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Effect of surface hydrophilicity on the alignment of lambda DNA molecules

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NOTE TO USERS

EFFECT OF SURFACE HYDROPHILICITY ON THE ALIGNMENT OF LAMBDA DNA MOLECULES

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ABSTRACT

EFFECT OF SURFACE HYDROPHILICITY ON THE ALIGNMENT OF LAMBDA DNA MOLECULES

By Vikram Sisodiya

The field of bio-inspired materials chemistry has emerged as a major area of interest. Of particular interest is the development of novel approaches and substrates for aligning and adsorbing single DNA molecules. Recent studies have suggested that due to their ability to interact with other biomolecules and chemicals, single DNA molecules can be successfully used as templates for metallic structures such as nanowires and nanotubes. In this thesis, the hydrophilicity of poly (methylsilsesquioxane) thin films was controlled with UV/Ozone exposure and the resulting interaction of the surface with λ-DNA was evaluated. It was found that the optimal surface for DNA adsorption is a very hydrophilic surface resulting from a 70-90 minute exposure to UV/Ozone (static contact angle around 20 to 30 degrees). The optimal surface for efficient DNA alignment is a mildly hydrophilic surface resulting from a 40-50 minute exposure to UV/Ozone (static contact angle around 50 degrees).

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CHAPTER 1
INTRODUCTION

1.1 Bio-inspired Materials Chemistry

The field of bio-inspired materials chemistry has emerged as a major area of interest [1]. The integration of biological concepts, mechanisms and functions with the development of novel synthetic materials and devices with advanced structure and function has had an impact in a variety of research areas ranging from biosensors to self-assembly of biologically driven materials for use in optics and electronics.

A particular area of interest is the development of novel approaches for aligning and adsorbing single DNA molecules. Recent studies have suggested that due to their electrical conductivity properties and ability to interact with other biomolecules and chemicals, single DNA molecules can be successfully used as templates for metallic structures such as nanowires and nanotubes [2]. This area of research is important since the templating of metallic structures at the nanoscale level has raised many hopes, such as new transistors and the development of electronics at a molecular level [2]. In the field of biotechnology, the alignment of single DNA molecules from multiple genomes on the same substrate could also lead to the development of new biosensors, allowing a rapid evaluation of biomolecular interactions [1].

1.2 Lambda DNA

The majority of researchers working in this area have utilized lambda DNA (λ-DNA) for alignment and adsorption. Lambda DNA is a long, double stranded DNA, which is isolated from bacteriophage lambda and contains 48,502 base pairs. Due to the long length of each molecule, single λ-DNA molecules can be easily visualized using fluorescent microscopy after staining with fluorescent dyes. Researchers have found that due to the long length of the λ-DNA molecules, there is some unwinding at the ends of the strand, exposing a negatively charged region that binds to a positively charged surface either due to hydrophobicity and/or an electrostatic interaction between the negatively charged DNA and the positively charged surface [3].

As a result, λ-DNA has played a major role in numerous scientific and technological advances such as the templating of metallic nanostructures, single molecular diffusion behavior studies and the development of methods to align single DNA molecules on various surfaces.

1.3 DNA Alignment

Many methods of aligning single λ-DNA molecules on a substrate have been proposed, one of which is called "molecular combing", a technique which utilizes a meniscus force to align single λ-DNA molecules on a surface. Common substrates used for this area of research include chemically modified glass slides and mica surfaces.

Bensimon et al. first coined the term molecular combing to a technique they used to anchor λ-DNA to a silanated glass surface. Silanation is the vapor deposition of molecules on a surface by "self assembly" resulting in R'-Si-O-CH_3 groups exposed on a surface. As shown in Figure 1 below, the researchers extended the aligned λ-DNA molecule by using a receding air-water interface created during evaporation of a drop of fluorescent labeled λ-DNA solution placed on a silanated glass cover slip at ambient temperature [3]. In step 1 as shown in Figure 1, DNA in solution spontaneously binds to a silanated glass surface at one or both extremities. As the meniscus passes, the DNA molecule is aligned in a direction perpendicular to the movement of the meniscus (steps 2, 3, and 4) [3].

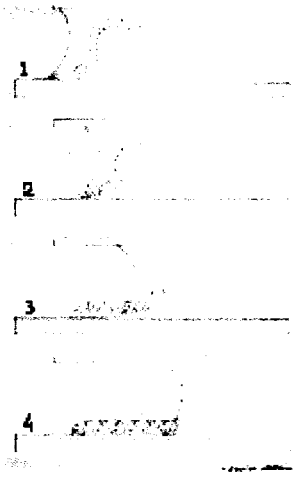

Figure 1. Schematic of molecular combing. A single molecule of lambda DNA is anchored to the surface at one extremity and aligned by a receding meniscus [3].

A fluorescence microscope image of λ-DNA molecules stained with fluorescence dye and aligned on a surface using the technique of molecular combing is shown in Figure 2 [3].

Figure 2. Fluorescence microscope image of "combed" Lambda DNA [3].

Other methods of DNA alignment include modified versions of molecular combing such as the use of nitrogen gas to push a droplet of λ-DNA in buffer solution across a surface. More recently, the use of flow through micro channels and the utilization of optical tweezers to align and manipulate λ-DNA on a substrate have also been developed [4, 5, and 6]. The most important factor in being able to successfully align single λ-DNA molecules utilizing any of these techniques is the ability to control the interaction between the substrate surface and the λ-DNA molecules by developing substrates with varying degrees of surface hydrophilicity.

1.4 Hydrophilicity-controlled Organosilicate Surfaces

Currently used methods of creating contrasting hydrophilic and hydrophobic areas on a surface include traditional lithography and imprinting, which deliver and tether either hydrophilic or hydrophobic molecules to a predefined hydrophobic or hydrophilic

region on a substrate either by use of a mask, or template [2]. These processes however involve multiple processing steps and chemicals, making them less desirable for use with biomolecules where the precise control of pH and ion properties is extremely vital.

Also, the ability to influence hydrophilic and hydrophobic properties of the substrate becomes very important due to the nature of DNA in a buffer solution [2]. Any means of creating different levels of hydrophilicity and hydrophobicity on a substrate is extremely important since it offers the ability to confine molecules of interest to specific areas of the substrate while restricting their presence in others. As a result, a simple yet effective method of tailoring specific levels of hydrophobicity or hydrophilicity on a substrate surface is very important for the adsorption and alignment of λ-DNA. In addition, a simple method of introducing hydrophilicity conditions on a substrate would offer a more cost effective solution since the utilizing of multiple chemicals and steps used in traditional methods are eliminated.

A simple method of controlling surface hydrophilicity is utilizing a combination UV/ozone treatment on thermally cross-linked organosilicate surfaces [2]. A schematic of UV/ozone treatment is shown in Figure 3 (a). In this schematic, a substrate was exposed to UV/ozone through a mask, resulting in a more hydrophilic region in the unmasked area. Static water contact angles of dense polymethylsilsesquioxane (PMSSQ) as a function of UV/ozone treatment time are shown in Figure 3 (b). In this study, researchers used UV and ozone to pattern a surface to show areas of hydrophilic and hydrophobic properties on the same sample. The nonporous film is masked before

exposure to UV/ozone, resulting in a selectively hydrophobic region on an otherwise hydrophilic surface.

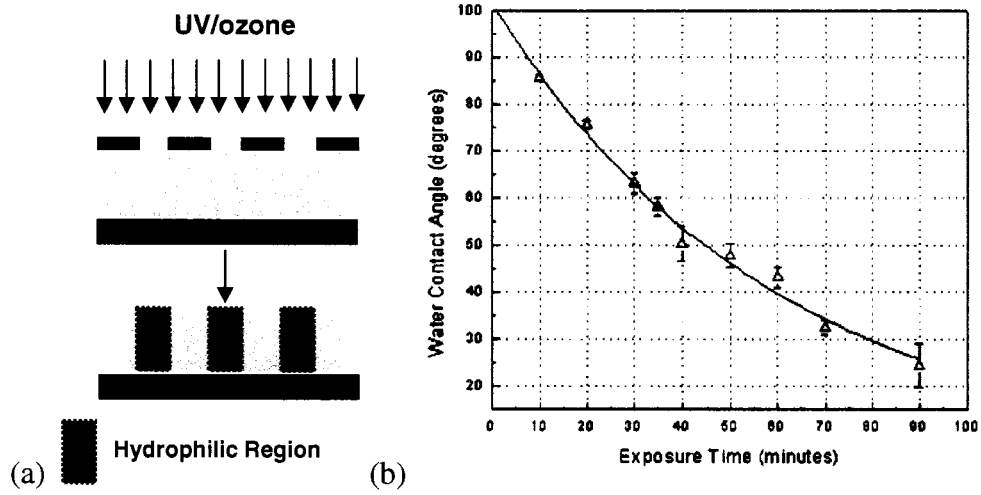

Figure 3. (a) Schematic of UV/ozone patterning process (b) Static water contact angles of dense PMSSQ surfaces as a function of UV/ozone treatment time at 30 °C [2].

Kim et al. used this technique to create hydrophilic and hydrophobic patterns on dense organosilicate thin films by selectively masking specific regions of the film substrate before exposure to UV/ozone. Dense (non-porous) organosilicate thin films of PMSSQ ($SiO_{1.5}CH_3$) can easily be manipulated by exposure to UV/ozone in order to tailor specific areas of hydrophilicity on an otherwise hydrophobic surface. The exposure to UV and ozone replaces methyl groups on the surface with –OH groups resulting in a more hydrophilic surface. In addition, the UV/ozone treated PMSSQ surface has been shown to be very stable over a period of time and a range of temperatures [2].

1.5 Significance

Published research in the area of DNA alignment has focused on surfaces such as mica, silanated glass and more recently silanated silicon, which are highly hydrophobic (static water contacts angle around120 degrees) [7]. No research has been done to assess the alignment and adsorption of DNA on surfaces with different levels of hydrophilicity. It would be important to see what level of surface hydrophilicity would result in maximum DNA adsorption as well as efficient DNA alignment on the surface. At optimum surface conditions, these thin films could be developed into substrates containing a high density of DNA adsorbed or aligned on the surface. Both of which are important characteristics for applications such as high throughput screening applications in genomics, which require the rapid screening of biological activity within a large chemical compound library attached to a solid surface. Also, optimizing DNA adsorption and alignment conditions would have a big impact in microelectronics research where the use of DNA as a template for carbon nano-tubes and metal templated nano-wires has gained a great deal of interest in recent years [1].

CHAPTER 2
LITERATURE REVIEW

The alignment of DNA on various substrates has been studied by researchers in fields ranging from genomic studies to more recently, microelectronics. The development of a simple method to control surface conditions such as hydrophilicity in order to provide optimal conditions for high density attachment of biological molecules would open up a vast area of future research in nanotechnology.

2.1 Molecular Combing

In developing the molecular combing method, Bensimon et al., placed a drop of dyed λ-DNA in buffer solution on a chemically treated glass surface and floated an untreated glass cover slip on top. The glass substrate used by the researchers was chemically treated with a grafted monolayer of silane molecules via molecular self assembly, which resulted in a surface with an exposed vinyl ($-CHX=CH_2$) end group. As shown in Figure 1, as the drying solution receded, the researchers observed that the λ-DNA molecules were attached to the substrate surface at one end and aligned due to the receding air-water interface [3].

The researchers found that the silanated glass surface had a high binding affinity only for the ends of the λ-DNA molecules. They concluded that this was due to the presence of a free protonated phosphate at the 5 prime end of the λ-DNA molecule which

suggested that the interaction between the λ-DNA molecule and the silanated surface was a case of electrophilic addition of weak acids to alkenes [3]. The molecular combing resulted in the extension in strand length of λ-DNA to 21.5 μm plus/minus 0.5 μm, versus the known un-stretched λ-DNA length in solution of 16.2 μm

The researchers found that in contrast to other techniques used to align DNA molecules such as viscous drag and electrophoresis, which act on the full length of the molecule, molecular combing seemed to be localized at the air-water interfaces. The combing was therefore independent of the length and conformation of the molecule [3]. Since the researchers were able to detect minute quantities of DNA by molecular combing, they suggested that molecular combing could open the way for faster genome mapping and the detection of small quantities of target DNA in a large group of molecules [3]. A disadvantage however, was that the silanation process resulted in substrates with varying properties that alter the surface's affinity for DNA.
Despite the above mentioned disadvantage of using silanated glass surfaces, many researchers have used this surface for molecular combing while at the same time, presenting a new modified version of the molecular combing technique than that developed by Bensimon et al. [3].

Michalet et al. applied a modified molecular combing technique to the field of genetic mapping in order to extend DNA representing hundreds of eukaryotic genomes on silanated glass surfaces [8]. The researchers incubated silanated glass cover slips in Teflon reservoirs containing dyed genomic DNA in buffer solution and then extracted the slides vertically out of the solution utilizing a mechanical device. According to the

researchers, during incubation, individual DNA molecules were able to bind spontaneously to the cover slip by the extremities only and were aligned as the meniscus exerted a constant force on a part of each anchored molecule as the molecule moved out of the solution [8]. The researchers used two different types of genomic DNA in this study. The cover slips were either immersed in a solution of total yeast genomic DNA in buffer, or total human genomic DNA in buffer. Figure 4 shows a schematic similar to their combing apparatus. In order to show the schematic clearly, the size of DNA has been exaggerated and DNA molecules in solution are shown in a coiled conformation. Binding of the molecule at the surface is shown at the air-water interface where the meniscus force exerted on the molecule aligns the strand.

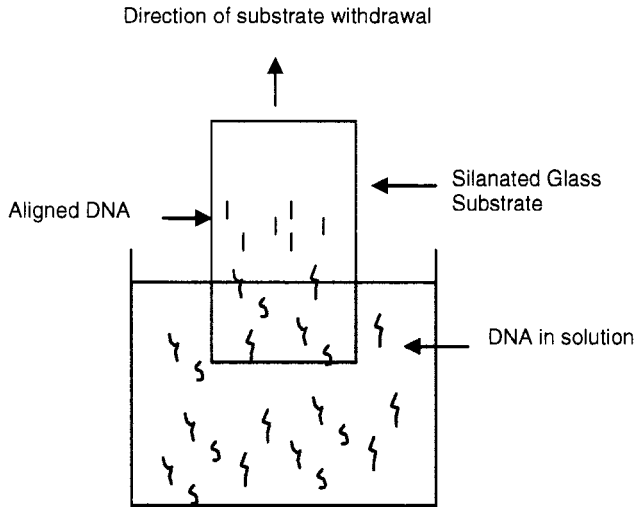

Figure 4. Schematic of modified molecular combing.

They concluded that molecular combing had the advantage of combing high concentrations of total genomic DNA while preventing the molecules from shearing [8]. Fluorescent probes were then hybridized with the DNA and combed on silanated glass

cover slips, resulting in a high density of DNA fibers detected on each cover slip, resulting in potentially hundreds of genomes per cover slip, a necessity for rapid genetic analysis studies.

Yokota et al. also used silanated glass cover slips to comb λ-DNA using a method similar to that described by Michalet et al., but modified the combing method by utilizing a computer to control the meniscus motion [9]. The silanated glass slides in this study were pre-coated with single stranded non-blocking DNA in order to increase the surface affinity for DNA. The substrate coated with the non-blocking DNA showed an improved DNA alignment compared to the nonspecific binding observed on the substrate without the non-blocking DNA treatment. The cover slips were extracted from the DNA solution at varying rates, controlled by a computer, which offered the advantage of controlled speed and the results showed a uniformity of DNA molecules combed on the surface.

Yi et al. used a similar but slightly modified technique than that developed by Yokota et al. for aligning human genome DNA on silanated mica surface. The researchers in this study used a solution of human genome DNA in buffer solution and placed a drop directly on the mica surface which was then dipped in distilled water before being extracted at a stable velocity controlled by a computer [10]. The researchers concluded that their particular modified technique had the advantage of using a smaller sample size and less sample contamination.

Whereas the previously mentioned studies showed silanated glass substrates to have uneven surfaces, Kwak et al. used a modified silanation technique on glass slides for both fluorescence microscopy and AFM analysis [11]. The glass slides were first treated

with only silane molecules as described by Bensimon et al. but the researchers found the density of combed DNA molecules to be relatively low using this substrate for AFM imaging. The researchers then treated the glass slides with a two component mixed silane, where the substrate was treated with a mixture of NH_2 and CH_2-CH-terminated silanes. The density of combed λ-DNA molecules on this surface correlated well with the molecular combing technique used by Michalet et al [10]. Whereas Yi et al. used a mica substrate for AFM analysis, the researchers in this study concluded that while the original silanated surface as described by Bensimon et al. was suitable for combing for fluorescent analysis; the two component silane treatment was a better substrate treatment for combed DNA imaging using both fluorescent microscopy and AFM since a higher density of combed DNA was observed [10].

Many other researchers have modified the molecular combing technique over the years. One modified method was the development of computer controlled capillary containing DNA solution as described by Otobe and Ohtani [12]. The researchers used a motor driven micromanipulator placed on an inverted fluorescent microscope to adjust the position of a capillary tip which was attached to a micro injector containing λ-DNA /dye solution. The microscope stage was adjusted using computerized controls to enable the λ-DNA drop on the capillary tip to drag on a silanated glass surface [12]. In a method developed by Petit and Carbeck, a flow of DNA/Dye solution through micro channels was used to create patterns of stretched and oriented DNA molecules by controlling the shape of the air-water interface [5]. A poly (dimethylsiloxane) (PDMS) surface was used as a substrate in this study. By manipulating the amount of pressure applied to the flow,

the researchers were able to create a flat, concave and convex air-liquid interface, which resulted in a difference in orientation of combed DNA molecules [5].

2.2 Molecular Combing and Microelectronics Research

Molecular combing has since gained interest not only in the biotechnology areas of research, but also in the field of microelectronics and materials research. Studies have shown that DNA molecules can be used to organize nanoparticles into arrays as a result of base pairing or as linear templates for the creation of metallic or semiconducting nanowires [13].

Deng and Mao used molecular combing to align λ-DNA on a mica surface and followed this by the metallization of the patterned λ-DNA to create ordered metallic nanowire arrays [13]. The researchers in this study placed a drop of DNA/dye solution onto a mica surface and then used compressed air to drive the solution flow in one direction, developing yet another modified molecular combing technique. This resulted in the alignment of DNA molecules in a one dimensional parallel pattern. The researchers repeated this experiment by flowing DNA in the other direction, resulting in a lattice 2 dimensional pattern of combed λ-DNA on the surface. The substrate was then checked under a fluorescent microscope to confirm the proposed pattern, which was then confirmed using AFM techniques similar to those described by Kwak et al. Following DNA deposition on the substrate surface, a solution of dissolved 10 μL of a saturated palladium ($Pd(Ac)_2$) solution in distilled water was then allowed to incubate with the

patterned DNA molecules for 10 seconds [13]. In another modified form of molecular combing, the palladium solution was removed from the substrate edge using filter paper. AFM images showed the presence of parallel or crossed metallic nanowires on the mica surfaces.

Research such as that conducted by Deng and Mao is very significant in this area of research since the controlled orientation and placement of biomolecules within predefined areas is a very challenging task and according to Dupraz et al., DNA alone is not a good conductor for electronics applications [13]. As a result, researchers have been working to adopt molecular combing as a technique to enhance the electrical properties of DNA by combing metal coated DNA over defined electrodes.

Dupraz et al. added λ-DNA modified with platinum nanoclusters to de-ionized water and incubated the solution at room temperature for up to 1 week [14]. The incubation allowed the platinum clusters to grow on the DNA strands, which following reduction reaction, resulted in the formation of closely spaced platinum particles along each DNA strand. The platinum modified λ-DNA was then stretched between two lithographically placed gold electrodes on a silanated silicon substrate using a modified molecular combing technique [14]. In this method, filter paper was used to remove a drop of DNA from the substrate surface, resulting in the alignment of the platinum modified λ-DNA. In order to increase the conductivity of the platinum modified λ-DNA strands, the substrate was incubated in a solution of $KAuCl_4$, KSCN and hydroquinone, which are the chemicals used for deposition of gold on platinum. When a current was passed through the electrodes, resistances between 100 ohms and 400 kohms were

obtained, depending on the number of aligned DNA strands present [14]. The researchers believe that this method of synthesizing continuous semiconductor nanowires along DNA strands would lead to the fabrication of a number of electronic components [14].

Besides the use of molecular combing, other more complicated methods of templating DNA with metallic nanoparticles have also been proposed, one of which was developed by Nyamjav and Ivanisevic [15]. The researchers in this study utilized imbedded magnetic nanoparticles (MNP) on substrates which were then used to attach DNA hybridized with complementary magnetic nanoparticles [15]. A process called Dip Pen Nanolithography (DPN) was used to pattern the surface utilizing an AFM tip to visualize and manually stamp a silicon substrate. DNA molecules were then incubated in a solution of MNP and combed onto the patterned substrate using a method similar to that developed by Otobe and Ohtani. The researchers were able to observe elongated DNA molecules on the DPN templated substrates which were stretched in a site-specific manner for the most part. The researchers did have a problem with non specific binding of MNP in the unpatterned area of the substrate and recommended the identification of a suitable blocking agent for that region on the silicon wafer [15].

More recently, Kinsella and Ivanisevic used molecular combing coupled with metal templating to show that aligned and metal templated DNA molecules maintained their biological properties [17]. The researchers electrostatically assembled magnetic nanoparticles on stretched and aligned DNA molecules before digesting the DNA molecules with a restriction endonuclease BAMH1 [17]. Using a similar approach,

Braun et al. assembled 3.5 nm gold nanoparticles on micron long DNA templates stretched on silanated silicon substrates [18]. Both of the above mentioned studies show that molecular combing is an invaluable tool to align and stretch DNA on solid surfaces without compromising the biological activity of the molecules once out of solution.

While molecular combing has been applied to areas of research ranging from the development of high throughput arrays for genetic mapping to the creation of metalized DNA templates for microelectronics, it has one main limiting factor. As mentioned by Nyamjav and Ivanisevic, unless the substrate surface is chemically treated and prepared to repel the nonspecific binding of combed DNA molecules, the method is limited [15]. Therefore, a simple method of creating an even surface substrate, treated to provide selectivity for binding molecules in some areas while repelling them in others is one possible solution to this problem.

2.3 Organosilicate Thin Films and UV/ozone Treatment

According to Kim et al., substrates with hydrophilicity contrast, which contain hydrophilic regions surrounded by hydrophobic areas, are ideal for confining biomolecules to discrete regions [2]. The methods currently used to create hydrophilicity contrasted areas include traditional lithography and imprinting, both of which deliver and tether molecules to defined areas on the substrate using either a mask or manually stamping the substrate surface using AFM as described by Nyamjev and Ivanisevic [15].

According to Kim et al., these methods generally involve the use of a series of chemicals such as photoresists and elastomers in their many processing steps [2].

Kim et al. developed a simple method of creating contrasting hydrophilic and hydrophobic areas on a nanoporous thin film surface using UV/ozone treatment. The researchers in this study used Poly (methylsilsesquioxane) (PMSSQ), a matrix material that is thermally crosslinked as the thin film for the substrate. What makes PMSSQ a promising substrate for microelectronics use is the fact that it has an inherently low dielectric constant, ranging from 2.7-2.9 and is thermally stable up to 500 ^{o}C [19]. According to Oh and Ree, PMSSQ is a reliable dielectric thin film in that it provides a high level of surface hardness and mechanical toughness, both key properties in order for the substrate to withstand the severe mechanical stress resulting from integrated circuit fabrication [20].

PMSSQ is intrinsically hydrophobic but Kim et al. developed a method to tailor surface hydrophilicity using a combination of UV irradiation and ozone exposure [2]. According to Kim et al., although the exact mechanism of UV/ozone treatment has been unclear in literature, it has been used widely as a method for cleaning and etching in the microelectronics industry. The researchers created the substrates by spin casting a mixture of PMSSQ and a porogen to form a homogeneous thin film on a silicon wafer [2]. The substrate is then subjected to heat in order for a thermal cross linking reaction to take place, which induces a phase separation between the PMSSQ and porogen. As a result, the organic porogen molecules are trapped within a cross linked PMSSQ matrix which upon further heating, decompose forming a porous structure within the PMSSQ

matrix [2]. A schematic of the substrate preparation and UV/ozone treatment process is shown in Figure 3.

Kim et al. found that UV/ozone removed the organic porogen materials within exposed areas of the thin film and more importantly, an observed decrease in static water contact angles with increasing treatment time led them to conclude that the treatment also affected the hydrophilicity of the PMSSQ matrix itself. Using photolithographic masks, the researchers used UV/ozone treatment to create patterns on the nanoporous PMSSQ substrates, leaving the surface with contrasting hydrophilic and hydrophobic characteristics. The researchers created hydrophilic patterns surrounded by hydrophobic PMSSQ and obtained images after spreading water on the patterned surfaces, shown in Figure 5.

Figure 5. Water droplets confined to UV/ozone generated pattern [2].

The researchers tested the quality of the patterns by attaching a fluorescent dye selectively within the hydrophilic patterns using linkers, shown in Figure 6.

Figure 6. Fluorescence micrograph of patterns attached with dye [2].

Well defined arrays of circular patterns were observed and the researchers concluded that the surface hydroxyl groups generated on the surface by the UV/ozone treatment could be used to covalently bond probe molecules such as DNA in an aqueous environment [2].

Recently, Zhang et al. described using UV/ozone to alter the hydrophilicity of poly (dimethylsiloxane) (PDMS) and using the treated surface to align DNA using the molecular combing technique [7]. The researchers concluded that the highly hydrophobic PDMS surface (120 degree static water contact angle) had a weak interaction with DNA and resulted in a very low density of aligned DNA on the surface after molecular combing whereas a more hydrophilic surface resulted in a higher density of aligned DNA [7].

2.4 Summary

There has been great interest in the adsorption and alignment of DNA for microelectronics purposes. Researchers have shown that aligned DNA molecules on a

solid surface can be used as a template for metal nanoparticles and nanowires while retaining their biological activity. Researchers have developed many different versions of the molecular combing technique pioneered by Bensimon et al. Through the use of many types of substrates such as silanated glass, mica and more recently PDMS, researchers have been able to adsorb and align DNA on highly hydrophobic surfaces with varying degrees of success. Recent work by Zhang et al. gives rise to the idea that varying degrees of surface hydrophilicity may play an important role in the ability to efficiently comb a higher density of aligned DNA on a surface. Therefore, treating PMSSQ substrates with UV/ozone treatment as described by Kim et al. in order to obtain surfaces with varying degrees of hydrophilicity would provide substrates which could be used to study the effect of surface hydrophilicity on DNA alignment and adsorption.

CHAPTER 3
RESEARCH OBJECTIVES

3.1 Research Objectives

The UV/ozone treatment method described by Kim et al. will be used to control the surface hydrophilicity of Poly (methylsilsesquioxane) (PMSSQ) organosilicate substrates. The primary research objective is to study the effect of PMSSQ surface hydrophilicity on λ-DNA adsorption. The secondary research objective is to study the effect of PMSSQ hydrophilicity on λ-DNA alignment and quantification of the alignment.

The majority of research done in the area of DNA interaction with solid surfaces has been done using highly hydrophobic surfaces such as glass, mica and silanated glass/silicon. Recent studies have shed some light on the effect of varying surface hydrophilicity on DNA adsorption/alignment. This study will provide a valuable insight into the effect of surface hydrophilicity on DNA interaction by quantifying optimal PMSSQ surface conditions in terms of static contact angles in order to obtain both a higher density of DNA adsorption and more efficient DNA alignment on UV/ozone treated thin films of PMSSQ.

CHAPTER 4
RESEARCH APPROACH

The overall scope of this experiment is to study the interaction between hydrophilicity controlled PMSSQ surfaces and λ-DNA. Surface hydrophilicity was controlled by exposing the PMSSQ substrates to varying conditions of UV/ozone. Surface hydrophilicity was measured using a video contact angle system to assign a static water contact angle for each UV/ozone treated surface. The treated PMSSQ substrates were then used to adsorb λ-DNA on the surface. A fluorescent microscope was utilized to image and analyze fluorescence intensity. The UV/ozone treated surfaces were then used to align single λ-DNA molecules using a dip coater in a method similar to that described by Michalet et al. [8]. A fluorescence microscope was utilized to image and analyze the single λ-DNA molecules. An orientation function was used to quantify the efficiency of DNA combing on the hydrophilicity controlled PMSSQ surfaces.

4.1 Experimental Design

The experiments were conducted according to Table 1. In part 1 of the experiments, UV/ozone treatment was applied to 10 PMSSQ substrates. The UV/ozone exposure time ranged from 0-90 minutes at 30°C. Static water contact angle measurements were taken for each sample. In part 2 of the experiments, the same number of samples (n=10) were used for UV/ozone treatment at the same UV/ozone

exposure times and those samples were then used for DNA adsorption. Fluorescence intensity was measured and the analysis of these samples was used to determine an optimal surface hydrophilicity for DNA adsorption. In part 3 of experiments, 10 samples were treated at the same UV/ozone conditions as in part 2 and used for DNA alignment. The orientation order parameter shown in equation 1 was used to quantify the efficiency of DNA alignment on the treated surfaces [16].

$$S = \left\langle \frac{3\cos^2 \Omega - 1}{2} \right\rangle \qquad \text{Equation 1}$$

S is the orientation order parameter and Ω is the angle between the axis of the DNA molecule and the direction of sample withdrawal out of DNA solution as shown in Figure 7. An S value of 1 ($\Omega = 0^{\circ}$) would show a zero deviation of the DNA molecule from vertical; therefore an S value closer to 1 would denote efficient combing.

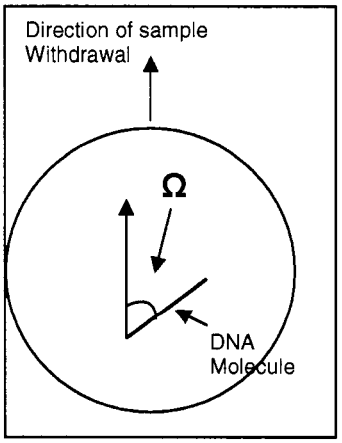

Figure 7. Schematic of DNA alignment and calculation of orientation order parameter.

Table 1. Experimental Scheme.

Sample Number	Uv/O3 exposure times (at 30°C) (min)	Contact Angle Measurement
1	0	√
2	10	√
3	20	√
4	30	√
5	40	√
6	50	√
7	60	√
8	70	√
9	80	√
10	90	√

Surface hydrophilicity quantified by static contact angle measurements.

Sample Number	Uv/O3 exposure times (at 30°C) (min)	DNA adsorption
1	0	√
2	10	√
3	20	√
4	30	√
5	40	√
6	50	√
7	60	√
8	70	√
9	80	√
10	90	√

DNA adsorption will be quantified by measuring the fluorescence intensity as a function of UV/ozone exposure time.

Sample Number	Uv/O3 exposure times (at 30°C) (min)	DNA combing
1	0	√
2	10	√
3	20	√
4	30	√
5	40	√
6	50	√
7	60	√
8	70	√
9	80	√
10	90	√

Efficiency of DNA alignment will be quantified using an orientation function to quantify alignment according to the direction of sample withdrawal from DNA/Dye solution.

4.2 Materials and Methods

4.2.1 (Poly) methylsilsesquioxane (PMSSQ) Film Preparation

PMSSQ (commercially available) has a chemical formula of $SIO_{1.5}CH_3$. PMSSQ was spin-coated at 3000 rpm onto clean 1" silicon wafers and then heated to 450^0 C under Argon gas. The silicon wafers were cleaned using UV/ozone. The prepared PMSSQ films were then treated at varying combination of UV/ozone conditions.

4.2.2 UV/ozone Treatment

A Samco UV-300H UV/ozone stripper/cleaner was used for UV/ozone treatment of PMSSQ organosilicate substrates. According to Kim et al., UV/ozone exposure replaces the methyl groups on PMSSQ with –OH groups, indicating a more hydrophilic surface [2]. As shown in Figure 8, substrates were loaded through a sliding drawer and then exposed to UV/ozone for a pre-determined amount of time and temperature. For this experiment, the UV/ozone exposure took place at varying exposure times and a temperature of 30^o C.

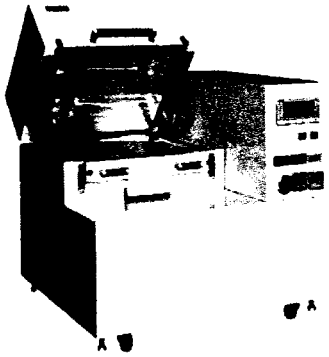

Figure 8. Samco UV-300H stripper/cleaner.

Static water contact angle measurements of the substrates were used to quantify the hydrophilicity conditions by assigning a static water contact angle to the treated substrates.

4.2.3 Static water contact angle measurements

AST video contact angle system VCA-2000 was used to study the hydrophilicity of PMSSQ samples treated with UV/ozone. As seen in Figure 9 (a), the video contact angle system uses a closed circuit camera to examine the profile of a droplet dispensed by a syringe on the substrate of interest and captures the image of the droplet profile on a computer screen. A line tangent to the droplet is then calculated and used to compute the contact angle as shown in Figure 9 (b). The contact angle is calculated using the equation $\gamma_l \text{Cos}\,\theta + \gamma_{sl} = \gamma_s$. A static water contact angle was recorded for 3 points on each substrate and the average of the 3 points was used to assign a contact angle to the substrate as well as to determine the deviation.

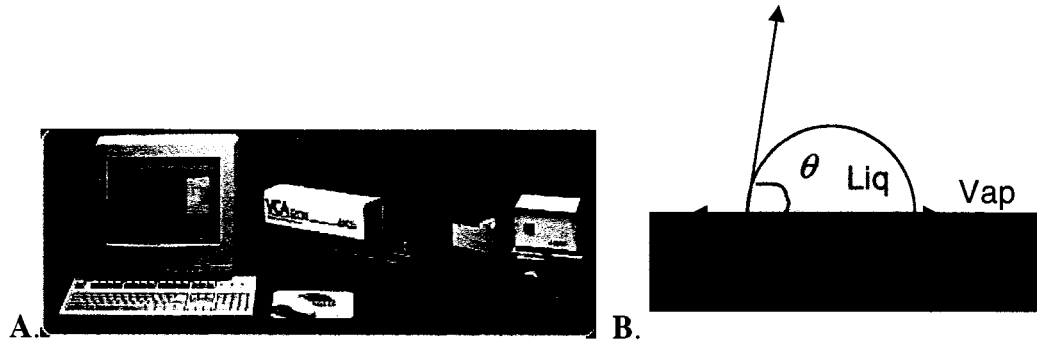

Figure 9. (a) AST VCA-2000 video contact angle system. (b) Computation of the static water contact angle.

The treated UV/ozone substrates were then used to study the interactions between hydrophilicity controlled surfaces and λ-DNA.

4.2.4 DNA Preparation and Adsorption on UV/ozone Treated PMSSQ

Lambda DNA (250μg) in a concentration of 500μg/ml was ordered from New England Biolabs. This particular λ-DNA is isolated from the purified λ phage and dialyzed against 10 mM Tris-HCL 1 mM EDTA (TE) buffer (pH 8). For this experiment, TE buffer (pH 8) was ordered from Ambion. The fluorescent dye YOYO-1 was used for fluorescence studies and ordered from Molecular Probes. YOYO-1 is an intercalating cyanine dye which inserts itself between DNA base pairs. For this experiment, 5 base pairs to 1 dye molecule ratio was used, along with a 1.5μg/ml concentration of DNA/YOYO-1 in TE buffer solution, both determined from literature. It is well known that 1 μg of λ-DNA has 1.38×10^{10} molecules. Therefore, 1.5μg/ml contains 2.07×10^{10} molecules per ml.

Lambda DNA with YOYO-1 fluorescent dye in pH 8 TE buffer solutions was adsorbed on UV/ozone treated PMSSQ surfaces. A 10 μl drop (2.07×10^7 molecules of λ-DNA) DNA/dye solution was incubated on the hydrophilicity controlled surfaces for 15 minutes and then washed off with TE buffer 5 times. The incubation time of 15 minutes is the same as the recommended incubation time for DNA combing according to literature. The samples were then observed under a fluorescent microscope, and an image of each sample was taken.

4.2.5 Lambda DNA Alignment on Hydrophilicity Controlled Surfaces

A molecular combing technique as described by Michalet et al. was used to align λ-DNA/YOYO-1 dye on hydrophilicity controlled substrates using a dip coater. A NIMA DC-mono-Dip Coater was used for the molecular combing of λ-DNA/Dye on hydrophilicity controlled PMSSQ substrates. This device, shown in Figure 10, enables a controlled immersion and withdrawal of substrates into solutions. The dipping cycles and maximum distance of immersion into solution can be programmed. The NIMA-DC can be programmed with dipping speeds from 0.6 to 60 mm/min.

Figure 10. NIMA DC-mono-Dip Coater.

A 1 ml sample of DNA/dye solution was placed in a Teflon block in place of the beaker shown in Figure 10. Teflon was used due to the fact that DNA sticks to glass and a nonstick surface is therefore necessary.

Earlier studies were performed with an increased incubation time (30 minutes – 2 hours) which resulted in more aggregation due to increased time for non specific binding of DNA molecules with the surface to occur. Therefore, the UV/ozone treated substrate was placed on the clip and lowered into the dye solution for an incubation time of 15 minutes and then withdrawn at a speed of 18 mm/min, the latter determined from literature.

4.3 Sample Analysis

Contact angle measurements of UV/ozone treated PMSSQ samples were compared with those reported in literature. A contact angle measurement was taken at 3 points on each sample, using an average of the 3 values as the contact angle of that particular sample. The variation in contact angles on each sample was reported. Variation in contact angle measurements on the same sample can be attributed to non uniformity of PMSSQ film and surface roughness.

The UV/ozone treated substrates with adsorbed λ-DNA and those with aligned λ-DNA were viewed under a fluorescent microscope (Olympus, BX51) with the intensity of images captured by a digital camera. The images from the adsorption phase of the experiment were quantified by measuring the fluorescence intensity in terms of the number of pixels in the green spectrum, as a function of UV/ozone exposure time. The adsorption of DNA on the sample surface was also quantified by measuring the diameter of the drop after the DNA had absorbed on the surface and the sample had been rinsed with buffer. The diameter and fluorescence intensity measurements were taken using Image Pro software manufactured by Media Cybernetics.

The images of aligned λ-DNA were analyzed and compared to those from literature in order to validate the presence of aligned single molecules of λ-DNA. The reported λ-DNA length of 21.5 μm plus/minus 0.5 μm was used to justify the presence of single λ-DNA molecules. For reproducibility, the images of aligned λ-DNA at each

surface condition from different runs were compared in a similar fashion. In addition, the efficiency of DNA combing was quantified using the orientation function described in Equation 1.

CHAPTER 5
EXPERIMENTAL RESULTS

5.1 Static Water Contact Angle Measurements

Figure 11 shows the static contact angles of dense PMSSQ films exposed to UV/ozone at varying times. The UV/ozone exposure took place at 30° C and for a period of time ranging from 0-90 minutes.

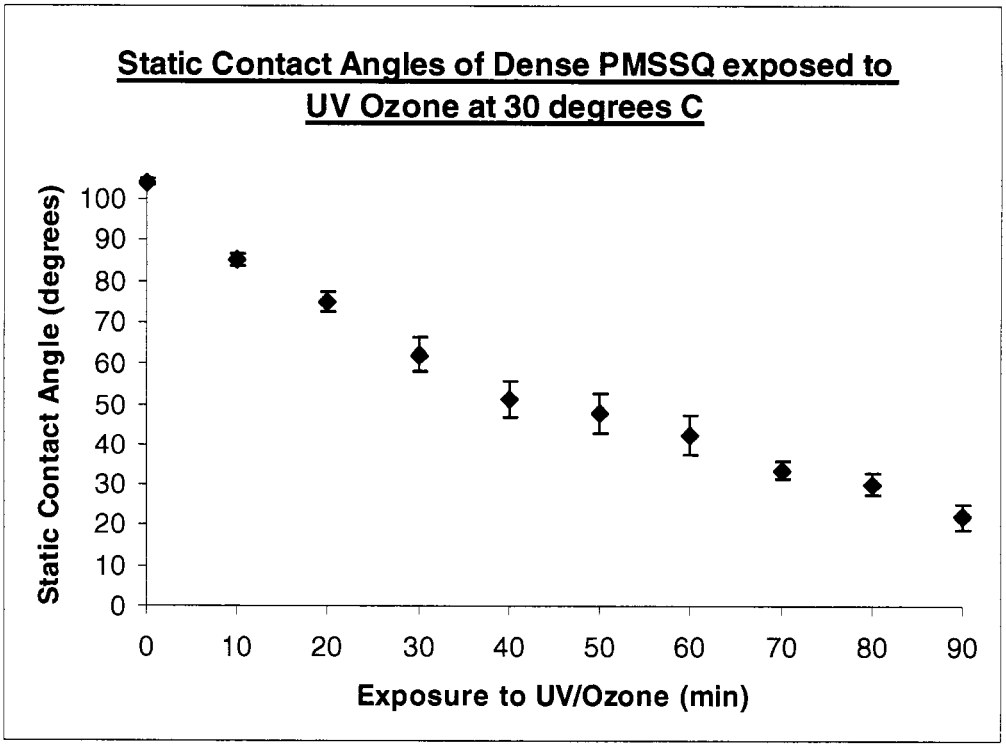

Figure 11. Static contact angles of dense PMSSQ films exposed to UV/ozone at 30°C for 0-90 minutes.

As shown in Figure 11, there was a decrease in static water contact angle as the substrates were exposed to UV/ozone for longer times. The film surfaces that were not exposed to UV/ozone (0 minute exposure) had a static contact angle of 104.2 degrees ± 0.57 degrees. The surfaces that were exposed to UV/ozone for 10 minutes had a static contact angle of 84.3 degrees ± 1.48 degrees. A 20 minute exposure to UV/ozone led to a static contact angle of 74.9 degrees ± 2.4 degrees. The static contact angle for a 30 minute exposure to UV/ozone was 62.25 degrees ± 4.2 degrees. The 40 minute exposed sample had a static contact angle of 51.4 degrees ± 4.4 degrees. The sample exposed for 50 minutes resulted in a surface with a static contact angle of 47.7 degrees ± 4.9 degrees. The 60 minute exposed sample had a static contact angle of 42.6 degrees ± 4.9 degrees. The sample exposed for 70 minutes resulted in a static contact angle of 33.7 degrees ± 2.1 degrees. The sample exposed for 80 minutes had a static contact angle of 30.3 degrees ± 2.8 degrees, and the surface exposed for 90 minutes to UV/ozone had a static contact angle of 21.9 degrees ± 3.3 degrees.

5.2 DNA Adsorption on UV/ozone Treated Samples.

Figure 12 shows the fluorescent microscope images of the adsorbed DNA drop on UV/ozone treated surfaces. The images were captured after a 10μl drop of DNA/dye solution was pipetted onto the substrate surface for 15 minutes, which was then rinsed 5 times with TE buffer and air dried.

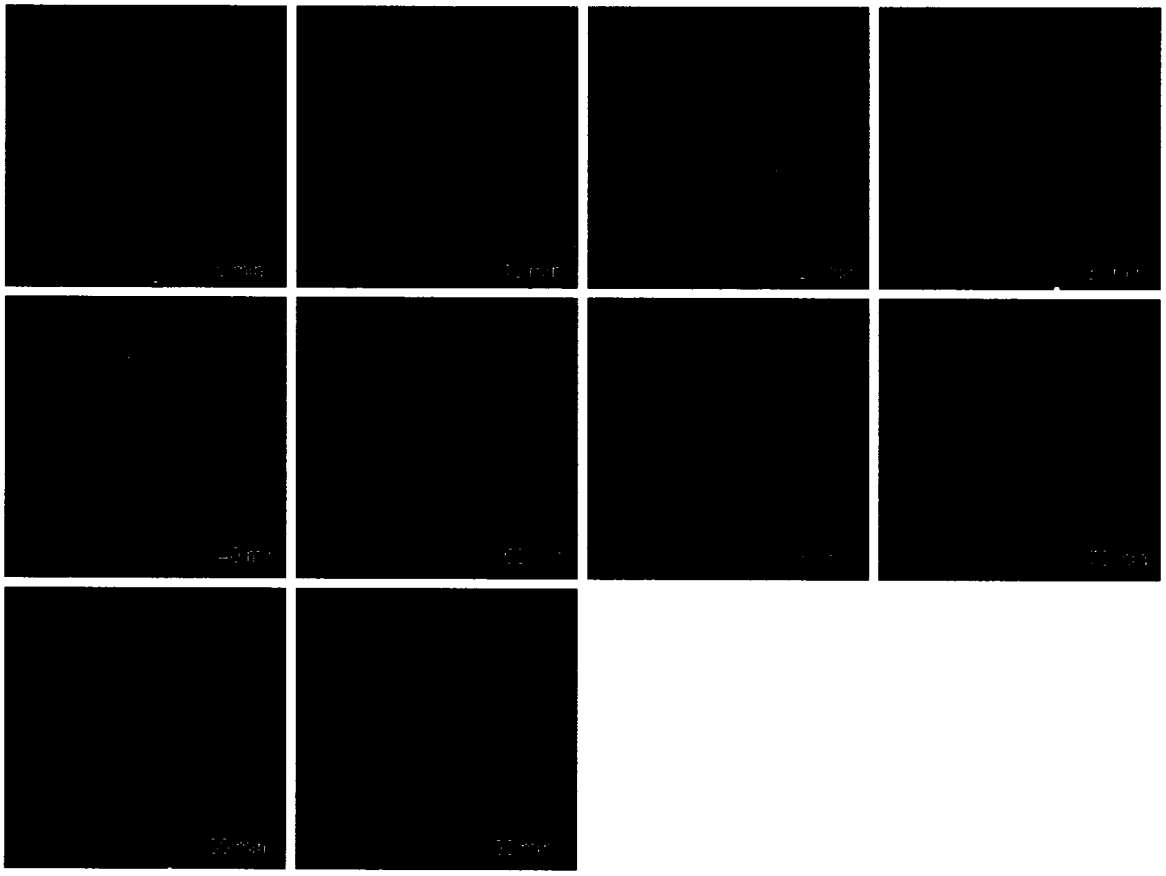

Figure 12. Fluorescent images of adsorbed 10 μl drop of DNA/dye on UV/ozone treated surfaces after 15 minute incubation and air drying of substrate.

As seen in Figure 12, the drop boundary increases as the surface hydrophilicity increases. Figure 16 shows a plot of adsorbed DNA drop diameter as a function of UV/ozone treatment time. The drop diameter was measured using ImagePro software.

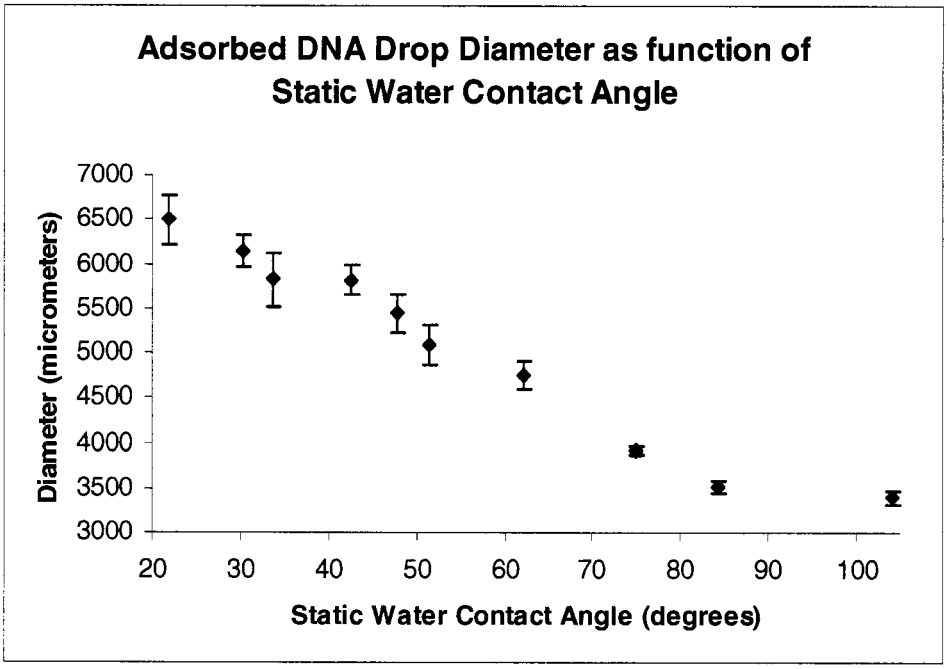

Figure 13. Adsorbed DNA drop diameter as a function static water contact angle.

As seen in Figure 13, the drop diameter increased as the surface hydrophilicity increased. The samples with an average static water contact angle of 21.9 degrees (90 minute UV/ozone exposure) resulted in an average drop diameter of 6495 ± 285.2 micrometers. The samples with an average static water contact angle of 30.3 degrees (exposed for 80 minutes) had an average drop diameter of 6144 ± 181 micrometers. The samples with an average static contact angle of 33.7 degrees (70 minute exposure) had an average drop diameter of 5824 ± 301.7 micrometers. The samples with an average contact angle of 42.6 degrees resulted in an average drop diameter of 5813.3 ± 166 micrometers. The samples with an average contact angle of 47.7 degrees (50 minute exposure) had an average diameter of 5440 ± 211.1 micrometers. The samples with an average contact angle of 51.4 degrees (40 minute exposure) had an average diameter of

5091.4 ± 219.6 micrometers. The diameter continued to decrease as the static contact angle increased. The samples with an average static contact angle of 62.25 degrees (30 minute exposure) had an average diameter of 4756.5 ± 152 micrometers. The samples with average static contact angles of 74.9 degrees (20 minute exposure) had an average diameter of 3914.7 ± 45.3 micrometers. The samples with an average static water contact angle of 84.3 degrees had a drop diameter of 3520.1 ± 60.5 micrometers. The fluorescent image of the samples not treated with UV/ozone (static water contact angle: 104.2 degrees) showed a DNA drop residue with an average diameter of 3401 ± 77.8 micrometers.

Figure 14 is a plot of the fluorescence intensity as a function of static water contact angle. The intensity is measured as the area under the curve of the green wavelength as detected by the fluorescent microscope and analyzed by Image Pro software. The intensity area is reported in arbitrary units (au).

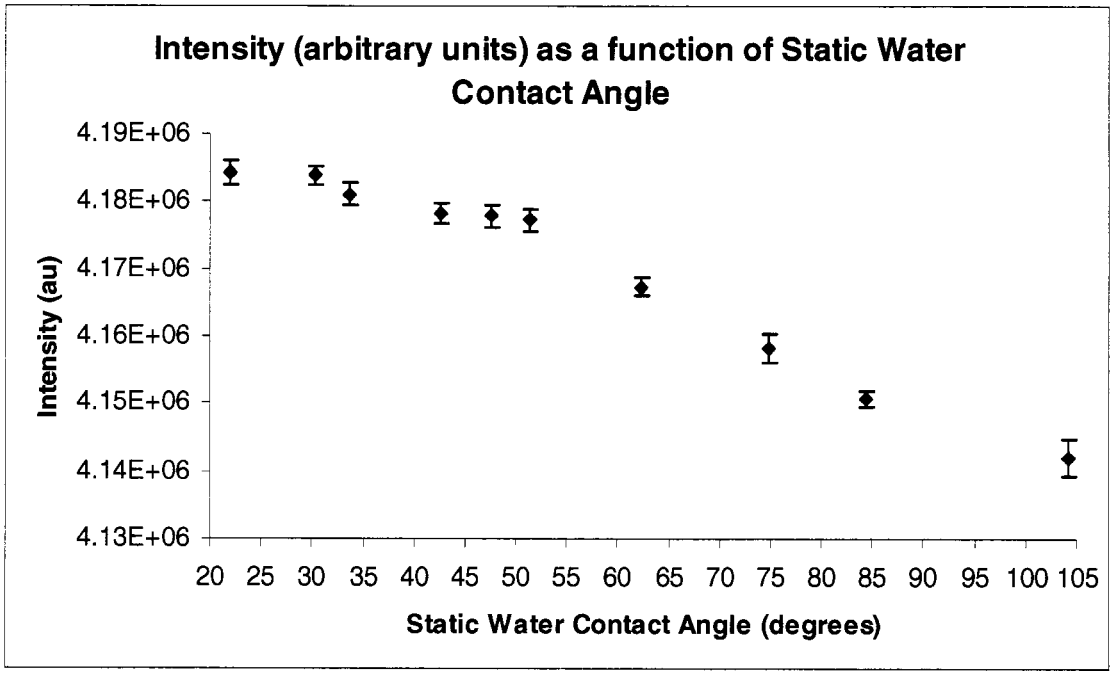

Figure 14. Plot of intensity as a function of static water contact angle.

As seen in Figure 14, there was a decrease in intensity as the surface hydrophilicity decreased. The hydrophilic samples had an average static water contact angle of 21.9 degrees (90 minute UV/ozone exposure) and showed an average intensity area of 4184293 ± 1814.9 au. The samples with an average static contact angle of 30.3 degrees (80 minute UV/ozone exposure) resulted in an average intensity area of 4183903 ± 1320.2 au. At a static contact angle of 33.7 degrees, the intensity area was 4181143 ± 1615.8 au. The intensity area continues to decrease as the static contact angle increases, at a static contact angle of 42.6 degrees, the intensity area is 4178324 ± 1543.2 au. At an average static contact angle of 47.7 degrees, the intensity area decreases to 4177879 ± 1646.6 au. The sample with an average static contact angle of 51.4 degrees showed an

average intensity area of 4177349 ± 1618.6 au. As the static contact angle increased to 62.25 degrees, the average intensity area decreased to 4167276 ± 1399.5 au. This trend continued with the average intensity area of the sample with a static contact angle of 74.9 degrees decreasing to 4158183 ± 2109.3 au. The more hydrophobic samples with static contact angles of 84.3 and 104.2 degrees had an average intensity area of 4150591 ± 1232.7 and 4142070 ± 2716.5 au respectively.

5.3 DNA Alignment on UV/ozone Treated Samples

Figures 15, 16, and 17 show the fluorescent images of aligned DNA on UV/ozone treated surfaces ranging in time from 0 minutes to 90 minutes exposure. The arrow represents the direction of withdrawal of the sample from DNA/Dye solution. The bar denotes 20 μm.

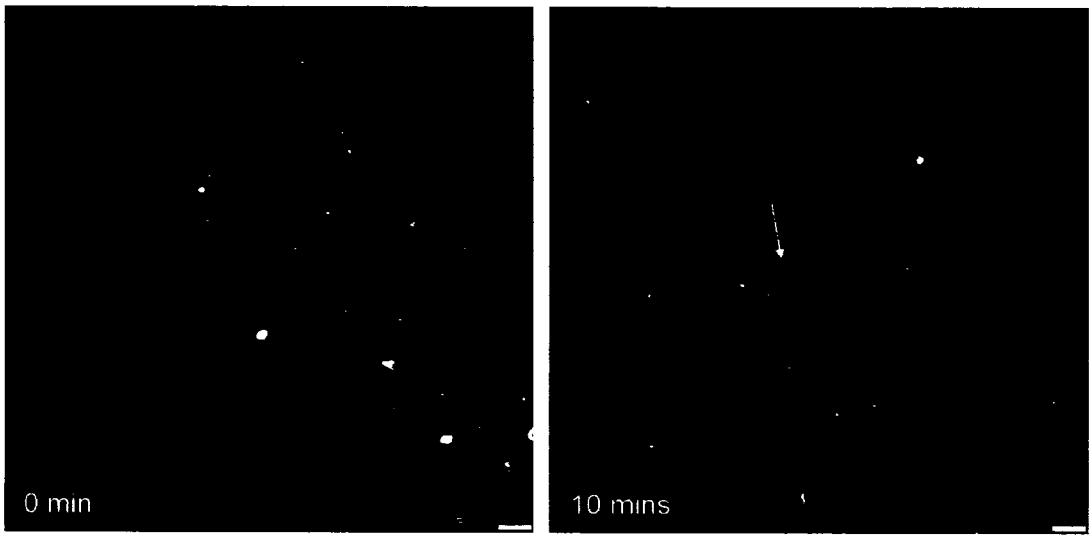

Figure 15. Fluorescent images of aligned DNA on PMSSQ surfaces exposed to UV/ozone for 0 and 10 minutes.

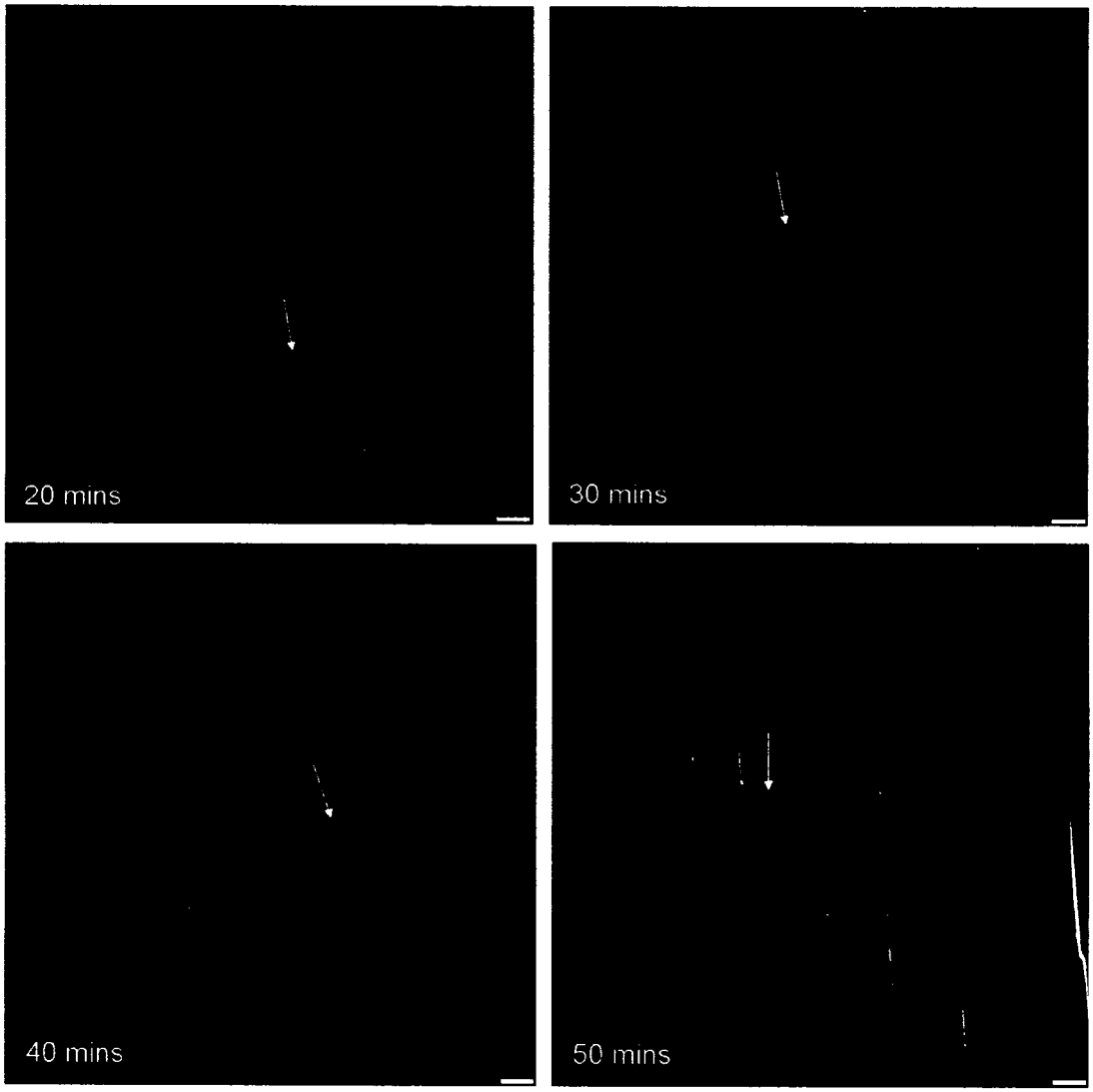

Figure 16. Fluorescent images of aligned DNA on PMSSQ surfaces exposed to UV/ozone for 20, 30, 40 and 50 minutes.

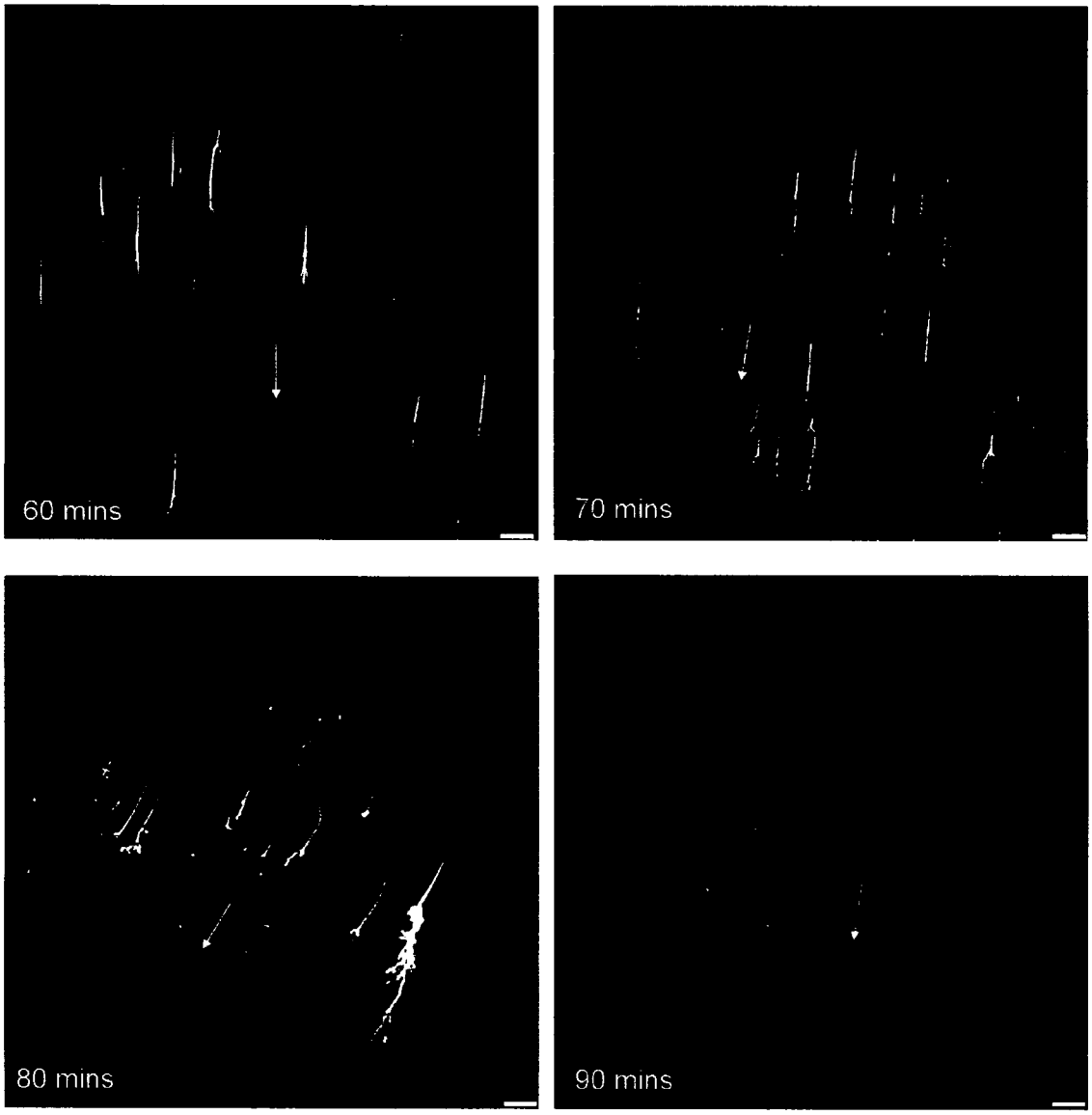

Figure 17. Fluorescent images of aligned DNA on PMSSQ surfaces exposed to UV/ozone for 60, 70, 80 and 90 minutes.

Table 2 is summary of the data for DNA alignment on UV/ozone treated surfaces. The angle between the stretched DNA molecules and a line normal to the air water interface (direction of substrate withdrawal out of DNA/Dye solution) for each sample is

listed for 10 randomly selected DNA molecules on each sample. An orientation order parameter, calculated using Equation 1 is listed in the 13th column.

Table 2. Summary of DNA Alignment on UV/ozone Treated Surfaces.

0	-	-	-	-	-	-	-	-	-	-	-	-
10	26	47	36	55	13	14	32	33	22	10	28.8	0.6519
20	6	9	10	17	35	29	25	6	8	4	14.9	0.9008
30	12	13	9	12	16	4	8	5	11	9	9.9	0.9557
40	7	8	3	1	5	6	4	6	8	7	5.5	0.9862
50	8	7	4	5	9	8	6	9	7	5	6.8	0.9790
60	11	7	10	5	14	4	6	5	9	10	8.1	0.9702
70	6	10	11	13	12	11	14	9	10	8	10.4	0.9511
80	17	11	9	25	7	10	11	9	10	11	12	0.9352
90	-	-	-	-	-	-	-	-	-	-	-	-

As seen in Figures 15 and 17, due to aggregation of DNA on the sample not exposed to UV/ozone (0 minutes) and the sample exposed for 90 minutes, it was difficult to observe linear DNA molecules; as a result, no orientation order parameter could be calculated. For the sample exposed to UV/ozone for 10 minutes, the average degree between the linear DNA molecules and a line normal to the direction of flow out of DNA/dye solution was 28.8 degrees. Based on this, the calculated orientation order parameter was 0.6519. This is also consistent with the fluorescent image of this sample in Figure 15 where a few single DNA molecules are visible but the alignment is inconsistent. The sample exposed for 20 minutes had an average angle of 14.9 degrees and an orientation order parameter of 0.9008. As seen in Figure 16, the samples treated

to UV/ozone for 20, 30 and 40 minutes showed an increase in the presence of individual DNA molecules with increasing UV/ozone exposure time. The sample exposed for 40 minutes resulted in an orientation order parameter of 0.9862 which was closest to the ideal alignment parameter value of 1. The sample exposed for 50 minutes had an average angle of 6.8 degrees and an orientation order parameter of 0.9790. On the other hand, the sample exposed to UV/ozone for 80 minutes had an average angle of 12 degrees and an orientation order parameter of 0.9352.

CHAPTER 6
DISCUSSION

6.1 Increasing Surface Hydrophilicity

An increase in surface hydrophilicity results in a decrease in static water contact angle as shown by Kim et al [2]. This part of the experiment was done to confirm the increase in surface hydrophilicity with exposure to UV/ozone as described by Kim et al., in order to study the UV/ozone treated PMSSQ surface interactions with λ-DNA. As shown in Figure 13, the static contact angle for the control sample (no UV/ozone exposure) was 104.2 degrees. On the other hand, the static contact angle for the sample treated to UV/ozone for 90 minutes was 21.9 ± 3.3 degrees. This marked decrease in static contact angle shows the gradual increase in hydrophilicity as a result of increased exposure to UV/ozone.

The sample exposed for 10 minutes was more hydrophilic than the control sample but still fairly hydrophobic, with a static contact angle of 84.3 degrees. This trend continues as the exposure of UV/ozone is increased from 10 minutes to 20, 30, and 40 minutes. Since Kim et al. reported that UV/ozone exposure to PMSSQ surfaces results in methyl groups on the surface being replaced with hydroxyl groups, it can be assumed that as the substrate is exposed to UV/ozone for longer periods of time, more methyl groups on the surface are replaced with hydroxyl groups, increasing the surface hydrophilicity [2].

The sample exposed to UV/ozone for 40 minutes had a contact angle of 51.4 ± 4.4 degrees, within the 50 ± 5 degrees described by Kim et al. under the same UV/ozone exposure. This sample showed a similar surface hydrophilicity to the sample treated for 50 minutes. The 50 minute exposed sample had a static contact angle of 47.7 ± 4.9 degrees which overlaps with the static contact angle for the 40 minute sample. Therefore, it can be assumed that these two surfaces would show similar interactions with DNA. There is a further decrease in static contact angle as the samples are exposed to UV/ozone for 60, 70 and 80 minutes, culminating with the static contact angle of 30.3 ± 2.8 degrees for the 80 minute exposed sample.

Since the λ-DNA is in an aqueous buffer/dye solution, a more hydrophilic surface would allow more DNA molecules to better diffuse to and interact with the sample surface since the aqueous solution would cover more surface area as it spreads due to a higher interaction between the water molecules and surface hydroxyl groups. Similarly, a hydrophobic surface would limit the surface area the DNA/dye solution could cover and therefore limit the interaction between the DNA molecules and the substrate surface. As a result, the control substrate (0 minute UV/ozone exposure) does not seem best suited for DNA adsorption when compared to a highly hydrophilic surface such as that resulting from a 90 minute exposure to UV/ozone.

6.2 DNA Adsorption on Hydrophilic Surfaces

As shown in Figure 13, the 10 µl drop of DNA/dye solution incubated on a UV/ozone exposed surface and washed off with TE buffer after 15 minutes resulted in the adsorbed DNA on the surface drying to form a circular pattern. The drop of DNA/dye solution was able to broaden out on the surface over time depending on the level of surface hydrophilicity. A more hydrophobic surface such as the control sample resulted in a drop that was restricted to a certain area whereas the same amount of liquid on the more hydrophilic surfaces spread farther. In addition, the intensity area in the green spectrum plotted as a result of UV/ozone exposure time in Figure 16 showed an increase in intensity as the UV/ozone exposure time was increased. Since the fluorescent dye YOYO-1 is an intercalating dye which embeds itself within DNA base pairs, the results from this phase of the experiment show that more DNA had interacted with and was adsorbed to the sample surface as the DNA/dye solution extended beyond the original drop boundary due to increasing surface hydrophilicity.

The samples treated with UV/ozone for 40 and 50 minutes had overlapping static contact angles with standard deviation taken into account and it was therefore expected that they would show similar surface characteristics for DNA adsorption. Taking deviation into account, this is true for both the drop diameter and intensity area as seen in Figures 15 and 16. On the other hand, the sample treated with UV/ozone for 60 minutes resulted in an intensity area similar to the 40 and 50 minute exposed samples but had a

bigger drop diameter. This could be attributed to non specific interactions between the DNA molecules and the surface causing aggregation. According to Otobe and Ohtani, highly hydrophobic surfaces allow DNA solutions to be sufficiently repelled [12]. In other words, a mildly hydrophilic surface would restrict how many DNA molecules could diffuse to the surface and bind specifically whereas a very hydrophilic surface would result in an even dispersion of the liquid on the surface enabling more DNA molecules to diffuse to the surface.

As seen in Figure 5, Kim et al. showed the confinement of water droplets to the hydrophilic patterned area surrounded by hydrophobic PMSSQ. Similarly in this study, although the same number of DNA molecules (2.07×10^7 molecules) in the 10µl of DNA/dye solution are present on the surface, the DNA solution was repelled on the more hydrophobic surfaces, being confined to a given space which resulted in a smaller drop diameter compared to more hydrophilic surfaces after the sample was washed off with buffer. On a more hydrophilic surface, the DNA solution was able to spread out over a bigger area resulting in a similar intensity but bigger drop diameter. As mentioned earlier, more surface hydroxyl groups are available to interact with the solution on the more hydrophilic surfaces.

This could also explain why the drop diameters for the 70, 80, and 90 minute samples increased with increasing exposure time. At the same time, it was observed that these three surfaces resulted in the intensity area leveling off while the drop diameter increased, confirming the need for more studies in this area to see the exact configuration of the DNA on the surface, especially since the probability of non specific binding due to

a strongly polar surface exists. It can however, be deduced that while increasing the hydrophilicity of the surface results in more DNA adsorbing on the surface, a highly hydrophilic surface might not be best suitable for individual DNA molecule alignment. Therefore the optimal surface condition for DNA adsorption may not necessary draw a parallel with the optimal surface condition when it comes to DNA alignment on UV/ozone treated surfaces.

6.3 DNA Alignment

Bensimon et al. along with other researchers who used hydrophobic substrates such as silanated glass and mica surfaces were successful in combing DNA on those surfaces although no studies have been done to try and quantitate DNA alignment on surfaces with varying levels of hydrophobicity/hydrophilicity. In other words, it has been shown that hydrophobic surfaces can be used for DNA alignment although the exact level of surface hydrophobicity for preferred DNA alignment has not been studied. Therefore, it was important to quantify the DNA combing efficiency using the orientation order parameter for each surface exposed to varying levels of UV/ozone.

As shown in the fluorescent microscope images in Figure 15, the sample not treated with UV/ozone resulted in no visible aligned single DNA molecules. This was also observed by Nakao et al. when they attempted to align λ-DNA on highly hydrophobic glass substrates [21]. The researchers observed only a few molecules that were partially stretched whereas the majority of the molecules were aggregated. Since a

highly hydrophobic surface has more positively charged groups on the surface and a DNA molecule is negatively charged, the aggregation of DNA on the surface can be attributed to nonspecific binding of DNA along its length to the surface.

This was also confirmed recently by Zhang et al. The researchers used molecular combing to align DNA on highly hydrophobic PDMS (static contact angle around 120 degrees) and found that very low densities of straight DNA molecules were aligned on the surface [7]. According to Zhang et al., the force acting on the air-water interface to align DNA is mainly due to surface tension. The researchers concluded that the larger the contact angle, the stronger the surface tension since surface tension is a linear function of the variation of the cosine contact angle of the liquid on the surface [7]. The resulting increase in surface tension led to the presence of non specific binding of DNA molecules on the hydrophobic surface. It can therefore be assumed that a strongly hydrophobic surface increases the surface tension, thereby increasing the interaction of the DNA molecules in solution to the substrate surface, resulting in non specific binding of the molecule to the surface and effecting proper molecule alignment during the withdrawal of the sample from DNA/dye solution.

DNA molecules were visible on the slightly more hydrophilic (and subsequently less hydrophobic) substrate treated with UV/ozone for 10 minutes although the resulting orientation order parameter listed in Table 2 of 0.6519 is not consistent with efficient combing. Efficient combing as described by the use of the orientation function occurs when the resulting orientation order parameter is closest to 1. Compared to the control sample, the sample exposed to UV/ozone for 10 minutes would have sparse areas where

the methyl groups would be replaced by hydroxyl groups as described by Kim et al [2]. Therefore the presence of negatively charged areas would discourage the non specific binding of DNA along its length due to repulsion and therefore less aggregation is observed.

It can be assumed that this trend would continue as the samples are exposed to UV/ozone for longer periods of time, introducing increasing areas of negative charges, resulting in more DNA binding to the surface by the extremities. This can be seen with the sample treated with UV/ozone for 20 minutes (static contact angle: 62.25 degrees) which had more visible aligned DNA molecules than the 10 minute exposed sample. With an orientation order parameter of 0.9008, the 20 minute exposed sample showed more efficient combing than the 10 minute combing but did not have a high density of individual DNA molecules that were within the known range of individual λ-DNA molecules (21.5 μm plus/minus 0.5 μm).

Continuing the trend, the images for the 30, 40 and 50 minute exposed samples showed the highest density of combed DNA molecules and with respective orientation order parameters of 0.9557, 0.9862 and 0.9790. Based on the orientation order parameter, it seemed these surfaces were most suitable for efficient combing. The combing efficiency of the 60 and 70 minute samples was better than the 20 minute sample with orientation order parameters of 0.9702 and 0.9511 but the images showed partially stretched and aggregated long chains of DNA molecules.

It can however be concluded that a more hydrophilic surface is not the best surface for the alignment of individual DNA molecules. This can be seen with the surface exposed to UV/ozone for 80 minutes, which showed increased aggregation and partial alignment of DNA and the trend continued with the highly hydrophilic 90 minute exposed sample. This was also seen by Zhang et al., who concluded that although DNA interaction with the surface increased as the surface hydrophilicity increased, the reduced contact angle decreased the surface tension force thereby resulting in partially stretched DNA molecules [7].

Based on the obtained results, it can be concluded that the most suitable surface for DNA alignment is a surface which is mildly hydrophobic. Nakao et al. observed that DNA interactions with strongly hydrophobic surfaces are weak and highly polar surfaces show strong though non-specific interactions with DNA [21]. Based on alignment quantification on this part of the experiment, a surface with a static contact angle of around 45-50 degrees (samples exposed to UV/ozone for 40, 50 and 60 minutes) is most suitable for the efficient combing of individual DNA molecules. Of these three samples, the sample exposed to UV/ozone for 40 minutes had the most efficient combing although the 50 minute exposed sample had a higher density of combed single DNA molecules on the surface. The orientation order parameter was calculated using 10 randomly selected aligned DNA molecules regardless of their size, therefore if the number of visible individual aligned DNA molecules (within known λ-DNA size) is taken into account; the surface exposed to UV/ozone for 50 minutes would be best suitable for DNA alignment.

CHAPTER 7
CONCLUSIONS

In this study, the surface of PMSSQ thin films was made more hydrophilic with exposure to UV/ozone and interaction of the resulting surface with λ-DNA was evaluated. The surface of PMSSQ was shown to increase in hydrophilicity with an increase in exposure to UV/ozone. Therefore, it was shown that UV/ozone treatment is a simple yet effective method of tailoring specific degrees of hydrophilicity on PMSSQ surfaces. It was also shown that this method of tailoring specific levels of hydrophilicity/hydrophobicity on surfaces creates a suitable surface for the attachment biomolecules such as DNA.

The interaction between hydrophilicity controlled PMSSQ surfaces and fluorescently dyed λ-DNA depends on the degree of surface hydrophilicity. The more hydrophilic surfaces allowed further dispersion of a drop of DNA/dye solution beyond the boundary of the initial drop. The drop was confined to a particular area depending on the hydrophilicity of the surface as quantified by static water contact angles. The control sample with a hydrophobic surface (104 degree static contact angle) had the smallest drop diameter and lowest intensity area on the sample whereas the sample treated with UV/ozone for 90 minutes (22 degrees static contact angle) had a higher drop diameter and intensity area. It was found however, that the intensity of the sample exposed to UV/ozone for 90 minutes had an intensity area similar to samples treated with UV/ozone for 60, 70 and 80 minutes even though the drop diameter was bigger. This proves that the level of interaction between the DNA molecules and the surface is limited by the

ability of the solution to disperse over a greater area, which in turn is limited by the hydrophilicity of the surface. More studies are needed to show the exact morphology of the DNA on the surface of these samples and whether there is non specific binding between individual DNA molecules instead of between the DNA molecules and the surface. The study would help explain why the intensity of the 70, 80 and 90 minute samples is similar even though the drop diameter increases as the surface hydrophilicity increases.

This study also showed that the optimal surface hydrophilicity for DNA adsorption is not optimal for the alignment of individual DNA molecules. The DNA combing phase of this study showed that a surface which is mildly hydrophilic (static contact angle around 40 to 50 degrees is most suitable for efficient alignment of individual DNA molecules. The efficiency of molecular alignment was quantified using an orientation function. The control sample (0 min UV/ozone exposure) showed no visible aligned DNA molecules whereas increasing the surface hydrophilicity (> 50 degrees) resulted in aggregation of DNA molecules.

In conclusion, the method developed by Kim et al. to control the hydrophilicity of PMSSQ thin films creates a suitable surface for interaction with λ-DNA. The optimal surface for DNA adsorption is the very hydrophilic surface resulting from a 70-90 minute exposure to UV/ozone. On the other hand, the optimal surface for efficient DNA alignment is one which is mildly hydrophilic such as that resulting from a 40-50 minute exposure to UV/ozone.

CHAPTER 8
FUTURE STUDY

Optimizing the molecular combing process is a very important study to undertake. In order for this technique to one day be implemented in large scale manufacturing, the exact parameters and conditions for successful DNA alignment need to be studied and optimized. Also as mentioned earlier, more studies need to be done to visually see the interaction between the DNA molecules and UV/ozone treated surfaces. It is important to determine the level of interaction between the DNA and the surface during the adsorption phase of this study. An increase in the interaction and non specific binding of DNA molecules instead of between the DNA molecules and the surface might explain why there is an increase in drop size but no change in intensity values for the highly hydrophilic samples. This study could be done using AFM imaging.

In order to apply the results of this research to a field such as microelectronics, the logical next step would be to couple DNA alignment with patterned surfaces. This would allow the placement of individual aligned DNA molecules on a predetermined nano-patterned area with contrasting hydrophilic/hydrophobic regions. Therefore a study in this area could help determine whether UV/ozone treated patterned surfaces are suitable for use in microelectronics, eventually giving rise to aligned DNA molecules serving as nanowires and as templates for carbon nanotubes. This study would depend on the availability to manufacture a mask with intricate patterns on a nanoscale in order to tailor hydrophilic areas using a UV/ozone dry stripper/cleaner.

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