IMMOBILIZATION OF POLY(N-ISOPROPYLACRYLAMIDE) ON HYDROXYLATED SURFACES USING CROSS-LINKED ORGANOSILANE NETWORKS

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ABSTRACT

Poly (N-isopropylacrylamide) (pNIPAAm), a thermo-responsive polymer that exhibits a lower critical solution temperature (LCST) of 32 °C in water has found an extensive use in tissue engineering and bioengineering applications in general. Since it is soluble in water, one of the main challenges that limit its applications in an aqueous environment is the tedious and expensive electron beam or plasma based procedures to retain it on a substrate. In this study, we report the use of various types of organosilanes to form siloxane networks for immobilizing pNIPAAm onto Si-wafer and silica glass substrates in a simple two-step approach: spin coating followed by thermal curing. Attempts are made to elucidate the entrapment mechanism and factors that affect such entrapment.

It was found that the entrapment occurs via the segregation of high surface tension organosilanes towards the substrate at a temperature higher than the glass transition temperature (T_g) of pNIPAAm and simultaneous cross-linking of the segregated organosilane molecules that form siloxane networks. Organosilanes having low surface tension were found to segregate towards the air-film interface leading to poor entrapment. Factors such as polarity and hydrogen bonding were found to influence the retention of those organosilanes in the blend film during spin-coating and thermal annealing and subsequent film retention after 3 days of soaking in cold water. Additionally, organosilanes that are allowed to hydrolyze and oligomerize in the blend

solution prior to spin-coating also resulted in higher organosilane retention and subsequently, thicker retained blend films compared to solutions that were spin-coated immediately after preparation.

Substrates utilizing those organosilanes to entrap pNIPAAm resulted in stable films that exhibited thermo-responsive behaviors that were verified by wettability measurements. Rapid cell sheet detachment (<5 min) of embryonic mouse fibroblast cells were obtained on all substrates apart from pNIPAAm entrapped via a methyl-terminated organosilane due to poor cell adhesion. An epoxy based organosilane that is capable of chemically reacting with pNIPAAm was also used to chemically graft pNIPAAm. The resulting films were thicker than those obtained by the organosilane network entrapment approach; however these chemically grafted substrates showed poor cell adhesion and loss of thermo-responsiveness with the increase in its ratio to pNIPAAm.

This study illustrates the simplicity and versatility of the entrapment approach utilizing network forming organosilanes, which could broaden the usage of pNIPAAm. The approach could possibly be used to entrap other functional polymers on hydroxylated substrates that might otherwise dissolve away in solution or when exposed to a particular solvent, or need laborious methods to be immobilized on the substrate.

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CHAPTER I

INTRODUCTION

Stimuli responsive “smart” materials have gained an increased interest in recent years due to their ability to change their physical structure, hydrophobicity, electrical, mechanical and optical properties when subjected to a certain stimulus such as electric field, temperature, light and pH. These properties have proven to be quiet lucrative in fields of drug delivery, catalysis, photolithography and tissue engineering[1]. In tissue engineering, the use of thermo-responsive polymers to harvest cells offers a non-invasive approach compared the use of enzymes to disaggregate the extracellular matrix or by mechanical scrapping, which can be damaging to the cell-surface proteins[2–4].

A study by Fujioka et al.[5] showed that mechanical scrapping results in a loss of intercellular DNA in addition to the total reduction of harvested cells compared to other detachment techniques. The detachment via proteolysis results in the cleavage of important transmembrane proteins which can lead to cells losing their functionality[4]. Additionally, a study reported by Fan et al.[6] demonstrated that human mesenchymal stem cell (hMSC) harvested using temperature-responsive substrates better maintained their ability to differentiate compared to trypsinized cells. The recovery of intact cell sheets is also greatly desired for tissue engineering applications.

Poly(N-isopropylacrylamide) (pNIPAAm), a thermo-responsive polymer that exhibits a lower critical solution temperature (LCST) of 32 °C in water, has been used for

regenerating various types of cells such as endothelial cells, urothelial cells, keratinocytes, cardiomyocytes, mesenchymal stem cells, retinal cells, epithelial cells, hepatocytes and fibroblast cells[7,8]. Above 32 °C, pNIPAAm is slightly hydrophobic and compact, whereas below 32 °C, pNIPAAm chains are extended and fully hydrated[9]. The processes of harvesting cell sheets using pNIPAAm typically involves seeding the cells and incubating them at 37 °C which is above the LCST of pNIPAAm. Upon cooling below the LCST, the polymer layer hydrates which promotes the detachment of cells.

There are various methods to prepare pNIPAAm-based thermo-responsive surfaces. These methods can be categorized into two main categories: non-grafted and grafted. The non-grafting approach involves coating pNIPAAm on a substrate where pNIPAAm is physically adsorbed. This approach, although simple, is not applicable due to the poor cell adhesion on pNIPAAm coatings[10]. To overcome this obstacle, researchers have used approaches such as using copolymers or incorporating extracellular matrix proteins such as collagen in order to enhance cell adhesion. However, the incorporation of proteins could introduce disease transfer risk which is undesirable especially for therapeutic tissue engineering applications[10]. Additionally, the dissolution of the non-grafted pNIPAAm into the cell medium during the detachment process is undesirable. As a result, several grafting techniques have been developed.

The grafting of pNIPAAm can be done via electron beam irradiation[11], gamma irradiation[12] plasma/plasma graft polymerization of N-isopropylacrylamide[13–15] and photo-grafting[16]. While these techniques allow for the grafting to occur on tissue culture polystyrene (TCPS) dishes, the often inaccessible nature of these techniques (e.g.

electron beam irradiation) hinders their applicability[17]. Additionally, the cell detachment times are quite long (20-120 mins)[8]. In a previous study, we showed that pNIPAAm can be entrapped on Si-wafers and glass slides by utilizing the cross-linked network formed by 3-aminopropyltriethoxysilane (APTES)[17] as illustrated in Figure 1.1.

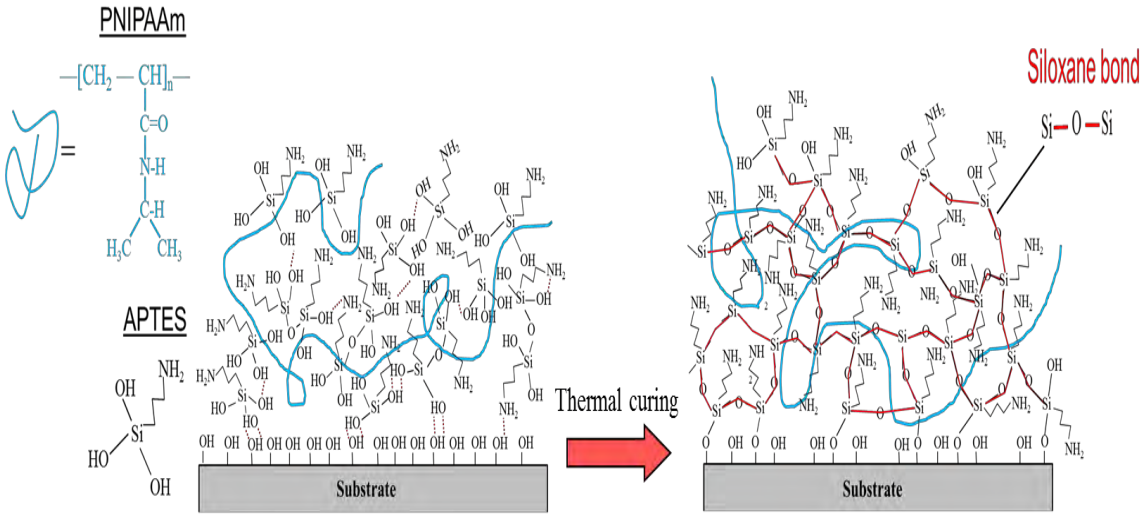


Figure 1.1 pNIPAAm immobilization via the siloxane network (Si-O-Si, shown in red) formed by APTES after thermal curing at 160 °C. The blue line represents the pNIPAAm chain and the dotted red line represents hydrogen bonding. Upon heating, the hydrogen bonds that are formed between the silanol groups (Si-OH) convert to the much stronger covalent siloxane bonds (Si-O-Si), hence forming the cross-linked network.

The entrapped pNIPAAm by APTES exhibited thermo-responsive behavior and showed rapid cell sheet detachment (< 5 mins), however, the exact mechanism on how APTES molecules are retained in the film to form the network for entrapping pNIPAAm has not been carefully investigated. In this study, the effects of experimental conditions such as curing time and pNIPAAm/APTES ratio on the film thickness and % film retention are examined. Additionally, other organosilanes bearing various functional groups are used and the entrapment of pNIPAAm is compared to that obtained using

APTES. X-ray photoelectron spectroscopy (XPS), ellipsometry and contact angle measurements are utilized for depth profiling, thickness and wettability determination, respectively. The results from this study allow us to elucidate the details behind the retention of organosilane molecules and the formation of their networks for immobilizing polymers on a substrate following a simple blending and thermal annealing approach. These results are presented in Chapter 4. The background on pNIPAAm and existing methods for grafting of pNIPAAm, the orangosilanes used for this study, along with the characterization methods are presented in Chapter 2. Chapter 3 details the materials and methods used for the study, and the thesis ends with an outline of the future direction of the project in Chapter 5.

CHAPTER II

BACKGROUND

2.1 poly(N-isopropylacrylamide) (pNIPAAm) in tissue engineering

pNIPAAm exhibits a lower critical solution temperature (LCST) in water at 32 °C. Below this temperature, pNIPAAm is soluble in water showing a clear one phase solution, whereas above this temperature, the pNIPAAm chains collapse and phase separate to form a two-phase opaque suspension. The phase change can be visually observed as shown in Figure 2.1.

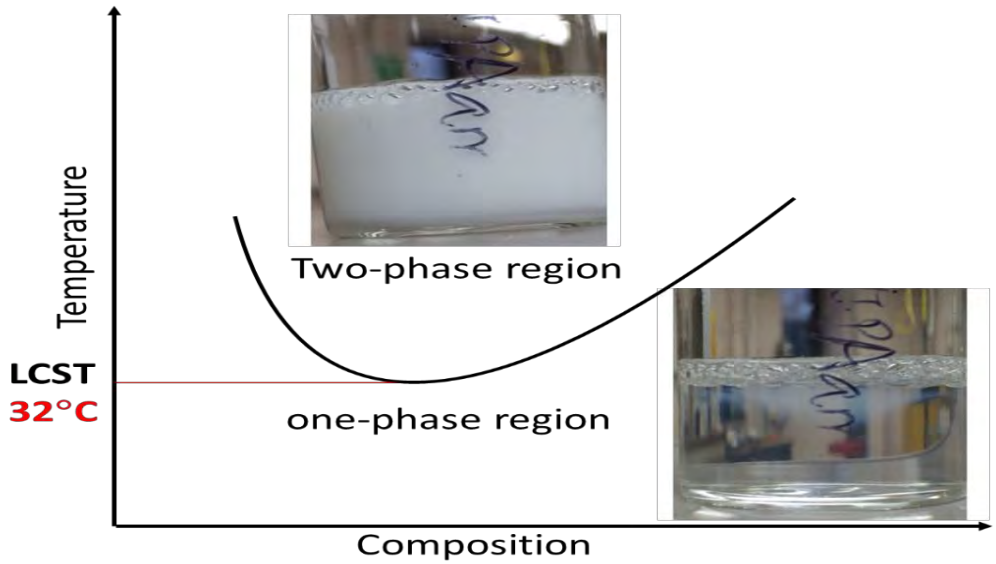


Figure 2.1 The phase separation of pNIPAAm/water solution above the LCST.

The physiological relevance of pNIPAAm's LCST has led to the development of thermo-responsive surfaces that enable cell (individual and sheets) harvesting[17]. In

general, the processes of harvesting cell sheets using pNIPAAm-based thermo-responsive surfaces involves seeding the cells on a thermo-responsive substrate, allowing the cells to grow and proliferate above the LCST, and finally, detaching cell sheets upon cooling the substrate below the LCST as illustrated in Figure 2.2.

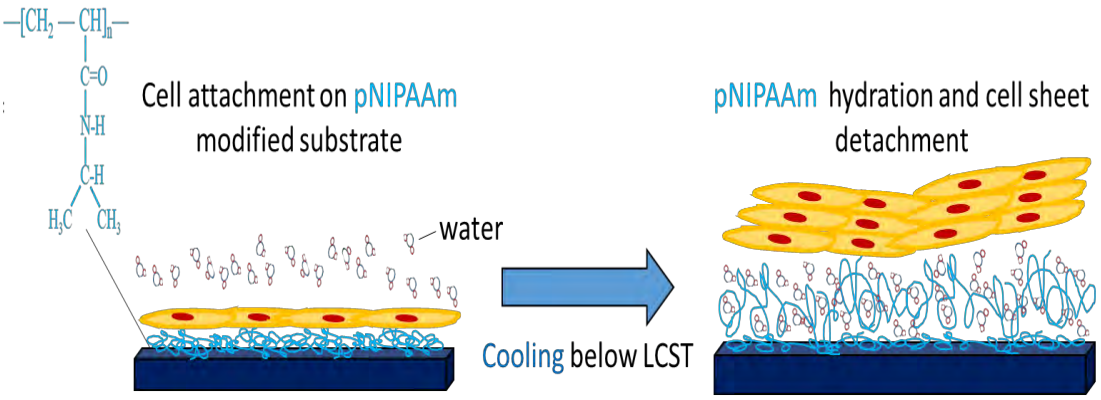


Figure 2.2 Schematic illustration of cell sheet detachment from pNIPAAm-modified surface. Above the LCST, pNIPAAm chains are collapsed and slightly hydrophobic. Below the LCST, pNIPAAm chains are hydrated mediating the cell sheet detachment.

The use of these thermo-responsive substrates provides an alternative, non-invasive approach to harvesting individual cells or cell sheets. Thermo-responsive polymers have been used for regenerating various types of cells such as endothelial cells, urothelial cells, keratinocytes, cardiomyocytes, mesenchymal stem cells, retinal cells, epithelial cells, hepatocytes and fibroblast cells[7,8]. The conventional approaches for detachment whether by mechanical scraping or via proteolysis often result in negative effects on the detached cells.

2.2 Grafting of pNIPAAm

The grafting of pNIPAAm has been primarily done via the use of irradiation such as electron beam (e-beam) irradiation, UV irradiation as well as plasma-based graft-

polymerization. pNIPAAm e-beam grafting is done by coating tissue culture polystyrene (TCPS) dish with NIPAAm monomers (N-isopropylacrylamide) dissolved in isopropanol. The coated TCPS dishes are the irradiated with 0.25 MGy electron beam (210 kV, 19 mA, $4x10^{-4}$ Pa)[3]. The cell adhesion on the e-beam grafted pNIPAAm is reported to be sensitive to the thickness and grafting density[8,18]. A Thick layer (29.3 ± 8.4 nm), with a high grafting density was found to have no cell adhesion whereas a thin layer (15.5 ± 7.2 nm), with a low grafting density allowed for cell adhesion and detachment above and below the LCST, respectively[18]. The detachment time for cells grown on e-beam grafted pNIPAAm TCPS dishes is around 1 hour at 20 °C.

UV irradiation has also been used to graft pNIPAAm where polydimethylsiloxane (PDMS) is first treated with plasma and then exposed to air for 15 min to generate peroxides on its surface. The PDMS then is immersed in NIPAAm monomer solution in water. UV irradiation (for 2h) is then used to decompose the peroxides and initiate the polymerization of NIPAAm[19]. The cell adhesion was found to be independent of pNIPAAm thickness and the detachment time was found to be around 30 min at 20 °C, which is faster than the detachment obtained using the e-beam grafting approach.

Another way that has been used to graft pNIPAAm is through vapor phase plasma polymerization, where NIPAAm vapor was used to deposit pNIPAAm onto various substrates such as Si-wafer, glass and TCPS dishes[20]. The deposition process consists of methane plasma deposition followed by NIPAAm plasma deposition and a gradual reduction in power (80 to 1 W for 30 min). Similar to the UV grafting approach, the pNIPAAm substrates obtained using this plasma grafting method appeared to have good cell adhesion regardless of film thickness. The cell detachment time, however, is quite

long (2 h)[8]. One issue with the plasma-based graft polymerization is the possible fragmentation of the monomer and loss of material functionality[8,20].

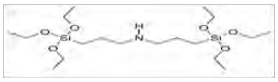
Some common issues that are present in all these grafting techniques include long detachment times as well as the use of often inaccessible equipment. In a previous study published by our group[17], we reported a simple method to entrap pNIPAAm onto silica surfaces by utilizing the disordered siloxane network that is formed by aminopropyltriethoxysilane (APTES), which is a commonly used organosilane. The process simply consists of spin-coating a blend of pNIPAAm and APTES onto a silica substrate and then curing the films at 160 °C to form the APTES network for trapping pNIPAAm chains as shown previously in Figure 1.1. The resulting films showed excellent cell adhesion regardless of film thickness and rapid cell detachment (< 5 mins).

2.3 Organosilanes

Organosilanes are chemical compounds that contain both organic and inorganic functionalities. This property has led to their wide use as coupling agents and adhesion promoters[21]. The use of organosilanes as adhesion promoters involves coating the surface with an organosilane followed by thermal treatment and then the polymer of interest is coated on top of the organosilane layer. The adhesion at the substrate-organosilane interface is a result of the chemical reaction between the silanol groups (Si-OH) and the hydroxyl groups present on the substrate. On the other hand, the adhesion promotion at the polymer-organosilane interface is a result of interdiffusion between the polymer chains and the organosilane leading to an interpenetrating network as verified by

Gellman et al.[22]. In this study, the organosilanes are blended with pNIPAAm and then coated onto a substrate rather than following the adhesion promotion approach mentioned above. The chemical structures and abbreviation of the organosilanes used in this study are summarized in Table 2.1.

Table 2.1 The abbreviation and chemical structure of the organosilanes used for entrapping pNIPAAm in this study

Name	Abbreviation	Chemical Structure
(3-Aminopropyl)triethoxysilane	APTES	
n-Butyltrimethoxysilane	BTMS	
[3-(2-Aminoethylamino)propyl]trimethoxysilane	AEAPTMS	
bis(3-Triethoxysilylpropyl)amine	BiAPTES	
triethoxysilylbutyraldehyde	TESBA	
(3-Mercaptopropyl)trimethoxysilane	MPTMS	
3-(2,3-Epoxypropoxy)propyltrimethoxysilane	EPPTMS	

Organosilanes first undergo a hydrolysis reaction when exposed to moisture, where the methoxy or ethoxy groups are hydrolyzed to form a silanol (Si-OH) and release methanol or ethanol, respectively. The silanol groups are reactive to various hydroxylated substrates and can be used to modify surfaces like metals, glass, minerals and cellulose[21]. In addition, the silanol groups can polymerize with themselves to form oligomers via siloxane bonds (Si-O-Si). The oligomerization of organosilanes often leads to the formation of multilayers as opposed to monolayers when modifying hydroxylated

surfaces such as glass. In this study, we exploit this disorder to immobilize pNIPAAm in an interpenetrated network as illustrated in Figure 2.3.

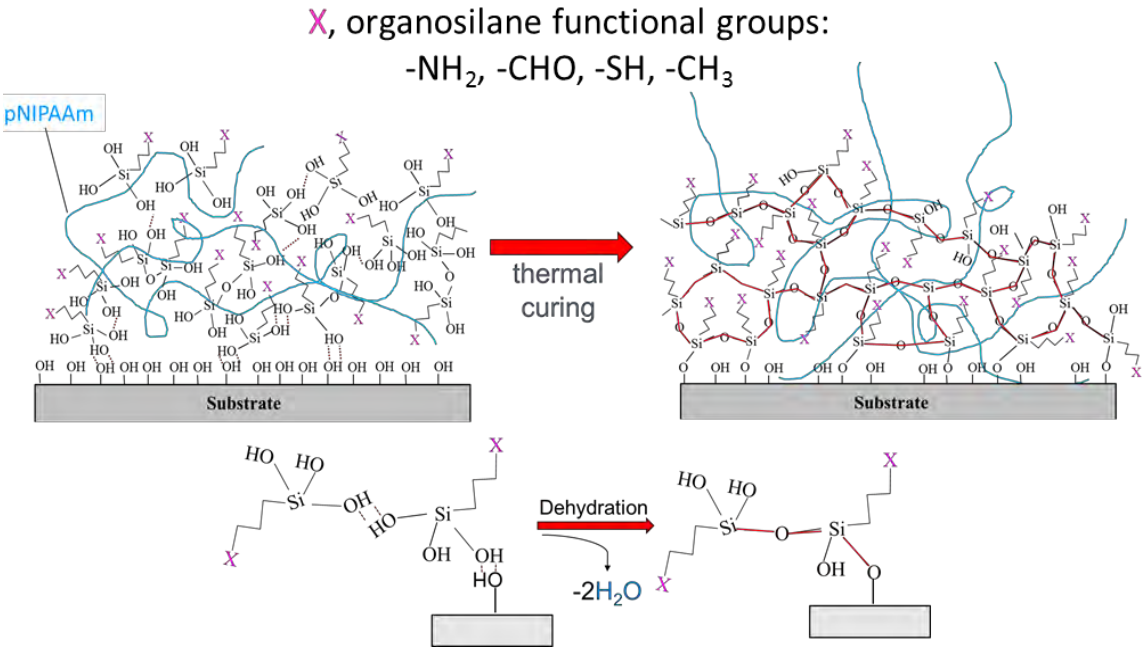


Figure 2.3 Schematic illustration of the siloxane network formation by organosilanes upon thermal curing and subsequent dehydration of the silanol groups (Si-OH) to form siloxane (Si-O-Si, shown in red) bonds.

The networks formed by the selected organosilanes are compared to that of APTES. BTMS was chosen as a low surface tension organosilane to investigate the possible segregation that is driven by the surface tension difference between pNIPAAm and BTMS. AEAPTMS, TESBA, MPTMS were selected to investigate the role of polarity on the retention of those organosilanes during processing. BiAPTES was selected to test whether the size of the molecule plays a role in the retention of organosilanes during spincoating. EPPTMS was selected in order to chemically graft pNIPAAm via the reaction between the epoxy groups in EPPTMS and the secondary amine group in pNIPAAm as illustrated in Figure 2.4. The grafting approach will be compared to the network entrapment approach.

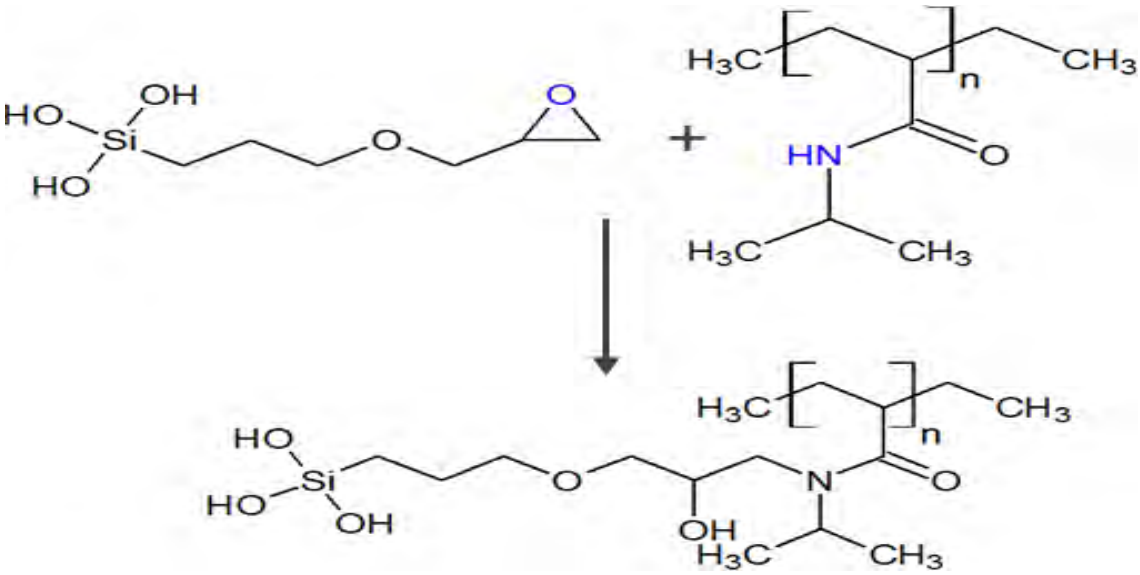


Figure 2.4 A schematic illustration of the possible reaction between the epoxy group in EPPTMS and the secondary amine group in pNIPAAm. The silanol (Si-OH) groups can then react with the substrate resulting in the chemical grafting of pNIPAAm to the substrate.

2.4 Surface characterization

Various surface characterization tools were used throughout this study. X-ray photoelectron spectroscopy (XPS) was used to obtain the sample composition as a function of depth (depth profiling). Ellipsometry was used to determine the film thickness, which was necessary for determining the percentage of film removed/retained after prolonged soaking as well as estimating the amount of organosilane retained in the processed film. Contact angle was used to evaluate whether the resulting films exhibited hydrophobic to hydrophilic transition above and below the LCST. These characterization methods are briefly described in their respective sections below.

2.4.1 X-ray Photoelectron Spectroscopy (XPS)

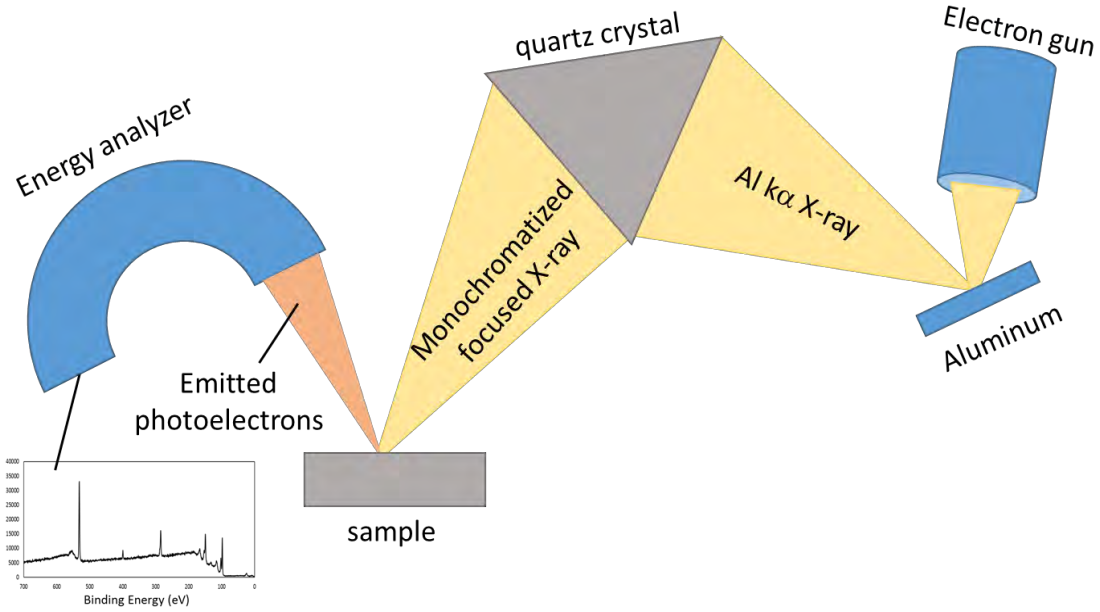


Figure 2.5 Schematic illustration of the XPS spectrum generation process

X-ray photoelectron spectroscopy (XPS) is a technique that is widely used for surface analysis especially chemical composition and chemical state. As shown in Figure 2.5, the low energy X-ray is generated by aiming a 10 keV electron gun at an aluminum target. An Al kα X-ray is formed with an energy of 1486 eV and is directed at a quartz crystal where it is focused and monochromatized before hitting the sample of interest. The X-ray energy excites the sample electrons and if those electrons have a binding energy that is lower than the X-ray energy (1486 eV), the electrons are ejected. An energy analyzer analyzes the kinetic energy of the emitted photoelectrons and the binding energy is then determined by subtracting the kinetic energy from the Al kα X-ray energy (1486 eV). The binding energy of an electron is dependent on the element as well as orbital that the electron is ejected from. The stronger attraction between the electron and

the nucleus would result in a higher binding energy. As a result, electrons further from the nucleus would have lower binding energy than electrons closer to the nucleus[23]. PHI VersaProbe II Scanning XPS Microprobe was used in this study with lateral resolution range of 8-100 μm and a vertical resolution of 5-7 nm.

In general, a survey scan can be obtained to determine the elements involved in the sample based on the binding energy. The binding energy from the high-resolution scan of each element can then be used to decide the chemical state and composition based on the shift in binding energy and the area under the peak of each binding energy. A higher resolution scan can be obtained by scanning multiple times over a small range of binding energies. In this study, samples were etched to various thicknesses using argon plasma in order to determine the silicon content as a function of depth and then determine the content of organosilane in the film as a function of depth.

2.4.2 Ellipsometry

Ellipsometry is a sensitive optical technique that utilizes a laser light with a known wavelength to detect changes in the variation of the elliptically polarized light in order to investigate properties such as film thickness and refractive index. The apparatus can measure thicknesses with an accuracy of ±0.3Å[24]. The setup consists of a light source (L), a polarizer (P), a quarter-wave plate (compensator) (C), an analyzer (A) and a detector (D) as shown in Figure 2.6.

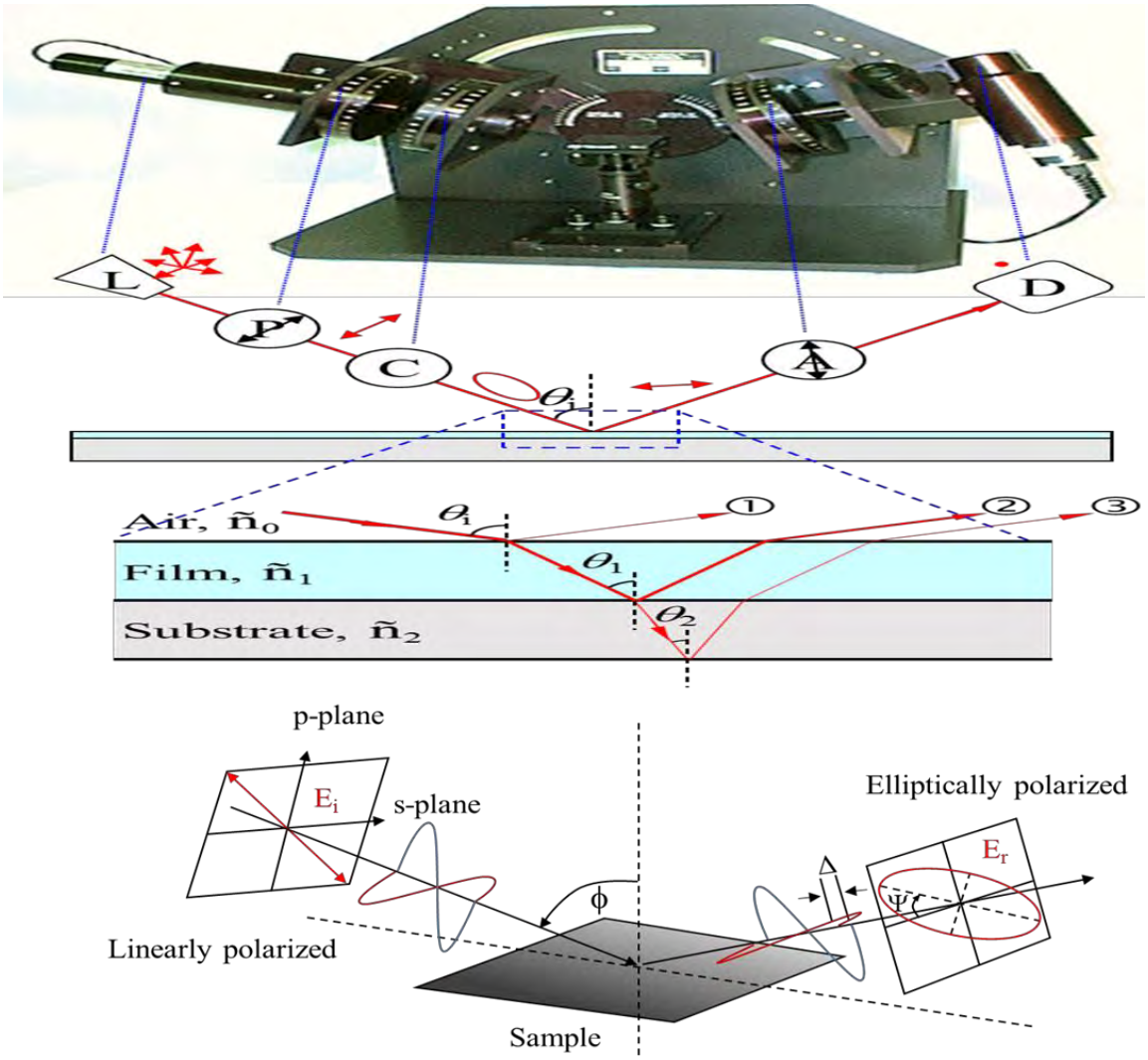


Figure 2.6 A manual ellipsometer along with schematic illustrations of the polarization processes. The analyzer and polarizer values are used to determine the relative amplitude change (Ψ) and relative phase change (Δ), respectively, of the laser passes through a film and reflects from the film surface as shown, respectively, as ② and ① in the sketch above.

The laser is aimed at the sample at an incident angle (ϕ) where it passes through a polarizer and the light is converted to a linearly polarized light. The compensator then converts the linearly polarized light to an elliptically polarized light before it hits the sample (e.g., the film in Figure 2.6). The orientation of the polarizer and compensator is chosen so that light is completely linearly polarized after it is reflected off the sample.

The polarizer and analyzer are rotated so that the light detected is minimized, which occurs when the analyzer crosses with respect to the polarization axis of the reflected light. The polarizer and analyzer angles are then used to determine
Δ and Ψ, respectively[24]. From the values of Ψ and Δ, the thickness can be calculated with the aid of a software.

In this study, ellipsometry was used to determine the film thickness, which was used to determine the percentage of film removed/retained after rinsing and prolonged soaking as well as estimating the amount of organosilane retained in the processed film

2.4.3 Contact angle

The solid-liquid contact angle (θ) is the angle that is formed by a liquid drop on a solid surface at the solid-liquid-vapor contact line which is also known as the three-phase contact line[25–27] as illustrated in Figure 2.7. In general, a large contact angle value suggests that the liquid does not wet the solid surface, whereas a low contact angle value suggests that the liquid wets the surface. The contact angle is related to the interfacial tension (γ) between the three phases (S: solid, V: vapor, L: liquid) via the Young's equation[26,28]:

$$cos\theta = \frac{\gamma_{SV} - \gamma_{SL}}{\gamma_{SV}} \tag{2.1}$$

The sessile drop technique is one of the most commonly used techniques for determining the contact angle, where a liquid drop is placed on a surface and the angle is measured with the aid of camera and an angle-measuring software. In this study, the

sessile drop technique was used to determine the static and advancing (measured as the edges of the liquid drop are advancing) contact angles at various temperatures in order to assess the thermo-responsive behavior of the prepared pNIPAAm films as illustrated in Figure 2.7.

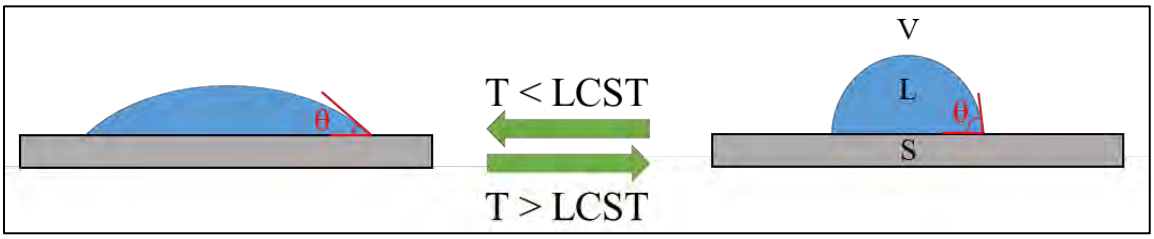


Figure 2.7 A schematic illustration showing the reduction in contact angle (θ) when the temperature is lowered below the LCST.

Above the LCST of pNIPAAm, the surface is slightly hydrophobic and the formed water contact angle is large. Cooling the substrate below the LCST induces a hydrophobic to hydrophilic transition resulting in a reduction in the contact angle.

CHAPTER III

EXPERIMENTAL APPROACH

3.1 Materials and Equipment

Poly(N-isopropylacrylamide) (pNIPAAm) with a number average molecular weight of 20,000 – 40,000 g/mol and 99% (3-Aminopropyl)triethoxysilane (APTES) were from Sigma-Aldrich. (3-Mercaptopropyl)trimethoxysilane (MPTMS), [3-(2-Aminoethylamino)propyl]trimethoxysilane (AEAPTMS), triethoxysilylbutyraldehyde (TESBA), bis(3-Triethoxysilylpropyl)amine (BiAPTES), 3-(2,3-Epoxypropoxy)propyltrimethoxysilane (EPPTMS) and n-Butyltrimethoxysilane (BTMS) were from Gelest. 200 proof ethanol was from EMD, and DI water was purified in house with a conductivity of ~ 0.5 μS/cm. Other chemicals used included 30 % hydrogen peroxide from BDH, 98% concentrated sulfuric acid and concentrated acetic acid from VWR. The cell culture medium used was MEME (minimum essential medium eagle) +10% FBS (fetal bovine serum) + 1% of antibiotic antimycotic solution (100x). 1x trypsin/EDTA, and phosphate buffered saline (PBS), were purchased from Sigma-Aldrich. Unless otherwise mentioned, all reagents were purchased from Sigma-Aldrich. STO cells were Dr. Liya Yin from Northeast Ohio Medical School (NEOMED). Argon gas with a purity of 99.999% was from Praxair. Microscope glass slides were from VWR, silicon wafer (P type P<100>) from Silicon specialist. Treated 35 mm culture dishes were from Greiner bio-one.

Basic equipment used for this study involved a spin coater (p-6000 Spin Coater, Specialty Coating System Inc., Indianapolis, IN), a plasma chamber (Harrick Plasma PDC-32G), a UV/ozone cleaner (model 42, Jelight), analytical balances with an accuracy of 0.1 mg, a vacuum oven (VWR) and its pump (Welch), a humid CO_2 cell incubator, a contact angle goniometer (Ramé-Hart Instrument Co., Netcong, NJ with a CCD camera attached), an ellipsometer (Rudolph Instruments, Inc., Fairfield, NJ equipped with a λ = 632.8 nm laser), a home built heating/cooling stainless steel stage with a thermo-meter attached from its side for real time mentioning of the stage temperature, a digital camera, an optical microscope (OM) (Olympus IX 70) with an eye-piece digital camera, a humidifier, and XPS (PHI VersaProbe II Scanning XPS microprobe) with an Al Kα excitation source.

3.2 Films Preparation

Glass slides and Si-wafers were cut into ~ 1 cm × 1 cm pieces, and then cleaned using a freshly prepared piranha solution followed with copious DI water rinsing. The slide or wafer was dried with a stream of dry air, and then oxidized for 8 minutes in the UV/Ozone chamber.

1.5-2 wt.% APTES and 1.5-2 wt.% pNIPAAm in 200 proof ethanol were prepared separately, and then they were mixed to form 1.5-2 wt.% solutions containing pNIPAAm/organosilane mass ratios of 20/80, 40/60, 50/50, 60/40, 70/30, 80/20, and 90/10. For making the pNIPAAm/EPPTMS blend, pNIPAAm was added to EPPTMS before adding the ethanol to make the final solution. Each freshly prepared solution was spin-coated (~ 80μL) on to a freshly cleaned and oxidized glass slide or Si-wafer at a

spin-speed of 2000 rpm for 30 seconds. The spin-coated slides and wafers were placed inside glass petri-dishes for at least ~ 30 minutes under ambient conditions, and then placed inside the vacuum oven to be cured for 1-3 days at 160°C.

3.3 Film Characterization

Film thickness, water contact angle and film composition on those films were characterized as detailed in the following sections.

3.3.1 Film Thickness

The film thickness for each sample was measured after the sample was removed from the oven, after it was thoroughly rinsed with DI water, and after it was soaked in DI water for 3 days in ambient conditions. The film thickness was measured on Si-wafer via an ellipsometer (before/after rinsing, and after 3 days soaking in ambient conditions). The thickness values were used to assess the retention of the films as well as estimating the amount of organosilane retained in the film.

3.3.2 Thermo-responsive Behavior (water contact angle)

The advancing and static water contact angles on the thoroughly rinsed films were measured at ~ 42°C and 26°C using a contact angle goniometer equipped with a home built heating/cooling sample stage. DI water was dispensed as drops from a Gilmont syringe through a G22 needle with a blunt tip. A humidifier was turned on next to the stage to minimize water evaporation from the drop. The images of the contact angle were

taken every 2-3°C increment. The contact angles were measured from the captured images using ImageJ software.

3.3.3 Composition and Depth Profiling (XPS)

The 50/50 blend films were used to profile the chemical compositions at different depths of the films using XPS. After rinsing off the loose (i.e., un-entrapped pNIPAAm) top layer, the film was either un-treated or further etched by Ar plasma to obtain a thickness of ~ 25 nm, 10 – 20 nm, and 5 – 10 nm. These films, along with pure APTES and pNIPAAm, both cured for 3 days under vacuum at 160°C, were de-gassed in the vacuum oven overnight prior to XPS scanning. A separated set of un-cured 50/50 APTES/pNIPAAm films with a thickness of ~ 25 nm, 10 – 20 nm, and 5 – 10 nm, was also used for the comparison study. Survey scans and the high resolution scans of C1s, O1s, N1s and Si2p were obtained for all the samples.

3.4 Cell Behavior and Detachment

The cured films were thoroughly rinsed with cold DI water to remove excess pNIPAAm and then were placed in a 35 mm dish. ~ 300K cells/ml were seeded and then incubated for 2-3 days until the cells reached at least 50% confluence. The dish was then placed on the microscope stage, and the warm medium was replaced with ~ 3 mL of 4°C cold medium. A thermo-couple was placed inside the medium to monitor the temperature, and in most cases the temperature reached 15 – 20°C. The cell detachment process was followed via a microscope-video system.

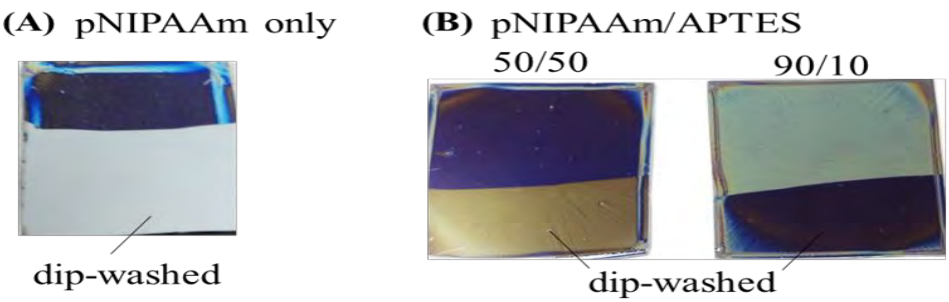
CHAPTER IV

RESULTS AND DISCUSSION

4.1 pNIPAAm Entrapment via APTES

The films prepared using APTES and pNIPAAm blends resulted in thicker films after curing and dip-washing in cold water (~23 °C) compared to using pNIPAAm only as shown visually in Figure 4.1 and the accompanying table. The final thickness for cured pNIPAAm only and 50/50 pNIPAAm/APTES was found to be 4 nm and 25-35 nm, respectively, prepared from 1.5 wt.% of initial spin-coating solution. Additionally, curing results in thicker films for both pNIPAAm only and pNIPAAm/APTES films. The non-cured films were almost completely washed away after rinsing with cold water. The residual films were ~ 0.5 and ~ 1 nm thick, respectively, for the pNIPAAm and the 50/50 pNIPAAm/APTES blend.

This result indicates that the presence of APTES in the film as well as curing is necessary in order to retain a thicker pNIPAAm layer on Si-wafer. The difference in thickness is also shown visually in Figure 4.1 by the difference in color with 90/10 pNIPAAm/APTES showing a thicker film (~ 110 nm) after dip-washing compared to 50/50 pNIPAAm/APTES (~ 64 nm).



films		rinsed film thickness (nm)	water contact angle (°) at 40°C
pNIPAAm	noncured	0.4 – 0.5	7.4 ± 0.5
	cured	~ 4	58.5 ± 2.1
50/50 pNIPAAm/APTES	noncured	0.9 – 1	28.7 ± 0.8
	cured	25 – 35	56.9 ± 4.4

Figure 4.1 The appearance of the 3-days cured films after dip-washing in DI water. (**A**) pNIPAAm only and (**B**) 50/50 and 90/10 pNIPAAm/APTES by weight. For imaging purposes (to have better contrast in color), some films were prepared using a higher solute concentration (2 or 3 wt.%) as opposed to 1.5wt.% used in this study. The accompanying table shows the thickness values and water contact angles of the rinsed films prepared using 1.5 wt.% solutes in ethanol.

This illustrates that the initial and final thickness for the 90/10 and 50/50 blend films is influenced by the ratio of pNIPAAm/APTES used, which is likely due to the higher viscosity of the 90/10 pNIPAAm/APTES solution. The influence of pNIPAAm/APTES ratio on the final retained film thickness after rinsing and prolonged soaking in cold water is discussed in the following section.

4.1.1 Effects of pNIPAAm/APTES ratio and Curing Time on Film Retention

The % film removal (~ 40%) after rinsing appears to be the same regardless of curing time with films containing more APTES having less % film removal as shown in Figure 4.2 A, B&C.

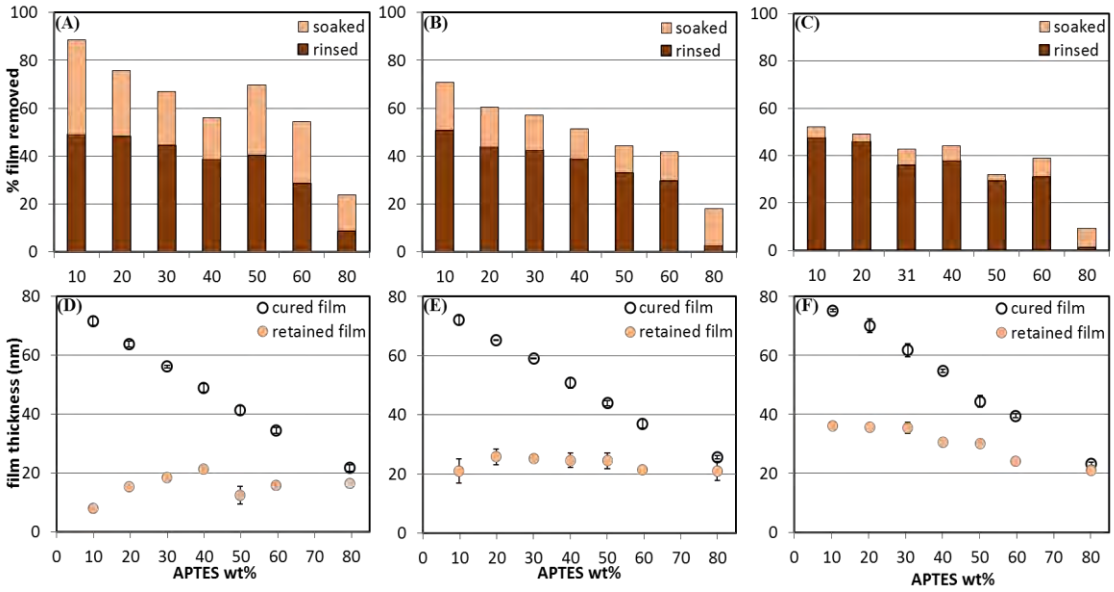


Figure 4.2 % film removal (upper row) and film thickness (lower row) before/after rinsing and further soaking for 3 days in cold water (~23 °C) for films prepared using different pNIPAAm/APTES ratios and cured at 160 °C for 1 day (A &D), 2 days (B&E) and 3 days (C&F).

After soaking for 3 days, all films lost additional thickness, with films cured for 3 days having the least % (~ 5%) film removal, compared to ~ 15% and ~ 27% for the 1 day and 2 days curing, respectively. This result suggests that the APTES network forms within 1 day of curing, although the network might not be tight (free Si-OH groups remain uncross-linked), which results in water penetrating the network and freeing pNIPAAm (i.e. 27 % film removal) in the case of 1 day curing compared to 3 days curing. The presence of un-crosslinked Si-OH groups could increase the water penetration into the network due to hydrogen bond formation compared to cross-linked siloxane bonds (Si-O-Si). The presence of Si-OH groups also results in protonation of the amine groups present in APTES, which is detected using XPS as shown in Figure 4.3A.

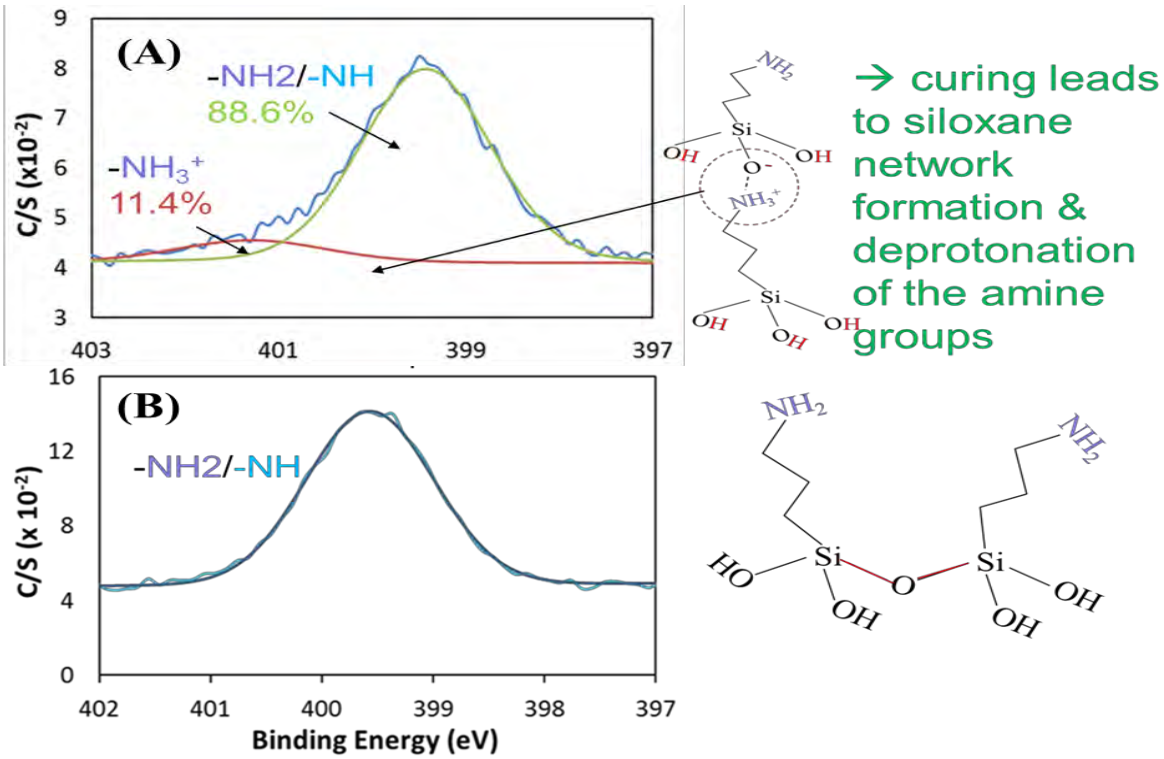


Figure 4.3 High resolution N1s scan showing (A) uncured 50/50 pNIPAAm/APTES and (B) cured 50/50 pNIPAAm/APTES.

By looking at Figure 4.3A, it is possible to see that the overall shape of the amine peak is asymmetric compared to the peak shown in Figure 4.3B. The asymmetry indicates that the N1s peak could be decoupled into multiple peaks, where one peak having a binding energy of ~400 eV could be assigned to the primary and secondary amines found in APTES and pNIPAAm, respectively, and another peak having a slightly higher binding energy (~402 eV) could be assigned to the protonated/hydrogen bonded primary amine ($-NH_3^+$) found in APTES in the presence of neighboring Si-OH groups. The disappearance of the NH_3^+ peak from the 3-day cured film (Figure 4.3B) indicates that the majority of the Si-OH groups are cross-linked to form Si-O-Si bonds leaving the primary amine groups unable to be protonated.

The final film thickness after 3 days of soaking shown in Figure 4.2D, E&F seems to indicate that the amount of APTES used does not have a significant effect on the final thickness for films cured for 1 and 2 days (with average final thickness of 15 - 20 nm). For films cured for 3 days, the final film thickness decreases with the increase of APTES content starting at ~ 36 nm (10 wt.% APTES) and decreasing down to ~ 21 nm (80 wt.% APTES) indicating that more pNIPAAm could be entrapped with longer curing time with less APTES used in the spin-coating solution. Additionally, the final film thickness after 3 days soaking for the 3 days cured sample appears to be correlated to the amount of APTES that is retained in the film during spin-coating and after curing in the oven at 160 °C as shown in Table 4.1.

Table 4.1 The wt.% of APTES retained in the cured film and % film retained after 3 days soaking in water (~23 °C)

pNIPAAm/APTES wt. ratio in solution	wt.% APTES in cured film	% film retained after 3 days soaking
90/10	8.5 ± 3.1	47.9 ± 0.6
80/20	11.2 ± 5.5	51.0 ± 3.2
70/30	13.0 ± 5.6	57.3 ± 7.1
60/40	14.0 ± 5.6	56.0 ± 4.5
50/50	15.6 ± 5.0	68.1 ± 5.8
40/60	21.0 ± 8.3	61.1 ± 3.8
20/80	40.6 ± 9.3	90.6 ± 6.1

Due to the constant % film removal after rinsing regardless of curing time shown in Figure 4.2A, B&C, we hypothesize that the entrapment/network formation occurs as APTES segregates downwards towards the film/silica substrate interface during the curing processes (i.e. above the T_g of pNIPAAm). Likely, this process occurs mostly within 1 day of curing. Subsequently, further cross-linking (2-3 days) occurs resulting in

a tighter network that leads to higher pNIPAAm retention. To investigate the segregation behavior of APTES in the blend films, XPS scans were obtained at various depths and the results are discussed in the following section.

4.1.2 Composition and Depth Profiling

In order to verify that APTES segregates towards the silica substrate during the curing processes, the cured pNIPAAm/APTES blend films were etched to different thicknesses using argon plasma followed by XPS scans. The survey scans at various depths of cured 50/50 pNIPAAm/APTES films are shown in Figure 4.4.

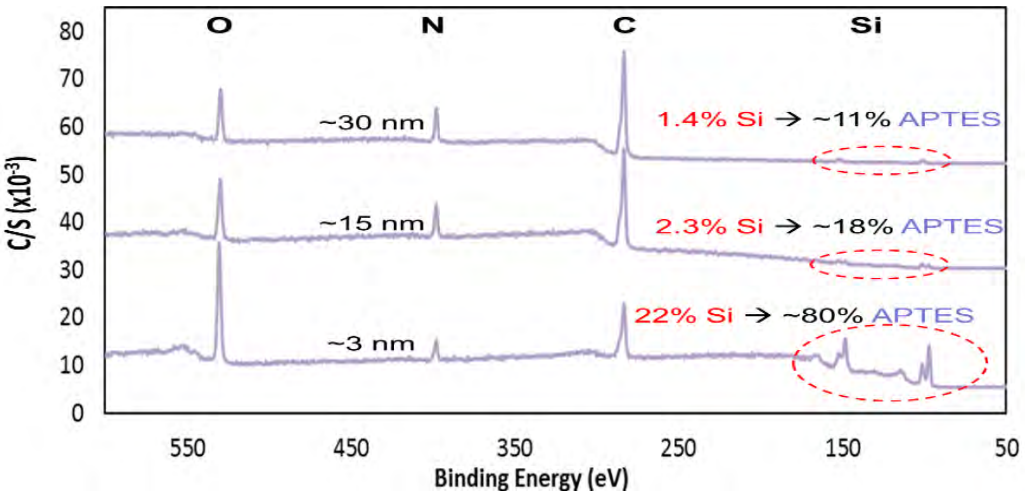


Figure 4.4 XPS survey scans showing the Si atomic percent for 50/50 pNIPAAm/APTES films etched to different thicknesses. The mol.% APTES shown is estimated from the atomic % of Si assuming that the APTES molecule is fully hydrolyzed. The silica layer on the silicon wafer has a thickness of 2.5 nm, and the average X-ray penetration depth of 6 nm was used for the estimation.

The increase in APTES content (from 11% to 80%) as a function of depth shown in Figure 4.4 verifies that APTES likely segregates down towards the substrate during the curing processes. One possible explanation is that above the T_g of pNIPAAm, the blend

might undergo some rearrangement in the vertical direction that is mainly driven by the difference in surface tension between pNIPAAm (with a surface tension, γ, of ~ 36 mN/m) and APTES (γ ~ 40 mN/m). To minimize the free energy, molecules with lower surface tension (pNIPAAm in this case) tend to segregate towards the air-film interface whereas molecules with higher surface tension (APTES in this case) tend to segregate towards the high surface tension substrate (Si-wafer/silica glass)[26,29]. The segregated APTES molecules would cross-link (i.e. polymerize) with neighboring APTES molecules to form the APTES network as they segregate down and reach each other's vicinities, and entrap the pNIPAAm present within the network. These APTES molecules would also graft to the hydrolyzed silicon substrate via siloxane bonds to anchor the network to the substrate. Eventually, the segregation process might slow down due to the cross-linking of APTES molecules, which prevents the complete segregation of APTES and leads to the entanglement of pNIPAAm as illustrated in Figure 4.5.

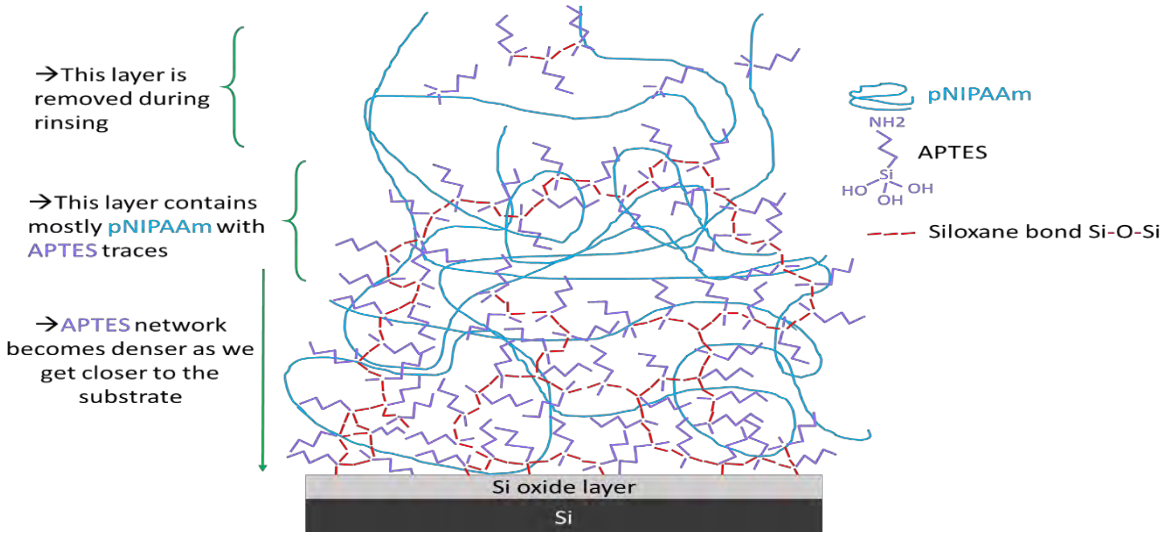


Figure 4.5 A schematic illustration of the network after the segregation of APTES in the pNIPAAm/APTES film during the curing processes.

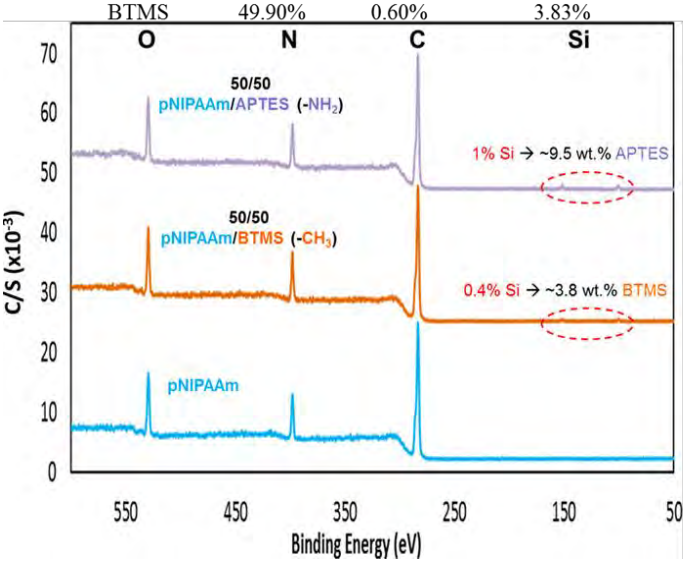
In a previous study published by our group[17], we reported that curing at 145 °C resulted in poor pNIPAAm entrapment and that curing at 160 °C was necessary. This is likely due to the glass transition temperature of pNIPAAm being round 120 °C to 142 °C depending on the molecular weight and tacticity[30], and as a result, curing at 145 °C might not provide sufficient mobility to allow APTES molecules to segregate down in the pNIPAAm/APTES film; whereas curing at 160 °C which is well above the T_g of pNIPAAm, could allow for easier segregation and network formation.

4.2 Effect of Surface Tension on Segregation and Network Formation

To verify the segregation of organosilane was indeed the result of the surface tension difference between organosilane and pNIPAAm, n-Butyltrimethoxysilane (BTMS), a methyl-terminated organosilane with a similar molecular size (Mw = 136 g/mol for the fully hydrolyzed BTMS) as APTES (Mw = 137 g/mol for the fully hydrolyzed APTES) but with a low surface tension (~29 mN/m) was used. It is hypothesized that due to the low surface tension of BTMS, the molecules would segregate towards the air-film interface, hence no anchoring to the substrate, which results in poor pNIPAAm entrapment. To verify this behavior, pNIPAAm/BTMS blend films from a 50/50 blend solution were prepared to compare to their APTES counterparts. The total amount of BTMS and APTES in the blend film was estimated based on the increase in thickness after curing compared to pNIPAAm alone. XPS scans were also generated on the cured but non-rinsed films at the top surface layer (~6 nm). The total

percent of organosilane in the cured film and the XPS survey scans are shown in Figure 4.6 and the accompanying table.

Organosilane	wt.% in the blend solution	wt.% in the cured film	wt.% in the top surface layer (5-7 nm)
APTES	50.20%	17.70%	9.53%
BTMS	49.90%	0.60%	3.83%



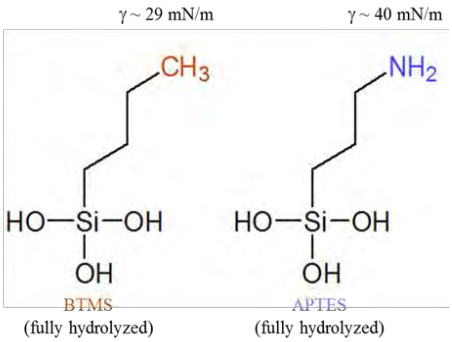


Figure 4.6 XPS survey scans showing the atomic percent of Si present in the top surface layer (5-7 nm) of cured non-rinsed 50/50 pNIPAAm/BTMS or APTES. The accompanying table shows the total wt.% of the organosilane in the original solution, in the cured film and in the top surface layer of the films. The chemical structure of fully hydrolyzed BTMS and APTES is shown on the right along with their surface tension values.

As shown in Figure 4.6 and the accompanying table, the BTMS content at the top surface layer of the cured non-rinsed pNIPAAm/BTMS film is much higher (3.8 wt.%) than the total content in the film (0.6 wt.%), confirming the likeliness of BTMS segregation towards the air-film interface compared to APTES. The APTES content in the top surface layer (9.5 wt.%) is lower than the content in the total bulk (17.7 wt.%), confirming that APTES does indeed segregate towards the bottom. This segregation behavior plays a major role in the entrapment of pNIPAAm. In the case of BTMS, the

segregation of the BTMS molecules towards the air-film interface would likely lead to the formation of a weak network mainly at the surface as illustrated in Figure 4.7.

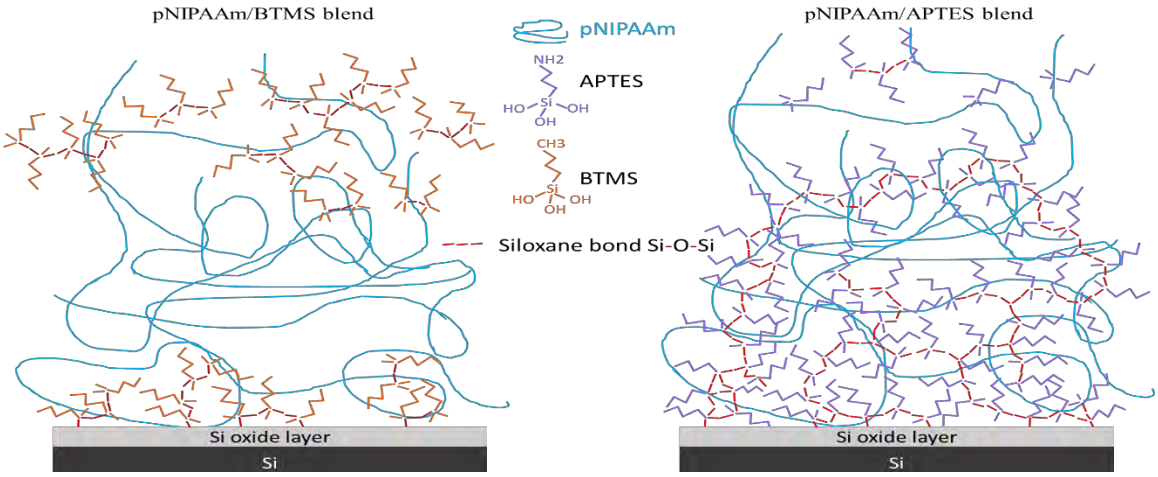


Figure 4.7 A schematic illustration of the network formed by BTMS (left) and APTES (right) after curing.

The weak BTMS network resulted in the film being washed away and losing about 90% of its original thickness just by simple rinsing (initial thickness ~ 50 nm and thickness after rinsing ~5.5 nm) compared to APTES which only loses ~ 30% of its original thickness after rinsing (from ~ 61 nm to ~ 44 nm).

4.3 Intermolecular Interactions and Retention of pNIPAAm in the Blend Films

It is important to note that even though the original blend solution contained ~ 50 wt.% of the organosilanes, the cured film (before rinsing) contains much less than the percent found in the solution (17.7 wt.% and 0.6 wt.% for APTES and BTMS, respectively) as shown in the accompanying table in Figure 4.6. The loss of organosilane is likely to happen during spin-coating and thermal curing inside the oven. The loss during spin-coating could be a result of poor intermolecular interaction between the

organosilanes and pNIPAAm resulting in the small organosilane molecules being removed with the solvent during spin-coating. Additionally, the evaporation of the non-polymerized organosilanes inside the oven could lead to further removal. For both BTMS and APTES, the boiling point at a reduced pressure (0.01 atm or less can be achieved in our vacuum oven) could be way below 160°C, and the evaporation of individual molecules of BTMS and APTES is expected during the thermal curing in the vacuum oven. In the case of BTMS, the lack of polar groups (aside from the silanol Si-OH groups) might limit the attraction (mainly hydrogen bonding) between BTMS molecules and pNIPAAm resulting in major loss of BTMS with the solvent during spin-coating. On the other hand, APTES molecules contain a primary amine group ($-NH_2$) that can both accept and donate hydrogen bonds with pNIPAAm resulting in higher APTES retention during spin-coating compared to BTMS.

To investigate the role of intermolecular interactions, three additional organosilanes were used. (3-Mercaptopropyl)trimethoxysilane (MPTMS), an organosilane with poor polarity but similar boiling point to that of APTES, [3-(2-Aminoethylamino)propyl]trimethoxysilane (AEAPTMS), an organosilane containing two polar amine groups, and triethoxysilylbutyraldehyde (TESBA), an organosilane containing a carbonyl group. In addition, due to the size of AEAPTMS being larger than APTES, and to rule out the possibility that molecule size influences retention during spin-coating as opposed to molecule polarity, bis(3-Triethoxysilylpropyl)amine (BiAPTES), a larger organosilane with one amine group was used. The chemical structures for those organosilanes are provided in the background section.

In order for pNIPAAm to be entrapped by the organosilanes, it is essential that those organosilanes are retained during spin-coating. It is expected that the interactions (mainly hydrogen bonding) between the organosilanes and pNIPAAm play a major role in the retention of those organosilanes during spin-coating. Organosilanes having polar functional groups are expected to be retained more than those with poor polarity. The retention of the four organosilanes after spin-coating and curing as well as the amount of blend film retained after rinsing and soaking are summarized in Table 4.2.

Table 4.2 The weight % and thickness of various 50/50 pNIPAAm/organosilane mixtures after spin-coating and curing using 2wt.% of solutes in ethanol. The organosilanes are ordered based on their ability to form hydrogen bonds (highest to lowest). Rinsing and soaking was done using room temperature water (~ 23 °C)

Organosilane	**wt.%**		**Thickness (nm)**		
	in blend solution	in cured film	initial	rinsed	3 day soaked
AEAPTMS	49.8%	35.1%	87.4±2.6	88.4±0.7	39.3±5.3
APTES	50.2%	17.7%	61.1±0.6	43.9±5.7	36.6±4.9
BiAPTES	49.8%	16.7%	60.4±3.0	42.8±5.5	23.5±3.2
TESBA	49.1%	4.3%	53.2±0.4	31.0±0.4	13.0±1.8
MPTMS	49.4%	4.9%	48.8±1.8	7.7±1.0	4.1±0.6
BTMS	49.9%	0.6%	49.9±0.4	5.7±0.7	2.9±0.39

As shown in Table 4.2, even though the wt.% of the organosilanes in the original blend solution was ~ 50%, the percent in the cured film (before rinsing) is much less with AEAPTMS having the highest wt.% (35.1%) and BTMS having the lowest wt.% (0.6%). APTES and BiAPTES have approximately the same wt.% in the cured film (17.7% and 16.7%, respectively), even though the BiAPTES molecules are larger. This possibly indicates that the size of AEAPTMS is not the main reason as to why it is retained to a higher degree compared to the other organosilanes. The presence of two amine groups (one primary and one secondary) in AEAPTMS indicates that this molecule can form

more hydrogen bonds (can donate 3 and accept 2) compared to APTES, which contains only one primary amine group (can donate 2 and accept 1), or BiAPTES, which contains one secondary amine group (can donate 1 and accept 1). As a result, more AEAPTMS can likely be retained during spin-coating than the other two amine-containing organosilanes. In addition to hydrogen bonds, all the amine-containing organosilanes used here are positively charged which could further enhance the electrostatic attraction between pNIPAAm and the organosilane used.

For TESBA and MPTMS, the ability of these molecules to form hydrogen bonds is a lot weaker than the amine-containing organosilanes, resulting in poor retention during spin-coating and thermal annealing in vacuum (4.3% and 4.9%, respectively). In the case of BTMS, this molecule cannot form hydrogen bonding due to the lack of polar groups and as a result, BTMS is almost entirely lost during spin-coating followed with thermal annealing in vacuum (only 0.6 wt.% is retained in the cured film).

The retention of the organosilane during spin-coating seems to have an influence on the final thickness of the retained film. After rinsing, the pNIPAAm/AEAPTMS film retains approximately 100% of its original thickness, APTES and BiAPTES retain ~ 70% of their original thickness, TESBA retains 58% of its original thickness, while MPTMS and BTMS retain the least (16% and 11% respectively) amount of their original thickness. After prolonged soaking (3 days in cold water), AEAPTMS retains 45% of its original thickness. APTES retains 60%, BiAPTES retains 39%, TESBA retains 24%, and MPTMS and BTMS as expected retain the least amount (8% and 6% respectively). The large reduction in the pNIPAAm/AEAPTMS film thickness after prolonged soaking could possibly be attributed to the hygroscopic nature of amine groups[31]. During

submersion, water can penetrate the AEAPTMS network, which might result in pNIPAAm chains being freed due to the rearrangement within the network. This behavior could also occur with APTES, although APTES contains less amine groups compared to AEAPTMS and the blend film contains less weight percent APTES (17.7%) compared to AEAPTMS (35.1%). Nevertheless, the final thickness value seems to be somewhat correlated with the content of organosilane that is retained in the film as shown in Figure 4.8.

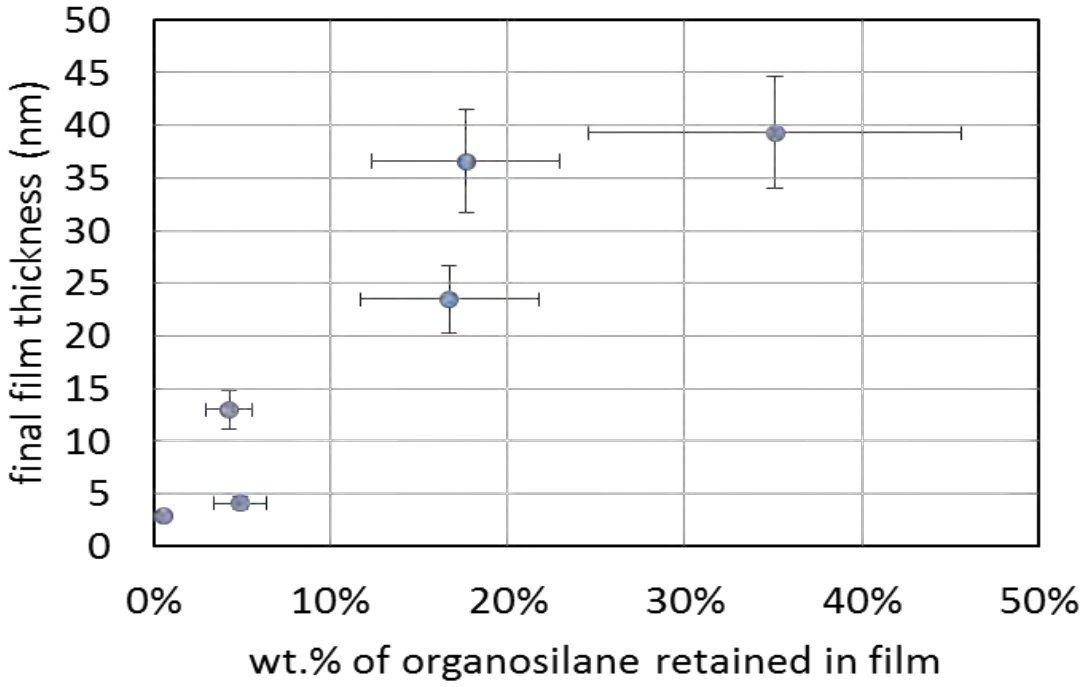


Figure 4.8 The final film thickness after 3 days soaking in water (23 °C) for films prepared using 2 wt.% pNIPAAm/organosilane solution in ethanol.

4.4 Thermo-responsive Behavior and Cell Detachment

Regardless of the amount of film that is retained, all films showed a change in wettability with temperature with the exception of films prepared using pNIPAAm/BTMS blends as shown in Table 4.3.

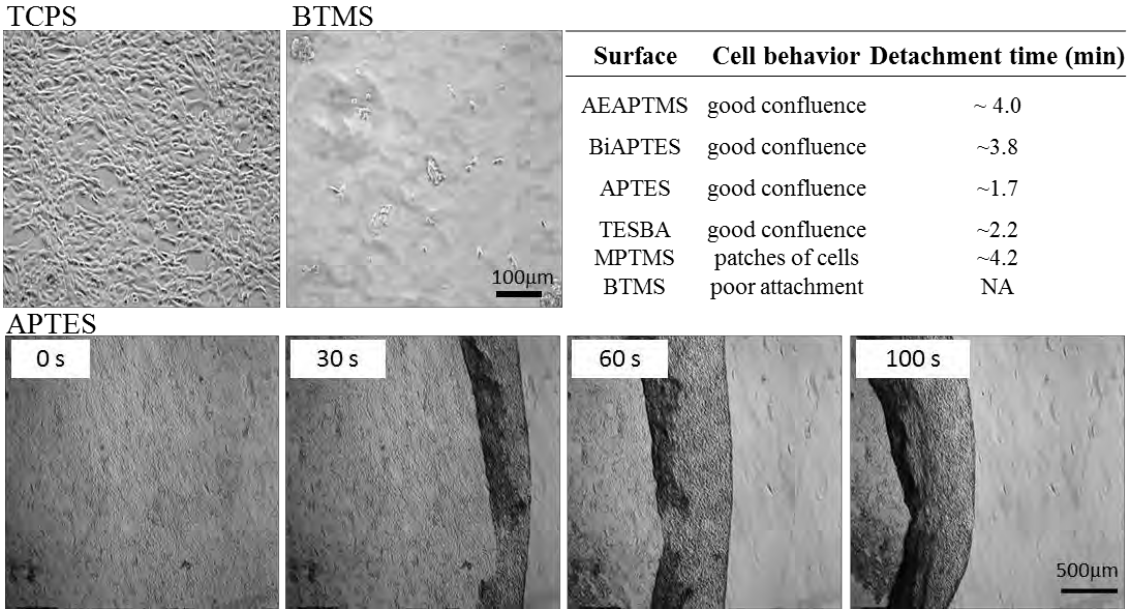
Table 4.3 The advancing and static water contact angles measured on 50/50 pNIPAAm/organosilane blend films at 42 °C and 26 °C.

	42 °C		26 °C	
	Advancing	Static	Advancing	Static
APTES	95.3 ± 1.7	68.9 ± 2.2	75.2 ± 2.2	59.5 ± 1.6
AEAPTMS	86.1 ± 0.8	73.5 ± 1.5	82.9 ± 2.8	67.4 ± 1.0
BiAPTES	101.7 ± 3.5	73.1 ± 3.1	87.2 ± 4.1	62.2 ± 0.4
TESBA	88.5 ± 2.8	70.7 ± 1.3	47.0 ± 2.7	25.7 ± 2.9
MPTMS	75.0 ± 4.0	55.8 ± 1.8	70.0 ± 4.0	29.4 ± 2.9
BTMS	66.4 ± 2.5	51.8 ± 2.7	65.8 ± 3.5	48.7 ± 1.5

The advancing and static contact angles shown in Table 4.3 show a clear change in the wettability of the thermo-responsive films. At 42 °C, the advancing water contact angle is ranged from ~66° to ~102° (52° to 74° for static angles). Lowering the temperature resulted in a transition to a less hydrophobic state and the advancing contact angle drops to a range of ~66° to ~87° (26° to 67° for static angles). One thing to note is that a change in wettability can be observed before the temperature reaches the LCST. As demonstrated in a previous section, rinsing resulted in the removal of films obtained using BTMS due to its poor network formation ability. As a result, the resulting substrate showed weak change in wettability with temperature indicating that the remaining residual layer is not sufficient to exhibit thermo-responsive behavior.

For APTES, AEAPTMS and BiAPTES, the change in contact angle was relatively small (6°-10° based on static angles) compared to that of TESBA and MPTMS (25°-41° based on static angles). One possible explanation is that the amine-containing organosilanes are able to form stronger networks due to their ability to form hydrogen bonds with pNIPAAm (demonstrated by higher film retention) and as a result, entrapping

pNIPAAm more strongly than the networks formed by TESBA and MPTMS. The stronger interactions between the amine-containing organosilanes could hinder/delay the ability of pNIPAAm to fully hydrate resulting in weaker wettability change. Even though the change in water contact angle is not very large for APTES, AEAPTMS and BiAPTES, the substrates showed excellent cell attachment and detachment. Representing attachment and detachment images are shown in Figure 4.9.



Surface	Cell behavior	Detachment time (min)
AEAPTMS	good confluence	~ 4.0
BiAPTES	good confluence	~3.8
APTES	good confluence	~1.7
TESBA	good confluence	~2.2
MPTMS	patches of cells	~4.2
BTMS	poor attachment	NA

Figure 4.9 The poor cell attachment of mouse embryonic fibroblast (MEF) cells on the pNIPAAm/BTMS blend film is shown compared to the attachment on a tissue culture polystyrene (TCPS) dish (top row). Both images were taken with a 10X phase objective. The detachment of MEF cells from a 50/50 pNIPAAm/APTES blend film cured for 3 days is shown in the bottom row. The images were taken using a 4X phase objective. The accompanying table summarizes the cell behavior and detachment time observed for the pNIPAAm/organosilane blend films.

As shown in Figure 4. 9, the detachment times for all the pNIPAAm/organosilanes were less than 5 minutes. Additionally, the type of organosilane used seems to influence the cell attachment. Cell adhesion and growth on pNIPAAm

coatings have been found to be very poor and attempts have been made to alter the wettability of pNIPAAm using copolymers in order to enhance the cell adhesion[32]. The MEF cell sheet harvested on the pNIPAAm/APTES blend films shown in Figure 4.9 indicates that the presence of APTES enhanced MEF cell adhesion. Additionally, by looking at the accompanying table in Figure 4.9, other organosilanes also enhanced MEF cell adhesion apart from BTMS. The lack of cell adhesion on the pNIPAAm/BTMS blend films could possibly be attributed to the presence of the hydrophobic $–CH_3$ end group in the BTMS molecule, which could enhance protein adsorption and alter the conformation of adhesion-aiding proteins such as fibronectin and vitronectin, and as a result, preventing the subsequent cell adhesion[33,34].

4.5 Chemical Grafting Using 3-(2,3-Epoxypropoxy)propyltrimethoxysilane (EPPTMS)

Through the use of an epoxy-based organosilane (EPPTMS in this case), it was possible to chemically graft pNIPAAm to Si-wafer/glass. The epoxy groups present in EPPTMS can react with the secondary amine groups in pNIPAAm (as illustrated in Figure 2.4 in the background chapter). It is important to note that when adding EPPTMS to a pre-dissolved pNIPAAm in ethanol, no grafting occurs. However, when adding EPPTMS to pNIPAAm in its solid form, a thick film is obtained, indicating that the reactivity of EPPTMS towards pNIPAAm is enhanced in the absence of alcohol. This could possibly be explained by noting that the epoxy groups are susceptible to a ring-opening reaction in the presence of alcohols or water. The film thickness and % retention using this grafting method is shown in Table 4.4.

Table 4.4 The thickness of cured, rinsed and 3 days soaked pNIPAAm film grafted using 10 wt.% and 50 wt.% EPPTMS (prepared using 2wt.% total solute in ethanol). Rinsing and soaking was done using room temperature water (~23 °C)

pNIPAAm/EPPTMS	Thickness (nm)			% film retention
	Cured film	Rinsed	3 day soaked	
90/10	88.1±2.2	67.6±1.7	57.2±1.5	64.9
50/50	49.8±1.3	44.0±1.1	41.3±1.1	82.9

The thickness of the pNIPAAm/EPPTMS films after 3 days of soaking are thicker than those of the other pNIPAAm/organosilane reported in the previous section. In addition, the % film retention is higher and seems to increase with the increase in EPPTMS content. This possibly indicates that the reactivity of the epoxy group towards pNIPAAm enhances the retention of the film due to the chemical reaction between the secondary amine in pNIPAAm and the epoxy group in EPPTMS. One major drawback to pNIPAAm grafted using EPPTMS is the poor cell adhesion compared to other organosilanes. The reason for this behavior is not yet understood.

In Addition, a reduction in thermo-responsiveness of pNIPAAm in the presence of large amounts of EPPTMS is observed as shown in Figure 4.10. The magnitude of the change in contact angle for the 50/50 pNIPAAm/EPPTMS blend is around 8 °C whereas for the 90/10 blend it is around 23 °C (Figure 4.10). Even though the resulting film is thick for both cases, the 50/50 film appears to have lost some of its thermo-responsiveness.

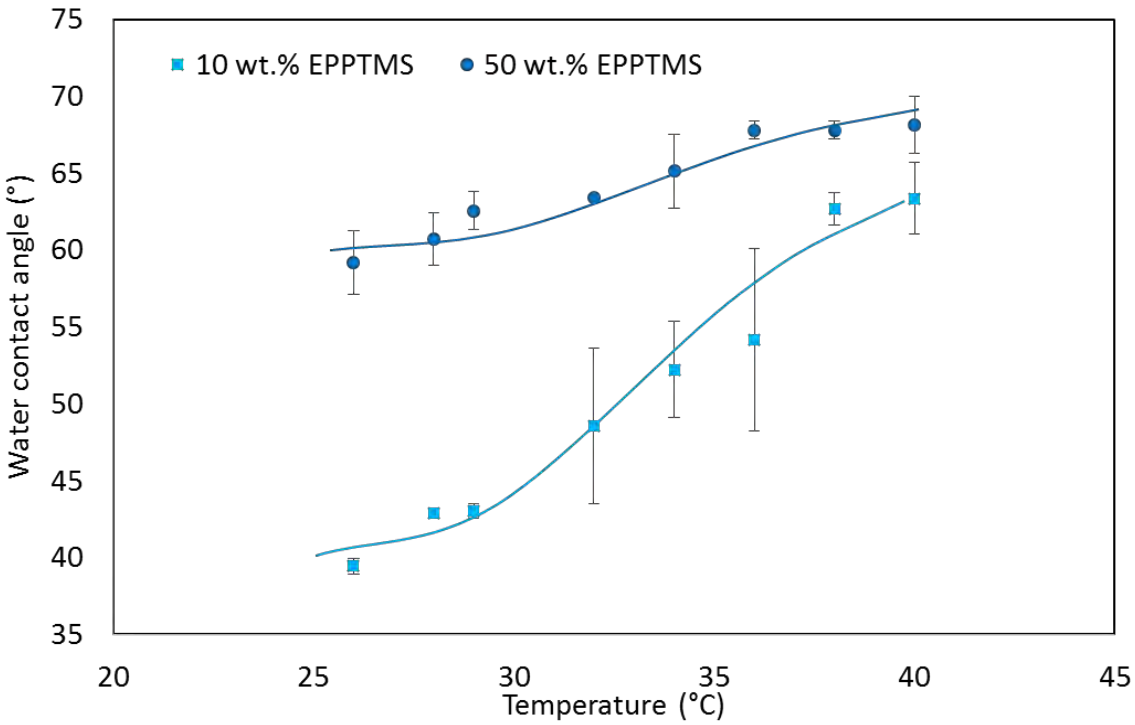


Figure 4.10 The thermo-responsive behavior of 50/50 and 90/10 pNIPAAm/EPPTMS films.

The amide group present in pNIPAAm is responsible for the hydration of pNIPAAm through hydrogen bonding with water below the LCST of pNIPAAm. However, the reaction of this amine group with the epoxy group present in EPPTMS could interfere with the thermo-responsiveness of the amide group leading to a smaller wettability change as shown in Figure 4.10.

4.6 Hydrolysis and Oligomerization

While the pNIPAAm entrapment approach using organosilanes is relatively simple compared to other approaches, one factor that should be taken into account is the hydrolysis and continuous oligomerization of the organosilanes present in the spin-coating solution. In the case of APTES, TESBA and MPTMS blends, (50/50

pNIPAAm/organosilane), solutions prepared days before spin-coating resulted in thicker films compared to freshly prepared solutions as shown in Figure 4.11.

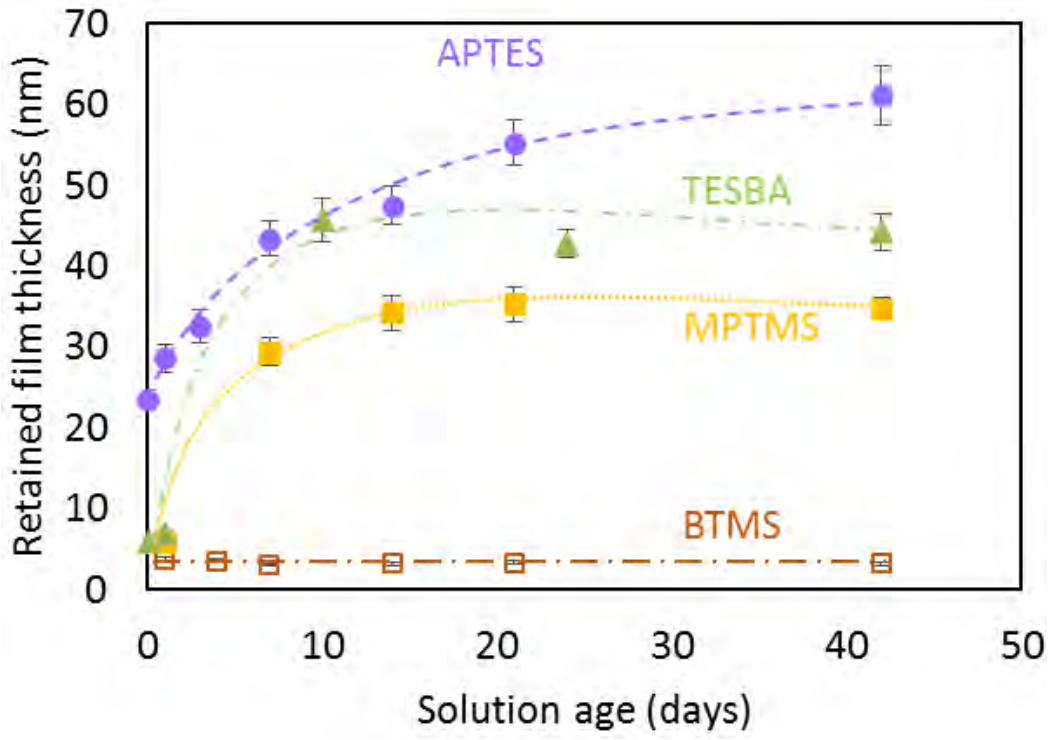


Figure 4.11 The retained film thickness after 3-days soaking for 1.5wt.% 50/50 pNIPAAm/organosilane spin-coated using blend solutions with various ages.

The thickness increase is likely due to the hydrolysis and oligomerization of the organosilanes especially for APTES which can be self-catalytic due to the presence of amine groups. In the case of freshly prepared solutions (freshly prepared and spin-coated within ~ 5 mins), organosilanes might not be fully hydrolyzed and most of the molecules present are individual molecules, and as a result, a large amount of the molecules were removed during spin-coating and subsequent evaporation during thermal annealing in vacuum. The small amount retained would still segregate and cross-link to form a

network for entrapping pNIPAAm with the exception of BTMS which does not favor segregation towards the substrate due to its low surface tension. However, when the solutions are left out and allowed to cross-link before spin-coating (up to 42 days), the resulting film thickness increased for all pNIPAAm/organosilane blends except for BTMS. The wt.% APTES retained in the film significantly increased (~48%) and approached the concentration in the original solution (50%).

This result suggests that the hydrolysis and oligomerization of organosilanes can have a significant effect on the final film thickness and the organosilane content in the resulting film. As a result, controlling the hydrolysis and oligomerization of the organosilanes is needed in order to obtain reproducible results. Factors such as solution age, humidity and water content in the solvent, would need to be taken into account as those factors are known to influence the hydrolysis and oligomerization of organosilanes.

CHAPTER V

CONCLUSIONS

5.1 Summary

The main purpose of this study was to understand the factors that affect the immobilization of pNIPAAm via the siloxane network formed by various organosilanes. APTES, BTMS, AEAPTMS, BiAPTES, TESBA, MPTMS and EPPTMS are the organosilanes that were investigated. pNIPAAm/organosilane blend films were prepared by spin-coating a solution containing 1.5-2wt.% total solutes in ethanol on silicon wafers and glass slides followed by thermal annealing. The effect of curing time, organosilane content, surface tension and chemical functionality of the organosilanes on the final film thickness were examined. Film retention was examined by rinsing and soaking the films for 3 days in water at 23 °C to remove loose molecules. X-ray photoelectron spectroscopy (XPS), ellipsometry and contact angle measurements were utilized for chemical composition and depth profiling, thickness and wettability determination, respectively.

It was found that curing time (1-3 days) for the pNIPAAm/APTES blend films had no effect on the thickness of the films that were retained after rinsing but affected the films that were retained after 3 days of soaking in water (~23 °C). Films cured for 3 days were retained more than films that were cured for 1 day. Additionally, the ratio of pNIPAAm/APTES used had an effect on the final thickness of the soaked films only after

3 days of curing. Films cured for 1-2 days appeared to reach a final thickness in the range of 15 to 20 nm after soaking regardless of APTES content, whereas films cured for 3 days appeared to vary in the final film thickness after soaking based on the APTES content, specifically a slightly thicker film was retained for the film prepared from a spin-coating solution containing a lower amount of APTES (i.e., a higher concentration of pNIPAAm or a more viscous solution).

It was also found that the difference in surface tension between pNIPAAm and APTES resulted in the segregation of APTES towards the substrate due to having higher surface tension compared to pNIPAAm. This segregation behavior led to the accumulation of a sufficient amount of APTES towards the bottom of the film for cross-linking and subsequent resulted in the entrapment of pNIPAAm. This result was verified by depth profiling using XPS and it was found that the APTES content increased as a function of depth. On the other hand, a low surface tension organosilane such as BTMS segregated towards the air-film interface resulting in poor pNIPAAm entrapment. In addition to surface tension, it was found that the functionality of the organosilanes and particularly, their intermolecular interactions (such as hydrogen bonding) with pNIPAAm was necessary for them to be retained during spin-coating and thermal annealing. The organosilane content in the cured film appeared to be dependent on the chemical functionality of the organosilanes and that organosilanes containing polar groups were found to be retained more than ones that are weakly polar or apolar.

Regardless of film thickness and retention, all pNIPAAm/organosilane blend films exhibited a thermo-responsive behavior, which was examined using water contact angle as a function of temperature. It was found that the organosilanes that resulted in a

tighter siloxane networks, such as APTES and AEAPTMS, had a smaller change in wettability with temperature compared to blend films obtained using weak network forming organosilanes, such as BTMS and MPTMS. Additionally, excellent cell attachment and proliferation was observed on all blend film systems except BTMS. which was attributed to the hydrophobicity of the methyl end groups resulting in unfavorable protein conformation. Rapid (less than 5 mins) cell sheet detachment was obtained on all pNIPAAm/organosilane substrates that supported cell adhesion.

Chemical grafting of pNIPAAm through the chemical reaction between the epoxy group present in EPPTMS and the secondary amine group present in pNIPAAm resulted in films that were thicker than all the films obtained by the siloxane entrapment approach. However, the reaction between EPPTMS and the thermo-responsive part of pNIPAAm resulted in reduction in thermo-responsiveness of the blend film, which was verified by water contact angle measurements. Additionally, films obtained using this grafting approach resulted in poor cell adhesion.

It was found that allowing APTES to hydrolyze and oligomerize in the pNIPAAm/APTES solution before spin-coating resulted in thicker films. Cured films that were prepared using solutions that are 42 days old resulted in a significant increase in film thickness as well as APTES retention in the film after 3 days of soaking in cold water compared to films that were prepared immediately after making the pNIAAm/APTES solution. As a result, controlling the hydrolysis and oligomerization of the organosilanes is needed in order to obtain reproducible results.

5.2 Future Work

The entrapment of pNIPAAm via organosilane resulted in thermo-responsive thin films that could be used for non-invasive tissue harvesting. However, there are many areas related to these thermo-responsive films that are not fully understood.

- In some cases, the detachment of the cell sheets appeared to initiate from one side of the glass slide and proceed to roll to the other side of the glass slide, whereas in other cases, cell sheets appeared to detach uniformly from all directions. The investigation of the rolling behavior could allow the use of these thermo-responsive films for harvesting, for example blood vessels when the endothelial cells, smooth muscle cells and fibroblast cells are seeded side-by-side.
- The poor cell adhesion on the chemically grafted pNIPAAm using EPPTMS can be investigated and potentially improved, since simple grafting via EPPTMS could alleviate the need for long thermal curing at elevated temperatures.
- The molecular interactions between the organosilanes and pNIPAAm can be investigated using thermodynamic models and systematically verified in order to better understand the retention and subsequent entrapment of pNIPAAm. In addition, the thermo-responsiveness of the entrapped pNIPAAm could be optimized for a better control over cell sheet detachment.
- Kinetics of the hydrolysis and oligomerization of organosilanes and effects of pH can be investigated in order to obtain reproducible film properties such as organosilane content and film thickness.

Apart from the thermo-responsive polymers, the entrapment of other functional polymers via organosilanes can be investigated as this approach offers a simple way to retain polymers that might otherwise dissolve away in solution/solvents or need more complicated procedures to be immobilized on a support.

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