SURFACE MODIFICATION OF POLYMERIC BIOMATERIALS FOR CELL/TISSUE INTEGRATION

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CERTIFICATION

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A THESIS APPROVED BY THE THEORETICAL & APPLIED PHYSICS DEPARTMENT

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ABSTRACT

This thesis presented the surface modification of polydimethyl-siloxane (PDMS) to enhance surface wettability and surface roughness. Physical and chemical modifications were performed on cured surfaces of PDMS and PDMS-based nanocomposites. Firstly, physical modifications were done by direct coating of PDMS-based substrates with biopolymers such as polyethylene glycol (PEG), poly lactic-co-glycolic acid (PLGA) and sodium alginate (SA). On the other hand, coatings were done with the biopolymers (PEG, PLGA, and SA) on pre-stretched PDMS-based substrates. Coated and uncoated, as well as treated surfaces of PDMS and PDMS-based nanocomposites, were characterized by aproscope optical microscope. Substrates wettability were characterized through contact angle measurements. The contact angle measurements were used to determine the hydrophilicity of the coated substrates. Coated PDMS-based substrates with SA have a contact angle of 12° showing high hydrophilicity and adhesiveness, followed by PEG with a contact angle of 25°, whilst PLGA recorded a contact angle of 42.2° for PDMS (10:1). For PDMS (40:1), biopolymer coatings also show a SA contact angle of 18.2 followed by PEG with a contact angle of 26 and the PLGA contact angle was 32.2°. There was a significant difference in the contact angle with the pre-stretched coatings. A pre-stretch contact angle of 8.5° was recorded for PEG, whilst SA was 36.7° for PDM (Fe_3O_4)10:1. Similarly, the contact angle for a pre-stretched PEG coating was 14.4 and SA was at 22.1° on PDM (Fe_3O_4) 20:1. SA coating was good for direct physical coating, whilst a PEG coating was good for pre-stretched coating. Also, the chemical modification improved whilst the boiling time decreased the contact angle as a result of creating a -OH bond. The PDMS-magnetite composites were found to induce heating for hyperthermia treatment. The surface's wettability and roughness were then discussed to determine the implications of substrates to be used for cell culture. The modified surfaces gave indications of how cells would grow or how substrates would integrate in body tissues.

Keywords: Surface modification, polymeric biomaterials, tissue integration, contact angle, chemical modification.

ACKNOWLEDGEMENT

"But seek ye first the kingdom of God, and his righteousness; and all these things shall be added unto you." Matthew 6:33

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God bless you all.

DEDICATION

This work is dedicated to God the Father, the Son, and the Holy Spirit for the strength, knowledge, support and cares.

Also,to my family, especially my parents for their prayers and encouragement.

Finally, to my supervisor Dr. Shola Odusanya and co-supervisor Dr. Danyuo Yiporo for their concern and advice that made this thesis a reality, and to all the Theoretical and Applied Physics Department.

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Table of Abbreviations

AFM	Atomic force microscopy
IARC	International Agency for Research on Cancers
OH	Of hydroxyl
PBS	Phosphate buffer saline
PEG	Polyethylene glycol
PLGA	Poly lactic-co-glycolic acid
SA	Sodium alginate
SEM	Scanning electron microscope
XRD	X-Ray Diffraction
BDH	
DCM	Dicloromethane
DI	De-oinized
ECM	Extracellular matrix
EDS	Energy dispersive detector
FTIR	Fourier Transform Infrared
HCL	Hydrocloric Acid
PAMI	Pan- African Material Iinstitute
PDMS	Polydimethylsiloxane
PVP	Polyvinylpyrrolidone
R.O.C	
SHELLAB	Sheldon Manufacturing

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CHAPTER ONE

1.1 INTRODUCTION

Cancer is the uninhibited growth of unusual cells in the body. Cancer presently remains the next prominent cause of death after heart disease [1, 2]. Research has revealed that there were 10 million new cases, 6 million deaths, and 22 million people living with cancer globally in the year 2000[3]. These statistics denote a rise of about 22% prevalence and death from that of the year 1990 [4]. It was estimated that the figure of new cases of all cancers globally would be

12.3 and 15.4 million in the year 2010 and 2020, correspondingly [3]. The treatment of cancer has drawn the attention of many physicians and scientists trying to fashion out treatment methods that can help solve the cancer problem.

1.2 CURRENT CANCER TREATMENT METHODS AND CHALLENGES

A present treatment method such as chemotherapy is a kind of cancer cure that administers drugs to kill cancer cells in the body. Chemotherapy does not only kill fast-growing cancer cells, but also kills or slows the progress of healthy cells that develop and divide rapidly. Damage to cells can lead to severe side effects, such as mouth sores, nausea, and hair loss [5]. Radiation therapy is another cancer cure that practices high dosages of radiation to destroy the uninhibited growth of unusual cells in the body and shrink tumors [6]. Radiation kills or slows the advancement of uninhibited growth of unusual cells in the body, and it can also affect neighboring sound cells [6]. Injury to healthy cells can cause side effects such as fatigue, which is, feeling exhausted and worn out [6].

Surgery, when used to treat cancer, is a procedure in which a physician eliminates tumors in a patient's body [5]. The above cancer treatment techniques with others such as home therapy, liposomes have severe side effects [6] and are costly for an average African [1]. This therefore calls for alternative treatment methods such as using microfluidic devices for localized drug delivery. However, issues of biocompatibility and surface modification of biomaterials are of great concern.

1.3 BIOMATERIALS

Medical implants can serve as life-saving and restoring means to the health of cancer patients [6]. A biomaterial can be defined as any material used to create a device to replace a part or a role of the body in a harmless, dependable, cost-effective, and functional satisfactory way. Materials that are used for biomedical or medical uses are well-known as biomaterials. The key classes of biomaterials comprise; metals, ceramics, polymers, and composite materials [7]. Such materials are used as molded or machined parts, coatings, fibers, films, foams, and fabrics. Below is a chart on biomaterials summarized in **Figure1**.

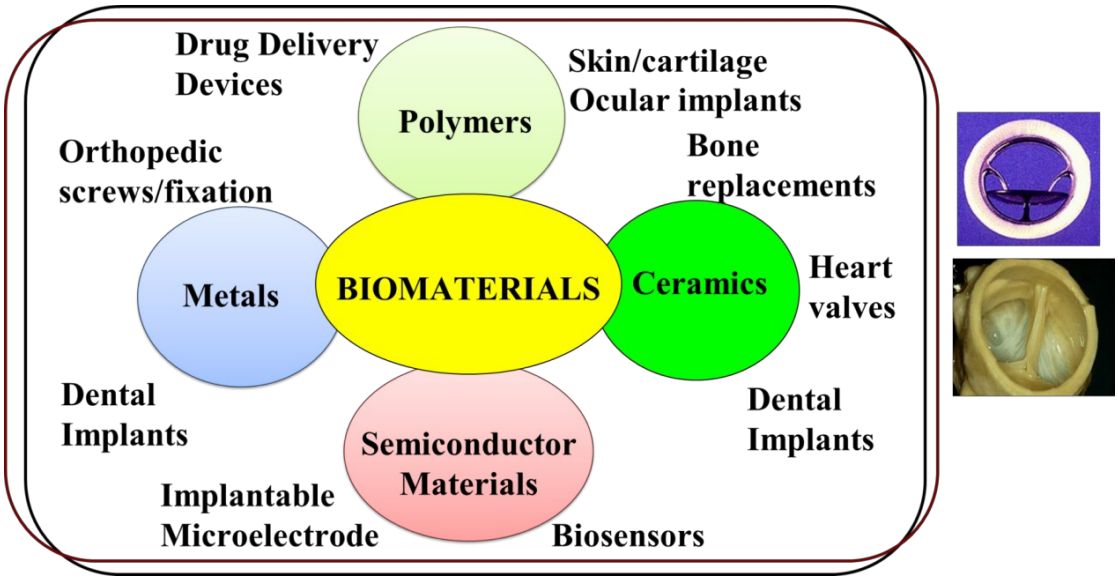


Figure Summary of biomaterials

1.4 REQUIREMENTS OF BIOMATERIALS USED AS IMPLANTS

Medical implants often do not adapt to the body tissues and sometimes are reject as foreign objects. However, our body can be trapped with the exact kind of material to make it compatible with biomaterial. The surface of an implant can be altered in several ways, enclosing; plasma modification [8]; ozone treatment [9], chemical modification [10], physical modification [11] and spread on coatings to the substrate [8]. Surface modifications can be used to disturb surface energy [12]; measure the disturbance of intermolecular bonds that arise when a surface is formed [12], and interface adhesion [13]. Adhesion is the affinity of different atoms or faces to adhere to one another [14].A biomaterial is therefore expected to possess the qualities of biocompatibility, chemical inertness, lubricity, sterility, asepsis, thrombogenicity, vulnerability to corrosion, deprivation, and hydrophilicity [15]

Implantable materials should confirm the biocompatibilities of material to perform with a suitable host reaction in a particular application [16].Thus, biocompatibility comprises the approval of a man-made implant by the neighboring tissues and by the entire body. A biocompatible implant, therefore, should not, infuriate the surrounding structures [17], incite an unusual provocative response [18], incite allergic reactions [19], and must be cancer free [20]. Other compatibility characteristics that may be important in the function of an implant device prepared as a biomaterial are sufficient mechanical properties such as strength; stiffness, and fatigue properties, suitable visual assets if the material is to be used in the eye, skin, or tooth and appropriate density [21].

1.5 PROBLEM STATEMENT

Bio-integration is an absolute requisite for a man-made implant. The connections at the interface between an implant and the host tissues should be treated to guarantee that they do not prompt any harmful effects such as prolonged inflammatory reaction or the establishment of abnormal tissues [22]. Among biomaterials, maximum polymeric materials fulfil the necessities to be used for biomedical usage. Polydimethylsiloxane (PDMS) has been used in the production of maximum biomedical devices comprising drug delivery devices [22]. However, most polymeric biomaterials such as PDMS are restricted in most cases by the nonsufficient bio-connection properties of the polymer [22]. Surface modification creates nanosize-microsize layers with controlled chemical structure, structure and roughness, and hydrophilic/hydrophobic balance emerge as a simple, useful, and adaptable method to answer the problem [23]. Knowing the process of cell surface interaction is very vital for the scheme of polymeric implants surfaces with enhanced bio-interaction possessions to improve cancer cure.

1.6 MOTIVATION OF RESEARCH

The motivation for this thesis was derived from the Soboyejo Group Focus. The group aimed at developing a multi-modal implants device for drug delivery. This drug delivery device has its surfaces made up of polydimethylsiloxane (PDMS). The problem of this PDMS is its hydrophobic surface. Therefore several surface modifications techniques need to be applied to overcome this problem of hydrophobicity. Possible modifications include making the surface hydrophilic by using oxygen-plasma [24], or bonding functional groups to the surface capable of supporting the home-made proteins, antibodies and mammalian cells [25]. The advantages of bonding functional groups to the surface overcome a constant problem with modified PDM surfaces in that; they tend to recover their original hydrophobicity [26].

1.7 GOAL OF WORK

This work will focus on the patterning and wettability of PDMS to control cell adhesion. Given this, several surface modification techniques will be employed to test the effect of cell growth on modified PDMS surfaces. The modified surfaces will guide the development of implantable biomedical devices for both hyperthermia and controlled drug delivery for cancer treatment.

1.8 SCOPE OF WORK

In order to meet the above goal, the research is to be carried out in the following steps:

- Polymerization of PDMS slabs will be done along with coating of substrates with various approaches (polyethylene glycol, sodium alginate, polylactic glycolic acid).
- Other surface modifications such as boiling in deionized water will also be investigated.
- Coating with a thin layer magnetite nanocomposite (made of PDMS:Fe_3O_4) to serve for both cell adhesion and also to ensure hyperthermia cell death.
- Pre-stretch effect and surface coating on PDMS substrate.
- Study the wettability of the PDMS modified surface using drop-water contact angle measurement.
- Breast cancer cells will be cultured to study cell viability and cell attachment on the various modified surfaces.
- The modified surfaces will be characterized through various techniques including:
- Fourier Transform Infrared (FTIR) Spectrophotometer to determine the functional groups on the modified PDMS,
- X-Ray Diffraction (XRD) techniques to check the crystallinity of the PDMS and also the chemical elements present in the modified surfaces,
- Use scanning electron microscope (SEM) to examine the morphologies of the modified substrate. EDS on the SEM can also be used to study the elementary analysis qualitatively.
- The implications of the results will be discussed for the design of an implantable biomedical device, with the right surface modification, for tissue integration.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

This thesis focused on the use of polydimethyl siloxane (PDMS) to fabricate microfluidic devices for cancer drug delivery [1-3]. The use of PDMS in the current devices has been confronted by its hydrophobic nature which prevent the growth of cells [2]. There are a lot surface modification methods.Small and nano-technology based bio-analytical systems require constant progressive and transformed biomedical research fields, which offer many advantages comprising small structure sizes [4-6]. Specifically, PDMS has been progressively engaged for the production of these micro-devices used equally as cell culture stages.

The PDMS has a well-known demand over other materials owing to its physical attributes, optical transparency, durability, and flexibility. This chapter presents a review of earlier works concentrating on PDMS substrates. The result from previous works on the surface modification and mechanical properties of PDMS will also be presented. Wettability and contact angles measurement methods will also be revised.

2.2 POLYDIMETHYL SILOXANE (PDMS) SUBSTRATES

PDMS as a silicone elastomer has established much care as a popular material for developing substrate stages in mechanobiological [7-10] and microfluidic uses, [11-13] owing to its various benefits above other fabrication materials. The outstanding features of PDMS give rise to varied applications for example its tunable elastomeric properties, cheap, gas permeability, optical transparency, durability-, flexibility and it surface can be modified when fabricated [14- 16].

Though, the use of PDMS for cell culture frequently poses numerous challenges over continuing studies, its inherent high surface hydrophobic nature has been known by numerous research to be the primary factor that causes poor cell adhesion, creating and disconnecting bases of cell aggregates [17-19]. This constraint frequently hinders the usage of PDMS as a substrate material for cell culture lacking any additional surface treatments [20]. In biomedicine, PDMS substrates are used mostly for encapsulation of drugs [21]. Consequently, it is highly required to progress the surface biocompatibility of PDMS to enable lasting cell studies. The main focus of this thesis is a modification of the PDMS substrate surface for Cell/Tissue integrations.

2.3 SURFACE MODIFICATION AND CELL GROWTH

PDMS can easily be modified and changed for particular molecular interactions. This is so because PDMS has a highly hydrophobic nature in its intrinsic state that can be reduced hydrophilically via oxygen–plasma treatment [22], UV-ozone radiation [23], self-assembled monolayer coating [24], or polymer/peptide grafting techniques [24]. It is significant, however, to focus on the prospective problems that may arise when using PDMS substrates. One common concern, frequently ignored, is the physicochemical properties of PDMS surfaces which might affect suitable cell functions. Various kinds of cells that attach well to surfaces equally as tissue culture plastic do not attach well to modified PDMS surfaces. Surface modifications (active or passive) might seriously affect cell culture results for different cell types.

Cells react to specific surface properties in cell connection and positions. The study by Whiteside's and co-worker's (2006), confirmed that the different compositions of PDMS surfaces have an effect on cell connection and growth rates [25]. Another study by Toworfe and co-worker's (2004), reported that fibronectin-coated PDMS can improve and upgrade cellular purposes, mostly on its connection and spread [26]. The structure and stiffness of PDMS have micro-environmental effects on the differentiation of human cells [27-29]. Surface properties are known to affect stem cell attachment [30], but few studies have characterized the phenotypic stability of cancer cells on PDMS, which resultantly have become an important phase as the material is usually used in cancer research and medical applications. Mammalian cells must be involved onto either solid substrates, or supports in order to increase and purpose [31, 32].

2.4 SURFACE PROPERTIES AND CELL GROWTH

So far, it has been hard to predict how cancer affected directly or indirectly the elastic stiffness of substrates or a subordinate adsorbed molecule on the substrate. Numerous adsorbed proteins, similar to fibronectin or collagen, have been recognized to affect cellular connection [30]. Recent reports have shown stem cell differentiation on substrates with changing modulus of elasticity and changed pre-adsorbed proteins [30, 33]. A study by Weijia Zhang co-workers has shown that coating a PDMS surface with fibronectin and collagen maintained the breast cancer cell phenotype to be nearly identical to the culture of commercial polystyrenes petri-dishes [34].

Park *et al*., (2012) discovered the surface chemistry medication of PDMS elastomers with boiling water treatment. This was found to be a low-cost and suitable way of improving the hydrophilicity of PDMS. The process involves creating hydrophilic cell-necessary-sites on the surfaces of hydrosilylation cured PDMS elastomers. A gold nano-textured face gives more fibroblast growth when compared to flat PDMS surfaces [35].

2.5 MECHANICAL CHARACTERIZATION OF PDMS

When polymers are used for biomedical implants, the target concerns are the flexibility and stiffness, compared to surrounding tissue [36]. Elastomers are recognized to have changing mechanical properties, based on their curing operation temperatures [37]. The resistance of an elastomer to axial deformation is characterized by Young's modulus [38]. Research has shown that the Young's modulus plays an essential role in controlling cellular functions, such as proliferation [39], migration [40], differentiation [41], and apoptosis [42]. However, it is very crucial to characterize mechanical properties of PDMS substrate and study how they affect cell function. During cell/tissue integration, the extracellular metrics (ECM), and the PDMS substrate provide physical support for the cell/tissue attachment. The cell/tissue sense the PDMS substrate stiffness, and the stiffness of nano and micro posts, as well as the ECM coating on the texture surface [43]. The curing operational temperature has effects on the mechanical properties of PDMS substrates. Some research has been done on the mechanical properties of PDMS substrates by the Sylgard 184 kit [36]. From the study, a direct relationship occurs between the curing temperature and Young's modulus [37]. It was revealed that as the curing temperature rises so is the case for the Young modulus. This provided quantitative data for the design employing Sylgard 184 kit PDMS as engineering substrate material. The cell/tissue acknowledgment of the substrate hinge on the mechanical properties of the substrate [43]. The ability of cell/tissue to react to mechanical properties of a substrate is known as mechano-sensing [43]. In the course of cell/tissue reaction to mechanical properties of a substrate, there is both the action of the material on the cell and the action of the cell on the material [44].The first experimental proof of the ability of cell/tissue reaction to mechanical properties of a substrate was by Harris et al. [45].

Table 2. : Influence of curing temperature on the mechanical properties of PDMS [45].

Temperature (^{o}C)	**Young's Modulus (MPa)**	**UTS (MPa)**	**UCS (MPa)**
25	1.32 ± 0.007	5.13 ± 0.55	51.7 ± 9.60
100	2.05± 0.12	6.25± 0.84	40.1 ± 4.30
125	2.46± 0.16	7.65 ± 0.27	36.8 ± 3.84
150	2.59± 0.08	5.24± 0.82	28.4 ± 4.46
200	2.97 ± 0.04	3.51 ± 1.11	31.4 ± 2.04

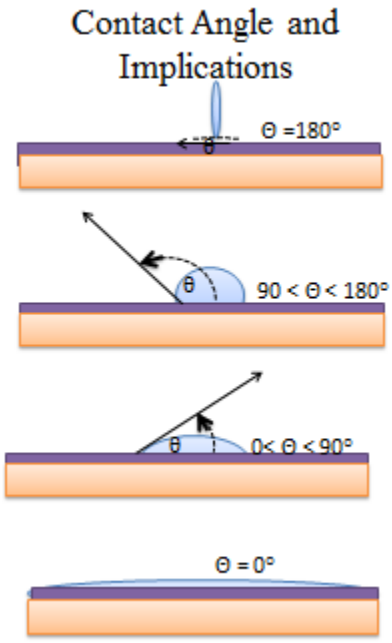
2.6 METHODS FOR STUDYING CELL/SURFACE ADHESION

The adhesion between cells and biomaterials is essential for the integration of biomaterial implants [46]. The significance of the cell/tissue adhesion has stimulated significant research efforts [47-48] in the development of experimental techniques for the measurement of cell adhesion. Cell adhesion has a significant effect on cellular functions, such as proliferation, differentiation, among many others. [46] Quantitative measurement of cell adhesion provides insights into these cell functions. It is also important to know that cell functions occur when cells attach to a substrate and begin to spread and form a planned actin cytoskeleton and to difficult transmembrane gesturing regions [49].

2.7 WETTABILITY AND CONTACT ANGLE

A surface is claimed to be wetted if a liquid spreads over the surface equally without the formation of droplets. Once the liquid is water and spreads over the surface without the formation of droplets, the surface is claimed to be hydrophilic. Regarding energetics, this suggests that the forces related to the interaction of water with the surface are larger than the cohesive forces connected to bulk liquid water such that property and hydrophilicity are relative terms [50]. The contact angle (θ), as shown in **Table 2.1**, is the angle at which the liquid--vapours interface meets the solid-liquid interface. The contact angle is determined by the balance between adhesive and cohesive forces. Static water contact angle is a criteria used for analysis of the hydrophobicity for the estimation of self-cleaning property [51].

Table 2. : Shows various contact angles and their implications



Degree of wetting	Strength of:	
	Solid/liquid interactions	Liquid/liquid interactions
Perfectly non-wetting	Weak	Strong
Low wettability	Weak	Strong
High wettability		
Perfect wetting	Strong	Weak

CHAPTER THREE

3.1 MATERIALS AND METHODS

3.1.1 MATERIALS

Sylgard 184 kit elastomer base and its curing agent which were used in the polymerization of polydimethylsiloxane (PDMS) were obtained from Sylgard Dow Corning Krayden Inc. (Midland, Michigan, USA). Poly lactic-co-glycolic (PLGA) with ratio of lactide to glycol ide; 85:15 (Mw = 75,000-120,000) were purchased from Sigma-–Aldrich (St Louis, MO, USA). Dichloromethane (DCM) and, polyvinylpyrrolidone (PVP) were also procured from BDH Chemicals (Poole Dorset, England). Sodium alginate (Mw = 14000-132000), polyethylene glycol (PEG) (Mw =940000-1060000), magnetite nanoparticles (Fe_3O_4) were obtained from the biomaterials laboratory, Kwara State University, Nigeria. Other consumables such as distilled water, deionized water, sodium chloride (NaCl), potassium chloride (KCl), sodium phosphate (Na_2HPO_4), potassium phosphate (KH_2PO_4) and hydrochloric (HCL) acid were purchased from a laboratory shop in Ilorin, Kwara State-Nigeria.

3.2 EXPERIMENTAL METHODS

3.2.1 Polymerization of PDMS

Polymerization of PDMS was done using Sylgard-184 elastomer kit alongside its curing agent–.The Sylgard elastomer kit was vigorously mixed with a cross-linker at different volume ratios as shown in Table 3.1.Subsequently, Fe3O4 was added to two--volume ratios of monomer-cross -linker (10:1 and 40:1) at weight percent's of 5,10,15,20,25 as shown in Table 3.2. Each mixture was vigorously stirred with a stirrer. The stirring was done for the cross- linker to ensure a homogenous mixture The blended polymer resins were then degassed with a SHELLAB vacuum oven (Sheldon Manufacturing, Inc.300N.26Th Avenue, USA) to remove bubbles without any heat, while venting at every 15 min interval and re-degassing for an hour at -72 cmHg. The degassed samples were gently poured into multiwall plates which served as molds and then left at room temperature to polymerize for 24 hours.

The PDMS substrates were enclosed in a petri dish and stored in a desiccator to prevent contamination and moisture interferences before further characterization. The transparency of the cured PDMS substrate allowed each sample to undergo a visual assessment to detect

possible tears, bubbles or other detectable defects that could affect the mechanical properties of the sample.

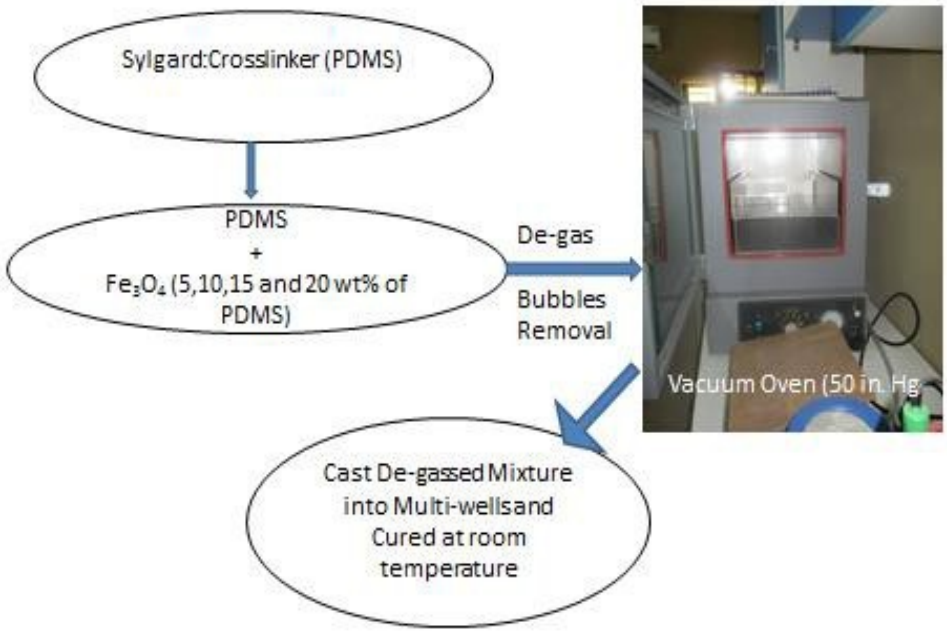


Figure 3.-: Illustration of Sample Preparation

Table 3. : Shows Sylgard and cross-linker ratios

Sample	Sylgard (ml)	Cross-linker (ml)
10: 1	5	1.0
20: 1	5	0.26
30: 1	5	0.16
40: 1	5	0.13
50: 1	5	0.10
60: 1	5	0.09

Table 3. : Shows Sylgard and cross-linker ratio with Fe_3O_4

Sample	Sylgard (ml)	Crosslinker (ml)	Weight % of Fe_3O_4
10: 1	5	1.00	-
10: 1	5	1.00	5
10: 1	5	1.00	10
10: 1	5	1.00	15
10: 1	5	1.00	20
10: 1	5	1.00	25
40: 1	5	0.16	-
40:1	5	0.16	5
40:1	5	0.16	10
40:1	5	0.16	15
40:1	5	0.16	20
40:1	5	0.16	25

3.3 MODIFICATION OF PDMS

The surfaces of the polymerized PDMS were modified via various methods to become more cell friendly. The methods used for this thesis include; boiling water treatment, surface coating with biopolymers [polyethylene glycol (PEG), poly lactic-co-glycolic (PLGA) acid and sodium alginate]. In addition, pre-stretching while coating was also investigated for the formation of micro-buckles.

3.3.1 Chemical Modification of Samples

Following the PDMS/PDMS-Fe_3O_4 sample preparation as described previously, selected samples were boiled in a Pyrex beaker deionized containing (DI) water for 1-5 hrs at hourly intervals. The boiled samples were removed at regular time intervals and then quickly dried for about 2-5 minutes before the contact angles were determined (see section 3.5). The reaction involved in the generation of hydroxyl (OH) group on PDMS substrate is given by [1]:

Figure 3.: Boiling PDMS Samples in Deionized H_2O

Reaction involved: $(\text{-SiH}) + H_2O \xrightarrow{Pt} (\text{-Si-OH}) + H_2\uparrow$ (1)

PDMS is cured by organometallic crosslinking reaction [1]. The cross-linker of PDMS contains platinum catalyst hydrosilylation of vinyl terminated PDMS and vinylated silica (their combination represented generically as PDMS-Vi) [1] with an oligomeric siloxane (PDMS-H) multifunctional in silicon hydride (SiH) groups to form an end-linked network of PDMS [1].

3.3.2 Surface Modification with Biopolymers

Polyethylene glycol (PEG) was dissolved in dichloromethane, while PVP was used as a binder to form a viscous fluid. Using a syringe, about 0.5 ml of PEG was deposited on the PDMS substrate with the aid of a spin coater at 4,000 revolutions per min (rpm). The spin coater (WS-650MZ-23NPP, Seal Punge, and USA) was set to rotate for 4000rev/min for the fluid to be uniformly distributed on the substrate surface. The process was repeated for PDMS substrates and those containing magnetite reinforced PDMS. Polylactic-co-glycolic acid (PLGA) was similarly dissolved in DCM and PVP to form a viscous fluid. This was then spin coated in a similar way using PEG. Moreover, sodium alginate (SA) was also dissolved in distilled water, while PVP stock solution was also added to form a viscous fluid. The substrates were also spin coated with the PLGA. **Table 3.3** details the quantities used in preparing the biopolymers for coating. **Figure 3.3** illustrates the process involved in the surface coating of PDMS substrates with hydrophilic biopolymers, pre- stretch and hydrophilic coatings.

Table 3. : Quantities used in the preparation of biopolymers for coating.

Biopolymer fluids	Solvent	Solute	Binder(PVP)
PLGA viscous fluid	2ml of DCM	1g of PLGA	0.5ml
SA viscous fluid	7ml of distilled H_2O	1g of SA	0.5ml
PEG viscous fluid	2ml of DCM	1g of PEG	0.5ml

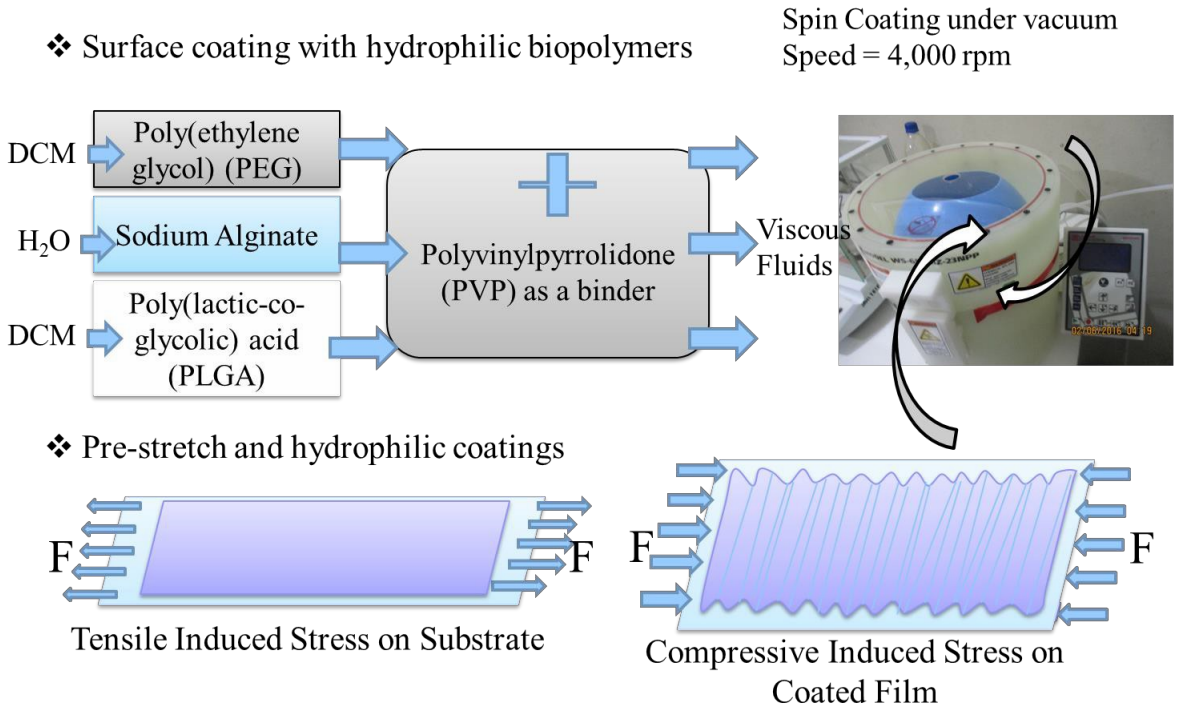


Figure 3.:Surface coating with hydrophilic biopolymers, pre-stretch and hydrophilic coatings.

3.3.3 Pre-stretching

The PDMS slabs were stretched with clips on a glass slide to about 5% strain. The stretched slabs were processed for spin coating as shown in **Figure 3** above. For about 5 hours the pre-strain was gently released. The relaxation of the pre-strain compressed the coated thin film leading to micro-buckling/wrinkling. The film at the PDMS substrate formed a sinusoidal pattern. This was as a result of the minimization of the system's potential energy by the out of plane deformation.

3.4 PREPARATION OF PHOSPHATE BUFFER SALINE

To mimic physiological conditions, phosphate buffer saline (PBS) solution was prepared by dissolving 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4 and 0.24 g of KH_2PO_4 in 800ml distilled H_2O. The volume was adjusted to 1000 ml with additional distilled water. The pH was then adjusted with distilled water and or HCl to 7.4. The PBS sample was sterilized by autoclaving for 20 minutes at 125˚C, at higher pressure (106kPa).

3.4.1 Optical Characterization of PDMS

Scanning electron microscope (SEM) (Sirius50/3.8, Aspex Corporation, UK) and pros-cope imaging system were used to obtain images of the unmodified surface, treated surface and pre-stretched surfaces of the PDMS. The Gwydion software package (2.34) was then used to determine the roughness of the coated or treated surfaces.

3.5 DETERMINATION OF CONTACT ANGLE

The apparatus for contact angle measurement consisted of a retort stand, lamp holder, 40W bulb, rectangular cardboard, convex lens, a lens holder, a firm stage for the PDMS sample, glass slides and a digital camera (12 megapixels with a magnification of 8x). The experimental setup involved a lighting system in addition to an optical system. The optical components consisted of a basic digital camera (Canon 8x megapixel) and an optical lens with a focal length (15.0 cm) that was situated between the camera and the sample (**Figure 3.4**). A lamp was positioned behind the samples to shine on the liquid drop which appeared dark. This was necessary for measurement precision and image processing. The carton box was positioned near but over the lens and the sample, thus rejecting stray light. The liquid drop did not reflect stray light that could affect the measurement. The carton box also prevented the drops from been polluted by dust particles.

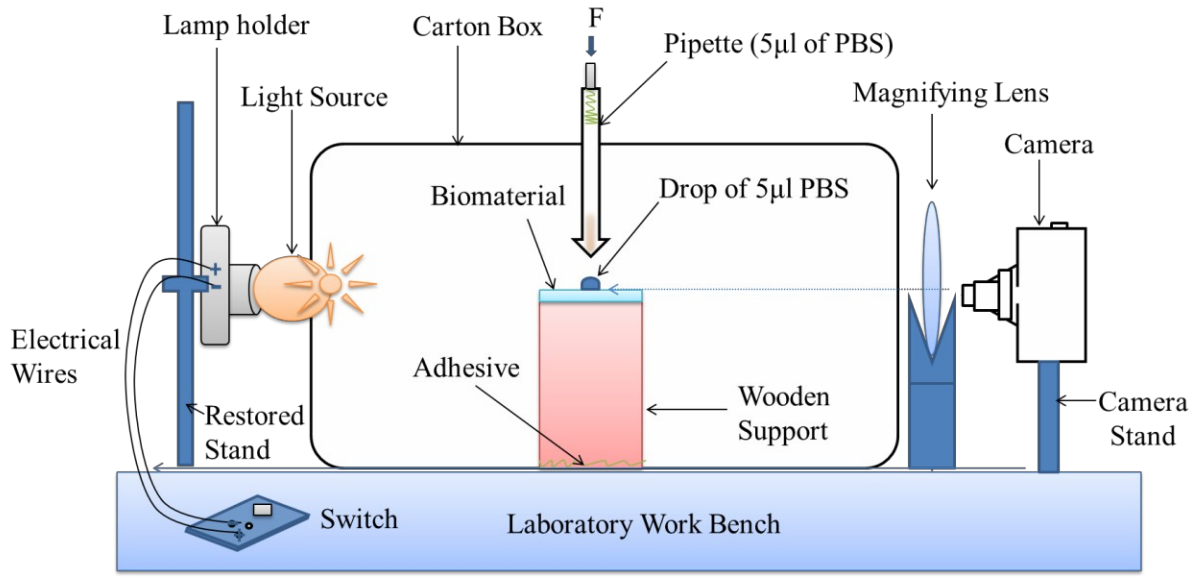


Figure 3.: Experimental setup for contact angle measurement

The above setup was guided by the Lenses formula:

$$\frac{1}{f} = \frac{1}{u} + \frac{1}{v} \tag{3}$$

where f = focal length, u = image distance, v = object distance

5 μl of phosphate buffered saline (PBS) solution was measured with a micro pipette and gently released on the substrate (mounted on a stage). Then the camera was used to capture the image of the liquid drop immediately when it fell on the substrate through the magnifying lens. Gwydion software package (2.34) was then used to determine the contact angle.

CHAPTER FOUR

4.1 RESULTS AND DISCUSSIONS

4.2 INTRODUCTION

This chapter presents the results and discussions of experiments carried out on modified PDMS surfaces.

4.3 OPTICAL CHARACTERIZATION

4.3.1 Morphologies of Substrates Coated with Biopolymers

A proscope optical microscope and a scanning electron microscope were used to examine the morphological characteristics of the samples. The optical images of plane PDMS are presented in **Figure 4.1a**. Coated PDMS surfaces with biopolymers such as polyethylene glycol (PEG), poly lactic-co-glycolic (PLGA), as well as sodium alginate (SA) are presented in **Figures 4.1b**, **4.1c,** and **4.1d**, respectively.

The controlled sample, which is a normal PDMS without any further modification (**Fig. 4.1a**), revealed a smooth surface. The control sample also indicated a transparent elastomeric structure with a higher degree of extension. The lack of porosity in PDMS substrate contributed to its hydrophobic nature. Also, the PDMS sample coated with PEG (**Fig. 4.1b**) indicated some degree of roughness with micro-ridges. This surface was found to be promising for cell growth based on its wettable ability. Moreover, the coated substrates with PLGA (**Fig. 4.1c**) indicated macro-voids on the PDMS substrates. Further analysis with the Gwyddion software package revealed the voids to be cup-shaped in nature. The macro-voids indicated pore diameters between 10.22µm-106.78µm. However; SA samples (**Fig. 4.1d**) revealed a balance of microspores on the substrates. The heterogeneous porous structure was obtained when the PDMS was coated with SA. The average pore diameters varied from 1.02µm-17.9µm

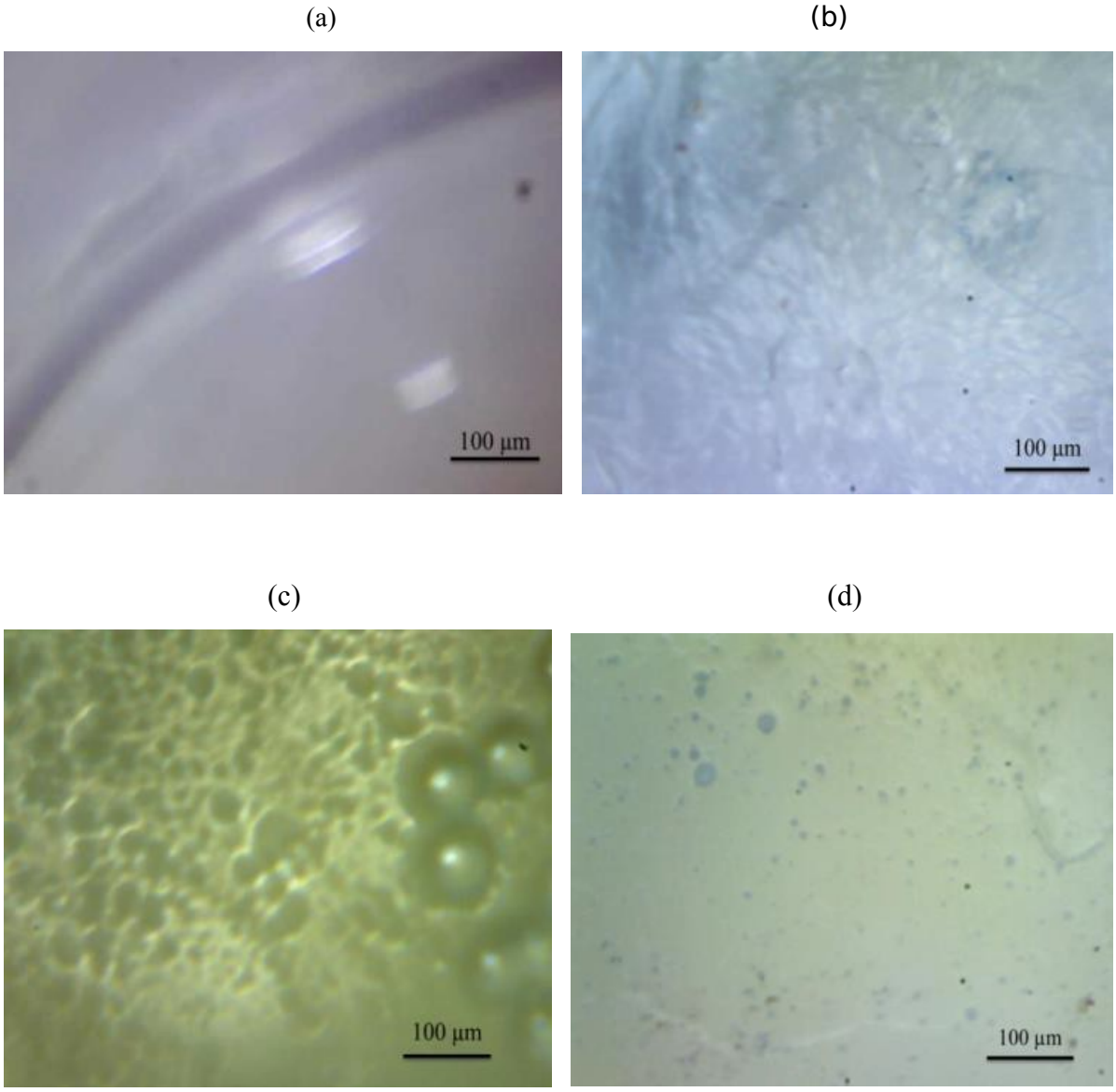


Figure 4.: PDMS Coated with Biopolymers: (a) Control Sample (uncoated PDMS), (b) Coated with PEG (c) Coated with PLGA-, (d) Coated with Sodium Alginate.

4.3.2 Effect of Coatings on PDMS: Fe_3O_4 Substrates

The surface of the PDMS-based nanocomposite (**Figure 4.2a**) was a bit rough as compared to the plain PDMS presented in **Figure 4.1a**. Meanwhile, coating biopolymers such as PEG, PLGA, and SA on PDMS-based nanocomposites improved the overall roughness as shown in **Figures** (**4.2b-d**). The coated substrates with PLGA produce spherical wells as indicated in **Figure. 4.2b**. General roughness and micro-cracks were observed when PEG was coated on PDMS-based nanocomposites. Similarly, microspores were observed on the

PDMS-based nanocomposites. The surface had micropores with an average pore diameter of 27.03µm

Figure 4.: Effect of 15 wt% Fe_3O_4 on PDMS Matrices via Coating with Biopolymers: (a) Control Sample (uncoated PDMS-Fe_3O_4), (b) PLGA (c) PEG (d) SA.

4.3.3 Effect of Pre-stretch on Surface Morphology

From the SEM micrographs, the pre-stretching revealed a lot of micro-ridges (**Figure 4.3b**) formed on the PEG coating on PDMS-based nanocomposites, while large ridges were formed on the sodium alginate coatings (**Figure4.3d**). These micro-ridges on the PEG coating were due to the close match of the modulus of the PEG to the modulus of PDMS substrate whilst, the macro-ridges on sodium alginate were due to modulus mismatch of sodium alginate PDMS substrate.

(a) (b)

(c) (d)

Figure 4.3: Scanning Electron Micrographs of before and after pre-stretch and coating on PDMS-based nanocomposites (a) Coated with PEG (b) Coated with PEG (c) Coated with Sodium Alginate

4.4 SURFACE ROUGHNESS

Figure 4.4 shows optical roughnesses of plane PDMS when coated with SA and PEG.3D images were obtained using Gwyddion software package (2.34).From the results, the surface of the plain PDMS (control) indicated homogeneous and smooth roughness as illustrated in **Figure 4.4a,** the roughness ranged from 0.45-0.79 µm. PEG coatings (**Figure 4.4b**) on PDMS shows an improved roughness between 0.2-0.90 µm. SA gave roughness ranging from 0.32-0.83 µm. Cells need rough surfaces to adhere on [1].These roughnesses are a good indication of how cells will adhere on PDMS-based composites when coated with PEG and SA. However, coating with PEG produces higher roughness on PDMS substrates as compared with SA.

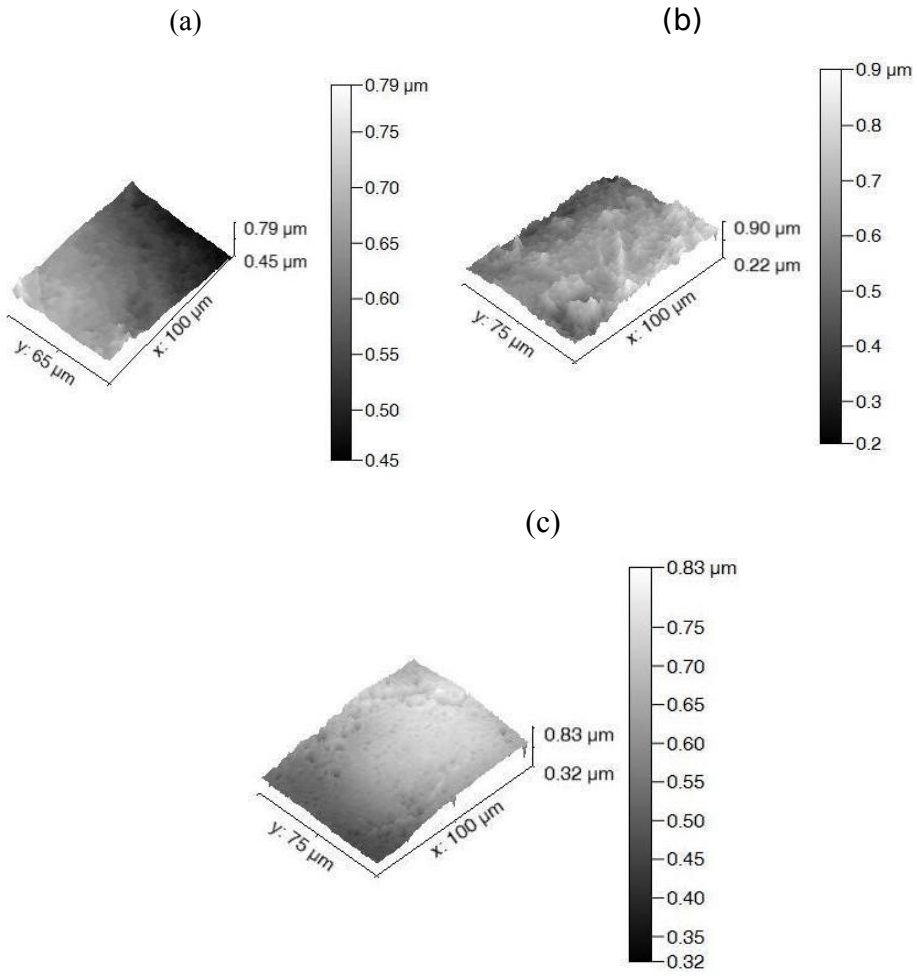


Figure 4.4: Three-dimensional (3D) optical roughness's various coatings on plane PDMS: (a) control (uncoated PDMS), (b) Coated with PEG (c) Coated with Sodium Alginate

4.4.1 Effect of Fe_3O_4 on the Surface Roughness

The roughness of the control sample (**Figure. 4.5**) was between 0.45-0.79 µm. However, the presence of Fe_3O_4 nanoparticles (nps) in PDMS resulted in increasing the roughness up to 1µm. Though the roughness of PDMS-based nanocomposites was improved, substrates wettability was key to our discussion. Roughness at nano and macro/micro-scales can support cell growth and cell spreading [2].

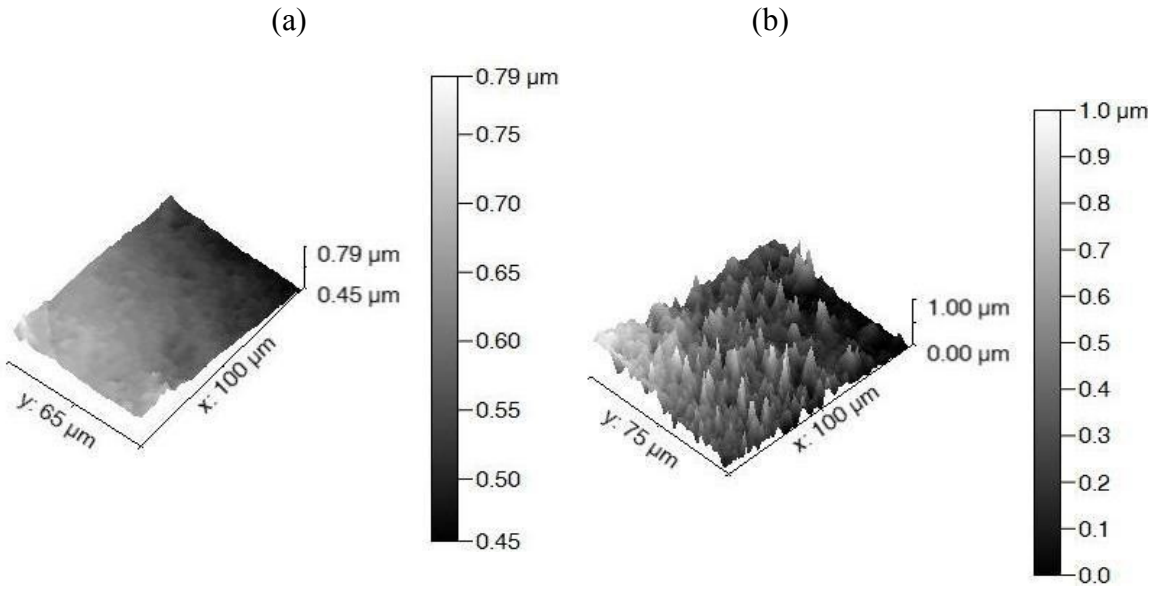


Figure 4.5: Effect of Fe_3O_4 on the Surface Roughness of PDMS-based nanocomposites (10:1 v/v %).

4.4.2 Effect of PDMS (10:1)-Fe_3O_4 Surface Roughness due to Biopolymers Coated

3D Surface roughness was determined with the Gwydion software package 2.34 version. Distinct features were observed for the various coatings. Results were then used for the wettability test to confirm the hydrophilicity of the modified surfaces. Meanwhile, PLGA coated surfaces produced large cup-shaped structures (multi-wells), which may not support contact guidance and cell spreading. The multi-wells structures of the PLGA allowed the 5 µl PBS/water droplets to stay cup- shaped instead of spreading, leading to higher contact angle and hence low wettability. Another challenge with the PLGA coated surfaces was delamination when any physical force was applied on the sample. SA roughness ranged from 0.0-1.0 µm which may support contact guidance and cell spreading [3]. PEG also showed a

roughness from 0.2 μm- 0.9 μm which may also support contact guidance and cell spreading, but sodium alginate is better off than PEG in terms of physical coatings.

(a) (b)

(c) (d)

Figure 4.6: 3D optical images of surface roughness due to biopolymers coated on the: (a) control (uncoated PDMS-based nanocomposite), (b) Coated with PLGA (c) Coated with SA and (d) Coated with PEG.

4.4.3 Effect of Polymer Coatings on Surface Wettability

Surface wettability via contact angle measurements on coated surfaces are presented in **Figure 4.7**. Extremely wetting surfaces were generated with angles less than 90˚ (i.e. $\theta < 90$ ˚). More improved results were reported for sodium alginate followed by PEG, and PLGA was least. The results also indicated the hydrophilic nature of the substrates which resulted in more contact area and surface spreading of water (PBS) droplets. From the results, it can be deduced

that, the adhesiveness of sodium alginate to PDMS substrate was very high which resulted in small contact angle. The contact angles on PDMS-based substrates coated with PLGA indicated low wettability with higher contact angle. This was due to the nature of the multi-well structures observed on the PDMS substrates when PLGA was coated. The multi-wells clearly allowed the liquid drops to stay in the "cup-shaped" structures instead of spreading.

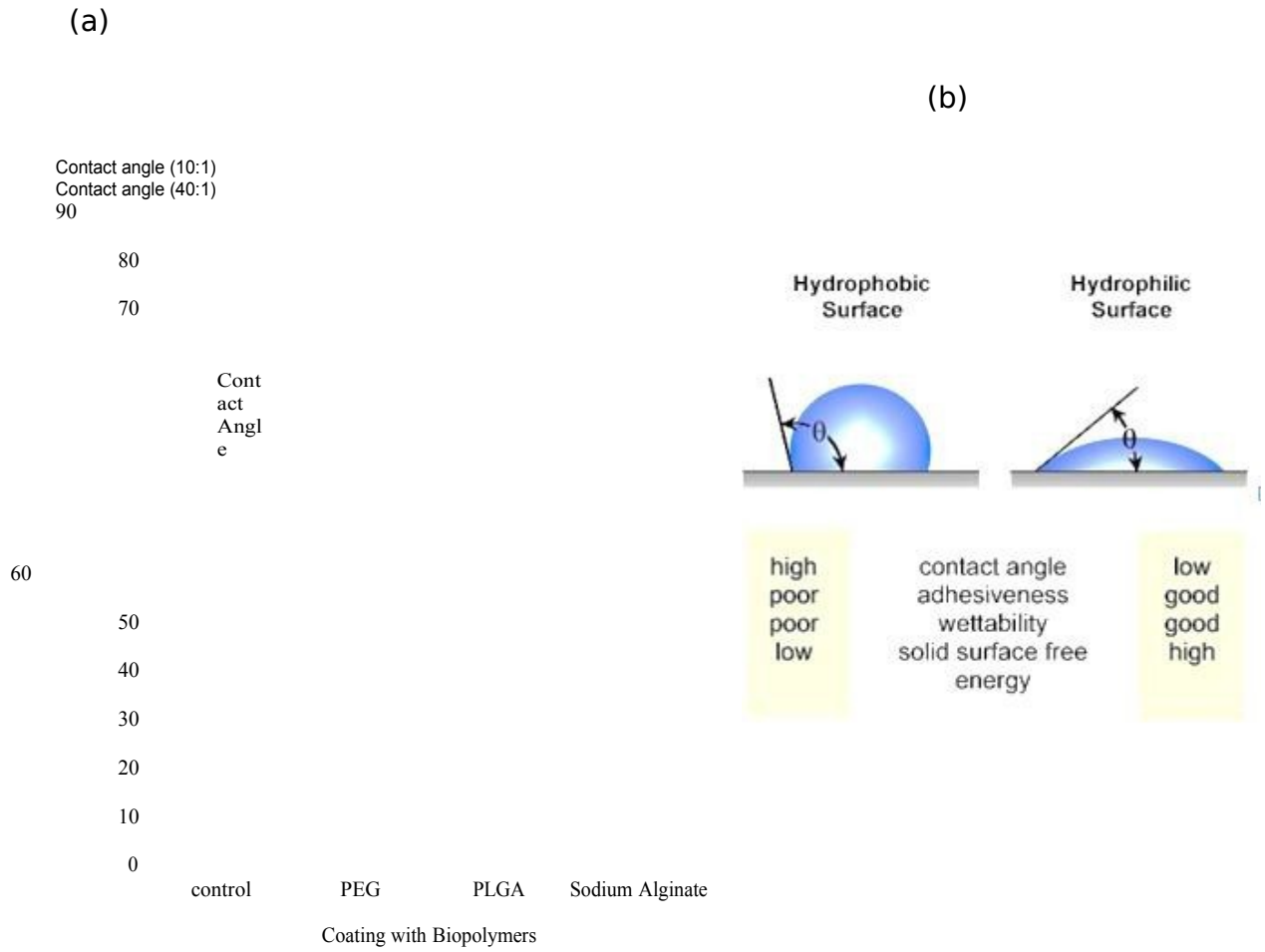


Figure 4.7: (a) Surface Wettability via Contact Angle Measurements on Coated Surfaces and (b) Interpretation of contact angle.

Substrate surface wettability is usually characterized using the Young's equation given by (Young's, equation):

$$\gamma^1 = \gamma^2 - \gamma^3 \cos\theta \qquad (4.1)$$

Where γ^1 is the solid − liquid interfacial energy, γ^2 is the solid surface energy-, γ^3 is the liquid surface energy and θ is the contact angle. From the Young's equation presented in equation (4.1), $\gamma^2 > \gamma^3$ implies spreading of the liquid due to greater

adhesive force between the liquid and coated surface. Small contact angle implies that the adhesive force between the

substrate and the coating was high. The unmodified PDMS has higher contact angle with poor wettability, poor adhesiveness, and low solid surface free energy as compared to the modified surfaces with good wettability, good adhesiveness and higher solid surface free

energies. However, there was no significant difference due to the cross-linker ratio in the substrate.

4.4.4 Wettability of PDMS-Based Nanocomposites

There was a significant improvement in the substrate wettability due to magnetite nanoparticles incorporated into PDMS matrix before coatings with PEG and SA. The result, however, showed good wettability with coated PEG than SA when Fe3O4 nanoparticles were incorporated as compared to **Figure 4.7**, where biopolymers were just coated on the plain PDMS. This could be due to the differences in substrate-free energies. Moreover, no significant differences were observed, when the cross-linker concentration was varied.

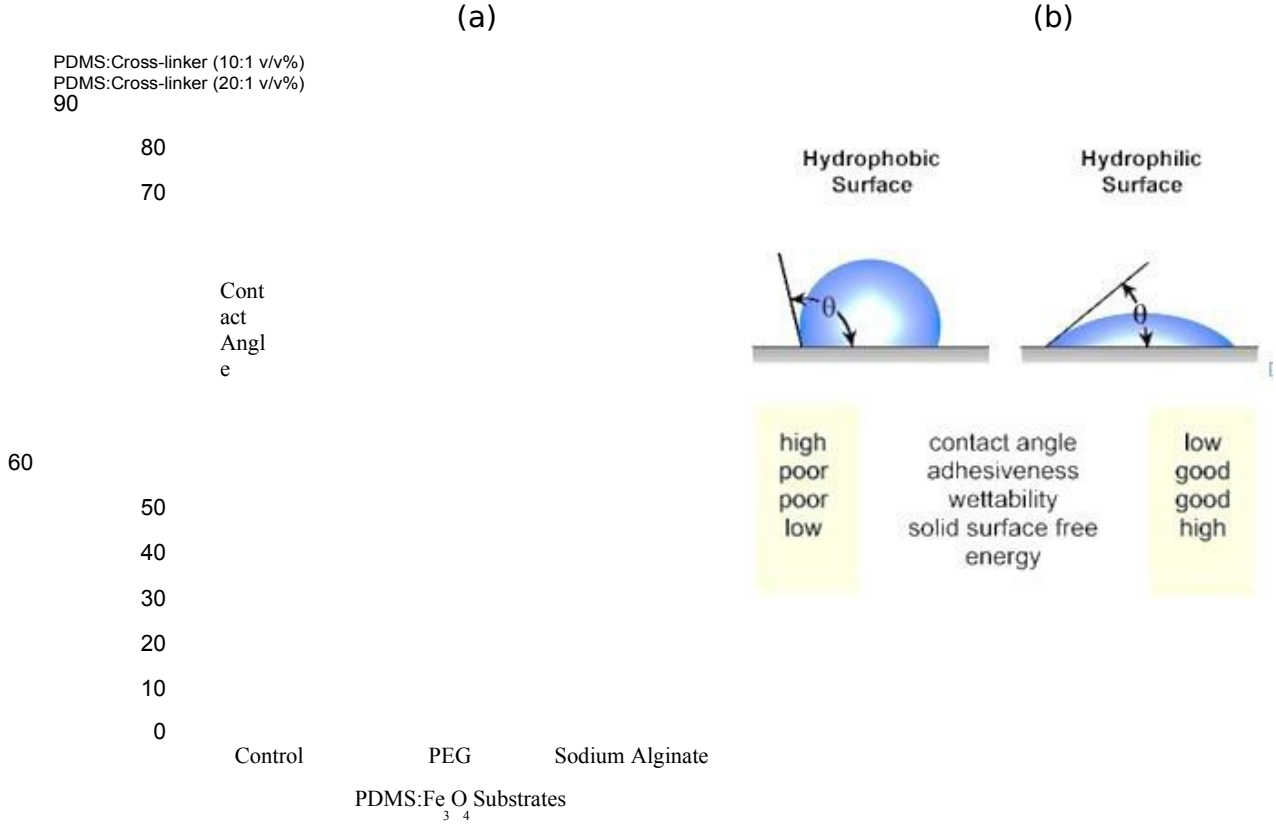


Figure 4.8: (a) The effect of Cross-linker on the Wettability of PDMS: Fe_3O_4 Substrates and (b) Interpretation of contact angle.

4.4.5 Kinetics of Substrate Wettability via Chemical Modification

Kinetics of substrate wettability via chemical modification was presented for both PDMS-substrate and PDMS-based nanocomposites (**Figure 4.9**). There was an improved result when Fe_3O_4 was reinforced with PDMS before treatment. The rate of chemical modification was exponentially determined with increasing boiling time (**Figure 4.9**). Meanwhile, there was no

appreciable change in contact angle after 4 hrs. However, surface wettability improved with PDMS-nanocomposites (reinforced Fe_3O_4 nps).The kinetics equation from the exponential dependency of contact angle on the chemically modified surfaces was determined to be:

(4.2)

Where $\theta(t)$ is the time dependent contact angle, A is the most stable part of the graph where the fitted graph almost remained horizontal (stable) even with increasing treatment time, t is the treatment time, θ is the rate of treatment and θ_i is the least contact angle determined in the data. Details of these values are presented in **Table 4.1**.

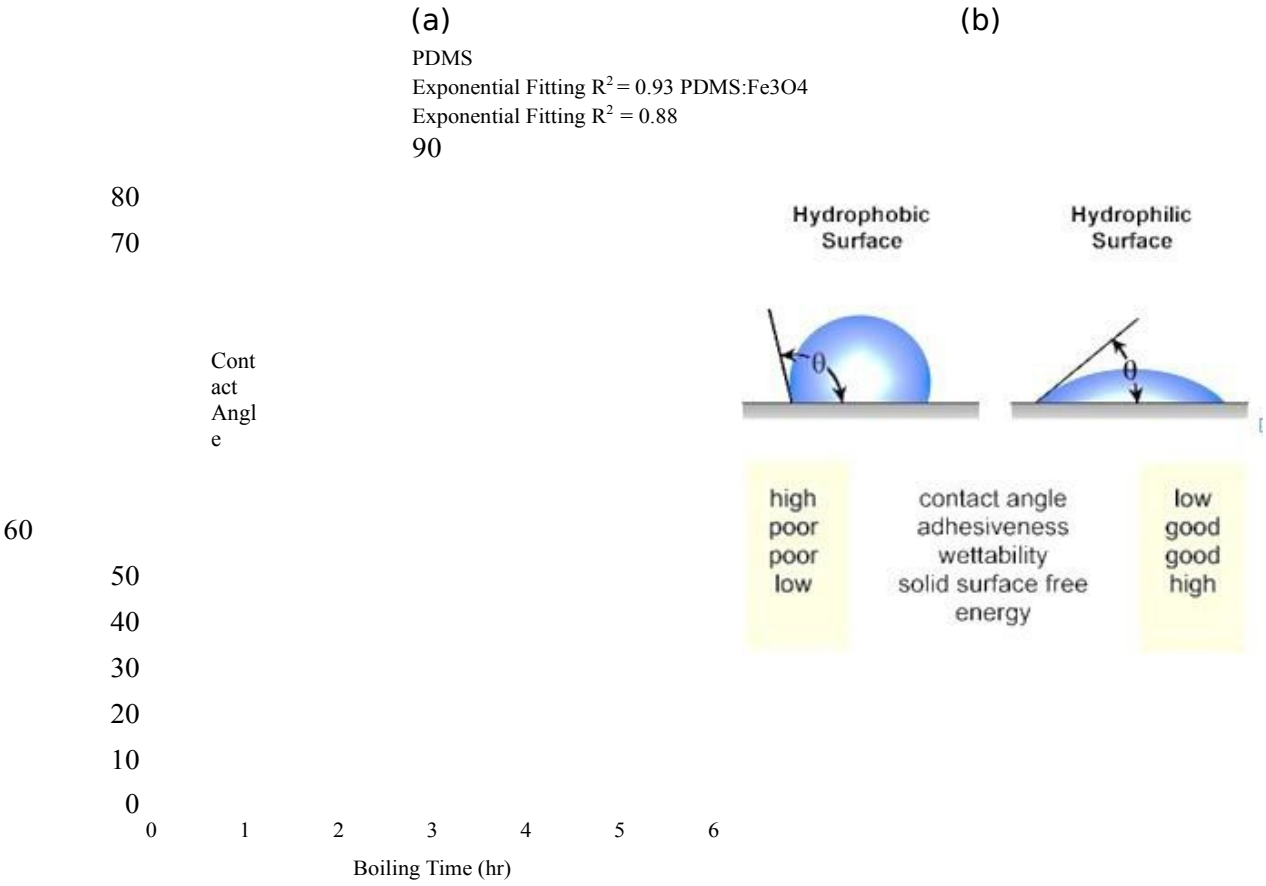


Figure 4.9: (a) Kinetics of Substrate Wettability via Chemically Modification and (b) Interpretation of contact angle.

Table 4.1: Values from the Exponential Fitted Equation.

Equation	$\theta^{(t)} = A_i * \exp(-\frac{t}{R}) + \theta_i$			
	Symbol	Value	Standard Error	R-Square
PDMS-Nanocomposite	θ_1	32.18	3.79	0.88
	A_1	46.25	7.61	
	t_1	1.41	0.51	
PDMS	θ_2	41.27	1.49	0.93
	A_2	40.76	4.98	

	t_2	0.71	0.17	

4.4.6 Effect of Physical Modifications on Substrate Wettability

The results show that sodium alginate does well than PEG during direct coating. The reverse was the case when pre-stretching was involved before coating. From the SEM analysis, it was observed that after pre-stretching, a lot of micro-ridges formed on the PEG coatings, whilst macro-ridges also formed on the Sodium alginate coating. These micro-ridges on the PEG-coated substrates was as a result of the similar modulus of the PEG compared to the modulus of PDMS substrate. However, macro-ridges were formed on sodium alginate due to modulus mismatch of Sodium alginate to that of the stretch PDMS substrate.

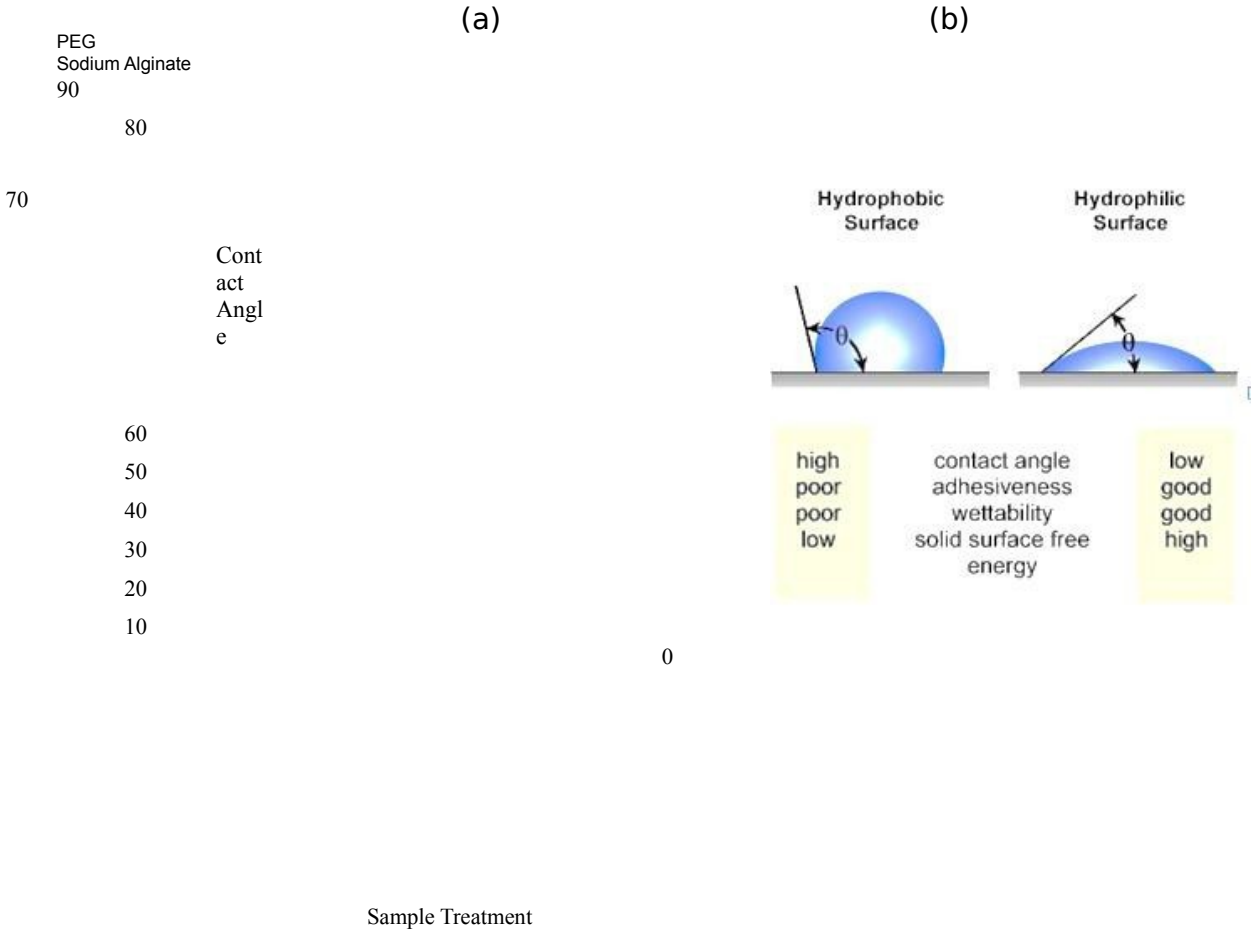


Figure 4.10: (a) Effect of Physical Modifications on Substrate Wettability and (b) Interpretation of contact angle.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION REMARK

5.1 CONCLUSION REMARKS

Polydimethyl siloxane (PDMS) is an approved biocompatible material for microfluidic device fabrications (Approved by the American Food and Drug Administration). This same PDMS was used to fabricate a microfluidic device for controlled drug delivery (Danyuo et al., 2014). The surface of PDMS was found by recent research to be highly hydrophobic (Danyuo et al., 2016). The surface wettability of PDMS needs to be improved by either chemical modification, physical modification or both. In this work, biopolymers such as PEG, SA, and PLGA were coated on the PDMS substrates, either before or after pre-stretching and lastly, chemical modification via boiling in deionized water with platinum as catalysts was also employed.

The physical modification stands the test of time and can be employed in coating biopolymers such as SA and PEG on the implantable device. The results show that biopolymers could be selected for hydrophilic modification of PDMS-based substrates. Highly wettable surfaces were formed with pre-stretching on PEG with micro-ridges which can support cell growth. Chemical modification can be an easy method though stability is not guaranteed. The PDMS-Fe_3O_4–based improved the results from coated substrates. The surface tension/surface energy places an important role in water spreading. The spreading of water droplets on modified images is an indication of how cells will function on the modified biomaterial.

5.2 RECOMMENDATIONS REMARKS

Though surface wettability was well established, cell culture is highly recommended to determine the effect of surface roughness on cell growth on the modified PDMS.

- ❖ We highly recommend other coatings with proteins such as extracellular matrix (ECM), fibronectin, collagen, among many others.
- ❖ We also recommend the development of analytical solutions for the contact angle and substrate surface energies effects on substrate wettability.
- ❖ Nano-indentation analysis is recommended on the mechanical properties of the coatings (modulus, bonding forces, etc.).
- ❖ X-ray diffraction (XRD) on elemental analysis and the crystalline nature of the substrates is also highly recommended.
- ❖ Atomic force microscopy (AFM) is highly recommended to characterize the roughness of the samples further.

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Chapter 3

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Chapter 4

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