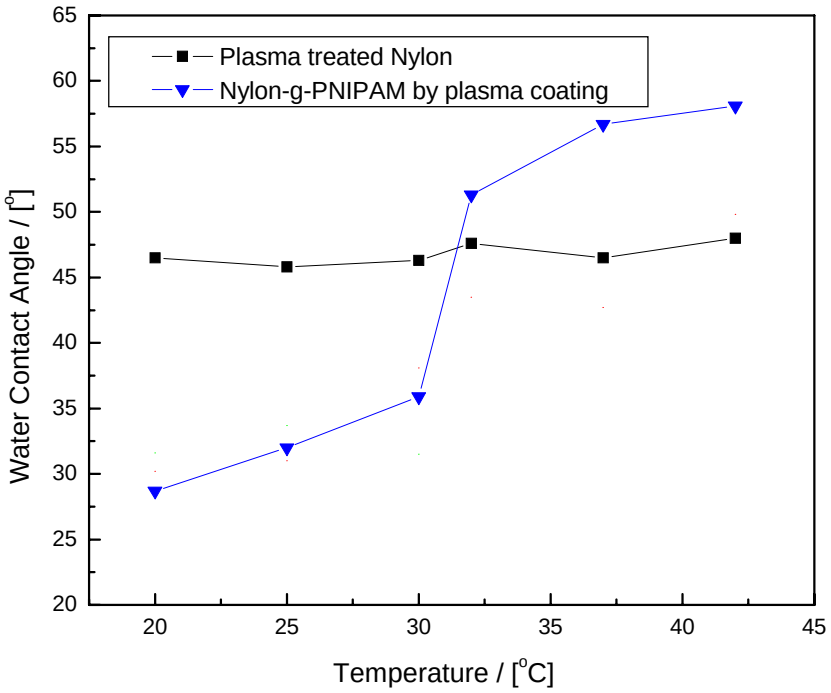


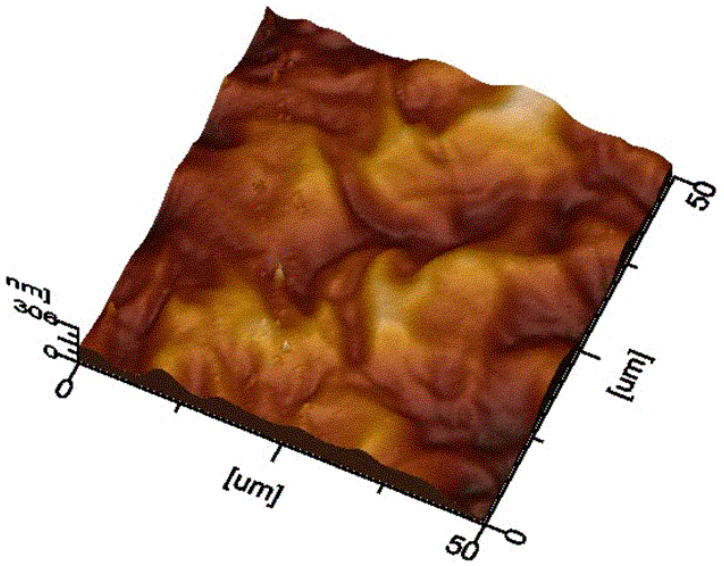
Figure 6. Contact angles for PNIPAM-grafted nylon.

water contact angles for PNIPAM grafted surfaces were determined using the sessile drop method at temperatures ranging from 20 to 42 ^{o}C and data are shown in Figure 5 and 6. The PNIPAM grafted PS plate sample was prepared by 1 min plasma pretreatment, 100 ul NIPAM solution coating, and 1 min post plasma treatment on the 60mm non-tissue culture treated polystyrene (PS) plates. The plasma treated PS and nylon have a very consistent contact angles in this temperature range, approximate 47 and 46 ^{o}, respectively. In contrast, the contact angles of PNIPAM grafted PS and Nylon are both thermoresponsive. They have 40 ^{o} (PS) and 30 ^{o} (Nylon) increases as temperature increases from 20 to 42 ^{o}C. The most significant increase occurs around 32^{o}C, which is the LCST of PNIPAM. Therefore, the grafting of PNIPAM results a thermoresponsive contact angle, i.e., thermoresponsive wettablility. The PNIPAM grafted surface is very hydrophilic at low temperature; however, above 32^{o}C, they become hydrophobic.

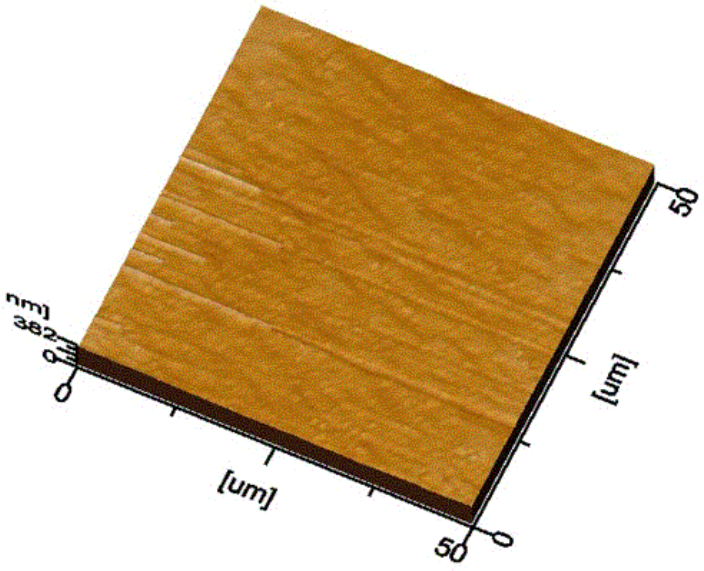
Therefore, wettability of PNIPAM grafted surface changes with temperature. Cells will respond to this surface wettability change as temperature changes. Their adhesion/detachment behavior on this surface will be different when temperature drops.

3.3 AFM images

The water contact angle change of PNIPAM grafted surface with temperature shows the surface wettability changes as temperature increases. This is due to the PNIPAM conformational changes. The conformational changes cause the changes in both wettability and surface topography. Therefore, PNIPAM grafted surface topography was studied using AFM at various temperature. Since PNIPAM can form a hydrogel with water, PNIPAM grafted surface topography was also studied with AFM in both dry and wet states.



a)



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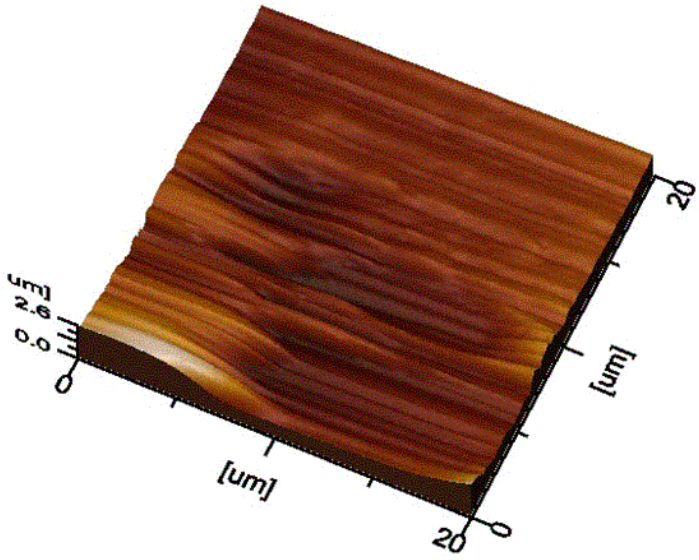

c)

Figure 7. AFM images of PNIPAM grafted surface: a) dry in vacuum at 22°C; b) wet in water at 22°C; c) wet in water at 40^{o}C.

Figure 7 shows the images of a PNIPAM grafted PS surface at different conditions. The PNIPAM grafted PS plate sample was prepared by 1 min plasma pretreatment, 100 ul NIPAM solution coating, and 10 min post plasma treatment on the 60mm non-tissue culture treated polystyrene (PS) plates. In the dry condition under vacuum at 22^{o}C (Figure 7 a), PNIPAM grafted PS has convoluted surface, indicating a condensed polymer network. However, in water at 22^{o}C, the surface convolutions disappear, and the surface appears smooth. At this temperature, the PNIPAM chains extend and form hydrogen bonds. PNIPAM is known to form a hydrogel with water below the LCST (32^{o}C). Furthermore, when the temperature increases from 22^{o}C to 40^{o}C, the surface become convoluted again even in a wet state. AFM images show some horizontal streaks on the surface on the grafted gel. PNIPAM undergoes a phase change at 32^{o}C and the grafted PNIPAM becomes more condensed at high temperatures. Hydrogen bonds decrease as temperature increases, hence the hydrophobic interaction between PNPAM hydrophobic chain segments dominates over hydrogen bonds[15, 16]. Then PNPAM chains aggregate together, and the gel becomes more viscous, creating drag on the top of the area and resulting in these horizontal striations

Table 1: Roughness of PNIPAM grafted PS surface at different condition

Condition	Average of RMS roughness	Average Peak-to-Valley roughness
Dry, 22^{o}C	538 nm	2520 nm
Fluid, 22^{o}C	10.9 nm	90.4 nm
Fluid, 40^{o}C	186.2 nm	1323 nm

The roughness of a surface can be obtained by AFM too. One of the most common roughness parameters is the root-mean-squared (RMS) roughness. Assuming a surface in the horizontal plane, this is the root mean squared value of all vertical deviations from the mean surface level. Another measure of surface roughness is Peak-to-Valley roughness, which gives the maximum peak-to-valley distance within the selected profiles. Table 1 shows the roughness difference of PNIPAM grafted surface at different states. In the dry state at 22^{o}C, RMS and peak-to-valley roughness are 538 and 2520 nm respectively, because the PNIPAM chains compact together randomly. In the fluid state at 22^{o}C, both roughnesses decrease to 10.9 and 90.4 nm, which are only 2% and 4% of the dry values. This is caused by the formation of gel and hence the smooth surface. In the wet state at 40^{o}C, both the roughnesses increase again to 186.2 and 1323 nm, which are 35% and 53% of the dry value. This is due to the fact that some of gel compacts, so the roughness increases again.

Overall, AFM images reveal distinctly different surface topographies for dry and fluid samples over a temperature range. The grafted surface exhibits gel like morphology below 32°C and solid like morphology above 32°C in water phase. It indicates that the grafted PNIPAM still has phase change properties of PNIPAM around 32°C. Since cells respond to surface topography and hardness variations, the cell adhesion behavior on PNIPAM grafted surface will change with temperature.

3.4 Cell detachment

Temperature dependent cell adhesion/detachment was investigated. HEPG2 cells were cultivated on PNIPAM grafted PS plates for several days. The PNIPAM grafted PS plate sample used in Figure 8 was prepared by 3 min plasma pretreatment, 25 ul NIPAM solution coating, and 10 min post plasma treatment on the 6-well non-tissue culture treated polystyrene(PS) plates. The cells adhere, spread, and proliferate on the surface at 37 ^{o}C (Figure 8a), which shows that the PNIPAM grafted PS is suitable for cell cultivation. In contrast, at 0 ^{o}C, the HEPG2 cells lift up from the PNIPAM grafted surface and there is no cell adhesion (Figure 8b). If the cell media is removed at 0 ^{o}C, none of cells are left on the PNIPAM grafted surface(Figure 8c), which further confirms that all the HEPG2 cells detached from PNIPAM grafted surface at 0 ^{o}C. Therefore, PNIPAM grafted PS surface can be used to grow HEPG2 cells at 37 ^{o}C and detach them at 0 ^{o}C. The cell adhesion/detachment is due to the surface phase change of PNIPAM grafted PS. Contact angle and AFM results show that the surface is very hydrophobic and rough at temperatures above the LCST (32 ^{o}C). However, below the LCST, the surface becomes hydrophilic and smooth because of the formation of PNIPAM gel. Cell adhesion responds to surface wettability and topography change, which causes the cell detachment at low temperatures.

Cell detachment on PNIPAM grafted surface has been studied by other groups [1-8]. However, this is the first time that the PNIPAM grafted surface prepared by atmospheric plasma was used and HEPG2 cells were detached from the surface. More importantly, the HEPG2 cells detach from the surface just by reducing the temperature without chemical treatment, which has great potential for application in development of artificial organs. HEPG2 cells are less expensive cheap and display similar characteristics to primary liver cells. Cell lines are used to work through model systems before trying to work with primary cells. Proof of principle can be shown by manipulating cells to get them to lift from materials grafted with PNIPAM, moving a step closer to using primary cells in a bioreactor, which can be recovered without chemical dissolution. A problem with all current bioreactors is fouling, in which the materials inhibit collection of the tissue without damage to the cell-to-cell contacts and structures. To make progress in tissue engineering, there has to be a way to grow tissue ex vivo and keep it intact when attempting to manipulate the system.

Therefore, the HEPG2 cell detachment provides proof there is a way to recover cultured tissue ex vivo without damage of the cells in collection stages.

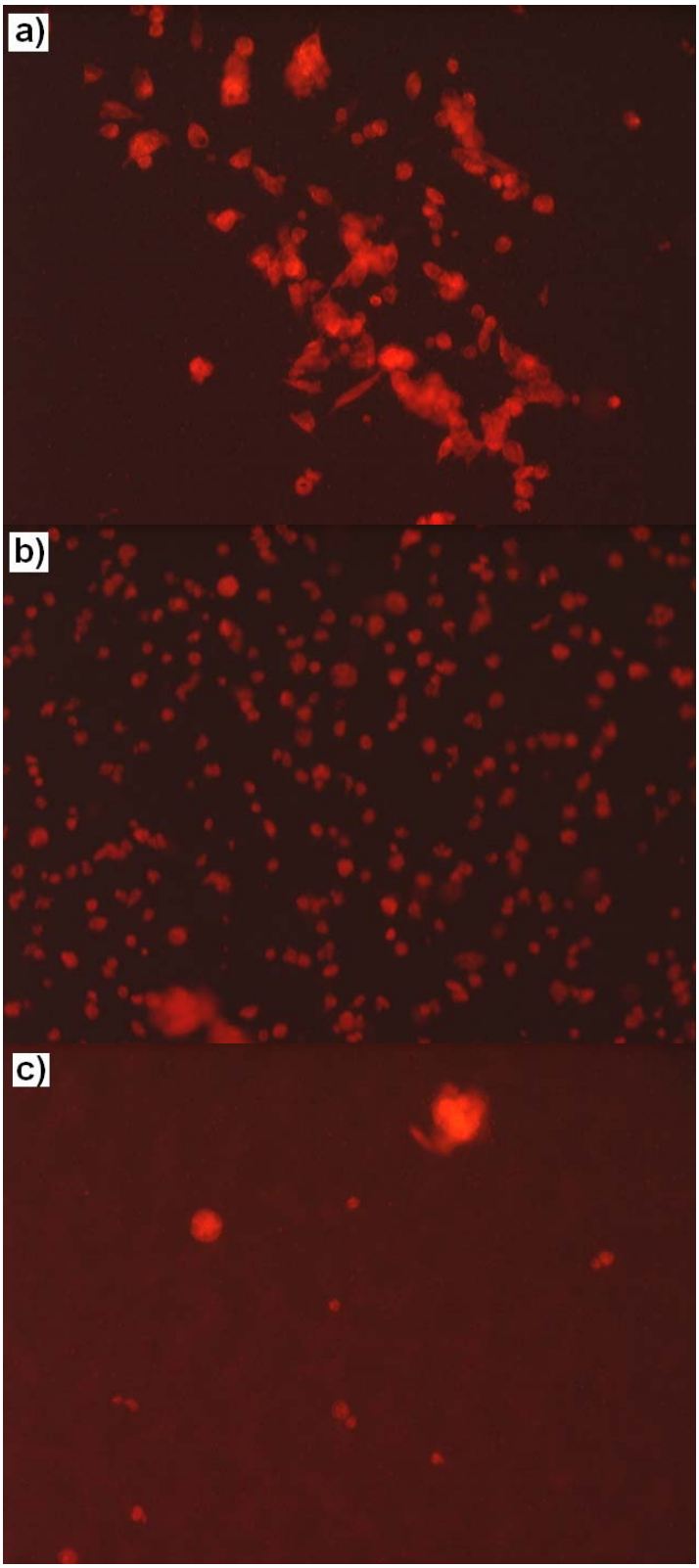

Figure 8. Microscipic views of HEPG2 cells on PNIPAM grafted PS plates: a) at 37^{o}C; b) at 0^{o}C; c) after removing the media at 0^{o}C.

similar characteristics of the primary liver cells. Cell lines are used to work through model systems before trying to work with primary cells. Proof of principle can be shown by manipulating cells to get them to lift from materials grafted with the NIPAAM, moving a step closer to using primary cells in a bioreactor, which we can be recovered without chemical dissolution. A problem with all current bioreactors is fouling, in which the materials inhibit collection of the tissue without damage to the cells-to-cell contacts and structures. To make progress in tissue engineering, there has to be a way to grow tissue ex vivo and keep it in tact when attempting to manipulate the system. Therefore, the HEPG2 cells detachment provides proof there is a way to recover cultured tissue ex vivo without damage of the cells in collection stages.

4. Conclusion

Temperature responsive PNIPAM was grafted on to nylon film and PS plates by atmospheric plasma treatment of NIPAM monomer coated surfaces. FTIR confirms the grafting of PNIPAM. Water contact angle shows the surface change from hydrophilic to hydrophobic as temperature increases. AFM images show that the PNIPAM grafted PS surface forms gel below 32 ^{o}C. However, the gel compacts above 32 ^{o}C. HEPG2 cells adhere well on PNIPAM grafted PS surface at 37 ^{o}C. As temperature decreases, the HEPG2 detaches from the surface automatically. The PNIPAM grafted surface may be used to culture and recover of tissue ex vivo.

5. References:

1. Y. Akiyama, A. Kikuchi, M. Yamato, and T. Okano, Ultrathin poly(N-isopropylacrylamide) grafted layer on polystyrene surfaces for cell adhesion/detachment control. *Langmuir,* 20 (13): 5506-5511, 2004.
2. Y. Tsuda, A. Kikuchi, M. Yamato, S. Yasuhisa, and U. Mitsuo, Control of cell adhesion and detachment using temperature and thermoresponsive copolymer grafted culture surfaces. *J. Biomed. Mater. Res. 69A*:70-78, 2004.

3. A. Kikuchi and T. Okano**,** Nanostructured designs of biomedical materials: Applications of cell sheet engineering to functional regenerative tissues and organs. *Journal of Controlled Release*, 101 (1-3): 69-84, 2005.
4. F. A. Tobiesen and S. Michielsen, Method for grafting poly(acrylic acid) onto nylon 6,6 using amine end groups on nylon surface. *J. Polym. Sci. Part A: Polym. Chem*., 40: 719-728, 2002.
5. M. Yamato, M. Utsumi, A. Kushida, C. Konno, A. Kikuchi, and T. Okano, Thermo-responsive culture dishes allow the intact harvest of multilayered keratinocyte sheets without dispase by reducing temperature. *Tissue Engineering*, 7: 473-480, 2001.
6. A. Kushida, M. Yamato, C. Konno, A. Kikuchi, Y. Sakurai and T. Okano, Decrease in culture temperature releases monolayer endothelial cell sheets together with deposited fibronectin matrix from temperature-responsive culture surfaces, *J. Biomed. Mater. Res.*, 45: 355-362**,** 1999.
7. X. Cheng, Y. Wang, Y. Hanein, K.F. Böhringer, and B.D. Ratner, Novel cell patterning using microheater-controlled thermoresponsive plasma films. *Journal of Biomedical Material Research Part A*, 70A (2): 159-168, 2004.
8. D. Schmaljohann, J. Oswald, B. Jorgensen, M. Nitschke, D. Beyerlein, and C. Werner, Thermo-responsive PNiAAm-g-PEG films for controlled cell detachment. *Biomacromolecules*, 4 (6): 1733-1739, 2003.
9. A. Kikuchi, M. OKuhara, F, Karikusa, Y. Sakurai, T. Okano, Two-dimensional manipulation of confluently cultured vascular endothelial cells using temperature-responsive poly(N-isopropylacrylamide)-grafted surfaces. *J. Biomater. Sci. Polym. Ed.*, 9:1331, 1998.
10. T. Okano, N. Yamada, h. Sakai, Y. Sakurai, A novel recovery-system for cultured-cells using plasma-treated polystyrene dishes grafted with poly(N-isopropylacrylamide). *J. Biomed. Mater. Res.*, 27:1243, 1993.
11. I. H. Loh, Plasma Surface Modification in Biomedical Application. *AST Technical Journal*, 2004 (www.astp.com).
12. Y.J. Hwang, thesis: Characterization of atmospheric pressure plasma interactions
13. with textile/polymer substrates. *North Carolina State University*, 2004.
14. Y. V. Pan, R. A. Wesley, R. Luginbuhl, D. D. Denton, and B. D. Ratner, Plasma polymerized N-isopropylacrylamide: Synthesis and characterization of a smart thermally responsive coating. *Biomacromolecules* 2: 32-36, 2001.
15. P. B. van Wachem, A. H. Hogt, T. Beugeling, J. Feijen, A. Bantijes, J. P. Detmers, W. G. van Aken, Adhesion of cultured human endothelial cells onto methacrylate polymers with varying surface wettability and charge. *Biomaterials*, 8: 323-328, 1987.
16. I.Y. Galaev and B. Mattiasson, Stimulus Responsive Surfaces: Possible Implication for Biochromatography, Cell Detachment and Drug Delivery. *In*: R. Freitag (ed) *Synthetic Polymers for Biotechnology and Medicine(p 116~129).* Georgetown, Texas: Landes Bioscience 2003.
17. X.Y. Wu, Q. Zhang, and R. Arshady, Stimuli Sensitive Hydrogels. In: R. Arshady (ed) *Introduction to Polymer Biomaterials*. London, UK: Citus, 2003.

Chapter Four: Novel Thermoresponsive Fabrics: Poly(N-isopropylacrylamide) Grafted Cotton using Atmospheric Plasma Treatment

Abstract

Temperature-responsive Poly(N-isopropylacylamide)(PNIPAM) is grafted onto cotton fabrics via two novel methods, i.e., atmospheric plasma followed with graft copolymerization (two-step method), and atmospheric plasma treatment of NIPAM monomer coated surface (coating method). FTIR confirms the surface grafting of PNIPAM via both methods and the graft yields of PNIPAM for these two methods are 1.47% and 15.36%, respectively. In order to increase the graft yield of two-step method, Mohr's Salt and Ammonium Persulfate (APS) were added in the graft copolymerization after plasma treatment. The graft yield increased from 1.47% to 7.8% and 11.95%, respectively. Tensile testing shows that the PNIPAM-grafted cotton (coating method) maintains the original tensile property. The PNIPAM-grafted cotton (the two-step method) without any additives keeps almost the same tensile properties. However, for the grafted cotton made in the presence of Mohr's salt and APS, the tensile strength and modulus decreased up to 27% and 19%.

A skin model sweating comfort test shows that PNIPAM-grafted cotton has temperature-responsive characteristics. At 10^{o}C in wet conditions, less heat transfers from the skin model to outside through PNIPAM-grafted cotton than the control. However, at 35^{o}C, more heat transfers through it than the control. This novel smart PNIPAM-grafted cotton may have potential applications in responsive clothing, such as sportswear.

1. Introduction

Stimuli-Responsive Polymers (SRP) are polymers which can react, adjust or modulate their physicochemical characteristics, i.e., in most cases, their water-solubility, in response to an external stimulus. The environmental stimulus can be pH, temperature, ions, solvents,

electrical field, magnetic field, light, pressure, or chemical/biochemical compounds [1]. Poly(N-isopropylacrylamide) (PNIPAM) is one kind of thermo-responsive polymers among SRP. It shows remarkable changes in aqueous swelling with change of temperature. The thermoresponsive polymer shows fully-hydrated and extended chain conformation below 32^{o}C. Over 32^{o}C, however, it extensively dehydrates and changes to compact chain conformation [2].

One type of stimulus-responsive fabrics can be prepared by covalently grafting SRP onto fabrics surface. If a SRP is grafted on the fabric surface, the conformational change of SRP upon external stimuli will occur on the fabrics surface, which will result in stimulus-responsive characteristics of the fabrics. For example, the swelling behavior, the wettability, the pore size, or the permeability of SRP grafted fabrics may change upon external stimuli. Because of this novel stimuli sensitivity, the SRP grafted fabrics may find applications including smart textiles, responsive clothing, chemical separation, bioseparation, chemical sensor, tissue engineering, etc.

SRP has been grafted on a cellulose surface using a two-step method, i.e., use of high energy radiation or ceric ion oxidation, to generate free radicals in the cellulose surface, and followed graft copolymerization of SRP. For example, pH-sensitive poly(acrylic acid) has been grafted on rayon fibers using both Cobalt-60 high radiation and ceric ion methods[3]. Poly(acrylic acid) is also grafted on cellulose fibers by ozone treatment[4]. Temperature-sensitive PNIPAM has been grafted onto cotton by γ radiation [5] and ceric ion methods [6].

In this investigation, two novel atmospheric plasma methods are used to graft temperature-sensitive PNIPAM onto cotton surfaces. One is atmospheric plasma treatment of the cotton surface followed with NIPAM free radical graft copolymerization. In order to increase the graft yield, Mohr's salt (ferrous ammonium sulfate hexahydrate) and Ammonium Persulfate (APS) were added, respectively, in some reactions. The other method is atmospheric plasma treatment of NIPAM monomer solution coated fabrics. This is a novel method and not yet published. It is believed that one advantage of PNIPAM-grafted fabrics is that the fabric provides mechanical support and dimensional stability to the PNIPAM gel. So the tensile

properties of the fabrics before and after grafting were studied. Finally, a comfort test measured the heat and moisture transport properties of PNIPAM-grafted fabrics at low and high temperature.

Thermoresponsive textiles are a very important category smart or environmentally responsive textile. There are numerous situations where these can be beneficial and find applications. including athlete's sportswear, a pilot's uniform in a fighter plane, a soldier's uniform in extreme climate zones, uniforms for workers working at extreme temperatures, fire fighters, tents and temporary structures in extreme climates, and automobiles, etc [7].

2. Experiment

2.1. Materials

N-isopropylacrylamide (NIPAM) (99% purity) was generously provided by Kohjin (Tokyo, Japan). It was recrystallized using hexane prior to grafting. The fabrics used were woven cotton (Greige, 122gm/m^2) cut into 12.7×12.7 cm^2 squares and washed with water before use. Ammonium Persulfate(APS, $(NH_4)_2S_2O_8$), and 2-propanol were purchased from Aldrich and used as received. Mohr's salt ($Fe(NH_4)_2(SO_4)_2.6H_2O$, EM Science) and hexane (95%, Acros Organic) were also used without further purification after purchase.

2.2. Atmospheric Plasma Treatment

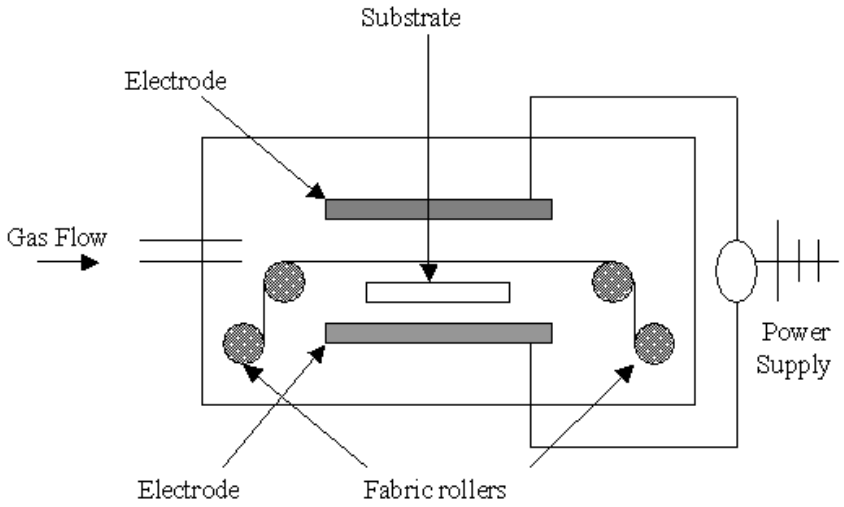

Figure 1. A Schematic of the Atmospheric Pressure Plasma System.

The atmospheric pressure plasma treatment system set up in the College of Textiles at North Carolina State University is an atmospheric pressure glow discharge (APGD) device. Figure 1 shows a schematic drawing of the experimental facility. It is a capacitively coupled chamber and contains two horizontal parallel electrodes. The radio frequency power coupled to plasma via electrode through an oscillating electro filed. Each electrode is covered with a dielectric material to limit the current in the discharge and force the charge to spread out over a large area instead of constricting to an arc. The dielectric barrier prevented the transition of the discharge to an arc when acoustic frequency voltage signal was applied. The device has two chambers. An inner plasma chamber is for batch treatment. The outer chamber is equipped with a fabric rolling system for continuous fabric modification treatments. Therefore, the device is capable of batch treatment of fabric pieces using a test cell, as well as continuous operation using the roller feed system for large fabric rolls or continuous filaments and yarns. Since the chamber is not pumped down, so it operates at atmospheric pressure.

In this investigation, fabric samples were treated in the batch chamber with atmospheric plasma generated from 100% He. The power level used was 4.8 kW, the frequency was 5 kHz. The flow rate of He was 10.18 L/min. All fabric samples were treated for 1 min.

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2.3. Graft Methods

2.3.1 Plasma treatment followed with monomer solution grafting(two-step method)

The cotton fabrics were first exposed to He gas plasma treatment for 60 seconds and then were immersed into a NIPAM aqueous solution (5%wt) in a reaction kettle immediately. The monomer solution was then degassed with N_2 for 30 min to remove the existing O_2. After that, the kettle was sealed under N_2 and placed in a 60 ^oC water shaking bath to begin the graft copolymerization. After 24 hour reaction, the reaction was stopped and the PNIPAM-grafted fabrics were washed by agitating in ultrapure water at room temperature for 24 h to remove unreacted monomers and ungrafted homopolymers. Then the samples was dried and weighted.

In order to increase the graft yields, additives Mohr's salt (ferrous ammonium sulfate hexahydrate) (0.345mM) and ammonium persulfate (APS) (0.01M) were added into NIPAM monomer solution, respectively, to compare the graft products made without additives.

2.3.2 Plasma treatment of NIPAM monomer solution coated samples (coating method)

In addition to the above methods, NIPAM was grafted onto the fabrics surface by one-step method, i.e., plasma treatment of NIPAM monomer solution-coated samples. First, the fabrics were pretreated by He plasma for 1 min, and then were coated with 45% NIPAM 2-propanol solution. After that, they were treated by He plasma again for 4 mins.

Similarly, the PNIPAM-grafted fabrics were washed by being agitated in ultrapure water for 24 h at room temperature to eliminate the unreacted monomers and ungrafted homopolymers.

2.4 Graft Percent of PNIPAM-grafted fabrics

The graft polymerization of PNIPAM was evaluated by weighing the fabric samples before and after the graft polymerization. The graft percent of PNIPAM-grafted on fabrics sample was calculated using

Graft Yield = $(W_1-W_0)/W_0$,

where W_0 is the weight of untreated fabric and W_1 is the weight of grafted fabric. The graft yield was an average of 3 for each sample.

2.5 FTIR characterization

Fourier transform infrared spectroscopy (Nicolet 510P FTIR spectrometer) was used to examine the surface chemistry of the PNIPAM-grafted and original cotton fabrics. The spectra were collected at 4 cm^{-1} resolution with an ATR-FTIR microscopic spectrometer over 32 scans. The sampling area was coupled with an attenuated total reflection accessory and a 45° KRS-5 crystal.

2.6 Comfort test

The heat and moisture transport properties of original and PNIPAM-grafted fabrics prepared in the presence of APS were characterized by a fabric comfort test. A thermolob II instrument with a sweating skin model located in the Textile Protection and Comfort Center (TPACC) at NC State University was used. The comfort test was conducted in wet condition at 10 and 35^oC under 65% RH. The standard specimen size of 12.7*12.7 cm^2 was used in three replications.

A Tabai ESPEC's Platinous Lucifer Model PL-2G, programmable low temperature and humidity chamber is used to obtain the required test conditions (Figure 2)[9].It contain three parts: 1) the environmental control chamber, 2) an insulated skin simulating guarded hot plate with sweating capabilities fit inside the chamber, 3) the testing fabric sample which is placed directly on the hot plate. To investigate the difference between PNIPAM-grafted cotton performance at low and high temperature, the environment chamber is controlled at 10^oC and 35^oC, respectively. At wet condition, four simulated sweating glands are supplying

water to the hot plate at the rate of 0.077ml/ min/ gland. Heat transfer from the plate through the test fabric (W) is measured in Watts at each condition.

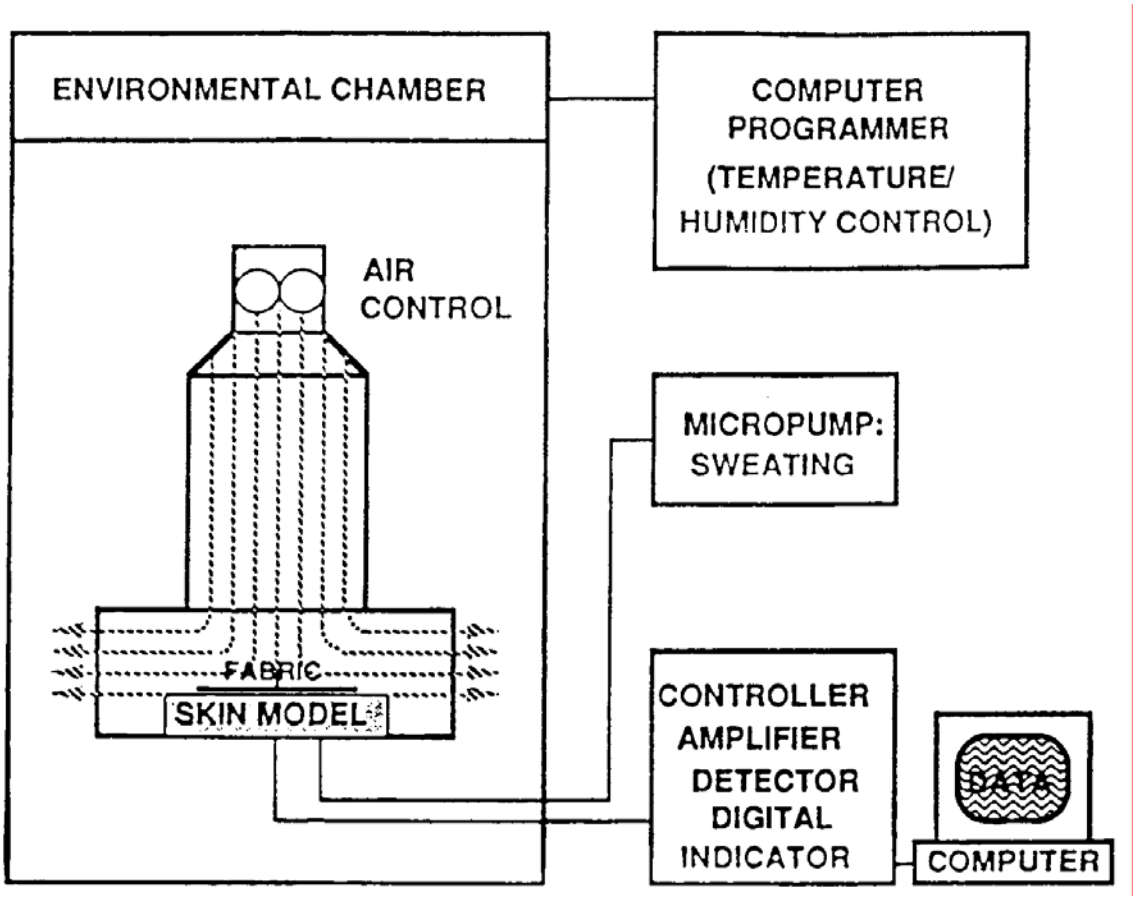


Figure 2. The chamber of skin sweating model comfort test.

2.7 Tensile property test

Tensile tests were performed on the original, plasma treated, and PNIPAM-grafted cotton fabrics at 21^{o}C and 65% relative humidity on a MTO Sintech (1/S) load frame was used to test them. The specimens were cut into 1×4 inch^2 strips. The thickness of the fabrics was 0.75mm. The gage length was 1.5 inch. The crosshead speed of the MTO Sintech (1/S) was set at 12 in/ min. The initial speed was also 12 in/ min.

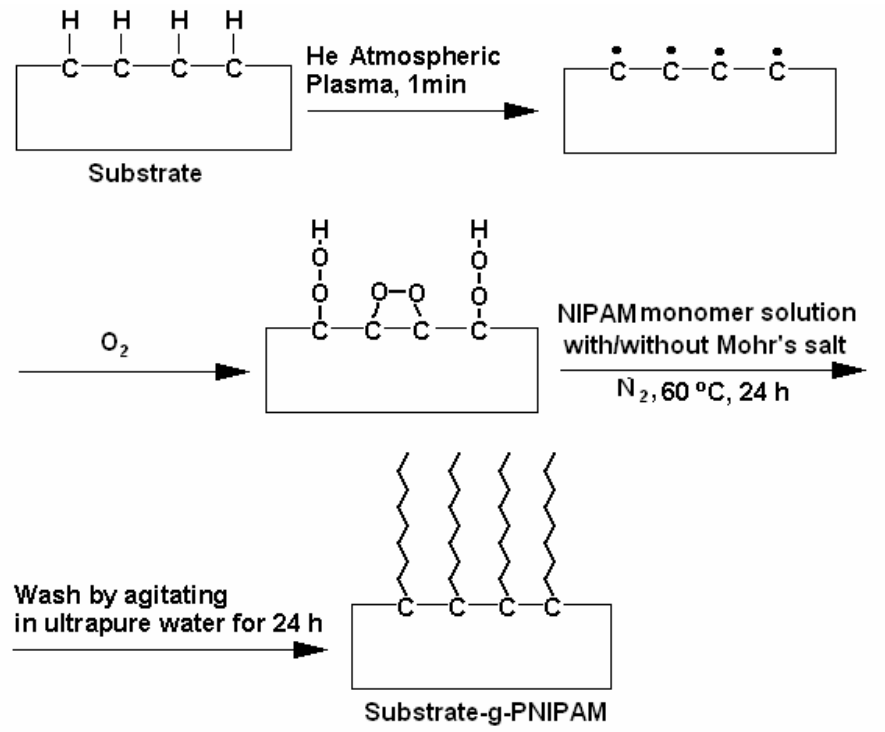
Three specimens were tested for each sample to ensure the reproducibility of the data. The tensile strength and strain at break were automatically calculated by computer. The slope of straight-line portion of the stress-strain curves was calculated as the elastic modulus of each

sample. The tensile strength and modulus were compared between the raw fabrics, the plasma treated fabric and the PNIPAM-grafted fabrics by different methods.

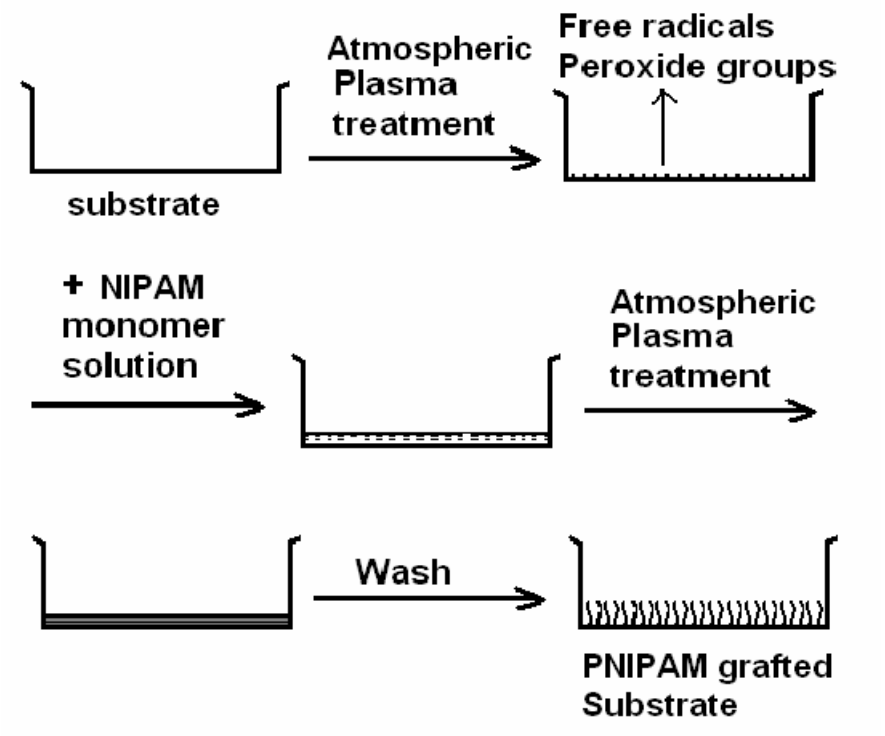
3. Results and Discussion

The PNIPAM was grafted on cotton fabrics surface by two methods: i) atmospheric plasma treatment activation and subsequent NIPAM monomer solution graft copolymerization and ii) atmospheric plasma treatment of NIPAM 2-propanol solution coated fabrics. A schematic presentation of both grafting method is depicted in Figure 3. In method I, the atmospheric plasma treatment activates the substrate surface and forms free radicals. Since the atmospheric chamber is not sealed, there is always oxygen in the system. Free radicals react with oxygen to form hydrogen peroxide groups as the main surface functionality in the plasma chamber or outside of the chamber. The hydrogen peroxide groups are thermally labile in nature and initiate the graft copolymerization of NIPAM in the NIPAM monomer solution at 60^{o}C under N_2. In order to increase graft yield, APS and Mohr's salt are added in Method i. The plasma treatment and subsequent graft copolymerization happen separately, so it is called a two-step method.

Compared to method I, method II is a one-step method because the plasma treatment and grafting happen simultaneously in the plasma chamber. It is a plasma in situ copolymerization. More specifically, the fabrics are pretreated by atmospheric plasma and active groups including free radicals and peroxide groups form on the substrate surface. After that, fabrics are coated by 45% NIPAM 2-propanol monomer solution. Finally, the coated fabrics are exposed to plasma for 4min. The free radials and peroxide groups will initiate the graft polymerization of NIPAM on cotton under plasma irradiation. Therefore, this method just needs previous plasma treatment and coating, and plasma treatment again. It does not require the reaction kettle, N_2 degassing, and water shaking bath, etc. Therefore, method ii is more straightforward and convenient than method i. All procedures of method ii can be done in a continuous process line, so it is very suitable for industrial scale up.



(I)



(II)

Figure 3. The schematic of graft polymerization of NIPAM on cotton fabrics methods: (I) Atmospheric plasma treatment followed by graft copolymerization (two-step method); (II) Atmospheric plasma treatment of NIPAM monomer solution coated fabrics (coating method).

3.1 FTIR spectra

FTIR spectra of the original and PNIPAM-grafted cotton are shown in Figures 4 a and b. PNIPAM-grafted cotton (coating methods) showed the strongest absorption bands of the PNIPAM characteristic peaks than cotton grafted by method I. They include 1650/1540cm^{-1} and 2970cm^{-1}, which are attributed to the secondary stretching of amide C=O groups and Asymmetric stretching of -CH3 groups in PNIPAM chains [10]. This indicates that PNIPAM has been successfully grafted onto the cotton surface by coating methods. The PNIPAM-grafted cotton using method I without any initiators has a similar FTIR spectrum to that of original cotton. One possible explanation is that the graft yield produced via this method may be very low, and not detectable with FTIR. However, the addition of APS or Mohr's salt increased the density of PNIPAM side chains on the surface and hence enhanced the intensity of the absorption bands at 1640cm^{-1} and 2970 cm^{-1}. Overall, the FTIR results confirm that grafting of PNIPAM on cotton surface and that method II results in greater graft yield comparing to method I. However, the additives (Mohr's salt and APS) increase the graft yield of PNIPAM in method I.

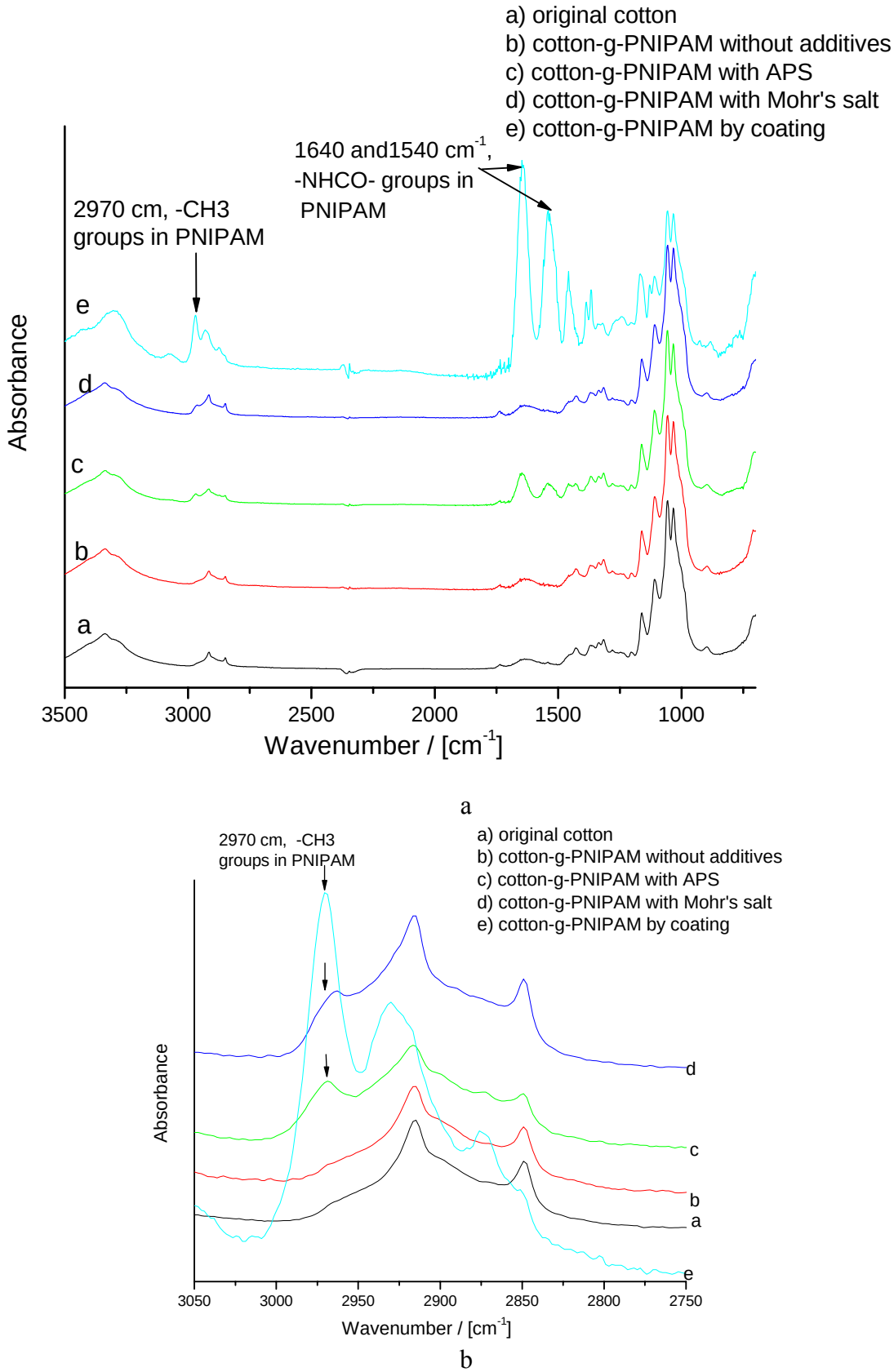


Figure 4. FTIR spectra of original and PNIPAM-grafted cotton.

3.2 Graft Yield

Table 1. Graft yield of PNIPAM onto cotton by various methods.

PNIPAM grafted cotton	Graft Yield
Two-step method (without any additives)	1.47%
Two-step method (with APS)	7.80%
Two-step method (with Mohr's salt)	11.95%
coating method	15.36%

The graft yield is calculated as $(W_1-W_0)/W_0$, where W_0 is the weight of untreated fabric and W_1 is the weight of grafted fabric. As seen in Table 1, the graft yield of PNIPAM-grafted cotton by method II is the largest, 15.35%. The graft yield of PNIPAM on cotton fabrics by method I without any additives is the smallest, 1.47%. FTIR of this PNIPAM-grafted sample doesn't show PNIPAM characteristic peaks because of its relatively small graft yield. After addition of APS and Mohr's salt, the graft yield increased from 1.47% to 7.80% and 11.95%. Therefore, both APS and Mohr's salt can improve the graft copolymerization. However, their mechanisms are very different. APS is usually used as an initiator for polymerization. After heating, its persulfate bonds are very easy to break down to free radicals. The free radicals can initial homopolymerization. It also induces free radical formation on the fabric surface by free radical transfer or break of peroxide groups, which initiate graft polymerization.

In contrast, Mohr's salt, by virtue of its reducing nature, modifies the usual thermal decomposition of hydrogen peroxide and suppress the formation of hydroxyl radical. Without Mohr's salt, the hydrogen peroxide group will form hydroxyl radicals (eq. 1), which are partly responsible for homopolymerization during the grafting reaction. With Mohr's salt, the hydrogen peroxide group is transformed to hydroxyl ion, and the primary radical PO·, which initiates the grafting reaction (see eq.2) [11]:

$$POOH=PO\cdot+OH\cdot \quad (1)$$

$$POOH+Fe^{2+}= PO\cdot+OH^{-}+Fe^{3+} \quad (2)$$

Therefore, Mohr's salt can suppress homopolymerization [eq.(1) overpowered by eq.(2)] and results in a higher degree of grafting.

3.3 Tensile Properties

One advantage of grafting PNIPAM on the fabric surface other than using PNIPAM alone is that the fabrics can provide a mechanical support and dimensional stability for the PNIPAM hydrogel. However, grafting of PNIPAM changes the surface chain structure of fabrics. Some additives used may also react with the fabric molecular chains. These two factors influence the mechanical properties of grafted fabrics since woven fabrics have a large surface area. Therefore, the tensile properties of original, plasma-treated, and the PNIPAM-grafted cotton are tested. The results are shown in Figure 5 and 6. The grafted samples were all washed with water for 24 hours after grafting. In order to compare the various samples' tensile properties, the original and plasma treated sample were also washed using water for 1 day prior to testing.

It is shown in Figure 5 that there is no significant difference in tensile strengths of original and plasma-treated one. The plasma treatment was 1min He atmospheric plasma. Early work in our group showed that 1 min He atmospheric plasma resulted little change of tensile strength of nylon fibers [12]. However, it is shown in Figure 6 that the modulus of plasma treated sample is about 40 MPa bigger than that of the original sample. This may be due to the crosslink effect of plasma. Plasma treatment, may have resulted in surface crosslink, decrease of chain mobility and strain, and increase of modulus.

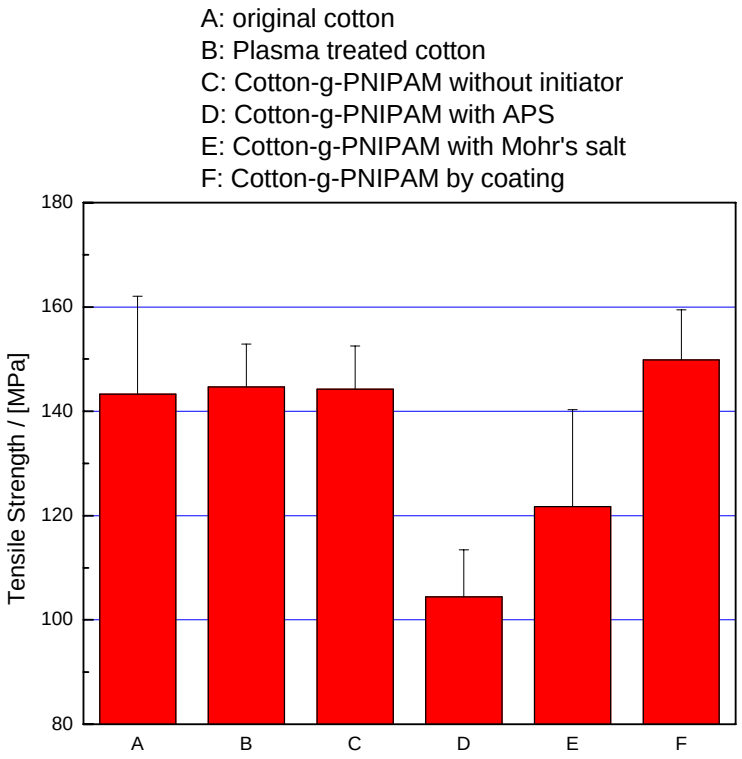


Figure 5. Tensile Strength of the original, plasma treated, and PNIPAM-grafted cotton.

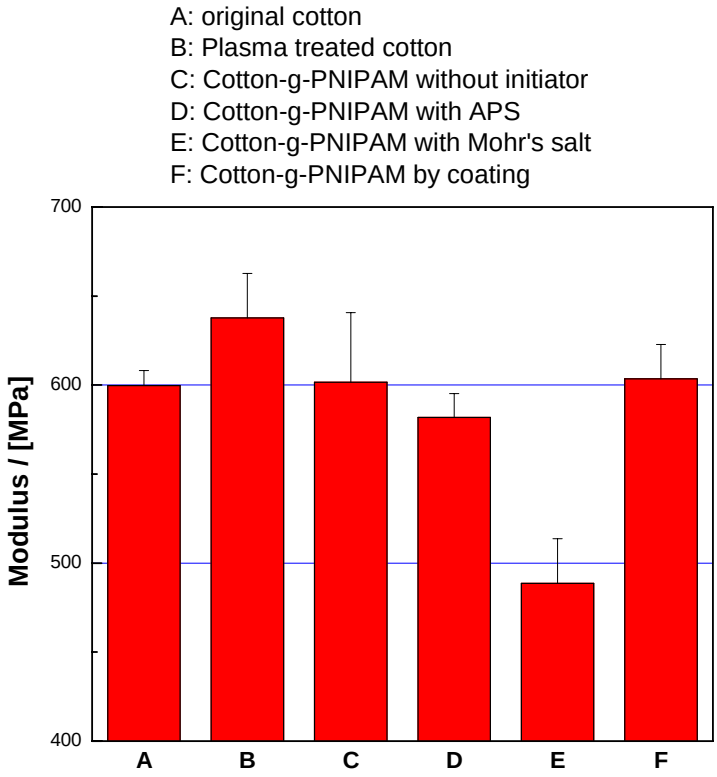


Figure 6. Modulus of the original, plasma treated, and PNIPAM-grafted cotton.

The PNIPAM-grafted cotton (two-step method) without any additives had almost the same strength and modulus as the original and plasma treated cotton. The possible reason is that although the PNIPAM is grafted, the graft yield is small, only 1.47%.

In contrast, the PNIPAM-grafted cotton sample by two-step method with the addition of APS and Mohr's salt have up to 27% and 19% loses in tensile strength and modulus. This is because their graft yields are 7.80% and 11.95%. The large amount of grafting decreased the tensile strength and modulus. McDowall et al[3] also found that as the graft yield increased, the tensile properties of poly(acrylic acid) grafted rayon fibers decreased. It was also found in literature that the tensile strength of PNIPAM-grafted polyethylene membranes gradually decreased to approximately half that of the original PE film with an increase in graft yield13]. One possible explanation for the decrease of the tensile properties is disruption of crystallinity and surface chemical structure[3].

The PNIPAM-grafted cotton (by coating) has very different tensile property with other grafted samples. Although the graft yield (15.36%) was higher than those from two-step methods, the tensile properties were not affected by grafting. This may be due to the fact that PNIPAM is grafted by plasma treatment of the NIPAM monomer solution coated fabrics. Since the grafting happens during the plasma (4min), there may be a lot of crosslinking of PNIPAM and cotton at the fabric surface. Crosslink increases the tensile properties.

In conclusion, PNIPMA-grafted cotton fabrics using various methods have good tensile strength. But the tensile properties of PNIPAM-grafted fabrics by plasma coating are superior to those achieved by two-step grafting method.

3.4 Responsive heat transfer property

PNIPAM alone undergoes a reversible phase transition in response to temperature; the phase change of PNIPAM occurs in the grafted surface too. Hence, graft copolymerization of PNIPAM makes a surface smart and temperature-sensitive, resulting in stimuli-responsive

characteristics of fabrics, e.g., the swelling properties or wettability, the pore size, or permeability.

To investigate the responsive characteristics of the PNIPAM-grafted fabrics, a skin model sweating comfort test was conducted on the original and PNIPAM grafted cotton fabrics made by method I with the addition of APS. The comfort test was conducted in wet conditions at 10 and 35^{o}C. The heat transferred (lost) from the skin model through fabric (W(watt)) was measured[9]. The high heat transfer indicates the poor insulation and high permeability. Therefore, the transferred heat can be used as a variable to test the relative permeability of the fabrics.

The heat transfer from the skin model through cotton fabrics in wet condition at 10 and 35^{o}C is shown in Table 2. The "control" is the original fabric. At low temperature (10^{o}C), the heat transfer through control and PNIPAM-grafted cotton were 9.24 and 8.49 watts, respectively. This means that the PNIPAM-grafted cotton is more heat insulating than the control at 10^{o}C. It may be due to the formation of the PNIPAM gel on the cotton fabric surface, which may block or reduce the pore size. However, at the temperature over the LCST of PNIPAM (at 35^{o}C), the heat transferred through control and PNIPAM-grafted cotton were 2.72 and 2.93 watts respectively. The grafted cotton transferred more heat than the control. This suggests that over the LCST, the PNIPAM-grafted cotton is more moisture vapor permeable and less insulating than the control one. This is because of the phase change of PNIPAM at 32^{o}C. Below 32^{o}C, PNIPAM forms a hydrogel with water. However, PNIPAM gel becomes more compact at temperature over 32^{o}C and the polymer chains aggregate because the hydrophobic interaction dominates. The compact of PNIPAM hydrogel on cotton surface should reduce the yarn diameters. Hence, the pore size of the woven cotton become larger, and the permeability increases.

The comfort test shows a thermoresponsive heat transfer ability of PNIPAM grafted cotton in the wet condition. Below 32^{o}C (e.g., 10^{o}C), the PNIPAM-grafted cotton has a lower heat transfer than control, i.e. it is more insulating; however, over 32^{o}C (e.g., 35^{o}C), the PNIPAM-grafted cotton has higher heat transfer than control, i.e., it is more permeable. This means that

this PNIPAM-grafted fabric may change its thermal insulative properties in response to the surrounding environment. If the temperature is below 32^{o}C, the PNIPAM-grafted cotton is more warm (insulating) than the control fabrics. However, over 32^{o}C, it is cooler (more permeable) than the control. This thermal response may have numerous potential uses in clothing. For example, the athletes can have warmer clothing in the cold temperature. However, after exercises and sweating, the warm clothes will become more permeable and cooler.

Table 2. Heat transfer rate of original and PNIPAM-grafted cotton in wet condition at 10 and 35 ^{o}C.

Cotton	**Heat transfer, W(watt)**	
	10^{o}C	**35^{o}C**
Control	9.24	2.72
PNIPAM grafted	8.49	2.93

4. Conclusion

Novel thermoresponsive fabrics were prepared by grafting thermoresponsive polymer Poly(N-isopropylacrylamide) onto cotton surface via two novel atmospheric plasma methods. FTIR confirms the grafting. Graft yields are calculated from weight change. Tensile tests indicate that PNIPAM grafting has no significant influence on the tensile properties of cotton fabrics. Sweating hot plate testing show a thermosensitivity of the PNIPAM-grafted cotton. In wet conditions (sweating), less heat was transferred from the skin model to outside through the grafted fabrics than through the control at 10^{o}C; however, more heat was transferred through it at 35^{o}C. PNIPAM-grafted cotton may be warmer at lower temperature and cooler at higher temperature than untreated cotton fabrics. These materials may have applications in smart textiles, such as sports wear.

5. Future directions

SEM or AFM will be used to study the PNIPAM-grafted cotton morphology at low and high temperatures in wet conditions. Morphology is the key factor to investigate for the permeability and tensile properties.

6. References:

1. I.Y. Galaev and B. Mattiasson, Stimulus Responsive Surfaces: Possible Implication for Biochromatography, Cell Detachment and Drug Delivery. *In*: R. Freitag (ed) *Synthetic Polymers for Biotechnology and Medicine (p116~129).* Georgetown, Texas: Landes Bioscience 2003.
2. N. Yamada, T. Okano, H. Sakai, F. Karikusa, Y. Sawasaki, and Y. Sakurai, Thermoresponsive polymer surfaces – control of attachment and detachment of cultured-cells. *Macromol. Chem. Rapid Commun.*, 11(11): 571-576, 1990.
3. D. J. McEowall, B. S. Gupta, and V. T. Stannett, A comparison of the properties of Ceric ion and preirradiated acrylic acid grafts to rayon fibers. *Polymer Journal*, 19(5): 643-648, 1987.
4. J. O. Karlsson, P. Gatenholm, Cellulose fibre-supported pH-sensitive hydrogels. Polymer, 40: 379-387, 1999.
5. J. Liu, M Zhai, H. Ha, Pre-irradiation grafting o temperature-sensitive hydrogel on cotton cellulose fabric. *Radiation Physics and Chemistry*, 55: 55-59, 1999.
6. J. Xie, Y. Hsieh, Thermosensitive poly(N-isopropylacrylamide) hydrogels bonded on cellulose supports. *J. Appl. Polym. Sci.*, 89: 999-1006, 2003.
7. Delhi, Environmentally responsive smart textiles – II, *Express Textile Articles, www. expresstextile.com*, 2005.
8. S. R. Matthews, Y. J. Hwang, M. G. McCord, M. A. Bourham, Investigation into etching mechanism of polyethylene terephthalate (PET) films treated in helium and oxygenated-helium atmospheric plasmas. *J. Appl. Polym. Sci.*, 94(6): 2383-2389.
9. Thermolabo Instrument and Method. *Textile Protection and Comfort Center Manual*, NC State University.
10. Y. V. Pan, R. A. Wesley, R. Luginbuhl, D. D. Denton, and B. D. Ratner, Plasma polymerized N-isopropylacrylamide: Synthesis and characterization of a smart thermally responsive coating. *Biomacromolecules*, 2: 32-36, 2001.
11. B. Gupta, J. G. Hilborn, I. Bisson, and P. Frey, Plasma-induced graft polymerization of acrylic acid onto poly(ethylene terephthalate) films*. J. Appl. Polym. Sci.*, 81: 2993-3001, 2001.
12. Y. Qiu, C. Zhang, Y. J. Hwang, B. L. Bures, and M. McCord, The effect of atmospheric pressure helium plasma treatment on the surface and mechanical properties of ultrahigh-modulus polyethylene fibers. *J. Adhesion Sci. Technol.*, 13(1): 99-107, 2002.
13. K. Yamada, T. Sato, S. Tatekawa, and M. Hirata, Membrane properties of polyethylene films photografted with hydrophilic monomers. *Polymer Gels and Network*, 2: 323-331, 1994.

Chapter Five: The Effects of Grafting Parameters on the Graft Yield of Poly(N-isopropylacrylamide) Grafted Polystyrene Surfaces Prepared by Atmospheric Plasma Treatment Coating

Abstract

Temperature-responsive poly(N-isopropylacrylamide) (PNIPAM) was grafted onto Polystyrene (PS) surfaces via atmospheric plasma treatment of NIPAM monomer coated PS surface. The PS was pretreated by plasma before coating. Fourier Transform Infrared Spectroscopy (FTIR) confirms the grafting of PNIPAM on PS surface. AFM images revealed distinctly different surface topographies of PNIPAM grafted PS compared to original and plasma treated PS. Water contact angles for PNIPAM grafted PS surface increased dramatically approximately at 32^{o}C, confirming the temperature sensitivity of the PNIPAM grafted surfaces. The effects of grafting parameters on the graft yield, i.e., pre-plasma treatment time, post-plasma treatment time, and coated monomer amount, were studied. The plasma pretreatment activated the surface and was beneficial to the graft copolymerization. However, more than 1 or 2 min pretreatment time decreased the graft yield indicating that plasma surface activation is not linearly related the treatment time. The post-plasma treatment induces the graft copolymerization. However, etching occurred and decreased the graft yield at higher post-treatment time (>2 min). 55% monomer solution higher than 109 μl decreased the graft yield

1. Introduction

Poly(N-isopropylacrylamide) (PNIPAM), a representative of thermoresponsive polymers, exhibits lower critical solution temperature (LCST) behavior around 32^{o}C in aqueous solutions. PNIPAM chains hydrate to form an expanded structure in water when the solution temperature is below its LCST and dehydrates to form more compact structure when heated to above the LCST [1]. Covalent grafting of PNIPAM on a material surface will endow that material surface with temperature sensitivity.

Different methods, including ultraviolet (UV) irradiation[2,3,4], vacuum plasma treatment[4,5,6,7], ozone treatment[8,9], electron beam[10,11,12,13], γ-irradiation[14], and chemical treatment[15], have been used to graft PNIPAM onto different polymers. For example, Wang et al [9] have grafted PNIPAM onto a fluorinated polyimide membrane surface using ozone treatment. Okano and his coworkers [13] have grafted PNIPAM onto polystyrene (PS) plates by electron beam irradiation. Among various irradiation methods, vacuum plasma treatment is of special interest because it offers flexibility, effectiveness, safety and environmental friendliness. However, this method still has some disadvantages, i.e., the need for a vacuum environment and relatively high cost. In light of the disadvantages of low-pressure plasma systems, atmospheric pressure plasma treatment has recently emerged as a novel technique. By using an atmospheric plasma treatment device, the machine can operate in a pre-existing continuous processing line. It can also save the cost of vacuum environment [16]. There is very little research about grafting of vinyl monomers using atmospheric plasma treatment.

Due to its temperature sensitivity, PNIPAM grafted surfaces have been utilized in new chromatographic separation methods for a variety of types of bioactive compound[17], a biofouling release coating, controlled release of drugs and growth factor. Okano et al. further applied the thermoresponsive surfaces for thermally regulated cell adhesion and detachment [10,11,13] and extended the idea to tissue engineering[12]. Confluently cultured cell monolayers on hydrophobic PNIPAM-modified surfaces at 37 °C detached as single cell sheets by lowering the culture temperature to 20 °C where the modified surfaces became hydrophilic due to PNIPAM's hydration/dehydration transition at 32 °C. However, graft yields of PNIPAM on the surfaces have a significant influence on cellular adhesion and detachment behavior [13, 18]. It has been found that bovine carotid artery endothelial cells (ABCs) adhere to the PNIPAM grafted PS with 1.4 $\mu g/cm^2$ graft yield and proliferate to form confluent cell monolayers. The cell monolayers were harvested as single cell sheets by a temperature decrease from 37 to 20 °C. On the contrary, BAECs did not adhere to the PNIPAM grafted surface with 2.9 $\mu g/cm^2$ graft yield. Because of the importance of PNIPAM graft yield for cell adhesion and detachment, the effects of various grafting parameters on the graft yield will be investigated in this paper. The grafting parameters include plasma

pretreatment time, monomer amount, and plasma posttreatment time, and choice of plasma gas.

2. Material and Methods

2.1 Materials

The substrates used for graft polymerization were 60mm non-tissue culture polystyrene (PS) plates (Corning). N-isopropylacrylamide (NIPAM, 97%, Acros Organic) was recrystallized using hexane (95%, Acros Organic) prior to grafting. NIPAM monomer solvent, 2-propan and hexane were purchased from Sigma-Aldrich.

2.2 Atmospheric Plasma System

The atmospheric plasma system set up in the College of Textiles at North Carolina State University is an atmospheric pressure glow discharge (APGD) device. Figure 1 shows a schematic drawing of the experimental facility. It is a capacitively coupled chamber and contains two horizontal parallel electrodes. The radio frequency power is coupled to the plasma via two copper electrode through an oscillating electrical field. Each electrode is covered with a dielectric material to limit the current in the discharge and force the charge to spread out over a large area instead of constricting to an arc.

In this experiment, treatments were conducted in a small test cell with plasma generated from 100% He or 99% He and 1% O_2 , or 99% He and 1% Forming gas. The power level used was 4.8 kW and the frequency was 4.5 kHz. The flow rate of 100% He was 10.00 L/min, and flow rates of 99% He and 1% O_2 or forming gas were 10.00 L/min and 0.11 L/min, respectively.

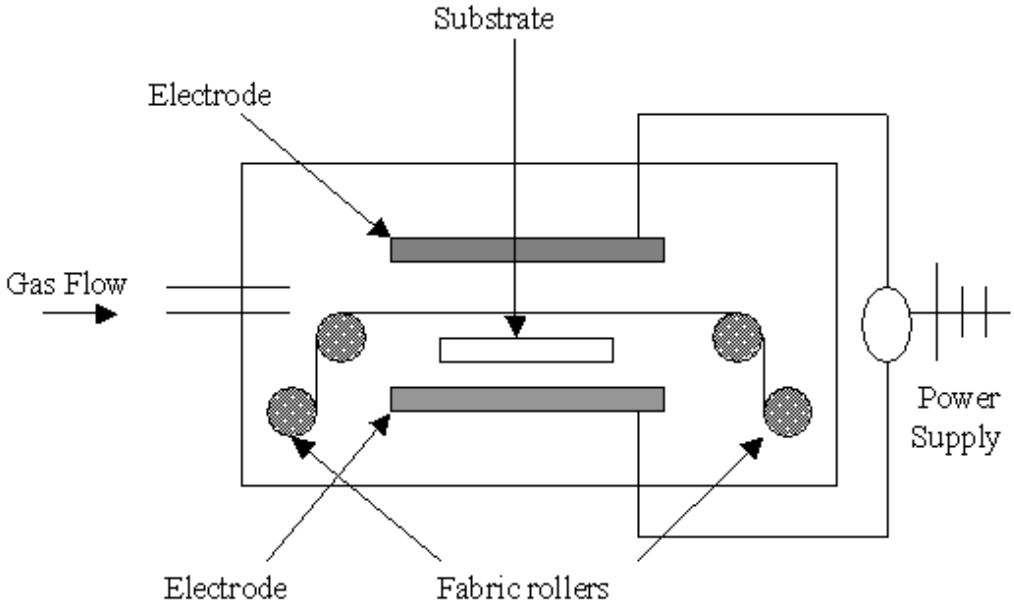


Figure 1. A Schematic of the Atmospheric Pressure Plasma System.

2.3 Grafting Copolymerization

The detailed process of grafting copolymerization is shown in Figure 2. The PS plates were exposed to He, He/O2 or He/Forming plasma for 0, 1, 2, and 3 min pretreatment. Then 109, 218, and 436 μl of a 55% NIPAM 2-propanol solution was added into the plates. After this, the plates were immediately exposed to a He, He/O2 or He/Forming plasma for 2, 5, or 10 min posttreatment. The treated samples were rinsed with ultra pure water and dried.

2.4 Graft Yield

The graft polymerization of PNIPAM was evaluated by weighting the PS samples before and after the graft polymerization. The amounts of PNIPAM grafted on the PS surfaces were calculated using

Graft Yield ($\mu g/cm^2$) = $(W_1-W_0)/A$,

where W_0 is the weight of untreated film, W_1 is the weight of grafted film, and A is the PS surface area.

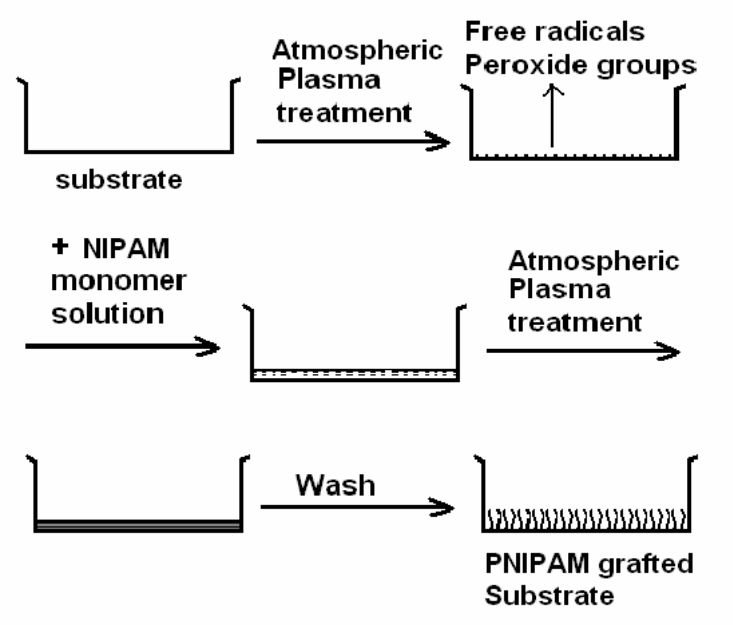


Figure 2. The schematic of graft polymerization of NIPAM on PS plates.

2.5 FTIR Measurement

Fourier transform infrared spectroscopy (Nicolet 510P FTIR spectrometer) was used to investigate the surface chemistry of the grafted PS. The spectra were collected at 4 cm^{-1} resolution with an FTIR microscopic spectrometer over 32 scans. The sampling area was coupled with an attenuated total reflection accessory and a 45° KRS-5 crystal.

2.6 Surface topography

Surface topography of PNIPAM-grafted PS (1min plasma pretreated, 109ul NIPAM monomer coated, and 10 min post plasma treated sample) was examined using an atomic force microscopy (JEOL JSPM-5200) in Tapping Mode in air. Measurements were carried out with a silicon probe (Olympus AC-160) at a scan area of 5μm^2.

2.7 Water contact angle measurement

The water contact angle of PNIPAM grafted PS was measured in air at 20, 25, 30, 32, 37, and 42 ^{o}C by the sessile method with a goniometer (Model A-100, Ramé-Hart, Inc.). The temperature of the test cell of the goniometer was controlled by a circulating water bath. Ultra pure water (12 μl) was placed on the sample surfaces using a syringe. The contact angle was then read after 1 min. The contact angle reported was an average of eight reading at different places on each sample.

3. Results and Discussion

3.1 FTIR

FTIR spectra of ungrafted PS and PNIPAM-grafted PS are shown in Figure 3. Absorption bands of 1540, 1650, 2970, and 3350 cm-1, attributed to the -C=ONH- and $–CH_3$ of PNIPAM chains [6], are found for the PNIPAM grafted PS samples, indicating that PNIPAM has been successfully grafted onto the PS surface.

Therefore, like vacuum plasma, atmospheric plasmas are also able to initiate grafting reactions of vinyl monomers. Atmospheric plasma can forms many active specials, including UV, free radicals, electrons, and ions, in the plasma. The dielectric barrier prevented the transition of the discharge to an arc when acoustic frequency voltage signal was applied. The resulting discharge was a nonequilibrium ionized gas that has many of the beneficial characteristics of low-pressure plasma treatment at increased pressure and without potentially damaging thermal energy [19].

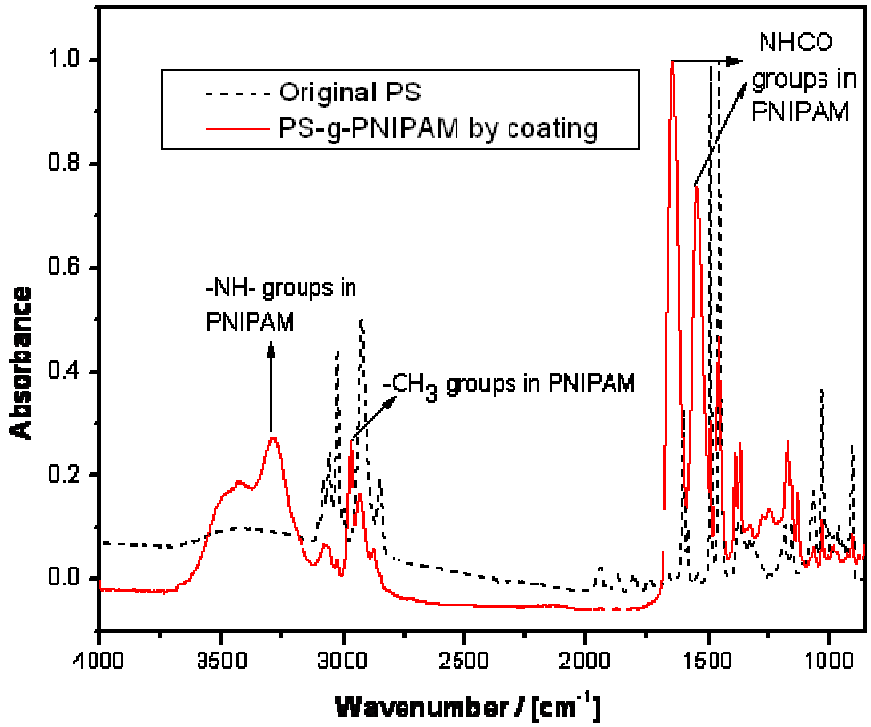


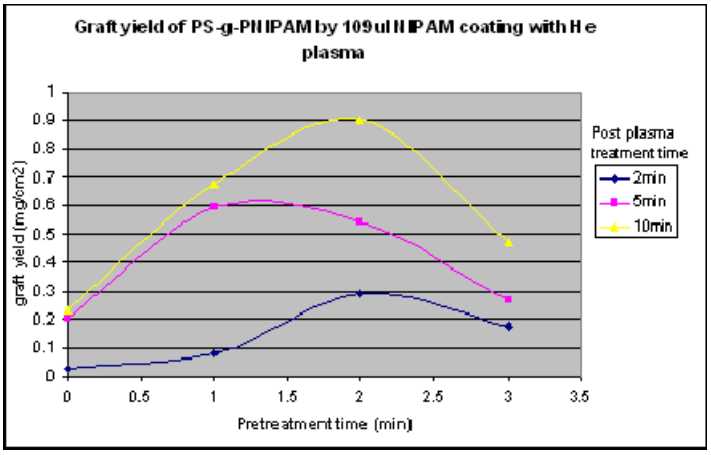
Figure 3. FTIR of original and PNIPAM grafted PS.

The possible mechanism of this coating method (Figure 2) is that the pretreatment of substrate activated the substrate surface by forming free radicals. The free radicals may react with oxygen to form peroxide groups. After coating with monomer solution, the substrate was exposed to plasma again. Since the monomer solvent, 2-propanol, is very volatile. During the post treatment, the peroxide groups break and react with NIPAM monomer or polymer segment, resulting in covalent grafting to the surface.

Vinyl monomers including NIPAM or acrylic acid are traditionally grafted on the polymer surface by two-step methods, i.e., irradiation of the substrates followed by the graft copolymerization in a heated sealed monomer solution. In contrast, this grafting method by atmospheric plasma coating is much more straightforward than traditional methods. It does not require vacuum, sealed reaction solution, or heating, and is therefore more suitable for continuous process.

3.2 Effects of grafting parameters on the graft yield

3.2.1 Plasma pretreatment time



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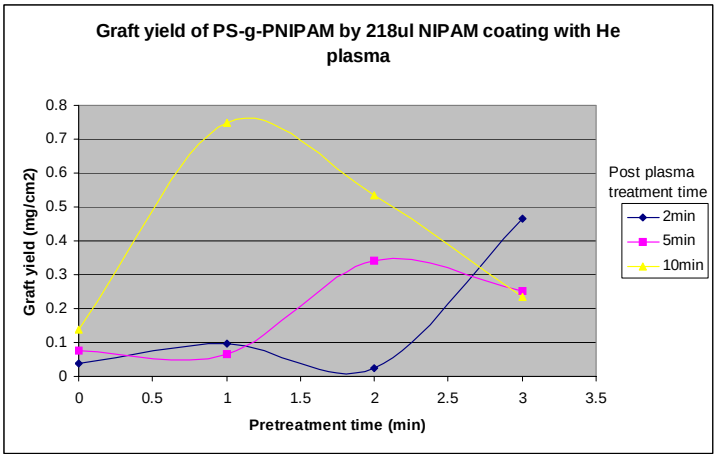

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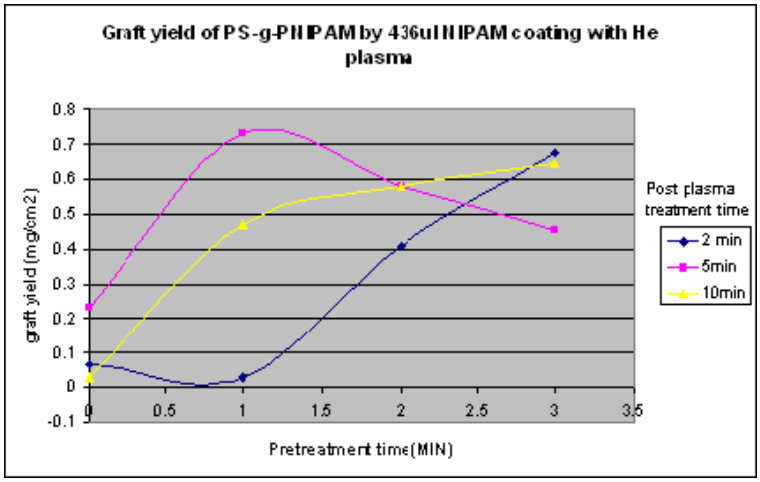

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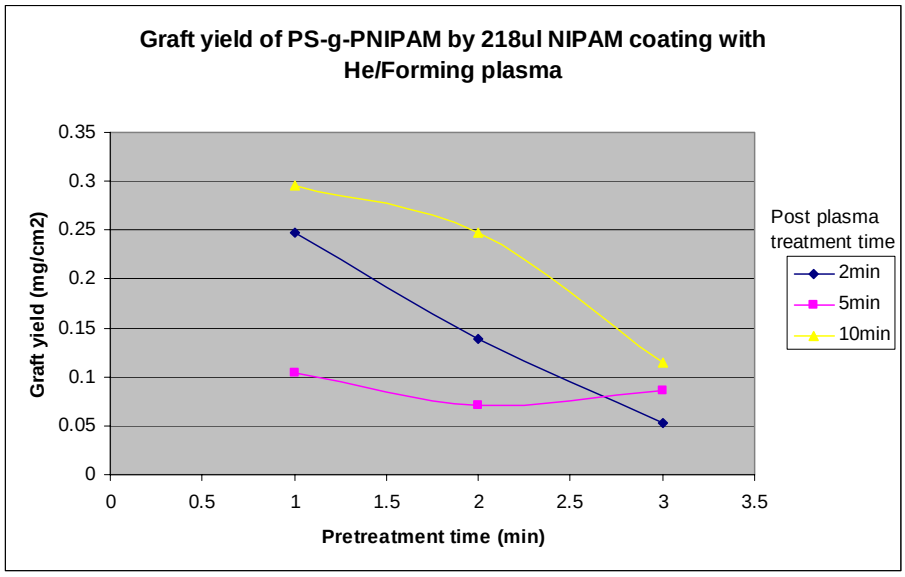


(d)

Figure 4. Relationship of Graft yield and plasma pretreatment time. a) He plasma, 109ul NIPAM coating; b) He plasma, 218ul NIPAM coating; c) He plasma, 436ul NIPAM coating; d) He/Forming plasma, 218ul NIPAM coating.

0, 1, 2, and 3 min pre plasma (He) treatment was used to activate the surface before coating. The graft yield with different plasma pretreatment time is shown in Figure 4 (a-c). The 0 min pretreatment still has graft yield around 0.1mg/cm^2, which indicates without plasma pretreatment, the PNIPAM can be grafted onto the PS surface to some degree. However 1, 2, and 3 min pretreatment time all increase the graft yield in Figure 4 (a-c). This is because the plasma pretreatment can activate the PS surface by forming some free radical and peroxide groups in the surface. However, when plasma pretreatment time is longer than 2 min, the graft yield of He plasma induced grafting begin to decrease. Therefore 2 min is the optimal pretreatment time for He plasma induced PNIPAM grafting on PS surface.

The same phenomenon was found for He/O_2 and He/forming plasma induced PNIPAM grafting. 1 min appears to be the optimal pretreatment time for He/O_2 and He/forming plasma induced PNIPAM grafting on PS surface (Figure 4d).The graft yield increased as the

pretreatment time increased but after 1 min the graft yield has no further increase or began to decrease.

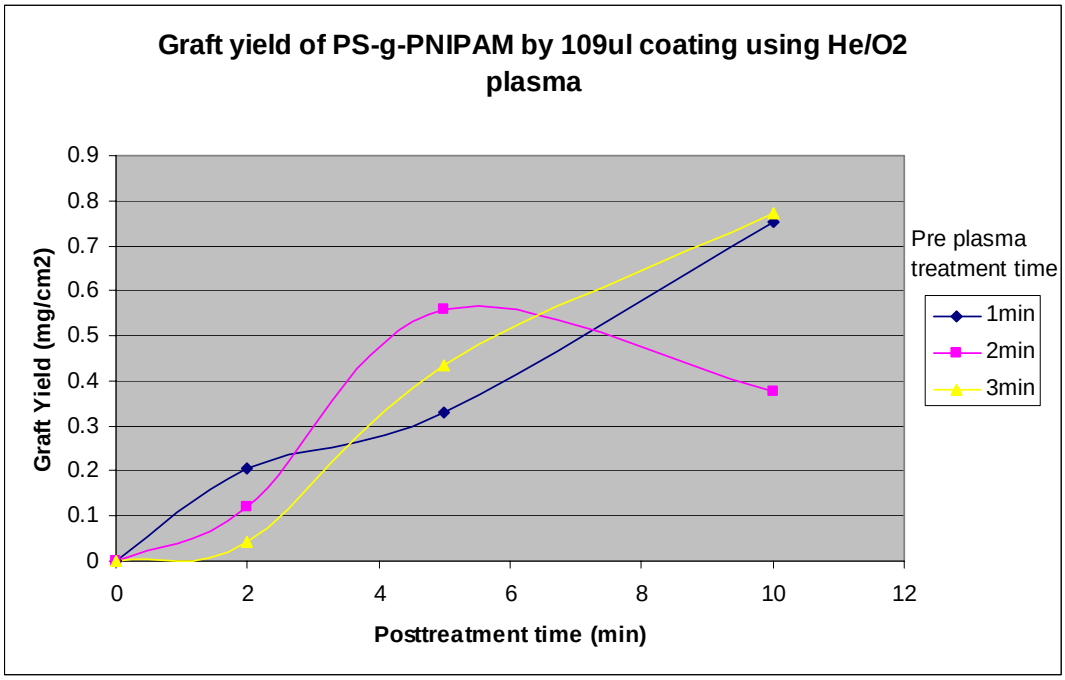
The decrease of graft yield when the plasma pretreatment time increased to more than 1 may be due to the etching and redoposition effect of plasma [16]. Initially, active species are likely to attach the PS surface, break polymer chains, and form free radicals by surface chain scission. Free radicals can react with O_2 and form peroxide groups. However, as the pretreatment time increase, it is possible that groups or atoms that volatilized due molecular chain scission and etching may be re-deposited on the surface. Therefore the amount of free radicals decreases, lowering the grafting yield.

3.2.2. Post plasma treatment time

The pretreated PS surface was coated by various amount of NIPAM monomer solution and then treated in plasma. Although the pretreated surface already had some active groups (free radicals and peroxide groups), there is little possibility for them to initiate graft copolymerization at room temperature and atmospheric pressure, hence, post plasma treatment is required for graft copolymerization. It was assumed that the graft yield was 0 when the post plasma treatment time was 0. The graft yield using He/O_2 gas at different post plasma treatment time is shown in Figure 5 (a, b, c). It shows that the graft yield increased when post treatment time increased from 0 to 5 min. After 5min, the graft yields had no further increase or began to decrease. The only two exceptions are shown in Figure 5a. With 1 and 3 min pretreatment and 109 ul monomer coating, the graft yields increase from 0 to 10 min. In order to get higher graft yields and save energy, 5 min was chosen as the optimal plasma posttreatment time for He/O_2 plasma grafting. The graft yield increase dramatically from 0 to 5 min is because the NIPAM monomer is continuously grafted to the PS surface by free radical copolymerization. It is though that after 5 min, the copolymerization ends and some etching occur, therefore decreasing the graft yields.

He plasma showed the same effects of plasma posttreatment time on the graft yields as He/O_2. However, He/Forming gas showed different results. In Figure 5d, as post plasma treatment

time increase from 0 to 2min, the graft yield increase dramatically. However, it is decreased at 5 min post plasma treatment, then increases at 10 min post plasma treatment. The reason is unknown.



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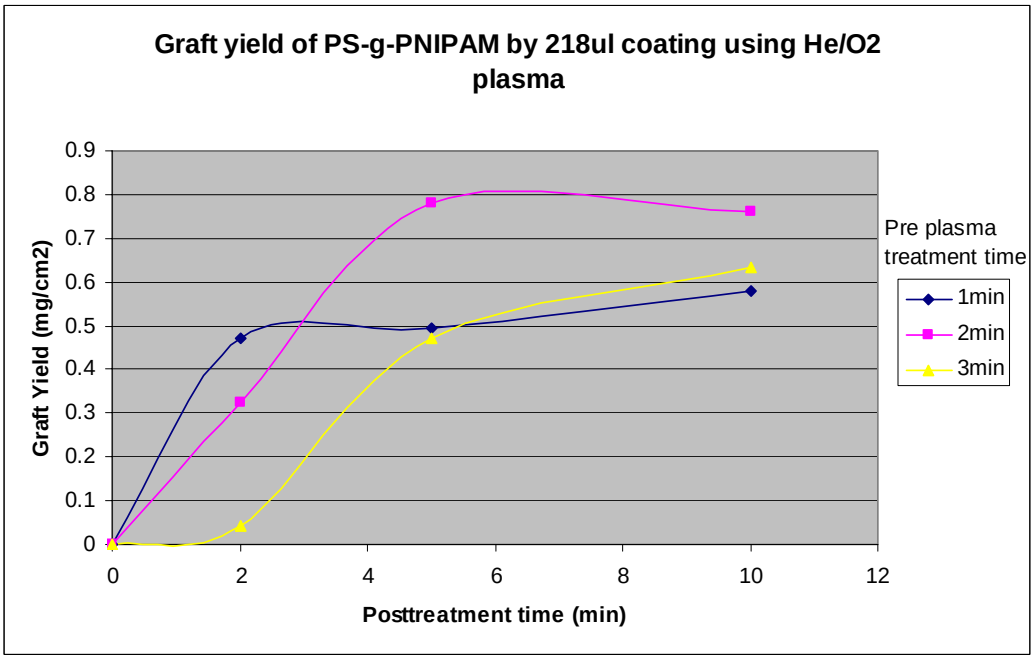

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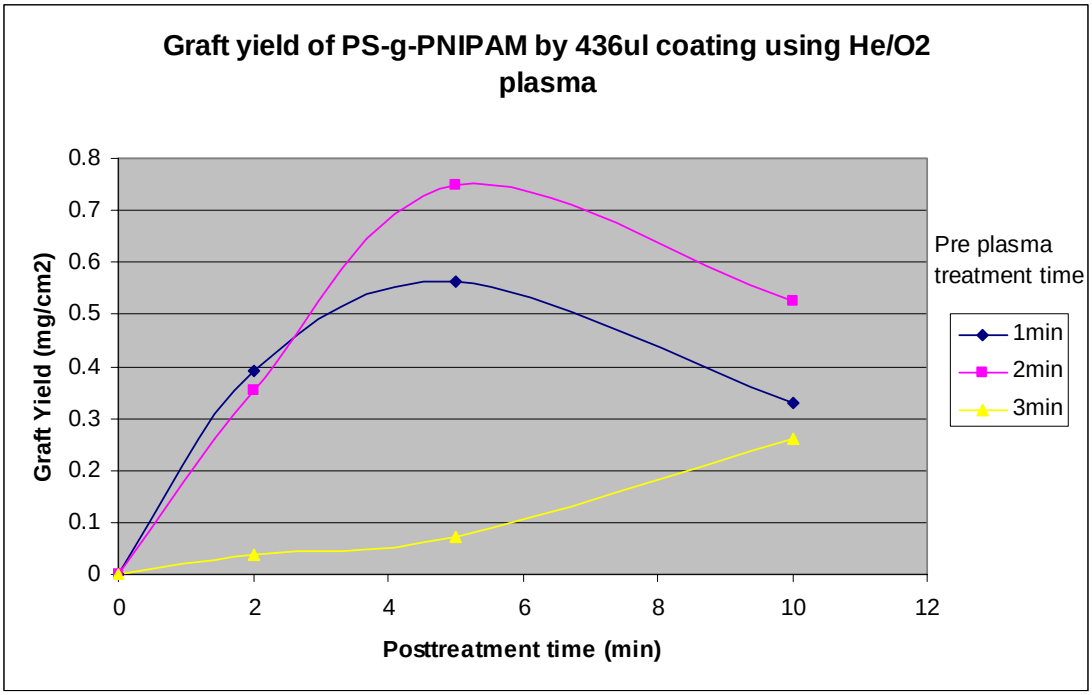

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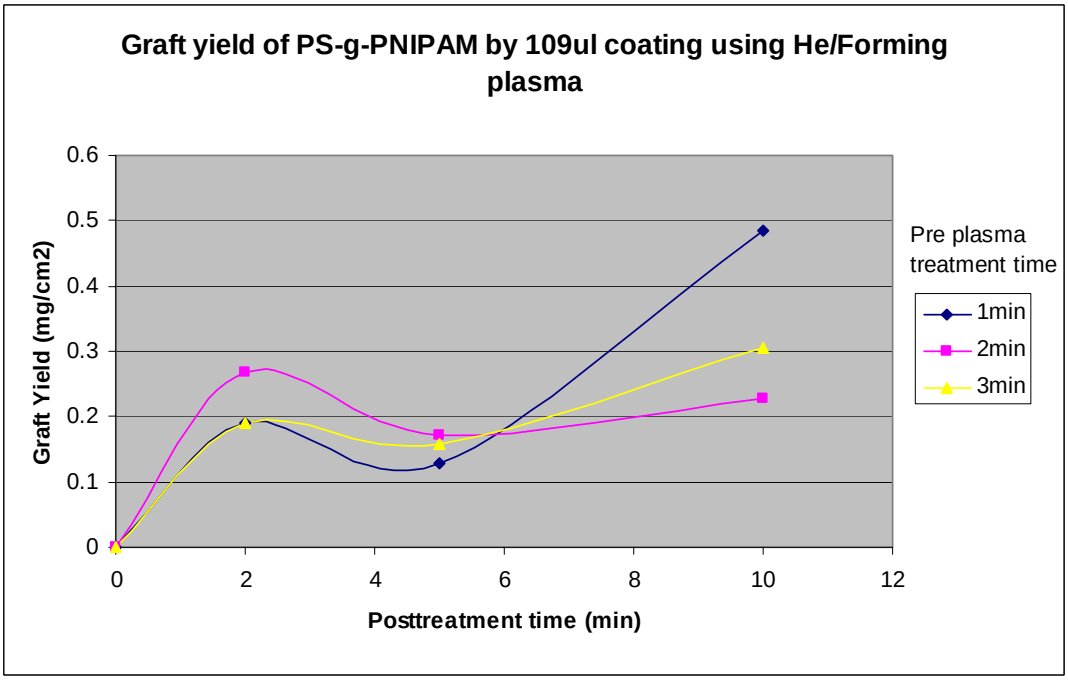

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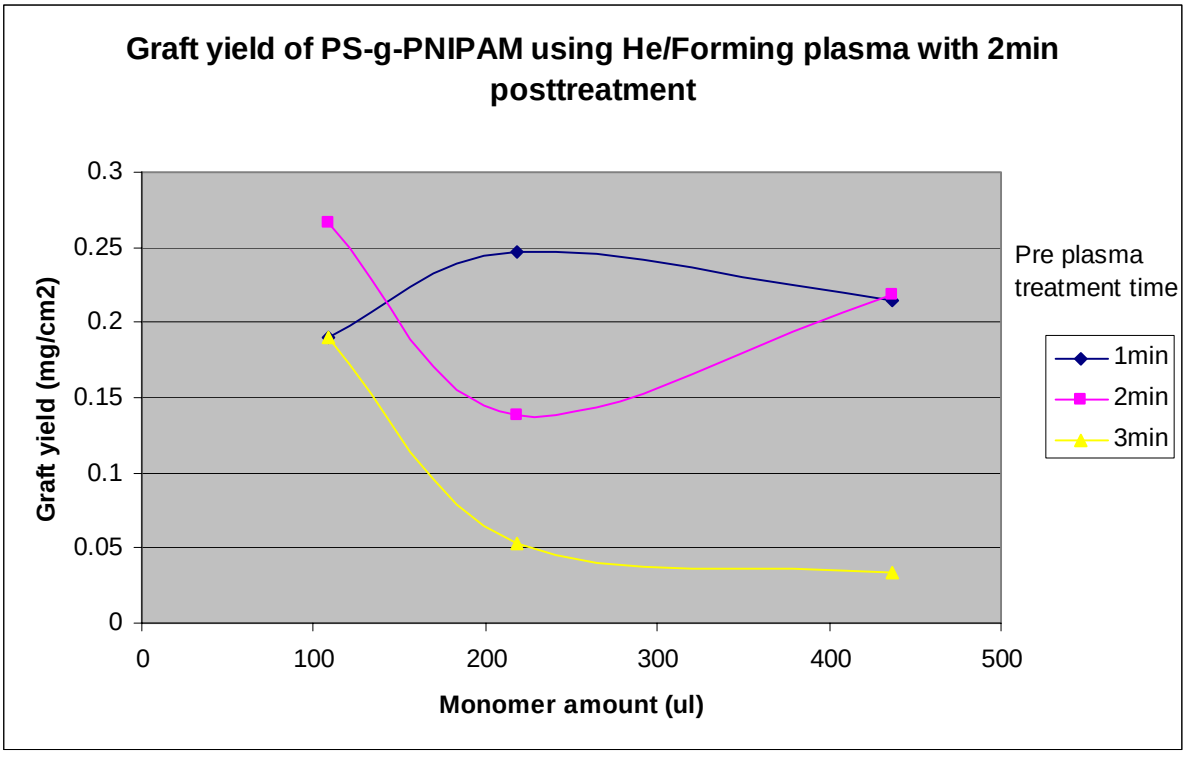
Figure 5. Relationship of Graft yield and post plasma treatment time. a) He/O2 plasma, 109ul NIPAM coating; b) He/O2 plasma, 218ul NIPAM coating; c) He/O2 plasma, 436ul NIPAM coating; d) He/Forming plasma, 109ul NIPAM coating.

3.2.3 Effect of NIPAM monomer amount on the graft yield

Different amounts of monomer solution coated on the surface will cause different graft yield. Usually the higher the volume of the monomer solution, the higher the graft yield. However, the graft yield decreases as the monomer solution increase from 109 to 436 ul in Figure 6. One explanation is that when a higher volume of monomer solution is used, the solution will may interfere with the interface between PS plates and monomer solution from plasma, reducing the interaction between the active species and the substrate interface. The optimal volume of monomer solution for He/Forming plasma induced PNIPAM grafting on PS is 109 ul or even less. This is also true for He and He/O_2 plasma induced PNIPAM grafting on PS surface.



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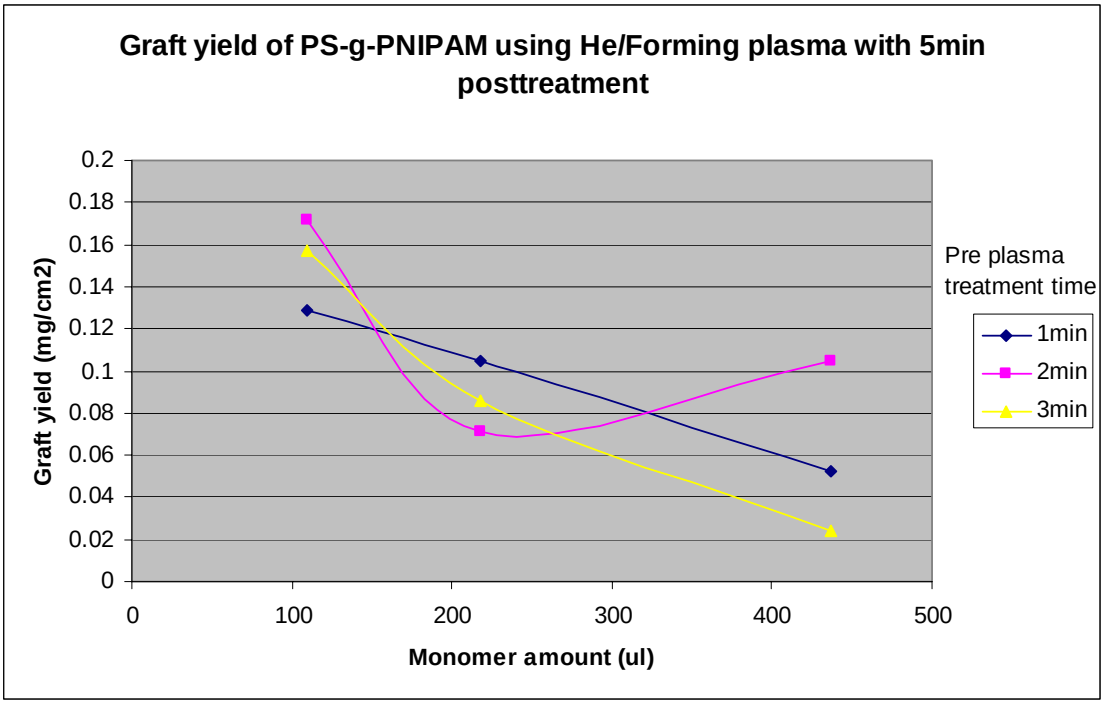

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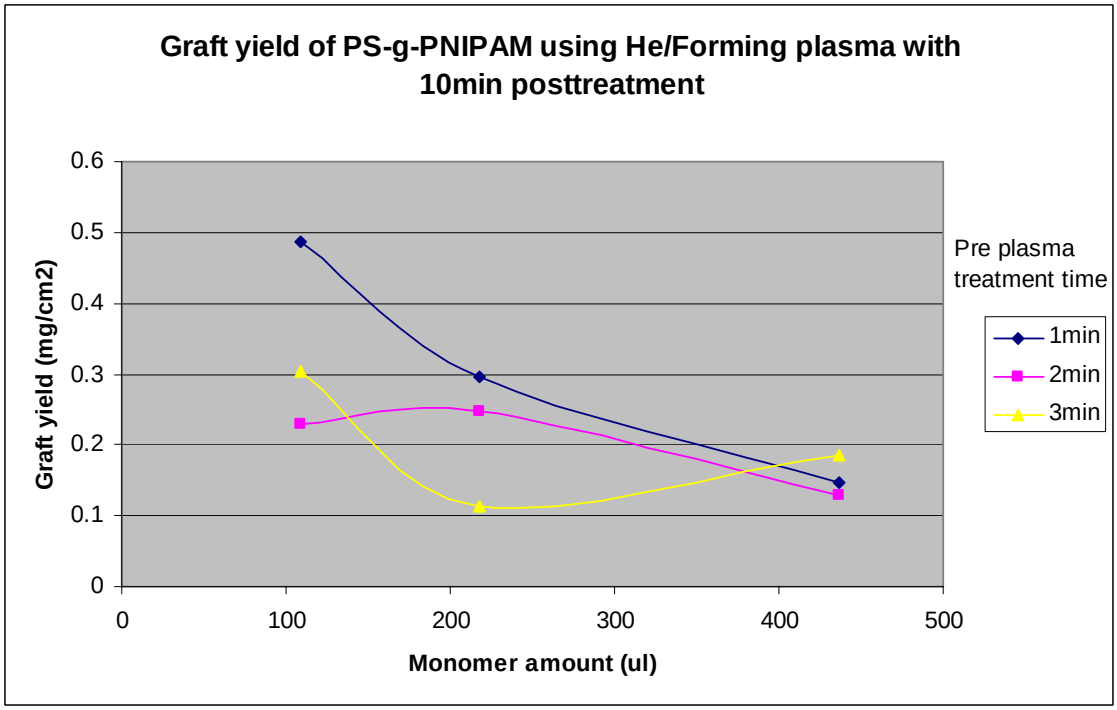

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Figure 6. Relationship of Graft yield and coated monomer solution amount using He/Forming plasma treatment. a) 2min postreatment; b) 5min posttreatment; c) 10min posttreatment.

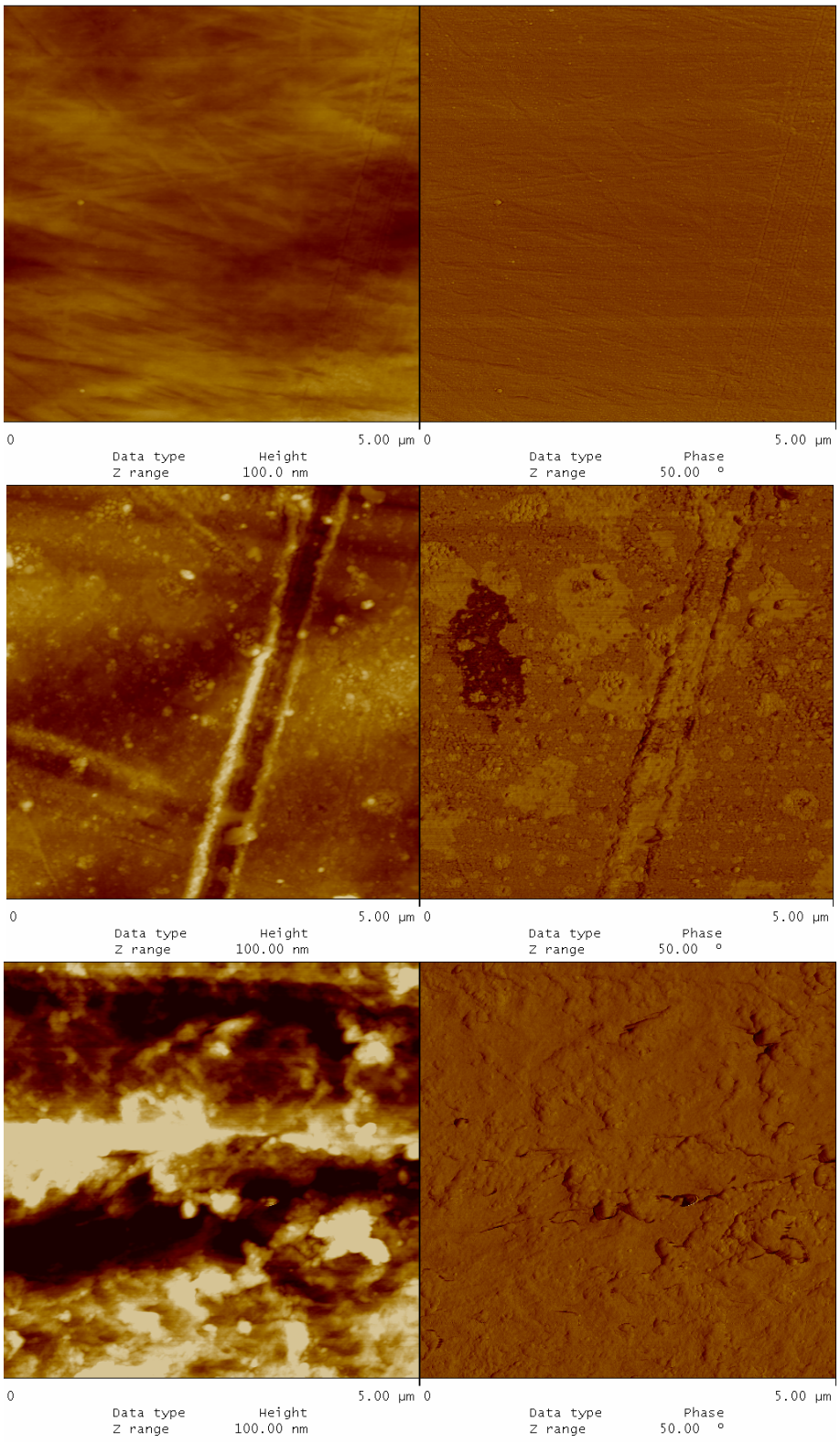

Figure 7. AFM images of PS: a) untreated PS; b) plasma-treated PS; c) PNIPAM-grafted PS by coating.

3.3 AFM images of PNIPAM grafted surface

Surface topographies of original, plasma treated, and PNIPAM-grafted PS samples were examined using atomic force microscopy (AFM). The original untreated PS has flat surface, while the topography changes by the surface treatment. The plasma-treated PS becomes has many isolated rough spots. Hwang *et al* [16] also found that atmospheric plasma treatment changes the morphology and roughness of polyester film and it was believed to be caused by the etching and redeposition effects of plasma. PNIPAM-grafted has very different topography, i.e., a skin-layer of PNIPAM spreads evenly on the PS surface. It indicates that the coating method produces a uniform PNIPAM layer. Akiyama et al [19] found similar surface topography while using electron beam to coat PNIPAM on PS plates.

3.4 Temperature Sensitivity

PNIPAM alone undergoes reversible phase transition in response to temperature; hence, graft copolymerization of PNIAPM makes a surface smart and temperature sensitive, i.e., the surface changes from hydrophilic to hydrophobic as temperature increases. This is confirmed by the contact angle measurement of PNIPAM grafted PS at various temperature from 20 to 42^{o}C. The result is shown in Figure 8. The plasma treated PS has a very consistent contact angle, about 46^{o}, at this temperature range. In contrast, PNIPAM grafted PS has a thermoresponsive contact angle. However, the contact angle of PNIPAM grafted increases from 18 to 56 degree at this temperature range. The biggest increase occurs at around 32^{o}C, which corresponds to the LCST of PNIPAM. Therefore, the grafting of PNIPAM results a thermoresponsive contact angle, i.e., thermoresponsive wettablility PNPAM grafted PS. As a result, the PNIPAM grafted surface is very hydrophilic at low temperature; however, over 32^{o}C, they become very hydrophobic.

Therefore, contact angle shows a thermoresponsive wettability of PNIPAM grafted surface. Cells will response this surface wettability change with temperature. Therefore, PNIPAM grafted PS surface can be used to control cell adhesion/detachment behavior.

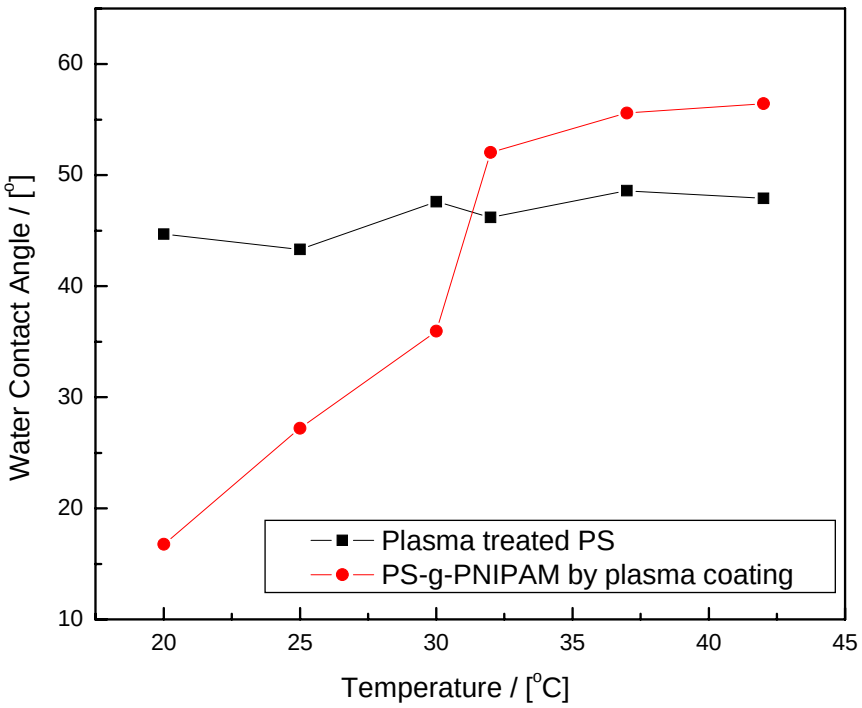

Figure 8. Contact angles of PNIPAM-grafted PS.

4. Conclusion

Temperature-responsive PNIPAM was grafted onto PS surface via atmospheric plasma treatment of NIPAM monomer coated plasma pretreated PS surface. FTIR confirm the grafting of PNPAM onto PS surface. The AFM images shows different surface topography of original, plasma treated, and PNPAM grafted surface. By changing different grafting parameters including plasma pretreatment time, post plasma treatment time, and monomer amount, different graft yields can be obtained. If high graft yield is needed, the optimal pretreatment time is 1 min for He/O2 and He/forming plasma, and 2min for He plasma. The optimal post treatment time is 5 min for He and He/O2, and 2 min for He/forming. The optimal monomer solution amount is 109ul or less for all three plasma gas. Water contact angle shows a thermoresponsive wettability of PNIPAM grafted surface.

5. References

1. Kim, S., Kanamori, T., Shinbo, T. Preparation of Thermal-Responsive Poly (Propylene) Membranes Grafted with N-Isopropylacrylamide by Plasma-Induced Polymerization and Their Water Permeation. *Journal of Applied Polymer Science,* 1168-1177, 2001.
2. S. Chen, J. Tsai, C. Chou, M. Yang, and J. Yang, Effects of additives on the photo-induced grafting polymerization of N-isopropylacrylamide gel onto PET film and PP nonwoven fabric surface. *Material Science and Engineering C*, 20: 203-208, 2002.
3. B. Yang and W. Yang, Thermo-sensitive switching membranes regulated by pore-covering polymer brushes. *Journal of Membrane Science,* 218: 247-255, 2003.
4. Y. M. Lee, S. Y. Ihm, J. K. Shim, and J. H. Kim, Preparation of surface-modified stimuli-responsive polymeric membranes by plasma and ultraviolet grafting methods and their riboflavin permeabion. *Polymer*, 36: 81-85, 1995.
5. D. Schmaljohann, J. Oswald, B. Jorgensen, M. Nitschke, D. Beyerlein, and C. Werner, Thermo-responsive PNiAAm-g-PEG films for controlled cell detachment. *Biomacromolecules*, 4 (6): 1733-1739, 2003.
6. Y. V. Pan, R. A. Wesley, R. Luginbuhl, D. D. Denton, and B. D. Ratner, Plasma polymerized N-isopropylacrylamide: Synthesis and characterization of a smart thermally responsive coating. *Biomacromolecules*, 2: 32-36, 2001.
7. S. Y. Kim, T. Kanamori, and T. Shinbo, Preparation of thermal-responsive poly(propylene) membranes grafted with N-isopropylacrylamide by plasma-induced polymerization and their permeation. *Journal of Applied Polymer Science*, 84: 1168-1177, 2002.
8. J.O. Karlsson and P. Gatenholm , Cellulose fiber-supported pH-sensitive hydrogels. *Polymer*, 40: 379–387, 1999.
9. W. C. Wang, G. T. Ong, S. L. Lim, R. H. Vora, E. T. Kang and K. G. Neoh, Synthesis and characterization of fluorinated polyimide with grafted poly(N-isopropylacrylamide) side chains and the temperature-sensitive microfiltration membranes. *Ind. Eng. Chem. Res.*, 42: 3740-3749, 2003.
10. N. Yamada, T. Okano, H. Sakai, F. Karikusa, Y. Sawasaki, and Y. Sakurai, Thermoresponsive polymer surfaces – control of attachment and detachment of cultured-cells. *Macromol. Chem. Rapid Commun.*, 11(11): 571-576, 1990.
11. T. Okano, N. Yamada, M. Okuhara, H. Sakai, and Y. Sakurai, Mechanism of cell detachment from temperature-modulated, hydrophilic-hydrophobic polymer surfaces. *Biomaterials,* 16: 297-303, 1995.
12. M. Yamato, M. Utsumi, A. Kushida, C. Konno, A. Kikuchi, and T. Okano, Thermo-responsive culture dishes allow the intact harvest of multilayered keratinocyte sheets without dispase by reducing temperature. *Tissue Engineering*, 7: 473-480, 2001.
13. Y. Akiyama, A. Kikuchi, M. Yamato, and T. Okano, Ultrathin Poly(N-isopropylacrylamide) Grafted Layer on Polystyrene Surfaces for Cell Adhesion/Detachment Control. *Langmuir*, 20: 5506-5511, 2004.
14. R. Mazzei, E. Smolko, D. Tadey, and L. Gizzi, Radiation grafting of NIPAAm on PVDF nuclear track membranes. *Nuclear Instruments & Methods in Physics Research B- Bam Interactions with Materials & Atoms,* 170: 419-426, 2000.

15. J. Xie, and Y. L. Hsieh, Thermosensitive poly(N-isopropylacrylamide) hydrogels bonded on cellulose supports. *J. Appl. Polym. Sci.,* 89:999-1006, 2003.
16. Y. J. Hwang, thesis: Characterization of atmospheric pressure plasma interactions with textile/polymer substrates. *North Carolina State University*, 2004.
17. H. Kanazawa, K. Yamamoto, Y. Matsushima, N. Takai, A. Kikuchi, Y. Sakurai, T. Okano, Temperature-responsive chromatography using poly(N-isopropylacrylamide)-modified silica. *Anal. Chem.*, *68*: 100-105, **1996.**
18. M. Yamato, C. Konno, S. Koike, Y. Isoi, T. Shimizu, A. Kikuchi, K. Makino, T. Okano, Nanofabrication for micropatterned cell arrays by combining electron beam-irradiated polymer grafting and localized laser ablation. J. Biomed. Mater. Res., 67A: 1065-1071, 2003.
19. B. L. Bures, thesis: Rapid mortality of pest arthropods by direct exposure to a dielectric barrier discharge. *North Carolina State University*, 2004.

Chapter Six: Grafting of Poly(acrylic acid) onto Nylon 6,6 Surface Using Atmospheric Plasma Treatment

Abstract

pH responsive poly(acrylic acid) (PAA) was grafted onto Nylon 6,6 film via a new procedure, i.e., He/O_2 atmospheric plasma treatment (APT) followed by free radical graft copolymerization. To increase graft yields, Ammonium Persulfate (APS) was added in the free radical graft copolymerization. The graft of PNIPAM was confirmed by Fourier transform infrared spectroscopy (FTIR) and water contact angle measurement. The graft yields increased as APS content increased. The hydrophilicity of the Nylon 6,6 film was increased by the He/O_2 atmospheric plasma treatment and was further increased by subsequent grafting of PAA. It is also found that higher graft yields resulted in more hydrophilic grafted surface. PAA grafted polymer materials have potential uses in drug delivery, tissue engineering, bioreactors, and responsive textiles.

I. Introduction

Synthetic polymer materials have been widely used in biomedical applications. However, they often result in a number of adverse physiological reactions such as thrombosis formation, inflammation and infection when they interact with natural tissue. Surface modification of polymers can improve their biocompatibility without changing bulk properties [1]. Various methods such as vacuum plasma treatment, ozone or photo-induced grafting, chemical treatment, electron-beam irradiation, etc., have been employed to graft hydrophilic and/or biologic materials to polymer surfaces to improve their biocompatibility. Among them, vacuum plasma treatment is of special interest because it offers flexibility, effectiveness, safety and environmental friendliness [2]. However, this method still has its own disadvantages, i.e., the need for a vacuum environment and relatively high cost.

This article reports a novel procedure for grafting poly (acrylic acid) (PAA) onto Nylon 6,6 film via He/O_2 atmospheric plasma treatment (APT) followed by free radical graft copolymerization. Compared with conventional vacuum plasma treatment, the APT method has several advantages, including no vacuum requirement, and therefore lower cost, and application in continuous processing [3].

2. Material and Methods

2.1 Materials

Nylon 6,6 film (McMaster-Carr) was used as the substrate for graft polymerization. Nylon film was cut into 3 x 3 cm^2 samples, which were washed with acetone, dried and weighed. Acrylic acid (99%) and ammonium persulfate (APS, $(NH_4)_2S_2O_8$) were purchased from Aldrich and used without further purification.

2.2 Atmospheric plasma treatment

The atmospheric pressure plasma treatment system set up in the College of Textiles at North Carolina State University is an atmospheric pressure glow discharge (APGD) device. Figure 1 shows a schematic drawing of the experimental facility. It is a capacitively coupled chamber and contains two horizontal parallel electrodes. The radio frequency power coupled to plasma via electrode through an oscillating electro filed. Each electrode is covered with a dielectric material to limit the current in the discharge and force the charge to spread out over a large area instead of constricting to an arc. The dielectric barrier prevented the transition of the discharge to an arc when acoustic frequency voltage signal was applied. The device has two chambers. An inner plasma chamber is for batch treatment. The outer chamber is equipped with a fabric rolling system for continuous fabric modification treatments. Therefore, the device is capable of batch treatment of fabric pieces using a test cell, as well as continuous operation using the roller feed system for large fabric rolls or continuous filaments and yarns. Since the chamber is not pumped down, so it operates at atmospheric pressure.

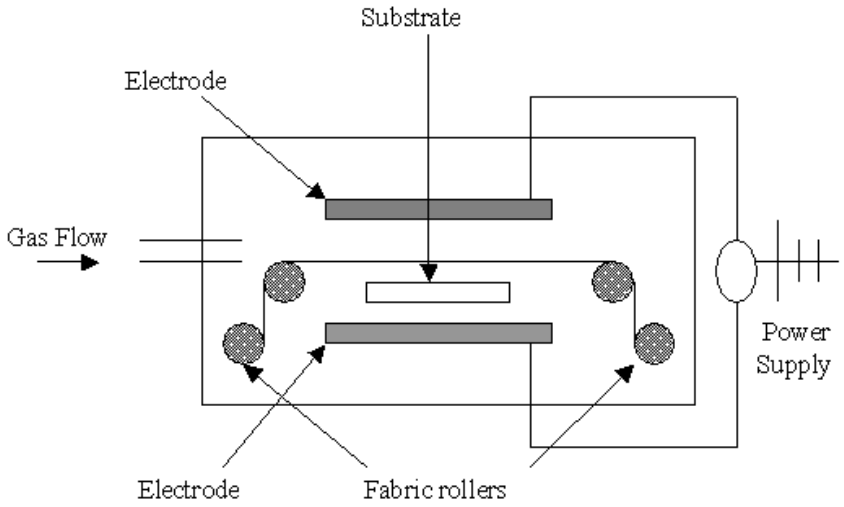

Figure 1. A Schematic of the Atmospheric Pressure Plasma System.

Atmospheric plasma treatments were used to activate the Nylon 6,6 surfaces. Treatments were conducted with plasma generated from the mixture gas of He and O_2. The power level used was 4.8kw, the frequency was 5 kHz. The flow rates of He and O_2 were 10.16 L/min and 0.13 L/min, respectively. The percentages of He and O2 in the mixture were 99% and 1%. Each nylon sample was treated for 1 min.

2.3 Graft Polymerization

Acrylic acid aqueous solutions (1 M) containing different ammonium persulfate (APS) concentrations (i.e., 0.01M, 0.05M and 0.1M) were prepared. A schematic of the grafting method is shown in Figure 2. Nylon samples were immersed into these monomer solutions immediately after 1 min He/O_2 atmospheric plasma treatment. The surface graft polymerization of acrylic acid was performed for 24 hours in a sealed reaction kettle under N_2 at 60 ^oC. The PAA grafted nylon film was washed by agitating in ultrapure water for 24 h at room temperature to eliminate the unreacted monomer and ungrafted photopolymers. The samples were dried at room temperature for 72 hours and weighted.

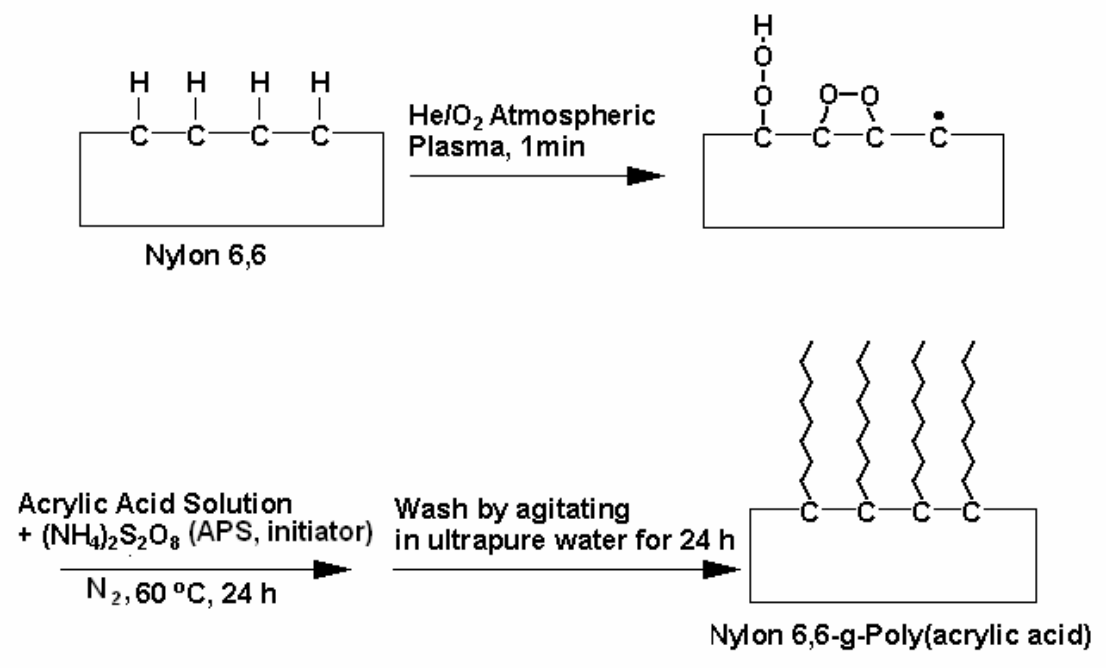

Figure 2. The schematic of graft polymerization of PAA on nylon surface.

2.4 Graft Yield of the PAA-g-Nylon Membrane

The graft polymerization was evaluated by weighting the nylon sample before and after graft polymerization. The graft yield of poly(acrylic acid) on the treated Nylon film was calculated using

$$\text{Graft Yield } (\mu g/cm^2) = (W_1 - W_0)/A$$

where W_0 represents the original weight of the samples, W_1 represents the weight of the samples after grafting and washing, and A is the surface area of nylon samples.

2.5 FTIR Measurement

Fourier transform infrared spectroscopy (Nicolet 510P FTIR spectrometer) was used to examine the surface chemistry of the grafted Nylon film. The spectra were collected at 4 cm^{-1} resolution with an ATR/FTIR microscopic spectrometer over 32 scans. The sampling area was coupled with an attenuated total reflection accessory and a 45° KRS-5 crystal.

2.6 Water Contact Angle Measurement

The water contact angle on the Poly(acrylic acid) grafted Nylon 6,6 surfaces was measured in air at 25 ^{o}C by the sessile method using a gonimeter(Model A-100, Ramé-Hart, Inc.). The contact angle was an average of 8 measurements on each sample.

3. Results and Discussion

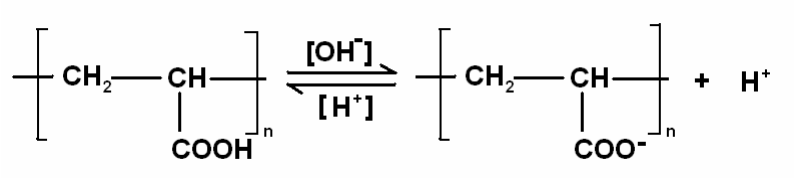

Figure 3. Chemical structure of PAA at different pH.

PAA has a carboxyl group in the polymer chain (Figure 3). The acrylic acid is a weak acid, which only dissociates at lower pH values. Its pKa is in the range of 4.2-5.5[4]. PAA becomes ionized above its pKa. Carboxyl groups can improve surface hydrophilicity and are very desirable for cell adhesion. They can also provide an end group for further functionalization, for example, further immobilize of collagen. Therefore, material grafted with PAA has many applications in tissue engineering and medical devices.

PAA is pH sensitive polymer. As shown in Figure 3, at high pH value (above its pKa), the carboxyl acids are ionized, and mutual repulsion by the negatively charged carboxyl groups forces the polymer chains apart, bringing large amounts of water into the polymer chains. PAA form a hydrogel with water at high pH. However, at low pH (below its pKa), the carboxyl groups lose their charge and the reduced repulsion allows the gel to collapse [5].

If PAA is grafted on material surface, the phase change will happen on that material surface, which endows the material with a new property, pH-sensitivity. It will have many potential uses, such as drug delivery, bioreactor, separation membrane, and responsive textiles, etc.

3.1 FTIR

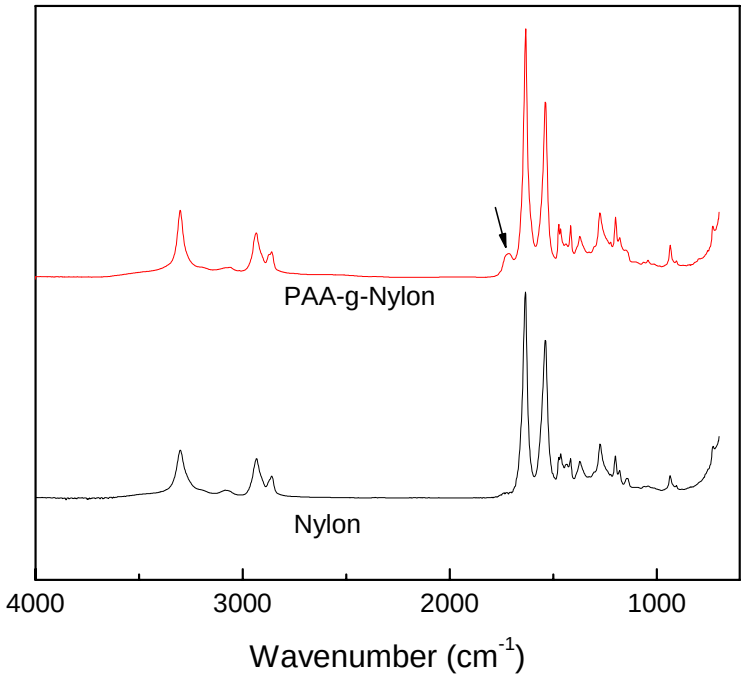

Figure 4. FTIR spectra of the Nylon 6,6 film grafted with poly(acrylic acid) and the original nylon 6,6 film.

Fourier transform infrared spectroscopy (FTIR) spectrum of PAA grafted nylon film shows the characteristic peak (1710 cm^{-1}) of the C=O bond of –COOH group of PAA[1], which indicates that the PAA has been successfully grafted onto the Nylon surface (Figure 4). It also shows that atmospheric plasma irradiation can also be used to activate the material surface and then initiate the grafting reaction as has been demonstrated previously. Since atmospheric plasma does require a vacuum environment, the cost is lower, and the process is more compatible with industrial continuous processing.

3.2 Graft yield

Graft yields of Nylon-g-PAA films with different APS contents were also measured. It was found that the graft yields increased with increases in APS content (Figure 5). The graft yield of PAA grafted nylon using atmospheric plasma treatment followed by free radical

copolymerization in the presence of 0.01M APS is 0.21 mg/cm^2. APS concentration of 0.05 and 0.10M resulted in increases in the graft yield to 0.69 and 0.79 mg/cm^2, which indicates that the addition of APS can enhance the grafting reaction. APS is usually used as an initiator for polymerization. After heating, its per sulfate bonds are very easy to break into free radicals. The free radicals can initial homopolymerization and may also induce free radical formation on the fabric surface by free radical transfer or peroxide group breakage, which initiate the graft polymerization.

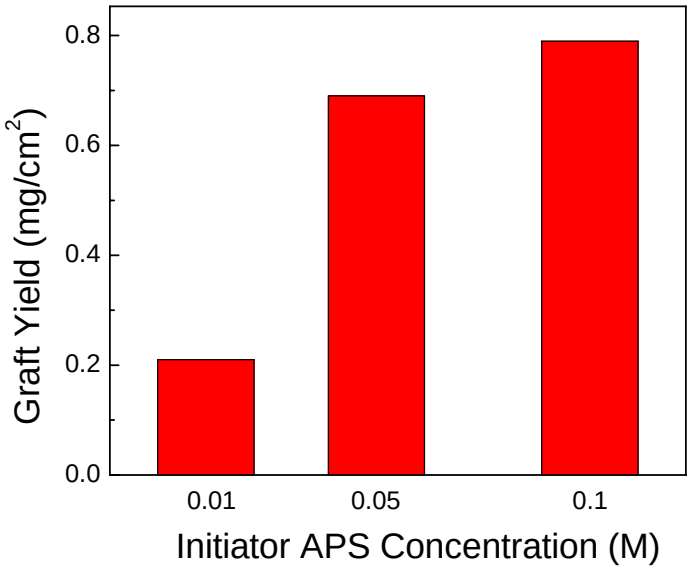

Figure 5. Graft yields of PAA grafted nylon with different APS concentration.

3.3 Water contact angle

To evaluate the changes of hydrophilicity of Nylon 6,6 film after graft copolymerization, the static water contact angle was measured. As illustrated in Figure 5, the static contact angles of the Nylon 6,6 film decreased from ca. 72^o to ca. 67^o after atmospheric plasma treatment. The reason is that the plasma treatment creates chemically active functional groups, such as radicals, carbonyl, peroxide, hydroxyl groups, which make the surface more wettable.

A further decrease of contact angle was obtained for the poly(acrylic acid) grafted Nylon 6,6 film, which also indicates the successful graft of PAA onto Nylon surface. As seen from Figure 5, the more PAA grafted onto Nylon 6,6, the lower the water contact angles are. It indicates that the graft of poly(acrylic acid) can further improve the hydrophilicity of Nylon 6,6 film.

Therefore, the hydrophilicity of the Nylon 6,6 film was increased by the He/O_2 atmospheric plasma treatment and was further increased by subsequent grafting of PAA, which also indicates the successful grafting of PAA onto Nylon surface. Hydrophilicity is directly proportional to graft yield.

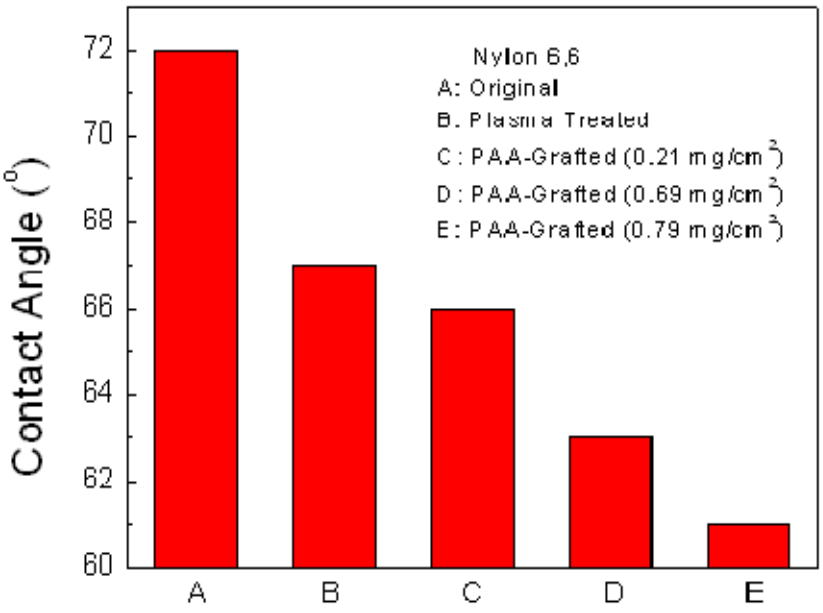

Figure 6. Water contact angel of original, plasma treated, and PAA grafted nylon.

4. Conclusion

pH responsive poly(acrylic acid) (PAA) was grafted onto Nylon 6,6 film via a new procedure, i.e., He/O_2 atmospheric plasma treatment (APT) followed by free radical graft copolymerization. The grafting was confirmed by FTIR and water contact angle measurement. The atmospheric plasma exhibits the activation capability to initiate graft

copolymerization. The addition of APS increased the graft yield. The contact angle of PAA was decreased by plasma treatment, and was further decreased by PAA grafting. PAA grafted polymer materials have potential uses in drug delivery, tissue engineering, bioreactors, and responsive textiles.

5. Further Direction

The introduction of PAA improves the hydrophilicity of Nylon 6,6 film, which may generate more favorable interaction with cells. Cell culture work can be done on the Nylon-g-PAA surface to investigate the effect of grafting on biocompatibility. Besides, biomacromolecules such as collagen, chitosan, and gelatin can be covalently immobilized through the –COOH groups in PAA, which will further improve the biocompatibility of Nylon 6,6. Also, poly(acrylic acid) is a weak polyelectrolyte and has a pH-sensitivity. The pH sensitivity of the PAA grafted nylon can be tested by swelling test, contact angle, AFM, permeability tests, or comfort tests. The plasma graft of poly(acrylic acid) onto nylon surface provide a way to fabricate novel pH-responsive polymer film, which has potential uses in bioreactors, drug delivery, tissue engineering, etc. PAA can also grafted on fabrics, which have the potential to improve the dyeability and anti-static ability and to be used as responsive textiles.

6. References:

1. S. D. Lee, G. H. hsiue, P. C. Chang, C. Y. Kao, Plasma induced grafted polymerization of acrylic acid and subsequent grafting of collagen onto polymer film as biomaterials. *Biomaterials*, 17: 1599-1608, 1996.
2. I. H. Loh, Plasma surface modification in biomedical application. *AST Technical Journal*, 2004 (www.astp.com).
3. Y.J. Hwang, thesis: Characterization of atmospheric pressure plasma interactions with textile/polymer substrates. *North Carolina State University*, 2004.
4. X.Y. Wu, Q. Zhang, and R. Arshady, Stimuli Sensitive Hydrogels. In: R. Arshady (ed) *Introduction to Polymer Biomaterials*. London, UK: Citus, 2003.
5. E.S. Gil and S. M. Hudson, Stimuli-responsive polymers and their bioconjugatess. *Progress in Polymer Science*, 29: 1173-1222, 2004.