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Celia Edith Macias

By

Nanoscale Properties of Poly(ethylene terephthalate) Vascular Grafts
ABSTRACT

Vascular grafts are prosthetic tubes that serve as artificial replacements for damaged blood vessels. Poly(ethylene terephthalate), PET, has been successfully used in large diameter grafts; however, small caliber grafts are still a major challenge in biomaterials. Due to surface forces, blood plasma proteins adsorb to the graft, resulting in inflammation, infection, thrombus formation, and ultimately, vessel occlusion. The objective of this project was to characterize and analyze the nanoscale surface properties of vascular grafts, and determine whether differences exist between blood plasma and protein adsorption. Specroscopic techniques were used to characterize the surface of the samples as well as associated differences in biomaterials. The study was performed on PET-coated and knitted heparin-bonded, all PET-based. The results indicate that differences exist in the surface properties of different commercial vascular grafts, as well as between blood plasma and protein adsorption. These differences could have significant implications for the performance of vascular grafts in vivo. Future research will focus on developing strategies to improve the biocompatibility of vascular grafts.
This research project was partially funded by the Undergraduate Research Opportunity Program (Class of 1973 Fund).

Their unconditional love and support for the past 21 years.

I want to thank my precious family for their appreciation to Mr. Joseph Adanto (DrSE). Last, I would like to thank my academic advisors, Prof. August Will and Prof. Bernhard J. Wenzel for believing in me every step of the way.

I would also like to thank the Ortiz Research Group, especially Miao Ye, Luan Li, and the students. I want to thank my mentor, Dr. Monica Rizman, for giving me the opportunity to learn from her. I want to thank my academic advisors, Kristin Domke, Zhe Chen, and Nan Yang. I would like to thank the Ortiz Research Group, especially Miao Ye, Luan Li, and the students. I want to thank my mentor, Dr. Monica Rizman, for giving me the opportunity to learn from her.

I would like to thank Prof. Christine Ortiz for her guidance and support throughout the research process. Very special thanks to my mentor, Dr. Monica Rizman, for giving me the opportunity to learn from her. I want to thank my academic advisors, Kristin Domke, Zhe Chen, and Nan Yang. I would like to thank the Ortiz Research Group, especially Miao Ye, Luan Li, and the students. I want to thank my mentor, Dr. Monica Rizman, for giving me the opportunity to learn from her.
Figure 1.1: Left: Adsorption of blood plasma proteins onto biomaterial surface: undesirable reaction. Right: Proteins do not adsorb to surface of biocompatible materials.

In thrombus formation, eventually resulting in thrombus formation and vessel occlusion [3].

Prostheses (Figure 1.1) upon blood flow exposure, triggering the activation of platelet
implant, it should be avoided in vascular grafts. Blood proteins adsorb to an arterial
adsorption can be desirable for certain applications, such as tissue engineering and bone
prevention of nonspecific, noncovalent surface adsorption of proteins [21]. While protein
a major challenge existing in the field of blood-compatible biomaterials is the

undesired response from the host's immune system [1].

If they are able to perform a specific function in biological conditions without causing an
function in intimate contact with living tissue. Biomaterials are said to be biocompatible
A biomaterial is a synthetic material used to replace part of a living system or to

1.1. BIOMATERIALS AND BIOMICROCOMPATIBILITY

CHAPTER 1

INTRODUCTION AND BACKGROUND
The adsorbed proteins' interactions with each other, and the adsorbed protein's orientation, can provide a foundation for understanding and incorporating protein adsorption at the molecular level. The adsorbed proteins' interactions with each other, and the adsorbed protein's orientation, can provide a foundation for understanding and incorporating protein adsorption at the molecular level. The adsorbed proteins' interactions with each other, and the adsorbed protein's orientation, can provide a foundation for understanding and incorporating protein adsorption at the molecular level.
Mechanical properties to cell surfaces compatible with the human body if they have similar chemistry, morphology and blood loss, and 

1) Abrasion resistant. Presumably; biomaterial surfaces will be more mechanically stable. 4) Prevent graft leakage which can lead to seroma formation and long-term difficulty. 

2) Biocompatibility, 2) Biocompatibility, 2) Biocompatibility, 2) Biocompatibility, 2) Biocompatibility, 2) Biocompatibility, 2) Biocompatibility, 2) Biocompatibility, 2) Biocompatibility, 2) Biocompatibility, 

A vascular graft should meet the following properties: 1) desirable challenge in biomaterials. successfully used in large diameter grafts; however, small caliber grafts are still a major replacement for damaged blood vessels (6). Synthetically, textile vascular grafts have been vascular grafts are prosthetic tubes that serve as artificial arteries; hindering blood flow. This situation leads to surgery in order to replace

The most common cardiovascular disease, atherosclerosis, reduces the caliber of
mechanical properties of the grafts are given in Table 1.1. Nonproprietary information provided by the manufacturer. The dimensions, physical, and cytotoxicod condustious sterilized by gamma irradiation. Following is a description of all the vascular grafts provided are soft, macroscopicallycmped (1GK0006-40H). The vascular grafts provided are soft, macroscopically cmped sample (1GK0006-40H), and (J) Knitted PET (GW0038-30), (K) Knitted collagen-coated PET available 2) woven collagen-coated PET (GW0038-30), (J) Knitted collagen-coated PET also known as PET or Daeron®. A very commonly used material for vascular grafts is polyethylene terphthalamidet sample in a wide one.

1.3. COMMERCIAL SAMPLES

Grafts. Small caliber grafts still show an unsatisfactory high percentage of failure in vivo biomaterials sciences [3] because a clot is more likely to obstruct blood flow in a narrow caliber grafts with successfully protein-resistant surfaces are still a major challenge for drue. While this method yielded satisfactory results in clinical trials, the design of small previen thrombus formation, grafts are sometimes bounded with heparin an anticoagulant inflammation, infection, thrombus formation, and ultimately, vessel reclosure. To Due to surface forces, blood plasma proteins adsor to the graft, resulting in
Types of interwoven grafts were studied: woven and knitted (Figure 1.3).

Two different manufacturers, does not define if integrity of the polyester fibers [7].

The graft's crimp are obtained by several materials, which (according to the

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Table 1.1: Dimensions, physical and mechanical properties of commercial vascular grafts used in this study.
Figure 1.4: LEFT: Distribution of Heparin and Collagen across the graft wall. RIGHT: Graft cross section.

The inside wall of the graft, as seen in Figure 1.4, contains a gradient of heparin concentration, with the highest concentration being near the inside. The heparin-bonded graft in the graft uses the inherent properties of the graft material and is used to prevent the formation of thrombus. Both woven and knitted collagen-coated grafts were used to seal the pores unmodified. The bare PET sample was left unsealed and it is clean and surface chemically. The bare PET sample was left unsealed and it is clean and

In addition to the differences in fiber structure, the graft samples also differ in

...
Currently, PET is the preferred polymer for medium and large caliber vascular grafts, because of the wide variety of types, linear densities, filament counts, filament structure, and surface topology of the polymer fibers [101]. This additive possibly affects the surface topology of the polymer fibers usually contain from 0.03 to 0.4 weight percent of titanium dioxide (used as a delusterant). Finished PET fibers are semi-crystalline (~50% crystallinity). PET fibers are then stretched, aligning the polymer molecules in the direction of their stretch. The resulting fibers are then extruded through small holes at slow speeds to form fibers. The usually, PET is extruded through small holes at slow speeds to form fibers. The

Figure 1.5: Chemical structure of polyethylene terephthalate

[01]

Chemical structure is shown in Figure 1.5. Its glass transition temperature is around 70°C. PET is a semi-crystalline polymer with a melt temperature that is higher than the crystalline melting point of the polymer chain [101]. PET's glassy solid, with amorphous links hindering the mobility of the polymer chain. PET's crystalline region is a clear, translucent material formed by the condensation of ethylene terephthalate units. The result is a clear, transparent material. PET is polymerized from ethylene glycol and terephthalic acid monomers. The

First step is the esterification of the acid with ethylene glycol. This is followed by

polymerization from ethylene glycol and terephthalic acid monomers. The

of the common organic solvents as well as humidity [6]. has a melting temperature of about 265°C and at room temperature it is resistant to most

poly(ethylene terephthalate), PET, is the most common of the polyester.
1.5 COLLAGEN

Collagen is a fibrous protein that occurs in almost all mammalian tissues. The
amino acid sequence of collagen consists of -Gly-Pro-Hyp-Gly- where X could be any
amino acid except for the total charge is zero of around 4.5 [15] and a contour
isoelectric point (pI) at which the total charge is zero. Table 1.2 shows the amino acid content of collagen. Collagen has an
amino acid X at position 14. Figure 1.2 shows collagen microstructures of human and
bovine structures [16].

Length of 309±1 mm [16].

Another major challenge encountered in the early years of vascular graft use was
order to improve their biocompatibility while preserving PET’s bulk characteristics.
Mechanical and physical properties, and chemical stability make it an attractive candidate
and thrombogenic, presumable due to its hydrophobic nature [6] (contact angle ~70
and 13°) however, its ease of needle penetration, handling characteristics, desirable
PET is known to prevent vascular healing and is considered to be immunomodatory
within the manufacturing process [12].

These factors, cross-sectional shapes and textured modifications that can be achieved during
1.6. Thrombosis

Vascular grafts, such as human serum albumin, govern the protein-polymer interactions ultimately resulting in thrombus formation and vessel reclosure. Low molecular vascular grafts, activating platelet adhesion and triggering the coagulation cascade [5], contract to devices such as vascular grafts. Plasma proteins adsorb onto the surface of the blood clot, also known as thrombus, a common problem observed in blood.

Table 1.2. Amino acid content of collagen [14].

<table>
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<th>Residue</th>
<th>Other</th>
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<tr>
<td>8.5-8.9</td>
<td>Basic polar (Lys, Arg, His)</td>
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<tr>
<td>11.5-12.5</td>
<td>Acid polar (Asp, Glu, Asn)</td>
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<tr>
<td>9.4-10.2</td>
<td>Hyp</td>
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<tr>
<td>11.7-13.8</td>
<td>Pro</td>
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<tr>
<td>31.4-33.8</td>
<td>Cys</td>
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mol/100 mol Amino acids
Figure 1.6. Chemical structure of heparin. The chemical structure is shown in Figure 1.6. Heparin is the most biologically relevant member of the family of sulfated glycosaminoglycans (GAGs), which are widespread in animal tissues and in the human body. The chemical structure of heparin is shown in Figure 1.6.

1.7. HEPARIN

Heparin is used extensively as an anticoagulant drug. Heparin, once the surface of the material has been immobilized on an anticoagulant drug, heparin, onto the surface of the material by an approach currently used in biomaterials to inhibit thrombus formation is the surface area, reducing platelet adhesion. However, commercial vascular grafts show a high degree of porosity, increasing the surface area available for protein adhesion and chemistry, charge, and topology [5, 6, 18]. A smooth surface results in a small molecule with higher surface affinities as predicted by the Vroman Effect [4].
Leakage

prevent rapid release of the heparin from the graft surface [7], and also prevent blood

The heparinized graft is also coated with collagen which acts as a barrier to

Figure 1. Chemical Structure of tri-decylammonium chloride (TDMAC) [7]

\[ \text{CH}_3-(\text{CH}_2)_2-\text{N}^+-(\text{CH}_2)_2-\text{CH}_3 \]

TDMAC

Heparin will bind ionically (noncovalently) to the positively charged nitrogen of the

hydrophilic tails [7]. The chemical structure of TDAC is shown in Figure 1.7. Heparin and binds with high affinity to the polyelectrolyte layer surface through its long

tri-decylammonium chloride (TDMAC) which forms an insoluble complex with

Heparin is coupled to the surface of PFA-based commercial vascular grafts using

or anti-thrombin, catalyzing their inhibition of thrombin and other coagulation factors.

congulation cascade [12]. It achieves this by binding to either plasma heparin cofactor II

anticoagulant properties by increasing the activity of anti-thrombin III (an inhibitor of the

Heparin is a negatively charged polysaccharide that possesses antithrombotic and
electron beam hits the sample, secondary electrons are released from its surface. As which focus the electrons. The focused beam of electrons scans across the sample. As the energy electrons, which travels toward the sample through a series of magnetic lenses placed inside an air-liquid chamber. Under vacuum, an electron gun emits a beam of high-energy electrons. Scanning Electron Microscopy (SEM) samples are then analyzed using the Scanning Electron Microscope (SEM).

2.1.1. Scanning Electron Microscopy

Surface properties studies will reveal the dependence of protein adhesion on biomaterial surface topography studies will reveal the dependence of protein adhesion on biomaterials. Through understanding of surface topography, complemented by high resolution force topography. Smooth surfaces exhibit small surface area, reducing particle adhesion. A flatter surface will result in lower surface energy is partially determined by surface area; texture, and response caused by the implantation of a synthetic biomaterial in the human body. Surface morphology plays a very important role in the immunological reaction.
of the cantilever. The computer then analyzes the data and converts it to an image.

deflection is measured by the photodetector by means of a laser diode reflected off the back
grooves, causing it to deflect as the sample surface is scanned. The cantilever
positions the sample with Au film accurately. The cantilever probe tip senses surface
properties and performs data acquisition, display, and analysis. The piezoelectric scanner
system and probe tip a photodetector detector and a computer (Figure 2.1). The computer controls the
imaging field. The AFM system consists mainly of a piezoelectric scanner, a cantilever
holder, and a computer (Veeco, Veeco Metrology).

2.1.2 Atomic Force Microscopy

sealed container prior to and immediately after imaging
the chips to the sample. The samples were then coated with gold and stored in an argon,
down. Colloidal graphite (Ted Pella, #16052) was used to bridge the conductivity from
features two spring clips to hold the sample down without the need to glue the sample
clean scissors. The pieces were then placed on a sample holder. The sample holder used
samples were prepared by cutting 1cm pieces from the different fabrics using
count of electrons emitted from the sample.
detector counts these electrons and emits a signal. SEM images are produced from the
to determine the effect of sample preparation. All images were taken in air under contact AFM imaging of the vascular graft samples were taken under different conditions stored in an airtight, sealed container prior to and immediately after imaging. To release the wrinkles in order to allow for surface engagement, the samples were glued to a magnesium sample holder using a thin layer of adhesive (much thinner than the clean scissors). The pieces were either mounted on a specially designed sample holder or samples were prepared by cutting 1 cm² pieces from the different grafts using.

Figure 2.1. Atomic Force Microscope Scheme:
vascular graft sample were measured and analyzed. The cantilever probe tip and the forces between the tip and the sample were measured in force versus distance curves. Blood proteins were then analyzed using the reflection of the cantilever probe tip. The computer while the photoacoustic measures the reflection of the cantilever probe tip. The tip follows (approaches) and away from the sample, (returns) at a constant rate, probe tip a photoacoustic scanner, a photoacoustic and a cantilever. The photoacoustic moves a cantilever tip by means of a system comprised of a laser diode, a cantilever displacement displacement \( \pm 3 \, \mu \text{m} \) by means of a system comprised of a laser diode, a cantilever force. In a manner similar to the AFM, the MFP measures force between a probe tip and a surface (limits of detection: force \( \pm 5 \, \text{pN} \)). Henceforth referred to as the MFP. In a manner similar to the AFM, the MFP measures force between a probe tip and a surface (limits of detection: force \( \pm 5 \, \text{pN} \)).

The molecular interactions with blood proteins will be studied using a single axis

2.2.1 Molecular Force Probe

The molecular interactions with blood proteins will be studied using a single axis

2.2 Protein-Grain Interactions
The cantilever length = 320 µm, resonant frequency = 850 Hz.

Force versus distance curves were measured at room temperature using a

response to intermolecular interactions with the surface.

Figure 2.3. LEFT: Typical HFRS force versus distance curve. RIGHT: Detection of a cantilever in

Figure 2.2. Molecular Force Probe Schematic
weight of HSA is 66.436 g/mol (calculated from the molecular mass of the 565 constituent 18 phenolic –OH, 60 amino, 16 imidazolyl, 24 guanidyl) [22]. The absolute molecular HS A is a single-stranded polypeptide. Its ionizable groups include 98 carboxyl.

interactions between HSA and the erythromycin

combined in one of the samples studied. The results will then show only the nonspecific

chosen over erythromycin (which is known to bind specifically to heparin, a drug

adsorbed to a blood-contacting implantable biomaterial) [4]. Due to this property, HSA was

contaminant in blood plasma [22]. Because of its low molecular weight, it is one of the first to

and most abundant plasma protein in the human body, accounting for 55% of the protein

Human serum albumin is a highly water-soluble plasma protein. It is the smallest

(HSA) was chosen.

simply nonspecific protein adsorption studies, a model protein, human serum albumin

Blood plasma contains thousands of different types of proteins. In order to

2.2.1.4. TIP FUNCTIONALIZATION

Figure 2.4. Microfabricated cantilever probe used in AFM experiments
detected (DI) water. The tips were then incubated for 60 seconds in a 0.01% (v/v) solution of gluteraldehyde for 30 minutes and then rinsed with copious amounts of 0.1 M PBS (phosphate buffer solution) before being immersed in a 2.5% (v/v) aqueous solution of ABDDMS for 2 hours and then rinsed in methanol followed by 10 W UV power immediately preceding modification. They were then immersed in a 4% Slots and probe tips were cleaned and oxidized in oxygen plasma for 10 seconds at 30 Pa and 8552, 102.1% (TA-9300), and HSA (7A9111, 102.1#1269322) were purchased from Sigma.

Aminoethyl(dimethyloctyl)silane (ABDDMS. #SS65320), gluteraldehyde (#G).

Figure 2.5. Chemical reaction scheme for the covalent immobilisation of HSA to a silicon nitride cantilever.

The square pyramidal probe tip found at the end of the cantilevers was chemically modified with HSA using the chemical reaction scheme [23] shown in Figure 2.5.

The square pyramidal probe tip found at the end of the cantilevers was chemically modified with HSA using the chemical reaction scheme [23] shown in Figure 2.5.

The square pyramidal probe tip found at the end of the cantilevers was chemically modified with HSA using the chemical reaction scheme [23] shown in Figure 2.5.
High resolution force spectroscopy experiments were performed on retinal non-adhesive events (point). Figure 2.7 shows typical force versus distance curves for the different events observed in and distances of adhesion under each of the experimental conditions was performed. Inherent in the non-adhesive adhesion, instead, statistical analysis of the maximum forces the data was not averaged because of the large adhesion force and distance distributions surface were averaged and the standard deviations were calculated. For retinal curves, force versus distance curves on approach from three different sites on the sample were measured using the MFP.

Following the experimental setup described in Figure 2.6, the retinal molecules of the protein were measured using the MFP, as a function of the separation distance between HSA and the different retinal molecules. For some flexibility and retention of the native movements of the protein, globularly shaped molecules provide a short linker for the HSA of the probe tip, allowing solution in 0.01M PBS, then rinsed with and stored in 0.01M PBS. The ABDMs and
Significance of the statistical analysis was calculated using student's t test which was statistically different with p<0.05.

Figure 2.7 Typical protein-surface interaction profiles. TOP: Schematic of protein extension. BOTTOM: Typical non-adhesive force vs. distance curve exhibiting surface adhesion. LEFT: Typical non-adhesive force vs. distance curve. BOTTOM CENTER: Typical force vs. distance curve exhibiting protein extension. BOTTOM: Force vs. distance curve exhibiting protein extension. Figure 2.8 Typical protein-surface interaction profiles. TOP: Schematic of protein extension. BOTTOM: Typical non-adhesive force vs. distance curve exhibiting surface adhesion. LEFT: Typical non-adhesive force vs. distance curve exhibiting protein extension. Figure 2.9 Typical protein-surface interaction profiles. TOP: Schematic of protein extension. BOTTOM: Typical non-adhesive force vs. distance curve exhibiting surface adhesion. LEFT: Typical non-adhesive force vs. distance curve exhibiting protein extension.
sample used above. The left image agrees with the reasonable PET fiber diameter [18] of Image 3.2, both left and right, shows SEM images of fibers found in the same

![SEM images of PET vascular graft, inside wall (woven, collagen-coated)](image)

Image 3.1. SEM images of PET vascular graft, inside wall (woven, collagen-coated).

\[\text{(due to sample damage caused by applied voltage)}\]

average diameter = 620 ± 493 µm \(n=10\) are believed to be the degrading collagen film 105 µm \(n=6\). It should be noted that the bubble-like features located between crimps average fiber bundle width is 250 ± 38 µm \(n=15\), and the average crimp width is 1180 ± 55 µm. The shows the inside (blood-contacting) wall of the woven collagen-coated sample. The revealed the surface morphology described from the schematics in Figure 1.3. Image 3.1.

3.1. Scanning Electron Microscopy

3.1. SURFACE ANALYSIS

RESULTS AND DISCUSSION

CHAPTER 3
Image 3.3: SEM image of PET fibers covered with collagen film (Woven, collagen-coated vascular graft).

Hence the damage on the uniformity of the collagen film.

Bulk sample and close to the edge where the sample was cut from the rest of the felt, deposited on the non-circular PET fibers. This image was taken on a slide away from the film bundle (non-circular diameters).

Image 3.2: LEFT: SEM image of single PET fiber (~20 µm diameter). RIGHT: SEM image of PET fiber bundle (see section 1.7).

...could potentially be undesirable, as it does not possess the smoothness of a circular caused by the stretching of the fiber bundles after the extrusion process. This feature 55 fibers. The image shows the non-circular geometry of the PET fibers, probably 20 ± 5 µm (n=10). The right image shows a fiber bundle. This fiber bundle consists of
oversetched, therefore it shrunk, due to the damage in the sample.

Collection of fibers pulled out of the edge of the graft. The collagen film has been

diameter [18]. The image on the right was not taken from a bulk sample, but from a

with a 1.5 mm diameter, once again, consistent with reasonable expected PET fiber

Image 3.5. Left and right show individual fibers. The left image reveals a fiber

Image 3.4. SEM image of PET vascular graft inside wall (Knitted)

260 ± 17 mm (n=4), and the chimp width is about 920 ± 180 mm (n=3).

the inside wall of a knitted PET vascular graft. The diameter of a fiber bundle is around

samples should show similar characteristics. Image 3.4 shows the surface morphology of

achieved by the SEM used here, only one knitted sample was studied assuming both

were taken. Since it would be impossible to distinguish heparin at the magnifications

After careful examination of the woven sample, images of the knitted sample
collagen section near the external wall of the graft

Image 3.6. SEM image of PET Vascular Graft (Knitless, Heparin bonded) cross-section, showing denser

visible layer of collagen film is observed closer to the red line in the bottom left of the
added to the top right of the image denotes the interior wall of the graft. A denser, more
Image 3.6 shows a cross-section of the graft wall, with a thickness of about 0.50

Image 3.5. SEM images of PET fibers used for knitted vascular graft.
holder, however, the glass surfaces did not stay attached to the holder using this technique. To use a thin, commercial layer of adhesive to glue the sample onto the magnetic grafts had to be prevented in order to allow for proper lip-surface engagement. Usually practice was to image the samples using the AFM in the contact mode.

All AFM images were taken in an air under contact mode. Of the sample preparation, and scan angle. All AFM images were taken in air under different conditions to determine the effect of the sample preparation procedure (that with the least damage in the sample). For the MIF experiments to surface topology of the grafts, but they also revealed the optimal sample preparation (which could potentially lead to contamination). The images not only revealed the AFM images of the vascular graft samples were taken to identify smaller features with better resolution than SEM, while eliminating the need to coat the surface with gold.

3.1.2. ATOMIC FORCE MICROSCOPY

Image: 3.7. SEM images of PET vascular graft, external wall (kinked) contact imaging. Blood flow (yellow) shown in Image 3.7. there is a dramatic difference between the clearly defined topology of the interior and wall shown in the previous images and the less defined fibers on the exterior (not well shown in the previous images).
Previously studied samples. The surface morphology of this fiber is similar to that of the samples were left under the weight. Image 3.9 shows an image taken from a sample of the samples; therefore a series of images were compared over a range of times in which the weight to damage the surface. This method could potentially damage the surface of the weight than the initial weight thickness. The glued sample was left to dry under a 5 kg weight to avoid surface contamination with cement. The adhesive layer was considerably thicker than the initial weight thickness. The glued sample was left to dry under a 5 kg weight to avoid surface contamination with cement. The adhesive layer was considerably thicker than the initial weight thickness.

After thorough analysis of the unglued sample, images were taken from samples imaged is not smooth at the nanoscale, featuring an RMS roughness of 1.47 nm. Image 3.1 shows the surface topology of the fibers at 5 and 10 nm scans. The surface of the fibers shows the surface topology of the fibers at 5 and 10 nm scans. The surface of the fibers shows the surface topology of the fibers at 5 and 10 nm scans. The surface of the fibers shows the surface topology of the fibers at 5 and 10 nm scans.

**Figure 3.1**. Especially designed AFM sample holder

![Figure 3.1](image)

was designed to image a sample held down by two spinning clips. Therefore, a special sample holder (Figure 3.1) involves gluing the sample to the holder. Presumably, the least damaging mounting technique would be one that does not involve gluing the sample to the holder.
The image on the top shows an apparent flattening of the surface; however, an image 3.10 shows the surface of a fiber after being under the 5kg weight for one minute. Sample was blu ed and left under 5 kg weight for 5 minutes.

Image 3.9 AFM images of PET fiber surface. Sample was mounted on spring chip sample holder (not shown).
Image 3.10. AFM images of PET fiber surface. Sample was glued and let under 3 kg weight for 60 minutes.

Below used be.

The fiber diameter in the direction normal to the longitudinal direction of the fiber must be used for contact with the surface during the scan. Therefore a scan smaller than 20 μm (about 0.15 μm, Image 3.10) This image revealed a surface practically similar to the previous fibers in the sample. A different fiber on that sample was imaged over a larger scan area. In order to understand if this change in surface morphology was universal to the different fibers having different degrees of roughness, RMS roughness of top image is 1.45 μm, which actually turns out to be more akin to the previous images than it visually appears.
5 kg weight for one hour!

Image 3.12 AFM image of vascular graft (Kinetically collagen-coated) after 2 hours. Sample was fixed and left under 5 kg weight for 24 hours.

RMS Roughness is 1.94 μm.

Difference in surface morphology for the collagen-coated PET caused by the collagen performed i.e., therefore, only the collagen-coated layers were imaged. Image 3.12 shows the Surface-immobilized hepatin is not distinguishable at the scale of the scans.

Image 3.11 AFM image of PET after surfact. Sample was fixed and left under 5 kg weight for 24 hours.

RMS Roughness is 1.32 μm. This series of previously obtained images. RMS Roughness of this sample is 1.32 μm. This series of weight applied, the surface morphology shown in Image 3.11 is comparable to the previous sample was imaged after being compressed for one day under the 5 kg weight.

One last sample was imaged after being compressed for one day under the 5 kg weight.
a control.

The experiments reported in this section included a cantilever probe tip experiments were used as
stronghold studies (as described in Section 2.2). All MFP experiments, except the ionic
a sample holder (as described in Section 2.2), except the ionic
cantilever length = 320 μm, resonance frequency = 850 Hz) against ionic samples glued to
thermometers coated with chitosan acetate-β-glucose-Si3N4 cantilever probe tip (κ= 0.01 N/m).
Force versus distance curves were measured at room temperature using a

3.2.1. ON APPROACH

Probe tip and the flat surface is increased.
the forces and distances of adhesion observed as the distance between the cantilever
pulled away from the surface of the flat. The forces curves provide information about
ip and the surface decreased. Retract data is collected as the cantilever probe tip is
and repulsive forces experienced by the cantilever probe tip as the distance between the
near the surface of the flat. This data collection, when averaged, reveals the attractive
and retract data. Approach data is then collected as the cantilever probe tip is brought
The results for the MFP experiments are divided into two components: approach

3.2.1. MOLECULAR FORCE PROBE

3.2. PROTEIN-GRAB INTERACTIONS
different types of plate.

Figure 3.2: Average force vs. distance curves for an unfunctionalized SiN$_4$ cantilever probe tip against the
samples.

Of all the samples, with a repulsion starting around 25 nm, similar to the collagen-coated
and surface charge. The bare polyester sample experiences the least amount of repulsion
each other. Again, this is expected since both samples have a similar surface chemistry
interacting with the probe tip. Both collagen-coated samples show a behavior similar to
80 nm) repulsion than the rest of the curves, due to the presence of surface charges.

Figure 3.2 shows the average force versus distance curves for the experiments
Figure 3. Force vs. distance curves under varied ionic strengths.

Interactions as a function of environmental conditions. Results prove the validity of the MEP experiments by exhibiting a change in the force versus distance curves, 0.01 M PBS and 0.1 M PBS show a long range repulsion starting force vs a function of ionic strength, and shape of the curve.

Figure 3 shows the effect of ionic strength on the magnitude and shape of the interaction. Over 3 different orders of magnitude. To do this, the ionic strength of the PBS solution was varied experiments with varying environmental conditions, excepting to see an effect on the behavior of the interaction. To do this, the ionic strength of the PBS solution was varied. The second approach was to run a series of probes hip is much shorter than the surface. The second approach was to choose a cantilever with a low spring constant, so that the cantilever surface forces and not nanotip curvature the sample. Two approaches were taken. The first

In order to prove that the experiments being performed were actually measuring...
Figure 3.4: Average force versus distance curves between HSA-functionalized ip and different vascular.

Graph sample:

Figure 3.4 shows that, indeed, PET experiences a
stronger protein interaction [4]. The lower two (collagen-coated and heparin) are knitted, unmodified PET was
expected to show the least repulsive character due to its high hydrophobic nature.

PET grafts. The other two (collagen-coated and heparin) are knitted. Unmodified PET was
should be noted that the bare PET graft is woven, as well as one of the collagen-coated
approach and 2) surface microtopography plays a more significant role in these interactions. If
experiments: 1) surface chemistry determines the magnitude of the forces experienced on
(HSA) as explained in Section 2.3.1. Two hypotheses were proposed before starting the
The collagen probe ip was then functionalized with Human Serum Albumin.
Percentage of surface adhesion, with the majority of interactions being based on pulling.

It should be noted that the heparin sample showed a significantly lower

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Table 3.1. Frequencies of interactions between HSA/Et-gold capped probe tips and different protein samples

section 3.2.1. Table 3.1 shows the percentage of occurrence for each entry observed: (1) surface adhesion, (2) pulling (3) surface adhesion followed by pulling (See forces and distance observed were measured. Three different types of interactions were after the tip was brought in contact with the surfaces, it was retracted and the

3.2.1. ON RETRACT

surface, since HSA was actually interacting with only collagen in all three cases.

Therefore, heparin should not affect the force experiment between the probe tip and the

As explained in Section 1.7, heparin binds specifically to antithrombin, not HSA. However, these results could also be explained by the specific nature of heparin bridging inhibited because of degradation and/or blocking of binding sites from proteins [24].

Interestingly, the surface-modified PTFE samples all exhibited a very similar behavior on approach. A possible answer could be that heparin was included during the surface attachment process. According to the literature, heparin bioactivity may be lost or

All of the samples exhibit a long range repulsion starting at around 50 nm.
By the hepatic negatively-charged nature.

This could be due to a potential change in the HSA adhesion mechanisms brought about...
Similar behavior for the different events. The magnitude of the force on surface morphology of the graph. The rest of the graphs show a correspondence in the kinked samples. These results clearly show the dependence of the compared using student's t-test. It is important to note that the outlier averages extension (pulling). The numbers are considered extremely statistically different at <P value > of The most prominent feature of Figure 3.6 is found in the graph for C. The peak force vs. distance was analyzed individually, measuring the distance and

The peak distance is corrected by 0.4548 nm. Figure 3.5 shows the distribution of peaks in AFM force/distance experiments.
Figure 3.6 Average forces and distance of adhesion and pulling (extension) for HSA-gelled protein lip.
attaching interactions; however, these properties are almost unsuccessful when it comes to Heparin's antithrombotic properties have been shown to affect specific

presumably an intrinsic to their nano- and microscopic features.

SEM and AFM images displayed the characteristic topographies of the samples

the surface morphologies and their respective interactions with human serum albumin.

analysis of the graft's physical properties provided the basis to draw conclusions relating

to measure the forces between these surfaces and blood plasma proteins. A thorough

specroscopy techniques were used to characterise the surface of the samples as well as

Scanning Electron Microscopy, Atomic Force Microscopy and High Resolution Force

to ascertain differences in bioocompatibility due to surface coating and morphology.

properties of PTFE-based commercial vascular grafts. The study was performed in order

The objective of this project was to characterise and analyze the nanoscale surface

experienced by vascular grafts.

in an effort to elucidate the molecular origins of the biological interactions.

combine standard characterisation techniques with new advancements in nanotechnology

surface has not been achieved. Consequently, the goal of this thesis project was to

performed for many years, still, a positively bioocompatible synthetic vascular graft

have been damanged. A variety of macroscopic in vivo and in vitro studies have been

biomaterials science. Every year, vascular grafts save the lives of patients whose arteries

Vascular grafts are an important field of study both in clinical medicine and in

CONCLUSIONS AND RECOMMENDATIONS

CHAPTER 4
is certainly an important factor determining the mechanisms of protein adsorption
human body. However, surface morphology and chemical modification of the PET fibers
chemistry and morphology do not necessarily dictate the fate of the biocatalyst in the
surface adhesion (adsorption of low molecular weight molecules, e.g., HSA) surface
The results here presented have the potential to prove that during the first stage of
surface chemistry
Knitted samples were significantly different to those of woven samples, regardless of
played an important role on these interactions. The forces of protein pulling of the
heparin-bonded graft was characterized by protein pulling. Surface morphology also
surface. While the collagen-coated samples exhibited mainly surface adhesion, the
surface. The bound heparin seemed to change the attachment mechanism of HSA on the
A very different situation was observed once the protein was in contact with the


morphology and chemistry
pharmaceutical or the relationships of these interactions with the biomaterial surface
not representative of either the interactions observed between other plasma proteins and
interactions between proteins and surfaces on approach. Nevertheless, this behavior is
nature of heparin binding to anilinophenol have little or no effect on the nonspecific
exhibit a very similar behavior on approach. Proving that HSA was actually interacting
The forces observed between HSA and all the surface-modified PET samples
during the first stage of the coagulation cascade.
non-specific interactions, which are in fact, important governing the activation of platelets
Biosocompatibility:

Alternative approach to pursue work in the design of vascular graft surfaces with better plasma proteins is needed. The results presented here provide an insight into an understanding of the nanoscale interactions between the biomaterial surface and blood.

In order to engineer the next generation of synthetic vascular grafts, a thorough range from a flat to an extremely rough surface.

Interestingly to also study the interactions between these proteins and a set of surfaces experiments performed using albumin instead of human serum albumin. It would be similar studies include a comparison between these results and those obtained by similar.

A next set of experiments is recommended to support these results. The studies
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