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Fabrication and Characterization of Plasma Polymer Thin Films from Monoterpene Alcohols for Applications in Organic Electronics and Biotechnology

Thesis submitted by

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in December 2011

for the degree of Doctor of Philosophy

in the School of Engineering and Physical Sciences

James Cook University

Supervisors: A/Prof Mohan Jacob, A/Prof Bruce Bowden, Prof Jeffrey Loughran

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institute of tertiary education. Information derived from the published and unpublished work of others has been acknowledged in the text, and a list of references is given.

Kateryna Bazaka

December, 2011

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ABSTRACT

After more than twenty years of basic and applied research, the use of nanotechnology in the design and manufacture of nanoscale materials is rapidly increasing, particularly in commercial applications that span from electronics across renewable energy areas, and biomedical devices. Novel polymers are attracting significant attention for they promise to provide a low−cost high−performance alternative to existing materials. Furthermore, these polymers have the potential to overcome limitations imposed by currently available materials thus enabling the development of new technologies and applications that are currently beyond our reach. This work focuses on the development of a range of new low−cost environmentally−friendly polymer materials for applications in areas of organic (flexible) electronics, optics, and biomaterials. The choice of the monomer reflects the environmentally−conscious focus of this project. Terpinen−4−ol is a major constituent of Australian grown *Melaleuca alternifolia* (tea tree) oil, attributed with the oil's antimicrobial and anti−inflammatory properties.

Plasma polymerisation was chosen as a deposition technique for it requires minimal use of harmful chemicals and produces no hazardous by−products. Polymer thin films were fabricated under varied process conditions to attain materials with distinct physico−chemical, optoelectrical, biological and degradation characteristics. The resultant materials, named polyterpenol, were extensively characterised using a number of well−accepted and novel techniques, and their fundamental properties were defined. Polyterpenol films were demonstrated to be hydrocarbon rich, with variable content of oxygen moieties, primarily in the form of hydroxyl and carboxyl functionalities. The level of preservation of original monomer functionality was shown to be strongly dependent on the deposition energy, with higher applied power increasing the molecular fragmentation and substrate temperature. Polyterpenol water contact angle contact angle increased from 62.7° for the 10 W samples to 76.3° for the films deposited at 100 W. Polymers were determined to resist solubilisation by water, due to the extensive intermolecular and intramolecular hydrogen bonds present, and other solvents commonly employed in electronics and biomedical processing.

Independent of deposition power, the surface topography of the polymers was shown to be smooth (R_q <0.5 nm), uniform and defect free. Hardness of polyterpenol coatings increased from 0.33 GPa for 10 W to 0.51 GPa for 100 W (at 500 μN load). Coatings deposited at higher input RF powers showed less mechanical deformation during nanoscratch testing, with no considerable damage, cracking or delamination observed. Independent of the substrate, the quality of film adhesion improved with RF power, suggesting these coatings are likely to be more stable and less susceptible to wear.

Independent of fabrication conditions, polyterpenol thin films were optically transparent, with refractive index approximating that of glass. Refractive index increased slightly with deposition power, from 1.54 (10 W) to 1.56 (100 W) at 500 nm. The optical band gap values declined with increasing power, from 2.95 eV to 2.64 eV, placing the material within the range for semiconductors. Introduction of iodine impurity reduced the band gap of polyterpenol, from 2.8 eV to 1.64 eV, by extending the density of states more into the visible region of the electromagnetic spectrum. Doping decreased the transparency and increased the refractive index from 1.54 to 1.70 (at 500 nm).

At optical frequencies, the real part of permittivity (k) was determined to be between 2.34 and 2.65, indicating a potential low-k material. These permittivity values were confirmed at microwave frequencies, where permittivity increased with input RF energy – from 2.32 to 2.53 (at 10 GHz) and from 2.65 to 2.83 (at 20 GHz). At low frequencies, the dielectric constant was determined from current−voltage characteristics of Al−polyterpenol−Al devices. At frequencies below 100 kHz, the dielectric constant varied with RF power, from 3.86 to 4.42 at 1 kHz. For all samples, the resistivity was in order of 10^8−10^9 Ωm (at 6 V), confirming the insulating nature of polyterpenol material. *In situ* iodine doping was demonstrated to increase the conductivity of polyterpenol, from 5.05×10^{-8} S/cm to 1.20×10^{-6} S/cm (at 20 V).

Exposed to ambient conditions over extended period of time, polyterpenol thin films were demonstrated to be optically, physically and chemically stable. The bulk of ageing occurred within first 150 h after deposition and was attributed to oxidation and volumetric relaxation. Thermal ageing studies indicated thermal stability increased for the films manufactured at higher RF powers, with degradation onset temperature associated with weight loss shifting from 150 ºC to 205 ºC for 10 W and 100 W polyterpenol, respectively. Annealing the films to 405 °C resulted in full dissociation of the polymer, with minimal residue.

Given the outcomes of the fundamental characterisation, a number of potential applications for polyterpenol have been identified. Flexibility, tunable permittivity and loss tangent properties of polyterpenol suggest the material can be used as an insulating layer in plastic electronics. Implementation of polyterpenol as a surface modification of the gate insulator in pentacene-based Field Effect Transistor resulted in significant improvements, shifting the threshold voltage from + 20 V to –3 V, enhancing the effective mobility from 0.012 to 0.021 cm^2/Vs, and improving the switching property of the device from 10^7 to 10^4. Polyterpenol was demonstrated to have a hole transport electron blocking property, with potential applications in many organic devices, such as organic light emitting diodes.

Encapsulation of biomedical devices is also proposed, given that under favourable conditions, the original chemical and biological functionality of terpinen−4−ol molecule can be preserved. Films deposited at low RF power were shown to successfully prevent adhesion and retention of several important human pathogens, including *P. aeruginosa, S. aureus,* and *S. epidermidis,* whereas films deposited at higher RF power promoted bacterial cell adhesion and biofilm formation. Preliminary investigations into *in vitro* biocompatibility of polyterpenol demonstrated the coating to be non−toxic for several types of eukaryotic cells, including Balb/c mice macrophage and human monocyte type (HTP−1 non-adherent) cells. Applied to magnesium substrates, polyterpenol encapsulating layer significantly slowed down *in vitro* biodegradation of the metal, thus increasing the viability and growth of HTP−1 cells. Recently, applied to varied nanostructured titanium surfaces, polyterpenol thin films successfully reduced attachment, growth, and viability of *P. aeruginosa* and *S. aureus*.

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LIST OF PUBLICATIONS

The following is a list of publications by the candidate during the period of this thesis.

Jacob, M. V., Bazaka, K., *Fabrication of electronic materials from Australian essential oils*, Rural Industries Research and Development Corporation 2010. (Ch. I)

Bazaka, K., Crawford, R. J., Ivanova, E. P., Do bacteria differentiate between degrees of nanoscale surface roughness? *Biotechnolology Journal* 2011, 6, 1103-1114. (Ch. II)

Bazaka, K., Crawford, R. J., Nazarenko, E. L., Ivanova, E. P., Bacterial extracellular polysaccharides, in: Linke, D., Goldman, A. (Eds.), *Bacterial Adhesion*, Springer Netherlands 2011, 213-226. (Ch. II)

Bazaka, K., Jacob, M. V., Crawford, R. J., Ivanova, E. P., Plasma assisted surface modification of organic biopolymers. *Acta Biomaterialia* 2011, 7, 2015-2028. (Ch. II)

Bazaka, K., Jacob, M. V., Crawford, R. J., Ivanova, E. P., Efficient surface modification of biomaterial to prevent biofilm formation and the attachment of microorganisms. *Applied Microbiology Biotechnology* (in review). (Ch. II)

Bazaka, K., Jacob, M. V., Synthesis of radio frequency plasma polymerized non-synthetic terpinen-4-ol thin films. *Materials Letters* 2009, 63, 1594-1597. (Ch. III)

Bazaka, K., Jacob, M. V., Bowden, B. F., Optical and chemical properties of polyterpenol thin films deposited via plasma-enhanced CVD. *Journal Materials Research* 2011, 26, 1018-1025. (Ch. III)

Bazaka, K., Jacob, M. V., Nanotribological and nanomechanical properties of plasma polymerised polyterpenol thin films. *Journal Materials Research* 2011, 26, 2952-2961. (Ch. IV)

Bazaka, K., Jacob, M. V., Post-deposition ageing reactions of plasma derived polyterpenol thin films. *Polymer Degradation and Stability* 2010, 95, 1123-1128. (Ch. V)

Bazaka, K., Jacob, M. V., Solubility and surface interactions of RF plasma polymerized polyterpenol thin films (in review). (Ch. V)

Bazaka, K., Jacob, M. V., Complex permittivity measurements of RF plasma polymerized polyterpenol organic thin films employing split post dielectric resonator. *Journal of Polymer Engineering* 2011, 31, 73-75. (Ch. VI)

Bazaka, K., Jacob, M. V., Investigation of electrical conduction in plasma polymerised polyterpenol thin films (in review). (Ch. VI)

Bazaka, K., Jacob, M. V., Effect of iodine doping on surface and optical properties of polyterpenol thin films. *Material Science Forum* 2010, 654-656, 1764-1767. (Ch. VI)

Bazaka, K., Jacob, M. V., Effects of iodine doping on optoelectronic and chemical properties of polyterpenol thin films (in review). (Ch. VI)

Jacob, M. V., Bazaka, K., Weis, M., Taguchi, D., et al., Fabrication and characterization of polyterpenol as an insulating layer and incorporated organic field effect transistor. *Thin Solid Films* 2010, 518, 6123-6129. (Ch. VII)

Bazaka, K., Jacob, M. V., Taguchi, D., Manaka, T., Iwamoto, M., Investigation of interfacial charging and discharging in double-layer pentacene-based metal-insulator-metal device with polyterpenol blocking layer using electric field induced second harmonic generation. *Chemical Physics Letters* 2011, 503, 105-111. (Ch. VII)

Bazaka, K., Jacob, M. V., Taguchi, D., Manaka, T., Iwamoto, M., Effect of organic gate dielectric material properties on interfacial charging and discharging of pentacene MIM device. *Physics Procedia* 2011, 14, 62-66. (Ch. VII)

Jacob, M. V., Bazaka, K., Taguchi, D., Manaka, T., Iwamoto, M., Electron−blocking hole−transport polyterpenol thin films. *Chemical Physics Letters* (http://dx.doi.org/10.1016/j.cplett.2012.01.031). (Ch. VII)

Bazaka, K., Jacob, M. V., Ivanova, E. P., Plasma polymerisation and retention of antibacterial properties of terpinen-4-ol, in: Mendez-Vilas, A. (Ed.), Science and technology against microbial pathogens. *Research, development and evaluation*. World Scientific Publishing Group 2011. (Ch. VIII)

Bazaka, K., Jacob, M. V., Ivanova, E. P., A study of a retention of antimicrobial activity by plasma polymerized terpinen-4-ol thin films. *Material Science Forum* 2010, 654-656, 2261-2264. (Ch. VIII)

Bazaka, K., Jacob, M. V., Truong, V. K., Ivanova, E. P., Radio frequency plasma enhanced synthesis of antifouling polymeric coatings from monoterpene alcohols. *IEEE TENCON Proceedings* 2010, 1486-1491. (Ch. VIII)

Bazaka, K., Jacob, M., Truong, V. K., Crawford, R. J., Ivanova, E. P., The effect of polyterpenol thin film surfaces on bacterial viability and adhesion. *Polymers* 2011, 3, 388-404. (Ch. VIII)

Bazaka, K., Jacob, M. V., Truong, V. K., Wang, F., et al., Effect of plasma-enhanced chemical vapour deposition on the retention of antibacterial activity of terpinen-4-ol. *Biomacromolecules* 2010, 11, 2016-2026. (Ch. VIII)

Publications not directly related to this thesis:

Bazaka, K., Jacob, M. V., Shanks, R. A., Fabrication and characterization of RF plasma polymerized thin films from 3,7-dimethyl-1,6-octadien-3-ol for electronic and biomaterial applications. *Advanced Materials Research* 2010, 123-125, 323-326.

Jacob, M. V., Olsen, N. S., Bazaka, K., Shanks, R. A., RF plasma polymerisation of linalool and the characterisation of the polymer thin films (in review).

CHAPTER I

INTRODUCTION

I.1 RATIONAL

There is an ever increasing demand for novel and advanced materials that can support ongoing technological development. In electronics, global semiconductor market is currently dominated by silicon based technology, with basic limitations of device fragility and significant cost of production. These limitations significantly hinder the advancement of novel and existing electronics applications, especially those that demand low-cost, lightweight and flexible circuit materials. Organic conducting, semiconducting, and insulating materials have the potential to address this need for low-cost, reliable and efficient materials by adopting innovative approaches for fabrication of novel and enhancement of existing materials. Indeed, recent significant progress in flexible large-area self-illuminating displays based on Organic Light Emitting Diodes (OLEDs) and Organic Field Effect Transistors (OFETs), low-cost organic solar cells (OSCs) and photovoltaic devices (OPVs), sensors and "plastic" electronics have been enabled by contemporaneous advancements in materials research. In addition to enormous economic significance, advances in organic electronics materials and devices will have far reaching implications in shaping novel and advanced technologies for future applications. Furthermore, controlling electronic confinement in increasingly miniaturised integrated circuit technology is increasingly exigent as the dimensionality and size scale of structures are reduced and the operating frequencies of communication and electronic systems increase. An appropriately chosen insulating polymer material with a lower dielectric constant can be employed to minimise signal delay, cross talk and lower device power consumption. Thermally stable dielectric interlayers can assist in dissipation of heat from the metal interconnecting lines, while optically transparent encapsulating coatings can protect electronic devices from the unfavourable environmental conditions and extend device performance.

Similarly, progress in materials research plays a crucial role in the advancement of regenerative medicine and tissue engineering, as well as technologies used for the detection and treatment of diseases. Over the last several decades, a number and complexity of available biomaterials have increased dramatically. In spite of impressive bulk material performance, many synthetic biomaterials frequently cause adverse physiological reactions when applied *in vivo.* Inflammation, infection and prolonged healing may result when surface properties of biomaterials are poorly matched to their *in vivo* environment. Currently, there is an increasing interest in economical technologies that allow for surface modification of biomaterials by which biocompatibility and biofunctionality can be attained, while preserving the favourable bulk characteristics of the biomaterial, such as strength. Amongst the numerous surface modification techniques available, application of organic coatings enables tailoring of the surface morphology, structure, composition, and properties of the material to a specific need. Functional organic coatings have the potential as biosensitive interfaces, connecting inorganic/metallic electronic devices with their physiological environments. Without materials that posses properties necessary for the advancement of current and development of new devices, avenues for advancement may become restricted.

Environmentally friendly materials and manufacturing technologies are attracting significant research and commercial attention globally. Precursor chemicals and fabrication methods commonly employed for fabrication of electronics materials and devices are frequently harmful to humans and the environment. Employment of technologies that require minimal use of harmful chemicals and produce no hazardous by-products can enable environmentally sustainable development. Furthermore, use of alternative monomer sources that are non-synthetic and can be polymerised to produce materials with properties comparable or superior to those of conventional materials is highly attractive. These materials may also possess desirable biodegradation properties.

Based on the aforementioned consideration, an Australian essential oil product, terpinen-4-ol, was chosen as a monomer for this project. RF plasma polymerisation was selected as a manufacturing method for it is an environmentally friendly technique capable of producing thin films of high quality and properties that can be tuned to the desired application.

I.2 RESEARCH OBJECTIVES

This project aims to fabricate a range of environmentally friendly low-cost materials from monoterpene alcohols for advanced and emerging applications, particularly in electronics and biomedical fields. This is achieved by addressing the following objectives:

- To develop a novel plasma-aided technique for highly controlled nanofabrication of functional organic materials and devices.
- To develop organic polymer thin film structures from synthetic (as a benchmark) and renewable resources using the RF plasma-aided techniques.

- To characterise these films using appropriate methodology and to compare their properties against materials currently in use within a chosen application.
- To identify most favourable deposition conditions and to further optimise most promising polymer thin films for a given application.
- To test performance of the developed thin film structures within the desired application to access feasibility and effectiveness of such implementation.
    - To design, develop and realise new organic electronic devices based on multi-layer polymer thin-films using plasma-assisted nanofabrication, leading to the realisation of a fully flexible electronic circuit.
    - To enhance performance and long-term stability of organic electronic devices via surface modification of device components and device encapsulation using plasma-assisted nanotechnology.
    - To improve biocompatibility of biomaterials via polymer encapsulation and to impart antifouling and antibacterial properties of monoterpene alcohols onto biomaterials using plasma-assisted surface modification.

This project builds on the preliminary work conducted by lead supervisor, A/Prof Jacob and the author, results of which has been published as Jacob, M. V., Bazaka, K., *Fabrication of electronic materials from Australian essential oils*, Rural Industries Research and Development Corporation 2010.

The outcomes of this project are expected to contribute to the field of materials development and characterisation. The developed technology is also expected to benefit the advancement of existing and novel applications in the areas of organic electronics and biomaterials. As an organic semiconducting material, polyterpenol has the potential to assist in design and realisation of environmentally friendly fully-flexible low-cost electronic devices. As antibacterial and antifouling coatings, polyterpenol has the potential to enhance biocompatibility of implantable biomaterials and biomaterial-based devices. In addition to immediate applications, the knowledge obtained in the course of this project will likely allow for achieving a better understanding of the relation between materials and device performance. From socio-economic perspective, this project will provide a framework for fabrication of essential-oil based polymer materials for advanced applications, potentially leading to the extension of utilisation of such Australian resources in high-tech applications.

I.3 THESIS ORGANISATION

This thesis comprises nine chapters, each representing a manuscript or a series of manuscripts at various stages of publication joined by a common theme. Given that each article is expected to stand on its own, a summary is provided at the beginning of each chapter to ensure cohesion and flow when reading chapters in sequential order.

Chapter I: This chapter provides the aim and objective, and also introduces the rational for this project. It builds on the preliminary findings published by *Jacob, M. V., Bazaka, K., Fabrication of electronic materials from Australian essential oils, Rural Industries Research and Development Corporation 2010.*

Chapter II: In this chapter, current progress in the fields of organic electronics and biomaterials is reviewed. In Section II.1, recent developments in the area of flexible electronics are discussed, with particular focus on the role plasma polymer thin films play in the advancement of these technologies. The potential of plasma-assisted modification to enhance biocompatibility of implantable medical devices and materials is discussed in Section II.2, and have been published as *Bazaka, K. et al., Plasma assisted surface modification of organic biopolymers. Acta Biomaterialia 2011, 7, 2015-2028.* These have also been reviewed, along with other surface modification tools for prevention of bacterial adhesion and biofilm formation, in *Bazaka, K. et al., Efficient surface modification of biomaterial to prevent biofilm formation and the attachment of microorganisms. Applied Microbiology Biotechnology*. The bases for cell−abiotic surface interactions are also provided in order to determine critical surface properties for which materials have to be optimised. These have been published as *Bazaka, K. et al., Do bacteria differentiate between degrees of nanoscale surface roughness? Biotechnolology Journal 2011, 6, 1103-1114*. The role of extracellular polymeric substance in colonisation, survival and virulence of bacteria are further investigated in *Bazaka, K. et al., Bacterial extracellular polysaccharides, in: Linke, D., Goldman, A. (Eds.), Bacterial Adhesion, Springer Netherlands 2011, 213-226.*

Chapter III: This chapter introduces the thin film fabrication procedure and outlines the details of the experimental apparatus employed throughout this work. Section III.1 presents the results from initial characterisation of polyterpenol deposited under a single set of fabrication conditions, as published in *Bazaka, K., Jacob, M. V., Synthesis of radio frequency plasma polymerized non-synthetic terpinen-4-ol thin films. Materials Letters 2009, 63, 1594-1597.* In Section III.2, studies of fundamental properties on a range of polymers fabricated under varied process conditions are reported. The outcomes of this study have been published in *Bazaka, K.*

et al., Optical and chemical properties of polyterpenol thin films deposited via plasma-enhanced CVD. Journal Materials Research 2011, 26, 1018-1025.

Chapter IV: In this chapter, film-substrate adhesion, mechanical and tribological properties of polyterpenol thin films are discussed. The findings were published as *Bazaka, K., Jacob, M. V., Nanotribological and nanomechanical properties of plasma polymerised polyterpenol thin films. Journal Materials Research 2011, 26, 2952-2961.*

Chapter V: Polyterpenol stability under ambient conditions and when subjected to environmental factors are investigated in this chapter. In Section V.1, ambient and thermal stability of the plasma polymers is discussed, as published in *Bazaka, K., Jacob, M. V., Post-deposition ageing reactions of plasma derived polyterpenol thin films. Polymer Degradation and Stability 2010, 95, 1123-1128.* Section V.2 explores the wettability and solubility of polyterpenol. The results of this investigation have been published as *Bazaka, K., Jacob, M. V., Solubility and surface interactions of RF plasma polymerized polyterpenol thin films*.

Chapter VI: This chapter reports on the electromagnetic properties of the polymer thin films fabricated under varied input power conditions. In Section VI.1, the dielectric properties of polyterpenol are characterised at optical and microwave frequencies, published as *Bazaka, K., Jacob, M. V., Complex permittivity measurements of RF plasma polymerized polyterpenol organic thin films employing split post dielectric resonator. Journal of Polymer Engineering 2011, 31, 73-75*. The insulating properties are also investigated at low frequencies, with the findings presented in Section VI.2 and in *Bazaka, K., Jacob, M. V., Investigation of electrical conduction in plasma polymerised polyterpenol thin films*. In Section VI.3, iodine doping is discussed as a potential means to enhance polyterpenol conductivity, with the outcomes published as *Bazaka, K., Jacob, M. V., Effect of iodine doping on surface and optical properties of polyterpenol thin films. Material Science Forum 2010, 654-656, 1764-1767* and communicated as *Bazaka, K., Jacob, M. V., Effects of iodine doping on optoelectronic and chemical properties of polyterpenol thin films*.

Based on the attained fundamental properties of polyterpenol, a number of potential applications for polyterpenol are reviewed.

Chapter VII: In this chapter, polyterpenol as a potential gate insulating layer in organic electronic devices is investigated. In Section VII.1, performance of polyterpenol-incorporated pentacene-based organic field effect transistors is compared against a non-modified counterpart. The improvement is shown with respect to device mobility, threshold voltage and switching ratio, with the study published as *Jacob, M. V., Bazaka, K., et al., Fabrication and characterization of polyterpenol as an insulating layer and incorporated organic field effect transistor. Thin Solid Films 2010, 518, 6123-6129*. Sections VII.2 and VII.3 investigate the carrier transport in double-layer p- and n-type metal-insulator-metal devices using electric field induced second harmonic generation, where polyterpenol was used as an insulating blocking layer. The carrier behaviour in polyterpenol-incorporated p-type MIM structures has been published in *Bazaka, K. et al., Investigation of interfacial charging and discharging in double-layer pentacene-based metal-insulator-metal device with polyterpenol blocking layer using electric field induced second harmonic generation. Chemical Physics Letters 2011, 503, 105-111*. The influence of polyterpenol material properties on the carrier transport within MIM structures has been published as *Bazaka, K. et al., Effect of organic gate dielectric material properties on interfacial charging and discharging of pentacene MIM device. Physics Procedia 2011, 14, 62-66.* The respective carrier behaviour in polyterpenol-containing n-type MIM devices has been reported as *Jacob, M. V., Bazaka, K.et al., Electron−blocking hole−transport polyterpenol thin films. Chemical Physics Letters http://dx.doi.org/10.1016/j.cplett.2012.01.031.*

Chapter VIII: This chapter investigates polyterpenol potential as an antifouling and antibacterial coating for encapsulation of medically-relevant devices. In Section VIII.1, the biological activity of the polymer against *Pseudomonas aeruginosa* is experimentally demonstrated, with the results published as *Bazaka, K. et al., A study of a retention of antimicrobial activity by plasma polymerized terpinen-4-ol thin films. Material Science Forum 2010, 654-656, 2261-2264.* Theoretical modelling is used to substantiate the observed phenomena, as published in *Bazaka, K. et al., Effect of plasma-enhanced chemical vapour deposition on the retention of antibacterial activity of terpinen-4-ol. Biomacromolecules 2010, 11, 2016-2026*. The activity of polyterpenol coating against other human pathogenic bacteria is further investigated in *Bazaka, K. et al., Radio frequency plasma enhanced synthesis of antifouling polymeric coatings from monoterpene alcohols. IEEE TENCON Proceedings 2010, 1486-1491* and *Bazaka, K. et al., Plasma polymerisation and retention of antibacterial properties of terpinen-4-ol, in: Mendez-Vilas, A. (Ed.), Science and technology against microbial pathogens. Research, development and evaluation. World Scientific Publishing Group 2011.* The role of polyterpenol surface architecture in controlling bacterial attachment and proliferation is studied in Section VIII.2, as published in *Bazaka, K. et al., The effect of polyterpenol thin film surfaces on bacterial viability and adhesion. Polymers 2011, 3, 388-404.*

Chapter IX: Concluding remarks and a recommendation for future work are provided in this chapter.

The work outlined in this document was performed by the candidate, Kateryna Bazaka, in discussion with supervisors and experts in respective fields of research. The contribution of others has been therefore acknowledged in the authorship of the publications. Project supervisors A/Prof Mohan Jacob (all Chapters) and A/Prof Bruce Bowden (Section III.2) contributed funding and time through training and feedback. Prof Elena Ivanova (Section II.2, Chapter VIII) and Prof Russell Crawford (Section II.2, Chapter VIII) provided expertise and access to facilities needed for biological characterisation, specifically antibacterial properties of polyterpenol. Vi Khanh Truong assisted in the collection of scanning laser microscopy and confocal laser scanning microscopy data (Chapter VIII). In Section VIII.1, Prof Feng Wang and Anoja Pushpamali assisted with chemical modelling, Prof Chris Berndt and Dr James Wang provided access to scanning electron microscope, and A/Prof Amanda Ellis assisted with the collection of Raman spectroscopy data. In Chapter VII, Prof Mitsumasa Iwamoto, A/Prof Takaaki Manaka and Dr Dai Taguchi provided knowledge on the electrical analysis of the polymers, specifically electric field induced second harmonic generation, and access to necessary equipment. Dr Martin Weis (Section VII.1) provided his expertise in fabrication and characterisation of pentacene-based organic field effect transistors. Where possible, all experiments were performed by the candidate.

CHAPTER II

LITERATURE REVIEW

This chapter provides scientific background for the research presented in this thesis.

Section II.1 gives a succinct overview of the current progress within the area of organic electronics. Limitations imposed by currently available technology, and consequent demands for novel semiconducting and insulating materials are discussed. Organic polymer materials are discussed as an alternative to conventional materials, with specific attention given to those materials fabricated using plasma polymerisation.

Section II.2 details recent advancements in the development of biomaterials with tailored surface properties which afford them enhanced biological performance. Plasma-assisted surface modification of biomaterials as a tool to effectively limit bacterial attachment and improve biocompatibility with host tissues is reviewed. Two review articles have been published by the author detailing various aspects of interactions between cells and native and nanostructured surfaces, including those modified using plasma-based technologies, − *Bazaka, K. et al., Plasma assisted surface modification of organic biopolymers. Acta Biomaterialia 2011, 7, 2015-2028*, and *Bazaka, K. et al., Efficient surface modification of biomaterial to prevent biofilm formation and the attachment of microorganisms. Applied Microbiology Biotechnology.*

The fundamentals of cell-surface interactions have been reviewed in *Bazaka, K. et al., Bacterial extracellular polysaccharides, in: Linke, D., Goldman, A. (Eds.), Bacterial Adhesion, Springer Netherlands 2011, 213-226*, and *Bazaka, K. et al., Do bacteria differentiate between degrees of nanoscale surface roughness? Biotechnolology Journal 2011, 6, 1103-1114.*

II.1 CURRENT PROGRESS IN ORGANIC ELECTRONICS

II.1.1 Introduction

After more than twenty years of basic and applied research, the use of nanotechnology in the design and manufacturing of nanoscale materials is rapidly increasing, particularly in commercial applications that span across many areas, including electronics, renewable energy, cosmetics, automotive and medical products. There are currently in excess of 1,000 manufacturer-identified nanotechnology-based consumer products on the market, and this number is rapidly increasing as a result of high levels of investment in research, development and commercialisation, together with an equally high demand for novel and advanced products from manufacturers and consumers. Nanotechnology is unprecedented in that it allows for the production of smaller, lighter, less expensive materials and devices that are significantly more efficient compared to conventional micro- and macrotechnologies. By allowing greater degrees of manufacturing control at the molecular and atomic scales, nanotechnology promises to supersede existing manufacturing technologies by tackling issues that for a long time were far beyond our reach.

In electronics, the advancement of nanoscale fabrication tools and materials facilitated a significant growth in the area of communication and optoelectronic device, also introducing new challenges. In the pursuit of fast computation, controlling electronic confinement in the solid state is increasingly difficult as the dimensionality and size scale of structures are reduced, covering the atomic, molecular and macromolecular length scales [1,2]. Having advanced at exponential rates in both performance and productivity, silicon semiconductor technology almost reached its fundamental, material, device, circuit, and system limits, with its remaining potential highly dependent on the ability to develop and economically mass produce double-gate MOSFETs with gate oxide thickness of ~ 1 nm, silicon channel thickness and length of 3 and 10 nm, respectively, and interconnecting wires to connect them [3]. Even if nanodevices with suitable performance become available, only economically viable nanotechnology solutions will find practical use [2].

II.1.2 Limitations associated with downsizing electronic devices

Indeed, device fabrication at this scale is challenging. Since the resolution of lithography is depended on the wavelength of the light sources, sources producing increasingly shorter wavelengths are required. Furthermore, a concomitant development of suitable photo resist, masks, and aligned systems is also needed. Although some technologies are successful at producing nano-features in the lab settings, their feasibility in commercial applications remain questionable. Another challenge in nanoscale device fabrication lies with doping, a process widely used to render semiconducting materials more conducting. Ion implantation and diffusion commonly engaged for the introduction of the impurity into the bulk of the material is in itself limited by the solubility of the semiconducting solid. In practical terms, this means that in a very small semiconducting layer, only several atoms of the doping element may be present, resulting in fuzzy boundaries and reduced device performance.

Further to consider, there are limitations imposed by the device operation. In current IC technology, complementary $n-$type and $p-$type metal oxide semiconductor field effect transistors (MOSFETs) are employed to realise the logic functions. In these devices, the speed of major carriers, electrons or holes in the case of $n-$ and $p-$type semiconductors, respectively, is linked to the carrier mobility in the channel, which in turn is inversely connected to the lateral electric field across the channel. As the electric filed is increased by the device downsizing, carrier velocity in the channel is no longer linearly related to the field. The increased scattering rate of highly energetic electrons causes the carrier velocity saturation and hence increases the transit time of carriers through the channel. Furthermore, in the inversion layer, the mobility is noticeably lower compared to the bulk material, owing to the electron wavefunction extending into the oxide where the carrier mobility is lower. The reduction in the device dimensions leads to the higher electric field across the channel, which displaces the electron wavefunction further into the oxide layer, and thus decreases the mobility and operating speed of the device. Increasingly thinner, the gate oxide layer is more susceptible to degradation from the increased tunnelling of carriers from the channel, eventually leading to the time-dependent destructive breakdown of the oxide layer.

The miniaturisation of the device further reduces the length of the channel along which the carriers are transported. As a consequence, the depletion region of the drain created by the application of the drain voltage may increase, ultimately merging with the source depletion region. In this state, the field at the gate is strongly dependent on the drain−source voltage to the extent where an increase in drain−source voltage may result in a rapidly increasing (leakage) current, with the carriers being transported from the source to the drain. Named ‘punch trough’, this phenomenon limits the maximum voltage at which the device operates and increase the output conductance of the MOSFET. Breakdown at a lower drain−voltage can also result from the synergistic relationship between the avalanche breakdown at the drain and the parasitic bipolar action, whereby holes resulting from the avalanche breakdown are transported from the drain to source under the inversion layer, positively contributing to avalanche multiplication.

In small scale MOSFETs, the parasitic capacitances inherent to the physical structure of the device become on the same scale as the intrinsic channel capacitance, resulting in a source of error in the analysis of these devices [4]. Furthermore, the potential increase in the parasitic capacitance of the gate−drain, gate−source, drain−bulk, and source−bulk capacitors stemming from the increase in the surface−to−bulk ratio of the components limits the performance of the MOSFET [5]. It is therefore evident, that scaling of MOSFET is a complicated endeavour that necessitates not only a reduction in the dimensionality of the gate, but also requires a concomitant miniaturisation of the gate−source and gate−drain alignment, appropriate scaling down of the oxide and the depletion layer widths, and matching of the substrate doping density.

II.1.3 Alternative polymer technologies for electronics advancement

The search for functional nanometer-scale structures has led to the exploration of many alternatives, including self-assembling monolayers, quantum dots, organic molecules, carbon nanotubes, nanowires, and single atoms or molecules [6,7]. Organic materials-based electronic devices offer unique attractions compared to silicon electronics, which include high-throughput and inexpensive production, mechanical flexibility, light weight and efficient integration within the supply chain [8]. Organic polymer semiconductors exhibit favourable material properties that include light emission, luminescent sensing, ferromagnetism, photoconductivity and photovoltaic effect, photonic patternability, optical nonlinearity, optical power limiting, to name but a few [9,10]. Many semiconducting conjugated polymers are soluble, and therefore can be solution-processed and printed onto large substrates, enabling low-cost and simple fabrication of a wider range of devices [11-13]. Owing to their synthetic nature, fundamental material properties of these polymers can be optimised to suit the identified application, by varying the nature of the monomer material and the polymerisation conditions [14]. A comprehensive summary of commonly used materials is available from a technical periodical Materials Matters published quarterly by Sigma Aldrich.

As such, organic electronics present exciting new possibilities for fundamental research and cutting-edge applications, such as large-area self-illuminating displays based on organic light emitting diodes (OLED) and organic field effect transistors (OFET), low-cost organic solar cells (OSCs) and photovoltaic devices, sensors and "plastic" electronics [15-19]. The market for printed organic, inorganic and composite electronics is expected to increase from $1.92 billion in 2009 to $57.16 billion in 2019, driven by a substantial growth of PV, OLEDs (on glass) and e-paper displays, followed by mixed organic and inorganic TFT circuitry, flexible OLEDs, sensors and batteries [20]. In addition to enhanced capabilities, the interest in these low-cost technologies stems from an increasing global interest in the development of renewable resources and advancement of technologies used for efficient energy conversion.

The International Energy Agency predicts the global investment in energy supply to reach estimated 22 trillion dollars by 2030, with photovoltaics accounting for a growing proportion (estimated at 10–20%) of this investment [20]. By converting sun light into electricity, organic photovoltaic cells aim to provide an environmentally sustainable alternative to burning fossil fuels [21]. Conversely, the research into OLEDs comes from the ability of these devices to generate light from energy in a highly efficient manner [22]. Other advantages of organic LEDs include their self-emitting characteristic, high luminous efficiency, full colour capability, wide viewing angle and high contrast [9]. These two complementary technologies can address a rapidly growing global need for energy. In a similar fashion, organic transistors and memory devices have the potential to attend to an ever expanding demand for technologies that enable high-speed computation and communication, and facilitate storage of rapidly increasing data sets [23].

Emerging technologies can also be realised with organic thin film materials characterised by useful functionalities. Recently, attempts have been made to integrate electronic systems with neural host systems, where an i*n vivo* neurotransmitter is used to effectively control the delivery of ions and other bio substances to the host thus modulating the sensory function in a living animal. Organic electronic ion pump technology was demonstrated to mimic the chemical and electronic signalling of native neurons, with attractive prospects in the field of electronically controlled diffusive non-convective biomolecule delivery for neural system interfacing and the management of neurological disorders [24].

A wide array of organic semiconducting and insulating materials is currently being intensely investigated for their potential applications in electronics and photonics [25]. For instance, linear acenes, thiophenes and their derivatives offer higher mobilities, better on-off ratios, improved environmental stability, and better reliability than most other organic semiconductors, while in graphene charge carriers exhibit giant intrinsic mobility, have zero effective mass, and can travel for micrometers without scattering at room temperature [26-28]. These materials vary significantly in their performance and the characteristics they possess. Historically, significant progress has been achieved in the advancement of p-channel semiconducting materials, such as functionalised acenes [29-33] and thiophene derivatives [34-39], whereas the development of high-performing n-channel materials was less successful [40]. Recently, however, several promising n-type semiconductors have been identified, such as dicyanomethylene-substituted tetrathienoquinoids, rylene and fullerene [41-47].

Bulk heterojunctions, currently the primary configuration for highly efficient organic solar cells, can be formed using bicontinuous polymer/fullerene donor-acceptor composites or phase-separated blends of [6,6]-phenyl C_{61}-butyric acid methyl ester and poly(3-hexylthiophene), the latter having power conversion efficiency of above 5% [9,48,49]. OFET devices based on solution-processed poly(3-hexylthiophene) demonstrated high hole mobilities of ~$0.10\,cm^2\,V^{-1}\,s^{-1}$, while the mobility of devices featuring air-stable 2,9-didecyldi-naphtho[2,3-b:2’,3’-f]thieno[3,2-b]thiophene exedeed 10 cm^2 V^{-1} s^{-1} [38]. Devices incorporating ambipolar architectures that are capable of supporting both n- and p-type conduction are investigated for these devices can enable complementary-like inverters without the need for sophisticated nanoscale patterning [9]. Numerous expansive review papers outlining various aspects of organic electronics materials and devices design, fabrication, characterisation and operation exist in the literature [4,5,7,9,14,22,23,40,47,50-54].

It is evident that the performance and lifetime of these devices are critically reliant on the properties of both active materials and their interfaces with inorganic electrodes used to inject the carriers and dielectric layers [51]. Interfacial characteristics that can be modified to impact on the device stability and behaviour range from free surface energy and surface morphology of individual layers, to adhesion between different layers within the device, to modifications of the actual electronic structure of the layers. For example, the surface energy and work function characteristic significantly influence the carrier injection, transportation and charge extraction. The employment of surfactant-modified cathodes [55], hole-transporting buffer layers [56], and self-assembled monolayer-modified anodes [57,58] has been demonstrated to directly affect the performance of electronic devices.

The nature of the interface between source and drain electrodes and the organic semiconductor will have a direct influence onto the overall performance of the OFET structure by affecting the charge carrier injection and the formation of the conduction channel, respectively [59-63]. Chemical optimisation of these interfaces with self-assembled monolayers and passivating the surface of widely employed SiO_2 or Al_2O_3 dielectrics have been demonstrated to positively contribute to the performance of OFET structures by increasing the crystallinity for the growth of organic semiconductors and decreasing charge carrier traps and tailor the surface energy of gate dielectric. In OPV devices, a bulk heterojunction configuration requires a precise control over the charge transport and carrier recombination across donor–acceptor interfaces [64]. The fill factor and consequently the power conversion efficiency are dependent on the device series resistance, which in turn is related to the contact resistance between the device layers [65,66]. Furthermore, surface modification can potentially be employed to further enhance OPV device performance via passivation of charge trap states, controlling energy level alignment, improvement of charge extraction, and enhancement of materials surface and work function compatibility [67-70].

Despite the exceptional properties of the organoelectronics materials, the realisation of high-performance, ambient-stable polymeric semiconductors remains challenging. Current application of organic structures in electrical devices is susceptible to short circuits and the devices are mechanically fragile at high voltages. Susceptibility to degradation upon exposure to operational environmental conditions is one of the foremost issues that hinder organic electronic circuits. Furthermore, realisation of a complete electronic circuit based on organic polymers, analogous to conventional silicon chips, requires the devices to carry negative charge [71,72]. The discovery of high-performance, stable and simple to process polymeric semiconductors would represent a major step towards polymeric complementary circuit technologies, where the combination of p- and n-channel transistors results in far greater circuit speeds, lower power dissipation and more stable operation [71]. Currently, plasma-assisted fabrication of intricate nanostructures and complex nanoassemblies with attractive semiconducting properties is attracting significant attention, both for fundamental research and for existing and potential industrial applications [73-75].

II.1.4 Plasma based nanotechnologies in deterministic nanoassembly of organic electronic materials and devices

While industrial applications of low-temperature plasmas in microelectronic manufacturing and film deposition are well-established, employment of low-temperature plasmas for material synthesis and processing at nanoscale and fabrication of nanodevices is a very new and emerging area at the frontier of nanoscience and nanotechnology. Plasma-assisted nanomodification allows for highly controlled modification of surfaces, including deposition of ultra-thin functional pin-hole free layers, activation and passivation of surfaces, and precise and selective nano-etching and nano-patterning of electronic devices. It is also unique in its ability to produce polymers from organic compounds that do not polymerise under normal polymerisation conditions [76,77]. As a result, plasma-assisted techniques can be used for the development of organic polymer thin films and high-quality and high-resolution functionalisation of materials at defined positions, with large-scale control of location and orientation, with the performance of plasma-based nanotools being proved superior to competing nanofabrication techniques [78]. A particular advantage of non-equilibrium plasmas is the possibility of directed motion of charged species under the influence of the electric field, which allows for anisotropic etching of substrates and controlled deposition of nanoassembly building units, including processing of temperature-sensitive materials such as polymers, plastics and ultra-thin nanolayers, and low-temperature crystallization. Application of non-equilibrium plasmas is also highly advantageous where directionality of fluxes and penetration of species is necessary and where controlled amounts of specific radicals are required. Furthermore, these plasmas are employed in cases where pre-formed building blocks are needed, for ionisation of otherwise neutral species, and for highly specific surface modification. Use of cold plasma is an efficient way to enhance catalyst activity, and owing to its low temperature is a process suitable for high-rate fabrication of delicate nanoassemblies. Thus, exploitation of plasma-enhanced nanofabrication presents great opportunities, both for pure research in understanding mechanisms of nanostructure formation, in applied research for the development of novel processes, and in new industrial applications.

Low-temperature plasma systems contain neutral and charged, reactive and non-reactive species, with the chemical structure and other properties that make them indispensable for nanoscale fabrication of exotic architectures of different dimensionality and functionality, and places uniquely among nanofabrication tools [78]. The species range from individual atoms and molecules to macromolecules, clusters, and mesoparticles. In these plasmas, electrons typically are at temperature of 1– 2 eV, whereas ions and neutrals are often at room temperature of approximately 0.026 eV, due to relatively low degree of ionization (in the range 10^{-5}–10^{-2}). The ‘coldness’ of the neutral gas makes the process ‘solid-state-friendly’ as it increases the chance of formation and preservation of large species, such as clusters and nanosized grains, in a solid state, and makes the environment ideal for temperature-sensitive nanofabrication , such as microchip fabrication and polymer processing. For plasma polymerisation, monomers that possess functionalities associated with conventional polymerization (such as unsaturation) undergo a more complex recombination process, combining conventional polymerization with fragment-recombination mechanisms triggered by the plasma and surface-attached active species, ions and free radicals. Low-temperature, weakly ionised plasmas also facilitate high level of control over delivery and consumption of building units, which is essential for the synthesis of delicate nanoassemblies, such as low-dimensional epitaxial semiconductor nanostructures. Such controlled delivery and high and uniform densities of species involved can also be used for controlled and highly uniformed atomic layer etching.

In spite of superior performance of plasma-based nanotools compared to many other competing nanofabrication techniques for the fabrication of nanoscale structures and devices, understanding of the most effective features of plasma-based nanoscale processes for precisely controlled and balanced delivery and consumption of plasma-generated building units is yet to be sufficient. There is a certain degree of debate regarding the roles of specific functional plasma-generated species in nanostructure formation or surface preparation. The stability, reproducibility and high-uniformity of the densities, fluxes and temperatures of the species required for the specific plasma-assisted nanoassembly has also been questioned, as it is highly dependent on the precise control of major process determinants of plasma-assisted growth kinetics, including monomer supply/consumption, the residence time of building units, magnitude of energy and operating pressure, substrate charge and temperature, etc.

The field of plasma-based nanofabrication will benefit from further experimental, theoretical and computational efforts required to provide additional detail regarding the influence of charges on plasma-exposed surfaces, electric potentials in the vicinity and across the nanoassemblies, ion fluxes and ion-flux-related temperature effects on the properties of the resultant materials. A deeper understanding of the effectiveness of process parameters contribution to the plasma-assisted nanostructure growth kinetics of organic nanostructures will be instrumental in achieving fully deterministic nanoassembly. Such knowledge will also contribute to the continuous goal of increasing resolution and selectivity in plasma etching of nanoassemblies and nanofeatures to supersede the limits of currently available lithographic tools.

II.1.5 Conclusion

Improvements in OLEDs and OFETs are crucial for the implementation of flexible electronic circuits and expansion of novel roles in electronics that cannot be filled by silicon technology alone. However, for these improvements to take place, materials with advanced semiconducting properties are essential. Furthermore, the performance and stability of these devices can be substantially enhanced by controlling the semiconductor–dielectric interfacial properties, such as by introducing a modification layer [79-82]. Due to their tunable surface chemistry and diverse properties, polymer dielectrics are ideal candidates for fabrication of OFETs with desirable properties and improved efficiency. Plasma-assisted polymerisation is a tool highly suited for production of thin semiconducting and insulating coatings with tunable material properties.

II.2 NANOBIOTECHNOLOGY AND BIOMATERIALS

In spite of significant advancement that have been made in the area of biomaterials and biomaterial-based devices, the real-life applicability of these materials is still hindered by the adverse physiological responses these materials may cause when in contact with biological environment. Numerous synthetic biomaterials that possess physical properties that are comparable or even superior to those of natural body tissues, frequently fail to perform once *in vivo* due to infections, inflammation and poor tissue integration. Most of the currently available biomaterials have been chosen for their favourable bulk characteristics, with little regard paid to the surface properties of the material. Recent developments in the microscopic and biochemical characterisation tools have enabled exploitation of certain nanoscale surface phenomena by providing valuable information as to how these affect interactions between living and created systems.

Section II.2.1 discusses plasma-assisted surface modifications as an economical and flexible tool that allows for tailoring of the surface morphology, structure, composition, and properties of the material to a specific need. A variety of materials can be modified using this technique, including temperature-sensitive polymer biomaterials. Given the antibacterial properties of terpinen-4-ol, modifications that limit bacterial adhesion and biofilm formation on indwelling medical devices are of particular interest to this project, and hence are reviewed in detail.

Section II.2.2 provides an overview of fundamental principles that govern cell-surface interactions at nanometre, molecular and atomic length scales. In-depth knowledge of how surface characteristics of biomaterials influence attachment, propagation and function of pathogenic and eukaryotic cells is required for adequate design of implantable devices and cell scaffolds. Chemical functionality and architecture of biomaterial surface can then be used to effectively promote or prevent the attachment of living cells. Furthermore, these surface properties can be imparted onto already available biomaterials, affording them with enhanced biocompatibility and bioactivity, with little detriment to their bulk characteristics.

II.2.1 PLASMA ASSISTED SURFACE MODIFICATIONS OF BIOMATERIALS

Abstract

Despite many synthetic biomaterials having physical properties that are comparable or even superior to those of natural body tissues, they frequently fail due to the adverse physiological reactions they cause within the human body, such as infection and inflammation. The surface modification of biomaterials is an economical and effective method by which biocompatibility and biofunctionality can be achieved, while preserving the favourable bulk characteristics of the biomaterial, such as strength and inertness. Amongst the numerous surface modification techniques available, plasma surface modification affords device manufacturers a flexible and environmentally friendly process that enables tailoring of the surface morphology, structure, composition, and properties of the material to a specific need. There are a vast range of possible applications of plasma modification in biomaterial applications, however the focus of this review paper is on processes that can be used to develop surface morphologies and chemical structures for the prevention of adhesion and proliferation of pathogenic bacteria on the surfaces of indwelling medical devices. As such, the fundamental principles of bacterial cell attachment and biofilm formation are also discussed. Functional organic plasma polymerized coatings are also discussed for their potential as biosensitive interfaces, connecting inorganic/metallic electronic devices with their physiological environments.

Keywords: bacterial adhesion; biofunctional coatings; plasma modification.

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II.2.1.1 Introduction

For decades, biomaterials have played an important role in disease management and the advancement of health care. Their applications range from coatings for tablets or capsules in pharmaceutical preparations to being essential components of extracorporeal devices such as contact lenses or kidney dialyzers, and indwelling devices and implants. Many of these materials were not originally designed for medical applications, and while they addressed many important medical issues, their use frequently led to complications, such as poor biocompatibility, time-dependent material degradation and subsequent mechanical failure, infection, inflammation and blood clot formation. The biomaterials were often selected only for their bulk properties, such as mechanical strength and inertness, and as a result, many widely used biomaterials exhibited significant drawbacks. Many possessed sub-optimal surface biological properties such as high hydrophobicity and high friction, resulting in deleterious effects such as inflammation and irregular tissue response. Recently, advanced surface characterisation techniques have allowed a better understanding of the reactions occurring at the interface between the biomaterial surface and host tissues. This has allowed an insight into the important role that the surface properties of biomaterials play with regard to the response of the biological environment to the indwelling medical devices [83]. As a consequence, novel techniques have been developed that can impart desirable chemical, physical, and biological properties to the biomaterials. This can occur through the synthesis of a new material with desirable properties built directly into its matrix, or by the modification of materials already being used by the medical industry [84]. The surface modification of biomaterials is an economical and effective method by which biocompatibility and biofunctionality can be achieved, while preserving the favorable bulk characteristics of the biomaterial, such as strength and inertness. One such modification technique is plasma surface modification. This provides device manufacturers with a flexible and environmentally friendly process that allows for tailoring the surface properties of the material to suit a specific need [85-90]. In addition, exposure to plasma has been shown to irreversibly damage bacterial cells, allowing for *in situ* sterilisation of the biomaterial during the surface modification process. For example, plasma sterilisation has been demonstrated to be effective against *Escherichia coli* [91,92], *Staphylococcus aureus* [91,93], *Pseudomonas aeruginosa* [92], *Bacillus cereus* [92], *Bacillus subtilis* [94] and *Geobacillus stearothermophilus* [95]. The resultant plasma coatings have been shown to possess spatial uniformity and strong adhesion to the substrate. They result in a smooth, defect-free surface with sound chemical and physical stability [96-98]. Furthermore, coatings manufactured using plasma technologies display interesting optical and electrical properties, making them suitable candidates for integration into a range of electronic devices that can interface between organic/inorganic electronics and physiological environments [99]. This paper discusses the processes used to develop plasma-modified surfaces with morphologies and chemical structures that prevent the adhesion and proliferation of pathogenic bacteria.

II.2.1.2 Plasma modification

A plasma is defined as a partially or wholly ionised gas, with approximately equal amount of positively and negatively charged particles. Near-equilibrium plasmas are formed under high temperature conditions and are characterised by a thermal equilibrium of its entire range of species. Temperatures required to generate near-equilibrium plasmas generally range between 4000 K to 20 000 K, depending on the ionisation potential of the element. These extreme conditions are not likely to be appropriate for the surface modification of biomaterials constructed from polymers [83], although they can be used for the evaporation and deposition of bioactive metals and ceramics, such as natural hydroxyapatite-based bio-glass–ceramics [100,101] and zirconia coatings [102,103] for artificial bones and hard tissues. Non-equilibrium plasmas, on the other hand, can be initiated at substantially lower temperatures, enabling their application for the surface cleaning and functionalisation of polymer surfaces. The ion mobility in a low-temperature plasma is significantly lower than that of the electrons that transport the energy through the electric field [104]. The plasma can also be classified according to the pressure at which it is initiated, or according to the energy source used to energise the gas [105].

During plasma surface treatment, the substrate is exposed to a reactive environment of a partially ionised gas comprising large concentrations of excited atomic, molecular, ionic, and free-radical species. The nature of the interactions between the excited species and the solid surface will determine the type and the degree of the chemical and physical modification that will take place. The processing conditions, such as power, pressure, gas etc., and the nature of the substrate will determine whether the surface modification is one of film deposition, substitution, or ablation. Plasma polymerisation can take place when a monomer, either in vapour phase or at the surface, is fragmented into reactive species that can then recombine and be deposited onto the surface of the substrate. Monomers that do not necessarily contain functionalities associated with conventional thermo-chemical polymerisation, such as unsaturation or ring structures, can be deposited in this way.

In plasma treatment, gases that do not fragment into polymerisable intermediates upon excitation are used. These include air, nitrogen, argon, oxygen, nitrous oxide, helium, tetrafluoromethane, water vapor, carbon dioxide, methane, and ammonia. Exposure to such plasmas can lead to the introduction of chemical functionalities, with the nature of the functionalities being highly dependent on the chemical composition of the biomaterial and the process gas. For instance, plasma oxidation, nitration, hydrolyzation, or amination will increase the surface energy and hydrophilicity of the biomaterial, therefore changing the way in which the biomaterial interacts with its immediate physiological environment. Free radicals are also created on the surface, since the surface is being bombarded by energetic particles and high energy UV radiation.This can lead to surface ablation, cross-linking or surface activation. Ablation is a process by which lower molecular weight species, such as volatile oligomers and monomers, are desorbed. Cross-linking occurs when radicals from one chain on the surface of the polymer combine with radicals from another polymer chain to form a bond. Surface activation, however, involves the recombination of surface radicals with atoms or chemical groups that are different from those that were originally present at the surface of the biomaterial.

The surface functionalities that arise as a result of plasma treatment can serve as a platform for further surface modification processes, such as the grafting of biomolecules and other functional structures. Further surface modification can be performed in order to tailor the properties of the biomaterial for a specific application.

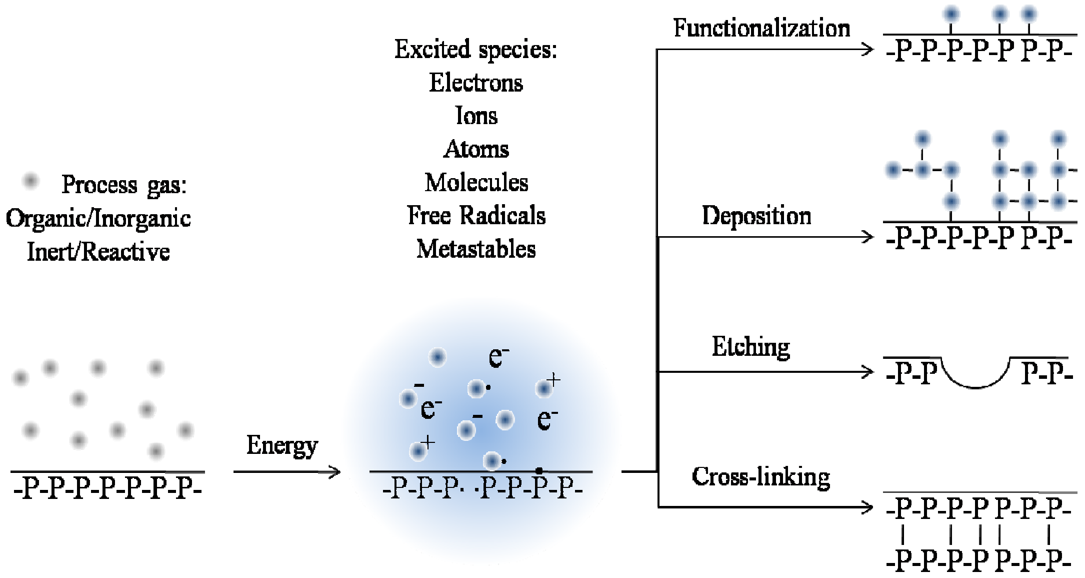

Figure II.2.1.1. Surface modification processes that can be achieved using the plasma technique.

Despite the many advantages associated with use of conventional plasma techniques for surface functionalisation, polymer thin films fabricated using this method are typically characterised as highly cross-linked and amorphous. Furthermore, these films retain only a limited amount of the original monomer functionality due to the high degree of fragmentation and recombination that takes place during the plasma polymerisation process. If low input power deposition and low levels of substrate heating are used, the original chemical structure of the monomer can be retained to a large extent, however a relatively low degree of cross-linking results, rendering these coatings inferior in terms of their mechanical properties and dynamic stability, hence limiting their *in vivo* applicability [106]. A number of papers have been published that detail the use of a pulsed plasma technique. This technique allows the precise control of chemical functionality and surface morphology and results in a coating with good stability [106-110] [111]. The plasma duty cycle was found to be an important determinant in controlling the degree of retained surface functionality [112], and hence a greater degree of compatibility with biomolecules, bacterial and host cells, and liquid media [113]. Moreover, the surface properties of the coating could be varied using this technique by changing the duty cycle between the 'pulse on' (ion implantation) and 'pulse off' (plasma exposure) periods during treatment, with a high ion implantation/plasma exposure time ratio being achieved by increasing the pulsing frequency and elongating the duration of the pulse [114].

II.2.1.3 Principles of bacterial attachment and biofilm formation

Designing a coating that will be effective in controlling bacterial adhesion and proliferation requires an in-depth understanding of the forces that govern these processes, the attachment and colony formation dynamics, and the consequences for both the coloniser and the abiotic target as a result of adhesion. Furthermore, the development of biomaterial-associated infections can arise in several ways, the most common being the introduction of aetiological agents from direct contamination of the implant during surgery [115] or post-operative care [116]. In addition, microorganisms that originate from an infection site elsewhere in the body can spread through the blood, causing the late haematogenous infection of the implant, particularly when medical devices are directly exposed to the blood stream, such as in the case of artificial valves [117,118].

II.2.1.3.1 Mechanism of initial attachment of planktonic bacteria to surfaces

The process of bacterial adhesion is complex, with initial reversible physicochemical interactions being followed by intricate irreversible molecular and cellular interactions. Bacterial cells move, or are moved, by the flow towards the surface of the material through and by the effects of physical forces, such as Brownian motion, van der Waals attraction forces, gravitational forces, the effect of surface electrostatic charge and hydrophobic interactions [119]. Bacterial motility mechanisms, including swimming, swarming, and twitching, are known to play important roles in bacterial attachment and biofilm formation, with directed motility being influenced by chemotaxis functions [120]. Chemotactic sensing is prevalent in almost all bacterial species and can influence bacterial colonisation of surfaces via regulation of expression of certain cellular adhesion components and bacterium-bacterium and bacterium-surface interactions [121,122]. Approaching a so-called chemoattractant, such as an amino acid, sugar, or an oligopeptide, encourages a bacterium to move in frequent runs, whilst a decreasing concentration of attractant and/or increasing concentrations of repellent such as an extreme pH, certain metal ions, or a hydrophobic amino acid instigates increased tumbling by the microorganism [123]. Haptotaxis is a mechanism that relates the cell speed and/or random turning behaviour to the magnitude of the adhesion ligands in the substratum, and the net direction of the cell movement to the gradient of adhesion [124]. It has also been suggested that haptotaxis also influences the attachment preferences of the microorganisms [125]. When the distance between the bacterial cell and other cells or abiotic surfaces is larger than 50 nm, the interactions between these two entities are nonspecific and are directly related to the distance and free energy characteristics pertinent to these two surfaces [119]. The nature of these forces, i.e. whether they are attractive or repulsive, will either facilitate the bacterial attachment or prevent the cell from moving into the molecular or cellular phase of adhesion. Studies have shown that bacterial adhesion and settlement increases with increasing surface roughness, due to the presence of a greater surface area for colonisation. In addition, the so-called ‘valleys’ on rough surfaces that provide a protected habitat, with reduced shear forces [126]. It has also been demonstrated that bacterial cells attach more favourably and rapidly to hydrophobic and non-polar surfaces rather than those with more hydrophilic properties [127]. When the distance separating these surfaces becomes less than 5 nm, chemical interactions such as hydrogen bonding, ionic and dipole interactions, hydration and/or hydrophobic interactions become significant, resulting in a more stable adhesion of the microorganism to the surface [128]. Various polymeric structures such as capsules, fimbriae, pili, and slime that can be present on the surface of the bacterial cell engage in the molecular specific irreversible reactions with the chemical features of the tissue or abiotic surface.

II.2.1.3.2 Influence of physiological status and substrate specific biological response on bacterial attachment

Bacteria secrete an elaborate variety of extracellular polymeric substances, including polysaccharides, proteins, and nucleic acids, that perform a wide range of biological functions, including shielding the cell surface, affording the cell protection from major bacterial pathogens [129], providing resistance to desiccation [130], and impeding antibody opsonization and phagocytosis [131]. Importantly, these substances play a significant role in mediating the bacterial colonization of surfaces by facilitating cell adhesion to biotic (i.e., epithelial and endothelial cells) and abiotic surfaces (i.e. mineral surfaces or medical implants) and cohesion to each other via dipole interactions, covalent or ionic bonding, steric interactions, and hydrophobic association [132-137].

For example, components of free extracellular polymeric substances released onto the surfaces that may otherwise be regarded as unfriendly for settlement by the bacterial cells will pre-condition the target surface by adsorbing to it hence making it more appropriate for bacterial attachment. The temperature, solution pH, electrolyte and macromolecule concentration, and adsorbent surface chemistry will directly affect the chemical composition and structure of the polymeric substances produced by the bacteria [138,139]. Cell adhesion to biotic targets such as host tissues has also been shown to be strongly associated with the presence of extracellular polymeric substances. *Streptococcus pyogenes,* for example, colonises the pharynx and is associated with infections such as necrotizing fasciitis and pharyngitis [140,141]. During the colonisation, the hyaluronic acid capsule of *S. pyogenes* attaches to CD44 receptor on human cells. CD44 is a hyaluronic acid-binding protein that mediates human cell-cell and cell-extracellular matrix–binding interactions, hence facilitating the colonisation of the pharynx keratinocytes *in vivo* [142,143]. Furthermore, the presence of the bound (capsular) and free (slime) extracellular material may significantly increase the chances for survival of the attached microorganism in the environment by acting as a permeability barrier that facilitates selective transportation of nutrients, whilst at the same time providing a protective barrier that excludes harmful substances, including systemic antimicrobial agents [144-149].

Factors such as the solution chemistry, abundance of nutrients, and the cell growth phase will exert a significant influence over the nature and distribution of the extracellular polymeric substances produced in these conditions[150]. *In vivo* studies on mice model involving acapsular mutant stains of *S. pyogenes* showed a spontaneous excision of the transposon from the capsule-synthesis region of the bacterial chromosome upon injection into a host, producing a high number of encapsulated revertants in subjects inoculated with the revertible mutant stains, resulting in mortality levels similar to those caused by parental encapsulated *S. pyogenes* [151]. In addition to secreted polymeric substances, lipopolysaccharides present on the outer leaflet of the outer membrane of gram-negative bacteria also affect the adhesive behaviour of the pathogen [152]. A carbohydrate structure comprised of a core oligosaccharide and a polysaccharide known as O-antigen is anchored to the bacterial membrane with the lipid A [153]. Although the O-antigen is flexible and can extend outwards depending on the ambient environmental conditions that surround the microorganism, the preferred conformation is thought to position the O-antigen to lie flat on top of the cell surface, covering the saturated fats and phospholipids of the lipid A and possibly non-polar sites of the surface of the pathogen [152]. As such, absence or attenuation of the O-antigen has been demonstrated to enhance the extent of bacterial attachment to hydrophobic surfaces [152].

The adhesive interactions between the microorganism and its environment have also been shown to depend on the length and heterogeneity of the O-antigen [153,154]. In *E. coli*, for example, the lipopolysaccharide core and O-antigen have been identified as the key components that mediate bacterial binding with inorganic surfaces and facilitate aggregation with other cells [155], with hydrogen bonding having been shown to be an important factor in controlling O-antigen adhesion to inorganic molecules such as Si_3N_4, TiO_2, SiO_2, and Al_2O_3 [153].

II.2.1.3.3 Coloniser proliferation and biofilm formation

Biofilm formation can be initiated by the multiplication of the primary coloniser without release of progeny cells and/or the recruitment of co-aggregate members of the same or different species, boosting their individual potentials for colonisation of various ecological niches [125,128,156]. A biofilm can comprise bacteria, algae, fungi and protozoa enfolded in a dynamic aggregation of polymeric compounds that are predominantly polysaccharides, but also contain proteins, nucleic acids, lipids, and humic substances. These extracellular polymeric substances (EPS) mediate the interspecies co-aggregation within the biofilms by providing a matrix for formation and stabilization of the film’s architecture. The composition and quantity of the extracellular polymeric substances that form the matrix of the biofilm will change according to the type of microorganism, the age of the aggregation and the environmental circumstances in which the formation exists, including oxygen and nitrogen levels, the extent of desiccation, temperature, pH, and availability of nutrients [128]. Charged non-carbohydrate components such as uronic acids or ketal-linked pyruvates present in the EPS further enhance the anionic nature of the surface polysaccharides of gram-negative bacteria, thus allowing the association of divalent cations (i.e., calcium, magnesium) to increase the binding forces within biofilm [157]. These non-carbohydrate components also strongly influence the tertiary structure and the physical properties of the EPS. Certain polysaccharide–surface combinations result in irreversible attachment. In these instances, the binding forces between the individual cell and the abiotic surface improve the overall stability of biofilm matrix [158,159]. Extracellular DNA has also been demonstrated to be an important component of the biofilm matrix via the introduction of favourable acid-base interactions. The removal of extracellular DNA from Gram-positive bacteria has been shown to reduce the initial adhesion and aggregation of bacteria on surfaces [160]. A combination of electrostatic interactions, hydrogen bonds and London dispersion forces are responsible for the initial attachment of the coloniser to the surface, and these forces also contribute to the subsequent biofilm formation and structural development.

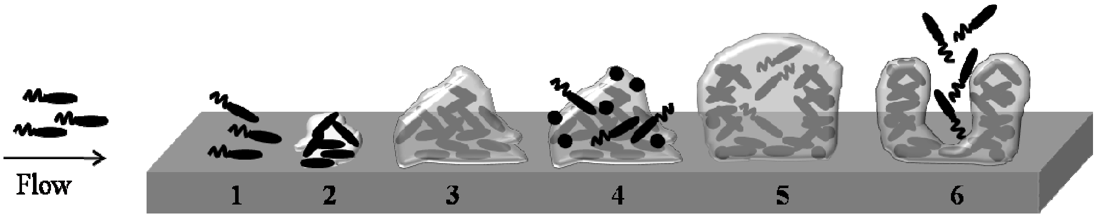

Figure II.2.1.2. A model of *P. aeruginosa* biofilm development in stages: (1) reversible attachment of the bacterial cells to the surface governed by electrostatic forces, Brownian motion and flagella-mediated locomotion; (2) irreversible attachment mediated by extracellular polymeric substances, loss of flagella-driven motility; (3) development of early biofilm architecture by bacterial reproduction, EPS secretion and twitching motility; (4) attachment of other organisms to the biofilm; (5) formation of a complex biofilm architecture and biofilm maturation; (6) dispersion of single motile cells from biofilm microcolonies into the medium.

Biomaterial-associated infections remain a major concern in the use of most implanted or intravascular devices, including orthopaedic prostheses, artificial valves, urinary tract and cardiovascular catheters, intraocular lenses and dentures [117,118]. Bacterial attachment and subsequent biofilm formation frequently results in indwelling device related infections, often resulting in device failure [161,162]. The state of the biofilm acts as a defence mechanism against predation by phagocytes, and that serves as a permeability barrier against harmful agents [163]. In the biofilm state, pathogenic bacteria are less susceptible to host defence mechanisms and systemic antibiotics. They are also more resistant to detachment under flow conditions, and as a result, the surgical removal of the infected device is often required [115]. For instance, the extracellular substances produced by Gram-negative *P. aeruginosa* cells limit the oxygen available to the microorganism, resulting in a reduction in the metabolic activity of the pathogen. Furthermore, recent studies on a mutant strain of *P. aeruginosa* showed that while they were still capable of forming biofilms with the characteristic *P. aeruginosa* architecture, they did not develop any high-level biofilm-specific resistance to three different classes of antibiotics. It was shown that periplasmic glucans synthesized by the bacteria interacted physically with antimicrobial agents, hence preventing the latter from reaching their sites of action [164,165]. In other words, in addition to the biofilm acting as a diffusion barrier for antibiotics, the bacteria within these films employed distinct mechanisms to resist the action of antimicrobial agents. The presence of a biofilm can also offer certain nutritional advantages to the bacteria over their planktonic state, in that the film acts as a ‘sorptive sponge’ which binds and concentrates organic molecules and ions close to cells [166]. While growing within the biofilm, *S. aureus* cells have been shown to synthesize and secrete an autoinducing peptide signal that accumulates in the extracellular environment. This is then used for cell-to-cell communication, otherwise known as quorum-sensing, a ubiquitous regulatory mechanism that controls the extent of *S. aureus* pathogenicity and biofilm development [167]. Upon reaching its critical concentration, the autoinducing peptide signal binds to a surface receptor, activating an agr regulatory cascade, which results in the increased expression of invasive factors, including toxins, hemolysins, proteases, and other tissue-degrading enzymes. Furthermore, the agr system also decreases the expression of surface adhesions, triggering a dispersal pathway and detaching cells from a surface-bound biofilm. Reverted to their planktonic state, these cells are then able to establish new colonisation sites elsewhere in the host, thus spreading the infection.

Nutrient depletion can also trigger cell detachment and drift. When a bacterial inoculum reaches a critical size and overcomes the local host defence, chronic infections can establish [125]. For instance, prolonged subclinical infections, that is bacterial presence without any signs of infection, have been linked to *Staphylococcus epidermidis* related capsular contracture formation around a silicone implant, the most common complication of augmentation mammaplasty and other procedures involving breast implants [168]. The biofilm formed on the outer surface of an implant triggers irritation and chronic inflammation, leading to accelerated capsular contracture. Another study involving *S. epidermidis* reported that in the presence of small colony variants on the surface of orthopaedic implants, osteoblasts initially adhered and spread on the surface of the implant, but were killed within 2 days [169].

II.2.1.4 Factors that influence bacterial adhesion

The stability with which a cell can attach to a solid surface and the degree of subsequent colonization have been shown to vary with the surface properties of the abiotic target. These surface properties include surface architecture and energy, the nature of the medium, and the surface characteristics of the microorganism itself. Microorganism-specific factors influencing the rate and degree of attachment to the surface include the hydrophobicity and surface energy of bacterial cell, the presence of fimbriae and flagella, the extent of EPS production and the type of polymeric materials being produced by the cell [126]. The hydrophobicity of bacteria is commonly inferred from water contact angle measurements on bacterial lawns deposited on membrane filters or from bacterial adhesion to hydrocarbons, whereas the electrical properties are determined by the zeta potential, which is related to the electrophoretic mobility of the microorganism [170]. In addition to these surface properties, the ability of the protruding EPS chains to reconfigure in terms of their spatial arrangement upon approaching the solid surface will determine if the subsequent interactions between the cell and the surface are attractive or repulsive [171]. The irreversible adhesion of Gram-negative *Stenotrophomonas maltophilia* to glass was shown to be facilitated by the attractive interactions of the long chain polysaccharides within the surface of the substrate. This interaction, known as bridging, resulted in a higher affinity between these surface structures and the surface of the solid. Lipopolysaccharide polymers, on the other hand, displayed a higher affinity for the medium than the substrate, and hence lower levels of attachment were observed as a result of the steric repulsion between the surface and the microorganisms [172]. Bridging is generally observed in instances where both the surface of the bacterium and the surface of the substrate are hydrophobic [173,174].

The ambient environment can promote or hinder colonisation by exerting selective pressure on the coloniser. This can be done by regulating its size, shape, growth rate and the substances the bacterium secretes, and also by directly affecting the surface properties of the abiotic target. Medium characteristics such as temperature, time of exposure, bacterial and antibiotic concentration, the degree of host immunity defence mechanisms activation, and the chemical composition and fluid flow in proximity of the surface also directly influence the dynamics of bacterial adhesion and biofilm development, with the latter often regarded as the most prominent factor. The pH and ionic strength of the medium can alter the surface hydrophobicity of the bacterium, and therefore the strength of the electrostatic interactions within the forming biofilm, hence affecting the stability and development of the biofilm architecture [125].

Cations such as magnesium and calcium actively contribute to biofilm cohesion and matrix development. They act as cross-linkers, contributing to the integrity of the outer membrane of the cell and the lipopolysaccharides. They also facilitate a physiology-dependent attachment process by acting as essential cellular cations and enzyme cofactors [175]. The presence of iron has also been shown to be a crucial factor for bacterial growth and biofilm formation; hence, targeting iron uptake systems may present an effective way by which the extent of biofilm formation can be restricted [176]. A study on the urinary tract biofilm-forming *E. coli* cells showed that biofilm formation can be impaired by the addition of divalent metal ions, such as Zn(II) and Co(II), which inhibit iron uptake by virtue of their higher-than-iron affinity for the master controller protein of iron uptake [176]. Biofilm formation can also be initiated in order to protect the bacteria from the presence of toxic compounds. *E. coli* bacterial cells, for example, have been shown to change from a planktonic to biofilm state in order to mitigate the harmful effects of a sub-inhibitory concentration of nickel [177]. In this case, the nickel-induced biofilm formation in *E. coli* was an adaptation process, occurring through a transcriptional effect on genes coding for adherence structures. Silver is also known to suppress bacterial growth and biofilm formation in a wide range of pathogenic bacteria, including *E. coli*, *Serratia proteamaculans*, *Serratia liquefaciens*, *P. aeruginosa*, and *P. chlororaphis* [178].

The pH of the medium has been shown to directly influence the surface hydrophobicity of the bacteria. Recent studies of the electrostatic potential and pH of bacteria upon adhesion to a solid surface indicated that the proton concentration at the surface of an adhered bacterium can vary greatly from that of one existing in the bulk medium, impacting cellular bioenergetics [179].

Mass transport conditions are also important factors that determine the efficiency of bacterial deposition and irreversible microbial adhesion, controlling the rate that the organisms arrive at the solid surface during adhesion [180]. Furthermore, time resolved studies of adsorption, desorption and transmission within biological systems have shown that desorption probabilities of microorganisms decrease by several orders of magnitude within 1 to 2 min after contact with a substratum surface, with microbial adhesion forces strengthening exponentially over time by progressively invoking acid–base interaction forces [180,181].

II.2.1.5 Effect of material properties of the substrate

As mentioned previously, bacterial attachment to a solid surface is highly dependent on the surface properties of the material, such as its chemical composition and reactivity, surface energy and hydrophobicity [182], surface roughness [183,184], and porosity. Furthermore, bacterial attachment is a competitive process where microorganisms race alongside the host proteins and cells for the colonisation surface [185]. A study using a microfluidic device for real time imaging of osteoblasts in response to the presence of very limited numbers of *S. epidermidis* showed that during the early stages of culture osteoblast adhesion, spreading and proliferation were not adversely affected. Towards the end of the culture, however, the osteoblasts became damaged because the *S. epidermidis* actively proliferated in the co-culture channels and formed small clusters on the alloy surface. This changed the microenvironment so that it was no longer favourable for the sustainance of osteoblasts [186]. Therefore, the ideal surface configuration of the biomaterial would be one that actively promoted the binding and attachment of host cells, while promoting tissue healing. This would encourage the mediation of host biomolecule attachment only to a level that facilitates the integration of the biomaterial into the host systems without generating an excessive immune response. In addtion, this would concomitantly prevent bacterial attachment and biofilm formation, the latter being the foremost cause of device related infections and device failure [161,162,187].

II.2.1.5.1 Chemical composition, hydrophobicity and surface free energy

Functional groups presented on the surface of the biomaterial will determine the hydrophobicity and surface charge of the abiotic target. Surface free energy is an important indicator of the type of interactions that occur at the solid-liquid interface, such as surface wettability [182]. It has been shown that the surface events that take place immediately after the insertion of a material into biological fluids will predetermine the subsequent response to the material. Surface events include the wetting of the material by physiological liquids, and the adsorption of proteins and cells to the surface [188]. There is a correlative relationship between surface wettability and blood-, cell-, or tissue-compatibility [189-191], with higher degrees of wettability corresponding to higher levels of cell attachment and subsequent spreading rates [192]. In addition, the friction behavior of an implantable tribological system is greatly affected by the extent of surface wettability, with higher wettability levels generally resulting in better tolerance of the biomaterial by the body [193]. A functional group, such as $–CH_3$, is inert in terms of protein and cell adsorption, whereas charged groups such as –COOH and $–NH_2$ encourage cell and protein attachment [194].

Plasma polymerization is frequently employed for the functionalisation of surfaces where hydrophobic behaviour is required. It allows for an introduction of a wide range of functional moieties and/or combination of moieties, including carboxyl (−COOH), amino ($−NH_2$), and alkene (−C=C) groups to name but a few, over a broad assortment of substrates [108]. Plasma polymer coatings derived from allylamine interact favorably via their amine functionality with both DNA and mammalian cells, making this material an attractive option for applications requiring biomolecule manipulation, such as on indwelling devices, whereas poly(ethylene glycol) films are being investigated for their ability to reduce protein fouling and limit cell attachment [195]. Films fabricated from maleic anhydride retain anhydride group functionality that can be then used for further modification via attachment of amino-functionalized alkyl chains, the polymerization of styrene, and protein attachment [112,113]. Nitrogen plasma treated bacterial cellulose improves the adhesion and proliferation of microvascular and neuroblast cells by increasing the porosity and changing the surface chemistry of the material, without affecting its wettability [196]. Argon plasma treatment has been demonstrated to reduce bacterial attachment, resulting in reduced levels of *S. epidermidis* adhesion to Ar-treated polyethylene [197]. According to Kumar et al., a surface engineering approach to the prevention of biofilm formation on surfaces of biomaterials involves designing a surface that is hydrophilic and with high surface energy; hydrophobic and inert with a low surface energy; or decorated with tethered antimicrobial self sterilizing agents which are attached directly to the surfaces of the devices [198].

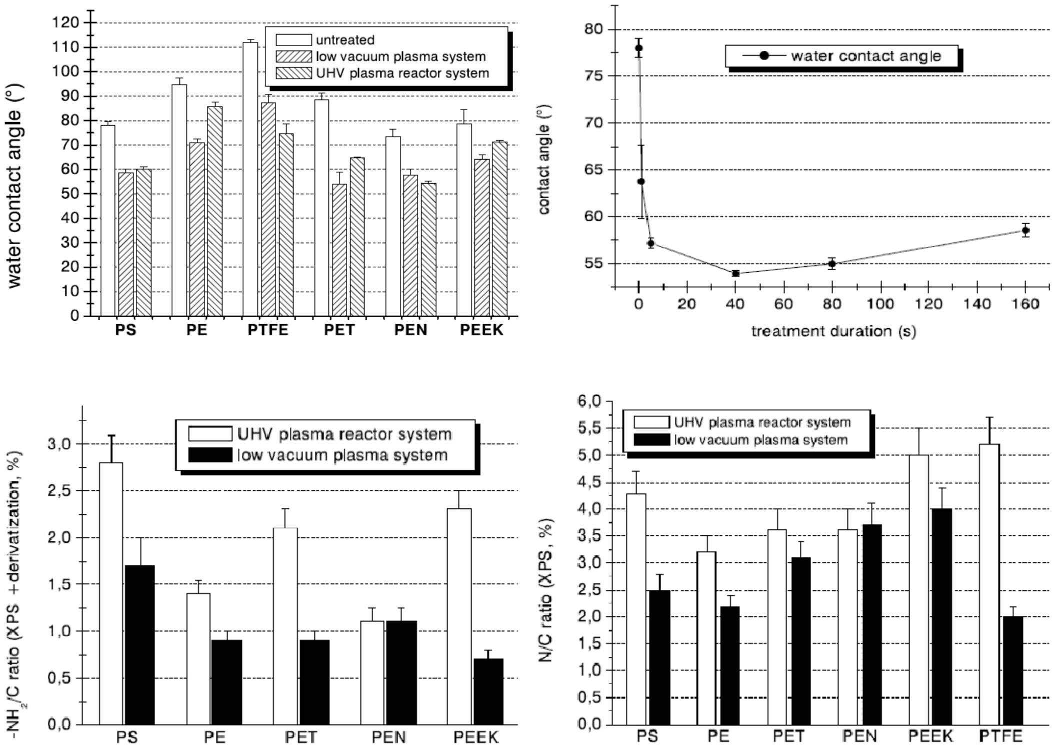

Figure II.2.1.3. Hydrophobicity and chemical composition of polystyrene (PS), polyethylene (PE), polyetheretherketone (PEEK), polyethyleneterephthalate (PET), polyethylenenaphthalate (PEN), polycarbonate (PC), polymethylmethacrylate (PMMA) and fluorinated (PTFE) polymers amino functionalised using ammonia plasma treatment: (1) water contact angle and (3) respective N/C and $–NH_2/C$ ratios after treatment of polymers in the low vacuum and the ultra high vacuum plasma reactors; (2) time dependence of the functionalisation effects in low pressure plasma system [199].

Our recent studies on fabrication of thin film coatings from essential oils and their individual constituents showed their potential in limiting bacterial attachment and proliferation. Coatings were deposited from terpinen-4-ol, a majour component of tea tree oil responsible for the oil's broad spectrum antimictobial and anti-inflammatory properties, using RF plasma polymerisation under varied input power conditions [200]. When produced at 10 W, the surfaces inhibited adhesion and growth of both *S. epidermidis* and *S. aureus* (Figure II.2.1.4), however, when fabricated at higher power, the coatings promoted attachment, adhesion and metabolic activity of the pathogens, and encouraged biofilm formation.

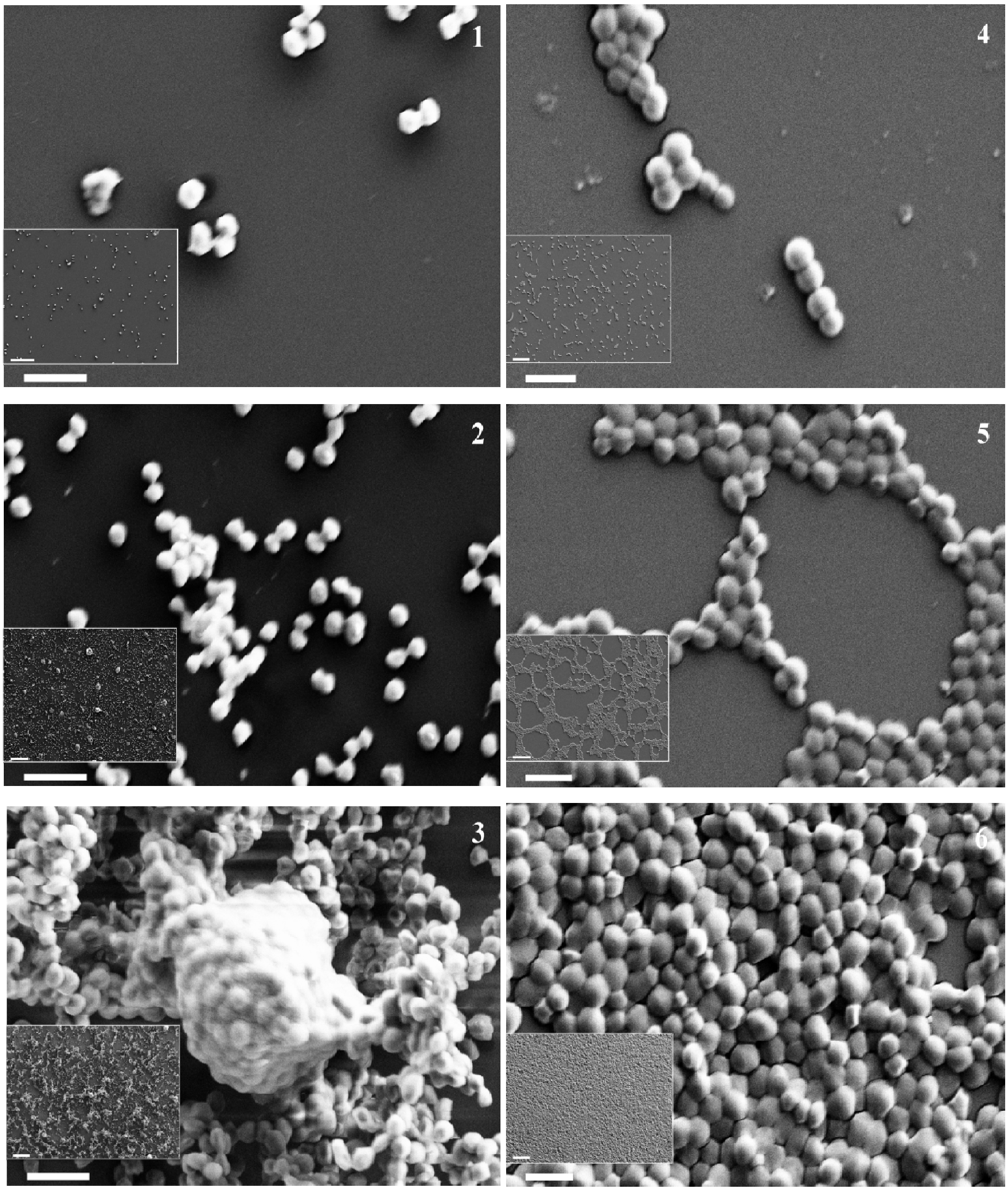

Figure II.2.1.4. Scanning electron microscopy images of attachment and proliferation of *Streptococcus epidermidis* (left) and *Staphylococcus aureus* (right) after 18 h of incubation on surfaces treated with monoterpene alcohol plasma deposition under varied input power conditions: (1,4) 10 W; (2,5) 25 W; (3,6) 50 W. Scale bar 2 µm; scale bar 20 µm for inset.

Hydrophobic material surfaces, such as those possessed by many polymers, are thought to be more attractive in terms of colonisation to hydrophobic microorganisms such as *S. epidermidis,* with the hydrophobicity of the material surface being identified as the more detrimental factor in the bacterial adhesion process than the hydrophobicity of the bacteria [125]. Similarly, metal surfaces are frequently hydrophilic due to the presence of surface hydroxyl groups on the surface oxide layer of the material. These surfaces are more attractive to the hydrophilic *S. aureus* bacerial cells [201]. A real time investigation of *S. epidermidis* adhesion dynamics to hydrophilic glass and hydrophobic dimethyldichlorosilane-coated glass highlighted that the number of adsorption and desorption events occurred, with a two-fold higher number of bacteria attaching to the hydrophilic surfaces [202]. The modes of adhesion were also found to differ, with approximately 20% of cells sliding over the surface of glass prior to either the fixed adhesion or desorption event, whereas a comparable mobile adhesion mechanism was virtually absent (1%) on the hydrophobic substrate, with only 2% of all *Staphylococci* desorbing from their adhesion site. The presence of high affinity sites on the surface of the hydrophobic coating was shown to initiate an attractive acid–base interaction with the surface of the cell, thus facilitating a closer approach and enhanced extent of immobile adhesion. Low affinity sites were associated with desorption and sliding of the bacterial cells.

A number of studies have reported that positively charged surfaces exhibit increased levels of bacterial attachment compared to their negatively charged counterparts, yet the subsequent growth was found to be more prominent on the latter surfaces [201]. Previous reports of antimicrobial activity increasing in the presence of positively charged surface sites [203] have been explained by a recent study that linked adhesion onto differently charged surfaces to changes in the charge-regulation process and cellular bioenergetics of the coloniser [179]. This study proposed that changes in the proton concentration at the cell surface can affect the periplasmic space, altering the levels of metabolic activity of the adhered bacteria. It has been shown that Gram-negative *E. coli* and the Gram-positive *Bacillus brevis*, when attached to a negatively charged glass surface, exhibited a decreased surface pH. This resulted in an enhanced proton motive force and an increased extent of ATP production, which may have assisted the cells to colonise the surface [204]. When these bacteria were attached to a positively charged surface, however, the effect was the opposite, resulting in a drop in metabolic activity, and possibly cell death, which may explain the antibacterial effect frequently reported for such surfaces [179].

Ultra hydrophobic hydrocarbons exhibit extremely low water solubility, are poorly bioavailable for bacterial colonisation, and can be toxic to bacterial cells due to their permeabilizing effect on the cytoplasmic membranes, leading also to a loss of ATP and a decrease in the proton gradient. In order to colonise such surfaces, bacteria may modify their cellular energetics through activation of their electron transport phosphorylation systems, allowing homeostasis of the ATP level and energy, which also results in a reduced growth yield [205].

Since most pathogens are hydrophilic under physiological conditions, decreasing the water contact angle of the material may improve its antibacterial properties. Indeed, a d.c. oxygen treatment of medical-grade poly(vinyl chloride) yielded a 70% reduction in bacterial adhesion for the four strains of *P. aeruginosa* [206]. However, this reduction was unlikely to be sufficient to prevent the *P. aeruginosa* colonization of endotracheal intubation devices [207]. Oxygen plasma-treatment of plasma deposited diamond-like carbon coatings resulted in the formation of superhydrophilic surfaces, but the presence of this surface did not increase the bactericidal properties of the material [208]. In addition to oxidation, the surface polarity of a substrate can also be increased by the plasma polymerisation of a coating that is not subject to hydrophobic recovery using an appropriately chosen monomer and carrier gas [209]. Hydrophobic recovery is a process of reorientation of the surface functionalities after oxidation with time attributed to the tendency to minimize the surface energy of the oxidized polymer and facilitated by the flexibility of polymer chains that allows for such a movement.

Treatment of polyethylene terephthalate with helium and 20% of oxygen in helium (He/O_2) plasma were demonstrated to significantly reduce *S. epidermidis* bacterial adhesion compared to the untreated material, however, the ageing effect and the subsequent decrease in the surface free energy of the substratum surfaces with time, particularly in the case of He treated surfaces, were found to favour bacterial adhesion and aggregation [210]. The surface energy and hydrophobicity of the substrate are greatly influenced by the chemical composition and pH of contact medium, consequently affecting the free energy of solvent-mediated interaction between the cell and the substrate. For instance, attachment of *S. aureus* to glass was predicted to be at its maximum at pH 3 and pH 11, whereas the higherst adehsion to Teflon should be observed at pH 5 [211]. The same study found that adhesion of *S. aureus* to glass was mediated by both short range forces (Lewis acid–bases forces) and by long range forces (van der Waals forces), whereas the attachment of the bacteria to Teflon was likely governed by short range forces only.

Plasma polymerized functional coatings are particularly susceptible to changes induced by the chemical composition of the liquid medium, such as the aqueous solution and body fluid, which can pose limitations on the potential applications of these structures as biomaterial or biocompatible surfaces [212]. Even before implantation, plasma polymers are vulnerable to degradation under ambient conditions, which may affect the storage and shelf life of the plasma deposited coating and undermine their usefulness. Upon immersion into a liquid, the swelling behaviour commonly observed in plasma polymers can cause the coating to increase in thickness and in volume. For instance, plasma polymerised maleic anhydride films have been shown to swell in water to form what is probably a polyelectrolyte film [113]. Interactions between ionisable functional groups of plasma polymers, such as acids and amines, and the ions of the liquid medium as a function of pH and ionic concentration of the solution will affect the swelling and degradation dynamics, and ultimately will influence the stability and bioactivity of these coatings. Leaching of small molecular weight compounds from the coating can also take place, a phenomenon that can be successfully utilised when designing a biodegradable or the diffusion-controlled release of a biocidal ingredient from the polymer system.

Pulsed plasma polymerised allylamine films deposited onto silicon showed a pronounced pH dependence of the magnitude of the average pull-off forces which was attributed to protonation of the amino groups, with the pull-off forces decreasing significantly for pH values below 5.5 [213]. The same study demonstrated that by varying the duty cycle of the deposition, coatings with controlled content of amino and nitrile groups can be achieved, creating a heterogeneous local environment in terms of chemical functionality and hydrophobicity on the nanometer scale. The adhesion behaviour of a product of pulsed plasma modification of polydimethylsiloxane substrates with maleic anhydride, with subsequent hydrolysation to promote the formation of dicarboxylic acid groups showed clear dependence on pH and electrolyte nature and concentration. The adhesion force was demonstrated to almost vanish under high pH in the presence of monovalent cation K^+ (due to condensation of counterions on the carboxylate groups), whilst it was oserved to increase slightly under high pH in the presence of divalent cation Ca^{2+}, due to ions bridging between two carboxylate groups [108]. The patterns of substrate-liquid medium interactions will therefore impact the substrate-biomolecule interactions.

Since proteins are regarded as the primary and the most significant player in mediating biomaterial–host interactions, the status of the proteins which adhere to the material surface will determine the ultimate biocompatibility of the given material, and the extent of bacterial cell attachment to such a surface [214]. For instance, globular proteins, such as fibronectin, adsorbed onto polymer films of various hydrophobicity, charge density and swelling characteristics have been shown to differ in terms of their adsorption and displacement patterns, which in turn affected their functional characteristics due to an altered availability for molecular interactions attributed to the conformational changes, orientation and/or the anchorage of the surface-confined proteins [215]. Fibronectin is a key protein of the extracellular matrix that enables cell adhesion and an important prerequisite for the differentiation of the cells, with the latter being dependent on the binding strength of the protein. In order to achieve specific cell responses, the coatings should be designed so as to reduce the non-specific protein adsorption that may lead to undesirable side effects, such as surface-induced thrombosis, while induce specific protein adsorption and anticipated cell responses by decorating the material surface with specific chemical functionalities [214].

The adhesion of the coating to the biomaterial substrate is also greatly affected by the properties of the ambient fluid, with partial loss of adhesion or full delamination of the coating being a serious hindrance to *in vitro* plasma polymer application. The adhesion can be significantly improved by pre-treatment of the substrate prior to film deposition, with the specific treatment dependent on the properties of the substrate and the coating. Exposing polymer substrates to oxygen or nitrogen plasma for short time facilitates energetic species mediated hydrogen abstraction and polymer bond breakage, and hence allows for activation of the substrate surface. Adhesion promoting layers, such self-assembled monolayers and silicon oxide film, are an effective solution for adhesion improvement between plasma polymerised coating and an inorganic or metallic substrate. In addition to improving stability of plasma films in aqueous environment, such an interlayer may enable more precise and more reproducible chemical reactivity of plasma deposited coatings for biomaterial applications [216].

A radio frequency oxygen glow discharge was used to pre-functionalise medical-grade poly(vinyl chloride) prior to sodium hydroxide and silver nitrate wet treatment and monovalent silver incorporation in order to reduce *P. aeruginosa* adhesion and colonisation [217]. Oxygen plasma pre-functionalisation step was demonstrated to be a necessary step to ensure reproducible biomaterial surfaces amongst production lots, as well as to increase the amount of ether/alcohol, esters and carboxyl functional groups. The resultant modification completely inhibited bacterial adhesion of four strains of *P. aeruginosa* and efficiently prevented colonization over longer periods. Plasma-modification was also used to successfully enhance the adhesion and uniformity of an electroless silver coating to polyurethane catheter surfaces [218].

Certain types of plasma polymers, such as fluorocarbon-based coatings, have been demonstrated to be stable and impermeable in a medium reproducing the physiological conditions, and can therefore be successfully applied as protective encapsulating coatings for biomaterials used for long-term implantation, such as intravascular stents and other metallic devices. Upon prolonged exposure to blood and other body fluids, these biomaterials can undergo degradation in terms of their mechanical properties, with a high potential for the release of toxic metallic compounds, such as nickel-based oxides and metal ions [219]. The application of a strongly adherent plasma-polymerised fluorocarbon coating can serve as a barrier against ion release, while being biocompatible with demonstrated thromboresistance properties and protein retention capability

[220,221]. Furthermore, the *in vivo* stent implantation to support the narrowed lumen of atherosclerotic stenosed arteries requires *in situ* stent expansion, a step that generates local plastic deformation of up to 25% and may cause coating failures, including cracking and delamination [222]. Fluorocarbon coatings with a thickness below 100 nm exhibited the required cohesion and interfacial adhesion to resist the stent expansion without cracking or delaminating [223].

Recently, surface-grafted stimuli-responsive polymers, such as poly (*N*-isopropylacrylamide) have attracted notable attention due to their ability to change their physicochemical characteristics upon induction of environmentally-triggered phase changes [224]. Of particular interest is the possibility to control biomolecular adsorption, bacterial cell attachment and release, and cell function, such as production of extracellular substances by the adsorbed microorganisms, using these materials. For instance, attached bacterial cells can be released from the surface due to changes in the anchorage strength of cells brought about by the physico-chemical changes of the surface upon induction of environmentally-triggered phase change [225]. Plasma immobilised thermo-responsive poly(*N*-isopropylacrylamide)-*co*–*N*-(1-phenylethyl) acrylamide films were demonstrated to successfully modulate initial attachment and adhesion strength of the diatom, *N. perminuta* [226].

II.2.1.5.1.1 Surface architecture and porosity

There is much debate as to the extent to which the surface topography of a solid substrate influences bacterial attachment and their subsequent proliferation to form biofilms, particularly on a nano-scale level [183,184]. Several early studies concluded surface roughness to be a ‘minor factor’ in the attachment mechanism of bacteria, with cells demonstrating no preference for adhesion to surface features such as scratches or grooves [183,184]. Subsequently, Scheuerman et al. described preferential adherence of bacteria to grooved and braided surfaces, with the increased adhesion effect being attributed to the increase in contact surface area [227]. It was reported that, where the size of the surface features were comparable with the size of the individual microorganism, such situation increased the binding potential of the bacteria by maximising bacteria-surface contact area [228], whereas features appreciably smaller than bacterial size led to reduction in the binding as a result of the decrease in the contact area [229]. Examination of the adhesion preferences of *P. aeruginosa* to poly(methyl methacrylate) contact lenses indicated that surfaces with root-mean-square roughness parameter of 14 nm or above increased the extent of micro-organism attachment [230]. Studies on the attachment behaviour of human pathogens, *Pseudomonas fluorescens* and *S. aureus*, concluded that the topography of micro-rough titanium surfaces affected the extent of cell attachment and preferential growth along the trenches in long rows [231], whilst the attachment response of these bacteria towards smooth surfaces did not follow a distinct pattern [227,232].

Furthermore, the surface architecture of the abiotic target has also been demonstrated to affect the metabolism and morphology of the coloniser [233,234]. Nano-patterning of gold surfaces has been shown to enhance *P. fluorescens* localized attachment in the trenches of the surfaces compared to native gold surfaces, with cells showing limited EPS synthesis and reduced cell size compared to those attached to non-nano-patterned surfaces [232]. Our recent investigation on pathogenic strains of *S. aureus* and *P. aeruginosa* have shown evidence of increased adhesion to “nanosmooth” glass, polymer and titanium surfaces, with concurrent elevation in cellular metabolic activity, augmented production of EPS, and increased number of bacterial cells undergoing attachment [234-236]. It has been proposed that as anisotropic topographies such as ridges and grooves affect the individual cell behavior (cells align along the anisotropic direction), isotropic topographies, such as evenly or randomly distributed peaks and valleys influence collective cell behaviors [194].

In general, porous materials are associated with higher infection rates compared to dense and smooth materials. A recent study of biofilm formation on bone grafts and bone graft substitutes reported a shorter biofilm detection time and a 10-fold (*S. epidermidis*) or 100-fold (*S. aureus*) higher bacterial counts on porous samples (β-TCP, processed human spongiosa) compared to smooth samples (PMMA and PE) [237]. It is assumed that the shear forces are significantly lower inside pores even under high bulk fluid velocity allowing for a protected environment for bacteria to attach and grow [238]. The dynamics of microbial attachment and biofilm formation within the pores of the substrates will be affected by the degree of the porosity, pore size and permeability distribution of the porous network [239]. For instance, recent studies of osteoconductive hydroxyapatite and biphasic calcium phosphate ceramic materials with pores ranging in size from 50 to 300 nm, with a mean pore diameter of 200 nm, demonstrated that this pore size is not sufficiently large to allow the internalization of *Staphylococci* due to the rigid structure of the cell wall of Gram-positive bacteria [240].

The morphology of biofilms in porous media will also depend strongly on the bacterial species and the prevailing hydrodynamic and nutritional conditions, ranging from continuous, smooth films to discontinuous, highly irregular colonies [241]. For porous substrates, the biofilm development involves initial formation of smooth biofilms on the pore walls, inducing changes in the geometry and topology of the porous medium, hence impacting the macroscopic properties of the porous medium, including its porosity and the permeability, drastically changing the fluid flow and mass transport through the porous medium. Gradually, the smooth biofilm would morph into more irregular biofilm forms, creating biofilm strands spanning the pores and separated by water channels (web-like structure) [241]. Plasma polymerisation can be used to decrease the size of the accessible pores, making those unavailable for colonisation. Furthermore, surface roughness and porosity are also known to affect friction behaviour of the material, an important property for surfaces that undergo insertion into body conduits such as blood vessels or urethra or for high wear applications, such as a replacement for articular cartilage in joints [242].

Plasma treatment with inert gasses such as argon or helium can facilitate the formation of a highly cross-linked and smooth surface layer, hence improving the friction and wear properties of the biomaterial, as is the case with radio frequency glow discharge surface treatment of the silicone rubber covering of electrical heart pacemaker which leads to a significant improvement in their slip properties [243]. Argon plasma sputtering of rough and smooth surfaces with amorphous carbon and titanium films to improve their biocompatibility showed an increased number of colony forming units on rough surfaces, especially on the a-C surfaces, with the degree of adhesion also dependent on bacterial taxa and surface chemistry of the coatings [244].

II.2.1.5.1.2 Plasma mediated grafting of surfaces

Plasma activation, film synthesis, ion implantation and grafting are tools frequently utilised for assembly of complex functional structures. For instance, covalent attachment (*i.e.* “tethering”) of antimicrobials and antifouling agents to a component of the coating system can be used to significantly extend service lifetime of the device, forcing compatibility and uniform dispersion of the active ingredient throughout the polymer matrix even in cases where some preferred drugs and polymer carriers may be incompatible [245]. Polymer cushions prepared using plasma polymerisation have also been used to assemble various types of polymer-supported lipid bilayer membranes by tethering of a lipid monolayer containing reactive anchor lipids onto the surface of the plasma polymer [246]. Tethering quaternary ammonium salts (QASs) to a crosslinked polysiloxane matrix produced a hybrid antifouling/fouling-release coating with biocidal activity toward marine *Cellulophaga lytica*, with 4 wt% QAS moieties resulted in approximately 50% reduction in *C. lytica* biofilm retention without any leachate toxicity [247].

Bottom-up chemical synthesis of quaternary ammonium groups on stainless steel and filter paper surfaces using low-pressure ethylenediamine plasma functionalisation generated films rich in secondary and tertiary amines [248]. The pre-treatment of the surfaces with oxygen and hexamethyldisiloxane plasma ensured covalent attachment of quaternary ammonium structures.

Modified steel surfaces exhibited greater than a 99.9% and 98% decrease in *S. aureus* and *K. pneumoniae* counts, respectively, whereas porous filter paper surfaces with immobilized QAS groups inactivated 98.7% and 96.8% of *S. aureus* and *K. pneumoniae*, respectively. The antibacterial properties of plasma treated surfaces can be further improved, such as in the case of the plasma-treated polymethyl methacrylate which was further modified with transparent TiO_2 films. These surface exhibited excellent photoinduced antibacterial effect against *S. aureus* and *E. coli* for the sterilisation of pathogen under indoor natural light, with approximately 100% of bacteria being inactivated within 2 h of illumination [249].

Plasma modification was used to activate poly(dimethyl siloxane) elastomer commonly used as a biomaterial, and to sequentially promote the attachment of Pluronic® F-68 synthetic surfactant or poly(ethylene glycol) methyl methacrylate to improve material hydrophilicity and bacterial cell repulsion properties [250]. The modification resulted in an increase of the oxygen content at the surface, with all materials found to be non-haemolytic and displaying no cytotoxicity. Asadinezhad et al. used surface activation by diffuse coplanar surface barrier discharge plasma followed by radical graft copolymerization of acrylic acid through surface-initiated pathway to render a structured high density brush on the surface of medical-grade polyvinyl chloride [251]. The brush modification was found to be remarkably effective to diminish the adherence of *E. coli*. Subsequent coatings with antibacterial agents, including bronopol, benzalkonium chloride, and chlorhexidine, were demonstrated to induce up to 85% reduction in adherence of *E. coli*, however only chlorhexidine coating was capable of retarding the adhesion of *S. aureus*, with a reduction of 50%. Active screen plasma alloying treatment of medical grade stainless steel has been demonstrated to produce highly durable antimicrobial surfaces with concomitant increase in surface hardness and sliding wear resistance; the nanocrystalline silver alloyed S-phase steel surfaces achieved 93% reduction in *E. coli* after 6 h contact time compared to untreated steel samples [252]. Silver ions introduced into plasma sprayed 57% SiO_2/3% Al_2O_3/ 34% CaO/6% Na_2O glass coating on titanium alloy and stainless steel substrates demonstrated *in vivo* antimicrobial action against *S. aureus*, while maintaining its biocompatibility, and has been suggested as a suitable coating for bone healing and prosthetic devices [253].

Plasma immersion ion implantation has been used by several teams to modify medical-grade poly(vinyl chloride) to enhance its antibacterial properties. Zhang et al coated triclosan (2, 4, 4P-trichloro-2P-hydroxydiphenylether) and bronopol (2-bromo-2-nitropropane-1,3-diol) on oxygen plasma activated poly(vinyl chloride) surfaces, followed by an argon plasma treatment to improve the antibacterial properties of the triclosan and bronopol-coated poly(vinyl chloride) samples [254]. The modification resulted in enhanced antibacterial properties against *S. aureus* and *E. coli*, with triclosan treated surfaces being more effective against *E. coli* compared to those modified with bronopol. The antibacterial efficacy of both coatings, however, was demonstrated to decrease with time. Kwok et al. reported plasma immersion ion implantation of polycarbonate and polytetrafluoroethylene using argon and oxygen, respectively, under varied pulse and frequency conditions [114]. High energy oxygen treatment resulted in super-hydrophobic polytetrafluoroethylene surface that was characterised by higher affinity for human cell and *S. aureus* attachment. Acetylene (C_2H_2) plasma immersion ion implantation used to treat polyethylene terephthalate increased the hemocompatibility and antibacterial properties of the biomaterials, with a significant decrease of bacteria adhesion and growth reported for *S. aureus, S. epidermidis, E. coli*, and *P. aeruginosa* [255].

The plasma immersion ion implantation technique can also be successfully used for modification of orthopedic nickel-titanium shape memory alloys and cardiovascular materials with diamond-like carbon containing nitrogen and phosphorus doping agents [256]. The coating was found to possess adequate surface mechanical properties and host tissue compatibility, enhancing the biocompatibility of the materials, effectively mitigating nickel out-diffusion, whilst allowing the NiTi rods to retain their shape recovery properties. Biocompatibility of polyurethane undergone acetylene plasma immersion ion implantation was also reported to improve, while argon plasma was used to pre-treat surface for subsequent grafting with heparin, albumin or polyethylene oxide bindings [255,257].

II.2.1.6 Concluding remarks

The utilisation of implantable materials and devices to replace missing tissues or restore a function has progressed rapidly over the past several decades. Continuous research efforts in the field of surface technology are directed toward enhancing tissue/surface interactions and advancing long-term performance of these materials. Furthermore, the ability to subtly modify surface properties can be potentially utilised to enrich our knowledge regarding the immune response, particularly the highly complex processes that govern the covalent binding of biomolecules, such antibodies and enzymes. Equally so, the intricate interactions between an abiotic surface and different types of living cell, including that of bacteria and fungi, can be investigated in greater detail in order to improve our ability to predict the biological responses to changes in surface properties of these biomaterials.

In this paper, we have reviewed the advantages of the family of plasma-assisted techniques for the production and modification of biomaterials. The plasma surface modification of biomaterials is an economical and effective method by which biocompatibility and biofunctionality can be achieved, while preserving the favorable bulk characteristics of the biomaterial, such as strength and inertness. This provides device manufacturers with a flexible and environmentally friendly process that allows for tailoring the surface properties of the material to suit a specific need. In addition, exposure to plasma has been shown to irreversibly damage bacterial cells, allowing for in situ sterilisation of the biomaterial during the surface modification process. Despite numerous auspicious results reported in literature, the real life applications are frequently hindered by limited understanding of the influence of process parameters, including among others geometry of the reactor, input energy, and pressure. The combination of these parameters determines the nature of the reactive species and ultimately the surface modifications produced. To the same extent, further advancements in the areas of immunology, biology and analytical techniques are necessary for the successful design and implementation of biomaterials.

II.2.2 CELL RESPONSE TO SURFACE PROPERTIES OF MATERIALS

Abstract

Although certain nanoscale surface phenomena are being exploited in an effort to promote or prevent the attachment of living cells, our appreciation of the nature of bacterial cell–surface interactions is not yet advanced to a level where effective optimisation of the nanoscale surface architecture of materials and devices can be performed in order to achieve the desired result. This review explores current research investigating living cells interactions with surfaces, both native and nano-structured, and the role surface properties play at different stages of cell attachment.

Keywords: nanoroughness; bacterial attachment; bacterial adhesion; nanobiotechnology; nanoarchitecture

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II.2.2.1 Introduction

After more than twenty years of basic and applied research, the use of nanotechnology in the design and manufacture of nanoscale materials is rapidly increasing, particularly in commercial applications that span electronics, renewable energy, cosmetics, automotive and medical products. There are currently in excess of 1,000 manufacturer-identified nanotechnology-based consumer products on the market, and this number is rapidly increasing as a result of high levels of investment in research, development and commercialisation, together with an equally high demand for novel and advanced products from manufacturers and consumers [258,259].

In spite of many years of intense experimental and theoretical investigation into understanding the physical, chemical and biological aspects of cell–surface interactions, our knowledge of these phenomena is mostly confined to interactions on the macro- and micro- length scales, whilst limited information is available on the fundamental behaviour of cells when they are in contact with the nanoscale environment on a surface. An increasing number of researchers have dedicated their time and efforts to gain a fundamental understanding of the principal mechanisms by which cellular systems are ultimately linked with their environment. Indeed, since the adhesion sites of the cell (focal adhesions) are in the range of 5–200 nm, these very small cellular components are likely to be greatly influenced by any nanoscale features on a surface, rather than microscale surface structures [258]. Nature presents us with many examples of surfaces that have evolved to exploit a combination of micro- and nanoscopic topographies, remaining remarkably clean of fouling in highly bacteria-rich aquatic environments. Nanostructured surfaces created to mimic that of a lotus leaf, or whale and shark skins, for example, have also been shown to exhibit desirable properties, such as preventing bacterial adhesion and biofilm formation without the need to use bactericidal agents [259-261]. Other possible applications for such materials are endless, from preventing marine fouling on ships and pontoons, to averting biofouling of desalination membranes and aquatic scanners, to preventing the attachment of microorganisms to surfaces in hospital settings. Importantly, these materials can be studied in order to further understand the relationship between the organisation and function of polymer complexes at cell and bacterial surfaces when they are in contact with the nanoscale surface features of materials [262]. This knowledge is essential for the effective design and fabrication of biomaterials, scaffolds, through the exploitation of differences in attachment responses between eukaryotic and prokaryotic cells. The aim of this review, therefore, is to outline current research investigating living cells interactions with surfaces, both native and nano-structured, and the role surface properties play at different stages of cell attachment.

II.2.2.2 Surface properties of a material as a determinant in cell–surface interactions

Given the mounting body of evidence highlighting the importance of the nanoscale surface configuration of both the living cells and the abiotic substrata in cell–surface dynamics, much research has been performed to demonstrate the significance of surface chemistry and surface free energy of substrata for the attachment, proliferation and differentiation of cells, whereas the contribution of the surface nanoarchitecture on these processes remains unclear [263]. This is not surprising, since environmental sensing by cells involves specific binding between cellular receptors and extracellular matrix ligands. To some extent, the paucity of data reporting of the role of nanotopography on cell behaviour can also be attributed to the inherent relation between the surface architecture and chemical functionality of native surfaces, which is particularly profound on the nanometer and sub-nanometer scale. Wenzel [264] first acknowledged the significant effect of surface roughness on the measurement of contact angle and proposed a model that described the roughness-induced hydrophobicity, expressed as $\cos \theta_{rough} = r \cos \theta_{smooth}$, where θ_{rough} and θ_{smooth} are fluid contact angles on rough and smooth surfaces, and r is the roughness factor. According to this equation, the wetting tendency of a surface with hydrophilic characteristics($\theta < 90°$) will increase with increasing surface roughness, whereas the wetting tendency of a surface with hydrophobic characteristics ($\theta > 90°$) will decrease with roughness, hence making the surface more water repellent. Subsequent theoretical and experimental studies have confirmed that nanoroughness is partially responsible for superhydrophobicity and low adhesion, similar to two-layer morphology of *Nelumbo nucifera* (lotus) leaf where the hydrophobic chemistry of the leaf produces a high contact angle, which is further enhanced by the two-layer morphology comprised of micro- and nanoroughness [265-268].

Scardino et al. [269] integrated hydrophobic materials with micro- and nanoscale architectures to investigate the influence of the nanoarchitectured roughness on the potential fouling resistance of the resultant superhydrophobic surfaces. The results of the settlement assays indicated that the nanoroughness of the surfaces alone deterred the settlement of all the tested fouling organisms, whereas the surfaces possessing nano- and micro-scale architectures were characterised by selective settlement. The superior performance of nanorough coatings was attributed to a noticeably larger amount of unwetted interface when immersed in an aqueous environment, due to the comparatively high work of adhesion, 60.77 mJ m^{-2} compared to 5.78 mJ m^{-2} for the microrough surfaces. In another study, the self assembly of hydrophobic three-dimensional alkane crystals was used to modify flat and microstructured surfaces to create hierarchical nanostructures to mimic the surfaces of superhydrophobic plants [265]. The lotus leaf morphology was also mimicked using hydrophobic chemistry and a two-layer topography, with a base layer of silica for microroughness and a top layer of intrinsically nanostructured polyhedral oligomeric silsesquioxanes particles, the latter structures having size of 1.5 nm [266,268]. These studies found that sufficient nanoroughness was essential to obtain the superhydrophobic properties of the surfaces, with the coatings displaying significantly inferior levels of hydrophobicity when used as a single layer. In addition to increasing the contact angle, the surface nanoroughness was also found to influence the magnitude of the interfacial adhesion between the liquid drop to a polymer surface of a given composition, together with the angle at which a water drop of a given mass began to slide down a tilted plane [267]. Most recently, Fadeeva et al. [259] created a lotus leaf-like morphology using femtosecond laser ablation to fabricate two-tier micro- and nanoscale quasi-periodic self-organized structures on titanium surfaces (Figure II.2.2.1A). A combination of large 10 – 20 μm grainlike convex features, together with 200 nm wide irregular undulations transformed moderately hydrophobic titanium surface, $\theta \sim 73$ °, into superhydrophobic surfaces, displaying a water contact angle of approximately 166 °. These superhydrophobic surfaces were reported to be highly effective in preventing Pseudomonas aeruginosa colonisation, although they were unable to avert attachment and colonisation by the morphologically dissimilar *Staphylococcus aureus* cells.

II.2.2.2.1 Mitigation of microorganism attachment and fouling by native marine surfaces

In addition to plant leaf surfaces, a number of other naturally occurring surfaces, including those of marine organisms, possess topography-enhanced chemistry that allows for effective mitigation of microorganism attachment and proliferation and fouling. In aquatic environments, the biofouling process is initiated from the conditioning layer of absorbed organic carbon residues on wetted surfaces to which the microfouling organisms attach, building up a biofilm onto which further biofouling proceeds [260]. Biofouling can be detrimental to both the health of living aquatic organisms and to the performance and longevity of the man-made materials that are in contact with such environment [270,271]. Biological fouling resulting from the settlement and subsequent growth of vegetable and animal organisms have been identified as one of the most important and high-cost problems affecting structures immersed in seawater, including platforms, jetties and ship hulls, as well as marine and riverine sensors [272,273] and seawater reverse osmosis membranes [274]. Traditionally, the issue of fouling has been tackled using antifouling coatings containing toxicants which were gradually leached in the surrounding seawater, producing a laminar zone which retards fouling settlement [275,276]. As contemporary ecological legislation has progressively prohibited the use of these toxic materials, researchers turned their attention to innovative antifouling technologies based on an improved understanding of the biological principles of the biofouling process, often exploiting and imitating natural antifouling processes [277,278]. For example, the chemically heterogeneous, nano-rough gel-coated epidermal surface of the skin of the pilot whale, *Globicephala melas*, is self-cleaning, contributing to the ablation of traces of the biofouling process [279-281].

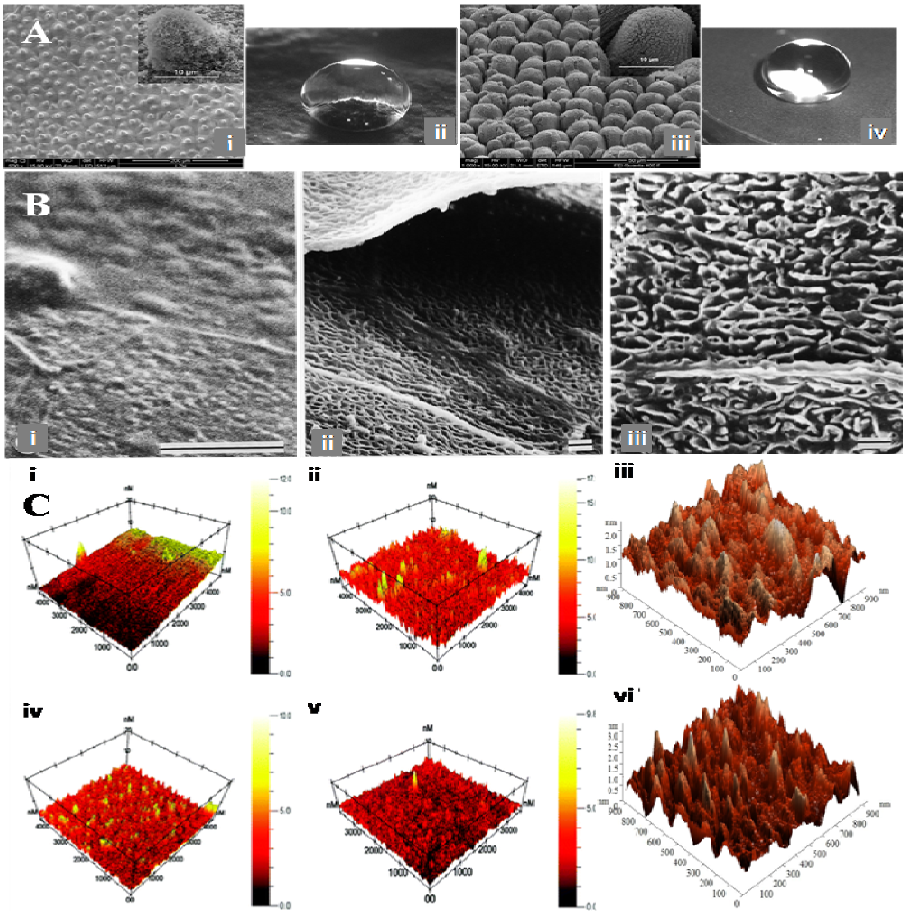

Figure II.2.2.1. Images of native and patterned surfaces. (A) Scanning electron micrograph of the adaxial leaf surface of the rough, water-repellent surface of *Nelumbo nucifera* (i) and a water droplet on a *Nelumbo nucifera* leaf (ii); and femtosecond laser structured titanium surface (iii) and water droplet on this surface (iv) [259]. (B) Cryo-scanning electron microscopic images of the skin surface of *Globicephala melas* pilot whale: (i) non-fixed, even, smooth surface with only tips of the nanoridges visible due to presence of smoothing medium (scale bar 10 µm); (ii) formol-fixed and trypsin hydrolysed samples and (iii) non-digested skin samples fixed in Bouin's fixative reveal an entangled network of nanoridges which enclose small pores (scale bar 1 µm) [260]. (C) Three-dimensional (3D) projections of typical AFM images of glass substrate (i) and 3 nm (ii), 12 nm (iii), and 150 nm (iv) titanium thin film surfaces deposited using magnetron sputtering [282], and polyterpenol thin films fabricated using RF plasma polymerisation at 10 W (v) and 25 W (vi) [283].

Analysis of the cryo-SEM of the pilot whale skin indicated the presence of nanoridge-enclosed pores with an average size of approximately 0.20 μm^2 (Figure II.2.2.1B), which is below that of most marine biofouling organisms, providing a substantially reduced available space for the attachment of biofouling organisms [260]. At certain developmental stages, the surfaces of *Dicathais orbita* mollusc eggs are also regularly ridged, which has been shown to prevent attachment and colonisation by a biofilm-forming *Pseudoalteromonas* sp. S91 in flow chambers [284]. Tropical sea stars *Linckia laevigata*, *Fromia indica*, *Cryptasterina pentagona* and *Archaster typicus* also have complex surface micro and nanotopographies that are thought to contribute to their surfaces remaining remarkably clean of fouling [285]. In spite of their value in illustrating the importance of nanoscale phenomena, the results obtained on the aforementioned native surfaces are insufficient to definitively determine the respective contribution of individual surface characteristics.

II.2.2.2.2 Patterned surfaces for investigation of nanoroughness contribution to cell adhesion

Nano-engineered surfaces have also been demonstrated as preferred candidates for the investigation of topography-dependent cell adhesion and settlement. Techniques such as ionic plasma deposition and ion implantation plasma deposition can be used to modify a wide range of commonly used biomaterials to create nanostructured surface features which mimic the natural cell environment *in vivo* [286]. Figure II.2.2.1C shows examples of surface topography of organic and metallic nanocoatings manufactured using RF plasma polymerisation and magnetron sputtering, respectively [282,283]. Recent advances in nanoscale patterning allow for controlled modification of surfaces, with the ability to alter individual surface characteristics, such as surface topography, without subsequently affecting surface chemical functionality. Nanoimprinting, colloidal and photolithography, transfer from self-assembled polymer films, and electrospinning can be used for the modification of topography with a lateral resolution of 40–100 nm [263]. For example, Lee et al. used photolithography to fabricate poly(ethylene glycol) hydrogel microstructures on alumina membranes with 200 nm nanopores to control spatial positioning of mammalian cells [287]. Chung et al. [261] used patterned silicon wafers to engineer a polymer elastomer containing a surface microtopography similar to that of the skin of sharks (Figure II.2.2.1). The structured surfaces effectively disrupted colonisation and biofilm formation by Staphylococcus aureus without the need to use bactericidal agents.

Other methods, such as anodic oxidation of metals and electron beam lithography can be used to produce an improved lateral resolution of 15 nm, whereas lateral resolution for polymer demixing and surface roughening techniques is on a larger sub-micron scale. Ranjan et al.

employed electron beam physical vapour deposition to fabricate patterned poly(dimethylsiloxane) films with periodic nano-grooves of 500 nm and alternating nano-and micron roughness to mimic the conditions of the micro-and nano-environment of vascular intima tissue [288]. Rat aortic endothelial cells displayed significantly higher elongation when adhered onto patterned films of the widest spacing and greatest surface area of nanoroughness compared to those seeded on narrower pattern spacings or non-patterned surfaces. Estrin et al. employed equal channel angular pressing to produce titanium surfaces with an average grain size in the low sub-micron range to investigate attachment of human bone marrow-derived mesenchymal stem cells as a function of grain refinement on the nanoscale (Figure II.2.2.2) [289].

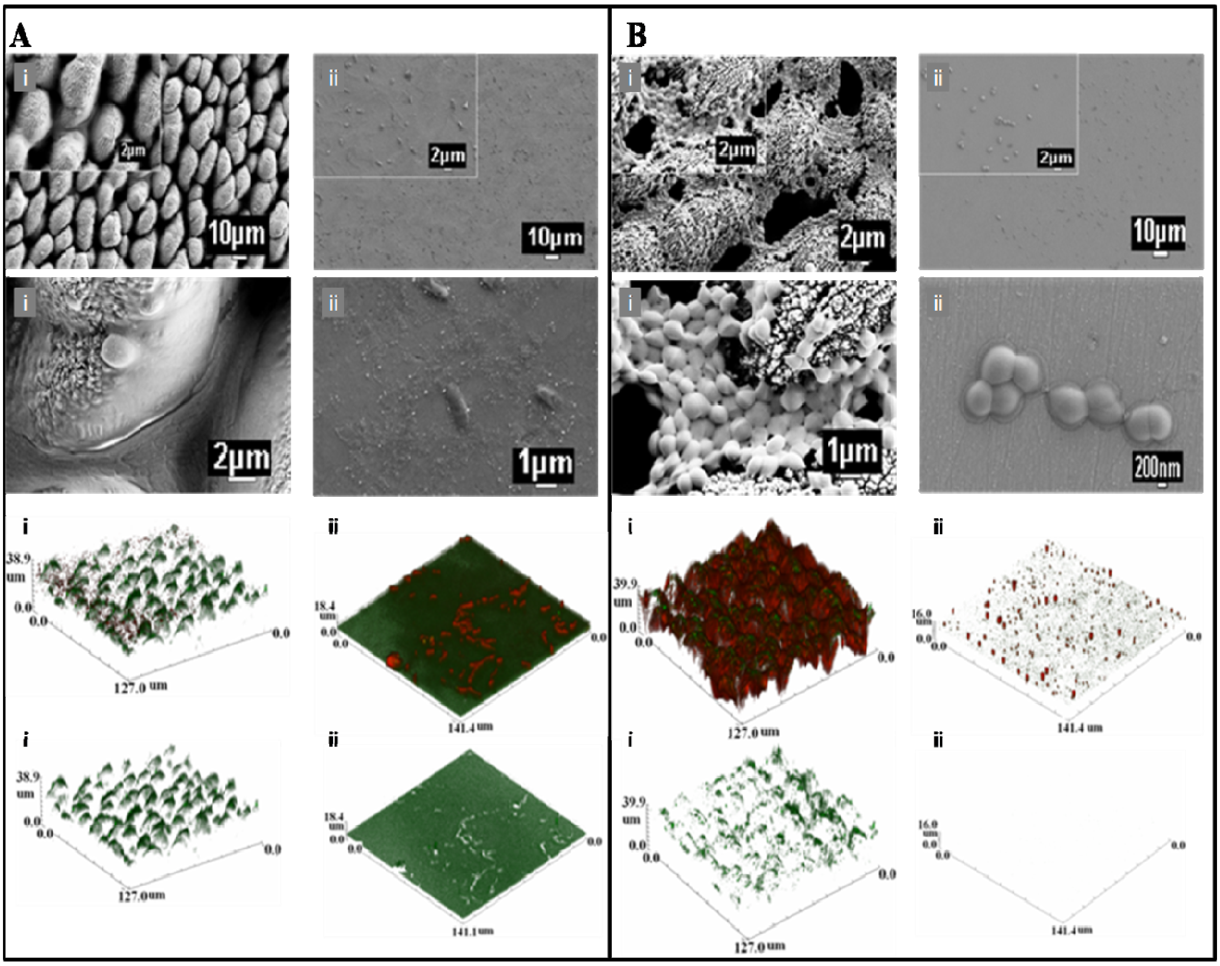

Figure II.2.2.2. Dissimilar attachment patterns of the taxonomically dissimilar human pathogens *Staphylococcus aureus* (A) and *Pseudomonas aeruginosa* (B) on the structured (i) and polished (ii) titanium surfaces after 18 h incubation visualised using SEM (top) and rCSLM (bottom). Viable cells stained red and the production of EPS is stained green [259].

Graded ethanol treatments were used to produce nanoarchitectured assemblies of silk fibroin protein membranes of 1–12 nm in height to investigate cytoskeletal organization, proliferation, and viability of primary rat bone marrow cells as a function of different nanotopographies [290]. Shen et al. [291] combined micropore and microgroove patterning with the plasma nanocoating of nitinol alloys to demonstrate an improved bovine aortic endothelial cell adhesion and found that surface endothelialisation of these surfaces could be accelerated with increased levels of surface roughness. Femtosecond laser structuring techniques have been shown to be an effective tool to achieve three-dimensional silicon surfaces with gradient roughness ratios on the micro- and nanoscale (Figure II.2.2.3) [292]. Hence, it is clear that nanoscale patterned surfaces would be eminently suitable for studies designed to provide a greater understanding of the mechanisms controlling eukaryotic cell–surface interactions, and provide an insight into the effect of size, morphology, organization and separation of nanofeatures on protein adsorption and cell response.

II.2.2.3 Detection and response of bacterial cells to nanoscale surface features

Despite significant advancements in cell manipulation and imaging techniques, the effect of micro and nanoscale topography on attachment and metabolic activity of bacteria remains controversial. As with eukaryotic cells, bacteria are believed to respond according to their spatial confinement and external environmental cues, including spatial and mechanical stimuli [183]. This is not surprising, since, surface attachment is an integral step in biofilm formation and in chemical signalling pathways within and between bacterial cells [293]. However, the nature and the extent of this response, and the scale at which these become profound are still the subject of debate. As is the case with mammalian cells, results reported in the literature regarding the role that surface nanofeatures play in bacterial cell adhesion, proliferation, phenotypic differentiation and specific cellular processes are inconsistent and at times contradictory. Given the numerous approaches taken to investigate these phenomena [184,230,234,236,294-303], comparative interpretation or even summation of these results is difficult in the absence of a clear framework [304]. Bacterial interactions with metallic surfaces present a good example of lack of consensus regarding whether increasing levels of surface roughness can be definitively correlated to the extent of bacterial attachment, despite the number of investigations, spanning the last few decades, that have been performed [184,230,300,301,303].

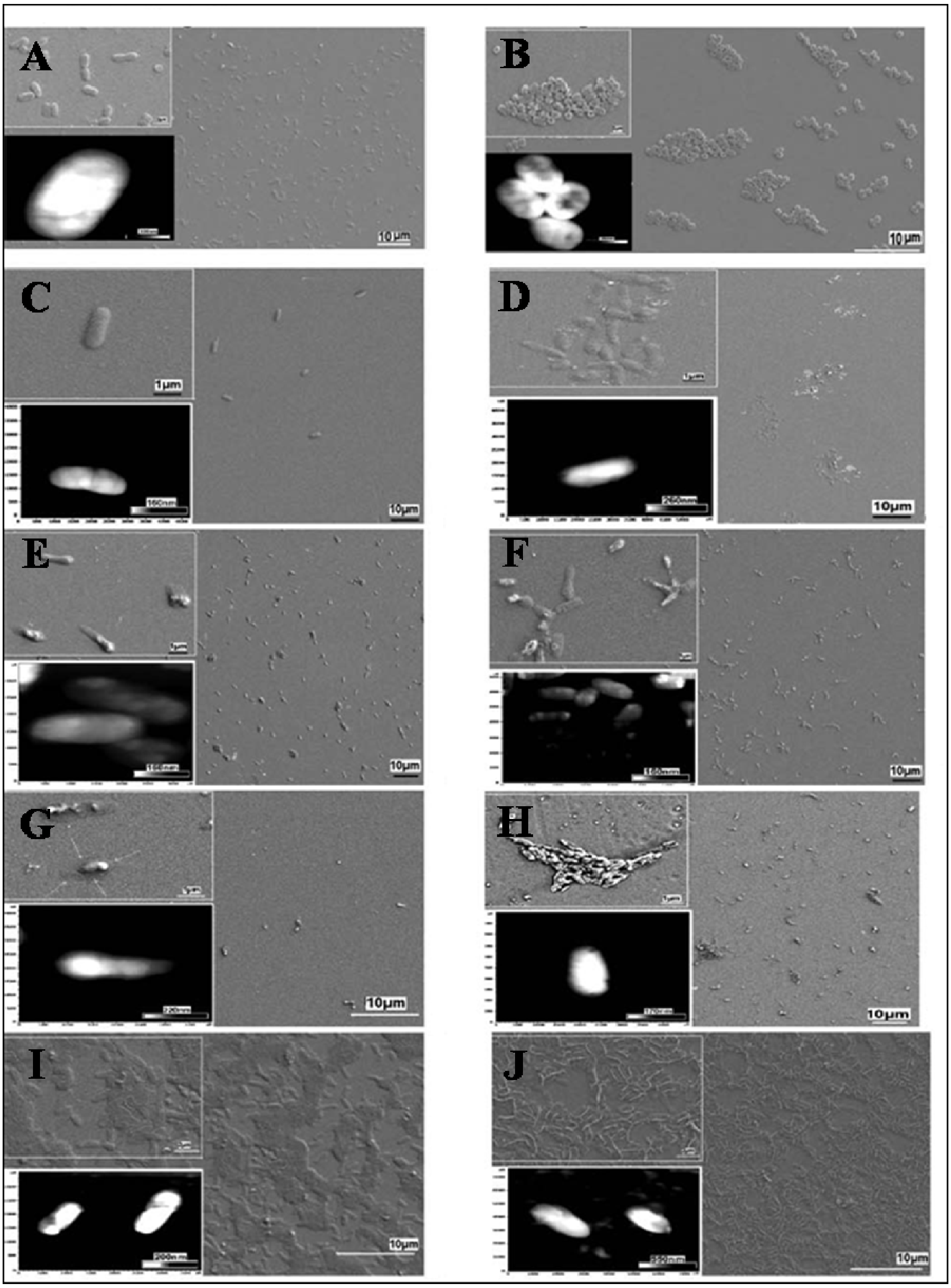

Figure II.2.2.3. SEM images of representative (A, B) *C. marina*, (C, D) *S. flavus*, (E, F) *S. mediterraneus*, (G, H) *S. guttiformis*, and (I, J) *A. fischeri* cell morphologies and attachment patterns on the non-etched (R_a~ 2.13 nm, left column) and etched (R_a~ 1.31 nm, right column) glass surfaces after 12 h incubation. Insets: zoomed SEM images (top) and AFM images (bottom) show typical cell morphologies. A considerable amount of EPS was observed on the etched glass surface [305].

II.2.2.3.1 Complexities with experimental design

Synergistic effect of surface chemistry

Many native irregular material topographies also benefit from possessing chemical and surface energy characteristics that assist in their ability to retard or promote certain bacterial cell behaviour. Even though studies on topographically or chemically patterned surfaces have the capability to be used to obtain adherence profiles against a single variable, the choice of substrate material and/or pathogen species limits the comparative scope of these observations. In addition to nanotopographic changes, patterned surfaces are frequently modified with regard to their surface chemical functionality in order to optimise such surface for desired application. For instance, *Escherichia coli* cells were effectively localised using micron-scale patterns of size 83 µm and 12 µm on polyethylene glycol [297] and 3 µm diameter holes of 0.5 µm depth on silicon oxide surfaces [296]. In both of these studies, respective chemical modifications with poly(ethylene glycol) and poly-L-lysine were also introduced to control *E. coli* cell attachment. Li and Logan also demonstrated that both topography and surface chemistry of nanorough glass and metal oxide substrates with R_a of 4.1–17.6 nm influences bacterial attachment [302], however the relative influences of individual surface characteristics could not be elucidated.

Genotypic and phenotypic variation of bacteria

The additional complexity in studying cell–surface interactions is introduced by the sheer abundance of bacterial species and phenotypes. Indeed, physical and chemical properties that define bacteria, such as cell shape and morphology, organisation of subcellular components, cell intracellular machinery and chemical composition and spatial configuration of cell extracellular features are highly dependent on the phenotype and the growth stage of bacteria (Figure II.2.2.2). Various polymeric structures such as capsules, fimbriae, pili, and slime that can be present on the surface of the bacterial cell engage in the molecular specific irreversible reactions with the chemical features of the tissue or abiotic surface. Work by Whitehead et al. on nanotopographically different titanium dioxide surfaces demonstrated that Gram-positive coccoid pathogen *Staphylococcus aureus* cells were able to attach in greater number to surfaces characterised by R_a of 8.7 nm compared to that of 43.6 nm, whereas Gram-negative rod shaped bacterium *P. aeruginosa* cells displayed an opposing tendency [303]. Recent study by Diaz et al. demonstrated that the attachment of *Pseudomonas fluorescens* aggregates onto surfaces to be strongly dependent on the surface topography of the substrate immersed in abiotic culture media [306]. Subsequent studies used patterned substrates to demonstrate different flagellar expression of *P. fluorescens* when trapped in the sub-microtrenches on patterned and nanostructured gold surfaces with a random orientation [307]. Flagellar expression of bacteria is considered to be an important factor in exopolysacharide production, bacterial attachment and biofilm development.

Cells in a planktonic state display a behaviour and features that are distinctly different from those displayed in organised in multicellular structures, such as colonies and biofilms [308]. Small molecule-facilitated coordination and synchronisation of activities within such a complex organism in itself has the potential to induce phenotypic differentiation amongst bacterial cells. The extent of production, together with the chemical composition of extracellular polymeric substances (EPS) produced by bacterial cells have also been demonstrated to be influenced by the surface architecture (Figure II.2.2.3) [232-234]. Nano-patterning of gold surfaces has been shown to enhance the localised attachment of *P. fluorescens* in the trenches of the surfaces compared to that obtained using native gold surfaces as the substrate [232]. Limited extracellular polymeric substance production and reduced cell size were also observed after attachment onto nano-patterned surfaces. Ivanova et al. and Mitik-Dineva et al. reported a similar cell metabolic behaviour for bacteria attached onto topographically dissimilar glass, polymer and metallic surfaces, with taxonomically different bacterial cells exhibiting greater propensity for adhesion and augmented EPS production on nanosmooth surfaces [234-236]. During attachment to poly(tert-butyl methacrylate) polymeric surfaces, for example, *Staleya guttiformis* cells were observed to secrete EPS for conditioning of the surface and formation of biopolymer network (Figure II.2.2.4) [236]. Oh et al. reported that different substrate materials influenced the morphology of *P. aeruginosa* biofilms, with increased surface roughness leading to an enhanced number of cells that could attach to the substrate [309].

Differences in experimental design and data interpretation

As highlighted by Anselme et al. [310], the experimental procedure itself can affect the results obtained via introduction of dewetting-related experimental artefacts. The authors elaborate that air or nitrogen drying, even in conditions of relative humidity, leads to liquid evaporation, leading to meniscus formation, convective flow, rupturing, dewetting, and capillary bridge formation, which in turn can modify bacterial localization, similar to colloidal particles. Despite the complexities underlying bacterial cell–surface interaction research, significant progress has been made in documenting nanotopography induced bacterial behaviour [235,236,296-299,305,311], driven by both technological advances that allow for atomic scale resolution imaging and nanoscale topography and the growing appreciation of the role that nanotopography plays in inducing behavioural changes in eukaryotic cells [233,293,294,304,312]. In addition to the clear implications this has for tissue engineering and in the design of biomaterials for controlled adhesion, differentiation and proliferation of mammalian cells, these discoveries are instrumental for the further development of essays for precise and rapid *in vitro* screening and the production of biomimetic antifouling surfaces.

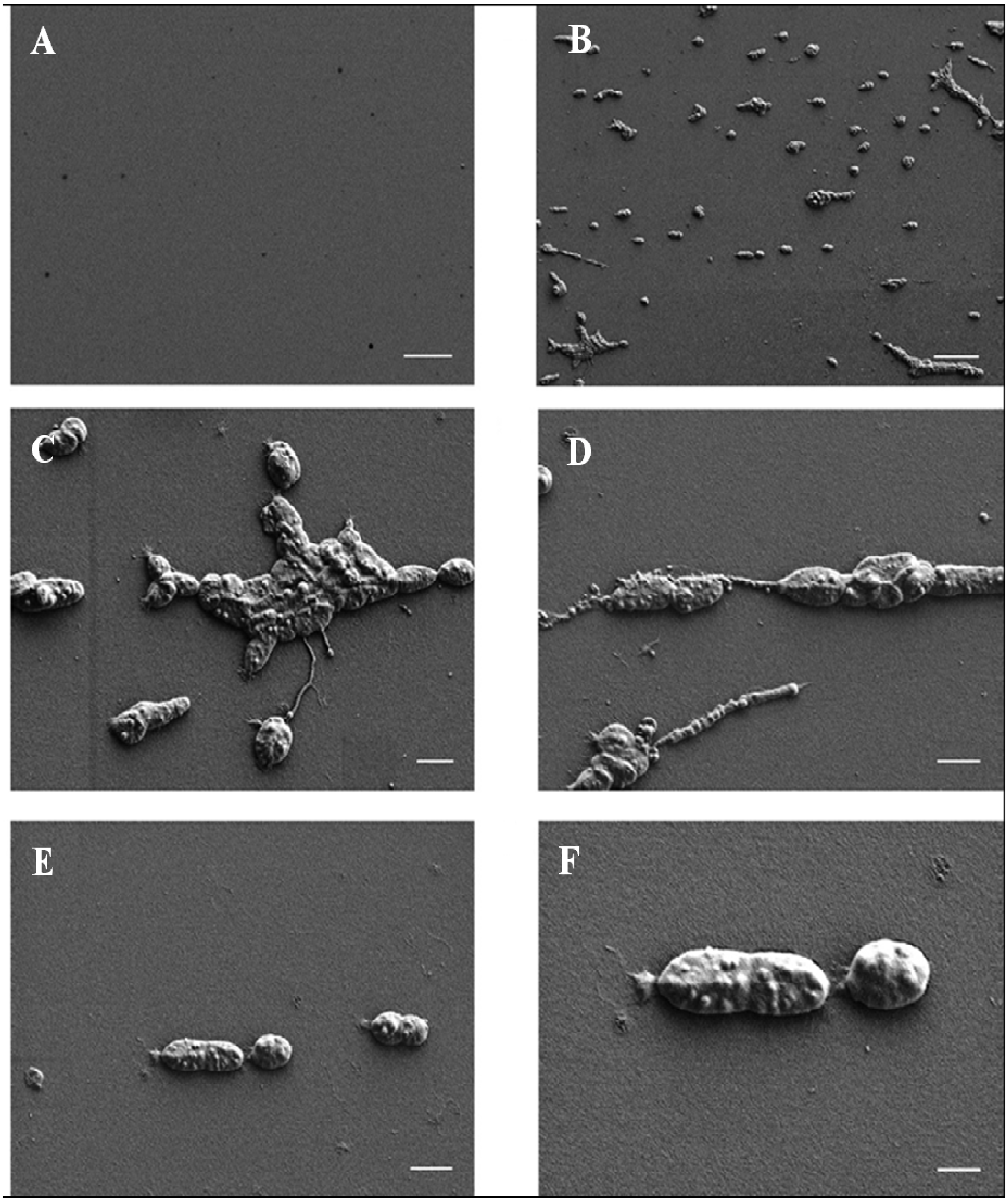

Figure II.2.2.4. EPS production and biopolymer network formation in *S. guttiformis* cells when attaching to poly(tert-butyl methacrylate) polymeric surfaces. Representative SEM images are given for (**A**) the polymer surface topography, R_a of 5.52 nm; (**B–F**) attachment pattern of after 24 h incubation showing the cell surface granular EPS structures and EPS-like material forming a biopolymer network to facilitate bacterial attachment. Scale bars: (A and B) 10 µm; (C–E) 2 µm; (F) 1 µm [236].

II.2.2.3.2 Current opinion on surface morphology-induced bacterial attachment

Several early studies identified surface roughness as a relatively minor factor in bacterial adhesion, with bacterial cells reported to have little preference for adhesion to surface features such as scratches or grooves [183,184,227]. Later studies indicated that the nanotopography of the surface became important in instances where the size of the surface features were comparable to the size of the bacterium. This conclusion was associated with issues concerning maximisation of the bacteria-surface contact area, followed by a consequential increase in the binding potential of the bacteria [228]. Regular submicron- and micron scale surface topographies with feature dimensions of above 100 nm were reported to modulate bacterial attachment [230,295-297]. For surfaces containing surface features that were significantly smaller than bacterial size, the contact area was believed to be reduced, resulting in a reduced extend of binding [229]. Mitik-Dineva et al. found that optical fibres etched with cylindrical wells 2.5 µm in diameter and 2.5 µm in depth would exhibit lower levels of bacterial attachment compared to that on the unmodified optical fibre surfaces, which were characterised by irregular topographies of R_a of 181 nm.[311] Whitehead et al. reported that *P. aeruginosa* and *Staphylococcus aureus* would preferentially adhere to surfaces characterised by regularly spaced pits of 1 µm and 2 µm but not attach to those surfaces containing irregularly spaced pits of 0.2 µm and 0.5 µm in size [230]. Similarly, *E. coli* was demonstrated to successfully attach and align when seeded on surfaces containing 1.3 µm wide and 120 nm deep microgrooves [295] yet unable to do so where surfaces contained grooves of 50 nm in height and period of 1.6 µm [294].

Studies of the attachment preferences of human pathogens *P. fluorescens* and *S. aureus* demonstrated that the extent of cell attachment and growth was influenced by the topography of micro-rough titanium surfaces, with cells showing a propensity for growth in the trenches of the surface in long rows [231,232], whereas the attachment of these bacteria onto smooth surfaces did not result in them following a distinct attachment pattern [227,232]. An et al. demonstrated that low micro- and nanorough titanium surfaces with an R_a of 0.43–1.25 µm did not allow the attachment of *Staphylococcus epidermidis* cells [184], whereas *Streptococcus thermophilus* bacterial attachment did not occur on stainless steel surfaces with an R_a of 0.01–1.04 µm [300]. A study by Medilanski et al. further investigated the cell adhesion and orientation of four bacterial species from three different phyla onto stainless steel surfaces with an R_a between 0.03–0.89 µm [301]. Minimal bacterial attachment was documented for surfaces with an R_a 0.16 µm, but for smoother or rougher surfaces, increased cell adhesion was observed. Recent studies by Ivanova et al. reported an increase in the extent of bacterial adhesion onto titanium surfaces of an R_a below 1.2 nm [282]. Given that the majority of the implant-associated infections are attributed to bacteria adhering to biomaterial surfaces in the form of complex communities, nanostructured metallic surfaces such as those that display opposing trends for osteoblasts *and Staphylococcus epidermidis* [313] or *E. coli* K12 [294] are of particular interest, because they have the capacity to influence the competitive colonization of eukaryotic over bacterial cells. In addition to their exciting potential for enhancement of biocompatibility and bacterial retardation of medical implants, these results highlighted the relevance and the necessity of analyzing eukaryotic cell and bacterial adhesion in a systematic and parallel way [314].

In spite of the growing body of evidence indicating that the extent and nature of bacterial cell attachment is influenced by surface nanometric scale roughness and that bacterial cells are capable of sensing changes in the average surface roughness within 1 nm, only a few studies have been published thus far that address attachment onto surface topographies on the sub-nanometer scale [233-236,282,305,311,315]. At this scale, it is becoming increasingly difficult to decouple the respective influences of chemical functionalities and atomic features. Magnetron sputtering has been demonstrated to be an effective technique for the controlled fabrication of metallic thin films containing atomic and nanoscopic surface roughness [316,317]. For example, titanium thin films fabricated on silicon wafers with an initial R_a of 0.29 nm were characterised by an R_a of 0.5 nm, 0.2 nm, and 0.18 nm. The taxonomically different bacterial cells, P. aeruginosa and Staphylococcus aureus, were seen to be able to differentiate between these surfaces, with a reduction in R_a from 1.22 nm to 0.58 nm resulting in a 2- to 3-fold increase in the number of attached cells and concomitant elevated levels of extracellular polymeric substance secretion.

The limitations brought about by a lack of data pertaining to the description of bacterial cellular responses to surfaces with various nanotopographic characteristics are also evident in the arguments frequently used to justify these phenomena. The commonly employed explanations for enhanced levels of bacterial cell adhesion and retention include the presence of an augmented surface area being accessible for attachment, surface rugosities providing a shield from shear forces and that there is an increase in the convection mass transport resulting from the presence of surface roughness and topographical features [263]. Investigations into the adhesion of *P. aeruginosa* to poly(methyl methacrylate) contact lenses indeed identified that surfaces with an R_q of 14 nm or above tended to result in an increased extent of micro-organism attachment [230]. However, a similar argument cannot be applied to adequately explain the increased level of attachment that has been reported for glass surfaces with an R_a of 1.3 nm, when compared to that obtained for surfaces with an R_a of 2.1 nm [234,305]. Anselme et al.

speculated that the attachment of bacteria in/on surface features bears associated costs to both the bacterial cells (in the form of elastic energy upon distortion) and the environment (as thermal energy) [263]. These substantial costs may lead to an energetic barrier to bacterial adhesion, which may be the reason why roughness and surface topographical features do not necessarily result in bacterial localization or alignment on the surface. On the microscale level, such an explanation has already been provided by Edwards and Rutenberg [229].

II.2.2.4 Concluding remarks

Nanotechnology is unprecedented in that it allows for the production of smaller, lighter, less expensive materials and devices that are significantly more efficient compared to conventional micro- and macrotechnologies. By allowing greater degrees of manufacturing control at the molecular and atomic scales, nanotechnology promises to supersede existing manufacturing technologies by tackling issues that for a long time were far beyond our reach, such as biomolecular and cell analysis, microfluidics, biomedical drug delivery and tissue engineering [318]. For this to eventuate, however, significant progress needs to be made in understanding cell–surface interactions. As novel and advanced characterisation techniques are developed, these will undoubtedly equip scientists with the tools necessary for precise molecular and atomic scale characterisation of both living cells and solid surfaces to which they attach. Yet, in order to truly appreciate the respective contribution of surface chemistry and topography, a clear and systematic framework needs to be designed to facilitate an accurate comparison between experimental results, since the literature published to date reports data obtained from studies performed under a wide range of experimental conditions. As a result, identifying a direct link between the extent of bacterial interaction and the chemistry/topography of a surface is difficult. Further, the competitive nature of eukaryotic compared to prokaryotic cell surface colonisation needs to be investigated, since addressing this issue is an essential step in advancing the understanding necessary for the design of biomaterials and in tissue engineering. As nanomodification technology approaches the atomic scale, we may be able to define a set of boundaries, or minimal dimensions at which a surface feature can exert influence over an attaching living organism.

CHAPTER III

THIN FILM FABRICATION

In this chapter, the feasibility of fabrication of thin solid films from terpinen−4−ol, the major constituent of *Melaleuca alternifolia* (tea tree) oil is examined.

In Section III.1, the experimental set−up and associated fabrication and characterisation procedures are provided in detail. Preliminary characterisations of polyterpenol films fabricated at a single set of deposition conditions indicate this material as a potential candidate for electronic and biomedical applications. The results are published as *Bazaka, K., Jacob, M. V., Synthesis of radio frequency plasma polymerised non-synthetic terpinen-4-ol thin films. Materials Letters 2009, 63, 1594-1597*. Based on the results of this initial study, the input power is identified as a major factor to influence material properties of the resultant thin films and the developed experimental procedure is further optimised in the course of a subsequent study.

Section III.2 reports on the influence of varying deposition process conditions, specifically the input RF power, on the resultant material properties of polyterpenol. Understanding of molecular and structural composition of a plasma polymer is an essential stage in material characterisation, providing important information regarding potential pathways along which fragmentation and polymerisation take place. In turn, this can be used for optimisation of material properties to fit a specific application. The results of chemical and optical characterisations of thin films deposited at different power conditions are reported, as published in *Bazaka, K. et al., Optical and chemical properties of polyterpenol thin films deposited via plasma-enhanced CVD. Journal Materials Research 2011, 26, 1018-1025*.

III.1 SYNTHESIS OF PLASMA POLYMERISED TERPINEN-4-OL THIN FILMS

Abstract

Recent advancements in the area of organic polymer applications demand novel and advanced materials with desirable surface, optical and electrical properties to employ in emerging technologies. This study examines the fabrication and characterization of polymer thin films from non-synthetic terpinen-4-ol monomer using radio-frequency plasma polymerization. The optical properties, thickness and roughness of the thin films were studied in the wavelength range 200 – 1000 nm using ellipsometry. The polymer thin films of thickness from 100 nm to 1000 nm were fabricated and the films exhibited smooth and defect free surfaces. At 500 nm wavelength, the refractive index and extinction coefficient were found to be 1.55 and 0.0007 respectively. The energy gap was estimated to be 2.67 eV, the value falling into the semiconducting E_g region. The obtained optical and surface properties of terpinen-4-ol based films substantiate their candidacy as a promising low-cost material with potential applications in electronics, optics, and biomedical industries.

Keywords: Plasma polymerization; thin film; optical properties; organic polymer;

As published in: Bazaka, K., Jacob, M. V., Synthesis of radio frequency plasma polymerized non-synthetic terpinen-4-ol thin films. *Materials Letters* 2009, 63, 1594-1597.

III.1 Introduction

Surface engineering is one of the most prominent areas of materials research and development and is widely implemented over a broad range of possible applications, to considerably improve performance and to add to the value of material manufacturing, and significant importance is placed on process and product innovation and cost management [85,86,88,319,320]. With regard to manufacturing, plasma based technologies are growing rapidly in terms of industrial application as they are highly engineering-friendly, deliver practically pollutant free surface chemistry, and can be easily integrated into manufacturing processes [86,319]. Early applications of plasma polymers made use of the physical nature of these highly cross-linked materials, the barrier properties in particular, and included electronics, metal coatings and membranes. With subsequent identification of non-equilibrium plasma conditions capable of delivering chemically functionalized polymer thin films with retention of some of the original chemistry and structure of the monomer and the inherent advantages of the substrates (e.g. their mechanical properties), the range of applications broadened from permselective membranes to include biomedical films [89,90]. Typically, the resulting thin films are characterized by smooth surface, ultrathin, pinhole free films possessing spatial uniformity, conformal coverage and high adhesion to the substrate [77,96,98,321,322].

With an ever increasing demand for novel low cost materials with specific electrical and optical properties for use in many emerging technologies, a number of organic materials have been examined as potential candidates for electronics and biomedical applications [85-88,319,320]. Radio frequency (RF) polymerization is one of the most commonly used deposition methods for fabrication of thin films from a wide array of organic materials, including the ones that do not contain the type of functionalities normally associated with conventional polymerization. The goal of this study was to evaluate non-synthetic material as a suitable source for RF polymerization of organic thin films with desirable optical and electrical properties. This paper reports on the fabrication of polymer thin films from non-synthetic terpinen-4-ol derived from *Melaleuca alternifolia* by means of RF plasma polymerization and optical, electrical and surface characterization of the polymer thin films.

III.1.2 Material and methods

RF polymerization process has been discussed in detail by many authors [85-88,90,319,320]. During the polymerization, the energy levels of particles that amass the gas within a deposition chamber increase significantly resulting in electrons being released and charged heavy particles (molecular and atomic species) formed [323]. The energy of most electrons is enough to break virtually any chemical linkage pertinent to organic molecules and organic structures containing main group elements, with higher energies needed to dissociate unsaturated bonds. A result of the plasma state processes, the high molecular weight networks generated from collisions and subsequent recombination of the charged and neutral species with each other and the surfaces that constrain the discharge, possess structural attributes that significantly differ from the ones of the original monomer. Such structures are not built from repeating monomer units and are likely to possess unsaturated, branched and cross-linked architecture. Conventional polymer formation employs plasma-generated functionalities and radicals, however the process takes place in the absence of plasma on plasma exposed substrate surfaces [85-88,90,319,320]. It is possible to control polymer material characteristics by changing the deposition conditions (e.g. power and frequency of the excitation signal, type and position of the substrate, geometry of the deposition chamber) or applying prior and post deposition treatments (e.g. subjecting the substrates to prefabrication RF plasma treatment) [85-87,320,324].

A custom-manufactured glass deposition chamber [325] was used to fabricate the RF polymer thin films from terpinen-4-ol monomer (supplied by Australian Botanical Products). Copper electrodes are capacitively coupled to the reactor so that RF (13.56 MHz) energy is delivered into the deposition chamber. The distance between the electrodes was varied to achieve optimal deposition conditions. A number of substrate cleaning processes were carried out. First, of all the superwhite glass substrates (25 mm × 75 mm) were washed in a solution of Extran and distilled water. Secondly, the substrates were subjected to 30 minute ultrasonic clean in distilled water heated to 50° C followed by a propan-2-ol rinse and air-dry. The cleaned glass substrate was placed into the deposition chamber and the chamber was flushed with argon for 1 minute. The monomer was released gradually into the chamber kept at a pressure of 150 mTorr – the glow was maintained by controlling the monomer flow by means of a vacuum stop cock. Deposition was performed at room temperature, pressure of 200 mTorr and RF power of 25 W. For every deposition, 1 ml of monomer was used.

III.1.3 Results and discussion

III.1.3.1 Film thickness

A spectrometer (Avantes 2048) was employed for preliminary investigations of the polymer films' absorbance and thickness. These parameters were also evaluated using a variable angle spectroscopic ellipsometer (model M-2000, J. A. Woollam Co., Inc.). The dependence of film thickness on the deposition time was studied on samples fabricated on the glass substrates for 2, 5, 10, 15, 20, and 30 minutes (4 samples for each deposition time). A film thickness of 99 nm was obtained with deposition time of 2 minutes. The sample thickness increased with time, approaching 874 nm for deposition time of 30 minutes. Linear thickness dependence on deposition time was observed. Therefore, assuming constant deposition conditions, the desired film thickness can be obtained by controlling the time of deposition so that the material can be tailored for a given application.

III.1.3.2 Surface properties

The surface morphology of the films was investigated using atomic force microscope (NT-MDT NTEGRA Prima) to understand topographical properties of the surfaces over scales ranging from angstroms to microns [87]. The non-destructive semi-contact AFM mode was chosen to minimize the damage to the surface of the films. A number of scans were performed on each sample to improve the accuracy of characterizations. Figure III.1.1 shows an example of the surface morphology of 1 x 1 µm scan of the terpinen-4-ol polymer thin film of thickness 874 nm. The results confirmed the polymer thin films as smooth, uniform and pin-hole free surface, implying that the plasma polymerization has taken place mostly on the surface of the substrate and not in the gas phase [326]. The average roughness of 0.3 nm obtained from the AFM data was in agreement with the value obtained from the ellipsometric studies of the films. Smooth surfaces are vital for optical and electrical applications, and also for implementing as a coating materials for surface protection or buffer layers [320].

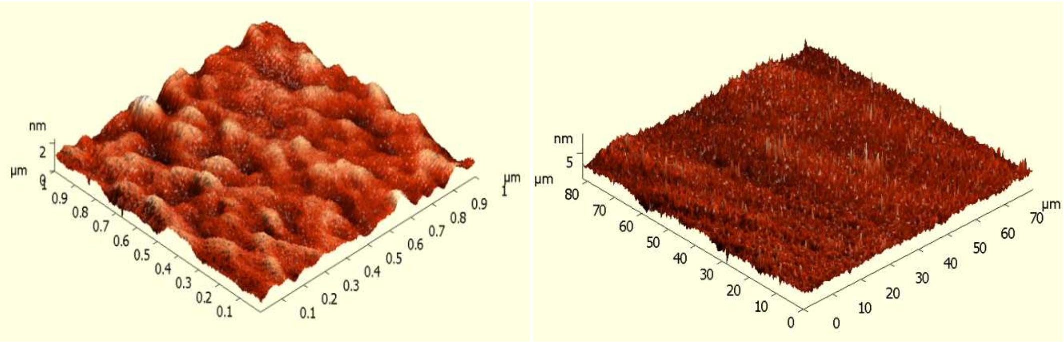

Figure III.1.1. AFM picture of film fabricated at 25 W, scan size 1 x 1 µm and 100 x 100 µm.

III.1.3.3 Optical properties

Optical properties of the films were derived from the ellipsometric data collected at three different angles of incidence (φ = 55º, 60º, and 65º) over the wavelength range of 200 – 1000 nm (6.2– 1.2 eV) by means of regression analysis (WVASE32 software package). First, Cauchy dispersion was applied to data within 500−1000 nm range assuming the films are optically transparent within this region (*k* approximates 0) and the film is homogenous [327]. A surface roughness layer was employed assuming the roughness being less than 10 % of the wavelength of the light used in probing the sample as greater roughness has a propensity to scatter and depolarize light [328]. Further, Gaussian oscillators were employed as harmonic oscillators [329,330]. Transmission data was appended to enhance the accuracy of the model used to determine optical constants of the material and understand the transparency range of the polymer films under investigation. The refractive index and extinction coefficient values range from 1.71 to 1.53 and from 0.1 to 4×10^{-7} respectively over the wavelength region of 200 to 1000 nm (Figure III.1.2).

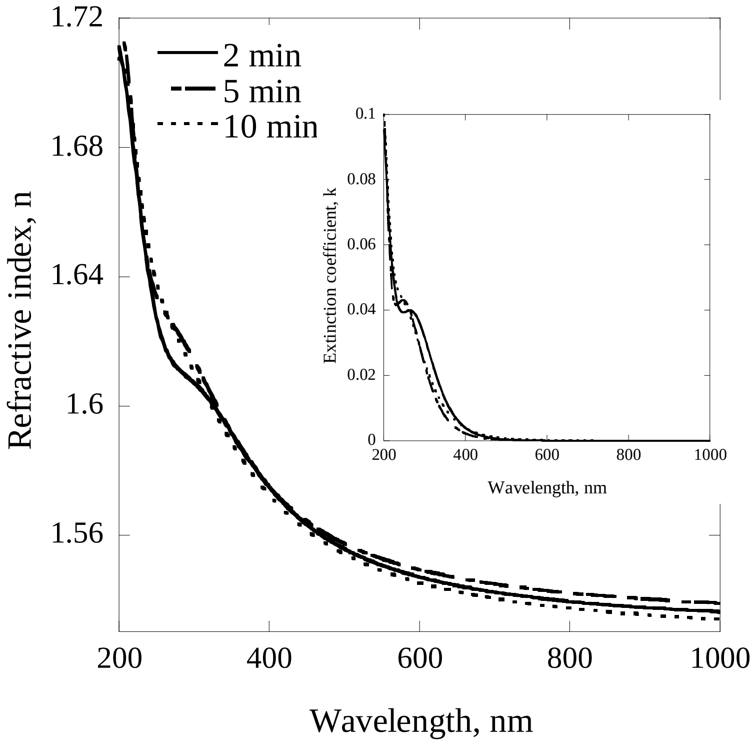

Figure III.1.2. Refractive index and extinction coefficient as a function of wavelength.

The effect of deposition time on the refractive index and extinction coefficient was insignificant with all curves exhibiting similar shape. Mean squared error (*MSE*) values used to assess the quality of the fit were below 3, and although it is preferential to have *MSE* values as close to zero as possible. *MSE* value tend to vary significantly depending on factors such as the amount and quality of the data available, or how precisely the chosen model mirrors the thin films under investigation [331]. UV-Vis absorption spectra of the samples deposited at 25 W RF power and 200 mTorr were studied. As is shown in Figure III.1.3, the polymer thin film was optically transparent across the deposition time range.

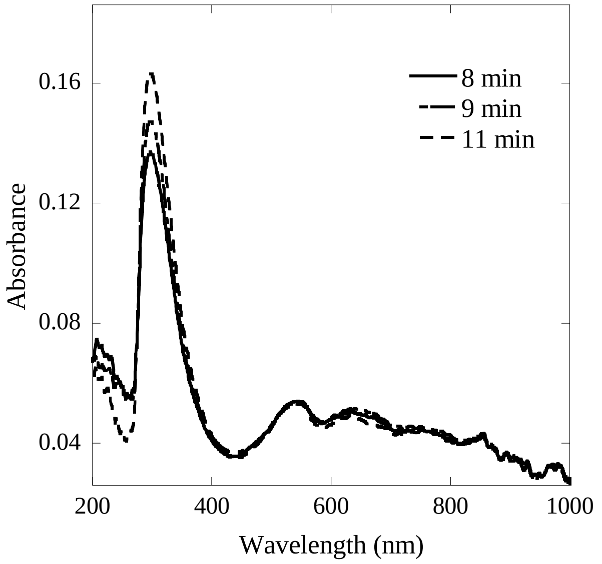

Figure III.1.3. UV-Vis absorption spectrum of films deposited at RF power of 25 W.

III.1.3.4 Energy gap

Energy gap estimates derived from ellipsometric and spectrometric data analyses were compared and conclusions on the conducting nature of the material were drawn. The optical band gap E_g is derived from the Tauc equation $\alpha h\nu = B\ (h\nu - E_g)^{\ n}$, where α is the optical absorbance, v is the frequency of light, and n and B are factors dependant on the type of transition and the length of localized state tails respectively [332]. The value of n is determined by the nature of the electronic structure of the material, with semiconducting plasma polymers generally attracting n of 2 which describes a parabolic function for the density of states [333]. Matlab software was employed to calculate the optical band gap values by extrapolating the linear section of the absorption curve to the abscissa. n = 2 (for indirect transition) were shown to provide the best fit for the polymer films under investigation [98]. The energy gap values (2.67 eV and 2.66 eV for UV-Vis spectrometric and ellipsometric data respectively) exhibits that the optical band gap of the material was in the semiconducting region. Although the optical band gap deviates from the value of E_g by the width of the range of localized states in the valence or conduction band, the degree of variation being dependent on the degree of disorder within the polymer film [334], this method provides important information about the electronic structure of amorphous materials [335]. Using the WVASE32 software, a combination of Tauc-Lorenz and Gaussian oscillators was fitted to the ellipsometric data during modelling to ascertain the values of E_g. An energy gap value of 2.68 eV was achieved in this study. This value is similar to the values obtained using the ellipsometric and spectroscopic UV-Vis absorption data and it falls within the semiconducting region of E_g.

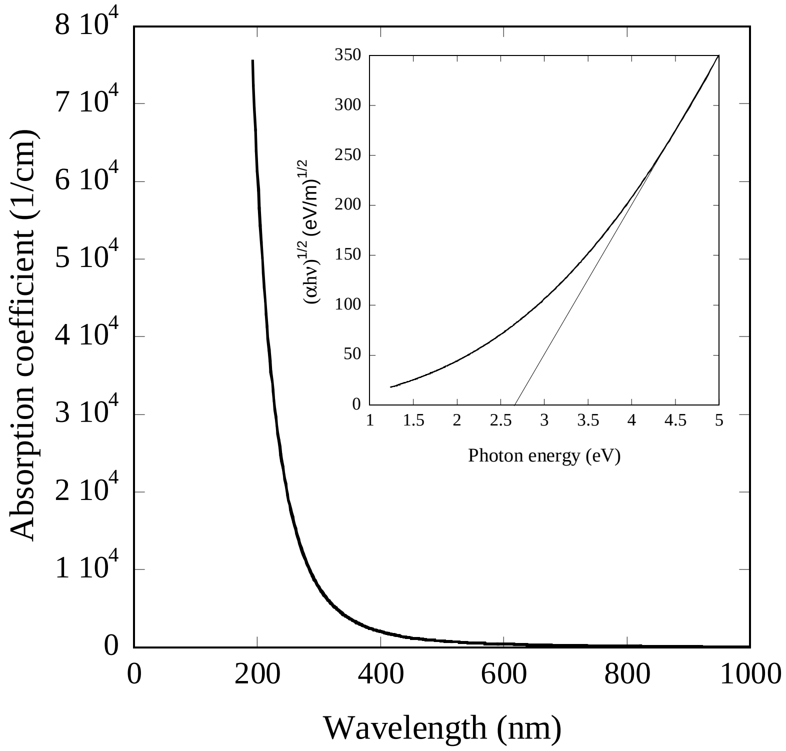

Figure III.1.4. Absorption coefficient as a function of wavelength of film deposited at 25 W.

III.1.4 Conclusions

The RF plasma polymerization of terpinen-4-ol monomer was studied with the objective to synthesize a smooth, defect-free and homogenous film from a non-synthetic source. UV-Vis, ellipsometry, and AFM characterizations demonstrated the plasma conditions had effect on morphology and properties of the resultant film. Transparent, smooth and uniform organic polymer films can be manufactured using RF excitation signal of 13.56 MHz and power of 25 W. The thickness could be varied from 100 nm to 1000 nm by controlling the deposition time. The optical properties studied in the wavelength range 200 – 1000 nm identified the films as potential candidates for applications in optics. At 500 nm wavelength, the refractive index and extinction coefficient were found to be 1.55 and 0.0007 respectively. The energy gap estimated to be 2.67 eV confirmed the possibility of using the polymer film in semiconducting applications. The obtained optical and surface properties of the fabricated films substantiate the candidacy of the material as a promising low-cost environmentally friendly material that could be used in many emerging technologies. We demonstrated the ability to manufacture uniform, defect-free non-synthetic terpinen-4-ol based films with desirable material properties in a reliable and reproducible fashion and their potential as candidates for electronics, optics, and biomedical industries applications.

III.2 OPTIMISATION OF DEPOSITION PARAMETERS

Abstract

The development of novel organic polymer thin films is essential for the advancement of many emerging fields including organic electronics and biomedical coatings. In this study, the effect of synthesis conditions, namely RF deposition power, on the material properties of polyterpenol thin films derived from non-synthetic environmentally friendly monomer was investigated. At lower deposition powers, the polyterpenol films preserved more of the original monomer constituents, such as hydroxy functional groups; however, they were also softer and more hydrophilic compared to polymers fabricated at higher power. Enhanced monomer fragmentation and consequent reduction in the presence of the polar groups in the structure of the high-power samples reduced their optical band gap value from 2.95 eV for 10 W to 2.64 eV for 100 W. Irrespective of deposition power, all samples were found to be optically transparent, with smooth, defect-free and homogenous surfaces.

Keywords: Plasma polymerization; thin film; optical properties; organic polymer;

As published in: Bazaka, K., Jacob, M. V., Bowden, B. F., Optical and chemical properties of polyterpenol thin films deposited via plasma-enhanced CVD. *Journal Matererials Research* 2011, 26, 1018-1025.

III.2.1 Introduction

Recent advancements in organic electronics systems such as flexible electronic circuits, displays, sensors, thin-film transistors and thin-film organic photovoltaic devices demand for the development of new high-performance organic materials with properties comparable to those of existing electronic materials[85,86,88,89,249,319]. Radio frequency (RF) plasma enhanced chemical vapour deposition (PE-CVD) is commonly used for fabrication of such materials to be applied in the microelectronic industry as they allow deposition of organic compounds that do not contain functionalities associated with conventional polymerization (such as C=C unsaturation [77]) on a variety of low-cost substrates, including glass, plastic, metal foils etc [86,319]. Thin films manufactured by means of RF PE-CVD exhibit high spatial uniformity and adhesion to the substrate and possess smooth and pinhole free surfaces [96,98]. In addition, the conditions of non-equilibrium plasma techniques allow for the formation of chemically functionalized polymer thin films that retain some of the original functionalities and structure of the monomer while taking advantage of inherent properties of the substrates [89,90].

In the process of PE-CVD, the gas-phase and surface-related plasma reactions are influenced by a number of conditions, such as the nature of the plasma, the mode of transferring energy from the generator into the reaction chamber, the geometry of the chamber, and the deposition conditions such as monomer flow, pressure, nature of the substrate and its temperature, and the input power. In the course of plasma enhanced synthesis, the monomer is dissociated with the degree of dissociation closely related to the input power applied [321]. Concurrently, there is a recombination process taking place inside and outside of the plasma region [323]. Monomers that possess functionalities associated with conventional polymerization (such as unsaturation) will undergo a more complex recombination process that will combine the conventional polymerization with fragment-recombination mechanisms triggered by the plasma-created and surface-attached active species, ions and free radicals. The resultant polymer will have chemical and physical properties notably different compared to its precursor [86,89,90].

Recently, fabrication of a novel organic polymer material, polyterpenol, from non-synthetic terpinen-4-ol was reported [336]. The optical and physical properties of the material were promising, with refractive index and extinction coefficient at 500 nm of 1.55 and 0.0007 respectively. Films fabricated from terpinen-4-ol were characterized by very smooth, defect free surface, high chemical inertness and thermal stability, and transparency across the visible wavelength range, and hence can find potential applications in organic optoelectronics as encapsulating layers for circuitry. Polyterpenol was also incorporated as an insulating interlayer into the pentacene-based organic field-effect transistor (OFET), which resulted in a significant shift in the threshold voltage and enhanced effective mobility attributed to lower charge accumulation on the pentacene-gate insulator interface. The inclusion of polyterpenol as a gate insulating material also improved the switching property of the pentacene based OFET [337]. Thus, polyterpenol has been shown to be an appropriate insulating material to enhance OFET output characteristics and hence is a prospective candidate for full flexible organic electronic circuits. Polyterpenol thin film coatings deposited on glass substrates have also been demonstrated to prevent adhesion and subsequent biofilm formation by selected human pathogenic bacteria, *Pseudomonas aeruginosa*, and hence can be potentially applied as antibacterial and antifouling coatings for indwelling medical devices and contact lenses [200].

To be considered for a given application, a material should possess a certain combination of properties. For instance, to serve as a protective coating for organic electronic devices, materials of high optical transparency, chemical stability and mechanical hardness may be desired. Similarly, material’s use within an electronic device will be largely guided by its conducting, semiconducting or insulating nature, and such physical properties as film uniformity and surface morphology. When choosing fabrication conditions to tailor for one particular property, it is therefore important to understand how these conditions will affect other properties of the material. In this study, the influence of RF deposition power on the optical, chemical and physical properties of polyterpenol thin films is investigated.

III.2.2 Experimental

Polyterpenol thin films were fabricated from non-synthetic terpinen-4-ol monomer sourced from Australian Botanical Products. The polymer thin films were manufactured at RF input powers of 10, 15, 25, 50, 75 and 100 W on a number of substrates, including glass and KBr disks. The details for the substrate cleaning and deposition procedures were described elsewhere [325]. Briefly, the thoroughly cleaned and dried glass substrates are placed in the custom manufactured deposition chamber, which is subsequently flushed with argon to produce an oxygen-free substrate surface. The chamber is then evacuated to achieve pressure of 50 mTorr. The monomer vapor is gradually released into the chamber at the rate of 2 cm^3/min. The deposition is performed at room temperature and pressure of 200 mTorr.

The optical constants, thickness and roughness of the polyterpenol film were estimated from Ψ and Δ ellipsometric data collected for $\varphi = 55^\circ$, 60°, and 65° angles of incidence over the 200 – 1000 nm wavelength range using a variable angle spectroscopic ellipsometer (model M-2000, J. A. Woollam Co., Inc.). UV-vis (Avantes AvaSpec 2048 UV-vis spectrometer) estimates of film thickness were used as the initial input to aid the fitting of the ellipsometric data. The optical band gap of the polymer thin films were determined by fitting a Tauc-Lorenz oscillator model to the ellipsometric data (Ψ, Δ). Absorption coefficient data from the modelling of optical constants (n, k) and UV-vis absorbance data provided complimentary estimations of the optical gap. Following Tauc power law that determines the optical energy gap in amorphous materials due to the band-to-band transitions, a number of possible values for n were tested [332]. The results were plotted in the y-axes and energy $h\nu$ in the x-axes and the optical band gap value was estimated by extrapolating the linear section of the curve to the abscissa, with the intercept representing the value of interest. Fourier transform infrared (FTIR) spectra of polyterpenol films and terpinen-4-ol monomer were acquired using Nicolet Maxim FTIR Spectrometer (Thermo Electron Scientific Instruments LLC, Madison, WI). For every sample, 32 scans were collected in transmission mode over the region of 4000 – 400 cm^{-1} at resolution of approximately 2 cm^{-1}. Background was measured prior to the measurements of the film samples in order to account for CO_2 and H_2O present in the air. Sessile drop water contact-angle studies were performed using a KSV 101 system (KSV Instruments Ltd, Helsinki, Finland). Images were recorded for 30 s at 1 s interval and image processing software was employed to establish the contact angle by fitting the Young-Laplace equation to the measured drop profile. Thin film samples were scanned using a NT-MDT atomic force microscope (AFM NT-MDT Co, Zelenograd, Moscow, Russia) in semi-contact (tapping) mode. The scan sizes of 1 x 1 µm, 10 x 10 µm, and 100 x 100 µm were used to quantify surface roughness and identify presence of defects. For nanoindentation analysis a Triboscope indenter system (Hysitron Inc., Minneapolis, MN) was employed with Berkovich indenter (70.3° equivalent semi-opening angle). Typical loads used in the indentation ranged from 100 µN to 700 µN with fixed loading time. The loading rate varied from 20 to 140 µNs.

III.2.3 Results and discussion

It is well accepted that the extent to which the chemical structure of the polymer differs from the one of the monomer is correlated with the power level used during the deposition. The degree of monomer fragmentation is determined to a large extent by the intensity of the plasma field, with the higher bond dissociation corresponding to the increased magnitude of RF energy input [338]. Furthermore, there is an increase in temperature of the substrate associated with higher applied power. According to Lopez et el, lowering the substrate temperature encourages condensation and adsorption of non-excited gaseous species and subsequent incorporation of increased number of non fragmented molecules or slightly fragmented precursors in the growth region of the film. This process of integration of these large fragments is driven by the reactive species that arise as a result of plasma field [339]. In addition, in line with a competitive ablation-polymerization concept proposed by Yasuda, the respective rates for ablation and polymerization may have differing temperature dependencies and therefore it is possible lower temperatures may result in reduction of energetic particle damage [340]. Hence, by choosing appropriate RF power conditions, polymer films with material properties tailored to the desired application can be manufactured.

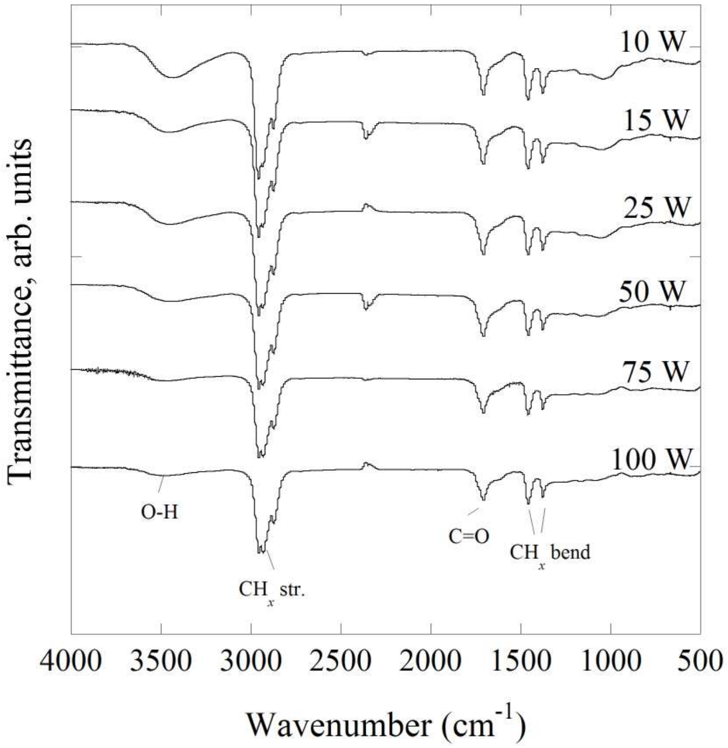

Figure III.2.1. Infrared spectra of polyterpenol films fabricated at varied RF power levels.

The bonding state of the plasma polymerized thin films was analysed by FTIR absorption over a range of 4000–500 cm^{-1}, as demonstrated in Figure III.2.1, with the absorption frequencies summarized in Table III.2.1. Compared to the spectrum for the monomer (not shown here), the number of absorption frequencies decreased significantly upon application of plasma field, particularly in the fingerprint region of the spectrum (1500 cm^{-1} and below). Infrared bands associated with C=C–H unsaturation and observed between 3150 and 3000 cm^{-1} completely disappeared even at the lowest of the deposition powers (10 W), which can be explained by C=C having the lowest value of dissociation energy among the bonds of the monomer. Bands at 1150 and 1050 cm^{-1} were characteristic of C–O stretching of alkyl-substituted ether. A peak at 1707 cm^{-1} which was not found on the monomer spectrum indicated the presence of carbonyl group (C=O stretch). From Figure III.2.1, the absorptions corresponding to methyl C–H asymmetric and symmetric stretching (around 2955, 2930, and 2875 cm^{-1}) and bending (around 1459 and 1380 cm^{-1}) showed diminution in magnitude with higher input RF power. Similarly, there was a substantial reduction in H–bonded O–H stretch related band magnitude with increase in deposition power. The absorption peaks at approximately 2400 cm^{-1} are associated with incomplete removal of CO_2 from the spectrum and are not pertaining to the polymer film itself.

Table III.2.1. Group frequencies for polyterpenol films.

Group frequency wavenumber cm^{-1}	Intensity	Assignment
~ 3460	medium to small	hydroxy group, H-bonded OH stretch
2955, 2930	very strong	methyl asymmetric C-H stretch
2875	medium	methyl symmetric C-H stretch
1707	medium	carboxylic (C=O) stretch
1459	medium	methyl C-H asymmetric bend
1380	medium	symmetric C-H bend
1150, 1050	small to non-existent	C–O stretching of alkyl-substituted ether

Dissociation of the terpinen-4-ol monomer is electron-generated rather than a result of thermal excitation or direct chemical reaction. Therefore, the nature of the chemically reactive species that engage in polymerisation and film formation is strongly dependent on the input RF power. Considering the bond orders for terpinene-4-ol molecule, C(6)–OH and C(6)–C(7) bonds are likely to dissociate first to form reactive fragments, and C(3)=C(4) double bond is likely to undergo saturation to produce single bonded C(3)–C(4). Increasing RF power, and hence the energy of the plasma field to which the monomer is exposed, will allow for dissociation of higher energy bonds and hence increase the resultant fragmentation of the precursor molecule. Furthermore, the temperature of the substrate will also increase, and as such will influence the recombination process taking place within and outside of the plasma region. Consequently, polymers fabricated at higher input power will bare less resemblance to the original molecule, and possess higher proportion of free radicals within the polymer framework compared to polymers fabricated at lower powers. On the other hand, recombination of plasma-created species generated at RF energy just sufficient enough to initiate dissociation will be more comparable to conventional polymerisation. In such polymers, more of the structure and functionalities of the monomer is preserved owing to the combination of weakly ionized plasmas and relatively low substrate temperature.

Since the intensity and the frequency at which a chemical bond absorbs infrared radiation varies depending on its environment, namely the type of neighbouring atoms and the presence of conjugation, the respective absorption peaks on the spectra for terpinene-4-ol monomer and polyterpenol films may differ in terms of their position and intensity. Similarly, broadening of the peaks is influenced by the density of a compound, or, more precisely, the extent of molecular contact between individual molecules. Such as in the case of O–H absorption, the individual molecules of the hydroxyl-containing compound will be characterised by a slightly different extent of hydrogen bonding hence giving rise to a broader O–H peak representing an average of these slightly dissimilar absorptions.

In general, polymer materials containing large portion of C=C, C=O and –OH groups are characterized by high dielectric constant because of their high polarizability, with polarizability of C=C being larger than that of C=O or –OH [341]. However, as both carbon-oxygen and oxygen-hydrogen bonds are polar due to oxygen being more electronegative than either of the two, polymer structures containing these functionalities are likely to be less chemically and mechanically stable compared to hydrocarbon polymers. Indeed, previous works demonstrated that polyterpenol films deposited at lower RF power to be softer and less stable when in contact with common processing solvents, such as acetone and alcohol. Higher applied RF power results in the reduction in or compete disappearance of such moieties, as clearly seen in IR spectra, rendering polyterpenol more conductive.

It is clear from the IR results that application of RF plasma field resulted in terpinen-4-ol ionization, where certain bond structures dissociated (such as C=C bond) while others emerged (e.g. C=O group). Polyterpenol materials were identified to be predominantly hydrocarbon dense, with oxygen containing groups being present within the structure in the form of alcohol and carbonyl groups. These results are in agreement with X-Ray Photoelectron and Raman spectroscopy studies of polyterpenol reported elsewhere. The study found that carbon and oxygen dominated the surface of the polyterpenol films (up to 99 at. %), with Si, Zn and N present in trace amounts (below 1%). An increase in deposition power from 25 to 100 W resulted in a significant reduction in oxygen content (from 22.8 to 12.2 %) and concomitant increase in the carbon fraction (from 76.0 to 87.1 %).

Typical surface morphology of polyterpenol thin films deposited at different RF powers is illustrated in Figure III.2.2. Independent of deposition power, polyterpenol samples were found to be smooth, uniform and pinhole free, suggesting the polymerization occurred predominantly on the surface of the glass substrate rather than in the gas phase [326]. There was a subtle change in surface topography of the samples with films manufactured at lower RF powers displaying broader, more rounded peaks and troughs while samples deposited at higher RF powers exhibiting narrower, sharper peaks. The number of peaks was also affected with higher number of peaks corresponding to higher deposition power. These observations were confirmed through surface skewness R_{skw} and coefficient of kurtosis R_{kur} which increased for samples deposited at higher RF power, although the increase was not statistically significant ($p > 0.05$). For every polyterpenol film independent of deposition power, surface skewness (R_{skw}) was found to be more than 0 and coefficient of kurtosis (R_{kur}) less than 3. Such values for R_{skw} and R_{kur} indicate a disproportionate number of peaks pertinent to the polyterpenol surfaces and a well spread out height distribution, respectively. Samples deposited at lower RF powers, e.g. 10 W, were characterized by slightly higher values of average (R_a) and RMS (R_q) surface roughness parameters and the maximum peak height (R_{max}) compared to higher RF power films, i.e. 100 W; R_a reduced from 0.4 to 0.33 nm, R_q from 0.53 to 0.51 nm, and R_{max} decreased from 5.4 to 5.1 nm. However, the statistical analysis of the R_a and R_q data collected for the polyterpenol films concluded that there was no significant difference between these parameters for the films deposited at different input powers ($p > 0.05$).

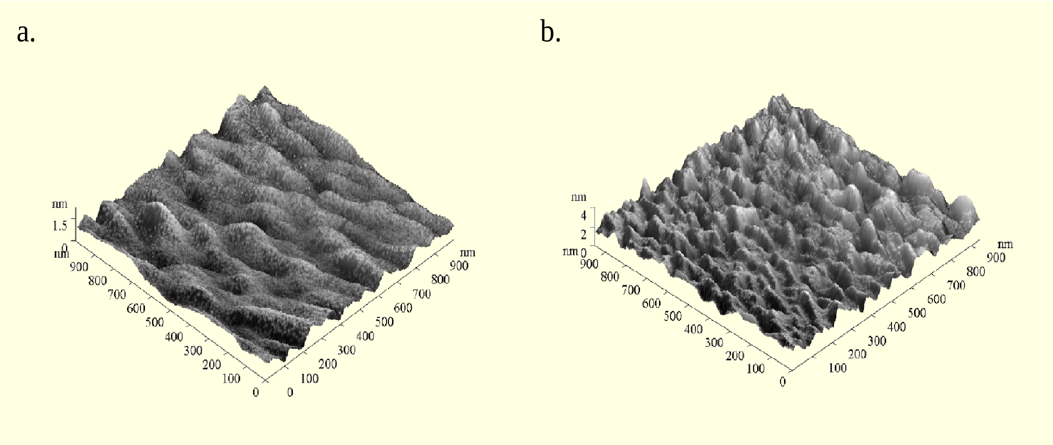

Figure III.2.2. AFM images of polyterpenol films fabricated at a) 15 W and b) 100 W, scan size 1 x 1 μm.

Water contact angle increased for samples deposited at higher RF power, as demonstrated in Figure III.2.3, from 62° to 76° for 10 and 100 W, respectively. Linear fit to the data had $R^2 = 0.99$, and the rate of change was in the vicinity of $0.02\ ^\circ s^{-1}$ compared to $0.2\ ^\circ s^{-1}$ for poly(acrylic acid) and therefore it was unlikely swelling or reorientation of polar groups were taking place at the liquid-solid interface [342]. Films became more hydrophobic as the RF power increased, due to the decrease in oxygen containing functional groups and increase in cross-linking [343], as demonstrated through FTIR analysis. Contact angle is known to be influenced by the chemical composition, homogeneity, and the morphology of the solid surface. Although films deposited at higher RF power were smoother compared to their low power counterparts, the difference was not statistically significant and hence is unlikely to be the major determinant in the increase of contact angle. A significant decrease in the oxygen containing moieties under increased deposition power conditions, illustrated by a decrease in the magnitude of C– –O/C=O and *O– –C=O bond peaks, at BE of ~532 eV and *O– –C=O at BE of ~533 eV, respectively, and an increase in relative concentrations of C–C/C–H bonding environment at BE of ~285 eV, indicates that a change in chemical composition of polyterpenol is responsible for the increase in water contact angle.

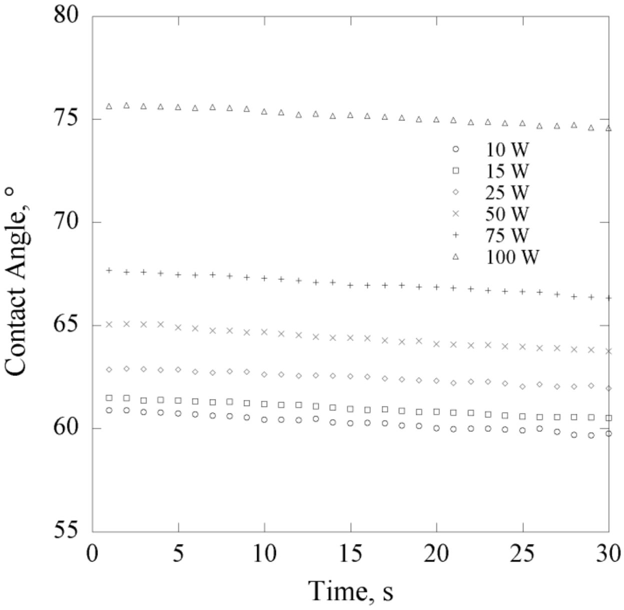

Figure III.2.3. Water contact angle as a function of time for polyterpenol thin films deposited at different RF power levels.

Polymers with higher hydrocarbon content are likely to be more stable when in contact with solvents compared to oxygen-rich polymers, and therefore are suitable for applications where solvent stability is required, such as thermally degradable sacrificial materials in IC fabrication where wet etching procedure is employed. On the other hand, more wettable polymer surfaces are desirable if the material is used as a bioactive coating, as such materials are generally better tolerated by the human body [193].

Ellipsometric studies of the polyterpenol films indicated a decrease in deposition rate from 38.2 to 19.8 nm/min for 10 and 100 W RF power settings, respectively; however, there was no effect on the surface roughness, which was determined to be approximately 0.3 nm of the sample films and was in agreement with estimates from the AFM analysis. Following Yasuda's competitive ablation-polymerization concept, the process of ablation is likely to be much more prominent in the case of higher applied plasma field compared to lower powers where, according to Lopez, the process is dominated by polymerization [339]. Furthermore, the reduction in film thickness can be attributed to a suspected increase in film density and degree of cross-link. This is also likely to be responsible for the increased in hardness, from 0.34 GPa for 15 W to 0.51 GPa for 100 W.

Optical absorption in solids takes place through a range of mechanisms in all of which the photon energy is absorbed by either lattice or by electrons where the transferred energy is conserved [344]. Photon absorption excites the electrons from a filled band to an empty band resulting in a noticeable increase in the absorption coefficient $\alpha(\nu)$. Fundamental absorption edge is the onset of this rapid change in the absorption coefficient and the optical band gap E_g is the corresponding energy. Several values of n were examined to understand the nature of transition with $n = 2$ offering the best fit, an indicator of indirect transitions taking place in the polyterpenol sample [98,336]. For amorphous materials, the frequently chosen n value of 2 is indicating a parabolic function for the density of states distribution [333,335]. High degree of disorder characteristic of plasma polymers means the value of the optical gap will diverge from the energy gap value by the width of the range of localized states in the valence and conduction band [334,345].

Optical band gap values were derived as presented in Figure III.2.4.The results showed consistency between estimation methods and are outlined in Table III.2.2. The optical band gap decreased with an increase in RF deposition power. Oppedisano et al suggests a decreasing trend of the optical gap in amorphous carbon-based materials is due to increasing sp^2 carbon content and varying hydrogen content [346]. The optical band gap values changed from 2.93 eV for 10 W to 2.64 eV for 100 W, falling within the semiconducting region of E_g. Hence, it is possible to control the optical band gap of the polymer film by selecting an appropriate RF input power in the process of film deposition.

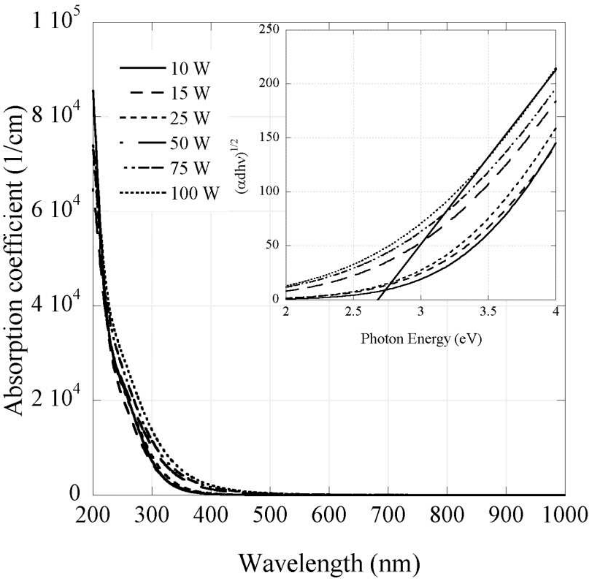

Figure III.2.4. Absorption coefficient of polyterpenol thin films fabricated at different RF input power.

Table III.2.2. Comparison of optical band gap estimates for polyterpenol films fabricated at varied RF input powers determined using UV-vis data, absorption coefficient data from the modeling of optical constants (*n, k*), and by fitting a Tauc-Lorenz oscillator model to the ellipsometric data (*Ψ, Δ*).

Power	Optical band gap eV		
	UV-vis	Absorption coefficient	Tauc-Lorenz
10	2.93	2.95	2.95
15	-	2.88	2.9
25	2.82	2.82	2.83
50	2.73	2.78	2.81
75	2.68	2.71	2.7
100	2.64	2.64	2.64

Figure III.2.5 and Figure III.2.6 demonstrate the effect of deposition power on the refractive index and extinction coefficient profiles of polyterpenol films. The overall shape of the curves was similar across all the samples. Below 250 nm, higher values of refractive index *n* corresponded to the lowest level of RF power which can be explained by the lower level of confidence pertinent to this region. However, above 250 nm, an increase in RF input power resulted in higher refractive index *n* values for the polymer. At 500 nm, the refractive index increased from 1.537 for 10 W to 1.544 for 25 W to 1.554 for 100 W. Similarly, the extinction coefficient values seemed to increase with higher RF power, with the exception of values corresponding to 10 W polymer samples. For all thin film samples, the magnitude of extinction coefficient *k* approached zero above 650 nm. Mean squared error (*MSE*) value varied slightly with the amount and quality of the data available, and the precision with which the chosen model described the film sample; MSE values of below 3 were obtained in the modeling process. It is therefore possible to control the values of optical indices *n* and *k* by manipulating the power of the excitation signal during the deposition process. A brief summary of material properties of polyterpenol thin films deposited at a range of RF input powers is presented in Table III.2.3.

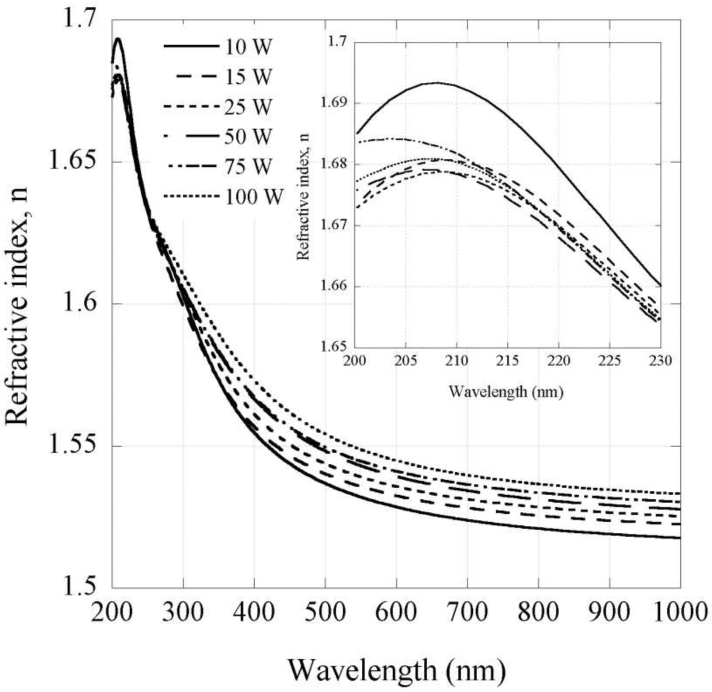

Figure III.2.5. Refractive indices of polyterpenol thin films manufactured at different RF powers as a function of wavelength.

Transmission spectra for the thin films were similar in shape and diverged slightly in the lower wavelength region (300-500 nm), which was consistent with *k* profiles for these film samples; otherwise, the transmission spectra curves were similar in shape indicating the RF deposition power had no significant effect on the transparency of the resulting polymer. Hence, by controlling the magnitude of the excitation signal, it is possible to manufacture coatings with different surface, chemical and physical properties while maintaining the transparency of the films. As previously discussed, polyterpenol samples deposited at 100 W displayed highest hardness and chemical and thermal stability [347], and as such can be successfully used as protective transparent coatings in organic optoelectronics. Films deposited at 10 W, however, have shown most biological activity due to preservation of original monomer functional groups [200], and therefore can be applied as transparent antibacterial and antifouling layers in intraocular contact lenses.

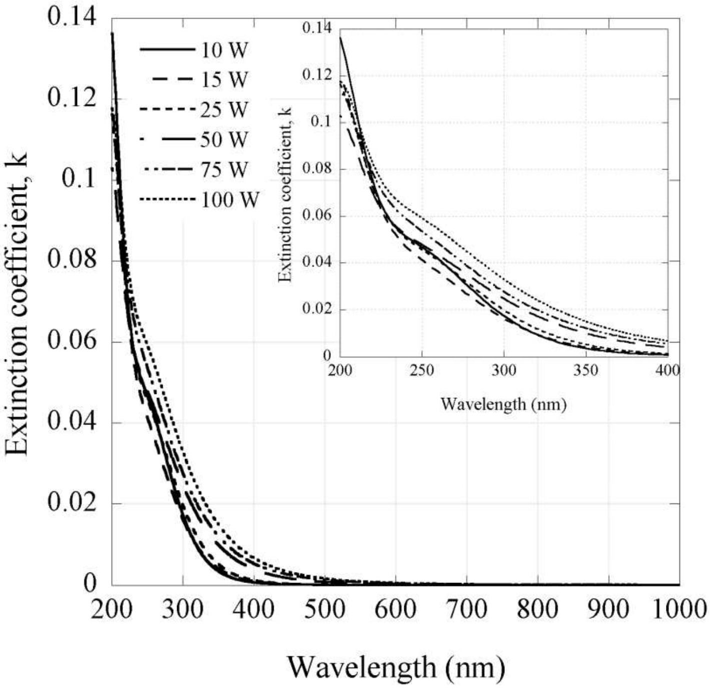

Figure III.2.6. Extinction coefficient of polyterpenol thin films fabricated at various RF powers as a function of wavelength.

Table III.2.3. Summary of properties of polyterpenol samples as a function of RF input power.

Power W	Thickness nm	Roughness nm	*n* at 500 nm	*k* at 500 nm × 10-5	E_g eV	Hardness GPa	WCA °
10	1151	0.44	1.537	2.67	2.95	0.32	60.29
15	1140	0.4	1.540	6.95	2.90	0.34	60.98
25	1045	0.4	1.544	8.25	2.83	0.35	62.48
50	730	0.35	1.548	75.01	2.81	0.42	64.40
75	700	0.33	1.550	130.03	2.70	0.49	67.04
100	593	0.33	1.554	160.74	2.64	0.51	75.18

III.2.4 Conclusion

Exposure to RF plasma field notably altered the chemical structure of the monomer, with polymer films retaining only some of the original monomer functionalities. Lower RF deposition powers favoured preservation of the high polarizability moieties, such as hydroxy group, and therefore was found to be more appropriate for fabrication of insulating films. The optical band gap values declined with increase in RF deposition power, from 2.95 eV for 10 W to 2.64 eV for 100 W, whilst the refractive index increased slightly. Films deposited at higher RF power were found to be harder due to the increase in the density and degree of cross-linking within the polymer thin film. Films also became less hydrophilic as the deposition power increased due to the reduction in the oxygen-containing functionalities. However, RF power had no significant effect on polyterpenol roughness or transparency, with all the samples characterized as smooth, defect-free, homogenous transparent films. Hence, RF power can be used as an effective control for fabrication of smooth and transparent organic polymer films with desired surface, chemical and physical properties with intended applications in electronics and biotechnology.

CHAPTER IV

SURFACE CHARACTERISATION

As a part of an electronic device or as a bioactive coating, the mechanical and morphological properties of polyterpenol influence the nature of application, performance and longevity of these materials. The morphology of the surface affects the wetting behaviour of the solid film, the latter being an important consideration when considering biomedical prospects of these materials. Similarly, the morphology of an insulating gate dielectric layer may affect the assembly and resultant conduction behaviour of the active layer within an organic electronic device. Therefore, in order to determine potential applications for polyterpenol thin films, and to optimise such a material for desired applications, an understanding of the relationship between the fabrication parameters and the resultant surface and mechanical properties of the plasma polymer is required.

In this chapter, mechanical and tribological properties of polyterpenol thin films fabricated on a range of common substrates at varied deposition power are outlined. Film hardness, elasticity, coating adhesion and susceptibility to wear are examined using nanoindentation and nanoscratch analyses. Surface architecture is examined in detail using images obtained by means of atomic force microscopy. The outcomes of these studies are reported in *Bazaka, K., Jacob, M. V., Nanotribological and nanomechanical properties of plasma polymerised polyterpenol thin films. Journal Materials Research 2011, 26, 2952-2961.*