Electrostatic Self-Assembly of Biocompatible Thin Films

by

Weiwei Du

Thesis submitted to the Faculty of
Virginia Polytechnic Institute and State University
In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE
in
Materials Science and Engineering

APPROVED:

Richard O. Claus, Chairman
Guo Quan Lu
Robert W. Hendricks

June 9, 2000
Blacksburg, Virginia

Key words: biocompatibility, electrostatic self-assembly (ESA), multilayer thin films, protein adsorption

Electrostatic Self-Assembly of Biocompatible Thin Films

by
Weiwei Du
Dr. Richard O. Claus, Chairman
Materials Science & Engineering

Abstract

The design of biocompatible synthetic surfaces is an important issue for medical applications. Surface modification techniques provide good approaches to control the interactions between living systems and implanted materials by modifying the surface characteristics.

This thesis work demonstrates the feasibility and effectiveness of the novel and low-cost electrostatic self-assembly (ESA) technique for the manufacturing of biocompatible thin film coatings. The ESA process is based on the alternating adsorption of molecular layers of oppositely charged polymers/nanoparticles, and can be applied in the fabrication of well-organized multilayer thin films possessing various biocompatible properties. ESA multilayer assemblies incorporating various biomaterials including metal oxides and polymers were fabricated, the uniformity, thickness, layer-by-layer linearity, and surface morphology of the films were characterized by UV/vis spectroscopy, ellipsometry, and AFM imaging.

Preliminary biocompatibility testing was conducted, concentrating on contact angle surface characterization and the *in vitro* measurements of protein adsorption. The use of Fourier Transform Infrared Reflection-Absorption Spectroscopy (FT-IRAS) for the investigation of the protein adsorption behavior upon the ESA multilayer films is presented.

Acknowledgements

First of all, I wish to thank Dr. Richard O. Claus, my supervisor, whose kindness and encouragement has made my research at the Fiber & Electro-Optical Research Center (FEORC) such a fun and rewarding experience. It was he who introduced to me the Electrostatic Self-Assembly (ESA) method. I owe him a great deal for his constructive suggestions and ideas. His flexible supervising style also allowed me to pursue a number of interesting projects, which proves to be quite beneficial to me.

I would also like to thank Dr. Youxiong Wang who has been working with me in this project. His expertise in the area of chemistry has helped me greatly. My thanks also go to the two readers of my thesis: Prof. G. Q. Lu and Prof. Robert W. Hendricks. I appreciate the time and effort they have put in. I wish to thank all the researchers and students at FEORC, Dr. Yanjing Liu, Dr. Yongqiang Wang, Dr. Kristie Lenahan, Fajian Zhang, Zhaoju Luo, and others, for the friendly and helpful discussions they have had with me, and Linda Jones and Ann, for their cheerful attitude and encouragements.

I also wish to thank my husband Hao for his love, for always being there with me through all the ups and downs.

Finally, I gratefully acknowledge the financial support provided by the OSER at Virginia Tech and by Dr. Claus.

Table of Contents

Chapter 1

Introduction 1

1.1 Biocompatibility 1

1.1.1 The concept of biocompatibility 1

1.1.2 Protein-surface interaction and biocompatibility 3

1.1.3 Factors that influence biocompatibility and possible improvements 4

1.2 Electrostatic self-assembly (ESA) method 7

1.2.1 Development of ESA process 7

1.2.2 ESA process description 7

1.2.3 The potential of ESA processing in biomaterials field 9

1.3 Motivation and objectives for this work 10

1.4 Thesis outline 11

1.5 References 12

Chapter 2

Fabrication and Characterization of ESA Thin Films 14

2.1 Fabrication process of ESA thin films 15

2.1.1 Materials 15

2.1.2 Substrate modification procedures 19

2.1.3 Multilayer dipping procedures 20

2.2 Characterization of ESA thin films 23

2.2.1 Tools for analysis 23

2.2.2 UV-vis absorption spectra of ESA thin films 25

2.2.3. Measuring thickness of ESA thin films 28

2.2.4 AFM images of the ESA multilayer thin films 30

2.3 Summary 32

2.4 References 33

Chapter 3

Biocompatibility Tests ***35***

3.1 Contact angle surface characterization 36

3.1.1 Contact angle and surface wettability 36

3.1.2 Contact angle measuring method 37

3.1.3 Results from ESA multilayer thin films 37

3.2 In vitro measurements of protein adsorption 38

3.2.1 Tool for the investigation of protein adsorption: FT-IRAS 39

3.2.2 Protein adsorption procedure on ESA multilayer films 41

3.2.3 Infrared spectra of adsorbed protein layer 43

3.3 Summary 48

3.4 References 49

Chapter 4

Conclusions and Future Work ***51***

List of Tables and Figures

Chapter 1

Figure 1.1. Two major biocompatibilities. 2
Figure 1.2. Interactions between living system and biomaterial. 2
Figure 1.3. Schematic illustration of cell adhesion to protein-adsorbed surface. 3
Figure 1.4. The approaches to achieve good biocompatibility. 4
Figure 1.5. Schematic for the buildup of multiplayer assemblies. 8

Chapter 2

Table 2.1. Biocompatible materials for use in the body 16
Table 2.2. Inorganic biomaterials used in ESA processing. 16
Figure 2.1. Molecular structure of poly(vinylpyrrolidone) (PVP) 17
Figure 2.2. Molecular structure of poly(methacrylic acid) (PMA). 17
Figure 2.3. Chemical structure of PDDA. 18
Figure 2.4. Chemical formula of PEI. 18
Figure 2.5. Synthesis of polyhydroxylated fullerene. 19
Table 2.3. Substrate materials. 19
Table 2.4. Deposition conditions. 20
Figure 2.6. Schematic for the layer-by-layer adsorption process 22
Figure 2.7. Principle of the ellipsometry. 24
Figure 2.8. UV/vis absorption spectra of PVP/PMA multilayer thin films. 25
Figure 2.9. UV/vis absorption spectra of PDDA/C60 multilayer thin films. 26
Figure 2.10. UV/vis absorption spectra of Al2O3/PMA multilayer thin films. 27
Figure 2.11. UV/vis absorption spectra of TiO2/PMA multilayer thin films. 28
Figure 2.12. Thickness growth of PVP/PMA, PEI/PMA, and PDDA/C60 films 29
Figure 2.13. Thickness growth of ZrO2/PMA, Al2O3/PMA, and TiO2/PMA films 29
Table 2.5. Thickness of multilayer assemblies determined from ellipsometry. 30
Figure 2.14. AFM image of 30 bilayer PVP/PMA multilayer thin films 31
Figure 2.15. AFM image of 30 bilayer ZrO2/PMA multilayer thin films 32

Chapter 3

Figure 3.1. Contact angle and the surface tension components. 36
Table 3.1. Contact angles of ESA multilayer films. 38
Figure 3.2. Infrared radiation and molecules on a metal surface. 41
Figure 3.3. The amide band intensity of protein layers from different ESA thin films. 43
Table 3.2. The amide frequency for bovine serum albumin on various ESA thin film surfaces. . 44
Figure 3.4. The infrared spectra of albumin adsorbing onto PVP/PMA with time. 45
Figure 3.5. The infrared spectra of albumin adsorbing onto PDDA/C60 with time. 45
Figure 3.6. The infrared spectra of albumin adsorbing onto PEI/PMA with time. 46
Figure 3.7. Plot of amide band area vs. time for PVP/PMA film surface 46
Figure 3.8. Plot of amide band area vs. time for PDDA/C60 film surface. 47
Figure 3.9. Plot of amide band area vs. time for PEI/PMA film surface. 47

CHAPTER 1
Introduction

The use of biomaterials to interface with living systems, such as fluids, cells, and tissues of the body, has played an increasingly important role in modern day medical applications. In particular, synthetic and natural polymers, metals, ceramics, composites, and tissue-derived materials have been applied in medicine and pharmaceutics [1]. These materials can be used in the permanent replacement of defective organs and tissues, temporary support of defective or normal organs, storage and purification of blood, and also control of drug delivery. The minimum requirements for biomaterials are: non-toxicity, functionality, sterilizability, and *biocompatibility*. Among these requirements, biocompatibility will be the central issue studied in this thesis.

1.1 Biocompatibility

1.1.1 The concept of biocompatibility

Biocompatibility remains the central theme for biomaterial applications in medicine. It is generally accepted that this term means not only absence of cytotoxic effect but also positive effects in the sense of biofunctionality, i.e. promotion of biological processes which further the intended aim of the application of a biomaterial [2]. In other word, a biocompatible material satisfies “biosafety”, which involves the exclusion of severe deleterious effects of the biomaterial

on the organism, and further, it has the ability to perform with an appropriate host response in a specific application.

Basically, biocompatibility can be divided into two groups [1], as presented in Figure 1.1. Mechanical compatibility is sometimes referred to as bulk compatibility, whereas interfacial compatibility often as biological compatibility.

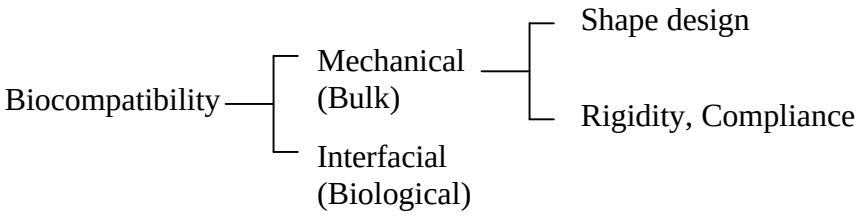


Figure 1.1. Two major biocompatibilities.

In this thesis, we use biocompatibility to refer to *interfacial biocompatibility* [1], which is more concerned with the physical and chemical interactions between the foreign material and the living body when they are brought into contact (Figure 1.2). When such a contact is made, the biomaterial may undergo detectable alteration in surface and bulk structure resulting in hydrolysis, deterioration, and fatigue, whereas the living body responds against the foreign material by invoking an immune reaction. The interfacially biocompatible materials, therefore, can be described as those in which the adverse biological responses occur at much reduced rates or in which the required tissue adhesion is promoted, depending on the objective of the biomaterial and whether tissue adhesion is desirable or not.

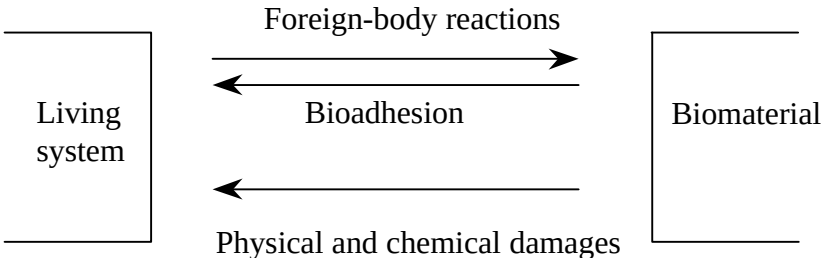
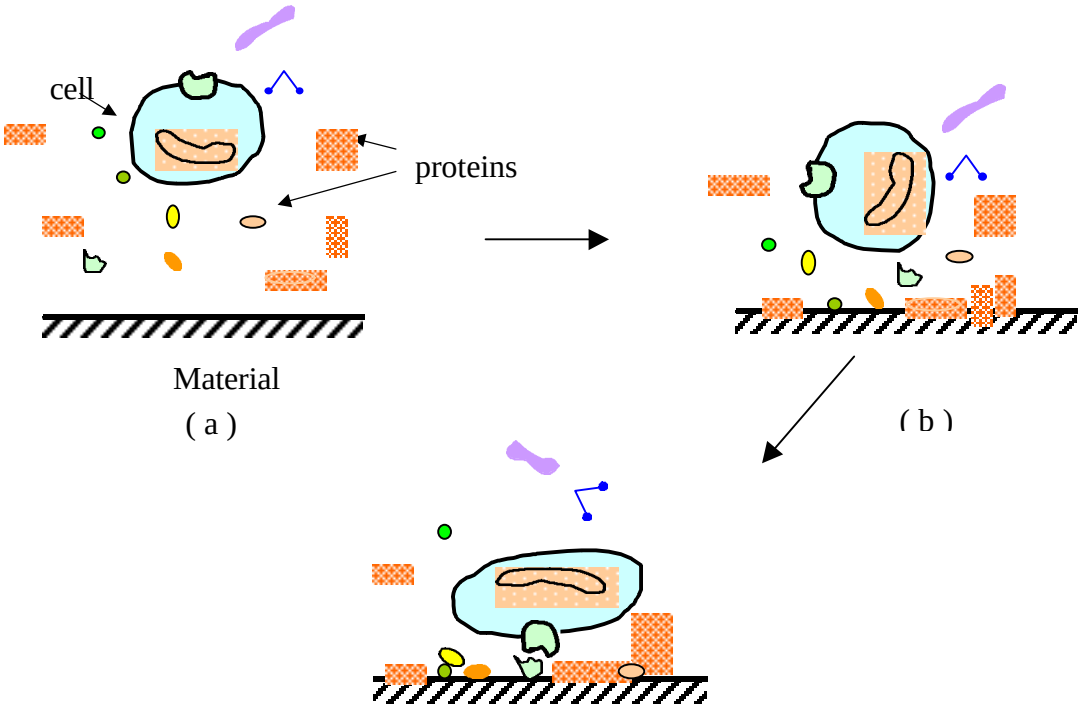


Figure 1.2. Interactions between living system and biomaterial.

1.1.2 Protein-surface interaction and biocompatibility

Biological recognition mediates cell and tissue interactions with biomaterials, whether the materials are employed in a bio-technical environment *in vitro* or a biomedical environment *in vivo*. These interactions are typically mediated by proteins that have adsorbed to the biomaterial surface [3, 18] (Figure 1.3).



(a) Contact of foreign material and body fluids. (b) Start of protein adsorption.
(c) Cell adhesion through receptor-ligand interaction.

Figure 1.3. Schematic illustration of cell adhesion to protein-adsorbed surface.

Protein adsorption is known to be the very first stage of the interactions between the foreign surface and the body tissue or fluids. Biocompatibility of materials is determined to a high degree by the characteristics of the formation of the irreversibly adsorbed protein layer during the initial stages of foreign-body contact, namely rate of the formation, thickness, and conformation of the adsorbed proteins [4]. Because proteins are dissolved in all body fluids and are present in most culture media, and they are a primary source of information for biological

recognition, protein adsorption to biomaterial surfaces is an important issue and one of the most widely used measuring sticks in biocompatibility analysis.

1.1.3 Factors that influence biocompatibility and possible improvements

i) How can biomaterials perform better

There are several ideas for achieving a high degree of biocompatibility:

- Make the material look like it belongs to the entire biological system (Figure 1.4a).

  The biomaterial surface is non-stimulative, and interactions between the foreign material and the living body are decreased to the minimum.

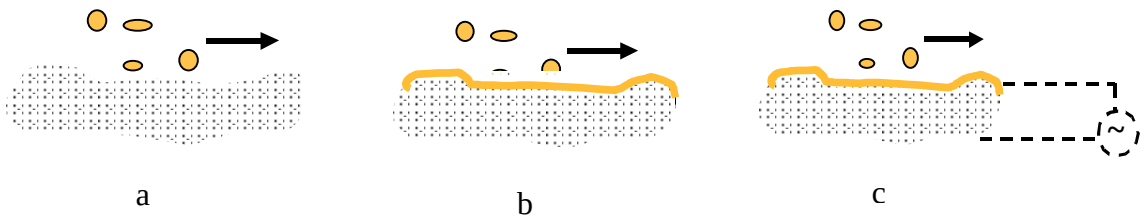


Figure 1.4. The approaches to achieve good biocompatibility.

- Make the material an attractive substrate for a biological layer to grow on (Figure 1.4b).

  In the first stage of foreign-body interactions, the proteins can be adsorbed exclusively and permanently, and no adverse reaction will be evoked by the host biological system, as the foreign surface will be covered with a layer of abundant proteins.

- Make the material conducting and apply an appropriate AC electric field (Figure 1.4c).

  The charge density and distribution on the foreign surface will be changed through the external AC electric field, in order to achieve the optimal foreign-body interactions.

Now it is well accepted that the biocompatibility of implant materials depends mainly on surface characteristics such as wettability (hydrophilicity/hydrophobicity or surface free energy), chemical composition, surface charge, charge distribution, and surface texture (i.e., roughness

and porosity) [19, 20]. This means that the surface structure of the material is regarded as a dominant factor of biocompatibility [20]. The primary approach widely adopted for investigating the biocompatibility of materials is to vary the molecular properties of the material surface while keeping other parameters constant.

ii) Techniques for surface modifications and treatments

Since surface characteristics are essential for the performances of implant materials in the living body, surface treatments and modifications [3] for biomaterials have been widely used to help obtain more "friendly" surfaces with good surface properties (i.e. high uniformity, low roughness, desired wettability) and appropriate chemical components.

The regular surface treatments for biomaterials include:

a) Surface grafting of polymers

Graft polymerization [3], in which radicals are generated onto a surface and it initiates polymerization of a monomer, has been the most popular methods for producing some biomedical polymers onto the bulk materials, so as to achieve the desired surface properties. Studies [5] have noted that the blood-contact properties can be improved with increasing polymer graft density. The thickness of the grafted polymer layer is usually a few micrometers.

- *Advantages*: The surface properties can be controlled by changing polymer graft density; permanency of surface grafting is critical for many applications involving biomaterials.
- *Disadvantages*: Some polymers have difficulties in surface grafting.

b) Adsorption of polymers and polymer multilayers

An alternative to surface grafting polymers onto biomaterials is the physical adsorption of amphiphilic molecules to hydrophobic polymers [3]. The amphiphilic molecules can be rearranged on a surface in an attempt to maximize packing on the surface, because the highest packing density allowable by steric exclusion principles is the lowest energy state [3]. Multilayer

systems are sometimes necessary to generate a dense layer of the polymer, and may enhance the biocompatibility even further.

- *Advantages*: High density packing of the thin films adsorbed on the surface can be achieved.

- *Disadvantages*: Shorter duration than grafted surfaces; after the formation, covalent attachment to stabilize these physisorbed dense layers is needed (radio frequency glow discharge, ionizing radiation or gamma irradiation are among the choices).

c) Self-Assembly

The successful use of surfactants to modify biomaterials leads to speculation about the applicability of supramolecular assemblies such as amphiphilic self-assembled, chemisorbed, multilayer, and nanostructured systems [3]. These systems are not covalently linked, but can build two- and three- dimensional structures based on noncovalent interactions. With self-assembly and chemisorption, the highly favorable enthalpy of association leads to very dense monolayers.

The Langmuir-Blodgett (LB)[6, 7] and self-assembled monolayer (SAM)[8, 9, 10] techniques are among the best-known methods for this purpose. With the LB technique, ultrathin films are formed by transferring mono-molecular films of lipids layer-by-layer from a water surface onto a solid substrate. Amphiphilic self-assembly relevant to the production of devices and implantable artificial organs has been examined using LB films. The surfaces of liposomes modified with PEG amphiphiles using self-assembly lessened recognition by the immune clearance mechanisms of the body [11, 12]. Some biological examples can also be found: the cell membrane is an example of a self-assembled system, and the extracellular matrix is an example of a nanostructured system, mostly of water-soluble polymers, organized in three dimensions [3].

- *Advantages*: Dense, uniform thin film coatings can be produced with easy processes.

- *Disadvantages*: Limitations in the flexibility of choosing molecules and substrates; films thicker than several monolayers are not easy to form.

1.2 Electrostatic self-assembly (ESA) method

1.2.1 Development of ESA process

In 1966, Iler's [13] demonstration of sequential deposition of negative silica colloids and positive alumina fibrils started the concept of alternate adsorption of oppositely charged polyions. This novel technique was ignored until 1990s, when Decher and co-workers [14] at Gutenberg University in Mainz, Germany, demonstrated optically transparent multilayer films of four different polyelectrolytes, extending the pioneering work of Iler et al. to a new preparative method of organized thin films by layer-by-layer adsorption of polyelectrolytes. Alternate adsorption of a polycation and a polyanion was achieved by excessive adsorption of polyelectrolytes on oppositely charged surfaces. UV/vis spectroscopy and small angle X-ray scattering (SAXS) [14] were used to verify the uniform growth of the thin films, and it was observed that aperiodic assemblies with no substrate size limitation were possible, which have significant advantages over the LB method.

1.2.2 ESA process description

The electrostatic self-assembly method is based on the alternating adsorption of molecular layers of oppositely charged polymers/nanoparticles (anionic and cationic polyelectrolytes), where the ionic attraction between opposite charges is the driving force behind the multilayer buildup. In contrast to chemisorption techniques [15] that require a reaction yield of 100% in order to maintain surface functional density in each layer, no covalent bonds need to be formed. Additionally, an advantage over the classic Langmuir-Blodgett technique is that a solution process is independent of the substrate size and topology. Multilayer thin films can be assembled by depositing alternating layers of polyelectrolytes of opposite charge. The result is a thin film capable of completely masking a surface.

The buildup process of multilayer films is shown in Figure 1.5 and is described as follows (The drawing is over-simplified, it is not implied that the symbols used for the polyelectrolytes represent their actual structure in solution or after the adsorption.)

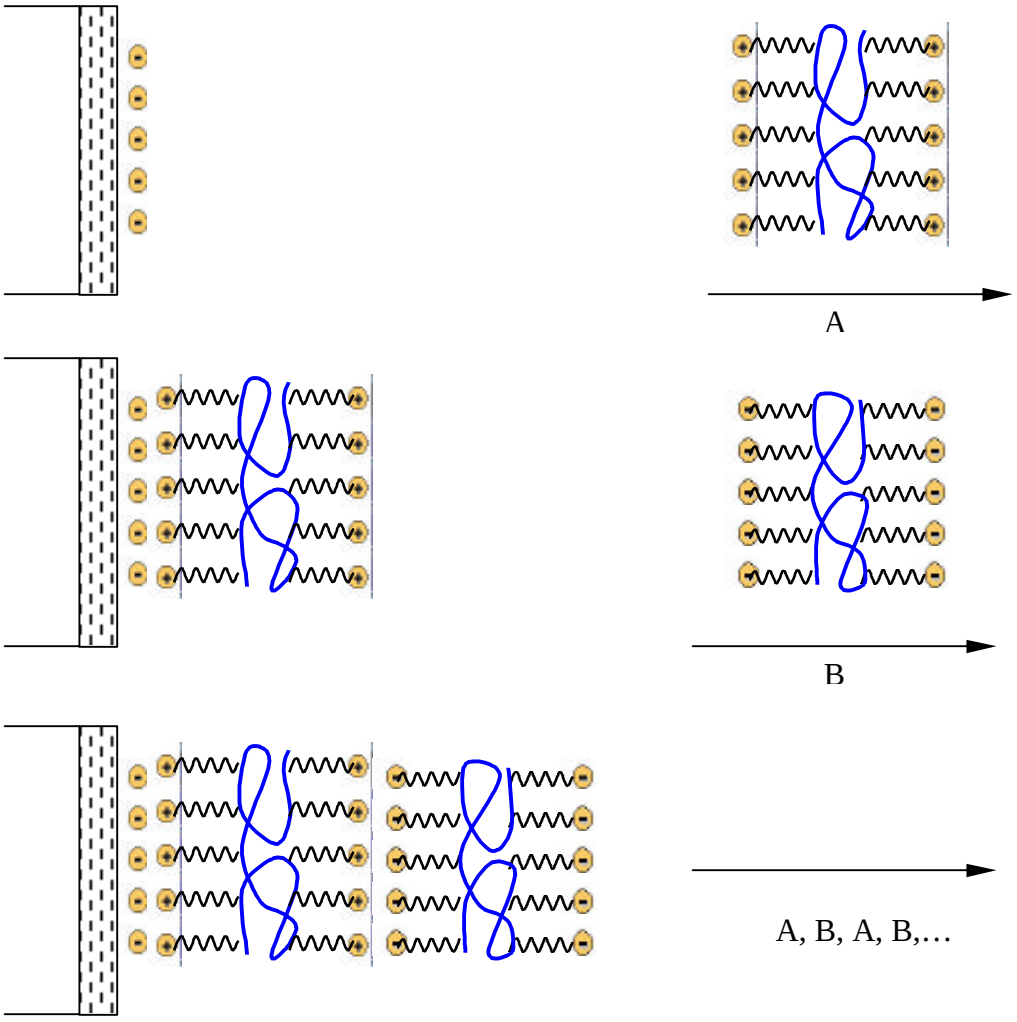


Figure 1.5. Schematic for the buildup of multiplayer assemblies.

A cleaned and functionalized substrate with net negative outer surface charge is immersed in the solution containing the cationic polyelectrolyte, and a monolayer of cationic polymer molecules with fixed positive functional groups along the polymer backbone are adsorbed (step A). The molecular order of such individual monolayers is nearly perfect, for the molecules automatically arrange themselves in a way so that the minimum total system energy is obtained. Since the adsorption is carried out at relatively high concentrations of polyelectrolyte, a number of ionic

groups remain exposed to the interface with the solution and thus the surface charge is reversed. After rinsing thoroughly in pure water to remove the loose molecules that may be held on the surface by weak van der Waals force, the substrate is immersed in the solution containing the anionic polyelectrolyte. Again a monolayer is adsorbed but now the original surface charge is restored (step B). By repeating both steps in a cyclic fashion (A, B, A, B…), alternating multilayer assemblies of both molecules are obtained.

This process of multilayer formation is based on the attraction of opposite charges, and thus requires a minimum of two oppositely charged molecules. Consequently, one is able to incorporate more than two molecules into the multilayer, simply by immersing the substrate in as many solutions of polyelectrolytes as desired, as long as the charge is reversed from layer to layer. Even aperiodic multilayer assemblies can be easily prepared [14]. In addition, individual nanoparticles may be incorporated into any or all of the monolayers, allowing wide design opportunities for thin films with specific or multifunctional properties [16].

1.2.3 The potential of ESA processing in biomaterials field

The electrostatic self-assembled multilayer thin films have generally been produced for their unique optical and electrical properties, but it can also be applied to biological systems. An example that has been used as a biomaterial for years is the coacervate of polylysine (a polycation) with alginate (a polysaccharide from seaweed with a negative charge). Alginate will gel in calcium-containing media, and subsequent adsorption of polylysine produces an isotropic coacervate. These membranes have been used extensively as microspheres to encapsulate cells for transplantation [3].

ESA processing of biocompatible thin films for biomedical engineering application has the following major advantages over traditional methods:

- **Excellent molecular level uniformity** permits the fabrication of high quality films with homogenous properties (i.e. hydrophilicity/hydrophobicity and charge distribution), which would reduce the intense biological response caused by surface heterogeneity of the implant materials.

- **Independent of substrate size or topology** makes possible the coating ESA biocompatible thin films on a variety of implant organs or devices with any sizes and complex shapes.

- **A large variety of materials** can be used, from polymers, proteins, to inorganic nanoparticles.

- **Self-assembly** and no additional processing is needed.

- **Easy synthesis and low-cost manufacturing –** room temperature and pressure, polyelectrolytes, counterions (e.g. NaCl), and water give us almost everything that is needed in ESA process, and the process may be upscaled in size and volume and automated easily.

- **Multilayer thin films** can be generated by continuing the adsorption of the membranes with polyelectrolytes, with almost no limit to the number of layers that can be generated. This scheme may help transform a heterogeneous charged surface to a homogeneous, dense coated surface after a few generations of layer building, and this treatment could be applied to any biomaterial derivatized by adsorption of a polyelectrolyte.

- **Long term stability –** the well-organized molecular order is stable due to the minimum system energy obtained through self-rearrangement of molecules adsorbed on the surface.

1.3 Motivation and objectives for this work

The use of devices and artificial implants made from biocompatible materials in the treatment of patients is increasing steadily in modern health care. Surface modifications or treatments of the implanted organs or devices have proved to be successful in improving surface properties that leads to better biocompatibilities. Among the various competing techniques, the electrostatic self-assembly (ESA) method offers a number of advantages over the others, as discussed in Section 1.2.3. The nanostructural nature of ESA thin films is compatible with the biological molecules, and the charged surface it provides may improve the inherence with plasma proteins, osteoblasts [17], the bone-forming cell and other cells. For instance, a dominant mechanism in

the cellular attachment to a biomaterial surface is electrostatic in nature, with the electrostatic characteristics of the surface encouraging the adsorption of special proteins to facilitate the initial attachment to biomaterial surface.

The major objective of this thesis is to demonstrate the feasibility and effectiveness of novel and low-cost ESA processing for the manufacturing of biocompatible thin films. The thesis work encompasses three major aspects:

- Investigation of ESA processing parameters and capabilities.
- Design, synthesis, and characterization of thin films with various biocompatible materials, including ceramic biomaterials and water-soluble polymers.
- Investigation of the details involved in protein-surface (coated with ESA biocompatible thin films) interactions with relevance to questions regarding biocompatibility.

1.4 Thesis outline

In Chapter 2, we describe our design, synthesis**,** and characterization of ESA multilayer thin films. We report in detail the materials selection, the ESA processing parameters and procedures used in our experiments, and show how we characterize the multilayer assemblies by UV/vis spectroscopy, ellipsometry, and AFM imaging.

Chapter 3 is concerned with the preliminary *in vitro* biocompatibility testing concentrated on the measuring of wettability and the degree of protein adsorption on the ESA thin films produced, using contact angle surface characterization for wettability testing and Fourier transform infrared reflection absorption spectroscopy (FT-IRAS) for protein adsorption analysis.

And finally, in Chapter 4, we draw some conclusions and give some suggestions for future work.

1.5 References

1. Y. Ikada, “Interfacial Biocompatibility”, *Polymers of Biological and Biomedical Significance* **540**: 35-48 (1994).

2. C.J. Kirkpatrick, F. Bittinger, M. Wagner, H. Kohler, T. G. van Kooten, C. L. Klein and M. Otto, “Current trends in biocompatibility testing”, *Proceedings of the Institution of Mechanical Engineers Part H-Journal of Engineering in Medicine* **212**: (H2) 75-84 (1998).

3. Donald L. Elbert, Jeffrey A. Hubbell, “Surface Treatments of Polymers For Biocompatibility”, *Annu. Rev. Mater. Sci.* **26**: 365-394 (1996).

4. Victor I. Sevastianov, “Role of Protein Adsorption in Blood Compatibility of Polymers”, *CRC Critical Reviews in Biocompatibility* **4** (2): 109-154 (1988).

5. K. Fujimoto, H. Tadokoro, Y. Ueda, Y. Ikada, *Biomaterials* **14**: 442-448 (1993).

6. I. Langmuir, *J. Am. Chem. Soc*. **39**, 1848 (1917).

7. K.B. Blodgett, *J. Am. Chem. Soc*. **57**, 1007 (1935).

8. J.P. Folkers, P.E. Laibinis and G.M. Whitesides, "Self-assembled monolayers of alkanethiols on gold: Comparisons of monlayers containing mixtures of short-chain and long-chain constituents with CH_3 and CH_2OH terminal groups," *Langmuir* **8**, 1330 (1992).

9. J.M. Tour et al., "Self-assembled monolayers and multilayers of conjugated thiols, alpha, omega-dithiols, and thioacetyl-containing adsorbates: Understanding attachments between potential molecular wires and gold surfaces," *J. Am. Chem. Soc.* **117**, 9529 (1995).

10. D. Li and O. Ramos Jr., "Molecular Self-Assemblies as Advanced Materials," in *Photonic Polymer Systems - Fundamentals, Methods and Applications*, ed. D.L. Wise et al., Marcel Dekker, New York (1998).

11. C. S. Cho, T Kotaka, T Akaike, *J. Biomed. Mater. Res.* **27**: 199-206 (1993).

12. M. Uchida, T. Tanizaki, T. Oda, T. Kajiyama, *Macromolecules* **24**: 3238-3243 (1991).

13. R.K. Iler, *J. Colloid Interface Sci.* **21**, 569 (1966).

14. G. Decher, J.D. Hong and J. Schmitt, "Buildup of ultrathin multilayer films by a self-assembly process: III. Consecutively alternating adsorption of anionic and cationic polyelectrolytes on charged surfaces," *Thin Solid Films* **210/211**, 831 (1992).

15. J. Gun, G. Sagiv, "On the formation and structure of self-assembling monolayers. 3. Time of formation, solvent retention, and release," *J. Colloid Interface Sci.* **112**, 457 (1986).

16. Katsuhiko Ariga, Yuri Lvov, and Toyoki Kunitake, "Assembling Alternate Dye-polyion Molecular films by Electrostatic Layer-by-Layer Adsorption", *J. Am. Chem. Soc.* **119**: 2224-2231 (1997).

17. Bengt Kasemo, "Biological surface science", *Current Opinion in Solid State & Materials Science* **3**: 451-459 (1998).

18. J.D. Andrade, "Interfacial phenomena and biomaterials", *Medical Instrumentation* **7** (2): 110-120 (1973).

19. Yoshito Ikada, "Overview of Current Blood-Compatible Polymers", *Advances in Polymer Science* **57**: 105-140 (1984).

20. Joseph D. Bronzino, *Biomedical Engineering Handbook*, CRC press (1995).

CHAPTER 2

Fabrication and Characterization of ESA Thin Films

The ESA technique, which produces multilayer thin films on solid supports by the spontaneous adsorption of oppositely charged polyelectrolytes from their dilute aqueous solutions, is a facile method in that it allows the structure, composition, and thickness of the film to be adjusted and controlled. Furthermore, this method has been developed and applied to other charged species, such as inorganic nanoparticles, dyes, proteins, nucleic acids, and functional polymers [1]. As discussed in Chapter 1, the ESA method for building up organic and inorganic thin films offers great advantages over other competing thin film fabrication methods, providing a good opportunity for the synthesis of unique biocompatible coatings with well-organized interfaces.

We have indeed fabricated ESA multilayer thin films from various biocompatible materials, including metal oxides such as zirconia and titania, and water-soluble polymers such as poly(methacrylic acid) (PMA), poly(vinylpyrrolidone) (PVP), and functionalized fullerenes. UV-vis spectroscopy was used during the ESA processing to monitor the film growth and uniformity. The measurements of film thickness were carried out with an ellipsometer. And finally, the surface morphology of the films was imaged by atomic force microscopy with nanometer resolution. Our experimental results showed that a large variety of biocompatible materials can be incorporated into ESA multilayer thin films and built up on different substrates with dense and uniformly distributed nanoscale structures.

2.1 Fabrication process of ESA thin films

There are numerous biocompatible materials used in artificial organs and devices as implants into the human body. These include metallic materials, composite materials, ceramics, and polymers. As long as the materials are water-soluble, and have charged functional groups (in the case of polymers) or are in the form of uniformly distributed charged nanoparticles in aqueous solutions (in the case of inorganic materials), they can be self-assembled to form ultra thin films using the ESA technique. Any charged surface is employable for ESA thin film deposition, regardless of its size and topology.

During ESA processing, for each molecule incorporated into the film, a particular combination of pH and concentration of the aqueous solutions must be determined to achieve both uniform growth and homogeneity of the film. Other important parameters affecting the film characteristics include salt concentration, dipping time, and the pH value of the ultrapure water used to rinse samples after each deposition cycle.

2.1.1 Materials

i) Conventional biomaterials

The widely used biocompatible materials can be classified into three groups: metals, ceramics, and polymers [2]. Table 2.1 provides a summary of some examples, with their applications and the advantages and disadvantages of using these three groups of materials in biomedical areas.

ii) Biomaterials suitable for ESA processing

For this thesis work, some biomaterials suitable for the ESA method, including ceramics and polymeric materials, were chosen. They are either charged colloidal nanoparticles or water-soluble polymers with charged functional groups on the backbone. Now we describe our work in more detail.

a) ceramic materials

Aluminum oxide (Al_2O_3), zirconia oxide (ZrO_2), and titanium oxide (TiO_2) were the inorganic biomaterials used in the ESA processing. They all provide uniformly dispersed cationic nanoscale particles in acidic solution. Their details are summarized in Table 2.2.

Table 2.1. Biocompatible materials for use in the body

	METALS	**CERAMICS**	**POLYMERS**
Examples	Titanium and alloys**,** Cobalt-Chromium alloys, stainless steels, Au, Ag, Pt, etc.	Aluminum oxide (Al_2O_3), Zirconium oxide (ZrO_2), Titanium oxide(TiO_2), carbon	Polyethylene(PE), polypropylene(PP), Poly(methylmethacrylate) (PMMA), Poly(ethyleneglycol)(PEG), Poly(vinylpyrrolidone)(PVP), Poly(ethelenimine)(PEI), Poly(*l*-lysine) (PL) etc.
Advantages	Strong, tough, ductile	Very biocompatible, inert, strong in compression	Resilient, easy to fabricate
Disadvantages	May corrode, dense, difficult to make	Brittle, not resilient	Not strong, deforms with time, may degrade
Applications	Joint replacements, bone plates and screws, dental root implants	Joint replacements, coating of dental and orthopedic implants	Contact lenses, blood vessels, hip socket, other soft tissues

Table 2.2. Inorganic biomaterials used in ESA processing

MATERIALS	**DETAILS**	**OBTAINED FROM**
Aluminum oxide (Al_2O_3)	F. W. 101.96, 20% in water, colloidal dispersion, density 1.19	Alfa Aesar
Zirconium oxide (ZrO_2)	F. W. 23.22, 20 wt.% in H_2O; colloidal dispersion	Alfa Aesar
Titanium oxide (TiO_2)	Synthesized at the Fiber & Electro-Optics Research Center of Virginia Tech	Virginia Tech

Titanium oxide (TiO_2) was synthesized in our lab. Titanium tetrachloride (99%, 44ml) was added into HCl aqueous solution (2M, 156 ml) very slowly with stirring at 0°C to obtain titania colloid. The aqueous solution was diluted to a desired concentration using Milli-Q water.

b) Polymeric materials

- Poly(vinylpyrrolidone) (PVP)

PVP is a water-soluble polymer with highly biocompatible properties; it is widely used in the medical field as a drug excipient to prepare artificial plasma [2]. By ESA processing, PVP was used to provide cationic polyelectrolytes in acidic aqueous solution. Figure 2.1 shows its molecular structure. PVP was obtained from the Aldrich Chemical Company and used as purchased.

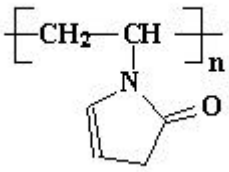


Figure 2.1. Molecular structure of poly(vinylpyrrolidone) (PVP)

- Poly(methacrylic acid) (PMA)

PMA acidic aqueous solution contains anionic polyelectrolytes, so it can be incorporated into ESA thin films by alternate adsorption with positively charged polymer molecules or inorganic nanoparticles. PMA is commercially available from Polysciences Inc. and can be used without any further purification. Its molecular structure is described in Figure 2.2.

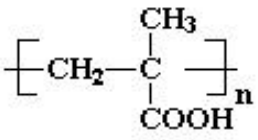


Figure 2.2. Molecular structure of poly(methacrylic acid) (PMA).

- Poly(diallyldimethylammonium chloride) (PDDA)

PDDA (Aldrich Chemical) has also been widely used in ESA processing. It can be dissolved in both acidic and basic solutions and produces cationic polyelectrolytes. Figure 2.3 gives the chemical structure of PDDA.

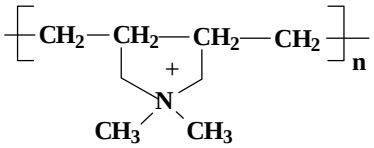


Figure 2.3. Chemical structure of PDDA.

- Poly(ethylenimine) (PEI)

PEI (Aldrich Chemical) is a newly reported biocompatible polymer. It offers polycations in acidic solution. The relevant chemical formula is given in Figure 2.4.

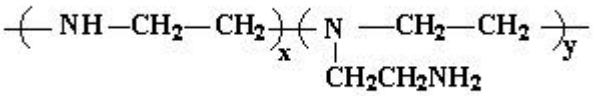


Figure 2.4. Chemical formula of PEI.

c) Functionalized fullerene

Pyrolitic carbon is considered as a biocompatible material and has been widely used for the fabrication of implants and surface coatings [3]. As a new form of carbon, fullerene (C_{60}) has generated considerable interest in the scientific community [3].

In our studies, C_{60} is polyhydroxylated fullerene, the synthesis procedure [4] and chemical structures are showed in Figure 2.5. In its basic aqueous solution, fullerene serves as an anionic polyelectrolyte provider.

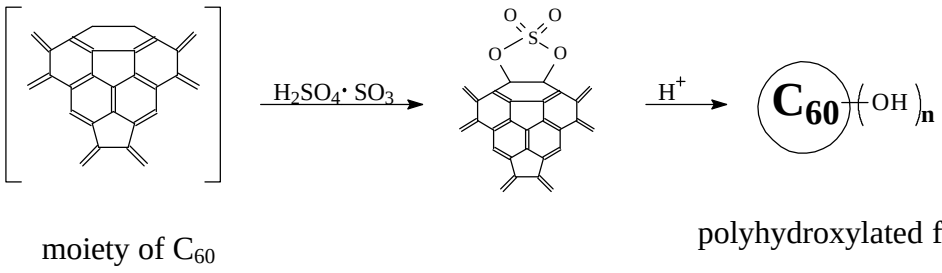


Figure 2.5. Synthesis of polyhydroxylated fullerene.

2.1.2 Substrate modification procedures

In our study, gold-coated glass, quartz, and single-crystal silicon with hydrophilic surfaces were used as substrates for ESA film deposition (Table 2.3). All the substrates used need to be cleaned to remove any impurities prior to the deposition of films. In addition, this procedure would modify the surface charge on the substrates.

Quartz and single-crystal silicon substrates were cleaned as follows: First, the substrates were immersed in a 30:70 mixture of 30% hydrogen peroxide (H_2O_2) and concentrated sulfuric acid (H_2SO_4) at ambient temperature for about 30 minutes. These substrates were then rinsed extensively with Milli-Q water and dried in an oven at 50°C for several hours. A substrate modified in this way has anionic charges generated on the surface.

Table 2.3. Substrate materials.

MATERIALS	EXPERIMENTS APPLIED	OBTAINED FROM
Gold-coated glass	Fourier-Transform Infrared Reflection Absorption Spectroscopy (FT-IRAS)	Chemglass, Inc.
Quartz	UV-vis spectroscopy, AFM	Chemglass, Inc.
Single-crystal silicon	Ellipsometry	EL-CAT, Inc.

Gold-coated glass substrates were first cleaned by ultrasonically agitating them in acetone. They were then immersed in ethanol at room temperature for 1 hour. This was followed by rinsing the substrates extensively in Milli-Q water.

2.1.3 Multilayer dipping procedures

In ESA processing, the surface charge must be reversed after every adsorption step. This is achieved by alternating the deposition of polyanions and polycations from their respective aqueous solutions. Under appropriate conditions, highly uniform and linearly growing multilayer thin films can be fabricated in a layer-by-layer fashion.

i) Deposition conditions

Table 2.4. Deposition conditions.

DEPOSITION PAIRS	CONCENTRATION OF SOLUTIONS (M)	PH VALUE OF SOLUTIONS	ADSORPTION TIME (MIN)	PH VALUE OF RINSING WATER
PVP/PMA	0.1	4.5	3.0	≈ 5.0
	0.01			
Al_2O_3/PMA	0.196	4.0	3.0	≈ 4.0
	0.01			
ZrO_2/PMA	0.162	3.5	3.0	≈ 4.0
	0.01			
TiO_2/PMA	0.16	2.5	3.0	≤ 3.0
	0.01	3.5		
PDDA/C_{60}	0.01	9.0	2.0	≈ 9.0
	2.5×10^{-4}		5.0	
PEI/PMA	0.02	4.5	3.0	5.0
	0.01			

The key to a successful buildup of multilayer thin film assemblies in such a layer-by-layer fashion is the inversion and subsequent reconstruction of substrate surface properties. For each molecule incorporated into the film, appropriate deposition conditions (i.e., solution concentration and pH value, adsorption time, and pH value of the Milli-Q water for rinsing) must be determined in order to achieve a linear growth of the film and high uniformity with the increasing number of adsorbed layers. Table 2.4 lists the optimal conditions in our experiments for all the deposition matches used in this work.

It is worth pointing out that the concentration of C_{60} solution should be made below 5×10^{-4}M, for the reason that aggregation will occur at a higher concentration, which could lead to non-uniformity of the films. On the other hand, a concentration that is too low ($< 1.25\times 10^{-4}$M) would not offer enough molecules for adsorption, thus failing the multilayer assembly.

Also, the pH value of TiO_2 solution should always be less than 3.0; otherwise the precipitation of the metal oxide would cause difficulty with uniform monolayer deposition and non-linear growth of film thickness, resulting in poor film quality. To adjust the pH value of the solutions and the rinsing water, diluted HCl, NaOH, and $NaHCO_3$ solutions were used.

The assembly matching is also very important. The charged function groups on polymers or the charged nanoparticles can only maintain the charge within a special range of pH value. Thus, similar pH values of the two solutions used in an assembly pair is necessary. Otherwise, the monolayer just deposited onto the substrate from one solution would "peel off" when it is immersed into the other solution with a different pH value, because the electrostatic attractions would have been lost. For the same reason, the Milli-Q water used for rinsing should also have a similar pH value with that of the solutions.

ii) Layer-by-layer adsorption process

Figure 2.6 gives a schematic description of the ESA alternate adsorption procedure. To initiate the growth of multilayer thin films, a clean, negatively charged substrate was immersed into a polycationic solution (PVP, PDDA, and PEI). After enough time to ensure the adsorption is sufficient, one monolayer of polycation molecules was adsorbed onto the surface, and the negative surface charge was reversed (Figure 2.6 (a, d)). The substrate (with one adsorbed monolayer) was then rinsed several times in Milli-Q water, for the purpose of removing the loosely attached molecules. It was then immersed into the other solution containing polyanions (PMA or C_{60}) to form a monolayer of polyanion molecules (Figure 2.6 (b, e)). This time, the original negative surface charge was restored. The polycation monolayer and polyanion monolayer together is called a bilayer [5]. Multilayer thin films were self-assembled by repeating this alternate adsorption cycle (Figure 2.6 (c, f, g)). Fabricated films were dried in flowing nitrogen gas in the final step.

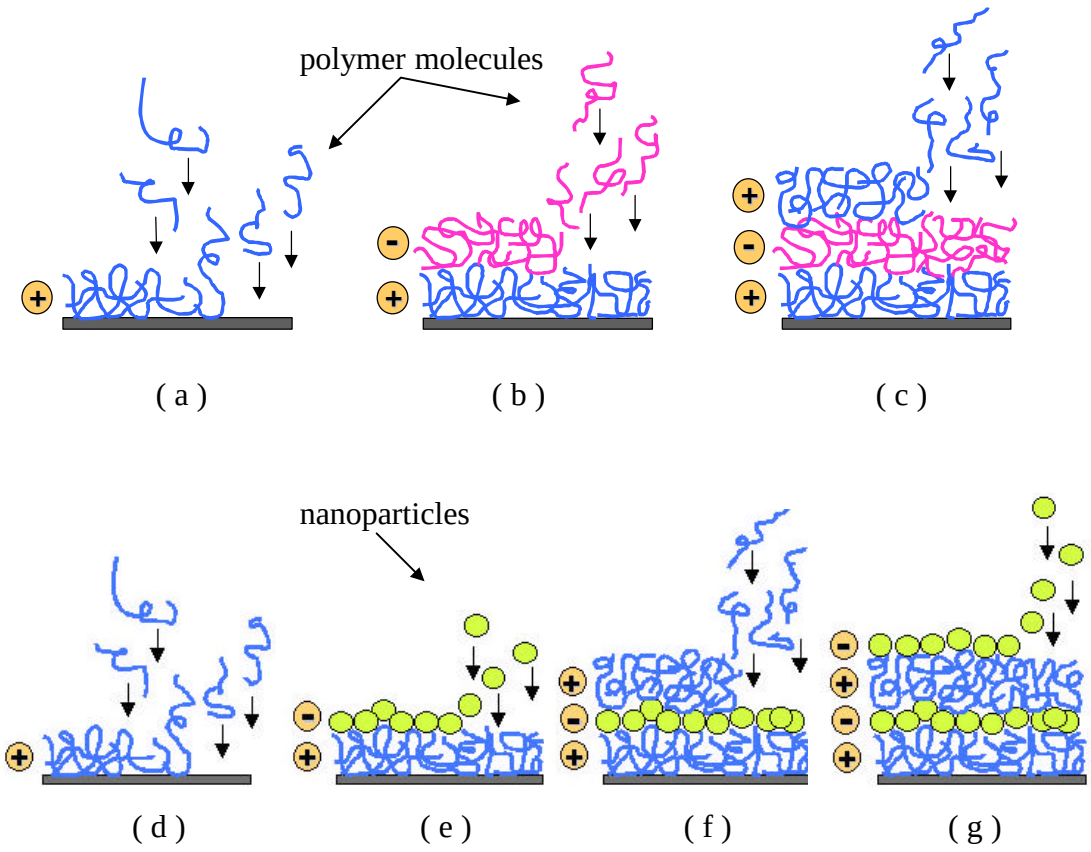


Figure 2.6. Schematic for the layer-by-layer adsorption process

Film deposition incorporating inorganic nanoparticles (Al_2O_3, ZrO_3 and TiO_2) is worth mentioning here. Prior to the adsorption of the first nanoparticle monolayer, a few bilayers of PVP/PMA were deposited on the substrate to promote the adhesion between the surface and the first monolayer of the films. Nanoparticle/polymer multilayer films were then constructed on these pre-coated polymer bilayers with a negatively charged uppermost surface.

The ultrapure water used for all the experiments and cleaning procedures was obtained from a Barnstead Nanopure III system, with a resistivity greater than 17 MΩ·cm (according to the manufacturer). Rinsing steps are very important. If they were omitted, an adhering layer of one solution would contaminate the next solution and eventually lead to co-precipitation of both compounds and the incorporation of precipitated particles into the following layers.

2.2 Characterization of ESA thin films

Several characterization methods have been applied to analyze the ESA multilayer thin film deposition technique and to visualize the quality and morphology of the resulting film structures [6]. During and after the fabrication process, the ESA thin films were characterized and evaluated to determine their structural characteristics with emphasis on uniformity, film thickness, layer-by-layer linearity, surface morphology, and optical characteristics.

2.2.1 Tools for analysis

- **UV/vis spectroscopy**

UV/vis spectroscopy is designed to perform qualitative and quantitative spectral analyses in the ultraviolet and visible regions. In the UV/vis absorption spectra of the multilayer assemblies, the increasing behavior of the film's optical absorption is explained by the Beer-Lambert Law (equation 2.1 and 2.2), which states that the change in the intensity of light passing through a material depends both on the pathlength and the concentration of analytes in the material, or

$$I_t / I_0 = 10^{-e \cdot c \cdot l} = t. \qquad \text{(eq. 2.1)}$$

This is most commonly written as

$$A = e \cdot c \cdot l = \log (I_0 / I_t), \qquad \text{(eq. 2.2)}$$

Where

e: absorption coefficient; *c*: concentration of analyte; *l*: length of absorption path; *t*: transmittance; *A*: absorbance (ABS).

The absorbance of a given sample is proportional to the absorption pathlength for a given concentration at any given wavelength. Thus, if a sample with thickness *l* produces absorbance *A*, then a sample with thickness 2*l* will produce absorbance 2*A* (The assumption here is the constant concentration.). Therefore, the stepwise growth of self-assembled multilayer films can be conveniently monitored by UV/vis spectroscopy.

- **Ellipsometry**

Single-wavelength ellipsometry, a precision optical technique, has been applied extensively over the years to determine the thickness of organic and biological films [7], and in particular, organized monolayers [8, 9]. It is based on the fact that, in general, a thin film affects the change in the polarization state of an elliptically polarized light beam when it is reflected by the interface. Figure 2.7 shows the basic principles of ellipsometry. This illustration shows the ellipsometer setup that was used in our studies. The instrument changes the polarizer angle *P*, until the reflected light from the sample is linearly polarized. The analyzer prism is then adjusted to an angle *A*, to extinguish the polarized light as detected by a photodetector. A prerequisite of this method is the use of surfaces with high and reproducible optical and chemical properties. Silicon wafers usually fulfill these criteria.

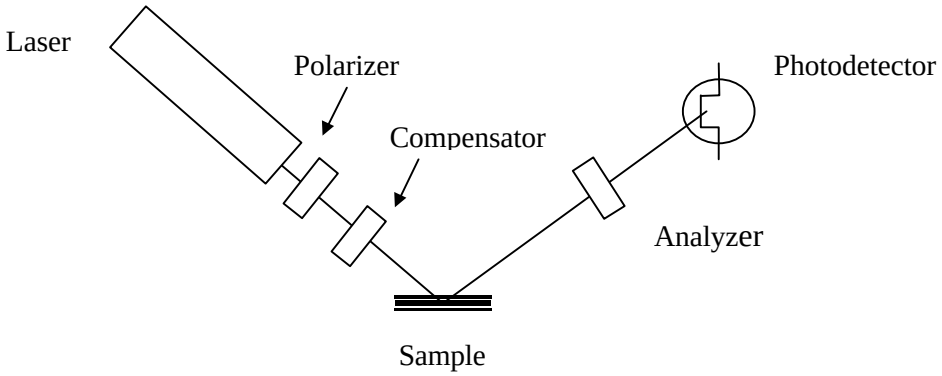


Figure 2.7. Principle of the ellipsometry.

- **Atomic Force Microscopy (AFM)**

The Atomic Force Microscopy (AFM) technique belongs to the family of scanning probe microscopy. It is a direct imaging technique that has a lateral resolution of approximately 100Å, and a vertical resolution of 1-2 Å [10]. Under optimal conditions (hard and smooth samples), AFM is capable of resolving surface detail down to the atomic level [11]. It is therefore ideal for imaging the structures of ESA thin films in three dimensions on a nanometer scale.

2.2.2 UV-vis absorption spectra of ESA thin films

We characterized our ESA multilayer thin films using a Hitachi Model U-2010 UV/vis spectrophotometer to monitor the growth of the multilayer structure, the relative amount of material deposited per layer, and the uniformity of the films during the deposition process. The optical absorption spectra measured at several points during film growth gave us a good indication of film quality and uniformity.

All the ESA thin films were fabricated on quartz substrates with up to 30 bilayers for the measurements of UV/vis spectra. Values reported are the average of the measurements taken at three locations on each sample.

Figure 2.8 and Figure 2.9 are the UV/vis spectra obtained from PVP/PMA, PDDA/C_{60} multilayer thin films, respectively.

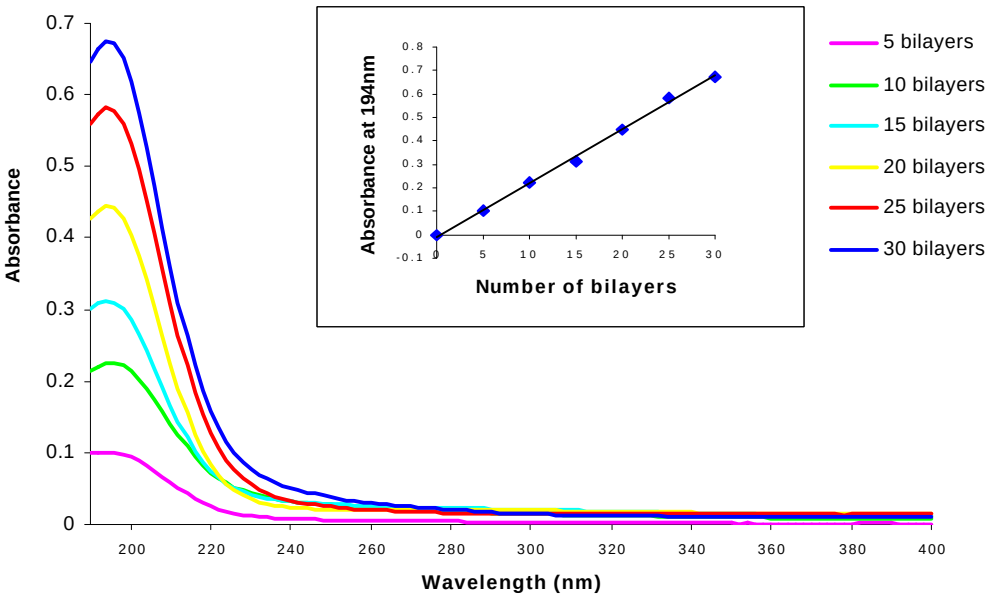


Figure 2.8. UV/vis absorption spectra of PVP/PMA multilayer thin films.

By UV/vis spectroscopy, we have demonstrated that the consecutive adsorption of layers is a stepwise and regular process. These two figures depict the optical absorbance spectra of

PVP/PMA and PDDA/C_{60} multilayer assemblies for the layer numbers from 0 to 30, with one recording after the growth of every 5 bilayers.

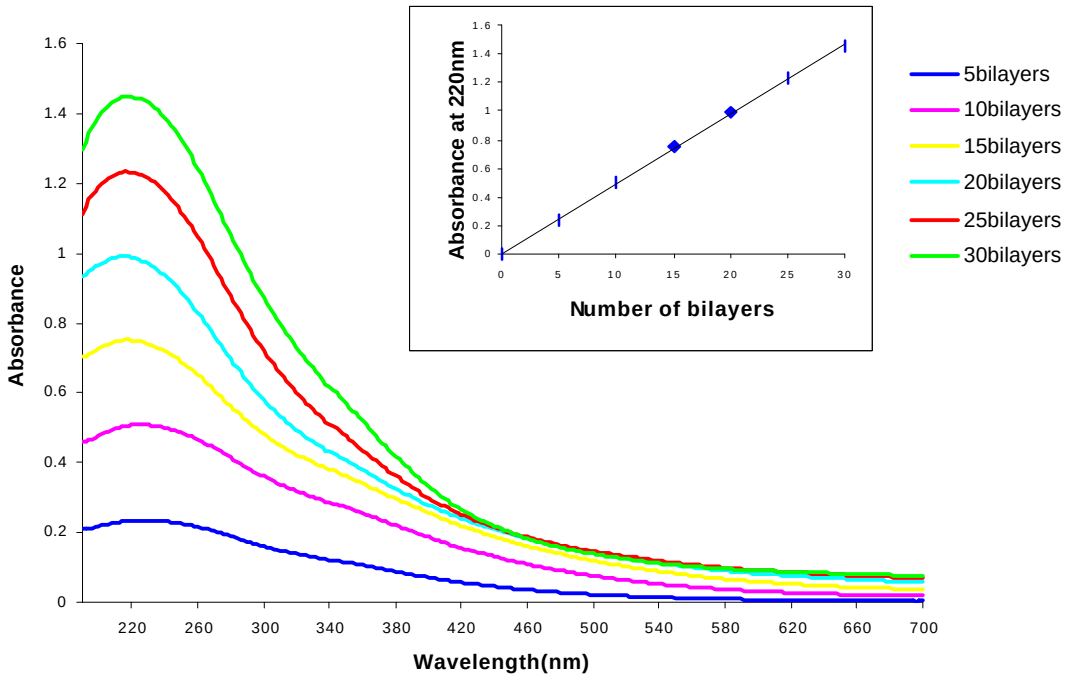


Figure 2.9. UV/vis absorption spectra of PDDA/C_{60} multilayer thin films.

The insets show us the plots of the optical density at the main absorption band (194nm for PVP/PMA and 220nm for PDDA/C_{60}) versus the number of bilayers. The points have a near-perfect linear fit, yielding an average optical density of 0.022 ± 0.001 per bilayer, in the case of PVP/PMA at 194nm and 0.049 ± 0.002 per bilayer, in the case of PDDA/C_{60} at 220nm. The linear nature of the plots suggests that each adsorbed layer contributes an equal amount of material to the thin films. There are no shifts in the main absorption bands, which indicates that the films are uniform and no molecular aggregation occurred between the adjacent layers.

Figures 2.10 and 2.11 are the UV/vis absorption spectra of Al_2O_3/PMA and TiO_2/PMA as examples of the nanoparticle/polymer multilayer assemblies. Same as above, the insets show linear increases of the optical absorbance versus the increasing number of bilayers deposited. The average optical density is 0.004 ± 0.0015 (at 260nm) for Al_2O_3/PMA multilayers, and 0.07 ±

0.01 (at 190nm) for TiO_2/PMA multilayers (in its UV/vis spectra, no apparent main absorption band was found, so 190nm was chosen for the analysis).

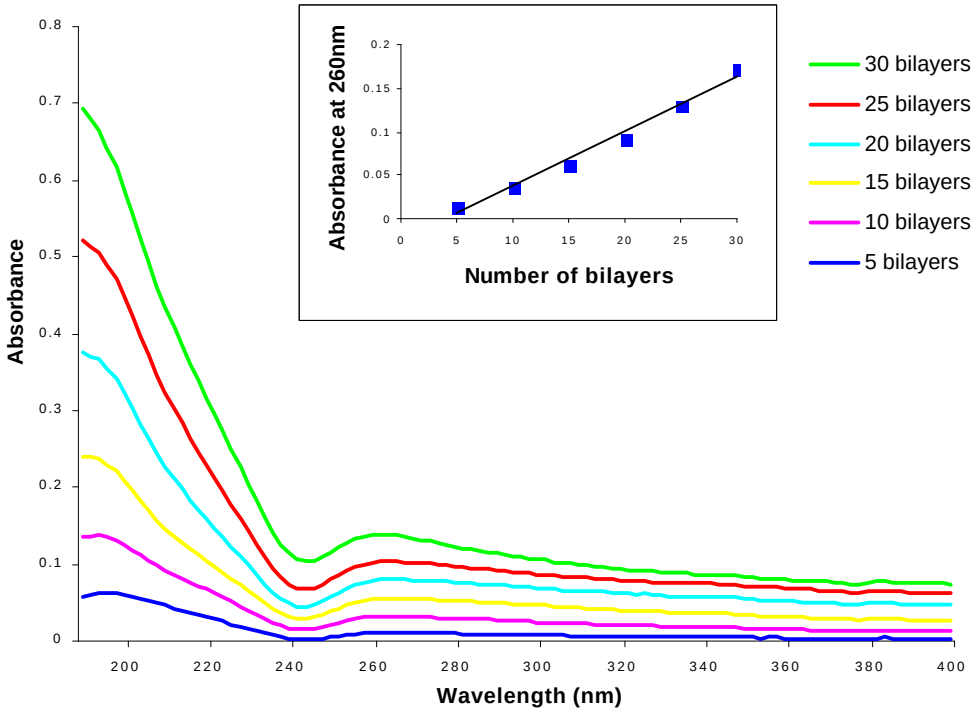


Figure 2.10. UV/vis absorption spectra of Al2O3/PMA multilayer thin films.

Slight deviation of some data points from the straight fit line in the inset plots of the optical density may be explained by the inhomogeneities in some of the layers. This is possibly induced by the formation of large clusters of nanoparticles in the solution which were finally incorporated into the multilayer films. When present they are easily visualized by the occurrence of more than one interference color in different regions of the substrate or by the observation of some opaqueness in the interference colors.

The multilayer assemblies which incorporate nanoparticles are more likely to show these defects than polymer/polymer thin films. And in fact, it is very difficult to build nanoparticle-incorporated thin films with up to hundreds of bilayers; while this can be easily achieved with polymer-pair assemblies. The uniformity of polymer/polymer films is also better than that of

nanoparticle/polymer films, both visually and quantitatively from the UV/vis spectroscopy measurements. These may be explained by the weaker inter-layer adhesion between nanoparticle and polymer monolayers. Polymer chains are soft and more flexible to arrange themselves into a stable "network." Also, aggregation could occur in nanoparticle colloid over time, which leads to a non-uniform distribution of the particle sizes.

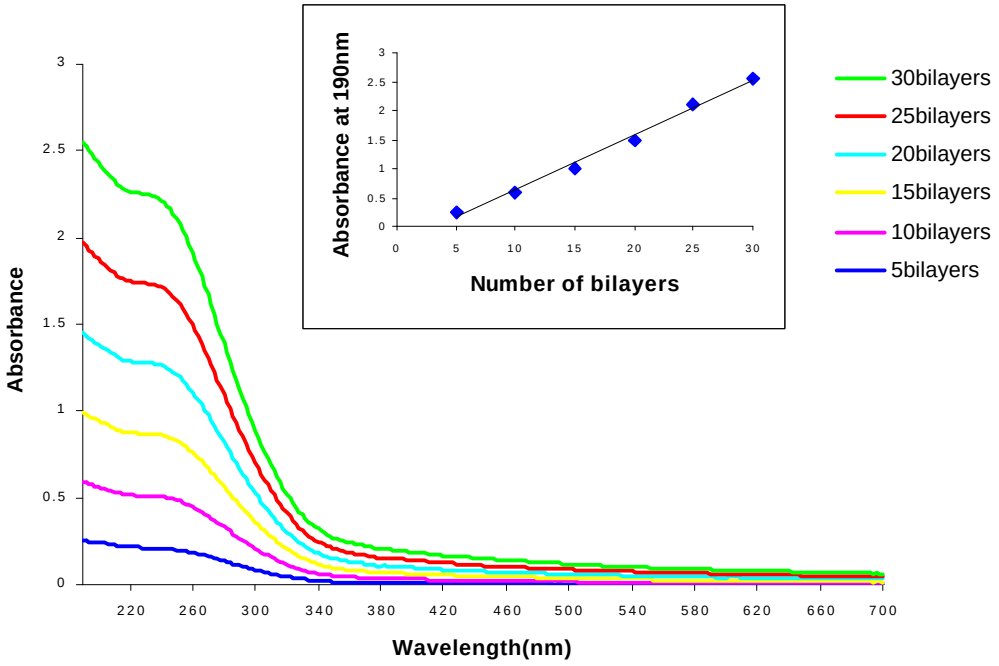


Figure 2.11. UV/vis absorption spectra of TiO_2/PMA multilayer thin films.

2.2.3. Measuring thickness of ESA thin films

Multilayer films assembled on single-crystal Si substrates (30 bilayers) were measured using a Rudolph Auto-EL ellipsometer to determine their thickness. Theoretically, the accuracy of the instrument can reach 1 Å. In our case, the instrument was calibrated using a standard silicon wafer with SiO_2 thickness 1097 ± 3Å and index 1.462 ± 0.002. The reported values in this study were the average of the measurements taken at three locations on the film surface, twice for each.

Figure 2.12 and 2.13 give us the dependence of the ellipsometry-determined thickness of the multilayer films on the number of deposited layers. The plots are linearly fitted, which confirms

the results from UV/vis spectroscopy that on average, the same amount of materials were deposited for each bilayer, and the linear growth of films continues up to tens of bilayers.

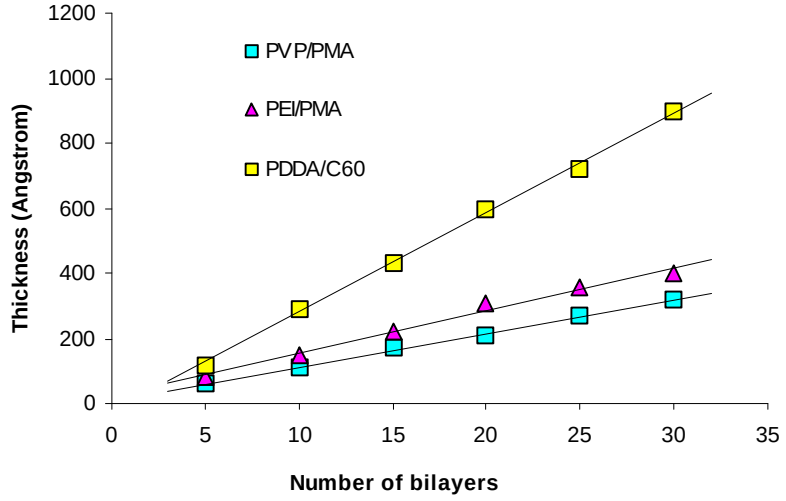


Figure 2.12. Thickness growth of PVP/PMA, PEI/PMA, and PDDA/C_{60} films

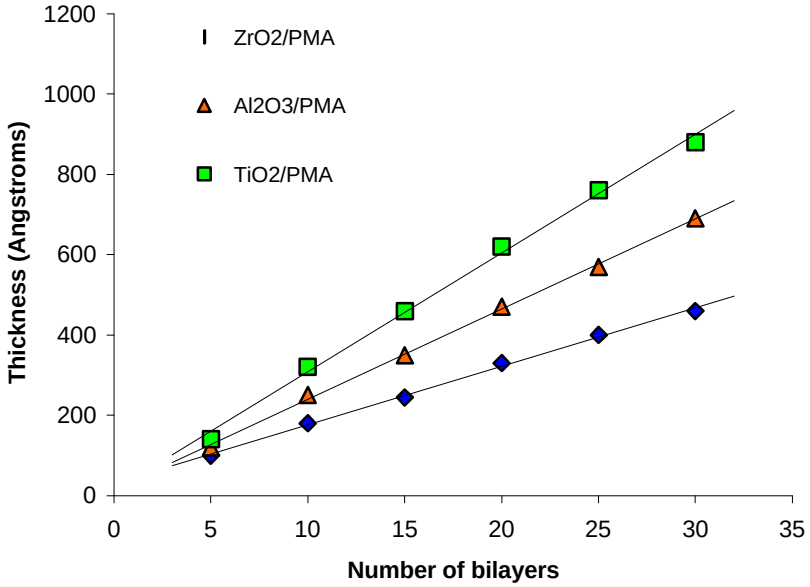


Figure 2.13. Thickness growth of ZrO_2/PMA, Al_2O_3/PMA, and TiO_2/PMA films

For each bilayer, polymer/polymer films are generally thinner than nanoparticle/polymer films. This phenomenon may be explained as follows. The inorganic particles dispersed in the colloids can be assumed as "hard" and spherical particles with a fixed size in the range of 2 ~ 10 nanometers in diameter. So the thickness of nanoparticle/polymer multilayer films is more dependent on the size of these particles (at least a few nanometers). Polymer chains, however, are more flexible to orient themselves in a way so as to achieve a minimum energy configuration. Consequently, each bilayer may obtain a thickness much less than the size of an inorganic particle. The thickness of PDDA/C_{60} is caused by the size of C_{60} on the polyhydroxylated fullerene. Table 2.5 summarizes the thickness data of all 30-bilayer multilayer assemblies.

Table 2.5. Thickness of multilayer assemblies determined from ellipsometry.

MULTILAYER ASSEMBLIES	TOTAL THICKNESS (ANGSTROMS)	THICKNESS OF EACH BILAYER (ANGSTROMS)
PVP/PMA	320	11
PDDA/C_{60}	896	30
PEI/PMA	393	13
Al_2O_3/PMA	672	22
ZrO_2/PMA	435	14
TiO_2/PMA	880	29

2.2.4 AFM images of the ESA multilayer thin films

The surface imaging technique of AFM yields information about uniformity, grain distributions, and defect formation on the film surface. The AFM images of ESA multilayer assemblies deposited on quartz substrates were obtained at ambient temperature with a Nanoscope III (Digital Instruments, Inc.).

The pyramidal AFM tips and cantilevers were made from etched silicon probes. The images were collected in the tapping mode in air, resonating the tip just below the oscillation frequency

of the cantilever (typically 250-325kHz). The oscillation frequency for scanning was set to 0.1~ 3 kHz below resonance. Typical images were obtained with the scanning rate of 1 Hz and the data collection resolution of 512× 512 pixels. Tips were changed frequently to maintain the resolution of the AFM images.

Figure 2.14 and 2.15 are the typical AFM images obtained from our ESA thin films, which were from PVP/PMA and ZrO_2/PMA film surfaces, respectively. The images indicate that the films are uniform, showing no apparent surface damages or defects. Also in the image in Figure 2.15, regular thin platelets of nanoclusters lying on the substrate plane can be observed; they are closely packed on the surface with an approximately uniform size of 20nm in diameter.

Observing the morphology of the films, we suggest that the strong electrostatic interaction between the anionic and cationic monolayers results in the appearance of a highly uniform nanostructured film formed by ESA deposition.

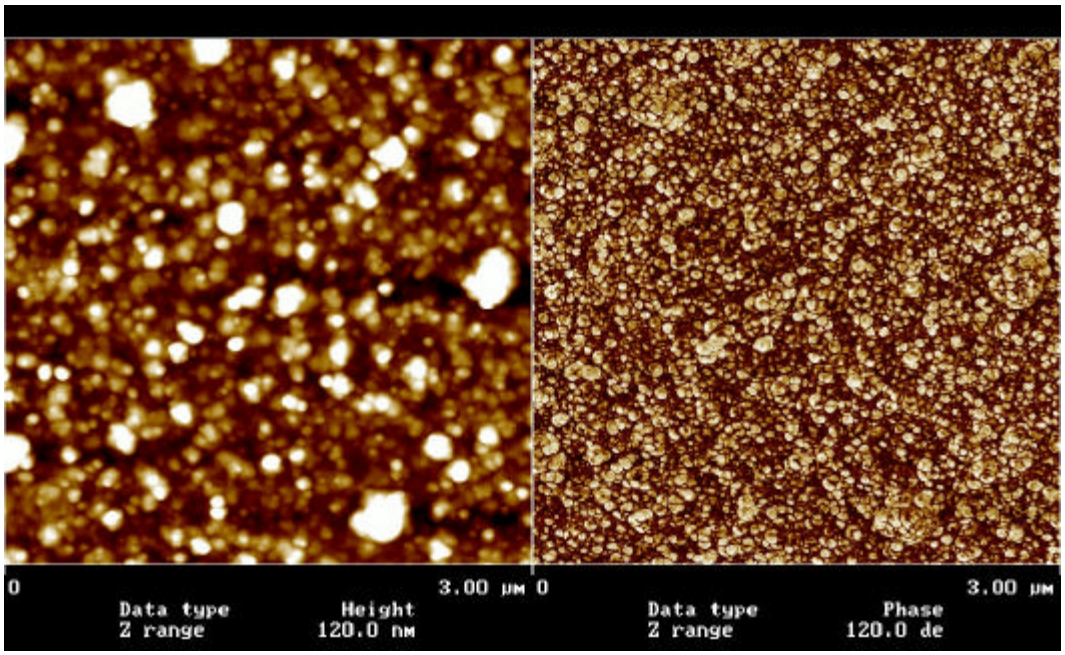


Figure 2.14. AFM image of 30 bilayer PVP/PMA multilayer thin films (left: height image; right: phase image).

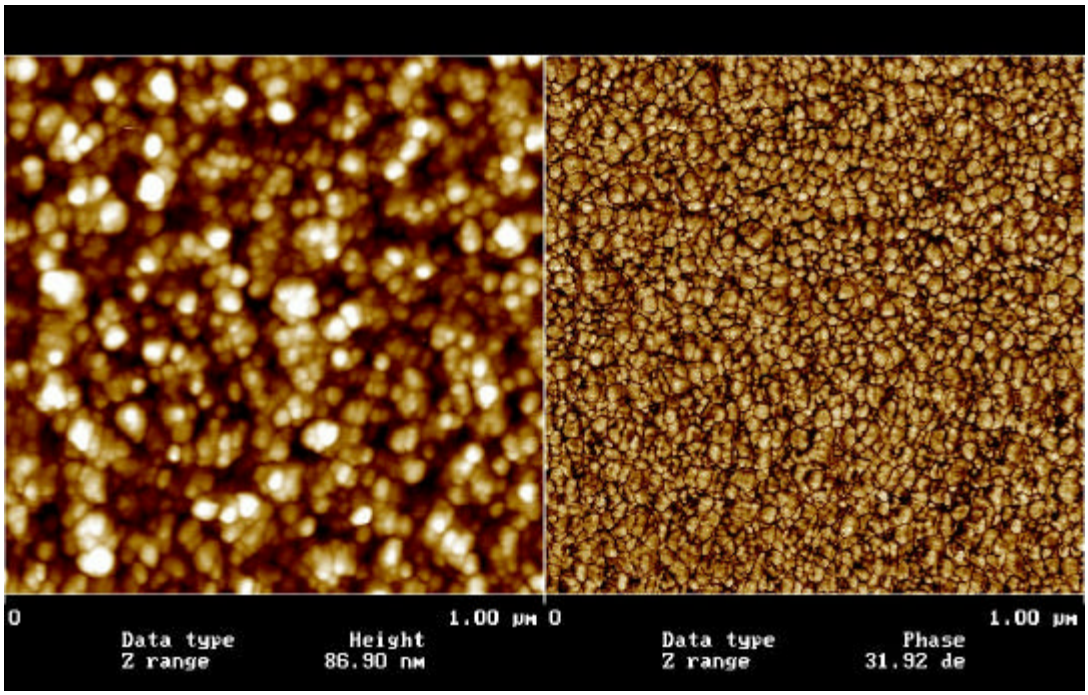


Figure 2.15. AFM image of 30 bilayer ZrO_2/PMA multilayer thin films (left: height image; right: phase image).

2.3 Summary

In this chapter, we investigated the application of ESA processing to the fabrication of ultra thin multilayer assemblies from various biocompatible materials including metal oxides and polymers. These materials were successfully fabricated into ESA multilayer films by an alternate adsorption process with appropriate assembly matching under the optimal processing conditions. The uniformity, thickness, layer-by-layer linearity, and surface morphology of the multilayer assemblies were characterized by UV/vis spectroscopy, ellipsometry, and AFM imaging. These experimental results demonstrated the feasibility of ESA processing to construct dense, highly uniform, and well-organized nanostructure biocompatible thin films on any charged surfaces.

2.4 References

1. Katsuhiko Ariga, Yuri Lvov, and Toyoki Kunitake, "Assembling Alternate Dye-polyion Molecular films by Electrostatic Layer-by-Layer Adsorption", *J. Am. Chem. Soc.* **119**: 2224-2231 (1997).

2. G. Carotenuto, L. Ambrosio, P. Natti, L. Nicolais in *Cambridge Healthtech Institute's meeting*, Boston, 1999.

3. Joon B. Park, Roderic S. Lakes, *Biomaterials: An Introduction*, Plenum Press, New York and London (1979).

4. L. Y. Chiang, L.Y. Wang, J. W. Swirczewski, S Soled, S Cameron, *J. Org. Chem.* **59**: 3960-3968 (1994).

5. Y. Liu, A Wang, R.O. Claus, "Molecular Self-Assembly of TiO2/Polymer Nanocomposite Films", *J. Phys. Chem. B* **101**: 1385 (1997).

6. Yanjing Liu, Aprillya Rosidian, Kristie Lenahan, Youxiong Wang, Tingying Zeng, R.O. Claus, "Characterization of electrostatically self-assembled nanocomposite thin films", *Smart Mater. Struct.* **8**: 100-105 (1999).

7. Rothen, In *Ellipsometry in the Measurement of Thin Films*. (E. Passaglia, R. R. Stromberg, and J. Kruger, Eds.) National Bureau of Standards Misc. Publ. **256**, Washington, DC, p7, (1964).

8. A. Ulman. *An Introduction to Ultrathin Organic Films From Langmuir-Blodgett to Self-Assembly*. Academic, New York (1991).

9. D.L. Allara and R. G. Nuzzo. *Langmuir*. **1**: 52 (1985).

10. Mingming Fang, David M. Kaschak, Anthony C. Sutorik, Thomas E. Malloouk, " A 'mix and match' Ionic-Covalent Strategy for Self-Assembly of Inorganic Multilayer Films", *J. Am. Chem. Soc.* **119**: 12184-12191 (1997).

11. P. K. Hansma, V.B. Ellings, O. Marti, C. E. Bracker, “Scanning tunneling microscopy and atomic force microscopy: application to biology and technology”, *Science* **244**: 209-216 (1988).

CHAPTER 3

Biocompatibility Tests

When a foreign material is placed in contact with blood, a number of complex reactions are known to take place, with the end result being the formation of blood clots (thrombus) which can be fatal [1]. These reactions are rapid and complex in nature under physiological conditions. In the intact tissue or blood system with the cells present, interaction analysis is most often severely compromised or impossible. Therefore, simple and rapid *in vitro* methods for studying these reactions have been developed [2].

In Chapter 2 we have demonstrated the ESA thin film fabrication technique and the characterization of multilayer thin films made from various biocompatible materials. In this chapter we present our results of some *in vitro* biocompatibility testing of ESA multilayer thin films. Our tests involve contact angle surface characterizations and *in vitro* measurements of protein adsorption.

The effect of water wettability of biomaterials is considered very important in the interactions of biological species with biomaterial surfaces [3]. The contact angle is a very useful inverse measure of wettability, and can be obtained by using contact angle goniometry.

Protein adsorption is the first step that occurs when a foreign surface is placed in contact with the blood. Because it is believed that these events influence subsequent interactions, we are attempting to study this protein adsorption phenomenon in real time as a preliminary step of biocompatibility testing. Fourier transform infrared reflection absorption spectroscopy (FT-IRAS) has been used to characterize the thin protein layers adsorbed onto our ESA biocompatible films.

3.1 Contact angle surface characterization

Contact angles, which provide a measure of relative surface energies, have long been thought to provide some insight into the potential biocompatibility [4]. Contact angle itself is not an indicator of biocompatibility of materials, but some possible correlations have been found between the protein adsorption behavior and the water contact angle of material surfaces [5].

Studies [3] have shown that protein adsorption and cell adhesion are enhanced on a surface with a moderate contact angle (in the range of 40°~65°). If the surface were extremely hydrophilic (water contact angle is less than 30°) or extremely hydrophobic (water contact angle is larger than 80°), protein adsorption and cell adhesion would be prohibited. Thus, from contact angle surface characterization, some useful information may be obtained for the research that relates surface wettabilities to blood- or tissue-compatibility.

3.1.1 Contact angle and surface wettability

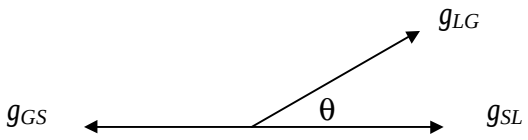


Figure 3.1. Contact angle and the surface tension components.

A basic expression necessary for the understanding of certain surface phenomena is the Young-Dupree or contact angle equation [6] (equation 3.1). Consider a drop of liquid on a solid substrate, characterized by:

$$g_{GS} = g_{SL} + g_{LG} \cos\theta \qquad \text{(eq. 3.1)}$$

Here $g_{GS,}$ $g_{SL,}$ and g_{LG} are the surface tensions (or surface energy) on a, solid-liquid interface, and θ is the contact angle of the liquid with respect to the solid substrate surface. The wetting characteristic of the surface can be described as follows

$\theta = 0$ complete wetting,

$0 < \theta < 90°$ partial wetting, and

$\theta > 90°$ non-wetting.

So the contact angle is a very useful inverse measure of wettability, as a smaller contact angle implies smaller surface tension, but higher surface wettability.

3.1.2 Contact angle measuring method

The measurement of water contact angle for 30-bilayer ESA thin films on quartz substrates were performed at room temperature and under static conditions using a contact angle goniometer (Rame-Hart, Inc.). Contact angles were measured at three different locations on each specimen. For each location, a 10μl Milli-Q water drop was deposited onto the surface, and the data was collected 30 seconds after the contact. The standard deviation between different specimens was within ± 4.0°.

3.1.3 Results from ESA multilayer thin films

Table 3.1 gives the water contact angles measured for our ESA thin films. It is known that surfaces with a higher concentration of oxygen atoms exhibit higher wettability (smaller contact angle) [3]. For polymer materials, if there were more oxygen-based polar functionalities (i.e., -OH, -C-O-, and -COO-) incorporated into the surfaces, the wettability would be higher. This is consistent with our experimental results. The assembly pairs like Al_2O_3/PMA, ZrO_2/PMA, and TiO_2/PMA all have water contact angles less than 30°, because of the high oxygen concentration caused by the –COO– groups in PMA and the oxygen atoms in the metal oxide particles. On the contrary, PVP, PDDA, and PEI do not have those oxygen-based functional groups, and this may be the reason why the three polymer assembly pairs they are incorporated into did not show good wettabilities.

Table 3.1. Contact angles of ESA multilayer films.

MULTILAYER ASSEMBLIES	WATER CONTACT ANGLE θ (°)
PVP/PMA	45 ± 4
PDDA/C_{60}	52 ± 4
PEI/PMA	55 ± 4
Al_2O_3/PMA	18 ± 4
ZrO_2/PMA	25 ± 4
TiO_2/PMA	28 ± 4

These results from the measurements of contact angles provide important information about the surface characteristics (i.e. surface energy) which are believed to influence the interactions between blood (or tissue) and the biomaterial surfaces. And, for our investigation of protein adsorption on ESA thin films in the next section, these contact angle data could be used as a reference when we consider the possibility of the surface energy influencing protein-surface interaction.

3.2 In vitro measurements of protein adsorption

Proteins are one of the most important classes of functional units in living organisms, e.g., as structural building blocks of tissue, as vehicles for transport of elements such as oxygen and CO_2, and for the catalytic-enzymatic processes that are central to life [7].

Currently, the adsorption behavior of proteins on various surfaces is an area of intensive research because of its relevance to biotechnology and medicine. Protein surface adsorption is the first step occurring after a foreign material, such as an implant, is brought into contact with the living

body. It plays an important role in processes such as protein binding to cell surface receptors, bacterial cell adhesion to surface, solid-phase immunoassys, fouling of food processing equipment, and protein adsorption to bioprobes. So it influences the biocompatibility of the implants essentially [8].

Biomaterial surfaces that are considered to be compatible with the biological systems in contact can be classified into two groups: non-stimulative (the least foreign-body reaction) surfaces and bioadhesion surfaces. The first group of surfaces is defined with the assumption that, if the material surface does not interact with the living body at all, in other word, if it does not adsorb any plasma protein then it must be biocompatible. A good example of this is contact lenses: protein adsorption on the lenses should be minimized to avoid clouding during long-term wear. So, various hydrogels have been used to make contact lenses because of their excellent feature of prohibiting protein adsorption.

A bioadhesion surface [5], from the second group, refers to a surface that facilitates a microscopic bonding between a biomaterial and a biological structure. Well-known examples include composite resins used in dentistry and bioactive ceramics for bone replacement. In this case, if the biomaterial is entirely covered with a dense and stable layer of proteins and cells, this material can acquire excellent interfacial biocompatibility.

As we have seen, for different purposes in medical applications, protein adsorption on biomaterial surfaces, either prohibited or enhanced, provides an insight into the basis for blood- or tissue-compatibility at the molecular level. *In vitro* evaluations of protein adsorption on biomaterial surfaces have been prescribed by the National Institutes for Health *Guidelines for Blood-Material Interactions* as a partial indicator of biocompatibility [9].

3.2.1 Tool for the investigation of protein adsorption: FT-IRAS

Today there exist a broad range of infrared spectroscopic techniques suitable for the characterization of ultra thin organic layers on solid supports. The infrared reflection-absorption spectroscopy (IRAS) is an external reflection technique especially useful for the characterization of organic thin films on highly reflecting (non-transparent) solids such as metals and doped

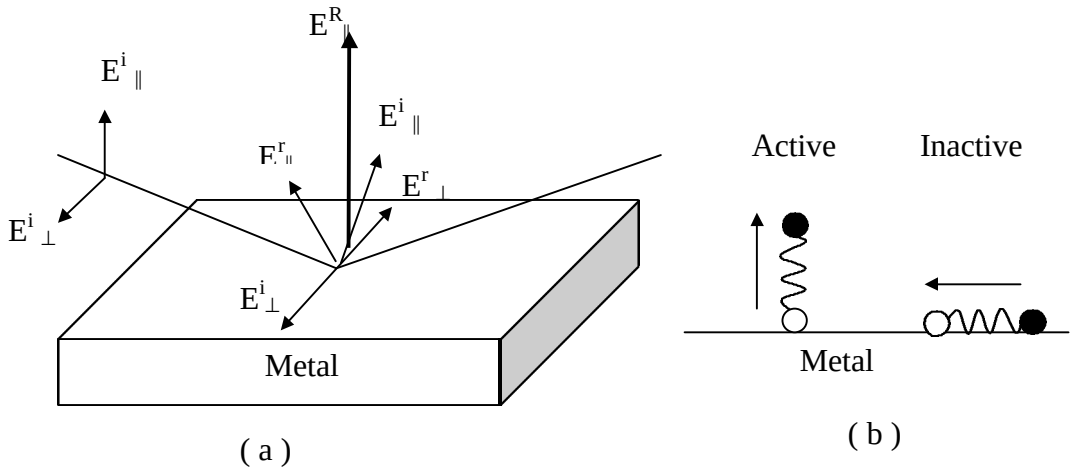
semiconductors. This technique was initially demonstrated in the late 1950s by Francis and Ellison [10] and was further developed in the 1960s by Greenler [11]. With the rapid development of stable and sensitive Fourier transform infrared (FTIR) spectrometers, IRAS has been developed into a routine technique in many surface physics/chemistry laboratories, and has contributed to the deeper understanding of various interfacial phenomena.

Due to its orientation sensing quality, IRAS is very often used for the characterization of highly organized and anisotropic monolayers and ultra thin films [2]. The sensitivity of IRAS is very high, typically 0.1-1 monolayers, depending on the molecular system, making it possible to study adsorbate-monolayer interaction phenomena [2].

IRAS is a direct reflection technique, similar to ellipsometry. The majority of IRAS experiments are performed in the mid-infrared region, 4000-400 cm^{-1} (λ = 2-25 μm). A molecule will generally be able to absorb infrared radiation if the molecular vibration is associated with a simultaneous change in dipole moment. In addition to this fundamental “selection rule”, Francis and Ellison [10] predicted that, in order to be infrared active, the molecule needs to have a component of its dipole moment change (transition moment) normal to the metal surface. This is due to the fact that the perpendicular component of the radiation undergoes an phase shift of approximately 180° for all angles of incidence upon a direct reflection, i.e., the electric field has a node at the surface for the perpendicular component, and, accordingly, no net electric field can interact with the oscillating dipoles at the metal surface and thus no adsorption can be observed. The parallel components, however, do not cancel because of the finite phase shift between the incident and the reflected components upon reflection. Therefore, a nonzero electric field normal to the metal surface exists (Figure 3.2), and only vibrational modes with transition moment normal to the metal surface can be excited by the infrared radiation. This requirement for the excitation of adsorbed molecules on metal surfaces is normally summarized in the “surface selection rule”[2], a unique and extremely important feature of IRAS, and it can be used to extract valuable information about the orientation of adsorbed molecules or groups with respect to the metal surface [12].

Infrared spectroscopy has been extensively used in studies concerned with the interaction between biological macromolecules such as proteins and foreign artificial materials [IV]. Protein

adsorbed on a material surface would show its feature absorbance band in the IRAS spectra. Some researchers [2] have found that there is an almost linear relation between the integrated area under the absorbance peak and the amount of molecules adsorbed on the surface. Thus, IRAS can be used as a tool to obtain semi-quantitative information about adsorbed thin layers of proteins on a highly reflective biomaterial surface.



(a) The electric field at the metal surface for both parallel and perpendicular polarization of the infrared radiation. The resulting electric field normal to the metal surface is indicated by $E^R_{\parallel}$. The electric field in the plane of the metal surface is almost zero, since $E^i_{\perp} = - E^r_{\perp}$

(b) Two hypothetical dipoles adsorbed in different orientations on the metal surface. The "surface selection rule" allows only the vibration with dipole moment change normal to the metal surface to be infrared active. The arrows indicate the direction of the transition moment

Figure 3.2. Infrared radiation and molecules on a metal surface.

3.2.2 Protein adsorption procedure on ESA multilayer films

We now describe the *in vitro* measurements of protein adsorption on our ESA thin films. Our procedure consists of three aspects:

i) Protein solutions

Bovine serum albumin (BSA) is commonly used as a model blood plasma protein and was chosen in our work. The BSA obtained from Alfa Aesar Chemical was used as received, without any purification or characterization. A 15mg/ml solution of BSA in phosphate-buffered saline (PBS) was stored at +4°C and used within 3 hours. The PBS was also purchased form Alfa Aesar, and its pH value is about 7.4.

ii) Adsorption procedure

Thirty bilayer ESA thin films were deposited on the surface of a piece of gold-coated glass (1 inch × 1 inch). The samples were mounted at their top ends in a lid made to cover a 400 ml beaker. The beaker was then filled with fresh protein solution, and placed in a water bath, 37 ± 1°C (the same temperature as the human body). Magnetic stirring was performed at 100rev/min. BSA was then adsorbed for 60 minutes.

Since water rinsing was necessary in order to remove buffer salts, which would otherwise crystallize on the sample surface and interfere with the protein spectra, the samples were rinsed in Milli-Q water for a few times after the adsorption. Then they were dried in nitrogen gas.

iii) Instrumentation

The infrared spectra were recorded with a FTS 6000 spectrometer (Bio-Rad Laboratories, Inc.), equipped with a VeeMax variable angle specular reflectance accessory. Higher detector sensitivity was achieved by using a high-sensitivity, narrow-range, liquid-nitrogen-cooled mercury-cadmium-telluride (MCT) detector. An opening on the top of the spectrometer detector compartment is used for filling liquid nitrogen into the MCT dewar using the funnel supplied by Bio-Rad. All experiments were carried out at a scan speed of 5 KHz and a constant resolution of $4cm^{-1}$.

3.2.3 Infrared spectra of adsorbed protein layer

We investigated the protein adsorption behavior on six different ESA multilayer assemblies: PVP/PMA, PDDA/C_{60}, PEI/PMA, Al_2O_3/PMA, ZrO_2/PMA, and TiO_2/PMA. The IRAS spectra were recorded before and after the albumin adsorption for each sample. Then the spectra before the adsorption were subtracted from the spectra obtained afterwards, and the subtracted spectra give the net result of protein adsorption on the film surface. The increase in the area under the absorbance peak can be used to determine the adsorbed amounts, and any changes in the shape of the peak can be correlated to structural changes in the molecules of the adsorbed layer.

Three of the assembly pairs, PVP/PMA, PDDA/C_{60}, and PEI/PMA, showed the albumin amide absorbance peak in the subtracted infrared spectra at the frequencies around 1580 cm^{-1} ~ 1630 cm^{-1}. But this peak was not observed in the other three assemblies (Al_2O_3/PMA, ZrO_2/PMA, and TiO_2/PMA), which indicates that no detectable albumin adsorption had occurred on those surfaces. Figure 3.3 gives the Amide band intensity from the IRAS spectra after 1-hour albumin adsorption on different ESA thin films.

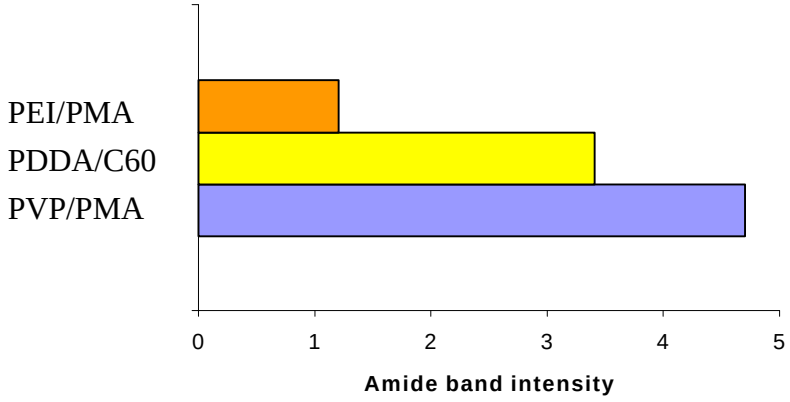


Figure 3.3. The amide band intensity of protein layers from different ESA thin films.

Since the amide band intensity has been found to be almost linear with the total amount of surface-bound protein, this chart indicates that, among the three films which did adsorb certain amount of albumin, PVP/PMA ESA film surface adsorbed the highest amount, and PEI/PMA adsorbed the least.

In the spectra obtained from the same surface, the amide bands with different adsorption time are almost at the same position. However, the amide band position showed obvious shifts in the spectra obtained from different surfaces. Table 3.2 lists the Amide frequencies for the adsorbed albumin on various ESA-formed thin film surfaces.

Table 3.2. The amide frequency for bovine serum albumin on various ESA thin film surfaces.

SURFACES	AMIDE FREQUENCY (cm^{-1})
PVP/PMA	1628
PDDA/C_{60}	1588
PEI/PMA	1593

The amide band of albumin layers are shifted in frequency upon adsorption on ESA thin films. Similar behaviors of this shift of amide frequency after adsorption have been observed previously for a large number of proteins [13]. The shifts are probably caused by the conformational change after the protein was adsorbed onto the solid surfaces [12].

The following IRAS spectra were obtained, with different adsorption time, for the albumin adsorbed on the three ESA films which showed amide band. Figures 3.4, 3.5 and 3.6 give the subtracted albumin IRAS spectra from PVP/PMA, PDDA/C_{60}, and PEI/PMA film surface, respectively.

These spectra with the frequency range of 1400 cm^{-1} ~ 1900 cm^{-1} are parts of the entire IRAS spectra in absorbance mode, showing the amide absorbance peak around frequency of 1600 cm^{-1}. The spectra indicate that there are some differences of the shape and position of the albumin amide band on different ESA film surfaces. These may correlate to the different protein conformational change when it is adsorbed onto the film surfaces.

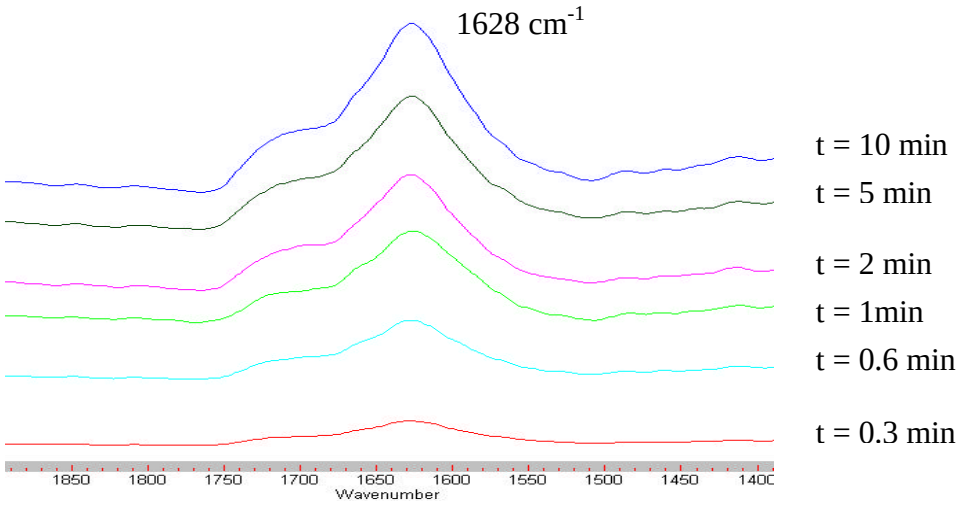


Figure 3.4. The infrared spectra of albumin adsorbing onto PVP/PMA with time.

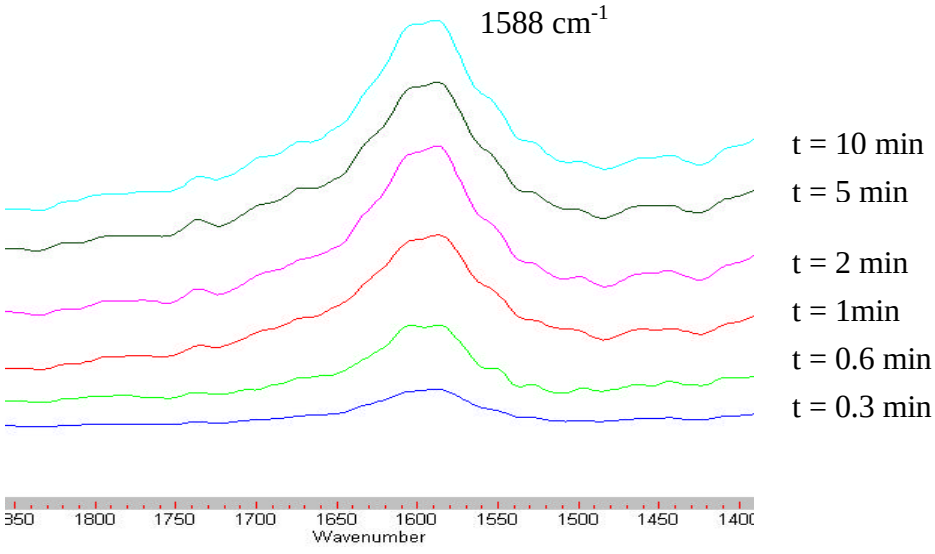


Figure 3.5. The infrared spectra of albumin adsorbing onto PDDA/C_{60} with time.

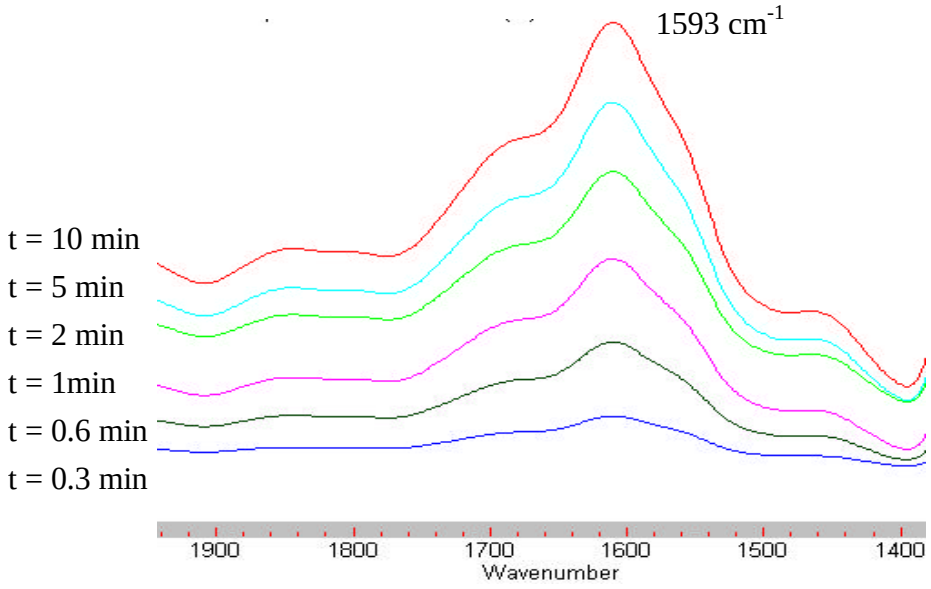


Figure 3.6. The infrared spectra of albumin adsorbing onto PEI/PMA with time.

Kinetic studies, obtained by plotting individual IR band intensities versus time, can be used to graphically demonstrate the amount of total protein adsorbed onto the surface. Figures 3.7, 3.8 and 3.9 are the kinetic plots of albumin adsorption onto PVP/PMA, PDDA/C60, and PEI/PMA, respectively.

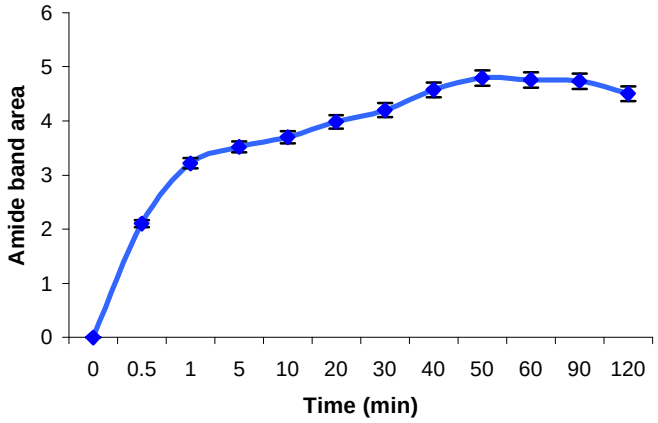


Figure 3.7. Plot of amide band area vs. time for PVP/PMA film surface.

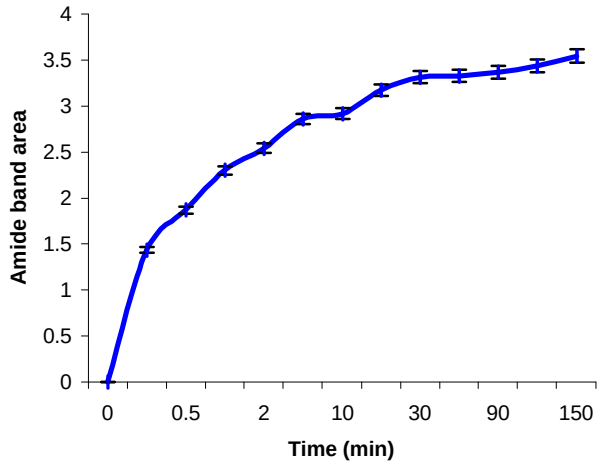


Figure 3.8. Plot of amide band area vs. time for PDDA/C_{60} film surface.

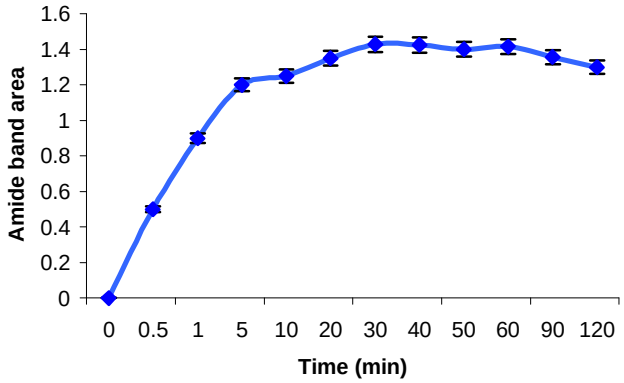


Figure 3.9. Plot of amide band area vs. time for PEI/PMA film surface.

The amide band intensities were calculated from the integrated areas under amide band absorbance peaks in each spectra. As one can see clearly, on PVP/PMA and PDDA/C60 film surfaces, initial albumin adsorption is extremely rapid for approximately 30 seconds after which it begins to slow down. PEI/PMA film surface adsorbed albumin at a slower speed than the other two surfaces, also, the amount of adsorbed albumin was also much less. After approximately 5-10 minutes, the adsorption nearly levels off and this low rate of adsorption has been found to last for several hours.

The theoretical model of protein adsorption on solid surfaces is a diffusion controlled process. The amount of protein present on the surface Λ is proportional to the concentration of that protein in solution C, and the square roots of the protein diffusion coefficient D, and time t, at least during the initial stages of the adsorption process:

$$\Lambda \alpha C (D t)^{1/2}$$

After a certain time, most area of the solid surface has been covered with protein molecules, the adsorption is near saturation, and the adsorbed amount increases very little with the time increasing.

The result that Al_2O_3/PMA, ZrO_2/PMA and TiO_2/PMA film surfaces did not adsorb detectable amount of albumin is probably due to the effect of surface wettability. Studies [5] have shown that protein adsorption and cell adhesion were enhanced on a surface with a moderate wettability, but were prohibited on some hydrophilic surfaces. These three film surfaces are all hydrophilic surfaces (contact angles are less than 30°), while the wettabilities of the PVP/PMA, PDDA/C_{60}, and PEI/PMA surfaces which adsorbed certain amount of albumin are in the moderate range (contact angles are about 40° - 50°). Another possible reason is the presence of amino groups on the PVP/PMA, PDDA/C_{60} and PEI/PMA film surfaces. Whether the amino group promotes protein adsorption is still not clear, but this may be a possibility for the enhancement of protein adsorption on biomaterial surfaces from the aspect of modifying surface chemical components, if the answer is positive after more investigations are included.

3.3 Summary

Simple and rapid *in vitro* methods for studying the interactions between biomaterials and biological systems have been developed, and they are also believed to be of great significance to the understanding of the *in vivo* behavior of implants. Contact angle surface characterization and *in vitro* protein adsorption measurements were performed on our ESA multilayer thin films as preliminary biocompatibility testing methods. FT-IRAS spectra were used to obtain the relative

amounts of adsorbed protein, and to analyze the kinetic adsorption behavior on certain film surfaces. The ESA film surfaces which have high wettability (Al_2O_3/PMA, ZrO_2/PMA, and TiO_2/PMA) prohibited protein adsorption onto the surfaces. Other surfaces possessing a moderate wettability (PVP/PMA, PDDA/C_{60}, and PEI/PMA) rapidly adsorbed a certain amount of protein in the first several minutes after contact, and the protein layers were stable over a long period of time. While surface wettability is a possible factor influencing protein adsorption on biomaterial surfaces, more investigations are needed to determine the role of the amino group in this adsorption behavior.

3.4 References

1. R. M. Gendreau, S. Winters, R.I. Leininger, D. Fink, C. R. Hassler, R. J. Jakobsen, "Fourier Transform Infrared Spectroscopy of Protein Adsorption from Whole Blood: Ex vivo Dog Studies", *Applied Spectros*copy **35** (4): 353-357 (1981).

2. Pentti Tengvall, Ingemar Lundstrom, Bo Liedberg, "Protein adsorption studies on model organic surfaces: an ellipsometric and infrared spectroscopic approach", *Biomaterials* **19**: 407-422 (1998).

3. Jin Ho Lee, Hai Bang Lee, "A wettability gradient as a tool to study protein adsorption and cell adhesion on polymer surfaces", *J. Biomater. Sci. Polymer Edn*. **4**: 467-481 (1993).

4. J. Black, *Biological Performance of Materials,* Marcel Decker, NY, USA, pp102-115 (1981).

5. Y. Ikada, "Interfacial Biocompatibility", *Polymers of Biological and Biomedical Significance* **540**: 35-48 (1994).

6. J.D. Andrade, "Interfacial phenomena and biomaterials", *Medical Instrumentation* **7** (2): 110-120 (1973).

7. Bengt Kasemo, "Biological surface science", *Current Opinion in Solid State & Materials Science* **3**: 451-459 (1998).

8. Fen-ni Fu, Michael P. Fuller, Bal Ram Singh, “Use of Fourier Transform Infrared/Attenuated Total Reflectance Spectroscopy for the Study of Surface Adsorption of Proteins”, *Applied Spectroscopy* **47** (1): 98-102 (1993)

9. National Heart, Lung, and Blood Institute Working Group, *Guidelines for Blood-Material Interactions*, National Institutes for Health (Publication No. 85-2185), Bethesda, MD, USA, 1985.

10. S.A. Francis, AH Ellison, “ Infrared spectra of monolayers on metal mirrors”, *J. Opt. Soc. Am.* **49**: 131-139 (1959).

11. R.G. Greeler, “Reflection method for obtaining the infrared spectrum of thin layer on a metal surface”, *J. Chem. Phys.* **50**: 1963-1968 (1969).

12. B. Liedberg, B. Ivarsson, I. Lundstrom, W. R. Salaneck, “Fourier transform infrared reflection absorption spectroscopy (FT-IRAS) of some biologically important molecules adsorbed on metal surfaces”, *Progress in Colloid & Polymer Science* **70**: 67-75 (1985).

13. J. L. Koenig, DL Tabb in Durig JR (ed) *Analytical Applications of FT-IR to Molecular and Biological Systems.* Nato Advanced Study Institutes Series C57. D. Riedel Publishing Company, Dordrecht Boston London, p 241 (1979).

CHAPTER 4

Conclusions and Future Work

The novel and low-cost electrostatic self-assembly (ESA) method for the fabrication of ultra thin films can be adapted to incorporate a wide variety of molecules, including metallic oxide nanoparticles, polymers, and biological molecules. The ESA multilayer thin films are water-stable, highly uniform, and have a well-controlled surface composition. This makes ESA thin films an excellent choice for use in applications covering a large variety of fields.

In this thesis work, we demonstrated the feasibility and effectiveness of the ESA technique for the fabrication of ultra thin multilayer assemblies from various biocompatible materials including metal oxides and polymers. These materials were successfully fabricated into ESA multilayer films by an alternate adsorption process with appropriate assembly matching under optimal processing conditions. The uniformity, thickness, layer-by-layer linearity, and surface morphology of the multilayer assemblies were characterized by UV/vis spectroscopy, ellipsometry, and AFM imaging. These characterizations confirmed that the ESA thin films have a dense, stable, homogeneous nanoscale structure, and a well-organized layer-by-layer thickness growth. These ESA multilayer assemblies can be used to modify surface properties of metals, polymers, and many other substrates, which is very useful in the design of biocompatible synthetic surfaces.

Some preliminary *in vitro* biocompatibility tests were performed for ESA multilayer assemblies. Contact angle surface characterization was used to measured the wettability of the different film surfaces, and Fourier Transform Infrared Reflection-Absorption Spectroscopy (FT-IRAS) was used for the investigation of protein adsorption behavior on these surfaces. Some hydrophilic film surfaces showed protein prohibition, while a certain amount of protein adsorption was

observed from the IRAS spectra for some surfaces with a moderate wettability. Kinetic studies implied that the protein adsorption occurred within 30 seconds on these ESA multilayer films, and continued at a rapid rate until the adsorption nearly levels off around 5-10 minutes. The adsorbed protein layers were stable in the PBS solution within a long period of time.

The reason why some surfaces resist protein adsorption may be correlated to the surface wettability. It has been well documented that hydrophilic surfaces generally prohibit the adsorption of various proteins, and also decrease the subsequent cell/tissue adhesion. These surfaces may be used in applications where cell adhesion is not desired, such as for small diameter artificial blood vessels and blood dialysis membranes. On the other hand, those surfaces promoting rapid adsorption of proteins may be very useful for the surface modification of implants used for teeth, bones, and soft connective tissues, where the cell adhesion is desired.

This thesis work can be considered as a preliminary step in the application of ESA technique for the fabrication and testing of biocompatible thin films. Future work on this topic may include the following.

- Quantitatively determine the amount of adsorbed protein on ESA multilayer films using other analysis tools such as ellipsometry, and compare them with other thin film coatings fabricated by alternative techniques.

- More research work is needed to investigate the role of amino group in the protein-surface interactions. We observed its presence on all the ESA film surfaces that adsorbed protein, so there may be a possibility for controlling protein adsorption through the modification of material surfaces with some special function groups.

- Cell attachment and cell adhesion involve biocompatibility at a higher level than protein adsorption, and more direct and accurate results may be obtained through these experiments. *In vitro* cell attachment and adhesion experiments can be performed on ESA thin film coatings, while AFM can be used to monitor the cell culture procedure and data collecting under cell living conditions.

VITA

Weiwei Du was born on March 17, 1974. She attended the First High School of Hefei, Anhui Province, in China. Graduated in 1991, she was admitted to Tsinghua University in Beijing, China, where she completed her Bachelor's degree in Material Science and Engineering. In the fall of 1998, Weiwei came to Virginia Tech to pursue her Master's degree. She studied in the Department of Material Science and Engineering and did research at the Fiber & Electro-Optic Research Center under the supervision of Dr. Richard O. Claus.