Lipid Vesicle Interactions with Plasma Polymers

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Abstract

Cell membrane models, such as supported lipid bilayers (SLBs) and adsorbed vesicle layers, have provided researchers with platforms to study a variety of processes, including cell signalling and drug-membrane interactions. Many surfaces do not facilitate vesicle adsorption and/or collapse and complex interactions at the interface are often poorly understood. Furthermore, the ability of such models to reproduce complex membrane architecture on both a micro- and nanoscale is limited. Plasma polymerisation provides a versatile, one step, dry method of creating thin films of different chemistries on almost any substrate. Successful lipid vesicle adsorption and collapse on such coatings would be beneficial for creating complex model platforms using patterned surface chemistries.

In this thesis, plasma polymers of varying chemistry were fabricated from acrylic acid (ppAAc), allylamine (ppAAm), 1,7-Octadiene (ppOct) and allyl alcohol (ppAAle) monomers and the dependence of film properties on substrate location during deposition was investigated. Contact angle, X-ray photoelectron spectroscopy (XPS), ellipsometry and atomic force microscopy (AFM) were used to characterise physiochemical properties of the films. Quartz crystal microbalance with dissipation (QCM-D) showed a dependence of swelling behaviour for ppAAc films on reactor position and electrochemical impedance spectroscopy (EIS) revealed different layered structures for ppAAc and ppAAm films depending on pH and ionic strength of the aqueous medium. The interaction of DOPC lipid vesicles with the plasma polymer films was investigated using QCM-D and fluorescence recovery after photobleaching (FRAP) techniques. pH was used to control electrostatic interactions and thus lipid structure at the surface of ppAAc films. ppAAm enabled vesicle adsorption at all pH levels investigated.

Lipid vesicle interactions with micropatterned dual ppAAm/ppAAc chemistries were also explored. The patterns were fabricated using standard photolithography techniques and physical characterisation was undertaken using AFM, scanning electron microscopy (SEM) and optical profilometry techniques. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) with multivariate analysis was used to confirm spatially controlled dual plasma polymer chemistries at the microscale. By using pH and a ppAAm/ppAAc patterned surface it was demonstrated that lipid position, structure and mobility could be spatially controlled using surface chemistry.
Finally, standard electron beam lithography (EBL) techniques were used to create dual ppAAm and ppAAC nanopatterns on both glass and silicon wafer substrates. Pattern resolution and quality was assessed using optical profilometry, SEM and AFM, which revealed line widths of 50-60 nm in size. Auger electron spectroscopy (AES) showed elemental differences between the patterned features greater than ~150 nm in size whilst high resolution ToF-SIMS imaging revealed differences in patterned regions for line widths sub 100 nm in size. These dual nanopatterns show great promise for a variety of applications including the development of complex, nanostructured cell membrane platforms.
This thesis is dedicated to my parents who have provided unwavering love and support over the last 26 years. Thank you.
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Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma and to the best of my knowledge contains no material previously published or written by another person except where due reference is made. Where the work is based on joint research or publications the relative contributions of the respective workers or authors is disclosed.

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1 Introduction

Today, engineering plays a key role in enabling researchers to mimic nature and deepen our understanding of complex natural systems. One such system is the cell membrane which encases and protects cellular components and plays an important role in transport, signalling and immune responses. These functions are facilitated by the complex organisation of membrane components including proteins, carbohydrates and lipids in a structure known as the phospholipid bilayer. Much is still unknown about membrane function due to the complexity of the environment and the vast number of components. Added challenges include the invasive nature of methods used to extract natural membrane architectures and the variability of the membranes from cell to cell. This has increased the demand for reliable membrane mimics that can provide a stable and reproducible platform for investigating membrane phenomena.

The supported lipid bilayer (SLB) is a widely used artificial membrane system that consists of a single lipid bilayer supported on a solid substrate. These systems can be formed using a range of techniques including lipid vesicle collapse. Traditionally, vesicle collapse has been achieved on a limited number of surfaces such as SiO$_2$, glass and mica. However, polymer cushion systems and conductive surfaces are desirable for sensing and cell interaction studies. Many approaches are also being used to pattern SLBs at both the micro- and nanoscale to enable recreation of the complex architectures that occur in native membranes.

A key challenge in the creation of SLBs is that the range and interplay of interactions that control vesicle adsorption and collapse are still not fully understood. In particular, electrostatic interactions are known to be a major driver (and inhibitor) of vesicle collapse with the substrate material, surface charge density and lipid charge all playing an important role. Buffer properties such as pH, chemistry and divalent ions are also known to be crucial in mediating interactions between the lipid vesicles and support surface.

The purpose of this thesis was to investigate the use of plasma polymer thin films as new surfaces for the development of increasingly complex supported lipid bilayer systems. The films can be deposited on almost any solid substrate in a one-step, dry process using a variety of monomers and deposition parameters to tune physiochemical properties. Plasma polymers can also be patterned to form micro- and nanoscale
chemical and topographical features.\textsuperscript{[20-23]} It is therefore surprising that literature exploring the interactions of lipid vesicles with plasma polymers is very limited.\textsuperscript{[24, 25]}

Therefore, the overall aims of this thesis were:

- To investigate plasma polymers of varying surface chemistry as substrates for cell membrane model systems, such as vesicle layers and SLBs.
- To develop and use micro- and nanopatterned plasma polymer surfaces as substrates to spatially control lipid structure and mobility.

To achieve these aims, the first stage of the project was to fabricate plasma polymer films with different surface chemistries. In order to understand how lipid vesicles may interact with the films, it was also necessary to perform detailed characterisation of the physiochemical properties of the films. As described in Chapter 4, characterisation was performed using a number of techniques, including quartz crystal microbalance with dissipation (QCM-D) and electrochemical impedance spectroscopy (EIS). These techniques enabled \textit{in situ} monitoring of plasma polymer behaviour in response to changes in ionic strength and pH of the surrounding aqueous solution.

The next stage was to investigate the interaction of lipid vesicles with plasma polymers of varying surface chemistry (Chapter 5). Given the importance of electrostatic interactions in the vesicle adsorption and collapse process, surface chemistry and pH were explored as tools for controlling vesicle interactions. Lipid vesicle interactions with gold surfaces were also investigated in Chapter 5. Gold substrates are important for the development of sensing systems and facilitate the use of characterisation techniques such as surface plasmon resonance (SPR). In this work the effect of buffer composition and pH on vesicle adsorption and collapse at gold surfaces was explored.

Chapter 6 describes the characterisation of plasma polymer micropatterns fabricated using photolithography and lipid vesicle interactions with patterned surfaces. Physiochemical characterisation of the patterned surfaces was important in order to understand how lipid vesicles may interact with the surface. Physical attributes of the patterns were characterised using optical profilometry, scanning electron microscopy
(SEM) and atomic force microscopy (AFM). Time-of-flight secondary ion mass spectrometry (ToF-SIMS), combined with multivariate analysis techniques, were used to spatially map chemically distinct regions on the patterned surfaces. Lipid vesicle interactions with micro-patterned surfaces of plasma polymerised acrylic acid and allylamine were then investigated using confocal scanning laser microscopy (CLSM) and fluorescence recovery after photobleaching (FRAP). The effects of patterned substrate chemistry and topography on lipid vesicle interactions are discussed.

Finally, in Chapter 7, the use of electron beam lithography (EBL) was explored for the fabrication of dual plasma polymer patterns at the sub-micron scale. Chemical patterning is useful for a variety of applications including the development of nanoscale membrane architectures in model systems. In this chapter the challenges of chemical characterisation at the nanoscale are highlighted and the use of auger electron spectroscopy (AES) and high spatial resolution ToF-SIMS imaging are explored as tools for chemical mapping of nanopatterned plasma polymers.
1.1 References


2 Literature review

2.1 The structure and function of the cell membrane

It was once thought the cell membrane was a passive barrier for encasing cellular components. In 1972, Singer and Nicholson proposed the fluid mosaic model. This model described the membrane as a 2D fluid within which membrane components were mobile. The main constituents of cell membranes are lipids and proteins organised into a bilayer structure composed of an upper and lower leaflet. These 3-5 nm thick membranes control transport of molecules in and out of the cell whilst playing important roles in cell signalling, cell-cell interactions and the regulation of inner cellular processes. The bilayer structure of the membrane contains three major classes of lipid: phospholipids, sphingolipids and sterols. Each exhibits amphipathic characteristics (hydrophilic head region and hydrophobic tail region) which enable orientation into a bilayer formation. Cholesterol is the main sterol found in mammalian cells. It plays an important structural role within the membrane and is thought to interact with phospholipids and sphingolipids to enable membrane function. Three classes of protein are partially or fully embedded within the bilayer structure. Integral proteins are linked permanently to the membrane by hydrophobic domains, peripheral proteins are bound via non covalent interactions (e.g. hydrophobic and electrostatic interactions) and lipid-anchored proteins are attached via fatty acids.

The difference between the diffusion coefficients of membrane components in native plasma membranes and their artificial counterparts suggests that specific organisation within the membrane acts to inhibit or slow diffusion. Proteins and lipids within artificial membranes have been observed to exhibit greater diffusion rates (by a factor of 5-100) than those within natural cell membranes. These observations propose that the motion of membrane components in natural cell membranes cannot be governed by Brownian motion alone. Indeed this behaviour does not comply with the two-dimensional continuum fluid mosaic model of Singer and Nicholson. The explanations for this discrepancy have been varied. It is now thought that the membrane is split into compartments with sizes from tens to hundreds of nm. Fujiwara et al. explored the organisation and structure of membrane compartments with regards to lipids. Actin contained in the membrane skeleton was found to be a main component
in compartment formation with no involvement from extracellular matrix, cholesterol-enriched rafts or the extracellular domains of membrane proteins.\[^{6}\] Lipid compartmentalisation was also observed by Murase \textit{et al.} in a variety of cell types suggesting a universal presence in mammalian cells. Boundaries were described as analogous to a fence with anchored proteins acting as ‘pickets’ and the membrane skeleton as the ‘fence’.\[^{7}\] Biologically, compartmentalisation could play an important role in separating or grouping membrane components and regulating and/or containing extracellular signalling.\[^{7}\]

In addition to compartmentalisation, other architectures exist within the phospholipid bilayer and thousands of different lipid species are present in a single membrane.\[^{3}\] This suggests that the role of lipids is more than a structural one, especially as it is well known that bilayer structures can be formed using a single lipid species.\[^{9}\] In order to understand the role of lipids in membrane function, a variety of theories have been developed. An important concept involves the formation of microdomains or rafts. These ‘small (10–200 nm), heterogeneous, highly dynamic, sterol- and sphingolipid-enriched domains’\[^{10}\] are thought to control signalling proteins by dispersing to prevent communication, or by clustering to activate several proteins simultaneously.\[^{11}\] Difficulties in extracting and analysing small membrane features meant that the raft theory became controversial.\[^{4}\] Advances in the past 10 years have revealed the importance of raft formation in immune responses such as T-cell signalling, disease processes (e.g. budding of the HIV virus from host cell membranes) and membrane transport.\[^{12}\]

Common techniques used to extract membrane components or domains, such as cold detergent or mechanical disruption, are highly invasive.\[^{13}\] Added to this is the sheer complexity of the membrane and the differences in observed architecture and behaviour from cell to cell. These factors have made the study of membrane processes very difficult and has created demand for an artificial membrane capable of simulating normal membrane functions. An effective membrane mimic should be:

- **Stable**: stability of the model will affect the processes which may be studied and also both the storage and distribution of such a system affecting overall cost.
- **Reproducible**: the model structure must be reproducible so that the effect of systematic changes can be investigated.
- **Flexible**: the model must enable tailoring of membrane composition and architecture to enable many processes to be simulated.
- **Functional**: the membrane should be capable of supporting a variety of membrane components whilst retaining their normal function.
- **Scalable**: if the model is difficult or time consuming to manufacture costs will be high.

### 2.2 Supported lipid bilayers (SLBs)

Supported lipid bilayers (SLBs) provide a model system that enables precise control of the membrane structure and complexity in order to study specific functions and interactions. In their simplest form, SLBs consist of a bilayer formed from a single lipid species that is separated from the planar substrate by a thin film of water. This water film typically varies between 5-10 Å in thickness, however, the film properties are highly dependent on both surface and membrane properties and greater values have been quoted. A key feature of SLBs is that the bilayer is not covalently attached to the substrate, preserving lateral mobility of the membrane. Electrostatic, hydration and long range van der Waals forces all play a part in coupling the bilayer to the substrate surface.

#### 2.2.1 Manufacturing a supported lipid membrane

Supported lipid membranes can be manufactured using a variety of methods. Two of the most common techniques are Langmuir-based approaches (Langmuir-Blodgett and Langmuir-Schäfer) and vesicle collapse. Traditional Langmuir-based approaches use the monolayer organisation of lipid molecules at the air-liquid interface. In the two-step process, both stable and reproducible bilayers can be formed by passing a hydrophilic substrate through the interface. Once the initial monolayer is attached to the substrate, a second monolayer can be applied by either dipping the substrate vertically back through the interface (Langmuir-Blodgett) or pushing the substrate horizontally through the interface (Langmuir Schäfer).

Vesicle collapse, as pioneered by McConnell, is one of the most common approaches to form supported lipid bilayers. Typically, small unilamellar vesicles (SUVs) are manufactured by first mixing lipid powder with an organic solvent such as chloroform.
The solvent is left to evaporate, leaving a lipid cake that can be hydrated in water or an appropriate buffer solution. Hydration yields a milky solution of multilamellar vesicles of varying size. Unilamellar vesicles with controlled size can then be formed using sonication or mechanical extrusion techniques.\[20\] Upon introduction to the substrate the vesicles spontaneously collapse, forming a bilayer.\[21\] As described in the following sections vesicle collapse is a complex process governed by a number of factors.

2.2.2 Mechanism of vesicle collapse

The mechanism involved in the process of vesicle collapse is highly complex. The first stage leading to rupture involves adsorption of vesicles onto the substrate. This process is driven by several interactions involving van der Waals and electrostatic forces amongst others.\[22\] Sometimes vesicles remain adsorbed without collapsing into a bilayer structure\[23\] or may not adsorb to the surface at all.\[21\] In cases where rupture does occur, the process may take place via different mechanisms including immediate rupture of single vesicles, fusion of adsorbed vesicles, presence of a free bilayer edge and vesicle-vesicle interactions.\[21\] Competition between the curvature energy of the vesicle (related to the bending rigidity) and the adhesion energy to be gained from the surface (related to the adhesion area) is an important factor involved in vesicle adsorption and collapse.\[21, 24\] In a typical two-stage bilayer formation process, as observed on SiO\(_2\) by quartz crystal microbalance with dissipation (QCM-D), vesicles will first adsorb to the substrate until a ‘critical vesicular coverage’ is met, before a rupture cascade is then initiated.\[25\] The presence of this critical concentration suggests that vesicle-vesicle interactions are important, although they are not necessarily a requirement on all surfaces. Critically, the literature clearly demonstrates that vesicle collapse is driven by a range of interactions.

2.2.3 Factors affecting vesicle adsorption and collapse

A number of factors have been found to influence both the structure formed on the substrate and the kinetics of bilayer formation. These include temperature, osmotic stress, buffer chemistry, surface chemistry, and vesicle composition.\[21, 25\] The major parameters are summarised in Table 2.1.

In particular, electrostatic interactions are known to be a major driver (and inhibitor) of vesicle collapse. The substrate,\[25, 26\] surface charge density,\[27\] and lipid charge\[23\] all play important roles in electrostatic interactions, however, buffer properties such as
pH\textsuperscript{[28-30]} buffer chemistry\textsuperscript{[27]} and divalent ions\textsuperscript{[31, 32]} have also emerged as useful tools for influencing surface-vesicle interactions. In particular, the lowering of pH to reduce electrostatic repulsion at the surface has been used to enable vesicle collapse on substrates such as maleic acid copolymer cushions\textsuperscript{[30]} and TiO\textsubscript{2}.\textsuperscript{[28]} The role of buffer chemistry and the counterions formed in solution is also of great importance. This was demonstrated by Cha \textit{et al.} who observed that larger molecular ions at a surface, such as HPO\textsubscript{4}\textsuperscript{2-} and H\textsubscript{2}PO\textsubscript{4}\textsuperscript{-} from phosphate-buffered saline (PBS), inhibited zwitterionic vesicle rupture at a –NH\textsubscript{3}\textsuperscript{+} terminated SAM surface by increasing entropic repulsion.\textsuperscript{[27]} The role of other ions in solution is also important. It is well known that ions such as Ca\textsuperscript{2+} can act as ‘bridges’ between negatively charged lipids and surfaces.\textsuperscript{[31]} This has been used for forming SLBs using anionic lipids on surfaces which do not normally support vesicle collapse such as TiO\textsubscript{2}\textsuperscript{[33]} and plasma polymerised maleic anhydride (ppMA).\textsuperscript{[34]}
<table>
<thead>
<tr>
<th>Variable</th>
<th>Effect</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vesicle properties</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Conc. of vesicles in solution</td>
<td>At low concentrations critical surface coverage may not be achieved reducing vesicle-vesicle interactions and bilayer formation (depending on the surface).</td>
<td>[21, 35]</td>
</tr>
<tr>
<td>- Vesicle size</td>
<td>Larger vesicles deform more towards the surface.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Does not affect vesicle adsorption and bilayer formation kinetics.</td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[36]</td>
</tr>
<tr>
<td>- Lipid charge</td>
<td>Lipid charge will affect electrostatic interactions. For example, a negatively charged surface and a positively charged lipid will form favourable electrostatic interactions.</td>
<td>[9]</td>
</tr>
<tr>
<td><strong>Buffer properties</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- pH</td>
<td>Dependent on charge of lipid and surface. For example, low pH has been used to promote collapse on TiO₂.</td>
<td>[28, 30, 37]</td>
</tr>
<tr>
<td>- Ionic strength</td>
<td>Increased ionic strength promotes charge shielding of both lipid and surface charges.</td>
<td></td>
</tr>
<tr>
<td>- Temperature</td>
<td>Lowers critical vesicular coverage expediting bilayer formation on surfaces that enable bilayer formation.</td>
<td>[25]</td>
</tr>
<tr>
<td>- Osmotic effects</td>
<td>Lowers critical vesicular coverage needed for collapse. The effect is most pronounced when there is a high concentration of NaCl outside the vesicle.</td>
<td>[25, 38]</td>
</tr>
<tr>
<td>- Size of counterions at surface</td>
<td>Large molecular counterions can increase entropic repulsion at the surface.</td>
<td></td>
</tr>
<tr>
<td>- Divalent ions such as calcium</td>
<td>Charge masking and electrostatic bridging.</td>
<td>[22, 31, 32]</td>
</tr>
<tr>
<td><strong>Substrate properties</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Surface chemistry</td>
<td>Functional groups, charge, charge density and surface energy all play an important role in interactions at the surface, in particular, influencing electrostatic interactions together with the other properties listed above.</td>
<td>[25-27, 39]</td>
</tr>
<tr>
<td>- Surface roughness</td>
<td>Affects bilayer spreading, edge features have been shown to contribute to, or induce, vesicle deformation and rupture (dependent on surface, feature and vesicle size.</td>
<td>[25, 40-45]</td>
</tr>
</tbody>
</table>
2.3 Substrate-dependent effects

Traditionally, vesicles have collapsed successfully on a limited number of surfaces such as SiO$_2$,$^{[25]}$ glass,$^{[37]}$ and mica.$^{[9]}$ As well as SLBs, several other outcomes such as monolayers, vesicle layers, hybrid layers and lipid-vesicle free zones can be created on different treated and untreated substrates. A range of surfaces examined for SLB formation have been summarised in Table 2.2. As previously mentioned, vesicle-substrate interactions are complex and in many cases experimental conditions such as pH or buffer chemistry must be carefully optimised to achieve the desired vesicle interactions.

Table 2.2 Lipid structures formed on a variety of substrates and coatings.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Outcome</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiO$_2$</td>
<td>SLB</td>
<td>$^{[23]}$</td>
</tr>
<tr>
<td>Glass</td>
<td></td>
<td>$^{[37]}$</td>
</tr>
<tr>
<td>Mica</td>
<td></td>
<td>$^{[9]}$</td>
</tr>
<tr>
<td>Gold</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxidised gold surface</td>
<td>Vesicular layer</td>
<td>$^{[39, 46]}$</td>
</tr>
<tr>
<td>Flame annealed</td>
<td>SLB</td>
<td>$^{[47]}$</td>
</tr>
<tr>
<td>Au(111) electrodes (flame annealed)</td>
<td></td>
<td>$^{[48]}$</td>
</tr>
<tr>
<td>TiO$_2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low pH</td>
<td>SLB</td>
<td>$^{[28]}$</td>
</tr>
<tr>
<td>pH 7</td>
<td>Vesicular layer</td>
<td>$^{[25, 28]}$</td>
</tr>
<tr>
<td>Cellulose</td>
<td>SLB</td>
<td>$^{[49]}$</td>
</tr>
<tr>
<td>Plasma-oxidised polydimethyl siloxane (PDMS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>Monolayer</td>
<td>$^{[50, 51]}$</td>
</tr>
<tr>
<td>Plasma-oxidised</td>
<td>SLB</td>
<td></td>
</tr>
<tr>
<td>n-octadecyltrichlorosilane (OTS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>Monolayer</td>
<td>$^{[52]}$</td>
</tr>
<tr>
<td>UV-exposed</td>
<td>SLB</td>
<td></td>
</tr>
<tr>
<td>Trehalose glass</td>
<td>SLB</td>
<td>$^{[53]}$</td>
</tr>
<tr>
<td>Indium tin oxide (ITO)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anionic vesicles and Ca$^{2+}$ ions</td>
<td>SLB</td>
<td>$^{[54]}$</td>
</tr>
<tr>
<td>Anionic vesicles, no Ca$^{2+}$ ions</td>
<td>Vesicular layer</td>
<td></td>
</tr>
<tr>
<td>Cationic vesicles and Ca$^{2+}$ ions</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.3.1 Gold

The ability to form cell membrane models on gold is useful for applications such as biosensing, but also enables the use of characterisation techniques requiring a gold substrate (e.g. surface plasmon resonance). Lipid interactions with gold are highly dependent on substrate preparation and buffer conditions. Despite the electrostatic potential of gold it is known that vesicles typically interact to form a stable vesicular layer without rupture.\textsuperscript{39, 46} To overcome this limitation the gold surface can be chemically modified using self-assembled monolayers (SAMs)\textsuperscript{59} or tethered thiolipid systems.\textsuperscript{60} Although successful, these modifications add complexity to substrate preparation and have only one mobile leaflet.

The Lipkowski group have undertaken extensive studies exploring the deposition of supported lipid bilayer systems on Au(111) electrodes.\textsuperscript{43, 61-64} Flame annealing of the gold surface and removal of chloride ions from the buffer solution were identified as major requirements for successful vesicle collapse.\textsuperscript{48} Flame annealing removes organic contaminants whilst flattening the gold surface, creating atomically flat terraces.\textsuperscript{43, 65} Chloride ions have a high affinity for gold, modifying the surface with chlorine atoms and changing the lipid/surface affinity.\textsuperscript{48, 66} In an alternative approach, Peng et al. fabricated nanogratings coated in gold which enabled vesicle rupture at the grating edges.\textsuperscript{44}

Whilst these approaches are successful, flame annealing of gold may not be practical for every substrate, such as delicate sensors (i.e. QCM-D crystals). Furthermore, the removal of certain buffer components such as chloride ions may mean the environment is no longer biologically relevant. This is particularly the case for some experiments on
Au(111) electrodes where the buffer used is NaF at pH 9.0, although Marques et al. has demonstrated SLB formation on flame annealed gold in HEPES buffer (without NaCl) at pH 7.4. The use of nanotopography for inducing rupture is promising however, such surfaces may not be suitable for every application, require a specialised topography or fabrication method and may not be commercially viable.

2.3.2 Plasma polymers as surfaces for model cell membranes

Plasma polymer deposition typically involves the introduction of an organic monomer, in vapour form, to a reaction chamber under vacuum at room temperature. A glow discharge is then generated by applying an electric field at radio frequency (RF), which ionises the organic vapour creating electrons, atoms, molecules, free radicals, positive and negative ions and photons (visible and UV range). Deposition can occur through a number of mechanisms, including the formation of reactive species and free-radicals at the substrate surface which react and polymerise with reactive species from the gas phase, enabling film growth at the substrate surface. The resulting film, unlike a conventional polymer, is a highly cross-linked network consisting of a variety of non-repeating chemical species. Plasma polymerisation therefore provides a versatile, one step, dry method for creating conformal thin films (which replicate the topography of the underlying substrate) of different chemistries on almost any substrate which has made them ideal candidates for use in many biomedical applications.

A wide range of monomers such as allylamine, 1,7-Octadiene, acrylic acid and allyl alcohol or combinations of monomers have been used in plasma polymer deposition. Optimisation of processing parameters such as those listed in Table 2.3, is crucial in determining physiochemical properties of the final plasma deposited film. Power is often used to manipulate the final physiochemical properties of film, as increasing power typically induces increased fragmentation of the monomer, producing more cross-link dense films. However, there is a trade-off because increasing fragmentation leads to reduced functional group density. One approach to overcome this limitation has been the use of pulsed discharge deposition.

With such a large range of parameters, one of the limitations of plasma polymer films is reproducing film characteristics from one laboratory to another. Plasma polymerisation setups are typically custom built and, even when using the same parameters such as discharge power and flow rate, other parameters such as reactor geometry mean the films must be fully characterised for each reactor.
As described later in this review, the spatial control of plasma polymer chemistries by patterning on both a micro- and nanoscale make plasma polymers versatile platforms for the development of increasingly complex membrane model systems. It is therefore surprising that the number of studies concerned with the use of plasma polymers as substrates for cell membrane model systems is limited. Plasma polymerised maleic anhydride (ppMA) has previously been used as a support for lipid bilayer system by vesicle fusion. ppMA has also been used to immobilize vesicles for a smart wound dressing for pathogenic bacteria detection and to study membrane-lysing toxins.

Table 2.3 Plasma polymer processing parameters.

<table>
<thead>
<tr>
<th>Processing parameter</th>
<th>Effect</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monomer</td>
<td></td>
<td>[77, 90]</td>
</tr>
<tr>
<td>Flow rate</td>
<td>Deposition rate, chemistry</td>
<td>[91]</td>
</tr>
<tr>
<td>Vapour pressure</td>
<td>Deposition rate</td>
<td>[92]</td>
</tr>
<tr>
<td>Non-polymerisable carrier gas (i.e. Ar)</td>
<td>Lower monomer fragmentation and higher retention of functional groups</td>
<td>[82, 83, 84, 85]</td>
</tr>
<tr>
<td>Discharge</td>
<td></td>
<td>[74, 91, 93]</td>
</tr>
<tr>
<td>Power</td>
<td>Chemistry, cross-linking and stability, deposition rate</td>
<td>[82, 83, 84, 85]</td>
</tr>
<tr>
<td>Continuous wave mode or pulsed</td>
<td>Pulsed plasma polymers have reduced fragmentation and retain greater functionality, deposition rate</td>
<td>[95, 96]</td>
</tr>
<tr>
<td>Reactor and electrode geometry</td>
<td>Chemistry, glow, film chemistry and thickness</td>
<td>[97]</td>
</tr>
<tr>
<td>Substrate</td>
<td></td>
<td>[98]</td>
</tr>
<tr>
<td>Material</td>
<td>Film stability and adhesion</td>
<td>[99]</td>
</tr>
<tr>
<td>Roughness</td>
<td>Chemistry</td>
<td>[100]</td>
</tr>
<tr>
<td>Position in reactor</td>
<td>Chemistry, film thickness</td>
<td>[100]</td>
</tr>
<tr>
<td>Temperature</td>
<td>Deposition rate (dependent on other processing parameters)</td>
<td>[100]</td>
</tr>
</tbody>
</table>
2.4 Patterned Supported Lipid Bilayers

2.4.1 Why pattern a bilayer?
A large number of techniques are now being used to tailor membrane structure on both a micro and nanoscale. The control of model membrane architecture is vital if truly complex membrane platforms are to be realised. Fortunately, the planar substrate provides an ideal template for both physical and chemical patterning. However, the small scale (nm) at which various membrane architectures form in cells mean the task of mimicking such environments is far from trivial. Aside from issues related to resolution (which also affect the choice of characterisation methodologies) other challenges arise from problems related to the air-liquid interface. The bilayer must remain submerged at all times to avoid destroying the membrane structure. Further difficulties relate to the use of very small volumes of solutions in some patterning processes which are susceptible to evaporation. These requirements must be considered before development of a successful patterning methodology.

2.4.2 Chemical patterning of the substrate
Manipulation of the substrate prior to bilayer formation eliminates any complications associated with preserving the integrity of the membrane structure during patterning. Two-part systems consisting of a patterned substrate and vesicle solution also enable components to be separated, increasing storage periods. Deterioration of the lipid or vesicle solution will not affect the substrate thus reducing both waste and cost. Flexibility is also introduced as the user is able to tailor lipid solutions to match the substrate properties. Reliance of such systems on vesicle collapse provides a simple process for bilayer formation when considering single or double lipid systems, however, collapse of vesicles with complex compositions and proteins has proved challenging.

To create compartmentalised bilayers, barriers are often used to restrict lateral movement of membrane components. Barriers can be formed from materials resistant to vesicle collapse such as aluminium oxide, fibronectin and bovine serum albumin (BSA). Patterning of protein barriers is of particular interest as they enable cell anchorage whilst effectively constraining and separating areas of fluid bilayer. Both fibronectin and BSA have been patterned using microcontact printing (µCP) to form defined bilayer regions with minimum dimensions of 40 and 25 µm, respectively.
Aluminium lift-off techniques (in conjunction with photolithography or electron beam lithography) have also been implemented to pattern fibronectin providing increased pattern quality when compared with µCP.\cite{103}

Of particular note is the nanoscale patterning of SLBs achieved using atomic force microscopy (AFM)-based nanoshaving where the tip is used to selectively remove material at the surface. Lines of bilayer, as small as 55 nm in diameter, were formed by nanoshaving a BSA monolayer on a borosilicate substrate and backfilling the glass areas using vesicle fusion. An attempt to form line widths of 36 nm was unsuccessful even when using smaller vesicles (~34 nm) or generating increased osmotic stress across the vesicle wall.\cite{56} This technique holds great promise for achieving high resolution membrane patterning in a field where much of the patterning is on the microscale.

Other materials enable vesicle attachment and/or vesicle collapse but inhibit diffusion via different mechanisms. Chrome is a highly hydrophilic material and acts as a diffusion barrier by strongly adsorbing vesicles and selectively immobilising lipid material.\cite{58} This effect has been exploited to create mazes of chromium lines (100-200 nm in width) for cell attachment studies, enabling the clustering of proteins on T-cells to be monitored.\cite{104} ITO exhibits similar behaviour. The hydrophilic surface enables vesicle fusion and collapse, however, the resulting bilayer is immobile.\cite{105} ITO also has the added advantages of being both transparent and conductive.

Some materials exhibit a range of behaviours dependent on specific preparation or cleaning protocols, which can be exploited when patterning. Gold is particularly useful in the patterning field as it provides a conductive surface suitable for sensor applications. Oxidised gold is known to strongly adsorb to vesicles but not enable bilayer formation.\cite{39} Despite this, both monolayers\cite{62} and bilayers\cite{48} have been observed to form on flame annealed Au(111) electrodes and more recently bilayers were formed on unmodified flame annealed gold.\cite{47} Functionalisation of gold has also proved useful. Substrates have been patterned using glass beads as a mask to form patches of SiO$_2$ (2-3 µm in size) surrounded by gold. Diffusion barriers were then formed by self-assembly of polymerisable thiol lipids onto the substrate. SiO$_2$ areas were backfilled using vesicle fusion to form fluid areas of bilayer.\cite{106} Lipids with a photocleavable moiety and hydrophobic termination have also been patterned using
photolithography to form bilayers on hydrophilic (cleaved) regions and monolayers on hydrophobic (uncleaved) regions.\textsuperscript{[107]}

Like gold, PDMS and OTS also exhibit different lipid-substrate interactions depending on the treatment used. In particular the sensitivity of lipid interactions with hydrophilic and hydrophobic surfaces has been explored. One study demonstrated that untreated PDMS enabled fluid monolayer formation. Low levels of plasma oxidation treatments resulted in lipid resistance, whilst increasing the level of plasma treatment further enabled vesicle collapse and bilayer formation. This property was used to pattern PDMS surfaces using an electron microscopy grid (line width 14 µm, gap width 56 µm) to generate alternate areas of monolayers and bilayers.\textsuperscript{[51]} In a similar manner lipid monolayers and bilayers have been formed on hydrophobic (UV masked) regions and hydrophilic (UV exposed) regions of OTS SAMs.\textsuperscript{[52]} These surfaces have been used to explore the role of disaccharides in protecting bilayers during dehydration.\textsuperscript{[53]}

Lithographic photopolymerization is a further method used to form diffusion barriers. Diacetylene phospholipids (Diyne PC) have been implemented with standard photolithography techniques to form corrals of fluid bilayer 50 µm in size.\textsuperscript{[108]} In a similar manner Poly (bis-SorbPC) can also be used to form cross-linked phospholipid patterns in silica capillaries down to 10 µm in width.\textsuperscript{[109]}

Incorporation of more than one vesicle composition into a patterned bilayer, within defined regions of the substrate, remains challenging particularly when using surface chemistry alone. Manipulation of both substrate surface chemistry and bulk buffer composition has provided a possible answer. Two different liposome compositions have been patterned on alternating regions of SiO$_2$ and TiO$_2$. In the absence of calcium, vesicles were collapsed onto the SiO$_2$ regions with the TiO$_2$ surfaces remaining lipid resistant. In the presence of calcium ions however a second lipid composition was encouraged to collapse on the TiO$_2$ regions.\textsuperscript{[110]}

These examples of substrate patterning demonstrate the huge variety of options available to those wishing to create complex SLB platforms. Despite this there are still many challenges to overcome. One challenge relates to overcoming difficulties associated with the processes and conditions related to the patterning process. In the case of fibronectin for example aluminium lift off has been shown to provide superior pattern quality when compared with µCP.\textsuperscript{[103]} However, a trade-off exists between the two techniques. The aluminium lift-off process is considerably more expensive because
the mask is destroyed each time. Furthermore, the conditions used for mask dissolution are very basic and therefore restrict the proteins and other materials that may be patterned.[103] In contrast, µCP is highly accessible because only a polymer stamp is required which can be fabricated with many designs, used repeatedly and is adaptable to stamping different materials.[111] Limitations include pattern quality[103] and PDMS contamination of the substrate (especially when using patterned stamps).[112]

A further very important challenge relates to the reluctance of complex liposomes and naturally derived membranes to collapse on simple surfaces such as glass, an affect that could be compounded when introducing a patterned surface. One way of overcoming this difficulty has been to use hydrodynamic shear forces to drive a fully formed bilayer towards an adsorbed native liposome within a microfluidic system.[102] Cellulose has also been shown to support selective spreading of native cell membranes and can be patterned using UV photolithography or BSA barriers to spatially control membrane spreading.[49]

2.4.3 Patterning a formed bilayer

Patterning a fully formed membrane by removal of lipid material has provided an alternative method to substrate-based techniques. Such an approach may alleviate complications related to collapsing a complex membrane onto a patterned substrate. Preservation of the SLB structure throughout the patterning process is a major consideration when undertaking this approach.

Polymer lift-off has emerged as a useful method for patterning SLBs. It involves coating the substrate with a polymer such as poly (p-xylene), which is then patterned and etched in selected areas. A bilayer is formed on the full surface of the coated substrate and upon removal of the polymer, the bilayer is left on the remaining etched areas. Lipid bilayers have been patterned down to a few µm using this approach for membrane compartmentalization studies[113] and bacterial toxin detection.[114] Patterns used for cell signalling studies,[115] cellular immunological studies[116] and functionalized membranes for a variety of other applications[117] have reached 1 µm. When used alone, lift-off technologies can only accommodate a single lipid composition or two if the pattern is backfilled with the second lipid.[118] Despite this, it has been noted that polymer lift-off can achieve more reliable pattern uniformity and increased resolution (< 1 µm) when compared with µCP.[116]
Blotting of lipid bilayers can also be implemented to pattern on the microscale. The reverse of µCP, the technique involves using a PDMS stamp to remove bilayer material. This method has been used to create alternating areas of bilayer and protein. A further technique implementing PDMS is the ‘squeegee’ method which uses a PDMS block translated across a microwell substrate. Lipid vesicles not contained within the wells are removed during repetitive translations. Although not strictly patterning of a single bilayer, 100 nm vesicles were squeezed into wells as small as 80 nm in diameter. The arrays were used to monitor ligand binding using neuronal lipid rafts. The squeegee method was also implemented to form arrays of bilayer coated SiO$_2$ beads (700 nm in diameter). All of the above are simple techniques to implement, however, as mentioned before, PDMS contamination must be considered and requires careful use and preparation of the stamp/squeegee.

Removal of lipid material can also be achieved using deep UV photolithography in aqueous conditions. This method of photodegradation involves the use of a physical quartz/chrome mask to expose select areas of the formed bilayer to short wavelength UV light. Following photochemical degradation of the lipids it is thought that hemimicelle formation at the energetically unfavourable edges creates a stable pattern. Backfilling of the lipid voids with a second liposome composition has also been demonstrated however the diffusion barrier did not remain and the two lipids mixed over time (28 mins). This effect may not be desirable for all applications, especially if specific areas of stable bilayer compositions must be maintained. Stable diffusion barriers have also been generated in formed bilayers under aqueous conditions, using focused femtosecond laser pulses. The technique uses a laser to create stable voids down to a diameter of 330 nm. The effect is reversible by re-illumination of the area using high repetition rates which, due to thermal effects, leads to bilayer healing. Similar patterning of spin coated lipid multiplayers (5-25 bilayers thick), with focused lasers, has also been conducted, however, not under aqueous conditions (humidity 40-60%). Using similar techniques line void diameters down to 1.1 µm exhibited stability under water for several hours, however, after 22 hours considerable degradation had occurred.

Finally, careful control of both pH and ionic strength of the bulk aqueous phase has enabled the creation of SLBs with two different vesicle compositions using nanoshaving techniques. Silica substrates patterned with chromium grids were first exposed to
vesicle fusion. Nanoshaving was implemented to remove 1 µm lines of bilayer from the silica surface before a second vesicle composition was introduced by further vesicle fusion.[128]

2.4.4 Direct application of lipid material

The final approach to pattern a lipid bilayer attempts to position lipid material at specific regions on the substrate. This technique avoids difficulties involving vesicle collapse (at least during patterning), however, there are certainly major considerations involving the structure of the bilayer generated and careful control of the patterning environment is required.

On the microscale, bubbles inked with lipid monolayers have been used to form bilayers on specific areas of the substrate using a technique known as bubble collapse deposition (BCD).[129] Empty areas of the substrate could then be backfilled using traditional vesicle collapse techniques. Areas of BCD bilayers showed limited mixing with the surrounding bilayer which was attributed to a region of disordered lipid molecules forming a boundary. However, the resolution of the technique is low (bubble deposited bilayer patches >200 µm).[129] BCD techniques have also been used to form bilayers on alumina.[130] This technique may provide a simple method of creating defined non-mixing areas of different lipid compositions on more challenging surfaces if resolution improves.

µCP has been successfully used to form multi-lipid systems by printing lipids with different transition temperatures. A higher transition temperature lipid was printed in gel-phase and then backfilled using vesicle collapse by a lipid in liquid-phase.[131] In a similar approach, µCP lipid barriers were implemented to contain nonspecific protein binding in 10 µm squares surrounded by 5 µm grids.[120] Simultaneous stamping of different lipids has also been demonstrated with agarose stamps requiring only picomolar amounts of each lipid on each stamp post (minimum diameter 200 µm).[132] In a similar method, simultaneous stamping of lipid multilayers and control of their height has been achieved by inking the stamp using dip pen nanolithography (DPN).[133] These examples exhibit both the versatility and accessibility of the technique which can be used to pattern large areas with high throughput.[3]

Robotic spotting has been used to create lipid membrane microarrays since 2003.[134] Initial challenges involved resolving issues with reproducibility, low resolution and evaporation of low volumes of lipid material.[3, 101] To overcome problems with
evaporation, spotting has been conducted at high humidity (~98%)\textsuperscript{[135]} and by dispensing vesicles onto a wet substrate,\textsuperscript{[136]} although the use of the technique for smaller membrane architectures may be limited.\textsuperscript{[101]} Ink-jet printing could also play a role in the manufacture of high throughput membrane arrays implemented as a method to both produce and deposit drug loaded vesicles (50-200 nm in diameter) for spontaneous bilayer formation.\textsuperscript{[137]} For direct application of bilayer material on a nanoscale, DPN can be used.\textsuperscript{[138]} The method is based on AFM whereby the cantilever tip is inked with lipid material.\textsuperscript{[139]} Phospholipid inks have been deposited at line widths down to 93±18 nm.\textsuperscript{[140]} Such inks form multilayers during deposition and when left in high humidity conditions for 1 hour they have been observed to spread slowly, forming a thin homogeneous layer.\textsuperscript{[140]} For simultaneous patterning of different lipid mixtures a 26-tip array with individual microfluidic wells has been used.\textsuperscript{[141]} The need for careful atmospheric control (especially high humidity) during and after deposition is a drawback of the technique together with low throughput. More recently DPN has been performed under water, thus eliminating the need for careful atmospheric monitoring once deposition is complete and enabling \textit{in situ} observations of the patterns in aqueous conditions.\textsuperscript{[138]} Other advances in the field include combining fluorescence intensity data and one-time AFM height measurements to eliminate the need for time consuming AFM-only height characterisation to improve throughput.\textsuperscript{[139]} DPN has also been used to form lipid multilayer diffraction gratings for optical label-free biosensing applications.\textsuperscript{[142]}

2.4.5 Transient/dynamic patterning and membrane component sorting

The patterning techniques described so far provide effective methods of partitioning the membrane, however, they are limited in their capacity to mimic the dynamic and transient nature of membrane structures. In order to recreate these temporary architectures different solutions have been developed. Perhaps the most commonly explored avenue for dynamic patterning has been the use of electric fields. When combined with SLBs, electrical fields can form systems similar to those of gel electrophoresis, enabling cell components to be separated in an environment similar to that of the membrane. The sensitivity of this approach has been demonstrated by separating isomers of fluorescently labelled lipids.\textsuperscript{[143]} Electric fields have also been used to control the migration of charged lipids\textsuperscript{[144]} and to control motion of tethered vesicles.\textsuperscript{[145]}

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Large alternating currents applied to gold electrodes patterned on silica have irreversibly formed diffusion barriers by electrochemically removing alkanethiol monolayers on their surface.\cite{146} In other work, tangential electric fields have been used to collect proteins at the edge of diffusion barriers which were created by mechanical scratching.\cite{147} Accumulation of proteins has also been achieved by applying AC electric fields to enable collection in topographical nested traps designed to restrict exit diffusion.\cite{148} Other methods of creating dynamic systems have included shear flow regimes\cite{149-151}, femtosecond laser pulses\cite{125} and surface acoustic waves (SAWs).\cite{152}

2.5 Plasma polymer patterning

Several techniques have been used to spatially control plasma polymer chemistries, as summarised in Table 2.4. A common approach is to physically mask the substrate prior to plasma polymerisation to prevent deposition of the film in spatially controlled areas. The simplest method is to place a physical mask such as a transmission electron microscope (TEM) grid on top of the substrate during deposition. This has been used to create dual plasma polymer patterns of plasma polymerised acrylic acid (ppAAc) / 1,7-Octadiene (ppOct)\cite{86}, ppAAc / plasma polymerised allylamine (ppAAm) and plasma polymerised maleic anhydride (ppMA)/ tetraglyme (ppTg) films.\cite{85} A significant limitation of the technique is diffusion of plasma species underneath the mask during deposition, which severely restricts the chemical resolution which can be reached.\cite{85}

To overcome this limitation, photolithography can be employed to selectively mask the substrate. Typically, an initial plasma polymer layer is deposited onto the substrate and photolithography is used to form a patterned resist mask on top of the coated substrate. A second plasma polymer film can then be deposited on top of the resist mask and finally the underlying resist is removed to reveal a dual plasma polymer pattern. This technique was first reported by Goessl et al. who fabricated ppTg/fluorocarbon plasma polymer micropatterns down to 5 µm in size.\cite{153} More recently Mishra et al. used photolithography to create a variety of dual plasma polymer patterns with chemical resolution down to 1 µm.\cite{85} These patterns were used to develop fouling/anti-fouling regions for spatial patterning of antibodies\cite{154} and in the development of surfaces for rapid isolation/pre-concentration of phosphopeptides and proteins.\cite{155} The technique has also been used to create cell adhesive/non adhesive micropatterns, using single plasma polymer chemistries and plasma treatments, to control cell attachment.\cite{156, 157} One limitation of the technique is direct contact of the resist with the underlying plasma
polymer leading to contamination,[85, 153] although, with the correct rinsing protocols and solvents, this problem can be addressed.[153] Sub-micron resolution is a further limitation, however, developments in photoresist patterning provide opportunities for high resolution photolithography approaches.[158] Colloidal lithography is another technique which employs physical masking and involves the self-assembly of spherical particles at the substrate surface which can be used as a mask. A plasma polymer film can then be deposited on top of the mask and the colloidal assembly removed via sonication to reveal the plasma polymer pattern. This approach has been used to create plasma polymerised n-heptylamine (ppHA) patterns for protein patterning[159] and dual plasma polymer chemistry patterns of ppAAc and a PEO-like material.[160] An alternative approach is to first deposit the plasma polymer thin film on the substrate before applying the colloidal mask. Unmasked plasma polymer film can then be etched away to reveal the substrate in specific regions. This was used by Valsesia et al. to create ppAAc zones on a silicon wafer substrate.[161] Colloidal lithography has also been combined with other approaches such as sputtering and evaporation to develop Au/SiO$_2$/ppOct patterns.[88] Whilst colloidal lithography provides a more cost effective approach to patterning plasma polymer chemistries it is not suitable where very intricate non-repeating patterning is required at high resolution. Electron beam lithography (EBL) has also been used for high resolution patterning of plasma polymer thin films. Standard EBL involves the exposure of a resist (typically poly(methyl methacrylate) (PMMA)) to an electron beam. Exposed areas are removed by using a developer solution to leave a patterned resist mask. A more unconventional approach has been to pattern plasma polymers by direct exposure to the electron beam without use of the PMMA resist. Bretagnol et al. used the electron beam to crosslink a water-soluble ppAAc layer in specific areas. The unexposed regions could then be removed by submersion in water leaving stable nanopatterned features.[162] Electron-beam lithography has also been used to tune ether bond concentration of a PEO-like plasma polymer film to form bio-adhesive nanopatterns.[163] Plasma polymers have also been explored as potential EBL resists[164, 165] as, unlike conventional resists, they can be easily deposited on substrates with existing topography in a conformal manner. This has led to the application of plasma polymer resists in 3D nanopatterning.[87] A limitation of the approaches involving direct electron beam exposure is the requirement
of carefully tuned plasma polymer films. Given that plasma polymer deposition systems are often home-built, with a variety of different configurations and film characteristics, [84] means that, unlike conventional PMMA resists, the coatings are not commercially available.

A further approach to plasma polymer patterning has been the development of a patterned upper electrode configuration to create a non-uniform plasma and thus form chemically distinct regions. [166] The one-step, solvent- and resist-free approach is highly advantageous, however, the chemical and physical gradient at the edges of the patterned features may not be desirable for some applications. It is also unclear what the resolution limits are for patterning of this nature.

Table 2.4 Techniques used for patterning plasma polymer chemistries.

<table>
<thead>
<tr>
<th>Patterning technique</th>
<th>Advantages</th>
<th>Limitations</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical masking</td>
<td>Simple, one-step process, solvent-free</td>
<td>Diffusion of plasma species under the mask</td>
<td>[85, 86]</td>
</tr>
<tr>
<td>▪ Physical grid on sample or at electrode</td>
<td>Micro- and nanopatterning, cost effective as no specialist instrumentation required</td>
<td>Limited pattern shapes and repeating patterns</td>
<td>[88, 159]</td>
</tr>
<tr>
<td>▪ Colloidal lithography</td>
<td>Established technique for reliable patterning of intricate features at the micron scale, large area, fast, cost effective, manufacturable</td>
<td>Microscale features (sub-micron resist patterning achieved using specialist techniques), photoresist and developer contamination, planar substrates</td>
<td>[85, 154, 155, 158]</td>
</tr>
<tr>
<td>Photolithography</td>
<td>High resolution patterning, intricate patterns and arrays, plasma polymer resist is conformal to topography of substrate</td>
<td>Requires precise optimisation of plasma polymer to act as a resist, time consuming and expensive for large areas of patterns</td>
<td>[87, 164]</td>
</tr>
<tr>
<td>Direct patterning of plasma polymer layer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>▪ Electron beam lithography (EBL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>One-step process, solvent-free</td>
<td>Requires patterned electrode, dependent on reactor design, lower resolution limits unknown</td>
<td>[166]</td>
</tr>
</tbody>
</table>
2.6 Physicochemical characterisation of plasma polymers

A number of techniques have been used to characterise plasma polymer thin films, many of which are summarised in Table 2.5. The performance of plasma polymer films for a particular application will be dependent on a broad array of film characteristics, such as mechanical properties, chemistry (both at the surface and bulk), surface wettability and topography. It is therefore often appropriate to use several techniques to reveal the plasma polymer film’s physicochemical properties. Each technique has its own strengths and limitations, with the latter including destructive sample preparation and/or analysis, substrate limitations, availability and expense, slow data acquisition and complex data analysis amongst others. Careful consideration is therefore required when selecting the most appropriate characterisation technique.

Table 2.5 Techniques used to characterise plasma polymer thin films.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Information obtained</th>
<th>Limitations</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>• X-ray photoelectron spectroscopy (XPS)</td>
<td>Chemical (elemental, functional groups), film stability and thickness, depth analysis possible using angle resolved XPS and/or organic depth profiling</td>
<td>High vacuum, functional group assignment can be ambiguous requiring derivatization</td>
<td>[72, 79, 167, 168]</td>
</tr>
<tr>
<td>• Time-of-flight secondary ion mass spectrometry (ToF-SIMS)</td>
<td>Chemical (molecular fragments, functional groups), high spatial resolution imaging</td>
<td>High vacuum, destructive, complex data interpretation</td>
<td>[85, 154]</td>
</tr>
<tr>
<td>• Near edge X-ray absorption fine structure (NEXAFS)</td>
<td>Chemical (elemental, functional groups on a range of edges)</td>
<td>Synchrotron, vacuum, limited availability and access.</td>
<td>[70, 79]</td>
</tr>
<tr>
<td>• Contact angle</td>
<td>Surface wettability</td>
<td>Clean sample preparation, affected by sample roughness/swelling</td>
<td>[75, 169]</td>
</tr>
<tr>
<td>• Fourier transform infrared spectroscopy (FTIR), attenuated total reflection (ATR) and grazing angle</td>
<td>Chemical (functional groups), spectroscopic and imaging modes</td>
<td>Deposition needs to be made on KBr crystals for high resolution and surface sensitivity</td>
<td>[91, 168, 170-172]</td>
</tr>
<tr>
<td>Method</td>
<td>Measurement parameters</td>
<td>Requirements</td>
<td>References</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Neutron reflectometry (NR)</td>
<td>Chemical, film thickness, roughness, water uptake, can detect levels of unsaturation</td>
<td>Requires neutron source, deuteration often required for resolution, large area of constant thickness required.</td>
<td>[173, 174]</td>
</tr>
<tr>
<td>X-ray reflectometry (XRR)</td>
<td>Chemical, film thickness</td>
<td>High vacuum</td>
<td>[173, 174]</td>
</tr>
<tr>
<td>Raman</td>
<td>Chemical bonding states</td>
<td>Not surface sensitive, issues with autofluorescence and background subtraction</td>
<td>[175]</td>
</tr>
<tr>
<td>Scanning electron microscopy (SEM)</td>
<td>Surface topography</td>
<td>High vacuum, requires destructive conductive coating</td>
<td>[168, 176]</td>
</tr>
<tr>
<td>Atomic force microscopy (AFM) imaging</td>
<td>Surface topography, roughness, coating thickness in dry or a variety of solvent systems</td>
<td>Tip artefacts and contamination, relatively slow imaging speeds</td>
<td>[75, 91, 173, 177]</td>
</tr>
<tr>
<td>AFM surface force measurements (Colloidal probe)</td>
<td>Surface charge and structure in solvents</td>
<td>Data interpretation requires modelling</td>
<td>[167]</td>
</tr>
<tr>
<td>Ellipsometry</td>
<td>Coating thickness, refractive index</td>
<td>Requires reliable model and knowledge of coating refractive indices</td>
<td>[81, 168]</td>
</tr>
<tr>
<td>Surface plasmon resonance (SPR)</td>
<td>Film thickness and stability</td>
<td>Plasmonic substrate (generally Au), requires refractive index of film to calculate thickness</td>
<td>[82, 91]</td>
</tr>
<tr>
<td>Nanoindentation</td>
<td>Mechanical, film hardness and modulus</td>
<td>Destructive, not suitable for sub 10 nm coating thickness</td>
<td>[169]</td>
</tr>
<tr>
<td>Quartz crystal microbalance with dissipation (QCM-D)</td>
<td>Swelling and stability in aqueous conditions in-situ, film mass and thickness, viscosity and elasticity</td>
<td>Requires specific QCM-D sensor substrate, more complex modelling required for viscoelastic films</td>
<td>[169, 173, 178]</td>
</tr>
</tbody>
</table>
In this thesis, several techniques were selected to characterise the physiochemical properties of plasma polymer films in both dry and aqueous conditions. The following sections describe the main techniques and how they can be used to characterise plasma polymers.

### 2.6.1 X-ray photoelectron spectroscopy (XPS)

XPS is a commonly used technique for the characterisation of plasma polymer thin films. X-rays of a specific wavelength are used to irradiate the sample, leading to the ejection of core and valence electrons (photoelectrons). The energy of the photoelectrons is unique to the element from which it is emitted and can also be used to identify functional groups due to the ‘electron withdrawing power’ of nearby atoms influencing the energy of the emitted electron. The intensity of the photoelectrons is proportional to the concentration of the element.\[180, 181\] The surface sensitivity of XPS arises because it detects unscattered photoelectrons, ~95% of which originate from a depth which is three times the inelastic mean free path (3λ) of the element being detected. For carbon, (which is typically quoted as the sampling depth for XPS) λ=3.3 nm, resulting in a sampling depth of ~ 10 nm.\[182\]

XPS has been used in a range of studies to look at the aging of plasma polymers in air,\[72\] film stability in various solvents,\[73, 93\] the effects of sterilisation,\[183\] film growth\[94\] and the effect of a variety of deposition conditions and reactor configurations on film chemistry.\[76, 84, 99\] XPS depth profiling using Ar cluster sources also holds great promise for the investigation of bulk plasma polymer chemistry.\[184\] A limitation of XPS is that the binding energies derived from some functional groups are too similar to be assigned with confidence. This is especially the case for plasma polymers with broader elemental peaks and highly complex chemical composition with functional groups and secondary structures not present in the starting monomer.\[79, 180\] In these cases, chemical derivatization for identification of specific functional groups can be
employed such as trifluoroethanol (TFE) labelling for acid functionalities.[185] XPS imaging has also been utilised to image DNA microarrays (100-150 µm features)[186] and ppTg micropatterns on silicon wafer,[187] however, the spatial resolution of XPS imaging is limited to the micron scale.[182]

2.6.2 Time-of-flight secondary ion mass spectrometry (ToF-SIMS)
ToF-SIMS is another surface sensitive technique used for the characterisation of plasma polymers and probes only the top 1-2 nm of material in the Static SIMS mode.[188] Primary ions collide with molecules in the sample, causing ionisation, fragmentation and bond breaking. At the collision site, it is predominantly atomic particles which are emitted, however, further away from the site, molecular fragments are emitted as the collisions have less energy. The mass to charge ratio (m/z) is analysed for each of the particles emitted in a charged state (≤ 1% of particles emitted). Positive and negative secondary ion mass spectra can then be plotted using the number of ions detected for each m/z.[188, 189] Molecular species at the surface of plasma polymer films can be investigated in this way.[83, 190]

One of the main advantages of ToF-SIMS is the improved spatial resolution for imaging at both the micron and sub-micron scale, which has been utilised to image a variety of single[88] and dual[85, 154, 155] plasma polymer patterns. A major challenge associated with ToF-SIMS imaging is the complexity and amount of data collected. For example, a 512 x 512 pixel image will be composed of 262144 mass spectra and each of these could include hundreds of peaks. Furthermore, molecular species relating to these peaks are related to each other, as they can be derived from fragmentation of the same molecule at the surface of the sample.[189] Various multivariate analysis techniques have therefore been developed as tools for data interpretation.[189] Mishra et al. used principal component analysis (PCA) to investigate the molecular properties of ppMA films and the influence of pulsed plasma conditions.[83] The McArthur group also used multivariate analysis to investigate the chemical boundary between chemically distinct regions of dual plasma polymer patterns fabricated using photolithography.[85]

2.6.3 Auger electron spectroscopy (AES)
In AES, an electron beam is used to initiate the Auger process. Electrons interact with atoms in the sample, causing an inner shell electron to be removed, leaving the atom in an excited state. De-excitation then occurs and an X-ray or Auger electron will be
emitted. The technique is therefore useful for elemental analysis of a surface (excluding hydrogen and helium), probing the top 0-3 nm of material.[191] One of the main advantages of AES is that it provides high spatial resolution and enables spatial mapping of elements at the surface,[192] which can be combined with high resolution SEM imaging.[193] However, Auger electrons can be generated from areas surrounding the spot where the electron strikes due to scattering effects and care must be taken to evaluate the analysis area.[194] AES can also be used to perform depth profiling of materials such as plasma polymerised trimethylsilane films.[195] A major limitation of the technique is that organic samples can be readily damaged by the electron beam.[196] Oxygen depletion can be a particular problem when analysing organic and insulating films, as their low electrical and thermal conductivities increase susceptibility to electron beam damage.[196]

2.6.4 Quartz crystal microbalance with dissipation (QCM-D)

QCM-D consists of a piezoelectric quartz crystal oscillated to resonance, using an AC voltage, which is continuously switched on and off throughout the measurement period.[197] Two properties are measured, the resonance frequency shift of the crystal ($\Delta f$) which is related to mass adsorbed at the surface and the dissipation shift ($\Delta D$), related to the softness of the adsorbed mass.

The Sauerbrey equation linearly relates $\Delta f$ to the change in mass ($\Delta m$).[25, 197]

$$\Delta m = \frac{C}{n} \Delta f$$

Where $n$=harmonic number and $C = 17.7 \text{ ng Hz}^{-1} \text{ cm}^{-2}$ for a 5 MHz crystal.

For the Sauerbrey relationship to hold, the added mass should be a rigid, thin and evenly distributed layer.[198, 199] For a nonrigid adsorbed mass alternative models are required such as Voight-based viscoelastic models.[199]

The dissipation is measured by switching off the driving power to the resonating crystal and observing the decay of the voltage across the crystal over time. This provides a decay time constant which is used to calculate the dissipation factor.[198, 200] An adsorbed mass which is more viscoelastic will induce greater damping of the crystal oscillations.
An advantage of the technique is the sensitivity, the E4 instrument (Q-Sense, Sweden) used in this thesis is stated as having a normal mass sensitivity in liquid of -1.8 ng cm\(^{-2}\).\(^{201}\) This together with the ability to conduct experiments under flow conditions \textit{in situ} has made the technique valuable for studying the kinetics of a wide variety of processes occurring at an interface.\(^{202}\) Although not widely used for the characterisation of plasma polymer films, QCM-D has been used to investigate the impact of deposition conditions on plasma polymer film stability and swelling in aqueous conditions.\(^{169, 173, 178}\)

2.6.5 \textbf{Electrochemical impedance spectroscopy (EIS)}

EIS can be used to investigate the electrical properties of plasma polymer films revealing changes in plasma polymer thickness, homogeneity, water content and ion content. During a typical EIS experiment an AC potential is applied to the system under investigation and the current response is then measured. These measurements are then repeated over a wide range of frequencies due to the dominance of different parts of the system at particular frequencies.\(^{203}\) The ratio between the applied voltage and the measured current provides the magnitude of the impedance (\(\mid Z \mid\)) whilst the phase shift between the measured current and applied voltage is represented by the phase angle (\(\omega\)). This data can be represented as a Bode plot where \(\mid Z \mid\) and \(\omega\) are plotted individually against frequency on the same graph. Low phase angles and constant impedance demonstrate resistive parts whilst high phase angles and a -1 slope for impedance show capacitive parts.

The data can be modelled using electric circuits with resistor and capacitor components.\(^{204}\) In this work plasma polymers were modelled using RCPE elements (a resistor (R) in parallel with a constant phase element (CPE)). In such a model, the plasma polymer is considered as a single insulating dielectric layer, which is a non-ideal capacitor (may have defects/inhomogeneities) where impedance is given by:

\[
Z_{CPE} = \frac{1}{CPE(j\omega)^\alpha}
\]

Where \(CPE\) is the constant phase element coefficient which describes the capacitance of the film and \(\alpha\) is a measure of homogeneity of the film (varying between 1 for an ideal capacitor and 0 for an ideal resistor).
A decrease in impedance can be related to an increase in conductivity which means charge can pass more readily through the film. In the context of a plasma polymer film this could be due to an uptake of ions into the film. An increase in capacitance can be related to an increase in H$_2$O entering the coating (due to an increase in the dielectric constant, $\varepsilon_r \approx 80$ for water) and/or a decrease in coating thickness according to the following equation:

$$C = \frac{\varepsilon_r \varepsilon_0 A}{d}$$

Where $C$ = capacitance, $\varepsilon_r$ = dielectric constant, $\varepsilon_0$ = vacuum permittivity, $A$ = surface area of capacitor and $d$ = film thickness.

Like QCM-D, a main advantage of EIS is the ability to perform experiments in situ in aqueous conditions, however, it is not commonly used for characterisation of plasma polymer films compared with other techniques such as XPS. Schiller et al. used the technique to investigate the swelling behaviour of ppMA films. It has also been used to investigate the effects of different deposition parameters on the stability and behaviour of plasma polymerised pyrrole (PPpy) films in phosphate buffered saline (PBS) solution.

### 2.6.6 Atomic force microscopy (AFM)

AFM consists of an atomically sharp tip mounted to a spring cantilever which is scanned across the surface of the sample. Interaction forces between the tip and sample cause deflection of the cantilever, which is measured using an optical system involving a laser reflected off the cantilever. The most common modes are contact mode and tapping mode. In the former, the tip is constantly in contact with the sample as it scans across the surface. In tapping mode the tip is intermittently in contact during scanning which reduces any adverse effects produced by lateral forces. AFM is not only used for topographical imaging but also provides a wide variety of information about surfaces such as micro- and nanomechanical properties of materials, surface chemistry via approaches such as tip functionalisation as well as combined approaches such as AFM-IR.

The major advantage of AFM is that it provides high spatial resolution and in situ measurements can be performed in air and liquid environments. For the characterisation of plasma polymers, AFM has been utilised to measure film thickness by scanning over
a step between the substrate and plasma polymer film\textsuperscript{[177]} and to image patterned plasma polymer films.\textsuperscript{[85]} It has also been used to investigate topographical differences of ppAAc films deposited onto different substrates\textsuperscript{[75]} and the effect of deposition parameters\textsuperscript{[173]} and ethanol extraction\textsuperscript{[91]} on film roughness and topography.

2.7 Characterisation of supported lipid bilayers and adsorbed vesicles

Techniques used for characterisation of cell membrane model systems are shown in Table 2.6. The list is by no means exhaustive given the continuous improvement and development of new techniques.\textsuperscript{[209, 212]} Fluorescent techniques have been vital in increasing the understanding of membranes, however, fluorescent labelling can affect membrane properties and have an impact on properties such as diffusion coefficients.\textsuperscript{[213]} Fortunately, there are several label-free techniques available for analysis with new developments in areas such as high resolution imaging.\textsuperscript{[209]} Further challenges when characterising model cell membrane systems are the speed at which processes occur, the size of the membrane components involved and the requirement for liquid environments (in many cases) which are not suitable for a variety of techniques requiring vacuum environments.

Table 2.6 Techniques used to characterise lipid bilayer model systems.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Information obtained</th>
<th>Limitations</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>▪ Fluorescent recovery after photobleaching (FRAP)</td>
<td>Lipid diffusion</td>
<td>Fluorescent labelling, assumptions in analysis may lead to misleading diffusion coefficients</td>
<td>[29, 214-217]</td>
</tr>
<tr>
<td>▪ Fluorescent resonant energy transfer (FRET)</td>
<td>Lipid domains</td>
<td>Fluorescent labelling, model-dependent</td>
<td>[218, 219]</td>
</tr>
<tr>
<td>▪ Fluorescence-lifetime imaging microscopy (FLIM)</td>
<td>Lipid domains</td>
<td>Fluorescent labelling, slow data acquisition during imaging means structure dynamics may not be adequately captured</td>
<td>[220-222]</td>
</tr>
<tr>
<td>▪ Fluorescent correlation spectroscopy (FCS)</td>
<td>Lipid diffusion, concentrations</td>
<td>Imaging depth much larger than membrane thickness, issues with statistical accuracy, stability, distortions of detection volume and photobleaching</td>
<td>[223-225]</td>
</tr>
<tr>
<td>Method</td>
<td>Technique/Imaging</td>
<td>Notes</td>
<td>References</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Single particle tracking (SPT)</td>
<td>Lipid diffusion</td>
<td>Some particles may cause perturbation affecting diffusion, unable to track nanoscopic dynamics, continuous tracking can be difficult and dependent on particle type and software</td>
<td>[215, 225, 226]</td>
</tr>
<tr>
<td>Near-field scanning optical microscopy (NSOM)</td>
<td>Optical and topographical imaging</td>
<td>Fluorescent labelling, low scan speed and fragile probes</td>
<td>[227]</td>
</tr>
<tr>
<td>Points accumulation for imaging in nanoscale topography (PAINT)</td>
<td>Sub-diffraction optical imaging of lipid phase domains</td>
<td>Requires fluorescent probe</td>
<td>[228]</td>
</tr>
<tr>
<td>Surface plasmon resonance (SPR)</td>
<td>Lipid mass</td>
<td>Plasmonic substrate, film thickness must be within limits for SPR signal detection, calculation of mass is dependent on system under investigation</td>
<td>[229-231]</td>
</tr>
<tr>
<td>Ellipsometry</td>
<td>Lipid bilayer thickness</td>
<td>Mode-dependent</td>
<td>[47]</td>
</tr>
<tr>
<td>Imaging ellipsometry (IE)</td>
<td>Lipid bilayer thickness, lateral organisation, defects and phase separation (IE)</td>
<td>Model-dependent</td>
<td>[232]</td>
</tr>
<tr>
<td>Attenuated total reflection fourier transform infrared spectroscopy (ATR-FTIR)</td>
<td>Lipid orientation, hydration, packing, physical state, orientation/conformation of hydrocarbon chain, thickness of water layer between the substrate and bilayer</td>
<td>Substrates may be limited due to transmission characteristics</td>
<td>[233-235]</td>
</tr>
<tr>
<td>Interferometric scattering microscopy (iSCAT)</td>
<td>Lateral and axial tracking of lipid diffusion</td>
<td>Sensitivity to scattering background and heating of nanoscopic scatterers</td>
<td>[226, 236, 237]</td>
</tr>
<tr>
<td>Coherent anti-Stokes Raman scattering (CARS)</td>
<td>Lipid chain order, packing, characterisation of selected leaflets</td>
<td>Unable to identify acyl chain length, cannot differentiate between chemically similar headgroups, selective imaging of leaflets requires deuterated lipids</td>
<td>[238] [209]</td>
</tr>
<tr>
<td>Method</td>
<td>Description</td>
<td>Nanoscopic metallic substrates</td>
<td>References</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Surface-enhanced Raman scattering (SERS)</td>
<td>Distribution of lipids</td>
<td></td>
<td>[239]</td>
</tr>
<tr>
<td>Tip-enhanced raman spectroscopy (TERS)</td>
<td>Chemical and topographical, lipid domains/distribution</td>
<td>Tip in contact with sample, diffusion of membrane components can cause spectral fluctuations</td>
<td>[240]</td>
</tr>
<tr>
<td>Sum frequency generation spectroscopy (SFG)</td>
<td>Lipid organisation, orientation and characterisation, structural dynamics</td>
<td>Interpretation of signal from complex systems</td>
<td>[241, 242]</td>
</tr>
</tbody>
</table>

**Mass spectrometry**

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
<th>Nanoscopic metallic substrates</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time-of-flight secondary ion mass spectrometry (ToF-SIMS)</td>
<td>Chemical, mapping spatial distribution of lipids, imaging of single lipid vesicles (~ 300 nm)</td>
<td>High vacuum, destructive sample preparation, trade-off between spatial and mass resolution, low yield of intact lipid species, extensive fragment peaks</td>
<td>[209, 217, 243]</td>
</tr>
<tr>
<td>Matrix-assisted laser desorption / ionisation mass spectrometry (MALDI-MS)</td>
<td>Lipid distribution (imaging)</td>
<td>Vacuum, destructive sample preparation, relatively low spatial resolution (um)</td>
<td>[244]</td>
</tr>
</tbody>
</table>

**Force methods**

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
<th>Nanoscopic metallic substrates</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atomic force microscopy (AFM)</td>
<td>Membrane topography and domains, interaction forces, elasticity, mechanical and chemical properties</td>
<td>Tip contact with the sample, slow imaging speeds</td>
<td>[245]</td>
</tr>
</tbody>
</table>

**X-ray and neutron reflectivity**

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
<th>Nanoscopic metallic substrates</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutron reflectivity (NR)</td>
<td>Structural information, bilayer ordering thickness, composition and roughness</td>
<td>Special facilities, low beam intensity means larger sample volume and measurement times, spatial resolution limited by high background signal, expensive deuterated molecules</td>
<td>[212, 246, 247]</td>
</tr>
<tr>
<td>X-ray reflectivity (XRR)</td>
<td>Structural information, bilayer thickness, associated water, asymmetric leaflet composition</td>
<td>Synchrotron, beam damage</td>
<td>[212, 248, 212]</td>
</tr>
</tbody>
</table>
In this thesis, fluorescence recovery after photobleaching (FRAP), and quartz crystal microbalance with dissipation (QCM-D) and AFM were selected to investigate lipid vesicle interactions with different substrates. The following sections describe the techniques and how they can be used to characterise lipid vesicle interactions with surfaces.

### 2.7.1 Fluorescence recovery after photobleaching (FRAP)

In FRAP, fluorescently labelled lipids within a membrane are exposed to a high intensity laser to destroy the fluorophores (bleach) in a specific region of the sample. If the components of the membrane have mobility, diffusion of lipids in and out of the exposed area will result in full or partial recovery of the fluorescent signal. The bleaching processes and subsequent recovery are imaged using laser confocal or

<table>
<thead>
<tr>
<th>Electrochemical</th>
<th>Coverage and defects, in-situ monitoring of vesicle fusion</th>
<th>Metallic substrate</th>
<th>[249, 250]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclic voltammetry</td>
<td>Coverage and defects</td>
<td>Metallic substrate, electroactive compounds could affect system under investigation</td>
<td>[47]</td>
</tr>
<tr>
<td>X-ray photoelectron spectroscopy (XPS)</td>
<td>Chemical composition (elemental, functional groups), lipid bilayer thickness with angle resolved techniques</td>
<td>Vacuum, requires freeze hydration sample preparation, functional group determination may be difficult without derivatization</td>
<td>[251, 252]</td>
</tr>
<tr>
<td>Quartz crystal microbalance with dissipation (QCM-D)</td>
<td>Lipid mass, mechanical properties, lipid structure (bilayer/vesicles), in-situ kinetics of bilayer formation and other processes</td>
<td>Data interpretation (film dependent)</td>
<td>[25, 28, 253]</td>
</tr>
<tr>
<td>Transmission electron microscopy (TEM) / Cryo-TEM</td>
<td>High resolution imaging of lipid layers</td>
<td>Vacuum, invasive sample preparation</td>
<td>[254]</td>
</tr>
</tbody>
</table>
epifluorescence imaging and this data can be used to calculate diffusion coefficients of
the lipids.\cite{212}
The technique has been commonly used on SLBs to confirm and evaluate mobility on a
variety of substrates such as glass,\cite{215,216} SiO$_2$,\cite{214,217} and TiO$_2$.\cite{29} One limitation of
the FRAP technique is that it relies on the use of fluorescent labelling of the lipid
headgroups or tails which has been shown to affect measured diffusion coefficients of
the lipids.\cite{213} Another limitation is that incorrect analysis of the data can lead to
misleading values for diffusion coefficients, especially if factors, such as the shape of
the bleach spot, signal/noise ratio and bleaching during image collection, amongst
others, are not carefully considered within the analysis.\cite{216}

2.7.2 Quartz crystal microbalance with dissipation (QCM-D)

QCM-D was first used to study the formation of lipid membranes via vesicle collapse
by Keller and Kasemo in 1998\cite{39} and is now a widely used technique for characterising
cell membrane model systems. The advantage of QCM-D is that it can measure both
mass and energy dissipation changes in real time, enabling label-free \textit{in situ}
characterisation of lipid vesicle interactions in aqueous conditions. Crucially, QCM-D
reveals the kinetics of bilayer formation on surfaces such as SiO$_2$\cite{25} and unlike
techniques such as SPR, bilayer and vesicle structures can easily be differentiated using
the dissipation component.\cite{253}

During a typical two-step adsorption and collapse process observed on SiO$_2$, vesicles
first adsorb to the sensor surface causing a decrease in resonant frequency (due to the
addition of mass, including lipid, liquid trapped inside the vesicles and water associated
with the vesicles).\cite{35} The dissipation increases as the vesicles create a viscous
environment which dampen oscillations of the crystal more readily. At a critical
coverage, signified by a minima and maxima of frequency and dissipation, respectively,
vesicles begin to collapse forming a bilayer at the surface which initiated further
collapse at the surface. The mass of the liquid trapped within the vesicles and water
associated with the vesicles is lost. This leads to a reduction in coupled mass at the
surface and a resultant increase in resonant frequency. Dissipation is reduced as the soft
layer of vesicles becomes a more rigid bilayer structure.\cite{35,255}

Bilayer formation is a complex process and other pathways have been monitored by
QCM-D. An example is one step vesicle collapse on SiO$_2$ using low pH conditions\cite{28}
and by changing vesicle charge.\cite{23} The technique has also been used to investigate
38
bilayer formation and other lipid structures such as monolayers and vesicle layers over a wide range of changing experimental conditions including substrate,[25, 28, 255] pH,[28] lipid charge,[23] presence of mono- and divalent ions,[32] osmotic effects,[38] vesicle size,[25] and temperature[25, 256] amongst others. The technique has also been used to investigate other phenomena including interleaflet distribution of lipids,[257] lipid exchange from vesicles[258] and the interaction of lipids with patterned surfaces.[259] The development of specialised chambers has also enabled QCM-D to be combined with other techniques such as EIS,[250] SPR[231] and optical reflectometry[260] for simultaneous measurements in real time.

An important aspect of QCM-D is data interpretation and analysis. For films that do not dissipate energy, such as an SLB, the Sauerbrey relation (described earlier in this chapter) is sufficient to quantify the lipid mass at the surface from the frequency shift. However, as described by Reviakine et al., dissipative films require more complex modelling methods depending on if the film is heterogeneous (such as an adsorbed vesicle layer) or homogeneous.[253] For example, in the case of a vesicular layer, the mass coverage at the surface cannot be linearly related to the frequency shift because, as more vesicles adsorb, the trapped liquid between them reduces. Other techniques, such as surface plasmon resonance (SPR) and ellipsometry, can be used to separate the overall mass change into contributions from the adsorbed mass and the solvent.[231] Dissipation properties of a vesicle layer are also complex and arise predominantly from the rocking and sliding of the adsorbed vesicles. Crucially, for useful and accurate quantification and interpretation of QCM-D data, a thorough understanding of the system under investigation and the use of complimentary techniques is essential.[253]

2.7.3 Atomic force microscopy (AFM)

AFM is one of the most widely used techniques for the characterisation of model cell membranes.[245, 261-264] As described in a previous section of this review, the main advantage of AFM is that the atomically sharp tip provides information of high spatial resolution as it is scanned across the surface. Furthermore, in situ measurements can be performed in liquid environments. For this reason the technique is ideal for imaging structural aspects of the membrane models such as defects,[265] leaflet symmetry,[266] domains and phase transitions[263, 267] and vesicle collapse processes.[23, 268] AFM force spectroscopy (FS), conducted in contact mode, can reveal nanomechanical properties of the cell membrane model under investigation by measuring the interactions of
functionalised or non-functionalised tips.\textsuperscript{[262]} Such interactions are typically represented as a force versus piezo displacement (Fv\(\Delta z\)) curves which can be analysed to reveal sample properties such as elasticity and surface forces such as electrostatic and van der Waals interactions. For example, AFM FS has been used to investigate the effect of ion-binding on PC lipid membrane nanomechanics\textsuperscript{[269]} and the nanomechanical characteristics of liquid and gel phases in SLBs.\textsuperscript{[270]} More recently, peak force quantitative nano-mechanics (PF-QNM) has enabled simultaneous imaging and high resolution nanomechanical mapping by oscillating the sample in the z direction which enables force-distance (FvD) curves to be produced every time the tip makes contact with the sample.\textsuperscript{[262]} This has been used to quantitatively map topographical, stiffness and deformation differences for fluid and gel phases within an SLB.\textsuperscript{[271]}

The combination of AFM with other techniques, such as fluorescence and mass spectroscopy, is enabling collection of complimentary information about the sample being analysed.\textsuperscript{[262]} Examples include the combination of ToF-SIMS and AFM to characterise lipid domains formed from mixed lipid systems (freeze-dried)\textsuperscript{[272]} and the combination of ATR-FTIR with fluid AFM to investigate thermal phase transition behaviour and protein insertion within multilamellar DPPC lipid bilayers.\textsuperscript{[273]}

A significant limitation of AFM is the time taken for data acquisition, especially when considering the timescales involved in membrane dynamics. However, the continual development of high speed AFM is providing faster scan rates.\textsuperscript{[262, 274]} Tip-induced damage and interactions with the lipid surface are also a consideration and may introduce artefacts upon contact of the tip with lipid material. Finally, care must be taken in the analysis of force curve data to obtain correct values for mechanical properties of the surface, especially as the absolute distance between the tip and surface is not known. Accurate analysis requires precisely defined tip geometry and an understanding of how the underlying substrate, tip chemistry and liquid medium may affect interactions with the sample.\textsuperscript{[262, 275]}
2.8 Summary

The complexity of the cell membrane has limited our understanding of many important processes and interactions. Models such as supported lipid bilayers are progressing research in the area by providing a versatile and controlled platform for studying this complex environment. A major challenge is the recreation of architectures involving precise spatial control of membrane components whilst retaining their functional properties. As discussed in this Chapter patterning using a variety of different techniques is being explored for this purpose on both a micro- and nanoscale. Further progress requires suitable substrates which support the development of stable, reproducible membrane models whilst facilitating the use of a range of fabrication and characterisation techniques. However, future developments will be determined by advances in new and/or enhanced characterisation techniques for adequately capturing membrane architectures only a few nm in size which may be transient in nature, forming and dispersing on timescales unavailable to many commonly used instruments.
2.9 References


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3 Materials and methods

3.1 Substrate preparation

Quartz crystal microbalance with dissipation (QCM-D) crystals (QSX301 gold coated quartz, 100 nm Au) were purchased from Q-Sense, Sweden. Silicon wafers (orientation <1-0-0>, thickness 500-550 µm, single side polished, resistivity 1-10 Ω·cm) were purchased from Micro Materials & Research Consumables Pty Ltd, Australia. Plain circular No. 5, 19 mm diameter glass coverslips were purchased from ProSciTech Pty Ltd, Australia. Isopropyl alcohol (IPA, 99.5% purity) and acetone (99.8% purity) were purchased from Chem-Supply Pty Ltd, Australia. Decon 90 (Decon Laboratories Limited, UK) and Milli-Q grade water (18.2 MΩ·cm, Millipore, Australia) were used for cleaning substrates.

Glass and silicon wafer: Silicon wafer and glass coverslip substrates were cleaned using the following sonication steps: 2% Decon 90 (30 mins), Milli-Q water (10 mins) and IPA (10 mins). Substrates were then rinsed with fresh IPA and dried under a stream of nitrogen before being treated for 10 minutes in a UV Ozone Cleaner instrument (Bioforce Nanosciences, Inc., USA) with constant settings set by the manufacturer which could not be adjusted (with the exception of time).

Gold QCM-D crystals: Unless otherwise stated gold QCM-D crystal sensors were wet cleaned in a heated solution (70°C) of 1:1:5 volume ratio solution of 30% ammonia, 30% hydrogen peroxide and Milli-Q water for approximately 10 minutes or until vigorous bubbling could be seen on the surface of the crystals. Following submersion in Milli-Q water the crystals were rinsed in IPA before being dried using a flow of nitrogen. Crystals were finally subjected to a 10 minute ozone treatment before use.

Electrochemical impedance spectroscopy (EIS) Au substrates: Au coated glass slides (37.5 mm × 25 mm) were used as substrates for EIS measurements. Prior to coating, the slides were rinsed with ethanol, sonicated in 2% Hellmanex cleaning solution and Milli-Q water before being dried under nitrogen gas. A 5-10 nm Ti adhesive layer followed by 50 nm of Au was deposited on the slides using evaporation or sputter coating.
3.2 Plasma polymerisation

Acrylic acid (99% purity) and allyl alcohol (≥ 99% purity) were purchased from Sigma-Aldrich, USA. 1,7-octadiene (98.5% purity) and allylamine (98%, extra pure) were purchased from Acros Organics, USA.

Plasma polymerisation was performed in a stainless steel T-piece reactor with an internal aluminium disk electrode (17 cm diameter). Plasma was ignited using a 13.56 MHz radio frequency power source generator (Coaxial Power Ltd, UK) coupled to the internal disc electrode by means of an impedance matching network (Figure 3.1A). Prior to coating, monomers were degassed using a minimum of three freeze-thaw cycles. Monomer flow rate was controlled using a fine flow needle valve (CMV-VFM-2-P-KK, Chell Instruments Ltd, UK) for allylamine, allyl alcohol and 1,7-octadiene monomers and a medium flow needle valve (CMV-VFM-3-P-KK, Chell Instruments Ltd, UK) for acrylic acid. Internal reactor pressure was measured using a Pirani Gauge (Edwards, UK). An aluminium sample stage was used to elevate samples to a height of 4.35 cm within the reactor chamber (Figure 3.1B). Prior to coating the chamber was pumped down to a base pressure of 1x10^{-3} mbar. Fixed deposition parameters used in the McArthur group[2, 3] were used for all monomers unless otherwise stated. The power was set to 20 W and the deposition time was 20 minutes.

Monomer flow rate was set to 1.5 sccm and calculated using the following equation:

\[
F = \frac{\Delta p}{\Delta t} \frac{16172 V}{T}
\]

Flow rate equation derived from ideal gas equation[4] where \( F \) = flow rate (sccm), \( p \) = pressure (mbar), \( t \) = time (s), \( V \) = volume of the reactor (L), \( T \) = temperature (K)
Figure 3.1 Plasma polymerisation setup. A) Schematic diagram of plasma polymerisation experimental set-up. 1. Monomer flask, 2. needle valve, 3. Pirani gauge, 4. reactor chamber, 5. electrode, 6. vacuum valve, 7. liquid nitrogen trap, 8. vacuum valve, 9. vacuum pump, 10. impedance matching network, 11. radio frequency (RF) power source. B) Photograph of the plasma polymerisation chamber including aluminium disc electrode and aluminium sample stage.
3.3 Photolithography

Photoresist AZ1505 and developer AZ726 were both purchased from Micro Materials & Research Consumables Pty Ltd, Australia. Acetone (99.8% purity) was purchased from Chem-Supply Pty Ltd.

Dual plasma polymer patterns were prepared using photolithography, as previously described.\cite{5, 6} A schematic of the photolithography patterning process is shown in Figure 3.2. Positive photoresist AZ1505 was spin coated onto plain or plasma coated round glass coverslips at 8000 rpm. Coverslips were heated to 200 °C for 3 minutes to harden the resist and remove any residual solvent. Substrates were brought into contact with a chromium photomask (Bandwidth Foundry International, ANFF, Sydney Australia). All samples described were exposed to ultraviolet radiation using a Series 200 mask aligner (OAI, USA) for 2-3 seconds depending on the measured light intensity. Exposed regions were removed via immersion in AZ726 developer solution for 6 seconds. Samples were rinsed with Milli-Q water before drying in a stream of N\textsubscript{2} gas. Following deposition of the second plasma polymer (see previous section for protocol) the underlying photoresist was removed using sonication in acetone for 3 minutes.
Figure 3.2 Schematic representation of the photolithography process for fabrication of dual plasma polymer patterns. 1) Base plasma coating, 2) spin coating of photoresist, 3) chromium mask brought into contact prior to UV exposure, 4) exposed areas removed during development, 5) 2nd plasma coating and finally 6) removal of remaining photoresist reveals dual plasma pattern.

3.4 Electron beam lithography (EBL)

For patterning on glass coverslips resist poly(methyl methacrylate) (PMMA) 950K (4% solids in anisole) and developer methyl isobutyl (MIBK) in a dilution ratio of 1:3 (MIBK:IPA) were purchased from Microchem Corp., USA. PMMA 950K (2% solids in anisole) and developer MIBK:IPA 1:3 were used for patterning silicon wafer substrates with PMMA and MIBK purchased from Microchem Corp., USA and ACROS Organics, Belgium, respectively. Acetone (99.8% purity) was purchased from Chem-Supply Pty Ltd.

A schematic of the nanoscale patterning process on glass coverslips is shown in Figure 3.3. The process was identical for the silicon wafer substrates however the conductive indium tin oxide (ITO) coating was not required. Clean No. 1 circular coverslips were coated with a 10 nm thick electrically conductive and transparent coating of ITO by plasma sputtering using an AXXIS instrument (Kurt J. Lesker, USA). The first plasma coating was deposited onto either the ITO coated coverslip or clean silicon wafer using the standard methods described previously. For the plasma polymer coated glass
coverslips, PMMA was spin coated at 2000 rpm and baked for 90 seconds at 180 °C. For plasma polymer coated silicon wafer, PMMA was spin coated at 4000 rpm and baked for 5 minutes at 170 °C. EBL was conducted using a RAITH150 Two instrument (Raith, Germany) for glass samples and an EBPG5000plus instrument (Vistec, Germany at the ANFF-Vic, Melbourne Centre for Nanofabrication) for silicon wafer samples. Figure 3.4 depicts the design used for fabrication of dual plasma polymerised allylamine (ppAAm) and acrylic acid (ppAAc) patterned surfaces. Lines ranging from 900 to 20 nm in thickness were used to investigate the resolution limits of the plasma polymer patterns. Electron-exposed patterned areas were removed via development in a methyl isobutyl (MIBK):IPA 1:3 solution and development was halted by dipping the sample in IPA. Glass and silicon wafer samples were then further rinsed in IPA and DI water respectively before being dried in a stream of N₂ gas. Following deposition of the second plasma polymer the underlying PMMA resist was removed using sonication in acetone for 3 minutes.

Figure 3.3 Schematic representation of the EBL process for fabrication of dual plasma polymer nanopatterns on glass substrates. 1) Conductive coating, 2) base plasma coating, 3) spin coating of PMMA, 4) EBL and development process, 5) 2nd plasma coating and finally 6) removal of PMMA by sonication in acetone.
Figure 3.4 Schematic of EBL pattern design with feature size labelled in red.

3.5 Buffers

Unless otherwise stated chemicals were of ACS grade or higher. (hydroxymethyl)aminomethane (trometamol) was purchased from Merck, USA. Sodium chloride was purchased from Riedel-de Haën, Germany. HEPES, free acid, molecular biology grade was purchased from Merck Millipore, USA. A standard phosphate buffered saline (PBS) solution (free of divalent ions) containing 137 mM NaCl, 2.7 mM KCl, 10 mM Na$_2$HPO$_4$ and 2 mM KH$_2$PO$_4$ was made using chemicals supplied from Merck, USA in distilled water. All other buffers were prepared using Milli-Q grade water (18.2 MΩ·cm, Millipore, Australia) and pH-adjusted using 1 M HCl and NaOH as required.

3.6 Exposure of plasma polymer films to aqueous conditions

Plasma polymer films deposited onto silicon wafer substrates were placed into 12 well plates (1 substrate per well) and exposed to 2 mL of the following solutions depending on the experiment being undertaken: Milli-Q water (MQ), PBS, 10 mM NaCl, 150 mM NaCl or 10 mM Tris + 150 mM NaCl (TNa pH 4, 7 or 10). For cycled experiments the substrate was submerged in each solution for 10 minutes. Solutions were removed and the next one added in the following order depending on the experiment: MQ → PBS → MQ → PBS → MQ, TNa: pH 7 → 4/10 → 7 → 4/10 → 7 → 4/10 or NaCl (mM): 10 → 150 → 10 → 150 → 10 → 150. Samples exposed to salt solutions, either for 1 hour or during a cycle, were prepared for analysis by removing the salt solution, submerging the sample in Milli-Q and then further rinsing the sample with a gentle flow of Milli-Q across the sample surface 10 times before being dried using a flow of nitrogen.
3.7 Vesicle preparation

1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (ammonium salt) (14:0 NBD PE), and 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt) (14:0 Liss Rhod PE) in powder form were supplied by Avanti Polar Lipids Inc., USA.

DOPC powder was dissolved in chloroform to a concentration of 25 mg/mL. 200 µL of the DOPC/chloroform solution was left to evaporate completely in the fume hood to form a lipid cake on the base of a glass vial. For fluorescence recovery after photobleaching (FRAP) experiments 1 wt% 14:0 Liss Rhod PE or 14:0 NBD PE was added to the DOPC mixture. Residual chloroform was removed by drying in a vacuum desiccator for a minimum of 2 hours. Lipid cakes were hydrated with a 10 mM Tris solution (pH 6.8-7.3) to a concentration of 5 mg/mL and the mixture was vortexed until the cake was fully hydrated. Chloride-free 10 mM HEPES or Tris-only buffers without pH adjustment were used for lipid cake hydration for the studies where the system was evaluated chloride ion free. Lipid vesicles were mechanically extruded by passing the lipid mixture through 100 nm polycarbonate filters (Whatman, UK) using either an Avanti Mini-Extruder (Avanti Polar Lipids Inc., USA) or a Lipsofast-Basic extruder (Avestin Inc., Canada) with 31 and 21 passes, respectively. Stock vesicle solutions were stored at 4 °C for no longer than one week. Immediately before use stock vesicles were diluted to a final concentration of 0.5 mg/mL in 10 mM Tris + 150 mM NaCl at the required pH. For experiments in NaCl-free conditions 10 mM Tris or 10 mM HEPES buffers were used for dilution depending on the experiment being undertaken.

3.8 Characterisation techniques

3.8.1 Surface wettability

The sessile drop method was used to measure static contact angles of Milli-Q water on plasma polymerised films deposited onto silicon wafer. Drops were deposited onto the sample using an FTA1000 instrument (First Ten Ångstroms Inc., USA). Images were captured with a Pelco Model PCHM 575-4 camera and analysed using a spherical fit in the FTA Windows Mode 4 software. Mean values were calculated from three individual samples (one analysis spot per sample) deposited in a single run placed at the same
distance away from the electrode. When comparing the effect of electrode distance on contact angle, values were considered significantly different when not overlapping by twice the error.

3.8.2 **Ellipsometry**

Ellipsometry measurements were undertaken using an M-2000IX® Spectroscopic Ellipsometry system (J.A. Woollam Co., Inc., USA) at ambient temperature. The angle of incidence was set to 65° and 70° using light of wavelength 210-1650 nm. A B-Spline model on top of a silicon substrate with native oxide layer was used to fit the acquired data using CompleteEASE software version 4.92 (J.A. Woollam Co., Inc., USA). The B-Spline model was selected as it is Kramers-Kronig consistent\[7\] accounting for adsorption in the sub 350 nm range. Quality of the fits was assessed using the ‘Uniqueness Fit’ option within the software to evaluate MSE values. Three analysis spots from each sample were analysed and a mean value was calculated. The standard deviation (SD) was calculated as a measure of the homogeneity of film thickness across the surface of an individual sample. The error is stated as ±1 nm when the standard deviation was < 0.5 nm. When comparing samples they were considered significantly different when there was no overlap of twice the error values.

3.8.3 **X-Ray photoelectron spectroscopy (XPS)**

XPS spectra were collected using a Kratos Axis Nova instrument (Kratos, Manchester, UK) with a monochromated Al K\textsubscript{α} source (Source energy 1486.69 eV) at a power of 150 W. Surface charging was compensated for by flooding with low-energy electrons. To minimise variations in the amount of surface charging (due to varying plasma polymer film thickness) silicon wafer substrates were mounted on a glass microscope slide using double-sided tape. Identification of elements was undertaken using survey spectra collected at a pass energy of 160 eV and a 1 eV step size. Coating chemistries were investigated using high resolution spectra collected at a pass energy of 20 eV and a step size of 0.1 eV. Data was converted to VAMAS format and analysed using CasaXPS software, version 2.3.15 (CASA Software Ltd., UK). Charge correction was applied by placing the hydrocarbon peak at a binding energy of 285 eV. The chemical shifts related to specific functional groups were taken from Beamson and Briggs Database of Polymer XPS spectra\[8\] unless otherwise stated. Instrument supplied sensitivity factors were used for quantification. Three analysis spots from each sample
were analysed and a mean value was calculated. The standard deviation (SD) was calculated as a measure of the homogeneity of the chemistry across the surface of an individual sample. The error is stated as ± 0.1% when the SD was < 0.05%. When comparing samples they were considered significantly different when there was no overlap of twice the error values.

3.8.4 Determination of carboxyl group density on ppAAc films using XPS derivatization

1-Ethyl-3-(3-Dimethylamino-propyl)carbodiimide hydrochloride (EDAC, 98% purity) and N-Hydroxy-succinimide (NHS, 98% purity) were purchased from Sigma-Aldrich, USA. 2,2,2-Trifluoroethylamine hydrochloride (TFEA, 98 % purity) was purchased from Alfa Aesar, USA.

Following deposition of the ppAAc films onto pieces of silicon wafer the samples were placed into a 24 well plate. Each sample was then submerged in 1 mL of a 0.1 M solution of EDAC in water for 30 minutes. Samples were then immersed in a 1 mL solution of NHS (0.125 M) + TFEA (0.126 M) in water for 2 hours. The samples were transferred to a separate plate for thorough rinsing with water before being dried with air and mounted for XPS analysis. Control samples were not exposed to EDAC and only submerged in the NHS + TFEA solution.

3.8.5 Dynamic light scattering (DLS)

Vesicle sizing was conducted using a Brookhaven 90Plus particle size analyser instrument (Brookhaven Instruments Corp., USA) at 25 °C using a 659 nm light source with scattered light detected at 90°. Data was collected and analysed using 90Plus Particle sizing Software (v. 3.85, Brookhaven Instruments Corp., USA).

100% DOPC vesicles were extruded in 10 mM Tris buffer and then diluted to the final concentration of 0.5 mg/mL using a 10 mM Tris or 10 mM Tris + 150 mM NaCl buffer solution at pH 7. Dynamic light scattering revealed that for both NaCl and NaCl-free dilution batches bimodal distribution was common. An example of this type of distribution is shown in Figure 3.5 where there is a population of vesicles varying from 197 to 308 nm in size and a second population varying from 33 to 43 nm in size. As previously shown such size variance does not affect collapse or fluidity of the resulting SLB.[9] The most frequently occurring vesicle size from the larger population of vesicles
was calculated as an average from three separate batches (Table 3.1). Generally vesicles diluted in buffer solution containing NaCl were larger than their NaCl-free counterparts. This was attributed to charge shielding effects enabling the vesicles to aggregate and/or fuse more readily. It is important to note that considerable variation between runs of a single batch existed and it is unclear if this was due to aggregation of the vesicles within the solution.

Table 3.1 Average vesicle diameter calculated for 100% DOPC vesicles diluted to a concentration of 0.5 mg/mL in 10 mM Tris or 10 mM Tris + 150 mM NaCl buffer solution at pH 7. For each batch two runs were conducted and an average calculated. The average diameter ± SD represents the variance between the three separate batches of DOPC vesicles.

<table>
<thead>
<tr>
<th>Dilution buffer</th>
<th>Average vesicle diameter (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mM Tris pH 7</td>
<td>169±4</td>
</tr>
<tr>
<td>10 mM Tris + 150 mM NaCl pH 7</td>
<td>258±4</td>
</tr>
</tbody>
</table>

Figure 3.5 Size distribution of DOPC vesicles diluted to a concentration of 0.5 mg/mL in 10 mM Tris + 150 mM NaCl buffer solution at pH 7.

3.8.6 Time-of-flight secondary-ion mass spectrometry (ToF-SIMS)
ToF-SIMS images of micropatterns on glass were collected using an ION-TOF ToF-SIMS IV instrument (ION-TOF GmbH, Germany) with all other spectra and images collected using an ION-TOF ToF-SIMS V instrument (ION-TOF GmbH, Germany)
with a bismuth manganese primary-ion source. The angle of incidence of the primary ion relative to the substrate was 45°. The analysis chamber was typically held at \( \sim 1.9 \times 10^{-10} \) mbar. Positive ion images were acquired for all samples. Images of micropatterned plasma polymers on glass were collected in spectroscopy mode using a Bi\(_3^{++}\) ion source at a kinetic energy of 50 keV over a 100 µm x 100 µm scan area (256 x 256 pixel). Images of plain plasma polymers and PMMA on Si were collected in spectrometry mode using a Bi\(_3^+\) ion source at a kinetic energy of 30 keV over a 100 µm x 100 µm scan area (128 x 128 pixels). Higher mass resolution images of nanopatterned substrates were collected in spectrometry mode using a Bi\(_1^+\) ion source at a kinetic energy of 30 keV. High spatial resolution images of nanopatterns were collected in ultimate or fast imaging modes using a Bi\(_3^{++}\) ion source at a kinetic energy of 60 KeV. Before analysis the positive ion spectra were calibrated using the CH\(_3^+\) (m/z 15.0229), C\(_2\)H\(_3^+\) (m/z 27.0229), C\(_3\)H\(_5^+\) (m/z 41.0385) and C\(_7\)H\(_7^+\) (m/z 91.0542) peaks for micro- and nanopatterned surfaces, C\(_2\)H\(_3^+\) (m/z 27.0229), C\(_3\)H\(_3^+\) (m/z 39.0229) and C\(_7\)H\(_7^+\) (m/z 91.0542) peaks for PMMA spectra, CH\(_3^+\) (m/z 15.023), CHO\(^+\) (m/z 29.0021), C\(_3\)H\(_7^+\) (m/z 43.0542) and C\(_7\)H\(_7^+\) (m/z 91.0542) peaks for ppAAc and CH\(_3^+\) (m/z 15.0229), C\(_2\)H\(_3^+\) (m/z 27.0229), C\(_3\)H\(_3^+\) (m/z 39.0229) and C\(_7\)H\(_7^+\) (m/z 91.0542) peaks for ppAAm. In some cases different sets of peaks were selected for calibration due to the saturation of signals for some standard peaks.

**Multivariate analysis of ToF-SIMS data**

Prior to multivariate analysis, data was prepared and exported using Surface Lab 6.4 (ION-TOF Gmbh, Germany). Multivariate analysis of ToF-SIMS image data was performed using MATLAB R2012b (MathWorks, Inc., USA), NBToolbox and ImageGUI (NESAC/BIO, The University of Washington, USA). NBToolbox can be accessed from the following link: http://www.nb.uw.edu/mvsa/multivariate-surface-analysis-homepage.

Raw measurement (.itm) files were opened in Surface Lab 6.4. Peaks with an intensity greater than 100 counts, over the mass range typically between m/z 0-100, were selected to create a peak list. The integration limits of each peak were checked to ensure suitable areas were assigned to each peak. The peak list was then used to reconstruct ToF-SIMS image data which was exported as a .bif6 file. The .bif6 file was exported into the ImageGUI of the NBToolbox operating within MATLAB. Before performing
multivariate analysis, data was normalised to total ion counts for each spectra/image and mean centred using the ImageGUI as described previously.[6]

3.8.7 Auger electron spectroscopy (AES)

AES was conducted using a PHI 710 Scanning Auger Nanoprobe instrument (ULVAC-PHI, Japan and Physical Electronics, USA). The main chamber was typically pumped down to $1.0 \times 10^{-9}$ torr. For analysis, an electron beam of 3 keV at 1 nA was used. For ion bombardment the sample was exposed to a 2 keV Argon-ion beam (2 mm x 2 mm raster). Data was acquired using SmartSoft-AES (ULVAC-PHI, Japan, USA) and analysed using MultiPak (ULVAC-PHI, Japan, USA). Line scan data was analysed using a binomial smooth function.

3.8.8 Scanning electron microscopy (SEM)

Samples were coated with a 10 nm thin coating of Au deposited by plasma sputtering using an AXXIS instrument (Kurt J. Lesker, USA). SEM imaging of plasma polymer micro- and nanopatterns was conducted using a RAITH150 Two instrument (Raith, Germany, EHT = 5 or 20 kV).

3.8.9 Atomic Force Microscopy (AFM)

Characterisation of plasma polymer film thickness

As shown in Figure 3.6 a polystyrene masking technique[10] was used to obtain a step between the plasma polymer film and silicon wafer substrate. A 100 mg/mL solution of polystyrene (Acros Organics, USA) in toluene (Sigma-Aldrich, USA) was applied to a small area of a clean silicon substrate. The sample was left under the fume hood overnight to enable complete evaporation of the solvent leaving a polystyrene mask. Following plasma polymer deposition the polystyrene film was carefully removed using tweezers. The samples were then sonicated in acetone for 3 minutes to remove any residual polystyrene on the samples.

1. Apply polystyrene mask
2. Plasma polymer coating
3. Remove mask

Figure 3.6 Schematic representation of the polystyrene masking process for plasma polymer thickness analysis.
AFM was conducted using a Multimode VIII instrument (Bruker, USA) under ambient conditions in air. Topography images were obtained in ScanAsyst (Peak Force Tapping) mode using ScanAyst-Air cantilevers with nominal spring constant 0.4 N·m⁻¹ (Bruker, USA). Images were flattened before analysis using the Nanoscope Analysis software (v.1.40, Bruker, USA).

Figure 3.7A shows an AFM image of the interface between a ppAAalc coating applied close to the electrode and the underlying silicon. To measure film thickness the vertical height difference was measured from a line scan as shown in Figure 3.7B. This process was repeated three times on each sample. A mean value and standard deviation (SD) was then calculated. Where the SD value was < 0.5 nm an error of ± 1 nm was stated. When comparing samples they were considered significantly different when there was no overlap of twice the error values.

Figure 3.7 Film thickness measurement of ppAAalc measured using AFM. A) AFM image of the step interface between a ppAAalc coating and silicon wafer substrate and B) a line scan across the step.

**Lipid interactions with Au QCM-D crystals**

All AFM measurements were acquired using a Bruker Multimode VIII AFM with NanoScope V controller and silicon nitride ScanAsyst-Air tips. The QCM-D gold sensor was freshly ultrasonicated in ethanol and placed into an AFM fluid cell and pH 4 buffer solution was introduced into the cell and left to equilibrate for an hour prior to imaging. Images and forces curves were collected of the gold substrate. Extruded DOPC (100 nm pore) vesicles (5 mg/mL) in pH 7 buffer solution were diluted 10 times
in pH 4 buffer solution and introduced into the fluid cell. The system was allowed to equilibrate for 1 h before AFM images and force curves were acquired in tapping mode with all the parameters including set-point, scan rate and feedback gains adjusted to optimise image quality and minimise imaging force. All force distance curves were acquired using a cantilever deflection of 100 nm and a z-scanner ramp size of 200 nm. Scanners were calibrated in the x, y and z using silicon calibration grids (Bruker model numbers PG: 1 µm pitch, 100 nm depth and VGRP: 10 µm pitch, 180 nm depth). Height images were analysed using NanoScope Analysis (v.1.40 Bruker, USA).

**AFM of micropatterns**

Topography images were obtained with a FastScan AFM (Bruker, USA) under ambient conditions in air. Quantitative nanomechanical mapping (PeakForce QNM) mode was used together with ScanAsyst-Air cantilevers (nominal spring constant 0.4 N·m⁻¹). Images were flattened before analysis using the Nanoscope Analysis software (v.1.40, Bruker, USA).

A comparative study was conducted to assess the best method for characterisation of feature heights on the dual micropatterned surfaces. The first method was to use line scans for assessing feature height as shown in Figure 3.8. Each line scan provided two edges which could be used to measure the height of the feature (Figure 3.8A and B).

These single line scan measurements were compared with the average from 10 line scans (twenty edges) (Figure 3.8C) and the depth analysis tool (across the single feature) in the NanoScope Analysis software (Table 3.2). The feature height measured using 10 line scans (20 points) and the depth analysis tool provided the same feature height. The line scan method was far more time consuming to conduct, therefore the depth analysis tool was deemed suitable for measuring individual feature heights on the micropatterned surfaces.
Figure 3.8 Measuring feature height using line scans across the feature. A) A single line scan across a ppOct circle patterned onto ppAAc, B) a single line scan can be used to measure the feature height at two points and C) 10 line scans were placed on the feature to measure the feature height at 20 different points.

Table 3.2 Comparing height measurements from section and depth analysis methods for a single feature. 10 µm diameter ppOct circles on ppAAc.

<table>
<thead>
<tr>
<th>Feature height in nm</th>
<th>Depth analysis (single line, 2 points)</th>
<th>Section analysis (average of 20 points) Mean value (± standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>121</td>
<td>120, 122</td>
</tr>
<tr>
<td></td>
<td></td>
<td>121±1</td>
</tr>
</tbody>
</table>
AFM of nanopatterns
AFM was conducted using a Dimension Icon instrument (Bruker, USA) under ambient conditions in air. Topography images were obtained in ScanAsyst (Peak Force Tapping) mode using ScanAsyst-Air cantilevers with spring constant 0.4 N m$^{-1}$ (Bruker, USA). Images were flattened before analysis using the Nanoscope Analysis software (v.1.40, Bruker, USA). Widths of nanopatterned features were approximated using FWHM methods. Three measurements were taken from each feature. The average of the values and the standard deviation (SD) were calculated. Where the SD < 0.5 nm an error of ± 1 nm was stated.

3.8.10 Quartz crystal microbalance with dissipation (QCM-D)
QCM-D measurements were conducted using a Q-Sense E4 instrument (Q-Sense, Sweden). Freshly cleaned sensor crystals or plasma coated crystals were placed in the measurement chambers before flooding with buffer solution. Prior to the measurement period resonant frequencies (overtones 3, 5 and 7) for all crystals were measured in air and then Milli-Q water. $\Delta F_5$ is presented throughout this thesis. The temperature of the QCM-D chamber was set to 37 °C unless otherwise stated. Once the baseline for both frequency and dissipation had stabilised the buffer and vesicle solutions were introduced into the measurement chambers. When operated in liquid the resolution of the frequency and dissipation is quoted as being on the order of ± 0.1 Hz and 1 x10$^{-7}$ respectively.$^{[11]}$

3.8.11 Electrochemical impedance spectroscopy (EIS)
EIS measurements were conducted using an Autolab PGSTAT 12 spectrometer (Eco Chemie). Impedance spectra were recorded for frequencies between 2 mHz and 30 kHz at 0 V bias potential with a 10 mV AC modulation amplitude signal. Customised polytetrafluoroethylene cells were used with an active area 0.283 cm$^2$ and a fluid volume of ~ 1 mL. A three electrode system was employed, consisting of the Au-coated glass slide as the working electrode, a leakless miniature Ag/AgCl reference electrode (eDAQ Pty Ltd) and a platinum wire (Sigma) as the counter electrode. After addition of a new electrolyte solution spectra were measured continuously until no further change in impedance was observed. Data analysis was performed using ZVIEW version 2.7 (Scribner Associates, Inc.). The raw data was normalised to the surface area of the working electrode and spectra were corrected for noise and electronic artefacts. The
data is represented as Bode plots where the absolute impedance (|Z|) and phase (θ) are plotted as a function of frequency. In this representation, ideal capacitors display a slope of \(|Z| = -1 \ \Omega \ \text{Hz}^{-1}\) and a phase shift of \(\theta = -90^\circ\) whereas ideal resistors display a slope of \(|Z| = 0 \ \Omega \ \text{Hz}^{-1}\) and a phase shift of \(\theta = 0^\circ\). To gain more detailed information on the properties of the plasma polymer films EIS spectra were modelled using electrical equivalent circuits (EECs) composed of resistors (R) and constant phase elements (CPEs). A smoothing spline fit (Origin) was applied to the model fits.

3.8.12 Optical profilometry

Samples were first coated with 10 nm of Au using an AXXIS instrument (Kurt J. Lesker, USA). Micro- and nanopatterned topographies were characterised using a Contour GT-K1 3D Optical Profiler (Bruker, USA). Phase shifting interferometry (PSI) and vertical scanning interferometry (VSI) modes were used depending on feature height with 50x and 115x objectives. Images were flattened by applying a Terms mask using the Vision software (v.4.20, Bruker, USA). Further image preparation and processing was conducted in Photoshop CS6 (Adobe, USA) and Image J (v.1.45s, National Institutes of Health, USA). Widths of nanopatterned features were approximated using FWHM methods as demonstrated in Figure 3.9.

![Figure 3.9 Example graph showing how feature width was approximated from 3D profilometry data using FWHM methods.](image-url)

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3.8.13 Confocal laser scanning microscopy (CLSM) and fluorescence recovery after photobleaching (FRAP)

Plain or plasma polymer coated glass No. 1 coverslips were mounted as shown in Figure 3.10 using ibidi Culture Inserts (ibidi GmbH, Germany). Glass controls were first rinsed in 10 mM Tris + 150 mM NaCl buffer (pH 7), fluorescent vesicles (diluted to 0.5 mg/mL immediately before experiment using 10 mM Tris + 150 mM NaCl pH 7) were then added to the chambers and left to incubate for 10 minutes. The individual chambers were then rinsed by removing 50 µL of the solution and replacing it with 50 µL of fresh buffer solution 10 times. For plasma polymers 10 mM Tris + 150 mM NaCl buffer (pH dependent on experiment) was first incubated on the surface for 10 minutes before applying the vesicle solution (diluted to 0.5 mg/mL immediately before use with 10 mM Tris + 150 mM NaCl buffer of appropriate pH for the experiment) for 10 minutes and then rinsing as previously described.

Confocal microscopy imaging and FRAP bleaching was conducted using an FV1000 IX81 confocal microscope (Olympus, Japan) using a 40x oil immersion lens (UPLAPO, NA 1.00, Olympus, Japan) and 300 µm pinhole. Images were collected using FLUOVIEW software (v. 1.7c, Olympus, Japan) and analysed using FLUOVIEW Viewer (v. 4.0a, Olympus, Japan). Areas were bleached using the Tornado scan setting at 100µs/pixel, images were typically collected using a fast scan speed (2.0 µs/pixel) to minimise bleaching of the imaging area overtime. Samples were also viewed using epi-fluorescence microscopy with filter cubes U-MNIBA (green emission, excitation: 470-490 nm) and U-MWIG2 (red emission, excitation: 510-550 nm).
For glass and unpatterned plasma polymer substrates incubated with DOPC vesicles doped with 1 wt% 14:0 NBD PE, excitation was conducted using a 488 nm laser which was used for both bleaching (100% laser intensity, 40 seconds) and imaging (2% laser intensity). Substrates incubated with DOPC vesicles doped with 1 wt% 14:0 Liss Rhod PE were excited using a 543 nm laser which was used for both bleaching (100% laser intensity, for 60 seconds) and imaging (4% laser intensity). For glass and unpatterned plasma polymer surfaces the bleach spot was kept constant throughout (diameter ~50 µm). Line profiles were generated from exported greyscale images using Image J software (v.1.45s, National Institutes of Health, USA).

For patterned substrates, areas were bleached using both the 488 nm and 543 nm laser simultaneously (100 % intensity, 60 seconds). Images were then collected sequentially by first exciting using the 488 nm laser (2% intensity) and then with the 543 nm laser (2% intensity). Images were typically collected every minute and up to 10 minutes after the initial bleach.
3.9 References


4 Characterisation of plasma polymers

4.1 Introduction

To understand how lipid vesicles interact with plasma polymer coatings it is necessary to investigate both the chemical and physical properties of the films. Furthermore, the use of plasma polymers in physiologically relevant settings necessitates an understanding of plasma polymer behaviour in aqueous environments. The following chapter describes the physiochemical characterisation of plasma polymerised acrylic acid (ppAAc), allylamine (ppAAm), allyl alcohol (ppAAlc) and 1,7-Octadiene (ppOct) films used in this thesis. The influence of substrate position relative to the electrode was investigated using a standard set of deposition parameters to fabricate the films. A number of techniques were used to characterise the films including contact angle to assess the surface wettability, X-ray photoelectron spectroscopy (XPS) to investigate chemistry and ellipsometry and atomic force microscopy (AFM) to measure film thickness. Quartz crystal microbalance with dissipation (QCM-D) was employed to explore film stability and swelling in-situ whilst electrochemical impedance spectroscopy (EIS) was used to examine structural changes occurring within the films upon exposure to solutions of different ionic strength and pH.

XPS data was collected by Dr. Thomas Ameringer (Swinburne University of Technology) and Dr. Robert Jones (ANFF-Victoria XPS facility, La Trobe University) and analysed by HJA. AFM data was collected by Dr. Hayden Webb (Swinburne University of Technology) and analysed by HJA. Ellipsometry data was collected and modelled by Dr. Thomas Ameringer (Swinburne University of Technology). EIS data was collected and analysed by Dr. Jackie Knobloch and Dr. Ingo Köper (Flinders University).

Parts of this chapter have been modified from the following papers:


4.2 Results

4.2.1 Surface wettability

Static water contact angles were measured for ppAAc, ppAAm, ppAAlc and ppOct coatings deposited onto silicon wafer substrates placed at varying distances from the electrode (Table 4.1). ppOct provided a hydrophobic surface at all positions within the reactor with a small but significant increase in contact angle from 92° at 3 cm to 97° at 21 cm. ppAAm and ppAAlc films were moderately hydrophilic with contact angles of 61° and 71°, respectively, at the position closest to the electrode with no significant change in the contact angle depending on reactor location. Acrylic acid produced the most hydrophilic films of all the monomer chemistries investigated. The films became more hydrophilic away from the electrode, measuring 50° at 3 cm and 44° at 20 cm. Contact angles reported in the literature vary considerably for plasma polymers deposited from the same monomers used here.[1-4] Deposition parameters and reactor design contribute to changes in plasma polymer properties making direct comparisons between coatings produced in different laboratories difficult.[5]

Table 4.1 Static water contact angles measured for plasma polymerised thin films at different positions within the reactor. The distance from electrode was measured between the electrode and the centre of the samples. The mean values are calculated from 3 individual samples (one analysis spot per sample) deposited in a single run with error of ± 1°.

<table>
<thead>
<tr>
<th>Distance from electrode (cm)</th>
<th>Contact angle (θ)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ppAAc</td>
</tr>
<tr>
<td>3-4</td>
<td>50</td>
</tr>
<tr>
<td>11-12</td>
<td>48</td>
</tr>
<tr>
<td>20-21</td>
<td>44</td>
</tr>
</tbody>
</table>
4.2.2 XPS of plain plasma polymer films
XPS analysis was performed to examine the chemical composition of ppAAc, ppAAlc, ppAAm and ppOct plasma polymer coatings deposited at various positions within the chamber.

4.2.2.1 ppAAc
The atomic composition of ppAAc coatings deposited at different positions within the reactor are shown in Table 4.2. The presence of both carbon and oxygen at the surface of the coatings was unsurprising given the molecular structure of the acrylic acid monomer (C\(_3\)H\(_4\)O\(_2\)). As the distance from the electrode increased, the oxygen content of the film also increased. This was reflected in the O/C ratio which rose from 0.27 to 0.38 as the sample moved from 3 cm to 20 cm away from the electrode. These values are consistent with the wide range of compositions for ppAAc films reported in the literature.\(^6\) Depending on the substrate position, the O/C ratio for these coatings was between 43-60 % lower than the theoretical value of 0.67. This can be attributed to the nature of plasma polymer films and deposition processes inside the reactor. The fragmentation of the monomer, subsequent deposition onto the substrate surface and the retention of functional groups is known to be dependent on many factors including reactor shape, sample position and deposition parameters such as flow rate and power.\(^5\) Small traces of Si contamination were also detected and likely originate from the mounting tape used to secure the samples onto the sample platen during XPS.

Table 4.2 XPS atomic composition and O/C ratio of ppAAc films deposited at different distances from the electrode onto silicon wafer at 20 W for 20 min at a flow rate of 1.5 sccm. The mean values (± standard deviation) are calculated from 3 analyses per sample.

<table>
<thead>
<tr>
<th>Distance from electrode (cm)</th>
<th>Atomic composition (%)</th>
<th>Atomic ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C 1s</td>
<td>O 1s</td>
</tr>
<tr>
<td>3</td>
<td>78.4±0.1</td>
<td>21.2±0.1</td>
</tr>
<tr>
<td>11</td>
<td>74.3±0.2</td>
<td>25.4±0.2</td>
</tr>
<tr>
<td>20</td>
<td>72.3±0.1</td>
<td>27.2±0.1</td>
</tr>
</tbody>
</table>

The survey scans for each position are shown in Figure 4.1. Clear C 1s and O 1s peaks were present for all coatings. Spectra for the 20 cm sample exhibited a rising background in the low binding energy region of the spectra. This indicated that the film was ~10 nm thick with the increasing signal associated with attenuation of Si from the substrate.
High resolution C 1s spectra were collected for ppAAc coatings to investigate the functionality retained in the films from the starting acrylic acid monomer and are shown in Figure 4.2. Five component peaks were fitted for each of the spectra and included hydrocarbon (C-C, C-H, at ~285 eV), β-shift from carboxylic acids and esters (C-COOH/R at ~285.7 eV), alcohols and ethers (C-O at ~286.6 eV), ketones/aldehydes (C=O at ~287.9 eV) and carboxylic acids and esters (COOH/R at ~289.2 eV). Analysis clearly indicated that the size of the COOH/R component was dependent on sample position. This difference was quantified in Table 4.3 where there is a clear increase in the COOH/R content from 9.2 % to 16.0 % as the sample was located further away from the electrode.

Analysis of the high resolution C 1s spectra provided some indication that carboxylic acid functionality of the acrylic acid monomer had been retained, however, the COOH/R component includes both carboxylic acids and esters. 2,2,2-trifluoroethylamine (TFEA) derivatization was used to confirm the presence of carboxylic acid species.\(^7\) The atomic composition measured for ppAAc coatings deposited at different reactor positions, following derivatization, is shown in Table 4.4. There was a clear trend with increasing N 1s and F 1s content of the films further away from the electrode.
from the electrode which correlates with an increase in the COOH/R component of the ppAAc films. Aside from one analysis spot, the control samples showed negligible levels of fluorine compared with the activated samples indicating successful binding of TFEA. However, the overall low levels of F1s suggested that the reaction had not gone to completion.

Figure 4.2 XPS C 1s spectra curve fitted for ppAAc deposited onto silicon wafer at 20 W for 20 min at a flow rate of 1.5 sccm. The functional groups related to each peak are hydrocarbon (C-C, C-H), β-shift from carboxylic acids and esters (C-COOH/R), alcohols and ethers (C-O), ketones/aldehydes (C=O) and carboxylic acids and esters (COOH/R).

Table 4.3 XPS C 1s peak assignment for ppAAc deposited onto silicon wafer at difference distances from the electrode. Functional groups: Hydrocarbons (C-H, C-C), alcohols and ethers (C-O), β-shift from carboxylic acids and esters (C-COOH/R), ketones and aldehydes (C=O), carboxylic acids and esters (COOH/R).

<table>
<thead>
<tr>
<th>Component (%)</th>
<th>Component (C-H, C-C)</th>
<th>Component (C-COOH/R)</th>
<th>Component (C-O)</th>
<th>Component (C=O)</th>
<th>Component (COOH/R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance from electrode (cm)</td>
<td>3</td>
<td>63.5</td>
<td>9.2</td>
<td>11.7</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>54.7</td>
<td>13.5</td>
<td>11.9</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>53.7</td>
<td>16.0</td>
<td>9.3</td>
<td>5.0</td>
</tr>
</tbody>
</table>
Table 4.4 XPS atomic composition of ppAAc films deposited at different distances from the electrode following TFEA derivatization. The mean values (± standard deviation) are calculated from 3 analyses per sample. \(^a\)0.7% F 1s measured at 1/3 analysis spots.

<table>
<thead>
<tr>
<th>Distance from electrode (cm)</th>
<th>C 1s</th>
<th>O 1s</th>
<th>N 1s</th>
<th>F 1s</th>
<th>Si 2p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>77.8±0.4</td>
<td>22.2±0.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>76.0±0.1</td>
<td>20.9±0.2</td>
<td>1.9±0.1</td>
<td>1.1±0.1</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>75.4±0.5</td>
<td>24.6±0.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>73.0±0.1</td>
<td>22.6±0.2</td>
<td>2.9±0.1</td>
<td>1.6±0.1</td>
<td>-</td>
</tr>
</tbody>
</table>

4.2.2.2 ppAAlc

The atomic compositions of ppAAlc coatings deposited at different positions within the reactor are shown in Table 4.5. As with ppAAc, all coatings contained both carbon and oxygen which is expected given the molecular structure of the allyl alcohol monomer (C\(_3\)H\(_6\)O). Oxygen content of the films increased between 3 and 12 cm away from the electrode with no significant increase between 12 and 20 cm. Traces of Si contamination (0.1-0.2%) were present, which was attributed to the adhesive tape used to secure the samples onto the sample platen during XPS.

Table 4.5 XPS Atomic composition and O/C ratio of ppAAlc deposited at different distances from the electrode onto silicon wafer at 20 W for 20 min at a flow rate of 1.5 sccm. The mean values (± standard deviation) are calculated from 3 analyses per sample.

<table>
<thead>
<tr>
<th>Distance from electrode (cm)</th>
<th>C 1s</th>
<th>O 1s</th>
<th>Si 2p</th>
<th>O/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>89.1±0.1</td>
<td>10.8±0.1</td>
<td>0.1±0.1</td>
<td>0.12</td>
</tr>
<tr>
<td>Control</td>
<td>87.5±0.2</td>
<td>12.4±0.2</td>
<td>0.2±0.1</td>
<td>0.14</td>
</tr>
<tr>
<td>Control</td>
<td>87.2±0.2</td>
<td>12.6±0.1</td>
<td>0.2±0.1</td>
<td>0.14</td>
</tr>
</tbody>
</table>
Figure 4.3 shows survey scans collected from ppAAlc films deposited at different distances from the electrode. O 1s and C 1s peaks are present for all of the samples. As observed with ppAAc, the spectra collected from the ppAAlc film deposited furthest away from the electrode (20 cm) exhibited rising background, in the low binding energy region of the spectra. This is due to the film thickness approaching the sampling depth of the XPS (~ 10 nm) when positioned 20 cm away from the electrode.

![Figure 4.3 XPS survey scans of ppAAlc films deposited at different distances from the electrode.](image)

As shown in Figure 4.4 high resolution C 1s spectra for the ppAAlc films were fitted with five component peaks including hydrocarbon (C-C, C-H) at 285 eV, β-shift from carboxylic acids and esters (C-COOH/R) at 285.7 eV, alcohols and ethers (C-O) at ~286.3 eV, ketones/aldehydes (C=O) at ~287.9 eV and carboxylic acids and esters (COOH/R) at ~289.2 eV. This is consistent with previous high resolution C 1s data of ppAAlc with dominant hydrocarbon and alcohol/ether components. [8]
Figure 4.4 XPS C 1s spectra curve fitted for ppAAle deposited onto silicon wafer at 20 W for 20 min at a flow rate of 1.5 sccm. The functional groups related to each peak are hydrocarbon (C-C,C-H), β-shift from carboxylic acids and esters (C-COOH/R), alcohols and ethers (C-O), ketones and aldehydes (C=O) and carboxylic acids and esters (COOH/R).
4.2.2.3 ppAAm

The atomic compositions of ppAAm films deposited at different positions within the reactor are shown in Table 4.6. The starting monomer allylamine (C₃H₇N) gave rise to coatings containing both carbon and nitrogen. The films also contained oxygen due to oxidisation of the coatings following exposure to the atmosphere. This has been previously reported for ppAAm films.[⁹] For all samples, the O/C ratio remained constant at 0.04. There was a minimal increase in nitrogen content of the films between 3 and 11 cm and no significant difference between the nitrogen content of the 11 and 20 cm films. A significant Si 2p component (1.1±0.1) was present for films deposited 20 cm away from the electrode. This was attributed to the technique probing the underlying silicon wafer substrate as the film thickness approached the sampling depth of XPS (~10nm). This was confirmed by the presence of a rising background in the wide scan spectra (Figure 4.5) and a doublet structure of the Si 2p signal, indicative of the substrate Si (99 eV) and SiOₓ (101 eV).

Table 4.6 XPS atomic composition and O/C ratio of ppAAm deposited at different distances from the electrode onto silicon wafer at 20 W for 20 min at a flow rate of 1.5 sccm. The mean values (± standard deviation) are calculated from 3 analyses per sample.

<table>
<thead>
<tr>
<th>Distance from electrode (cm)</th>
<th>C 1s</th>
<th>O 1s</th>
<th>N 1s</th>
<th>Si 2p</th>
<th>O/C</th>
<th>N/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>84.6±0.2</td>
<td>3.2±0.2</td>
<td>12.0±0.1</td>
<td>0.2±0.1</td>
<td>0.04</td>
<td>0.14</td>
</tr>
<tr>
<td>11</td>
<td>83.3±0.3</td>
<td>3.3±0.1</td>
<td>13.1±0.3</td>
<td>0.3±0.1</td>
<td>0.04</td>
<td>0.16</td>
</tr>
<tr>
<td>20</td>
<td>82.8±0.3</td>
<td>3.2±0.1</td>
<td>13.0±0.2</td>
<td>1.1±0.1</td>
<td>0.04</td>
<td>0.16</td>
</tr>
</tbody>
</table>
Figure 4.5 XPS survey scans of ppAAm films deposited at different distances from the electrode.

High resolution C 1s spectra for the ppAAm films were fitted with four component peaks,\textsuperscript{[10, 11]} including hydrocarbon (C-C, C-H) at 285 eV, amines (C-N) at 286.1 eV, alcohols and ethers (C-O), imines (C=N), nitriles (C≡ N) and the secondary shift from nitrile groups (C≡C=N) at ~287 eV and finally ketones and aldehydes (C=O) and amides (N\textsuperscript{C}=O) at ~288 eV.
Figure 4.6 XPS C 1s spectra curve fitted for ppAAm deposited onto silicon wafer at 20W for 20 min at a flow rate of 1.5 sccm. The functional groups related to each peak are hydrocarbon (C-H, C-C), amines (C-N), alcohols and ethers (C-O), imines (C=N), nitriles (C≡ N), secondary shift from nitrile groups (C=C≡ N), ketones/aldehydes (C=O) and amides (NC=O).
4.2.2.4 ppOct
Survey spectra of the plasma polymerised 1,7-Octadiene films showed clear C 1s and O 1s peaks (Figure 4.7). The atomic compositions of ppOct films, deposited at different positions within the reactor, are shown in Table 4.7. All films contained both carbon and oxygen. There was no significant dependence of oxygen content on sample position. As with the ppAAm films, the oxygen content can be attributed to the reaction between radicals trapped within the film and atmospheric oxygen. High resolution C 1s spectra for the ppOct films (Figure 4.8) were fitted with two component peaks including hydrocarbon (C-C, C-H) at 285 eV and a broader peak at ~286.2 eV, containing alcohols and ethers (C-O) and ketones and aldehydes (C=O).

Table 4.7 XPS atomic composition and O/C ratio of ppOct deposited at different distances from the electrode onto silicon wafer at 20 W for 20 min at a flow rate of 1.5 sccm. The mean values (± standard deviation) are calculated from 3 analyses per sample.

<table>
<thead>
<tr>
<th>Distance from electrode (cm)</th>
<th>C 1s (%)</th>
<th>O 1s (%)</th>
<th>Si 2p (%)</th>
<th>O/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>95.7±0.2</td>
<td>4.0±0.1</td>
<td>0.3±0.1</td>
<td>0.04</td>
</tr>
<tr>
<td>12</td>
<td>96.3±0.2</td>
<td>3.5±0.2</td>
<td>0.3±0.1</td>
<td>0.04</td>
</tr>
<tr>
<td>20</td>
<td>96.9±0.1</td>
<td>2.9±0.1</td>
<td>0.2±0.1</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Figure 4.7 Survey scans of ppOct films deposited at different distances from the electrode.
Figure 4.8 XPS C 1s spectra curve fitted for ppOct deposited onto silicon wafer at 20 W for 20 min at a flow rate of 1.5 scem. The functional groups related to each peak are hydrocarbon (C-H, C-C), alcohols and ethers (C-O) and ketones and aldehydes (C=O).
4.2.3 Film thickness measurements using AFM and ellipsometry

Films of ppAAc, ppAAm, ppOct and ppAAlc were deposited onto partially masked substrates placed at different positions within the reactor. Once the mask was removed to reveal the uncoated substrate (details described in Chapter 3), film thickness was measured by collecting AFM topography images of the film edge and placing three separate line scans across the feature in the image (Table 4.8). The results demonstrated reduced film thickness away from the electrode for all chemistries suggesting lower deposition rates at this position. Each of the plasma polymers deposited at different rates, with ppAAm producing the thinnest films at the end of the 20 minute deposition period.

Table 4.8 Plasma polymer film thickness measured using AFM line scan data from partially masked samples. The mean values (± standard deviation) are calculated from 3 analyses per sample.

<table>
<thead>
<tr>
<th>Distance from the electrode (cm)</th>
<th>Film thickness (nm)</th>
<th>ppAAc</th>
<th>ppAAm</th>
<th>ppOct</th>
<th>ppAAlc</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>51±2</td>
<td>43±1</td>
<td>83±5</td>
<td>67±3</td>
<td></td>
</tr>
<tr>
<td>11-12</td>
<td>42±1</td>
<td>26±1</td>
<td>57±1</td>
<td>43±1</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>20±2</td>
<td>16±2</td>
<td>30±1</td>
<td>20±3</td>
<td></td>
</tr>
</tbody>
</table>

The masking technique is invasive and can create edge effects during removal of the mask, introducing residual plasma polymer and polystyrene material. Furthermore, the use of acetone to remove residual polystyrene could affect film thickness. Ellipsometry was employed to measure film thickness before and after 3 minutes sonication in acetone in order to investigate these effects. Table 4.9 shows the untreated and treated film thickness measured for all plasma polymers at different positions within the reactor. The acetone treatment caused little or no difference to the thickness of all but one of the plasma polymer films, with a change in thickness of ± 1 nm often observed. This is within the range one would expect from both the technique and an individual sample and is therefore not a significant difference. The only film observed to exhibit a significant change in thickness was the ppAAc film deposited at 20 cm where film thickness was reduced from 23 nm to 16 nm. This suggested a less stable film and a loss of low MW materials.

Film thicknesses could vary between plasma polymerisation runs, however, the effect was most acute at the electrode. For example, in Table 4.8 ppAAm has a thickness of 100
43±1 nm 3 cm away from the electrode in contrast to 29±1 nm measured in a second run, as measured by ellipsometry after sonication in acetone (Table 4.9). Interestingly, in the latter case the film thickness at the electrode was the same as that at the middle of the electrode. For all of the other chemistries fabricated, the differences between film thicknesses for the two runs at different distances from the electrode were not significant. Film thickness variation at the electrode for ppAAm could be explained by greater differences in energy distribution over smaller distances at the electrode. It is known that there are different zones within a plasma glow with different characteristics, affecting the resultant plasma polymer film\cite{12}. Further investigation is required to explore the plasma characteristics for the reactors used in this work. Crucially, however, significant differences in chemistry between runs were not observed.

Table 4.9 Ellipsometry measurements of film thickness collected before and after sonication in acetone for 3 minutes. The mean values (± standard deviation) are calculated from 3 analyses per sample.

<table>
<thead>
<tr>
<th>Distance from electrode (cm)</th>
<th>Untreated thickness (nm)</th>
<th>Treated thickness (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ppAAc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>58±1</td>
<td>57±1</td>
</tr>
<tr>
<td>11</td>
<td>44±1</td>
<td>45±1</td>
</tr>
<tr>
<td>20</td>
<td>23±1</td>
<td>16±1</td>
</tr>
<tr>
<td>ppAAm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>30±1</td>
<td>29±1</td>
</tr>
<tr>
<td>11</td>
<td>28±1</td>
<td>29±1</td>
</tr>
<tr>
<td>20</td>
<td>18±1</td>
<td>18±1</td>
</tr>
<tr>
<td>ppOct</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>95±1</td>
<td>94±1</td>
</tr>
<tr>
<td>12</td>
<td>56±1</td>
<td>57±1</td>
</tr>
<tr>
<td>20</td>
<td>31±1</td>
<td>31±1</td>
</tr>
<tr>
<td>ppAAlc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>63±1</td>
<td>62±2</td>
</tr>
<tr>
<td>12</td>
<td>42±1</td>
<td>42±1</td>
</tr>
<tr>
<td>20</td>
<td>22±1</td>
<td>22±1</td>
</tr>
</tbody>
</table>
4.2.4 The dependence of reactor position on swelling behaviour and stability of plasma polymer films

Given the requirement for the use of aqueous environments in the formation of lipid structures using vesicle collapse, QCM-D was used to investigate both the swelling behaviour and stability of the plasma polymer films upon exposure to Milli-Q water and 150 mM PBS buffer in situ. XPS was used to characterise surface chemistry and film stability following exposure to aqueous conditions.

4.2.4.1 ppAAc

Figure 4.9 shows the frequency and dissipation plots for ppAAc films deposited at different positions within the reactor. A gold coated QCM-D crystal was used as a control surface. The results showed that the behaviour of the ppAAc films was highly dependent on the location of the sample within the reactor. Figure 4.9A shows how the introduction of the first PBS rinse step caused a decrease in the frequency for all of the surfaces. The film deposited at 3 cm exhibited similar behaviour to the gold control, with $\Delta f$ stabilising at -17 Hz. This response correlates with a buffer effect, caused by the sensitivity of the instrument, to a change in ionic concentration and pH as the solution changed from Milli-Q water to PBS.$^{[13, 14]}$ Both the films deposited at 11 cm and 20 cm showed decreases in $\Delta f$, indicating mass uptake at the surface. The kinetics and scale of the mass uptake were greatest on the sample positioned 20 cm from the electrode. Rinsing with Milli-Q water induced an increase in frequency for all of the surfaces with the 3 cm film returning to the Milli-Q baseline. The other films did not return to their initial frequency, with the effect most pronounced for the 20 cm sample. The kinetics of the frequency shift in Milli-Q water was slower than observed for the mass uptake during the PBS stage. Interestingly, the ppAAc films exhibited two distinct phases of mass loss with a faster initial stage and a slower second stage. This two-stage behaviour was not present during either of the PBS rinse stages for any of the surfaces.

The changes in dissipation for the 3 and 11 cm samples were similar to that of the gold control (Figure 4.9B), suggesting minimal or no changes in the nature of the coatings. Rinsing of the 20 cm film with PBS caused a dissipation increase of over $4 \times 10^{-6}$ (around double that of the other surfaces), indicating that the films were swelling through the uptake of water and ions from the buffer. Upon rinsing with Milli-Q water the dissipation of the film was reduced to less than $1 \times 10^{-6}$, demonstrating that the swelling behaviour of the film was pH and/or ionic strength related.
Figure 4.9 QCM-D frequency (A) and dissipation plots (B) for ppAAc films deposited 3 cm (dark grey), 11 cm (mid grey) and 20 cm (light grey) away from the electrode compared with an Au control (black) when exposed to Milli-Q water (MQ) or 150 mM PBS solutions at 37°C. Time markers show the point at which the pump was started with the new solution. The delay in response is due to the solution travelling through tubing prior to entering the chamber.

To further investigate the effect of Milli-Q and PBS exposure on film stability and surface chemistry, XPS was used to characterise the ppAAc films. Plasma polymers deposited at different distances from the electrode were exposed to a Milli-Q water/PBS cycle to simulate conditions in the QCM-D, or exposed to individual solutions for an hour (Table 4.10). For samples deposited 3 and 11 cm away from the electrode, exposure to Milli-Q water for 1 hour had no significant effect on the oxygen content of the films. For the 20 cm sample there was a loss of oxygen from the surface, ~3%. Trace silicon was also present (0.3%) which was not in any of the other Milli-Q water-exposed samples or the ‘as deposited’ 20 cm film. The widescan also exhibited an
increase in rising background when compared with the ‘as deposited’ 20 cm sample associated with scattered signal from the silicon substrate beneath the film, suggesting that exposure to Milli-Q water had thinned the film to within the sampling depth of the technique (~10 nm).

Exposure to PBS for 1 hour caused no significant changes in the oxygen content of the 3 cm sample. The 11 cm sample showed a small but significant decrease in oxygen with the 20 cm sample showing the largest reduction in oxygen (~3%). PBS exposure did, however, introduce sodium at the surface of the films. The sodium content increased further away from the electrode from 0.3±0.1 % at 3 cm to 3.2±0.2 % at 20 cm. It should be noted here that all samples were washed with Milli-Q water prior to XPS analysis, so the presence of buffer salts suggests that sodium has been trapped/bound within the ppAAc film. The silicon content of the 20 cm sample also doubled to 0.6 %, compared with the 20 cm sample exposed to Milli-Q, indicating that PBS exposure for 1 hour had removed more material than exposure to Milli-Q water alone.

Finally, the ppAAc films were exposed to a cycle of Milli-Q water and PBS solutions (MQ→PBS→MQ→PBS→MQ) for 10 minutes per solution. The 3 and 11 cm samples showed no significant changes in oxygen content, with the 20 cm showing a small but significant reduction of ~2%. Sodium was again incorporated into the 11 and 20 cm films, increasing from 1.5±0.1 % to 2.1±0.1 %.
Table 4.10 XPS atomic composition and O/C ratio for ppAAc films deposited at different distances from the electrode following different treatments. The mean values (± standard deviation) are calculated from 3 analyses per sample. Some samples showed trace N 1s levels typically < 0.2%.

<table>
<thead>
<tr>
<th>Distance from electrode (cm)</th>
<th>Atomic composition (%)</th>
<th>Atomic ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C 1s</td>
<td>O 1s</td>
</tr>
<tr>
<td>As deposited</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>76.6±0.5</td>
<td>23.4±0.5</td>
</tr>
<tr>
<td>11</td>
<td>73.4±0.4</td>
<td>26.6±0.4</td>
</tr>
<tr>
<td>20</td>
<td>70.7±0.3</td>
<td>29.3±0.3</td>
</tr>
<tr>
<td>Milli-Q water 1 hour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>77.4±0.3</td>
<td>22.6±0.3</td>
</tr>
<tr>
<td>11</td>
<td>74.7±0.2</td>
<td>25.3±0.2</td>
</tr>
<tr>
<td>20</td>
<td>73.3±0.2</td>
<td>26.4±0.2</td>
</tr>
<tr>
<td>PBS 1 hour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>77.2±0.2</td>
<td>22.5±0.1</td>
</tr>
<tr>
<td>11</td>
<td>73.5±0.1</td>
<td>25.1±0.1</td>
</tr>
<tr>
<td>20</td>
<td>69.7±0.2</td>
<td>26.5±0.1</td>
</tr>
<tr>
<td>Milli-Q water / PBS cycles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>77.7±0.7</td>
<td>22.3±0.7</td>
</tr>
<tr>
<td>11</td>
<td>73.1±0.5</td>
<td>25.4±0.4</td>
</tr>
<tr>
<td>20</td>
<td>70.2±0.3</td>
<td>26.9±0.2</td>
</tr>
</tbody>
</table>

Closer visual inspection of the samples, following exposure to the solutions, revealed that some of the films had optically different regions which varied depending on the sample. Distinct regions could be observed on the 20 cm sample after 1 hour in Milli-Q water, the 3 cm sample after 1 hour in PBS solution and the 11 cm sample after 1 hour in PBS solution (Figure 4.10). The 11 cm sample after cycling in PBS and Milli-Q also had some optical differences but these appeared over the whole sample surface.

XPS was used to perform region analysis of the films to investigate if the surface chemistry and stability varied across individual samples. As shown in Table 4.11, the ppAAc film deposited 20 cm away from the electrode and exposed to Milli-Q water did not exhibit significant differences in carbon and oxygen content for the films in regions a and b (Figure 4.10A). However, the sample appeared to exhibit uneven thinning, especially in region a which had silicon content ranging from 0.5 to 2.4%, depending on the analysis spot.
For the 3 cm sample after 1 hour in PBS solution (Figure 4.10B) the chemistry of the two regions on the sample was different (Table 4.11). Region c had an increased oxygen content of ~4 % compared with region d. This was attributed to an increase in the COOH/R component of the film (Figure 4.11), when compared with region d and the ‘as deposited’ film. It is unclear what mechanism may have caused this increase in the functional component of the film. Furthermore, all analysis points in region c showed an increased rising background in the widescan spectra which is not characteristic of untreated ppAAc films deposited at 3 cm. This showed thinning of the film after exposure to PBS for 1 hour. In contrast, region d exhibited reduced or no rising background within the widescan spectra, depending on the analysis point. However, variations in film thickness of the film across the region was indicated by large differences in silicon signal between analysis points (between 0.3 to 4.2 %). Although region c had a higher COOH/R component, sodium was not present at the film surface. Region d had 0.4±0.1 % Na.

Of all the samples, the 11 cm film exposed to PBS for 1 hour, exhibited the most visually distinct changes. In region e shown in Figure 4.10C the film had appeared visually to delaminate and this was confirmed by the XPS analysis (Table 4.11), revealing the underlying silicon wafer substrate (Si 53.8±0.9 %) and carbon content, which upon inspection of the C 1s spectra was in the form of hydrocarbon. In region f the film had not delaminated, however, there were variations in the film thickness across the region as indicated by differences in silicon signal between analysis points (0.2-2.3%).
Figure 4.10 Digital photographs of the treated ppAAc surfaces exhibiting optically different regions following exposure to Milli-Q water or PBS solutions. A) 20 cm sample after 1 hour in Milli-Q water (regions a and b), B) 3 cm sample after 1 hour in PBS solution (regions c and d) and C) 11 cm sample after 1 hour in PBS solution (regions e and f). The distance between each notch on the scale bar is 1 mm.
Table 4.11 XPS atomic composition for ppAAc films deposited at different distances from the electrode exhibiting optically different regions following exposure to Milli-Q or PBS solutions. Atomic composition is either listed as individual analysis points (3 analysis points collected per region) or as mean values (± standard deviation) calculated from 3 analyses per region. iCoating delaminated in this region.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Region</th>
<th>Atomic composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 cm</td>
<td>a</td>
<td>C 1s 74.1±2.2, O 1s 24.3±1.8, Si 2p 0.5, 0.7, 2.4, Na 1s -</td>
</tr>
<tr>
<td>1 hour</td>
<td>b</td>
<td>C 1s 73.5±0.8, O 1s 25.7±0.7, Si 2p 0.6±0.1, Na 1s -</td>
</tr>
<tr>
<td>Milli-Q</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 cm</td>
<td>c</td>
<td>C 1s 73.7±0.6, O 1s 25.2±0.4, Si 2p 0.7±0.3, Na 1s -</td>
</tr>
<tr>
<td>1 hour</td>
<td>d</td>
<td>C 1s 76.4±1.7, O 1s 21.4±0.2, Si 2p 4.2, 1.2, 0.3, Na 1s 0.4±0.1</td>
</tr>
<tr>
<td>PBS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 cm</td>
<td>e i</td>
<td>C 1s 16.6±0.9, O 1s 29.3±0.1, Si 2p 53.8±0.9, Na 1s 0.3±0.1</td>
</tr>
<tr>
<td>1 hour</td>
<td>f</td>
<td>C 1s 74±0.5, O 1s 24±0.1, Si 2p 0.2, 0.2, 2.3, Na 1s 1±0.4</td>
</tr>
</tbody>
</table>

Figure 4.11 Comparison of XPS C 1s spectra for ppAAc films deposited 3 cm away from the electrode as deposited and following exposure to PBS solution for 1 hour (regions c and d).
4.2.4.2 ppAAm

ppAAm films deposited at different distances from the electrode were exposed to PBS solution. As shown by QCM-D, the 3 cm coating and gold control behaved similarly, with $\Delta f$ stabilizing at around -6 Hz (Figure 4.12A). The 11 and 20 cm films experienced limited mass uptake, with $\Delta f$ reaching over -9 Hz before the Milli-Q water was introduced again. Overall, the 3 cm coating exhibited very similar behaviour to the gold control during the rinse stages, suggesting no mass uptake or swelling of the film, with the 11 and 20 cm coatings, demonstrating a small uptake of mass. The changes in dissipation for all films was similar to the response of the rigid gold control, indicating minimal if any changes in the viscoelastic properties of the films (Figure 4.12B).

Figure 4.12 QCM-D frequency and dissipation plots for ppAAm films deposited 3 cm (dark grey), 11 cm (mid grey) and 20 cm (light grey) away from the electrode compared with an Au control (black) when exposed to Milli-Q water (MQ) or 150 mM PBS (PBS) solutions at 37°C. Time markers show the point at which the pump was started with the new solution. The delay in response is due to the solution travelling through tubing prior to entering the chamber.
XPS analysis was used to further characterise the ppAAm films, following exposure to Milli-Q water and PBS solutions (Table 4.12). For the 3 and 11 cm films, exposure to the different solvent systems did not significantly affect nitrogen content. The 20 cm films showed a small but significant decrease in nitrogen (~1%) and a small but significant increase in oxygen (~1%). All of the 20 cm films (including the untreated surface) showed silicon content which was attributed to probing of the underlying silicon wafer substrate as the thin ppAAm films (deposited at this position) approached the sampling depth of the XPS technique (~10 nm). This was confirmed by the presence of a rising background in the widescan spectra for these samples. Samples exposed to PBS solutions (for 1 hour or when cycled with Milli-Q water) showed the presence of chlorine within the surface of the ppAAm films, derived from chloride ions in the PBS solution. The % of Cl in the film was independent of substrate location in the reactor.

Table 4.12 XPS atomic composition, O/C and N/C ratios for ppAAm films deposited at different distances from the electrode following different treatments. The mean values (± standard deviation) are calculated from 3 analyses per sample. a1/3 analysis spots with 0.4 % trace Si contamination. b2/3 analysis spots with 0.2 % and 0.1 % trace Cl respectively.

<table>
<thead>
<tr>
<th>Distance from electrode (cm)</th>
<th>Atomic composition (%)</th>
<th>Atomic ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>As deposited</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>82.4±0.3</td>
<td>5.5±0.1</td>
</tr>
<tr>
<td>11</td>
<td>82.8±0.3</td>
<td>5.5±0.3</td>
</tr>
<tr>
<td>20</td>
<td>81.8±0.1</td>
<td>4.8±0.1</td>
</tr>
<tr>
<td>Milli-Q water 1 hour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>81.8±0.1</td>
<td>6.6±0.1</td>
</tr>
<tr>
<td>11</td>
<td>81.5±0.5</td>
<td>6.1±0.2</td>
</tr>
<tr>
<td>20</td>
<td>81.3±0.3</td>
<td>6±0.1</td>
</tr>
<tr>
<td>PBS 1 hour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>81.7±0.2</td>
<td>6±0.2</td>
</tr>
<tr>
<td>11</td>
<td>81.9±0.4</td>
<td>5.9±0.3</td>
</tr>
<tr>
<td>20</td>
<td>81.7±0.2</td>
<td>5.9±0.2</td>
</tr>
<tr>
<td>Milli-Q water / PBS cycles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>81.8±0.1</td>
<td>5.9±0.2</td>
</tr>
<tr>
<td>11</td>
<td>81.7±0.1</td>
<td>5.9±0.1</td>
</tr>
<tr>
<td>20</td>
<td>81.6±0.3</td>
<td>5.8±0.1</td>
</tr>
</tbody>
</table>
4.2.4.3 ppOct

The response of ppOct films deposited at different positions within the reactor was also investigated. As shown in Figure 4.13A, the 3 cm film was stable throughout the experiment and did not exhibit any swelling or loss of material. The 12 cm and 20 cm films demonstrated some mass loss from the films during the PBS rinse stages, stabilising 2-3 Hz above the original baseline in Milli-Q. The dissipation response of all films was almost identical to that of the rigid gold control with no changes in viscoelastic properties of the films (Figure 4.13B).

![Figure 4.13](image)

Figure 4.13 QCM-D frequency and dissipation plots for ppOct films deposited 3 cm (dark grey), 11 cm (mid grey) and 20 cm (light grey) away from the electrode compared with an Au control (black) when exposed to Milli-Q water (MQ) or 150 mM PBS (PBS) solutions at 37°C. Time markers show the point at which the pump was started with the new solution. The delay in response is due to the solution travelling through tubing prior to entering the chamber.
XPS analysis of the ppOct samples (Table 4.13) revealed small but significant oxidisation for the 12 cm film exposed to the Milli-Q / PBS rinse cycle and the 20 cm films exposed to Milli-Q water for 1 hour and the Milli-Q / PBS rinse cycle (typically increasing the oxygen content of the films by less than 1 %). Trace silicon ~ 0.2% was present on most of the samples and this was attributed to contamination from the mounting tape used to secure samples to the analysis platen.

Table 4.13 XPS atomic composition and O/C ratio of ppOct films deposited at different distances from the electrode following different treatments. The mean values (± standard deviation) are calculated from 3 analyses per sample. 2/3 analysis spots showed traces of Si.

<table>
<thead>
<tr>
<th>Distance from electrode (cm)</th>
<th>C 1s</th>
<th>O 1s</th>
<th>Si 2p</th>
<th>O/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>As deposited</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>94.2±0.2</td>
<td>5.6±0.2</td>
<td>0.2±0.1</td>
<td>0.06</td>
</tr>
<tr>
<td>12</td>
<td>94.9±0.3</td>
<td>4.9±0.2</td>
<td>0.2±0.1</td>
<td>0.05</td>
</tr>
<tr>
<td>20</td>
<td>95.9±0.2</td>
<td>4.0±0.1</td>
<td>0.2±0.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Milli-Q water 1 hour</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>94±0.3</td>
<td>5.9±0.2</td>
<td>0.1±0.1</td>
<td>0.06</td>
</tr>
<tr>
<td>12</td>
<td>94.5±0.1</td>
<td>5.4±0.1</td>
<td>0.2±0.1</td>
<td>0.06</td>
</tr>
<tr>
<td>20</td>
<td>95±0.4</td>
<td>4.8±0.2</td>
<td>0.2, 0.3</td>
<td>0.05</td>
</tr>
<tr>
<td>PBS 1 hour</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>93.6±0.1</td>
<td>6.2±0.1</td>
<td>0.2±0.1</td>
<td>0.07</td>
</tr>
<tr>
<td>12</td>
<td>94.6±0.2</td>
<td>5.4±0.2</td>
<td>-</td>
<td>0.06</td>
</tr>
<tr>
<td>20</td>
<td>95.3±0.4</td>
<td>4.5±0.3</td>
<td>0.2±0.1</td>
<td>0.05</td>
</tr>
<tr>
<td>Milli-Q water / PBS cycles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>93.5±0.2</td>
<td>6.4±0.2</td>
<td>0.2±0.1</td>
<td>0.07</td>
</tr>
<tr>
<td>12</td>
<td>93.9±0.1</td>
<td>5.9±0.1</td>
<td>0.2±0.1</td>
<td>0.06</td>
</tr>
<tr>
<td>20</td>
<td>95.1±0.1</td>
<td>4.9±0.1</td>
<td>-</td>
<td>0.05</td>
</tr>
</tbody>
</table>
4.2.4.4 ppAAlc

The behaviour of the ppAAlc coatings deposited 3 and 20 cm away from the electrode were also compared with a gold control surface using QCM-D. Figure 4.14A shows very similar frequency responses for the 3 cm film compared with the gold control during the PBS and Milli-Q rinse stages, demonstrating limited if any mass change or swelling behaviour of the film. The 20 cm film exhibited different behaviour with a small change in frequency during the first PBS rinse stage. The loss of mass was confirmed when rinsing with Milli-Q where Δf stabilised above the initial baseline of 0 at over 2 Hz. The change in the film appeared to stabilise by the time of the second PBS rinse, suggesting that the initial change could be attributed to the loss of low molecular weight (MW) materials from the film. The dissipation response of both of the ppAAlc coatings was almost identical with no significant changes in the viscoelastic properties of the films when compared with the rigid gold control (Figure 4.14B).

Figure 4.14 QCM-D frequency and dissipation plots for ppAAlc films deposited 3 cm (dark grey), 11 cm (mid grey) and 20 cm (light grey) away from the electrode compared with an Au control (black) when exposed to Milli-Q water (MQ) or 150 mM PBS (PBS) solutions at 37°C. Time markers show the point at which the pump was started with the new solution. The delay in response is due to the solution travelling through tubing prior to entering the chamber.
XPS was used to characterise the ppAAlc films following exposure to the different Milli-Q water and PBS solvent systems (Table 4.14). Exposure to PBS, Milli-Q water or the cycled solutions did not significantly change the chemistry of any of the ppAAlc films. Trace silicon was present on all of the surfaces (~0.2 %). This was attributed to contamination introduced from the tape used to mount the samples prior to XPS analysis.

Table 4.14 XPS atomic composition and O/C ratio of ppAAlc films deposited at different distances from the electrode following different treatments. The mean values (± standard deviation) are calculated from 3 analyses per sample.

<table>
<thead>
<tr>
<th>Distance from electrode (cm)</th>
<th>Atomic composition (%)</th>
<th>Atomic ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C 1s</td>
<td>O 1s</td>
</tr>
<tr>
<td>As deposited</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>87.2±0.1</td>
<td>12.6±0.1</td>
</tr>
<tr>
<td>12</td>
<td>86.4±0.2</td>
<td>13.5±0.1</td>
</tr>
<tr>
<td>20</td>
<td>86.4±0.1</td>
<td>13.3±0.1</td>
</tr>
<tr>
<td>Milli-Q water 1 hour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>87.2±0.1</td>
<td>12.5±0.1</td>
</tr>
<tr>
<td>12</td>
<td>86.1±0.1</td>
<td>13.8±0.1</td>
</tr>
<tr>
<td>20</td>
<td>86.3±0.1</td>
<td>13.5±0.1</td>
</tr>
<tr>
<td>PBS 1 hour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>87.1±0.1</td>
<td>12.7±0.1</td>
</tr>
<tr>
<td>12</td>
<td>86.2±0.1</td>
<td>13.6±0.1</td>
</tr>
<tr>
<td>20</td>
<td>86.5±0.1</td>
<td>13.3±0.1</td>
</tr>
<tr>
<td>Milli-Q water / PBS cycles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>86.9±0.1</td>
<td>13±0.1</td>
</tr>
<tr>
<td>12</td>
<td>86.1±0.2</td>
<td>13.8±0.1</td>
</tr>
<tr>
<td>20</td>
<td>86.2±0.1</td>
<td>13.5±0.1</td>
</tr>
</tbody>
</table>
4.2.5 The effect of ionic strength and pH on ppAAm and ppAAc films: A comparative QCM-D and EIS study

EIS and QCM-D were used to monitor changes in the structural and electrical properties of ppAAc and ppAAm films *in situ* during exposure to aqueous environments of different ionic concentration and pH. The films were deposited close to the electrode, therefore the physiochemical attributes are comparable to the 3 cm coatings described previously in this chapter.

4.2.5.1 The effect of ionic strength

Figure 4.15 shows the QCM-D frequency and dissipation plots for ppAAm and gold control surfaces exposed to repeated rinsing cycles of 10 and 150 mM NaCl solutions. As the NaCl concentration was increased from 10 mM (pH 5.6) to 150 mM (pH 5.2), a decrease in frequency, corresponding to an increase in mass, was observed. The decrease in frequency occurred in two phases with a faster initial frequency shift from -1 to -4 Hz followed by slower frequency shift from -4 to -9 Hz over ~15 minutes. The fast initial decrease in frequency with a similar magnitude $\Delta f \sim 3$ Hz was also observed on plain Au. This decrease was associated predominantly with the ‘buffer effect’ due to the sensitivity of the technique when a change in ionic strength of the solution occurs above the sensor. The slower second phase in frequency shift was not observed on plain Au. Returning the NaCl concentration back to 10 mM caused a frequency shift from -9 to -7 Hz but unlike the Au control, the frequency of the ppAAm coated crystal did not return back to the initial frequency measured before the 150 mM solution was added. This indicated water and/or ions were retained in the film. The same pattern was observed over subsequent cycles, with the film adding more mass with each 150 mM rinse without reaching saturation. Dissipation changes of the ppAAm film measured at $\sim 1.5 \times 10^{-6}$ when rinsing at 150 mM were similar to those measured for the rigid Au control ($\sim 1 \times 10^{-6}$), suggesting limited changes if any to the viscous properties of the film.
Figure 4.15 QCM-D frequency and dissipation plots of a ppAAm thin film and plain Au crystal as the NaCl concentration is cycled between 10 and 150 mM. Time markers show the point at which the pump was started with the new solution. The delay in response is due to the solution travelling through tubing prior to entering the chamber.

EIS spectra were also collected from a ppAAm film exposed to cycles of 10 and 150 mM NaCl solutions (Figure 4.16). As shown in Figure 4.16A, a predominantly capacitive region between 0.1 and 100 Hz and a predominantly resistive region below 0.1 Hz can be observed. The data was modelled using an equivalent circuit composed of a single RCPE element, describing the film as a single insulating dielectric layer (Figure 4.17A). Figure 4.16B is a plot of the resistance, constant phase element coefficient (CPE) and α values measured whilst cycling between 10 and 150 mM solutions. The CPE describes the capacitance properties of the film and α is a measure of the homogeneity of the film. The resistance of the ppAAm film changed as a function of the ionic strength where film resistance was higher at 10 mM than 150 mM. This indicated the movement of ions in and out of the film and, as expected, a higher ion concentration within the film during the 150 mM cycles. The capacitance is dependent on both the dielectric properties of the film and the film thickness. Capacitance is reduced if film thickness increases or if water uptake into the film increases. Figure 4.16B shows that the capacitance was generally lower at 10 mM, suggesting a reduction in water content of the film (an increase in film thickness would also cause a drop in capacitance value, however, this is not consistent with swelling of the films at higher ionic concentration as observed with QCM-D). This trend was not observed for the second 10 mM rinse where the capacitance increased further, corresponding with a dip in α (due to a loss of homogeneity within the film). Such observations could be associated with structural changes occurring in the film and/or loss of low MW materials affecting film thickness.
Figure 4.16 EIS data collected from a hydrated ppAAm thin film as the concentration of NaCl in solution was cycled between 10 mM and 150 mM. A) EIS spectra where symbols represent data points and solid lines represent the EEC model fits and B) the resistance, constant phase element coefficient and $\alpha$ values obtained from the EEC model fit to the EIS data (Lines are a guide for the eye).

Figure 4.17 The electrical equivalent circuits (EECs) used to model the EIS spectra. The $R_{\text{elec}}$ component is not included in the results for comparison as it describes the resistance of the electrolyte solution and electrical leads.
XPS analysis of ppAAm films, deposited on silicon wafer pieces and exposed to the NaCl solutions, is shown in Table 4.15. There was no significant change in nitrogen content of the films upon exposure to the NaCl solutions. XPS also revealed that chloride ions had entered the films upon exposure to the NaCl solutions and were retained at the surface despite thorough rinsing in Milli-Q water prior to XPS analysis.

Table 4.15 XPS atomic composition, O/C and N/C ratios for ppAAm films deposited 3 cm from the electrode on silicon wafer following different treatments in NaCl solutions. The mean values (± standard deviation) are calculated from 3 analyses per sample.

<table>
<thead>
<tr>
<th></th>
<th>Atomic composition (%)</th>
<th>Atomic ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C 1s</td>
<td>O 1s</td>
</tr>
<tr>
<td>As deposited</td>
<td>82.4±0.3</td>
<td>5.5±0.1</td>
</tr>
<tr>
<td>10 mM NaCl</td>
<td>81.3±0.3</td>
<td>6.2±0.1</td>
</tr>
<tr>
<td>1 hour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150 mM NaCl</td>
<td>81.4±0.1</td>
<td>6±0.1</td>
</tr>
<tr>
<td>1 hour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mM / 150 mM</td>
<td>81.9±0.2</td>
<td>5.6±0.2</td>
</tr>
<tr>
<td>NaCl cycles</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Similar experiments were performed on ppAAc films. Figure 4.18 shows the QCM-D frequency and dissipation response from a ppAAc coated crystal and a plain gold control as the concentration of the NaCl solution was cycled between 10 and 150 mM. The behaviour of the ppAAc film was notably different to that of the ppAAm film. There was a frequency shift of around -5 Hz upon changing the concentration of the solution from 10 to 150 mM. The decrease in frequency was only a few Hz more than the decrease observed on the Au control, indicating limited uptake of water and/or ions into the film. Upon rinsing with the 10 mM solution, the frequency returned to a stable frequency close to the initial value. This response continued for subsequent cycles. The changes in dissipation of the film were almost identical to changes observed on the rigid Au control surface, indicating little if any changes to the viscous properties of ppAAc.
Figure 4.18 QCM-D frequency and dissipation plots of a ppAAc thin film and plain Au crystal as the NaCl concentration is cycled between 10 and 150 mM. Time markers show the point at which the pump was started with the new solution. The delay in response is due to the solution travelling through tubing prior to entering the chamber.

The response of the ppAAc film to changes in ionic strength of the NaCl solution was subsequently investigated using EIS methods. The response of the film was very different to that of ppAAm. As shown in Figure 4.19A, two predominantly capacitive regions (|Z| slope ~ -1 Ω Hz⁻¹ and high θ) between 10⁻¹ - 10³ Hz and 5×10⁻¹ - 2×10⁻³ Hz and two predominantly resistive regions (|Z| slope ~ 0 Ω Hz⁻¹ and low θ) between 5×10⁻¹ - 10¹ Hz and below 2×10⁻³ Hz were observed. In contrast to ppAAm, two RCPE elements were required to model the data, indicating the film was composed of two insulating dielectric layers (Figure 4.17B).

The element at higher frequencies (RCPE1) showed resistive values in the kΩ range and capacitive values fluctuating around 30 μF cm⁻², while the low frequency element (RCPE2) yielded resistance values in the MΩ range and capacitance values around 80 μF cm⁻². The resistance measured for the two layers was different as the ionic concentration of the solution was changed (Figure 4.19B). This suggested a difference in ion interaction between the two layers. Interestingly the capacitance followed the expected trend for both layers, increasing at higher NaCl concentration as water content of the film increased (Figure 4.19C). The alpha values for the two elements showed a different behaviour (Figure 4.19D). For RCPE1, the alpha value steadily increased over the course of the experiment from a value of 0.87 to 0.94, similar to the ppAAm films. RCPE2 remained relatively constant at around a value of 0.90 with a dip observed at the third 10 mM rinse.
Figure 4.19 EIS data collected from a hydrated ppAAc thin film as the concentration of NaCl in solution was cycled between 10 mM and 150 mM. A) EIS spectra where symbols represent data points and solid lines represent the EEC model fits. B) Resistance, C) constant phase element coefficients and D) $\alpha$ values obtained from the EEC model fit to the EIS data (Lines are a guide for the eye).
XPS analysis of ppAAc films deposited onto silicon wafer and exposed to the NaCl solutions is shown in Table 4.16. No significant changes in surface chemistry were observed for any of the NaCl solvent systems.

Table 4.16 XPS atomic composition and O/C ratio for ppAAc films deposited 3 cm from the electrode on silicon wafer following different treatments in NaCl solutions. The mean values (± standard deviation) are calculated from 3 analyses per sample. Some samples showed trace nitrogen levels typically < 0.2%.

<table>
<thead>
<tr>
<th>Atomic composition (%)</th>
<th>Atomic ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C 1s</td>
</tr>
<tr>
<td>As deposited</td>
<td>76.6±0.5</td>
</tr>
<tr>
<td>10 mM NaCl 1 hour</td>
<td>77.7±0.1</td>
</tr>
<tr>
<td>150 mM NaCl 1 hour</td>
<td>77.5±0.2</td>
</tr>
<tr>
<td>10 mM / 150 mM NaCl cycles</td>
<td>77.8±0.1</td>
</tr>
</tbody>
</table>

4.2.5.2 The effect of pH
The effect of pH on the behaviour of ppAAc and ppAAm was also investigated using QCM-D and EIS techniques. Solutions with constant ionic strength (10 mM Tris and 150 mM NaCl) were cycled between pH 7 and the pH when the surface of the individual films was neutral.

Figure 4.20 shows the frequency and dissipation response of a ppAAm coated QCM-D crystal and Au control as the pH of the solution was changed from pH 7 where the film is positively charged to pH 10 when the film is neutral (between pH 8.3-9.2).[6] Upon introduction of the first pH 7 solution, a limited response in frequency was observed with a frequency shift of -3 Hz. This was less than the frequency shift recorded for the Au control, suggesting swelling of the film was not occurring. Introduction of the pH 10 solution caused a dramatic positive frequency shift associated with a loss of mass from the film. Δf continued to increase past the initial baseline in Milli-Q water, a change attributed to expulsion of water from the film, dissociation of ions due to the neutral charge environment and a loss of low MW materials. Changing the solution back to pH 7 only partially reversed the loss of mass from the film with a gradual decrease in resonant frequency observed. With each successive pH 10 rinse more mass was lost from the film. A permanent change to the film was confirmed upon introduction of a
final Milli-Q water rinse. Instead of returning back to or close to the initial baseline of 0, established before the salt solutions were introduced (as observed with the Au control), further mass was lost from the film before stabilising at around 9 Hz. This confirmed permanent changes to the film following the pH cycles. This loss in mass was not significant enough to change the viscous properties of the film and the dissipation remained constant throughout the pH cycles and returned back to the baseline during the final Milli-Q water rinse.

Figure 4.20 QCM-D frequency and dissipation plots of a ppAAm thin film and plain Au crystal as the pH is cycled between 7 and 10. Time markers show the point at which the pump was started with the new solution. The delay in response is due to the solution travelling through tubing prior to entering the chamber.

Figure 4.21A shows the EIS data collected for the ppAAm film exposed to pH 7 and pH 10 solutions. At pH 7, one predominantly capacitive region (|Z| slope ~ -1 Ω Hz$^{-1}$ and high θ) between 0.05 and 1000 Hz and one predominantly resistive region (|Z| slope ~ 0 Ω Hz$^{-1}$ and low θ) below 0.05 Hz was observed. Interestingly at pH 10, a second element appeared in the spectra, with a dip in the phase shift at 20 - 30 Hz, suggesting a dual layer structure. The data collected at pH 7 and pH 10 was therefore modelled using an EEC containing one or two RCPE elements respectively. At pH 10, the element at high frequencies was clearly visible, but difficult to fit. The model parameters describing this element were simulated and held constant ($R_2 = 0.5 \text{kΩ cm}^2$ and $\text{CPE}_2 = 30 \mu\text{F cm}^{-2}$, $\alpha_2 = 0.85$) whilst the low frequency element was fitted.

Figure 4.21B shows the resistance, CPE and α values obtained for the ppAAm film as the pH was cycled between pH 7 and 10. The resistance ($R_1$) was lower at pH 7 and higher at pH 10. This is consistent with the reduced association of ions with a neutral
film at pH 10. Capacitance was greater at pH 7 which is consistent with a higher water content of the film. Unlike the QCM-D data, EIS showed reversible changes to the film during the pH cycles.

XPS analysis of ppAAm films deposited onto silicon wafer was conducted to study the stability and surface chemistry changes of the films. The films were submerged in 10 mM Tris + 150 mM NaCl solutions at varying pH for 1 hour or exposed to a buffer pH cycle (pH 7→10→7→10→7→10, 10 minutes for each solution) (Table 4.17). There were no significant changes in the nitrogen content of the films for any of the solvent
systems investigated. Chlorine was retained at the surface of all films following exposure to the NaCl solutions (<0.5%).

Table 4.17 XPS atomic composition, O/C and N/C ratios for ppAAm films deposited at the electrode on silicon wafer following different treatments in 10 mM Tris + 150 mM NaCl solutions at different pH. The mean values (± standard deviation) are calculated from 3 analyses per sample.

<table>
<thead>
<tr>
<th>Atomic composition (%)</th>
<th>Atomic ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C 1s</td>
</tr>
<tr>
<td>As deposited</td>
<td>82.4±0.3</td>
</tr>
<tr>
<td>10 mM Tris + 150 mM NaCl pH 7 1hour</td>
<td>81.8±0.1</td>
</tr>
<tr>
<td>10 mM Tris + 150 mM NaCl pH 10 1hour</td>
<td>82±0.2</td>
</tr>
<tr>
<td>10 mM Tris + 150 mM NaCl pH 7/ 10 cycles</td>
<td>82±0.3</td>
</tr>
</tbody>
</table>

In a similar experiment, ppAAc films were exposed to solutions at pH 7, when the film is negatively charged, and pH 4, when the film is neutral (neutral between pH 3-4.4). [6]

Figure 4.22 shows the frequency and dissipation response of a ppAAc coated QCM-D sensor and Au control. Changing from Milli-Q water to the pH 7 solution caused a frequency shift of -15 Hz which was -11 Hz more than the Au control surface. This was associated with the uptake of water and ions into the film. Rinsing at pH 4 caused the opposite effect with a rapid increase in Δf from -15 Hz to -6.5 Hz suggesting partial but not complete loss of ions and water from the film. The same behaviour was observed during successive pH 7 and 4 cycles and not observed for the Au control. The dissipation was similar to that of the gold control, suggesting a coating that remained rigid throughout the experiment.
Figure 4.22 QCM-D frequency and dissipation plots of a ppAAc thin film and plain Au crystal as the pH of the solution is cycled between 7 and 4. Time markers show the point at which the pump was started with the new solution. The delay in response is due to the solution travelling through tubing prior to entering the chamber.

EIS data collected from a ppAAc film as the pH was cycled from pH 7 to 4 is shown in Figure 4.23. Once again a dual layer was observed with two predominantly capacitive regions (between 0.005 – 5 Hz and above 200 Hz) and two predominantly resistive regions (below 0.005 Hz and between 0.005 – 200 Hz) (Figure 4.23A). The layer corresponding to the low frequency region had a higher resistance and was more homogeneous ($R_2 = 5.2(0.2) \, \text{MΩ cm}^2$, $\text{CPE}_2 = 11.78(0.09) \, \mu F \, \text{cm}^2$, $\alpha_2 = 0.870(0.003)$), whereas the layer corresponding to the mid-frequency range was less densely packed and less homogeneous ($R_1 = 5.2(0.1) \, \text{kΩ cm}^2$, $\text{CPE}_1 = 1.70(0.08) \, \mu F \, \text{cm}^2$, $\alpha_1 = 0.797(0.005)$). The presence of two layers each with resistance values in the MΩ and kΩ range, respectively, had previously been observed for ppAAc on response to changes in ionic strength.

Both layers showed lower resistance at pH 4 and a higher resistance at pH 7 (Figure 4.23B). This seemed counterintuitive given the QCM-D data which showed an increase in mass of the film at pH 7, associated with an influx of ions and water (Figure 4.22). It is probable that ions may be left trapped inside the film as water leaves thus increasing the ion concentration within the film. The two layers also demonstrated similar trends with regards to capacitance which increased at pH 4 and decreased at pH 7 (Figure 4.23C). This could be associated with a change in film thickness where an increase at pH 7 would generate a decrease in capacitance. Finally, the homogeneity of both the layers was shown to increase at pH 7 and decrease at pH 4 (Figure 4.23D).
Figure 4.23 EIS data collected from a hydrated ppAAc thin film as the pH of the solution is cycled between 7 and 4. A) EIS spectra where symbols represent data points and solid lines represent the EEC model fits. B) Resistance, C) constant phase element coefficients and D) α values obtained from the EEC model fit to the EIS data (Lines are a guide for the eye).
XPS analysis of ppAAc films deposited onto silicon wafer was conducted to characterise film surface chemistry and stability. The films were submerged in 10 mM Tris + 150 mM NaCl solutions at various pH for 1 hour or exposed to a pH buffer cycle (pH 7→4→7→4→7→4, 10 minutes for each solution) (Table 4.18). The 1 hour pH 4 and 7 samples showed a minimal (~1%) yet significant reduction in oxygen content whilst no significant change in oxygen was observed for the sample exposed to the buffer cycle. Close visual inspection of the films revealed that the film exposed to 10 mM Tris + 150 mM NaCl solution at pH 4 for 1 hour had two optically different regions (Figure 4.24). XPS analysis of the two regions revealed that they were not significantly different with regards to oxygen and carbon content (Table 4.19). Both regions contained silicon however neither of the samples exhibited rising background in the widescan spectra, therefore the Si content was not associated with thinning of the plasma polymer film. The contamination may have been introduced to the sample during rinsing and isolated to that one sample, or during mounting of that particular sample for XPS.

Table 4.18 XPS atomic composition and O/C ratio for ppAAc films deposited 3 cm from the electrode on silicon wafer following different treatments in 10 mM Tris + 150 mM NaCl solutions at different pH. The mean values (± standard deviation) are calculated from 3 analyses per sample. Some samples showed trace nitrogen levels, typically <0.2%.

<table>
<thead>
<tr>
<th>Atomic composition (%)</th>
<th>Atomic ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 1s</td>
<td>O 1s</td>
</tr>
<tr>
<td>As deposited</td>
<td>76.6±0.5</td>
</tr>
<tr>
<td>10 mM Tris + 150 mM NaCl pH 7 1 hour</td>
<td>77.9±0.1</td>
</tr>
<tr>
<td>10 mM Tris + 150 mM NaCl pH 4 1 hour</td>
<td>77.9±0.1</td>
</tr>
<tr>
<td>10 mM Tris + 150 mM NaCl pH 7/4 cycles</td>
<td>77.9±0.2</td>
</tr>
</tbody>
</table>
Figure 4.24 Digital photograph of the ppAAc film after 1 hour in 10 mM Tris + 150 mM NaCl solution at pH 4. Two optically different regions (a and b) were visible. The distance between individual notches on the scale bar is 1 mm.

Table 4.19 XPS atomic composition for ppAAc film after exposure to 10 mM Tris + 150 mM NaCl solution at pH 4. After exposure, the film exhibited optically different regions (a and b). Atomic composition is either listed as individual analysis points (3 analysis points collected per region) or as the mean (± standard deviation) calculated from 3 analyses per region.

<table>
<thead>
<tr>
<th>Region</th>
<th>C 1s</th>
<th>O 1s</th>
<th>Si 2p</th>
<th>Trace</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>78.1±0.3</td>
<td>21.1±0.4</td>
<td>0.5±0.1</td>
<td>Ca 2p 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N 1s 0.4, 0.4, 0</td>
</tr>
<tr>
<td>b</td>
<td>77.9±0.1</td>
<td>21.6±0.3</td>
<td>0.2±0.1</td>
<td>N 1s 0.4, 0.3, 0</td>
</tr>
</tbody>
</table>
4.3 Discussion

The results presented in this chapter provide an insight into the physiochemical properties of the ppAAc, ppAAm, ppAAlc and ppOct films used in this thesis. Several different techniques were used to characterise the films and investigate the influence of substrate proximity to the electrode on film properties. QCM-D combined with EIS also provided insight into the structure and behaviour of plasma polymers in aqueous environments.

The influence of substrate to electrode distance was most significant on films derived from the acrylic acid monomer. XPS data indicated that ppAAc films deposited furthest away from the electrode (20 cm) had a higher O/C ratio, increased COOH/R component and a larger carboxylic acid content when compared with samples deposited closer to the electrode (Table 4.2, Table 4.3 and Table 4.4). This higher carboxylic acid content was consistent with the small yet significant reduction in contact angle between the 3 cm and 20 cm coatings from 50° to 44° (Table 4.1). This variation in film chemistry can be related to the asymmetric setup of the plasma polymerisation unit. The plasma density at the electrode is higher (more energy per unit volume), as indicated by a brighter glow, leading to higher fragmentation of the monomer. Furthermore, the monomer inlet is located on the opposite side of the reactor to both the electrode and the vacuum pump. As the monomer travels from the inlet to the electrode the residence time increases and there is a higher likelihood of collision and fragmentation events.\[15\] As a result, samples positioned further from the electrode are expected to have a higher retention of monomer functionality. These observations are consistent with previous literature documenting a higher retention of oxygen in ppAAc films closer to the monomer inlet.\[16\]

Increased fragmentation may also provide an explanation for why the O/C ratio for ppAAlc (Table 4.5) and the N/C ratio for ppAAm (Table 4.6) are reduced close to the electrode. It has previously been observed that depositing these monomers at higher powers causes a reduction in retention of alcohol groups for ppAAlc\[8\] and primary amine density in ppAAm.\[2\] The deposition power of 20 W therefore goes some way to explain why the films are only moderately hydrophilic, measured at 71±1° and 61±1° for ppAAlc and ppAAm films deposited at the electrode, respectively. Unsurprisingly, the hydrocarbon-rich ppOct films were hydrophobic with contact angles above 90°. Oxygen content of the films was attributed to reactive species incorporated into the film during
deposition reacting with atmospheric oxygen upon removal of the samples from the reactor.\textsuperscript{[17]}

Film thickness was also shown to be highly dependent on both monomer chemistry and substrate position. As measured by AFM and ellipsometry, film thickness was reduced further away from the electrode for all monomers (Table 4.8 and Table 4.9), suggesting increased deposition rates at the electrode. This effect can be explained by the asymmetrical energy distribution throughout the reactor, generating more film forming species (due to increased collisions with electrons) at the electrode.\textsuperscript{[18]} A dependency between film thickness and monomer chemistry was also observed, with ppAAm providing the thinnest films and ppOct the thickest. Given identical deposition times for each monomer, these observations can be explained by the chemical structure and composition of the starting monomer. In aliphatic structures oxygen can be readily liberated to act as a ‘radical scavenger’.\textsuperscript{[19]} This means oxygen-containing groups have a poisoning effect which leads to reduced deposition.\textsuperscript{[19]} The presence of double bonds in the starting monomer is also important as they can form reactive species more readily, facilitating film deposition.\textsuperscript{[19]} This provides an explanation for the higher deposition of ppOct films as the 1,7-Octadiene monomer contains two double bonds but no oxygen species. Of the oxygen containing monomers, allyl alcohol demonstrated higher deposition rates than ppAAc close to the electrode. This may be explained by the poisoning effects of two oxygen atoms per molecule for ppAAc compared with one for ppAAc. However, film thicknesses for the two chemistries were not significantly different further away from the electrode, suggesting that when the monomer is less fragmented (i.e. near the monomer inlet) this effect is reduced. ppAAm provided the thinnest coatings at the electrode compared with the other chemistries, however, as the monomer does not contain oxygen this was attributed to previous observations of allylamine fragmenting less readily,\textsuperscript{[20]} leaving intact monomer within the film.\textsuperscript{[20, 21]}

A final consideration was the stability and behaviour of films in physiological-like aqueous conditions. As shown by QCM-D, it was unsurprising that the hydrocarbon-rich and hydrophobic ppOct coatings exhibited little if any interaction with the PBS solution, with all samples showing little evidence of mass change or swelling irrespective of deposition position within the reactor. The ppAAc films demonstrated ‘as deposited’ O/C ratios of between 0.12-0.14, depending on location within the reactor, which is less than reported elsewhere\textsuperscript{[8]} and together with high contact angles
over 70°, indicated a low retention of hydroxyl groups. For some coatings (ppOct 12 and 20 cm and ppAAlc 20 cm) small changes in the QCM-D frequency response may indicate loss of low MW materials (as previously observed on ppAAm films\textsuperscript{[22, 23]}, explained by lower cross-link densities further away from the electrode. XPS data revealed the ppAAalc 20 cm film underwent no significant changes in film surface chemistry following exposure to the Milli-Q / PBS rinse cycle. The 12 and 20 cm ppOct films showed minimal yet significant increases in oxygen content at the surface (~1%).

For ppAAc, the QCM-D studies revealed that when exposed to a 150 mM PBS solution at 37°C (pH 7.5) film behaviour was highly dependent on the location of the sample within the reactor. The further away from the electrode, the greater the increase in frequency and dissipation shifts and thus swelling of the film. This was attributed to the higher content of COOH groups in the 20 cm film which facilitated the uptake of water and ions into the film. This result is consistent with previous literature, where pulsed ppAAc films, deposited at lower power with a higher carboxylic content and lower contact angles, exhibited strong interactions with bi-distilled water, even when an ammonia plasma pre-treatment was used to improve film adhesion to the substrate.\textsuperscript{[24]}

Interestingly, the swelling behaviour of ppAAc is markedly different to that of grafted polyacrylic acid brushes where brush thickness reduces due to increased charge shielding at higher electrolyte concentration.\textsuperscript{[25]}

At pH 7, COOH groups would be dissociated forming COO\(^{-}\) species, providing ppAAc with a negative charge\textsuperscript{[6]} and driving the uptake of positive ions such as Na\(^{+}\) into the film. This was confirmed by XPS which showed an increase in sodium content of the films exposed to PBS for 1 hour from 0.3 % at 3 cm to 3.2 % at 20 cm (Table 4.10). Films exposed to a cycle of Milli-Q water and PBS also showed an increase in sodium content from 0 % at 3 cm to 2.1 % at 20 cm. Prolonged exposure to the PBS solution (without Milli-Q water cycles) enabled higher retention of sodium in the film. Na\(^{+}\) incorporation has previously been hypothesised for swollen plasma polymerised maleic anhydride films.\textsuperscript{[26]}

QCM-D data indicated no instability or delamination of the ppAAc films exposed to cycles of Milli-Q water and PBS solutions. In contrast, XPS revealed a thinning of all 20 cm films exposed to the solutions, either individually for a 1 hour period, or during
cycling of the two solutions. This effect can be attributed to the higher carboxylic acid content of ppAAc films deposited further away from the electrode and closer to the monomer inlet. The greater interaction of these films with water and/or ions may act to stretch the cross-linked structure of the film, enabling loss of low MW material from the film.

Following exposure of ppAAc films to PBS or Milli-Q water for 1 hour it was evident that some films had developed optically distinct regions and there appeared to be no clear trend between the films that did, or did not, develop the features. The 20 cm film treated with Milli-Q water for 1 hour did not exhibit significant changes in film chemistry, however, it appeared there had been uneven thinning of the film across the sample. Surprisingly, the 3 cm ppAAc film deposited close to the electrode, exhibited two regions with different COOH/R composition. There was also evidence of varied thinning of the film across the sample. It is unclear by what mechanism the carboxylic acid content of the two regions would be different, unless orientation or concentration of the functional groups changes throughout the thickness of the film. The 11 cm film exposed to PBS for 1 hour was the only sample which showed partial delamination of the film from the substrate. Indeed, it was somewhat surprising given that the 20 cm film, with the higher carboxylic acid content and increased swelling behaviour in PBS (as shown by QCM-D), did not delaminate in the same conditions.

In separate QCM-D studies of ppAAc films deposited 3 cm from the electrode, changes in ionic strength between cycles of 10 and 150 mM solutions of NaCl (at 25°C) had little effect on the films. XPS revealed no changes of the film surface chemistry following exposure to the salt solutions either through cycling or separately for 1 hour. Responses of the ppAAc films to changes in pH were more significant. As shown by QCM-D, films swelled at pH 7 when negatively charged and collapsed at pH 4 when neutral, ascribed to the charge behaviour of the films and association of ions and water, as described above. XPS revealed that exposure of the ppAAc film to the buffer cycle did not alter the surface chemistry.

Analysis of ppAAc using EIS indicated that the films contained two distinct layers in all conditions investigated, with a more resistive layer in the MΩ range and a less resistive layer in the kΩ range. The origins of this dual layer behaviour are unclear. The response of the layers to changes in pH and ionic strength was not always the same, suggesting
both structural and/or chemical differences between the layers. This dual layer structure may also go some way to explain the thinning observed for some ppAAc layers when exposed to PBS and Milli-Q water.

The layered structure could arise during deposition of the plasma polymer due to the formation of different cross-link densities or as a direct result of solvent exposure and the loss of low MW materials generating a more porous film. Interconnected voids (around 1 nm in size) have previously been observed in ppAAc films.\cite{27,28} These voids, attributed to the incorporation of carboxylic groups, enabled penetration of dye molecules throughout the thickness of the film.\cite{27,28} Such a network would play an important role in film behaviour in aqueous conditions. The increase in swelling of the ppAAc film deposited further away from the electrode could also be related to such a network generated from greater incorporation of COOH groups into the bulk of the plasma polymer.\cite{29} As EIS experiments were only undertaken on samples from the 3 cm position, it is unclear how the position within the reactor could affect the physicochemical properties of this dual layer structure. Further characterisation of both film structure and chemistry, using techniques such as AFM, XPS and/or ToF-SIMS depth profiling, would be required to investigate the complex composition of these films further.

As shown in the QCM-D results, when exposed to PBS solution at 37°C the ppAAm films showed little position dependent swelling behaviour. This was perhaps unsurprising given the small changes in both chemistry and contact angle observed for the films. The 11 and 20 cm coatings of ppAAm showed some swelling activity which was minimal compared with ppAAc. XPS revealed that all films exposed to PBS, either via cycling with Milli-Q water or during 1 hour prolonged exposure, had retained chloride ions from solution. This was not surprising given the positive charge of the ppAAm film.

ppAAm exhibited quite different behaviour in response to 10 and 150 mM concentrations of NaCl at 25 °C. The ppAAm film was modelled as a single insulating layer for EIS. QCM-D showed that the ppAAm film continued to swell with every 150 mM cycle (Figure 4.15) and water and/or ions appeared to be retained within the film, even when rinsing at 10 mM. This was confirmed by XPS which showed chloride ions
were still present at the surface of the ppAAm films, even after rinsing with Milli-Q water.

The QCM-D response of the ppAAm film to changes in pH showed limited, if any, swelling behaviour at pH 7 (when the film would be positively charged), however, upon rinsing at pH 10 (when the film is neutral) the film lost mass, a process which was repeated with each successive pH 10 rinse. The last rinse in Milli-Q revealed a permanent change in mass of the film. A structural, yet reversible change in the film at pH 10, was further revealed by EIS which showed the presence of a second layer within the deposited film, which was less distinct than observed for ppAAc (Figure 4.21).

The reason for structural changes within the ppAAm film can be partly explained by the loss of low MW materials from the coating, as previously documented. For example, N-heptylamine plasma polymers have been shown to form porous microstructures upon solvent exposure, an effect attributed to loss of low MW materials. Furthermore, the presence of interconnected pores, such as those found in ppAAc, have also been documented within dry amino-containing hydrocarbon plasma polymers, which allowed bulky dye molecules to penetrate the film. Why the loss of low MW materials may be encouraged at pH 10 is unclear, however, the expulsion of water together with the dissociation of low MW materials in a neutral charge environment provides some explanation.

XPS analysis of ppAAm films exposed to the pH 7 and 10 buffers during cycles, or separately for 1 hour, revealed no significant differences in nitrogen content of the films when compared with the untreated sample. Despite the structural changes revealed by EIS and QCM-D, there was also no evidence of rising background or substrate related peaks in the widescan spectra meaning the coatings were still greater than 10 nm in thickness. The atomic composition also revealed chlorine was retained at the surface of the ppAAm films exposed to the 10 mM Tris + 150 mM NaCl solutions.
4.4 Conclusion

This chapter has described the successful deposition of plasma polymer films from acrylic acid, allylamine, 1,7-Octadiene and allyl alcohol monomers. ppOct films provided a hydrocarbon-rich, hydrophobic environment and ppAAlc and ppAAm films provided moderately hydrophilic surfaces with alcohol/ether and amine functionality, respectively. ppAAc films provided the most hydrophilic films, of all the plasma polymers deposited, with a carboxylic acid functionality. By varying the position of the substrate within the reactor, films of varying physiochemical properties could be fabricated. In particular, thinner films were deposited further away from the electrode for all chemistries investigated.

The effect of substrate position on film chemistry was shown to be highly dependent on monomer chemistry with little or no effect evident when ppAAm, ppOct and ppAAlc films were being deposited. In contrast, ppAAc films with increasing carboxylic acid content could be fabricated by placing the substrate further away from the electrode and closer to the monomer inlet. This had significant implications on the behaviour of the ppAAc films in aqueous conditions. As the COOH content of the films increased a greater degree of both swelling could be observed by QCM-D upon exposure to PBS. XPS revealed that sodium ions were retained in the film more readily with increasing COOH content and some samples exhibited uneven thinning and even localised changes in chemistry upon exposure to PBS. Only one sample showed evidence of localised delamination but it was not the sample exhibiting the greatest swelling activity, suggesting a complex interplay between the substrate position, chemistry and mechanical properties of the films.

In a separate study using EIS, QCM-D and XPS, the response of ppAAm and ppAAc films, deposited 3 cm from the electrode, to changes in pH and ionic strength was investigated. Modelling of the EIS data revealed that in all conditions the ppAAc films comprised of a dual layer structure. These layers could respond differently to changes in both pH and ionic strength, suggesting structural and/or chemical differences in their composition. ppAAm was typically modelled as a single layer, however, at pH 10 a second layer appeared in the film, an effect which was reversible when cycling back to the pH 7 solution. QCM-D showed that an irreversible loss of mass had occurred from the ppAAm film following pH 7/10 rinse cycles, attributed to a loss of low MW
material. Interestingly, XPS revealed that these changes in the film, during the rinse cycle, had not affected the surface chemistry of the film.

These results highlight the importance of a multi-technique approach for characterising plasma polymers in both dry and aqueous conditions. In particular, EIS, which is not commonly used for plasma polymer characterisation, emerged as a powerful tool for investigating structural differences between ppAAc and ppAAm films when exposed to changes in ionic strength and pH. Importantly, the technique revealed that the plasma polymers were composed of one or two layers, each with different properties and behaviours dependent on the aqueous conditions. QCM-D also enabled mass and dissipation changes to be tracked in situ, whilst XPS provided complimentary information regarding changes in surface chemistry.
4.5 References


5 Lipid vesicle interactions with gold and plasma polymers

5.1 Introduction
Many surfaces do not facilitate controlled vesicle adsorption and/or collapse and processes at the interface can often be poorly understood. In this chapter lipid vesicle interactions with gold and plasma polymer films were investigated.

The ability to form artificial cell membrane models on conductive surfaces is important for the development of electrochemical sensing platforms to explore cell membrane phenomena. Furthermore, these surfaces facilitate the use of other characterisation techniques, such as surface plasmon resonance (SPR). In this study, the challenges associated with lipid vesicle collapse on commercially available gold substrates have been explored using quartz crystal microbalance with dissipation (QCM-D) and atomic force microscopy (AFM). The gold surface was characterised using X-ray photoelectron spectroscopy (XPS) and vesicle sizing was conducted using dynamic light scattering (DLS).

When surface conductivity is not required, plasma polymerisation provides a versatile, one-step, dry method of creating thin films (<100 nm) with different physiochemical properties on almost any substrate. Successful lipid vesicle adsorption and collapse on plasma polymer thin films could provide substrate independent films for the development of model cell membrane platforms whilst furthering our understanding of lipid vesicle interactions at surfaces. All plasma polymers described in this chapter were deposited close to the electrode and the physiochemical properties of these films have been described in Chapter 4 (3 cm samples). Lipid vesicle interactions with the plasma polymer films were investigated using QCM-D and fluorescence recovery after photobleaching (FRAP) techniques.

XPS data was collected by Dr. Thomas Ameringer (Swinburne University of Technology) and analysed by HJA. AFM images were collected by Dr. Renee Goreham (Flinders University) and analysed by Prof. Joe Shapter (Flinders University).
Parts of this chapter have been modified from the following papers:

Hannah. J. Askew, Renee V. Goreham, Joseph G. Shapter, Sally. L. McArthur, Investigating pH –driven assembly of supported lipid bilayers on commercial gold QCM-D crystals. (Submitted)

5.2 Results

5.2.1 Lipid vesicle interactions with gold

XPS was used to chemically characterise a clean gold QCM-D crystal (Figure 5.1). Both the widescan and high resolution Au 4f spectra (Figure 5.1A and B) were consistent with those reported elsewhere for gold.[1, 2] Oxidation (O 1s 5.5±0.2%) and the presence of adventitious sulphur (S 2p 2.7±0.4%) and carbon contaminants (C 1s 28.5±1.3%) were observed and attributed to post-cleaning atmospheric exposure during transport to the XPS and sample loading. Carbon contamination was predominately from hydrocarbon species as shown by the high resolution C 1s spectra (Figure 5.1C). The contact angle of the QCM-D crystal was measured at 55°. Surface wettability of gold surfaces is highly dependent on preparation and cleaning protocols, however, 55° is within the range previously reported.[3]

Figure 5.1 XPS A) widescan, B) high resolution Au 4f spectra and C) high resolution C 1s spectra for a clean gold QCM-D crystal. Inset displays mean (± standard deviation) of atomic % from 3 separate analysis points.
Figure 5.2 shows the QCM-D frequency and dissipation plot of DOPC vesicles adsorbing to the gold crystal surface. Time markers show the point at which the pump was started with each new solution. The delay in response is due to the solution travelling through tubing prior to entering the chamber. All vesicles in this chapter (except NaCl-free and HEPES conditions) were prepared using 10 mM Tris (~pH 7) as a hydration buffer and then diluted in a 10 mM Tris + 150 mM NaCl solution, at the appropriate pH for the experiment being undertaken, immediately before use. This was to create an osmotic stress across the wall of the vesicle which has previously been shown to reduce the critical coverage for vesicle collapse on SiO$_2$ surfaces.$^{[4]}$ In steps 1 and 2, the introduction of 10 mM Tris (pH 7) followed by a 10 mM Tris + 150 mM NaCl (pH 7) solution had minimal effect on the frequency and dissipation. Upon addition of the DOPC in step 3 large changes in both frequency and dissipation were observed with $\Delta f$ and $\Delta D$ stabilising at -250 Hz and $48 \times 10^{-6}$ respectively. As vesicles adsorbed to the crystal, the mass of the lipid, buffer trapped inside the vesicles and any water associated with the vesicular layer, increased mass at the surface causing a decrease in resonant frequency. The transition from the highly rigid environment of the gold crystal to the highly viscous vesicular layer caused an increase in dissipation.$^{[5]}$ No changes were observed when rinsing with buffer solution suggesting the presence of a stable vesicular layer at the gold surface which is consistent with previous literature.$^{[4-6]}$

Figure 5.2 QCM-D frequency and dissipation plot of vesicle adsorption at the gold sensor surface at neutral pH. Liquid additions: 1) 10 mM Tris 7.0 2) 10 mM Tris 150 mM NaCl 7.0 3) DOPC vesicles 4) 10 mM Tris 150 mM NaCl 7.0. Data collected at 37 °C.
Previous studies have identified the presence of NaCl and in particular Cl\(^-\) ions as an inhibitor of vesicle collapse on flame annealed gold surfaces.\(^7,8\) Whilst the surfaces in this work could not be flame annealed due to their fragile nature, the influence of NaCl was investigated by removing this component from the buffer solution. It is important to note that Cl\(^-\) ions were still present in the solution due to pH adjustment with HCl but at a much lower concentration (10 mM or less). Figure 5.3 shows how vesicles adsorbed to the gold crystal when NaCl was removed from the buffer system and lipids introduced (step 2). There was a decrease in Δf and increase in ΔD indicative of vesicle adsorption. At 68 minutes a sudden drop in ΔD and simultaneous drop in Δf were observed, which then recovered a minute later. This was associated with collapse of vesicles in isolated patches on the surface whilst vesicles continued adsorbing in other areas. Rinsing with salt-reduced buffer solution in step 3 caused a loss of mass from the surface of the crystal together with a simultaneous reduction in dissipation. Such an effect could be related to vesicle collapse at the crystal surface and/or loss of vesicles, however, the magnitude of the resting dissipation and frequency indicated that a high density of intact vesicles remained adsorbed at the gold surface.

![Figure 5.3 QCM-D frequency and dissipation plot of vesicle adsorption at the gold sensor surface at neutral pH with NaCl removed from solution. Liquid additions: 1) 10mM Tris 7.1 2) DOPC vesicles 3) 10mM Tris 7.1. Data collected at 37 °C.](image-url)
Cho et al. have previously demonstrated how lowering the pH could promote vesicle collapse on TiO$_2$ surfaces.\cite{9} The effect of low pH, NaCl-free Tris buffer on lipid vesicle interactions with the gold surface was therefore investigated. Figure 5.4A shows vesicles adsorbed to the gold surface at pH 4, in NaCl-free Tris buffer. Upon rinsing with the same buffer (Figure 5.4A, step 3), the vesicles remained adsorbed at the surface, however, a positive shift in frequency and simultaneous decrease in dissipation were observed which recovered after a few minutes. This was attributed to the rearrangement of vesicles at the surface or possible areas of isolated vesicle collapse. The attached vesicle layer was then rinsed with a pH 7.1 Tris buffer solution which resulted in a dramatic increase in $\Delta f$ and decrease in $\Delta D$ (Figure 5.4A, step 4). The change in frequency was indicative of a loss of mass from the surface of the crystal, while the changes in dissipation indicated a more rigid structure adsorbed to the sensor surface. The stable resting $\Delta f$ value at $-20$ Hz suggested possible bilayer formation across the crystal surface but is slightly lower than the previously reported $-25.5$ Hz.\cite{9} The resting dissipation observed at $2 \times 10^{-6}$ is certainly higher than $0.2 \times 10^{-6}$ expected for a uniform bilayer suggesting the presence of some residual vesicles at the gold surface.

The effect of using a low to neutral pH transition to promote collapse at the gold surface proved to be highly variable. In a separate experiment vesicles were shown to adsorb to the gold surface at pH 3.5 but remained firmly adsorbed when rinsed at pH 6.8 (Figure 5.4B). AFM imaging was used to further investigate changes in lipid structure at the gold surface. Features of around 160 nm in lateral size and 30-40 nm in height could be observed at the gold surface, following introduction of a DOPC vesicle solution at pH 4 (Figure 5.5). These features were associated with the presence of adsorbed vesicles at the gold surface. Rinsing at pH 7 caused a distinct smoothing of the surface suggesting structural changes and possible vesicle collapse at the crystal surface.
Figure 5.4  QCM-D frequency and dissipation plots of gold sensor surfaces. Different outcomes when inducing vesicle collapse using a low to neutral pH transition. A) Bilayer formation and B) stable vesicular layer. Liquid additions: A)1) 10 mM Tris pH 4.0, 2) DOPC vesicles, 3) 10 mM Tris 4.0, 4) 10 mM Tris pH 7.1 and B) 10 mM Tris pH 3.5, 2) DOPC vesicles, 3) 10 mM Tris 3.5, 4) 10 mM Tris pH 6.8. Data collected at 37 °C.

Figure 5.5 AFM images (1 µm x 1 µm) of a gold QCM-D crystal following adsorption of DOPC vesicles at pH 4 and rinsing at pH 7. Images collected at ambient temperature. The 0-70 nm Z-scale of the two images is the same.
AFM experiments were conducted at ambient temperature, therefore the effect of pH transition was further investigated using QCM-D at 25°C to investigate any temperature related effects. Figure 5.6 demonstrates that temperature did not affect the variability of the result. In identical conditions, partial collapse was apparent on one gold surface upon rinsing at pH 7 (Figure 5.6), whilst no changes occurred upon rinsing on the other (Figure 5.6B).

Figure 5.6 QCM-D frequency and dissipation plots of gold sensor surfaces. Different outcomes when inducing vesicle collapse using a pH 4.0 to pH 7.0 transition at 25°C. A) Partial collapse and B) stable vesicular layer. Liquid additions: 1) 10 mM Tris pH 4.0 2) DOPC vesicles 3) 10 mM Tris pH 4.0 4) 10 mM Tris pH 7.0.
Given the importance of buffer composition on vesicle collapse, chloride-free Tris and HEPES buffer solutions were used, without pH adjustment, to completely remove the effects of Cl\textsuperscript{−} on lipid vesicle interactions with the gold QCM-D surface. Stock vesicle solutions were made by hydrating the lipid cake using the respective HEPES or Tris chloride-free buffers which were also used for dilution to the final concentration of 0.5 mg/mL. As shown in Figure 5.7A, in chloride-free HEPES buffer (pH 5.3), vesicles were shown to readily adsorb to the gold surface and were stable upon rinsing, with no indication of collapse. In contrast, the chloride-free Tris buffer (pH 9.9) acted to inhibit vesicle adsorption at the gold surface (Figure 5.7B).

Overall, these results indicate the variability of interactions when using pH transitions to control vesicle interactions with gold surfaces and suggest that at low Cl\textsuperscript{−} concentrations both pH and buffer salt chemistry are important factors.

Figure 5.7 QCM-D frequency and dissipation plot of vesicle adsorption at the gold sensor surface in A) 10 mM HEPES solution and B) 10mM Tris solution in the absence of chloride ions. Liquid additions: A) 1) 10 mM HEPES 2) DOPC vesicles 3) 10 mM HEPES and B) 1) 10 mM Tris 2) DOPC vesicles.
5.2.2 Lipid vesicle interactions with plasma polymers

5.2.2.1 QCM-D
The interaction of DOPC lipid vesicles with ppOct, ppAAlc, ppAAm and ppAAc films was first studied in physiological-like conditions at a temperature of 37°C and neutral pH (~pH 7 in Tris NaCl buffers). In Figure 5.8 solutions of 10 mM Tris (step 1) and 10 mM Tris + 150 mM NaCl (step 2) were added in a stepwise manner to observe changes in film properties relating to the buffer ionic strength. DOPC vesicles in 10 mM Tris + 150 mM NaCl buffer were then introduced (step 3) before a final buffer rinse (step 4). Response of the films to the buffer solutions was minimal when compared with an uncoated gold QCM-D crystal in response to a 10 mM + 150 mM NaCl solution (Figure 5.2, $\Delta f \approx 4$ Hz, $\Delta D \approx 1.5$). The changes in frequency and dissipation were likely dominated by the buffer effect which arises from the sensitivity of the technique to changes in ionic concentration in the solution above the crystal.

Different lipid vesicle interactions were observed depending on plasma polymer film chemistry. The most hydrophilic of the films, ppAAc, provided the smallest response upon addition of the DOPC vesicles with a frequency and dissipation shift of $\sim 6$ Hz and $\sim 2 \times 10^{-6}$, respectively, indicating few vesicles attached to the surface. Rinsing at step 4 caused a gradual removal of mass from the surface with a simultaneous decrease in dissipation suggesting weak interactions between the adsorbed vesicles and ppAAc surface. In contrast, a strong interaction was observed with the ppAAm surface. Upon introduction of the vesicle solution large changes in both frequency ($\Delta f \approx 270$ Hz) and dissipation ($\Delta D \approx 44 \times 10^{-6}$) were observed. These changes indicated an adsorbed layer of vesicles at the surface$[4]$ which remained stable upon rinsing. Stable vesicle adsorption was also observed on ppAAlc, however, at a lower density than observed on ppAAm. Some vesicle interaction was also observed on the hydrophobic ppOct surface but upon rinsing a loss of mass and reduction in dissipation were observed with $\Delta f$ and $\Delta D$ reaching $\sim 25$ Hz and $9 \times 10^{-6}$, respectively. Rinsing caused a gradual loss of mass from the surface, lasting over 40 minutes with overall $\Delta f$ values of $\sim 9$ Hz associated with bound lipid. $\Delta D$ was also reduced from $8 \times 10^{-6}$ to $2 \times 10^{-6}$ during rinsing. Hydrophobic alkane thiol surfaces have previously been shown to enable monolayer formation which would be expected to have half the mass of a bilayer with an associated $\Delta f$ of $\sim 13$Hz and a dissipation close to 0.$[6]$ For ppOct the increase in dissipation associated with bound lipid was $\sim 1.5 \times 10^{-6}$ which is much higher than
expected for a rigid monolayer. Whilst lipid material remained adsorbed to the ppOct film it is unclear in what form.

Figure 5.8 QCM-D frequency and dissipation plots at neutral pH for ppAAc, ppAAm, ppAAlc and ppOct films. Liquid additions 1) 10 mM Tris 2) 10 mM Tris + 150 mM NaCl 3) Vesicles in 10 mM Tris + 150 mM NaCl 4) 10 mM Tris + 150 mM NaCl. 10 mM Tris (pH 6.8-7.3) and 10 mM Tris + 150 mM NaCl (pH 7.0-7.3).

Lowering pH has been used as a powerful tool for enabling vesicle collapse on surfaces such as TiO$_2$ that do not readily promote collapse at neutral pH.$^9, 10$ While there was limited lipid vesicle interaction evident with the ppAAc film at pH 7 the experiment was repeated at pH 4 to explore the role of solution pH on lipid interactions. Lowering of the pH was used to bring the ppAAc from a negative charge regime at pH 7 to a neutral charge regime at pH 4.$^{11}$ In Figure 5.9 vesicles readily adsorbed to the ppAAc film at pH~4, as indicated by Δf rapidly decreasing to -290 Hz and a simultaneous increase in dissipation of over 50x10$^{-6}$ (step 3). Rinsing of the surface at pH 4 demonstrated that the vesicle layer was stable. The effect of raising the pH was then investigated given the previously observed changes this promoted on gold surfaces (Figure 5.4A and Figure 5.5). Rinsing the vesicle layer with a pH 7 buffer resulted in a distinct spike in dissipation which was followed by a rapid reduction in dissipation,
stabilising at \( \Delta D \approx 7 \times 10^{-6} \). A simultaneous increase in \( \Delta f \) was also observed indicating loss of mass from the surface with \( \Delta f = \) stabilising at -30 Hz.

In the ppAAc control experiment without vesicles, shown in Figure 5.10, the change in pH caused an increase in the \( \Delta f \) value of \( \approx 10 \) Hz, suggesting the film increased in mass via water adsorption. Critically, there was no evidence of the spike in dissipation upon changing the pH from 4 to 7, as observed when the vesicles were present on the surface. This suggested that the feature was due to a structural change of the vesicle layer, associated with swelling and subsequent rupture of the lipid vesicles. The presence of the underlying plasma polymer and its response to changes in pH complicated the interpretation of the QCM-D data. The final resting values for \( \Delta D \) and \( \Delta f \), after rinsing at pH 7, were \( \approx 7 \times 10^{-6} \) and -30 Hz, respectively, however, these values include contributions from both the swollen film at pH 7 and lipid at the surface. If it is assumed that the film swells under the lipid vesicle layer in the same manner as the control film upon rinsing at pH 7, then the \( \Delta f \) and \( \Delta D \) values from the remaining lipid at the surface (subtracting \( \Delta f \) and \( \Delta D \) associated with the swollen film) would be approximately -15 Hz and \( 6 \times 10^{-6} \) respectively. This dissipation is much higher than one would expect for a rigid bilayer structure which suggests contributions from both the plasma polymer layer and sparsely adsorbed vesicles. The frequency contribution is also lower than one would expect for a uniform bilayer across the surface of a QCM-D crystal, however, the presence of a patchy bilayer across the surface of the crystal is a possibility. Once more, the behaviour of the plasma polymer layer makes interpretation difficult, requiring other complimentary techniques to confirm the type of lipid structure at the surface.
The effect of a low to neutral pH transition was next investigated for the ppAAm film. Figure 5.11 shows how the film swelled dramatically in step 2 upon introduction of the 10 mM Tris pH 4.0 2) 10 mM Tris + 150 mM NaCl pH 3.9 3) 10 mM Tris + 150 mM NaCl pH 7.0. Rinsing of the layer at pH 7.3 did...
not induce the same changes as observed with ppAAc and gold suggesting a stable vesicle layer at the surface of the ppAAm.

The experiments on ppAAc had demonstrated how pH could be used to adjust surface charge and influence vesicle interactions. An additional experiment was therefore conducted to explore vesicle interactions on ppAAm in a neutral charge regime at pH 10[11]. Figure 5.12 shows the swelling behaviour observed on ppAAm at pH 3.7, which was not observed at pH 10, and vesicles readily adsorbed to the neutral surface. The vesicle layer was rinsed at both pH 7.3 and then pH 3.7, however, the vesicle layer remained stable at the ppAAm surface. The drop in frequency following the pH 3.7 rinse in step 6 is attributed to swelling behaviour of the film beneath the vesicle layer, as observed in Figure 5.11, step 2.

Figure 5.11 QCM-D frequency and dissipation plot for ppAAm coating when using a two-step pH transition. Liquid additions: 1)10 mM Tris pH 3.7 2)10 mM Tris + 150 mM NaCl pH 3.7 3) Vesicles 4) 10 mM Tris + 150 mM NaCl pH 7.3.
Figure 5.12 QCM-D frequency and dissipation plot for ppAAm coating. Liquid additions: 1) 10 mM Tris pH 10.1 2) 10 mM Tris + 150 mM NaCl pH 10.0 3) Vesicles 4) 10 mM Tris + 150 mM NaCl pH 10.0 5) 10 mM Tris + 150 mM NaCl pH 7.3 6) 10 mM Tris + 150 mM NaCl pH 3.7.
5.2.2.2 Confocal microscopy and FRAP

To investigate the presence of lipid material at the surface of the ppAAm and ppAAc films, DOPC vesicles with a 1 wt% fluorescent lipid composition were incubated on the plasma polymers and confocal microscopy was used to image the surface. FRAP techniques were further used to characterise mobility of the lipid structures.

Glass control surfaces were first prepared in order to confirm the mobility of supported lipid bilayers formed from the collapse of DOPC vesicles with 1 wt% 14:0 NBD PE (green) or 14:0 Liss Rhod PE (red) fluorescent lipids. FRAP experiments were conducted by exposing a 50 µm diameter circular bleach area to a 100 % intensity laser (488 nm for NBD PE and 543 nm for Liss Rhod PE). Images were then collected immediately after bleaching and at one minute intervals for 10 minutes. Figure 5.13A shows a clean glass coverslip substrate incubated with 1 wt% 14:0 NBD PE (green) DOPC vesicles and rinsed at pH 7. A clearly defined bleach spot was formed following exposure to the intense laser, as imaged post-bleach at 0 min. Changes in fluorescent intensity within the bleach area were then monitored over a 10 minute period as a measure of lipid mobility. As shown in Figure 5.13A fluorescent, unbleached lipids, had diffused into the bleached area over the 10 minute period confirming mobility at the glass surface and the successful formation of a mobile SLB.

In a similar experiment another clean glass substrate was incubated with 1 wt % 14:0 Liss Rhod PE (red) DOPC vesicles and rinsed at pH 7. As in the previous experiment, a 50 µm diameter circular bleach area was exposed, this time, using the 543 nm laser. Lipid was clearly visible at the glass surface and a bleach spot was formed following exposure (Figure 5.13B). The bleach area was less defined than that observed for the bilayer incorporating 14:0 NBD PE lipids, suggesting differences between power of the two lasers (when set to 100% intensity) or that the diffusion was quicker for the bilayer incorporating 14:0 Liss Rhod PE lipids. The latter would mean faster recovery causing a less defined bleach area at the edges. As shown in Figure 5.13B, diffusion of unbleached fluorescent lipids into the bleach area over the 10 minute period (following exposure), confirmed the presence of a mobile SLB at the glass surface.
Figure 5.13 FRAP experiments demonstrating mobile SLBs on glass formed from vesicles of 1 wt% 14:0 NBD PE lipids (A) or 14:0 Liss Rhod PE (B) with 99 wt% DOPC. In both cases a 50 µm diameter bleach spot was exposed to the 100% laser. Vesicles were diluted to a concentration of 0.5 mg/mL in 10 mM Tris + 150 mM NaCl pH 7 buffer before use. Scale bar 20 µm.

To investigate lipid vesicle interactions and lipid mobility on plasma polymers, ppAAm and ppAAc films were deposited onto individual clean glass coverslips. The surfaces were then incubated with vesicles (1 wt% 14:0 NBD PE, 99 wt% DOPC) in varying pH conditions and rinsed with buffer.

Figure 5.14A shows a ppAAm film incubated with fluorescent vesicles and rinsed at pH 7. A 50 µm diameter bleach area was exposed to a 488 nm laser at 100 % intensity. The image collected immediately after exposure, at 0 min, shows lipid material adsorbed to the surface and the presence of a clearly defined bleach spot. Images were then collected every minute, for a total of 10 minutes, after the initial exposure, to monitor fluorescence recovery within the bleach spot. As shown in Figure 5.14A, after 10 minutes, fluorescence recovery was not observed indicating that lipid material adsorbed to the ppAAm film was immobile. The same experiment was conducted for ppAAm surfaces incubated with fluorescent vesicles at pH 10 (Figure 5.14B) and 4 (Figure 5.14C) which yielded the same observation of immobile lipid material adsorbed to the ppAAm film.
The effect of a pH transition was also investigated by rinsing the ppAAm surface and adsorbed lipid formed at pH 4, with a pH 7 buffer solution (Figure 5.14C). After rinsing, lipid remained adsorbed at the surface and was confirmed as immobile after performing a further FRAP experiment. This showed the absence of fluorescence recovery 10 minutes after initial exposure (Figure 5.14C). These results are in agreement with the QCM-D data which showed stable vesicular layers form at the surface of ppAAm over a wide range of pH (Figure 5.8, Figure 5.11 and Figure 5.12).

Identical FRAP experiments were also performed on ppAAc films deposited onto clean glass coverslips. FRAP could not be performed on the ppAAc film at pH 7 due to limited, if any, lipid material adsorption at the surface. Only at the edges of the cell insert wells were small amounts of fluorescent material observed. This indicated lipid material had not adsorbed to the surface. The result is in agreement with the QCM-D data showing limited interaction of DOPC vesicles with ppAAc at neutral pH (Figure 5.8).

Lowering the pH to 4 enabled lipid material to adsorb to the ppAAc surface, which could be bleached successfully as shown in Figure 5.14D at 0 minutes. No fluorescence recovery was observed in the bleach area in the 10 minute period after initial exposure. This indicated that lipid material adsorbed to the ppAAc surface (at pH 4), was immobile.

A previous QCM-D experiment had shown vesicle adsorption occurring at the ppAAc surface at pH 4 (Figure 5.9). The layer was then rinsed at pH 7, which appeared to initiate vesicle rupture, leaving lipid material adsorbed at the surface. These conditions were recreated on a ppAAc-coated coverslip and imaged using confocal microscopy. As shown in Figure 5.14D, after rinsing of the vesicular layer (formed at pH 4) with a pH 7 buffer, fluorescent lipid material remained at the ppAAc surface and could be successfully bleached. The lipid was immobile as shown by the absence of fluorescence recovery in the bleach area (10 minutes after initial exposure of the bleach area). Together with the QCM-D data, this result indicates that immobile lipid material remains at the ppAAc surface after rinsing at pH 7, but it is unclear in what form.
Figure 5.14 FRAP experiments on ppAAm and ppAAc plasma polymer films in various pH conditions. Vesicles of 1 wt% 14:0 NBD PE lipids and 99 wt% DOPC were incubated on the surface at the appropriate pH for 10 minutes before rinsing. Scale bar 20 µm.
5.3 Discussion

Vesicles adsorbed readily to the gold QCM-D crystal at neutral pH which was consistent with previous observations.\[^5, 6\] This is unsurprising given the high polarizability of gold providing an attractive potential.\[^6\] It is also known that chloride ions have a high affinity for gold surfaces and bind via a chemisorption bond modifying the surface with chlorine atoms.\[^8, 12\] Liu et al. described this as having several effects including a more positive charge at the gold surface\[^13\] which would provide more favourable electrostatic interactions with lipid vesicles. Facilitation of lipid interactions via chloride ions was further supported by the inhibition of vesicle adsorption to the gold in chloride-free non-pH adjusted Tris buffer conditions (pH 9.9). However, chloride-free HEPES buffer (pH 5.3) did enable vesicle adsorption. The difference in pH provides one possible explanation, however, the successful formation of bilayers on flame annealed gold in 100 mM NaF (pH ~9.0),\[^8\] suggests buffer chemistry might be playing a larger role than low chloride ion concentration in this instance. It is known that large counterions at a surface can inhibit vesicle collapse by increasing entropic repulsion and thus reducing lipid-surface interactions.\[^14\] It is possible that Tris ions may be more strongly associated with the gold surface than HEPES which would increase entropic repulsion, however, the nature of the interaction and how chloride ions would mediate this effect is unclear. NaF for example would generate smaller counterions, therefore reducing any effects of entropic repulsion. Overall, it is difficult to decouple effects from the buffer salt chemistry, pH and chloride ion presence in such a complex interaction.

A further consideration is the association of the primary amine of the Tris ion to the phosphate region of the phosphatidylcholine (PC) headgroup which has a more negative surface potential at higher pH.\[^10\] HEPES does not have a primary amine component which could reduce affinity with the gold surface and/or the lipid headgroup. Removal of NaCl from the buffer at neutral pH did not inhibit vesicle adsorption at the gold surface, however, it did appear to affect stability of the vesicles upon rinsing. This is likely related to the reduction in chloride ions affecting the interaction between the vesicles and gold surface.

Electrostatic interactions are known to be a major driver in vesicle adsorption and collapse. This was clearly demonstrated by the varied interactions of DOPC lipid
vesicles with the gold and plasma polymer-coated surfaces investigated in this work. QCM-D data showed that, in physiological-like conditions at 37°C and neutral pH (~pH 7), vesicle interactions with the ppAAc film were limited, with little evidence of vesicle adsorption and removal of those vesicles upon rinsing of the surface (Figure 5.8). This was also apparent under the epi-fluorescence microscope, where lipid material could not be observed. These results were explained by the repulsive electrostatic interactions between the negatively charged film and DOPC vesicles. At pH 7 COOH groups at the surface of the ppAAc film would be dissociated to COO⁻ and the PC headgroup of the lipids would exhibit a negative surface potential.\textsuperscript{[10]} In contrast, ppAAm is positively charged at neutral pH\textsuperscript{[11]} which created favourable electrostatic interactions with the slightly negatively charged vesicles and enabled stable vesicle adsorption, as shown by QCM-D (Figure 5.8). This vesicular layer was shown to be immobile using FRAP (Figure 5.14A). The dominance of electrostatic interactions was further demonstrated by QCM-D data clearly showing vesicle adsorption at ~pH 4 when ppAAc is within a neutral charge regime (Figure 5.9). FRAP showed that this vesicle layer was immobile (Figure 5.14D). Lipids also adsorbed to ppAAm at both pH 4, when the surface has an increasingly positive surface potential, and at pH 10 in a neutral charge regime (Figure 5.11 and Figure 5.12). As shown by FRAP, these vesicle layers were also immobile (Figure 5.14B and C).

ppAAlc also enabled vesicle adsorption at pH 7, however, with a lower vesicle density than on ppAAm (smaller mass increase) (Figure 5.8). In Chapter 4, high resolution XPS analysis of the C 1s peak from the ppAAlc film showed a larger component for the alcohol and ether contributions compared with smaller peaks derived from ketones and aldehydes and carboxylic acids and ester groups. The XPS O/C ratio of 0.12 together with the high contact angle of 71° suggested a low hydroxyl content for the film. Furthermore, oxygen contributions in the C 1s spectra can arise from both C-O-C and OH groups, which cannot be differentiated within the XPS spectra without using a derivatization technique. Overall, these results suggested that ppAAlc provided a lower negative surface potential when compared with ppAAc, with reduced electrostatic repulsion enabling limited but stable vesicle adsorption. However, other characterisation techniques such as XPS derivatisation and zeta potential measurements would provide greater insight into vesicle interactions at the ppAAlc surface.
The effect of pH transition as a means for inducing vesicle collapse was investigated on ppAAm, ppAAc and gold surfaces. Previous studies have shown that lowering pH can enable vesicle collapse on maleic acid copolymer cushions\cite{15} and TiO$_2$\cite{9}. In this work the same approach was taken for ppAAc to reduce electrostatic repulsion which enabled vesicle adsorption but not spontaneous bilayer formation at pH 4 (Figure 5.9). The effect of rinsing at pH 7 had a dramatic effect on the vesicle layer adsorbed on ppAAc. The peak in dissipation was particularly distinctive and not present on a control ppAAc surface (Figure 5.10). A possible explanation for this effect was the increased electrostatic repulsion between vesicles, caused by the increase in pH, leading to deformation of the vesicle walls and interbilayer stresses sufficient enough to initiate rupture.\cite{16} Given the osmotic difference across the vesicle wall (higher salt concentration on the outside of the vesicle) any pore formation occurring during deformation could have enabled rapid swelling of the vesicles prior to rupture giving rise to a spike in dissipation. The subsequent dramatic reduction in mass and dissipation suggested possible bilayer formation, however, the resting dissipation of 6x10$^{-6}$ is too high for a planar bilayer and indicated some vesicles may have remained intact. FRAP showed that vesicles adsorbed to the ppAAc surface at pH 4 were immobile (Figure 5.14D). Upon rinsing at pH 7, it was clear that lipid material remained at the ppAAc surface, however, this too, was immobile. It is not clear if the lipid was in the form of an immobile bilayer, a bilayer with sparsely adsorbed vesicles or lipid material in some other form. Further complementary techniques such as AFM are required to further investigate the lipid structure at the surface. In contrast, vesicles adsorbed to ppAAm remained stable during rinsing for all pH transitions investigated, despite the presence of tight vesicle packing at the surface (Figure 5.11 and Figure 5.12). FRAP revealed that at pH 4, 7 and 10 the vesicular layers were immobile (Figure 5.14 A, B and C). These results indicate that it was not increased electrostatic repulsion between vesicles alone which caused rupture on ppAAc, with interactions between the surface and vesicles also playing an important role. Important further work to explore the role of electrostatic interactions would be to investigate the interaction of charged lipid vesicles with the plasma polymers at varying pH.

In comparison, the outcome of a ~ pH 4 to 7 transition on gold in NaCl-free conditions proved to be highly variable at both 25°C and 37°C, however, the pH transition did initiate a loss of mass and dissipation on some crystals. This process was attributed to
structural changes occurring at the surface. This was later confirmed by AFM imaging which clearly showed a flattening of the surface upon rinsing at pH 7, however, the exact lipid structure at the surface requires further investigation. This is especially the case given that the interactions between the AFM tip and vesicles can introduce artefacts leading to displacement and even rupture of adsorbed vesicle depending on the tip properties and image settings.\cite{17} Despite this, the observations of vesicle collapse on gold at all were somewhat surprising given the previous literature describing stable vesicle adsorption on gold QCM-D crystals.\cite{4-6} As with ppAAc, increased electrostatic repulsion between the tightly packed vesicles may have played a role, however, the variability of the result suggested more complex interactions relating to the gold surface. Extensive work has been undertaken by the Lipkowski group to explore vesicle collapse on gold electrodes.\cite{8, 18} In particular, the elimination of chloride ions from solution together with a flame annealed surface were identified as requirements for collapse.\cite{8} As previously discussed, chloride ions play an important role in interactions at the gold surface, especially considering that chloride-free Tris buffer inhibited adsorption. These observations suggested a delicate balance of interactions at the surface where adsorption and possible collapse were controlled by a critical concentration of chloride ions. Flame annealing removes most organic contaminants whilst transforming the surface of the gold by flattening the topology via fusion of smaller domains into larger ones.\cite{19} For Au(111) electrodes this creates irregularly shaped atomically flat terraces with large monoatomic steps.\cite{18} QCM-D sensors are manufactured with an evaporated gold coating and optically polished to a surface roughness of less than 3 nm (RMS) during manufacture, however, some surface treatments such as flame annealing of the sensor are not practical due to the delicate nature of the sensors. In this work sulphur and carbon contaminants were detected at the gold surface by XPS which were attributed to atmospheric handling post cleaning. It is unclear if a more aggressive cleaning method and/or a clean room preparation environment would improve surface preparation and reproducibly of the result. This highlights the requirement for effective and reproducible surface preparation techniques for reliable bilayer formation on gold QCM-D sensors. Furthermore, the influence of the surface preparation method on surface topography of the gold is also interesting given that on SiO\textsubscript{2} topography does not appear to affect collapse of lipid vesicles.\cite{20, 21} The importance of surface preparation on gold may arise from the differences in how
lipids interact with the surface during the collapse process. Lipid molecules have been observed to orientate into flat lying monolayers following vesicle rupture which orientate into bilayers following the accumulation of further molecules at high vesicle concentration.\textsuperscript{[22]}

### 5.4 Conclusion

This chapter has investigated lipid vesicle interactions with plasma polymers and gold surfaces. Gold is a useful surface for sensing platforms and other characterisation techniques such as SPR. The interactions that govern vesicle adsorption and bilayer formation on gold are complex and not fully understood. This work has highlighted the importance of buffer composition, together with the previously known effects of chloride ions and surface preparation on vesicle interactions with gold. Whilst chloride-free HEPES buffer enabled vesicle adsorption, chloride-free Tris buffer prevented vesicle interactions. This is a surprising result which requires further investigation. The role of pH and in particular, the use of a pH transition, is also important. However, the results observed by QCM-D when using a pH 4 to 7 transition were highly variable. AFM revealed a structural change at the gold surface, from an adsorbed vesicular layer at pH 4, to a flattening of the structure at pH 7. These findings warrant further investigation to explore the effect of pH on lipid vesicle/gold interactions.

The interaction of DOPC lipid vesicles with plasma polymers was highly dependent on surface chemistry. At neutral pH, vesicles readily adsorbed to ppAAm, however, limited interactions were observed on ppAAc and ppOct. Vesicles also adsorbed to ppAAlc, however, not at the same density as seen on ppAAm. These differences were related to the electrostatic interactions between the vesicles and surface, governed by the functional groups at the surface and buffer conditions such as pH.

Stable and immobile vesicular layers could be formed on ppAAm over a wide range of pH. This makes it a useful coating for applications such as cell membrane models and encapsulation/delivery platforms. In contrast, ppAAc/lipid vesicle interactions proved to be far more complex, providing a more versatile coating. At pH 7, the surface provided a largely lipid-resistant surface, however, reducing the pH to 4 enabled vesicle adsorption. This was due to the reduced electrostatic repulsion between the neutral ppAAc surface (at pH 4) and vesicles. QCM-D indicated that a pH 4 to 7 transition could initiate vesicle rupture, which is interesting as a potential pH-controlled release
system. However, the structure of the remaining immobile lipid (as shown by FRAP) is not clear and requires further investigation using complimentary techniques such as AFM. Overall, plasma polymers such as ppAAm and ppAAC show great promise as new surfaces for increasingly complex cell membrane model systems.
5.5 References


6 Micropatterning of plasma polymers

6.1 Introduction

In Chapter 5 different lipid vesicle interactions were observed depending on plasma polymer chemistry and buffer pH. It was proposed that by spatially controlling plasma polymer chemistry, using patterning, different lipid structures could be formed on a single substrate. The following chapter describes the fabrication of plasma polymer micropatterns using photolithography techniques and the interaction of lipid vesicles with the patterned surfaces. Both base and patterned plasma polymer layers were deposited close to the electrode at 20W for 20 minutes. Physical characterisation of the patterns was performed using AFM, SEM and optical profilometry. XPS and multivariate analysis of ToF-SIMS image data was employed for chemical characterisation of the patterns and lipid vesicle interactions were studied using confocal microscopy and FRAP techniques.

Au coating and subsequent SEM of plasma polymer micropatterns was conducted by Pierrette Michaux (Swinburne University of Technology). XPS data was collected by Dr. Thomas Ameringer (Swinburne University of Technology) and analysed by HJA. AFM images of micropatterns were collected by Dr. Renee Goreham (Flinders University) and analysed by HJA. ToF-SIMS images were collected by Dr. Robert Jones (La Trobe University) and analysed and processed using multivariate analysis by HJA.
6.2 Results

6.2.1 Physical characterisation of plasma polymer micropatterns

AFM, optical profilometry and SEM techniques were used to assess the physical characteristics of dual plasma polymer micropatterns fabricated using photolithography techniques. Different chemical combinations were fabricated using ppAAc, ppAAm and ppOct films. Figure 6.1A and B shows 3D profilometry images of dual ppOct and ppAAc micropatterns fabricated using a photolithography mask consisting of 10 µm circles separated by 10 µm horizontal and vertical spacings. For both combinations (ppOct patterns on ppAAc or ppAAc patterns on ppOct) fabrication of circular features of around 10 µm in diameter is clearly visible. Prominent features causing ‘spikes’ in height can be observed at the edge of the patterns, an effect prominent on the ppOct patterns. These edges are clearly shown by a height profile of the surface in the x-direction (Figure 6.1C). SEM images further revealed the torn appearance of the ppOct plasma polymer resulting from the removal of the underlying photoresist layer by sonication in acetone (Figure 6.2). Further analysis by AFM confirmed the presence of torn edges for both pattern combinations (Figure 6.3). Using the depth tool, heights of the individual features on the image were analysed. Given the higher deposition rates observed for ppOct, compared with ppAAc, it was unsurprising that the average of the individual feature heights was 58±1 nm for ppAAc and 121±1 nm for ppOct (Table 6.1). The adhesion images demonstrated differences in tip interactions between the different plasma polymer materials and at the pattern edges. Both the optical profilometry and AFM images demonstrated that the patterns of ppAAc were sometimes irregular and not fully circular in shape but with reduced edge effects when compared with ppOct and ppAAm.
Figure 6.1 3D optical profilometer images of 10 µm ppOct and ppAAc micropatterns. A) ppAAc circles patterned onto ppOct, B) ppOct circles patterned onto ppAAc and C) x-direction height profile (red line) of a 3D optical profilometer image of 10 µm ppOct circles patterned onto ppAAc.

Figure 6.2 SEM images of 10 µm diameter ppOct circles patterned onto ppAAc. A) Image of several patterned features, B) higher magnification image of a single circular ppOct feature and C) image of the pattern edge.
Using the same photolithography mask as previously described, dual plasma polymer chemistry patterns were fabricated using ppAAc and ppAAm thin films. As with the ppAAc/ppOct patterns, there was evidence of tearing at the edges of the second plasma polymer layer, with both combinations of chemistry as shown by optical profilometry (Figure 6.4) and AFM (Figure 6.5). ppAAc patterned onto ppAAm exhibited more irregular circular patterns when compared with the inverse pattern of ppAAm circles on ppAAc. As indicated by the AFM images, in some cases the features could be considerably distorted (Figure 6.5B). Like ppOct, ppAAm formed more regular circular features with a higher incidence of edge effects (Figure 6.5A).
Figure 6.4 3D optical profilometer images of 10µm diameter circles patterned using ppAAm and ppAAc. A) ppAAm patterned on ppAAc and B) ppAAc patterned on ppAAm.

Figure 6.5 AFM height sensor (top) and adhesion images (bottom) of 10µm diameter circles patterned using ppAAm and ppAAc. A) ppAAm circles patterned on ppAAc and B) ppAAc circles patterned on ppAAm. Depth analysis for each feature has been labelled on the height sensor images.
Average feature heights from the dual patterns were measured at 45±1 nm for ppAAm patterns on ppAAc and 47±1 nm for ppAAc patterned on ppAAm (Table 6.1). Interestingly, ppAAc features were measured at 58±1 nm when patterned on ppOct. Whilst the film thicknesses for ppAAm and ppAAc were similar to those measured using AFM on unpatterned films in Chapter 4 (Table 6.1) they were not identical. As discussed in Chapter 4, variations in film thickness between runs have been observed close to the electrode, where these films were deposited. Other factors that can account for variation in film thickness include chemistry of the base plasma polymer, the substrate and finally, differences in the position of the substrate within the reactor. For the latter, whilst patterned substrates were coated close to the electrode, the size and number of substrates in a single deposition meant they could not be placed in exactly the same position as each other. The same factors also explain why the ppOct features patterned on ppAAc were different in height (121±1 nm) compared to the film thickness measured using AFM line scan data (83±5 nm).

Table 6.1 AFM depth analysis data of the average feature height for patterned substrates compared with the film thickness measured from plain films deposited on polystyrene masked substrates (data from 3 cm samples in Chapter 4). The mean values for feature height (± standard deviation) are calculated from all the features of the pattern in the AFM image. The mean values (± standard deviation) for film thickness for the masked samples are calculated from 3 line scan analyses per sample.

<table>
<thead>
<tr>
<th>Surface</th>
<th>Feature height from AFM depth analysis (nm)</th>
<th>Film thickness measured from masked sample (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ppOct circles on ppAAc</td>
<td>121±1</td>
<td>ppOct 83±5</td>
</tr>
<tr>
<td>ppAAm circles on ppAAc</td>
<td>45±1</td>
<td>ppAAm 43±1</td>
</tr>
<tr>
<td>ppAAc circles on ppAAm</td>
<td>47±1</td>
<td>ppAAc 51±2</td>
</tr>
<tr>
<td>ppAAc circles on ppOct</td>
<td>58±1</td>
<td></td>
</tr>
</tbody>
</table>

6.2.2 The influence of acetone sonication on plasma polymer chemistry

XPS was used to investigate the effect of sonication in acetone (required for resist lift-off) on film chemistry and stability. Table 6.2 shows that ppAAc was unaffected by the sonication process and the O/C ratio remained 0.27. ppAAm and ppOct showed a slight increase in oxygen content of the film indicating some oxidation of the coatings in acetone. The silicon substrate was not detected indicating no delamination or thinning of the films. Low levels of silicon contamination were detected on some samples which was associated with the adhesive mounting tape used to secure samples on the mounting platen prior to XPS analysis.
Table 6.2 Atomic composition for ppAAc, ppAAm and ppOct films untreated and after sonication in acetone for 3 minutes. The mean values (± standard deviation) are calculated from 3 analyses per sample.

<table>
<thead>
<tr>
<th></th>
<th>Atomic composition (%)</th>
<th>Atomic ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C 1s</td>
<td>O 1s</td>
</tr>
<tr>
<td>ppAAc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>78.4±0.1</td>
<td>21.2±0.1</td>
</tr>
<tr>
<td>Acetone</td>
<td>78.7±0.2</td>
<td>21.0±0.2</td>
</tr>
<tr>
<td>ppAAm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>84.6±0.2</td>
<td>3.2±0.2</td>
</tr>
<tr>
<td>Acetone</td>
<td>83.7±0.3</td>
<td>4.0±0.2</td>
</tr>
<tr>
<td>ppOct</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>95.7±0.2</td>
<td>4.3±0.2</td>
</tr>
<tr>
<td>Acetone</td>
<td>94.8±0.2</td>
<td>5.2±0.2</td>
</tr>
</tbody>
</table>

6.2.3 Chemical characterisation of micropatterns using ToF-SIMS

ToF-SIMS analysis was employed to map the spatial and chemical resolution of dual plasma polymer patterns at the microscale. Multivariate analysis was used to identify the secondary ion fragments generating the most variance within the images. In all cases 100 x 100 µm images were acquired in positive ion mode with a resolution of 256 x 256 pixels. The scores colour bar on the images for each principal component correspond to secondary ion fragments with positive loading (bright) and negative loading (dark). The fragments with positive and negative loading for PC1 have also been plotted individually.

6.2.3.1 Dual ppAAm and ppAAc micropatterns

Figure 6.6 presents 10 µm diameter ppAAm circles patterned onto ppAAc using photolithography. The image formed from PC1 shows clear contrast between the different patterned chemistries. The other components provide little or no contrast. The analysis revealed 16% variance for PC1 separating the surface into two chemically distinct regions. The positive loading plot and associated image display the patterned circles as regions rich in nitrogen containing secondary ion fragments. This supports the presence of a nitrogen containing ppAAm film. In contrast, the negative loading plot displays the background ppAAc as a film containing hydrocarbon and oxygen containing species with the dominant presence of Na⁺ and K⁺ contaminant species.

Removal of the Na⁺ and K⁺ peaks from the analysis is shown in Figure 6.7. Contrast between the patterned regions was reduced as reflected in the lower variance for PC1 at 5%. As in Figure 6.6, the ppAAc background material is dominated by hydrocarbon and oxygen containing secondary ion species and nitrogen containing species for the
ppAAm patterned circles. The image composed from the negative PC1 scores shows a less defined boundary between the regions of ppAAc and ppAAm plasma polymers, where nitrogen containing species seem to be concentrated at the ppAAm circles but also scattered on the ppAAc background plasma polymer.

Figure 6.6 PCA of ToF-SIMS images of ppAAm circles patterned onto ppAAc.
Figure 6.7 PCA of ToF-SIMS images of ppAAm circles patterned onto ppAAc following removal of Na$^+$ and K$^+$ peaks from the analysis.
Multivariate analysis of the inverse pattern consisting of ppAAc circles on ppAAm is shown in Figure 6.8. As observed previously using AFM (Figure 6.5), the quality of the pattern was reduced with more irregular circular structures formed during patterning of the ppAAc plasma polymer. There is clear contrast between the chemically different ppAAc and ppAAm regions, as shown by the PC1 image. The variance is lower, at 8% for PC1, when compared with the pattern of ppAAm circles on ppAAc. This is likely due to the Na\(^+\) and K\(^+\) peaks no longer dominating the loadings plot. The positive loading plot shows that the ppAAm regions display nitrogen containing secondary ion fragments. The negative loading plot contains various hydrocarbon and oxygen containing secondary ion fragments within the ppAAc regions.

Figure 6.8 PCA of ToF-SIMS images of ppAAc circles patterned onto ppAAm.
6.2.3.2 Dual ppOct and ppAAc micropatterns

Figure 6.9 shows multivariate analysis of a surface fabricated with 10 µm ppOct circular regions on a ppAAc film. The different regions of plasma polymer can be observed in the PC1 image with variance of 9%. Unsurprisingly, the PC1 loadings plot and associated images show that the ppOct regions are dominated by signal from various hydrocarbon species. The ppAAc regions expressed oxygen containing secondary ion fragments together with the dominant presence of Na\(^+\) and K\(^+\) contaminant species.

Figure 6.9 PCA of ToF-SIMS images of ppOct circles patterned onto ppAAc.
Upon inverting the chemical pattern to generate ppAAc patterned areas on a base layer of ppOct the dominance of the Na\(^+\) and K\(^+\) contaminant species from the loading plot was reduced (Figure 6.10). This was also observed when creating dual patterns using ppAAc with ppAAm (Figure 6.6 and Figure 6.8). This association between ppAAc as a base plasma polymer and the presence of Na\(^+\) and K\(^+\) contaminant species indicated interactions occurring during the photolithography stage. Reduced contamination when ppAAc is the upper pattern plasma layer suggests this interaction takes place when the photoresist is first applied as a liquid and then baked and developed and not during the acetone removal stage.

Figure 6.10 PCA of ToF-SIMS images of ppAAc circles patterned onto ppOct.
6.2.4 Lipid vesicle interactions with dual plasma polymer micropatterns

As described in Chapter 5, stable vesicle layers could be formed on ppAAm under a variety of pH conditions. In contrast, ppAAc showed limited vesicle interaction at pH 7 and vesicle adsorption at pH 4. This pH-dependent behaviour was used to explore spatially controlled lipid vesicle deposition on patterned surfaces of 10 µm diameter ppAAc circles on ppAAm. Figure 6.11 shows a schematic of the patterned surface and the stages involved in the experiments on patterned ppAAc/ppAAm substrates.

Figure 6.11 Spatial patterning of lipids on patterned surfaces. A) Schematic of 10 µm diameter ppAAc circles patterned on ppAAm and B) lipid vesicle incubation and rinse stages performed on the ppAAc/ppAAm patterns.

Figure 6.12 shows the patterned surfaces after incubation with 1 wt% 14:0 NBD PE, 99 wt% DOPC (green) vesicles and rinsing at pH 7. As expected, fluorescent lipid material could be imaged on the ppAAm patterned area but not on the ppAAc circles. A FRAP experiment was conducted on the surface by exposing an area of the sample to a 100% intensity laser to bleach the lipids on ppAAm in that region. As shown in Figure 6.12A a bleach spot is clearly visible immediately after exposure (0 min). Lipid mobility was
monitored by imaging the area every minute following exposure for a total of 10 minutes. Over the 10 minute period fluorescence recovery in the bleach region was observed (Figure 6.12A). This was associated with mobility of the lipids on the ppAAm surface. A second bleach spot was then exposed close to the initial bleach area. The second bleach spot was considerably darker, due to a loss of fluorescent signal compared with the initial bleach region, indicating that recovery had indeed occurred at the initial bleach site. This result was somewhat surprising given that immobile vesicle adsorption had been observed at pH 7 using QCM-D and FRAP in Chapter 5.

The surface was then incubated with 1% 14:0 Liss Rhod PE, 99 wt% DOPC vesicles for 10 minutes at pH 4. As expected, lowering the pH to 4 brought the ppAAc surface into a neutral charge regime, lowering electrostatic repulsion between the vesicles and surface, and enabling vesicle adsorption (Figure 6.13). For FRAP, the exposure area was placed over two of the ppAAc circular features and part of the ppAAm surface. As before, mobility was observed on the ppAAm surface (green channel) after 10 minutes. On the ppAAc features, very limited mobility was observed at the edges of the bleached areas but the fluorescent signal was very weak compared with the unbleached areas. This suggested predominantly immobile vesicles with a possibility of some mobile material with a very slow diffusion rate on the ppAAc regions. In a second FRAP experiment, a whole ppAAc feature and the surrounding ppAAm area were bleached (Figure 6.14A). Mobility was again observed on ppAAm. There also appeared to be some very weak signal coming from the ppAAc region after 10 minutes. This was associated with residual lipid fragments in solution adsorbing to the surface. These observations were confirmed by bleaching of a second area close to the initial bleach area (Figure 6.14B).

Finally, the surface was rinsed at pH 7 (Figure 6.15). This reduced the signal-to-noise ratio in the red channel, suggesting that less fluorescent material was present at the ppAAc surface. This observation supported the QCM-D data in Chapter 5 showing that a pH 4 to 7 transition caused significant mass and dissipation losses from the surface of ppAAc but that lipid material remained at the surface. Lipid mobility remained on the ppAAm surface, however, lipid material on ppAAc remained immobile during the 10 minute observation period.
Figure 6.12 FRAP experiments conducted on patterned surface of ppAAc circles on ppAAM after incubation with 1 wt% 14:0 NBD PE, 99 wt% DOPC (green) vesicles at pH 7. A) Confocal microscopy images of the surface after bleaching (0 minutes) and 10 minutes later and B) bleaching of a second area to demonstrate fluorescence recovery in the initial bleach area. Scale bar 10 µm.
Figure 6.13 FRAP experiment conducted on patterned surface of ppAAc circles on ppAAm after incubation with 1 wt% 14:0 NBD PE, 99 wt% DOPC (green) vesicles for 10 minutes at pH 7 followed by incubation with 14:0 Liss Rhod PE, 99 wt% DOPC vesicles for 10 minutes at pH 4. Confocal microscopy images of the surface were collected after bleaching (0 minutes) and 10 minutes later. Scale bar 10 µm.
Figure 6.14 FRAP experiments conducted on patterned surface of ppAAAc circles on ppAAm after incubation with 1 wt% 14:0 NBD PE, 99 wt% DOPC (green) vesicles for 10 minutes and rinsing at pH 7, followed by incubation with 14:0 Liss Rhod PE, 99 wt% DOPC vesicles for 10 minutes and rinsing at pH 4. A) Confocal microscopy images of the surface were collected after bleaching (0 minutes) and 10 minutes later, B) bleaching of a second area to demonstrate fluorescence recovery in the initial bleach area. Scale bar 10 µm.
Figure 6.15 FRAP experiments conducted on a patterned surface of ppAAc circles on ppAAm after incubation with 1 wt% 14:0 NBD PE, 99 wt% DOPC (green) vesicles for 10 minutes and rinsing at pH 7, followed by incubation with 14:0 Liss Rhod PE, 99 wt% DOPC vesicles for 10 minutes and rinsing at pH 4 and finally rinsing at pH 7. Confocal microscopy images of the surface were collected after bleaching (0 minutes) and 10 minutes later. Scale bar 10 µm.
On ppAAm areas exhibiting mobility, a dark ‘shadow’ could still be observed, after 10 minutes following exposure, in the green channel where the initial bleach area was located. An example of this is shown in Figure 6.15. This could be associated with partial or slow recovery, however, even at the edge of the initial bleach area, a defined dark edge is present. It has previously been observed\(^4,5\) that plasma polymers express auto-fluorescence associated with unsaturated carbon bonds suggesting that the underlying plasma polymer layer may also bleach upon exposure to the laser. The effect of intense laser exposure on the plasma polymers was therefore investigated by separating a ppAAc film into two isolated areas using an ibidi cell insert (ibidi GmbH, Germany, see Chapter 3 Materials and methods). One well was left lipid-free, with pH 4 buffer solution, whilst the other was incubated with green fluorescent vesicles and rinsed at pH 4 to form a vesicular layer. The data in Figure 6.16A shows that it is possible to bleach the plasma polymer layer. This is indicated by the reduced signal intensity in the bleach area immediately after exposure to the laser. The bleached area could still be imaged 9 minutes following exposure (Figure 6.16B). As shown by the line profiles associated with each of the images, the bleach area is not clearly defined from the background autofluorescence signal of the surrounding un-bleached plasma polymer.

For comparison, the fluorescent vesicular layer adsorbed to the ppAAc was bleached using identical bleach settings to that used for the lipid-free ppAAc film. A dark bleach spot could be observed as shown in the confocal image in Figure 6.16C. The loss of intensity in the bleach area was clearly indicated by the line profile. As expected, the vesicular layer was immobile as shown by the confocal image collected 9 minutes after bleaching (Figure 6.16D). Once again, the line profile showed a loss of fluorescent signal in the bleach area with improved signal-to-noise ratio when compared with the line profiles from the lipid-free surface.
Figure 6.16 FRAP experiments conducted on A) lipid-free ppAAc in pH 4 buffer and B) ppAAc film following incubation with 1 wt% 14:0 NBD PE, 99 wt% DOPC (green) vesicles for 10 minutes and rinsing at pH 4. Associated line profiles were conducted on greyscale images for each bleach spot. The same size bleach spot (50 µm diameter) was used in both cases. Scale bar 20 µm.
6.3 Discussion

The work presented in this chapter demonstrates the successful micropatterning of dual plasma polymer chemistries. As described previously, photolithography lift-off methods provide an effective way of masking the substrate to enable the fabrication of dual plasma polymer patterns.\textsuperscript{[5-8]} Physical characterisation of the patterns in this work revealed that edge effects were common. This is unsurprising given the lift-off process which effectively tears the plasma polymer at the pattern edge. This is shown in the SEM images collected of ppOct patterned onto ppAAc (Figure 6.2). ppOct produced the most prominent edges of all the plasma polymers patterned attributed to the thickness of the plasma polymer layer (121±1 nm) which was 2-3 times greater than that of the other films. As described in Chapter 4, ppOct more readily fragments, influencing both deposition rate and cross link density, properties contributing to the mechanical properties of the film and resistance to tearing. Several approaches could be taken to minimise edge effects. The first would be to reduce film thickness by lowering deposition time. Characterisation of the film would be required to ensure stability of the thinner films during the patterning process and sonication in acetone. A second strategy would be to reduce the thickness of the photoresist layer (~680 nm) by diluting the resist and/or optimizing the spin coating process. It could also be interesting to investigate the effects of using a negative photoresist to invert the patterning process.

ppAAc produced more irregular circular features when patterned on either ppOct (Figure 6.3) or ppAAm (Figure 6.5) base plasma polymers compared with ppOct and ppAAm. This was also apparent from some of the ToF-SIMS images (Figure 6.8). Given the similarity in film thickness between the ppAAc and ppAAm films, the involvement of film mechanical properties was possible. ppAAc films deposited at the electrode were stable during the acetone sonication process, however, in Chapter 4 EIS had revealed a dual layer structure for ppAAc which could contribute to mechanical differences between the films. Chemistry and adhesion properties were also likely contributing to more irregular patterning of the ppAAc features. Both ppOct and ppAAm adhered well to the ppAAc surfaces as indicated by the intact circular features following lift-off with residual material limited to the pattern edge (Figure 6.3B and Figure 6.5A). This was not the case for ppAAc, producing more irregularly shaped features (Figure 6.3A and Figure 6.5B). Given that the two very different chemistries of ppOct and ppAAm appeared to adhere well, this suggested specific attributes related to
the ppAAc coating. Carboxylic acid groups are present at the ppAAc surface providing a double bond structure which can more readily form reactive species during plasma polymerisation deposition. These reactive species could facilitate greater interactions and cross linking between the ppAAc base layer and the depositing plasma polymer layer. In contrast, the lack of reactive species generated at the surface of ppAAm and ppOct films within the plasma, could explain why interactions and possible cross linking between the two film chemistries is limited.

Given the importance of surface chemistry on lipid vesicle interactions ToF-SIMS was used for chemical characterisation of plasma polymer micropatterns. Multivariate analysis was employed to identify the secondary fragments generating the most chemical variance between the two patterned plasma polymers. For ppAAc, both as the patterned and base layers, C$_2$H$_3$O$^+$ and C$_3$H$_5$O$^+$ were dominant species. Na$^+$ and K$^+$ secondary ions were also present on the ppAAc films and attributed to contamination introduced during the patterning process. These results were consistent with ppAAc films patterned using photolithography in work by Mishra et al.\textsuperscript{[7]} The ppAAm regions were identified as containing a variety of nitrogen containing species whilst the ppOct regions were hydrocarbon-rich.

In Chapter 5 different lipid vesicle interactions were observed with plasma polymers depending on surface chemistry and pH. pH was therefore explored as a method for spatially controlling lipid deposition and structure on patterned surfaces. Green fluorescent vesicles were first incubated on a surface patterned with ppAAc circles on ppAAm at pH 7. Lipid material was clearly visible on the ppAAm but not the ppAAc patterned areas. This was an expected result because in Chapter 5 it was shown that vesicles adsorbed to ppAAm but had minimal interactions with ppAAc at pH 7. On ppAAm this is due to the favourable electrostatic interactions between the positively charged ppAAm and slightly negatively charged vesicles. On ppAAc, the electrostatic repulsion between the negatively charged ppAAc and vesicles limits adsorption. FRAP showed that the lipid adsorbed to ppAAm was mobile over a 10 minute period. This was surprising because FRAP on plain ppAAm films had previously shown immobile behaviour at pH 4, 7 and 10.

The presence of mobility suggests fluid SLB formation at the ppAAm surface. Crucially, this can be related to the presence of a patterned substrate, given that only
immobile vesicular layers form on plain ppAAm surfaces. There are two important properties of the patterned surface which could lead to induced vesicle rupture. The first is the difference in chemistry between the patterned areas and the second is the presence of pattern edges at the nanoscale, creating topographical features.

With regards to chemistry, one must consider the edge of the patterns where there is a transition from the positively charged amine-rich environment of ppAAm to the negatively charged carboxylic acid functionalised regions of ppAAC at pH 7. As shown in Figure 6.17A, several interactions could contribute to the rupture of vesicles on the ppAAC/ppAAm patterned surface. There is a strong electrostatic attraction between the vesicles and ppAAm which enables them to adsorb readily, however, at the edge of the pattern there is both attraction to the ppAAm but repulsion from the nearby ppAAC edge. It is likely that the attraction to ppAAm causes the vesicles to deform towards the surface, however, the added contribution of a repulsive interaction from ppAAC, together with tight-packing of nearby vesicles and the osmotic difference across the vesicle wall, could cause further deformation and interbilayer stresses sufficient to initiate localised rupture at the pattern edge.¹⁰ The presence of the energetically unfavourable bilayer edge could then initiate rupture of neighbouring vesicles causing a cascade effect across the ppAAm surface (Figure 6.17B). It is also important to note that vesicles have been described as having a rolling or sliding motion on substrates, which can influence vesicle-vesicle interactions.¹¹ Previous FRAP experiments on ppAAm in Chapter 5 showed that adsorbed vesicles did not express mobility on plain ppAAm films over a 10 minute period. This means that the electrostatic attraction between the ppAAm and vesicles, whilst not large enough to rupture the vesicles, may contribute to rupture on the patterned surface by immobilising the vesicles so that they cannot roll or slide away from neighbouring vesicles or from the repulsive ppAAC edge. A further important comment regarding chemistry of the substrate relates to potential modification of the ppAAm film during the patterning process. This could be investigated further by collecting ToF-SIMS negative ion spectra for the patterned surface and by exposure of a plain ppAAm surface to vesicles post removal of a photoresist film to monitor changes in response when compared with an unmodified ppAAm film.
Figure 6.17 Proposed vesicle rupture on the ppAAc/ppAAm patterned surface. A) A variety of interactions between the vesicles and surface can contribute to rupture including a) electrostatic repulsion between the vesicles and ppAAc pattern edge, b) electrostatic attraction between the vesicles and ppAAm surface, c) vesicle-vesicle interactions and d) osmotic difference across the bilayer wall. B) Localised collapse at the ppAAc edges could initiate a rupture cascade radiating out from the initial ‘rupture zone’ at the ppAAc/ppAAm pattern edge. Diagram not to scale.

The second important characteristic of the patterned surface which could contribute to vesicle rupture is the presence of nanoscale pattern edges. Previous work on SiO$_2$ revealed that nanostructured pits (110 or 190 nm pit diameters) could facilitate vesicle rupture but the effect was dependent on both topography dimensions and vesicle size.$^{[12]}$ It was found that pit edges caused a highly pre-stressed state in adsorbed 100 nm diameter vesicles that enabled rupture when combined with vesicle-vesicle interactions. Crucially, for the larger 100 nm vesicles, the edge alone was not sufficient to induce rupture, an effect related to ability of larger vesicles to deform more due to an increased average radius of curvature.$^{[12]}$ The contrast with this study and the work presented here is that the pits were coated with SiO$_2$ which is known to enable vesicle collapse without nanotopography present. In a separate study, it was demonstrated that vesicle rupture was induced at the edge of nanogratings on gold supports, enabling a double SLB structure.$^{[13]}$ The edge-induced rupture of vesicles is highly significant to this work given that the ppAAc edges would be ~50 nm in height, interacting with vesicles ~200-300 nm in diameter. However, given that the ppAAc would be negatively charged at pH 192
7 it is unclear if the edge would play a significant role given that the vesicles are unlikely to adsorb. Importantly, mobility was retained on ppAAm after rinsing at pH 4 and again at pH 7.

To enable vesicle adsorption on the ppAAc patterns the pH was lowered to 4, bringing the surface into a neutral charge regime. The red fluorescent vesicles were highly localised to the ppAAc areas and adsorption of vesicles to the bilayer present on ppAAm was not apparent. The high signal intensity in the ppAAc areas suggested vesicle adsorption of high density. There was some indication of possible mobility at the edges of the bleached areas, however, the diffusion coefficient would be very low. Upon rinsing of the surface at pH 7, to induce vesicle rupture, the fluorescence intensity in those regions appeared to drop, as signified by a reduction in the signal-to-noise ratio in the image, however, lipid material was still present across the surface. This was in agreement with both the FRAP and QCM-D experiments from Chapter 5. These showed that mass was lost from the surface upon rinsing at pH 7. Some immobile lipid material remained, although it is unclear in what form. This highlighted the importance of future work, involving the use of complementary techniques such as QCM-D and AFM, to investigate the lipid structures formed on the patterned plasma polymer substrates.

Finally, it is important to discuss the autofluorescence of plasma polymers. It was often the case that complete fluorescence recovery on ppAAm was not observed and a ‘shadow’ of the initial bleach area could be seen (Figure 6.15). This was attributed to bleaching of autofluorescence originating from the plasma polymer film.\textsuperscript{[4, 5]} The signal from the plasma polymer is considerably weaker than that of the fluorescent lipid and does not bleach as evenly and efficiently. However, bleaching of the underlying film clearly leads to reduced signal in the initial bleach area which can be imaged using confocal microscopy. This is significant for the quantification of fluorescence recovery on plasma polymers and in scenarios with low signal-to-noise ratio.
6.4 Conclusion

In this chapter dual plasma polymer patterns have been successfully fabricated using photolithography techniques. ToF-SIMS combined with multivariate analysis showed that the patterns formed spatially controlled chemistries which were dependent on the plasma polymers used. Due to tearing of the upper film during lift-off, edge effects were often present which was particularly evident for the thicker ppOct films. Further work is required to reduce these effects by optimising film properties and reducing the thickness of the resist. Pattern quality was also dependent on the combination of plasma polymers being patterned. This was related to the physiochemical properties of the films. In particular, ppAAc was more readily torn, sometimes leading to irregular pattern edges.

The pH-dependent interactions of lipid vesicles with plasma polymers were used to spatially control both vesicle adsorption and lipid structure on ppAAc/ppAAm patterned surfaces. At pH 7 green fluorescent lipids adsorbed to the ppAAm areas but not to the ppAAc patterns. Lowering the pH 4 enabled the deposition of a second red fluorescent lipid on the surface. This demonstrated that lipid location on the substrate could be controlled using patterned plasma polymer surface chemistries.

FRAP experiments performed on lipids adsorbed to the ppAAm areas showed mobility at both pH 7 and 4. Plain ppAAm films had previously shown immobile vesicle adsorption across a wide range of pH (Chapter 5). It was therefore hypothesised that the presence of electrostatically repulsive and attractive regions on the same substrate, at pH 7, appeared to initiate rupture of lipid vesicles on ppAAm (together with other interactions at the surface). This was significant as it enabled immobile lipid islands (on ppAAc), surrounded by fluid lipid regions (on ppAAm), to form on a single substrate. The spatial control of lipid mobility was therefore demonstrated using patterned plasma polymer surface chemistries together with pH.

In summary, this work has demonstrated the application of plasma polymer patterned chemistries for the spatial control of both lipid location and mobility. This is important for the creation of increasingly complex membrane architectures.
6.5 References


7 Nanopatterning of plasma polymers

7.1 Introduction

In Chapter 6, dual plasma polymer micropatterns were successfully fabricated using photolithography lift-off techniques. The next challenge was to create spatially controlled plasma polymer chemistries at the nanoscale. The following chapter describes the fabrication of sub-micron plasma polymer patterns using electron beam lithography (EBL). Atomic force microscopy (AFM), scanning electron microscopy (SEM), and optical profilometry were used to physically characterise the samples so pattern quality and resolution could be assessed. Chemical characterisation of the patterns was conducted using time-of-flight Secondary ion mass spectroscopy (ToF-SIMS) and auger electron spectroscopy (AES). Multivariate analysis of the ToF-SIMS data provided further insight into the chemistry of the patterned surfaces.

EBL on glass substrates, Au coatings and SEM of plasma polymer nanopatterns was conducted by Pierrette Michaux (Swinburne University of Technology). EBL on silicon substrates was conducted by Dr Fatima Eftekhari (Melbourne Centre for Nanofabrication, ANFF-Victoria). AFM images of nanopatterns were collected by Dr Hemayet Uddin (Melbourne Centre for Nanofabrication, ANFF-Victoria) and analysed by HJA. ToF-SIMS images were collected by Dr Robert Jones (ANFF-Victoria ToF-SIMS facility, La Trobe University) and analysed by HJA. AES data was collected and analysed by Dr Martyn Kibel (La Trobe University).

7.2 Results

7.2.1 Investigating the effect of electron dose and coating time on patterning of plasma polymerised acrylic acid (ppAAc)

The first step in creating the plasma polymer nanopatterns was to optimise poly(methyl methacrylate) (PMMA) resist patterning steps and investigate the effect of electron dose on pattern resolution. For this, a standard pattern with various shapes and sizes (from microns to tens of nanometers) was used. Figure 7.1 shows SEM images of patterns developed on silicon wafer coated with the PMMA resist. As shown in Figure 7.1A (low dose) and Figure 7.1B (high dose) the higher electron beam dose produced better defined patterns at the 200 nm feature size. When the feature size was reduced tenfold.
to 20 nm, it was evident that the resolution limits of the instrument were being reached with the high dose patterned features increasing in size (Figure 7.1D) and some of the lower dose features poorly defined or missing from the array (Figure 7.1C).

![Figure 7.1 SEM images of PMMA patterned using electron beam lithography at different electron dose levels. Following exposure to electrons and development, exposed areas of PMMA were removed revealing the silicon wafer surface beneath (darker features). 200 nm features patterned using A) low and B) high electron dose and 20 nm features patterned using C) low and D) high electron dose.](image)

In a subsequent experiment, the influence of both electron dose and plasma polymer coating time was investigated. Silicon substrates were coated with the PMMA resist and then patterned with EBL using both high and low dose electron densities. The patterned surfaces were then coated with ppAAc for 5 or 20 minutes to deposit two different thicknesses of plasma polymer film. Sonication in acetone was undertaken to remove the underlying PMMA resist leaving a ppAAc film patterned onto the silicon surface (see Chapter 3 Materials and methods). The 20 minute coating combined with low electron dose EBL PMMA mask provided the poorest pattern quality with incomplete lift-off of the ppAAc film (Figure 7.2C). Incomplete lift-off was also observed for the...
20 minute coating applied onto the high electron dose PMMA mask (Figure 7.2D) but the effect was minimal and only seemed to affect the outer rings of the larger circular features on the pattern. Whilst the 5 minute coating combined with the low electron dose EBL mask provided the best pattern quality for the larger circular band features (Figure 7.2A) it was also clear that some of the more intricate designs were missing completely. On closer inspection, patterned features 200 nm in size and smaller were clearly missing (Figure 7.3A). This was not the case for the 5 minute coating patterned using the high electron dose EBL (Figure 7.2B and Figure 7.3B). The combination of high electron dose with the thinner 5 minute coating was therefore selected for the first trial of dual plasma polymer nanopatterns.

Figure 7.2 SEM images of ppAAc patterned onto silicon wafer. 5 minute ppAAc coating patterned using PMMA exposed to A) low and B) high electron dose and a 20 minute ppAAc coating patterned using PMMA exposed to C) low and D) high electron dose.
Figure 7.3 SEM images of ppAAc patterned onto silicon wafer using a 5 minute coating time. Comparison of 200 nm feature sizes at A) low and B) high electron dose.

7.2.2 Physical characterisation of plasma polymer nanopatterns on glass substrates

7.2.2.1 3D Profilometry of dual ppAAc and ppAAm nanopatterns

Figure 7.4A shows an optical profilometry image of a 5 minute plasma polymerised allylamine (ppAAm) coating patterned onto ppAAc on an indium tin oxide (ITO)-coated glass substrate using standard EBL methods. At this resolution, patterned ppAAm lines, with width thicknesses designed from 900 nm down to 100 nm, were visible. Spiked features on some of the lines suggested residual edge effects left over from the PMMA removal stage. The 800 nm and 700 nm lines appeared to be joined by a bridge of plasma film on the left edge. Using a higher objective, the faint outline of the 50, 40 and 30 nm lines could be visualised (Figure 7.4B) however the lateral dimensions of these lines was less than the resolution of the objective (at around 200 nm). This means there were insufficient pixels to accurately capture the line dimensions using this technique. This effect is demonstrated in Figure 7.4C where vertical line scan data is plotted. Peaks in the graph indicated the presence of nine lines however features designed to be 50 nm in width or less, although faintly visible on the image, are lost in background noise.
Figure 7.4 3D optical profilometry images of ppAAm patterned onto ppAAc. A) Overview image of large ‘A’ feature with associated lines, B) line features designed to be 50, 40 and 30 nm in width, C) vertical line scan of patterned lines. Inset scale bar 10 µm.
A comparison between the actual and designed feature size was made by approximating patterned line width using a full-width-half-maximum (FWHM) measurement method (described in Chapter 3 Materials and methods) on line-scan data from the optical profilometry image in Figure 7.4C. Table 7.1 shows how the actual line width is larger than the designed line feature. For example, the 900 nm line is measured at 1.14 µm. Many of the patterned lines appeared to be of similar size due to the limited lateral resolution of the microscope objective. Although a useful and straightforward method for visualising the overall pattern, the technique proved unsuitable for accurately evaluating feature size. As described later in this chapter high-resolution techniques such as SEM and AFM were used for this purpose.

Table 7.1 Approximating patterned line width using FWHM methods on optical profilometry data collected for ppAAm patterned lines on ppAAc.

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<thead>
<tr>
<th>Designed feature size (nm)</th>
<th>FWHM (nm)</th>
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<tr>
<td>900</td>
<td>1140</td>
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<td>800</td>
<td>990</td>
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<td>200</td>
<td>620</td>
</tr>
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<td>100</td>
<td>510</td>
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Figure 7.5A shows a 3D profilometry image of ppAAc patterned onto ppAAm. The patterned lines are well defined with an imperfection on the 800 nm line which appears to be residual torn ppAAc film. Using a higher objective for the smaller line features, the 50 and 40 nm lines were faintly visible (Figure 7.5B). As previously observed, not all patterned line features could be identified using vertical line scan data (Figure 7.5C).
Figure 7.5 3D optical profilometry images of ppAAc patterned onto ppAAm. A) Overview image of patterned lines, B) line features designed to be 50 and 40 nm in width are faintly visible and C) vertical line scan of patterned lines. Inset scale bar 10µm.
7.2.2.2 SEM characterisation of dual ppAAc and ppAAm nanopatterns

SEM analysis was performed to provide further insight into the quality of the plasma polymer patterns and to more accurately evaluate the dimensions of the patterned lines. Quantification of patterned line widths was obtained by placing a grid over each SEM image and measuring the line width at three separate points along the length of the line as depicted in Figure 7.6A. For cases of poor pattern quality or edge effects two sets of measurements were taken. The first measurement, quantified line widths from the visible creases of the patterned line as depicted in Figure 7.6B. The second set of line width measurements was taken from the outer edges of the patterned line including any excess creased or folded material.

Figure 7.6 Measuring patterned line width. A) A grid was applied to the image using Image J software. The grid used was sufficiently large so that three vertical lines crossed the line of interest. The width of the patterned plasma polymer was then measured at these three points of intersection. B) In the cases of poorly defined pattern edges the line widths were first measured from defined creases. A second set of line widths was measured from the edges of the line including any residual materials.
Figure 7.7 displays the SEM line width data collected from ppAAc lines patterned onto ppAAm. Measured line widths for the three individual points on each patterned line were plotted against the designed line width. For all lines fabricated, the measured line width is greater than the designed line width. This can be attributed to a number of factors including increased initial size of the PMMA patterned features due to overexposure or overdevelopment,\cite{1} etching of the PMMA mask during plasma deposition and incomplete lift off. The lines designed to be 20 and 30 nm in width could not be located on this sample. Residual edge material was not present on the patterned lines therefore a second set of measurements was not required (Figure 7.8).

Figure 7.7 Line widths of ppAAc patterned onto ppAAm for A) 100-900 nm designed line widths and B) 40-50 nm designed line widths measured using SEM images. Three line width measurements are plotted per line.
Figure 7.8 SEM images of ppAAc lines patterned onto ppAAm. ppAAc is false coloured lilac to enhance contrast.
SEM line width data was further collected from ppAAm lines patterned onto ppAAc (Figure 7.9). Line quality was reduced due to the presence of residual ppAAm material at the edges of the lines designed to be between 200 and 900 nm in size. This is represented by the data plotted with hollow circles. The effect appeared reduced on the thinner lines (100 nm in width and below). As with ppAAc the measured line width was greater than the designed line widths for all features. An example of reduced pattern quality is shown in Figure 7.10. During the lift off process in acetone the upper ppAAm film was not adequately removed at the pattern edge leaving excess folded material.

Figure 7.9 Line widths of ppAAm patterned onto ppAAc for A) 100-900 nm designed line widths and B) 30-50 nm designed line widths measured using SEM images. In A the hollow circles represent the maximum measured width of plasma polymer material including excess and folded plasma polymer material.
Figure 7.10 SEM image of ppAAm patterned into ppAAc. This line was designed to be 400 nm in thickness. ppAAm is false coloured lilac to enhance contrast.

The smallest features observed using SEM for each pattern combination are shown in Figure 7.11. The smallest line feature of ppAAm film patterned onto ppAAc was designed to be 30 nm in width but measured 57 nm at the position shown in Figure 7.11A. Similarly, for the inverse pattern, the smallest line feature intended to be 40 nm in width measured 57 nm at the point highlighted in Figure 7.11B. SEM provided poor contrast for these samples, especially when imaging high resolution patterned features. Furthermore, the need to apply a 10 nm thick gold coating raised the probability that the coating process could affect the data interpretation. These factors made performing accurate measurements of line width difficult, especially for the smallest of features. As described in the next section further physical characterisation of plasma polymer nanopatterns was undertaken using AFM.
Figure 7.11 Smallest patterned plasma polymer line widths observed using SEM. A) ppAAm patterned on ppAAc and B) ppAAc patterned on ppAAm. The top layer patterned plasma polymer is false coloured lilac to enhance contrast. Due to very low contrast in these images, the false colouring serves as an approximate guide only. These images have been brightness adjusted.
7.2.3 Physical characterisation of plasma polymer nanopatterns on silicon substrates

Dual nanopatterns of ppAAc and ppAAm were further fabricated on silicon wafer. This was to demonstrate reproducibility of the patterning process on silicon substrates whilst providing a more suitable substrate for chemical analysis techniques such as AES.

7.2.3.1 AFM

AFM was used to measure the line widths of both the patterned PMMA and dual plasma polymer nanopatterns. Samples with ppAAc patterned onto ppAAm were selected for the study due to superior pattern quality generated from this pattern combination. Unlike SEM, no gold coating was required providing minimum interference with the patterned surfaces.

The second column in Table 7.2 shows the measured widths line of a PMMA mask following electron-beam lithography and development. The measured widths are very close and in some cases exactly matched with the designed line widths specified in the original design. As shown in Figure 7.12A the PMMA layer was measured at around 60 nm in thickness. At 20 nm it became apparent that the AFM tip was not reaching fully into the feature as indicated by the reduced height of the PMMA at < 60 nm as shown in the line scan of the feature in Figure 7.12D. This made FWHM measurements unsuitable for this feature size.
Table 7.2 Average measured line widths in nm of a patterned PMMA mask and 10 minute ppAAc coating patterned onto ppAAm as measured by AFM. The mean values (± standard deviation) are calculated from three FWHM analyses per sample.

<table>
<thead>
<tr>
<th>Designed line width (nm)</th>
<th>PMMA pattern</th>
<th>10 min ppAAc coating patterned onto ppAAm</th>
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<tbody>
<tr>
<td>900</td>
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<td>978±28</td>
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<tr>
<td>600</td>
<td>594±9</td>
<td>626±28</td>
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<tr>
<td>500</td>
<td>496±9</td>
<td>548±28</td>
</tr>
<tr>
<td>400</td>
<td>385±9</td>
<td>489±55</td>
</tr>
<tr>
<td>300</td>
<td>287±9</td>
<td>333±27</td>
</tr>
<tr>
<td>200</td>
<td>199±2</td>
<td>261±1</td>
</tr>
<tr>
<td>100</td>
<td>102±3</td>
<td>151±3</td>
</tr>
<tr>
<td>50</td>
<td>48±1</td>
<td>87±6</td>
</tr>
<tr>
<td>40</td>
<td>35±2</td>
<td>70±3</td>
</tr>
<tr>
<td>30</td>
<td>29±1</td>
<td>62±4</td>
</tr>
<tr>
<td>20</td>
<td>17±1</td>
<td>29±4</td>
</tr>
</tbody>
</table>
Figure 7.12 AFM images and associated line scans of patterned PMMA features designed to be A) 600 and 700 nm and B) 20 nm in width.

Further AFM characterisation was conducted on a 10 minute ppAAc coating patterned onto ppAAm. Figure 7.13A shows an AFM image of the largest of the patterned lines designed to be 900-500 nm in width. Both the image and the associated line scan across the features (Figure 7.13B) clearly showed residual plasma polymer material from the patterning process creating edge effects along the patterned lines. This was evident from the spikes in height on the edge of the lines such as the feature reaching over 60 nm in height on the 900 nm line.
Figure 7.13 AFM image (A) and associated line scan (B) of a 10 minute ppAAc coating patterned onto ppAAm.

It was also noticeable that the lines did not possess perfectly straight edges at the interface between the two chemistries. This is shown in Figure 7.14 where the top of the line feature is not flat but pointed with uneven edges on both sides. The line was designed to be 100 nm in width but is closer to 150 nm. It was also evident that the edge effects were not always consistent along the length of the lines meaning a large range of widths could be measured for a single patterned line as indicated by some of the standard deviation values in Table 7.2. The standard deviation values were reduced for the thinner line widths designed to be 200 nm or less. This is consistent with observations made with the SEM data from the first set of samples fabricated on glass and suggests the incidence of excess film material at the line edge reduces for smaller patterned line features. Overall, the average measured widths of the ppAAc lines were larger than the designed line width as shown in Table 7.2.
Figure 7.14 AFM analysis of a patterned feature designed to be 100 nm in width patterned from a 10 minute ppAAc film on ppAAm. A) AFM height image and corresponding line scan and B) 3D reconstruction of the AFM height data.

The smallest patterned line features measured by AFM are shown in Figure 7.15. The 30 nm line was measured at 62±4 nm in width and the 20 nm line at 29±4 nm in width however edge effects together with uneven and pointed features made useful FWHM approximations difficult. This was especially the case for the thinnest line, which was incomplete (Figure 7.15B). Missing sections had not been observed for the other lines including the 30 nm line (measured at 62±4 nm) suggesting a critical patterned line width limit between 30-60 nm.
Chemical characterisation of plasma polymer nanopatterns on silicon substrates

Chemical characterisation of the plasma polymer nanopatterns was performed using ToF-SIMS and AES. ToF-SIMS was employed to provide spatial chemical mapping of the dual patterns. Given the lateral resolution limits of ToF-SIMS, AES was also used in an attempt to characterise elemental differences in the plasma polymer patterns down to just tens of nm in size.
7.2.4.1 AES

AES data from plain plasma polymers deposited onto silicon wafer (20 minutes, 3 cm away from the electrode) were first collected to identify characteristic elements and atomic percentages. Figure 7.16 shows Auger survey spectra and high-resolution derivative spectra for a plain ppAAc plasma polymer. As expected both carbon and oxygen were present at the surface.

Figure 7.16 AES data for a ppAAc coating deposited onto Si wafer. A) Survey spectra and B) derivative spectra for carbon and oxygen regions.
In Figure 7.17 Auger survey spectra and high resolution derivative spectra for a plain ppAAm plasma polymer show the presence of carbon, oxygen and nitrogen at the surface, as expected.

Figure 7.17 AES data for a ppAAm coating deposited onto Si wafer. A) Survey spectra and B) derivative spectra for carbon, nitrogen and oxygen regions.
Although the identification of atomic species was consistent with previous XPS analyses (Chapter 4), the atomic percentages varied between the techniques. Table 7.3 shows the atomic percentage of elements measured by survey and high resolution spectra at two different regions for ppAAc and ppAAm using AES. In region 1 for ppAAm, the atomic % as measured by a survey scan is 88.2 % for carbon, 3.4 % for oxygen and 8.4 % for nitrogen. This is in contrast to XPS where 84.6±0.2, 3.2±0.2 and 12.0±0.1 was measured for carbon, oxygen and nitrogen, respectively. In the case of ppAAc the differences between the atomic % measured for XPS and AES vary to an even greater degree. In region 1 a survey scan measured 95.5 % carbon and 4.5 % oxygen. This is in contrast to XPS measuring 78.4±0.1 % and 21.2±0.1 % for carbon and oxygen, respectively. The difference can be attributed to electron beam damage causing oxygen depletion during AES analysis. This effect was further observed when considering the oxygen % measured from survey and high resolution Auger scans conducted on the same region (Table 7.3). In all cases (all regions and surfaces) the oxygen % measured is higher for the survey scan when compared with the high resolution scan. This is again attributed to increased oxygen depletion due to electron exposure because the high resolution scan was conducted following the survey scan for each region.

Table 7.3 Comparing the atomic % collected by survey and high resolution AES analysis for ppAAc and ppAAm films.

<table>
<thead>
<tr>
<th>Region</th>
<th>Atomic %</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ppAAm</td>
<td></td>
<td>C</td>
<td>O</td>
<td>N</td>
</tr>
<tr>
<td>1</td>
<td>Survey</td>
<td>88.2</td>
<td>3.4</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td>High res.</td>
<td>89.4</td>
<td>1.7</td>
<td>8.9</td>
</tr>
<tr>
<td>2</td>
<td>Survey</td>
<td>88.8</td>
<td>2.6</td>
<td>8.6</td>
</tr>
<tr>
<td></td>
<td>High res.</td>
<td>89.6</td>
<td>1.7</td>
<td>8.7</td>
</tr>
<tr>
<td>ppAAc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Survey</td>
<td>95.5</td>
<td>4.5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>High res.</td>
<td>96.6</td>
<td>3.4</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Survey</td>
<td>94.3</td>
<td>5.7</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>High res.</td>
<td>94.8</td>
<td>5.2</td>
<td>-</td>
</tr>
</tbody>
</table>
This effect was further explored by observing the atomic % of oxygen on a ppAAc coating following Auger analysis and ion bombardment on a single region (Table 7.4). As predicted oxygen % reduced to just 1.3% following a survey scan, high resolution scan and 30s ion bombardment.

Table 7.4 Comparison of atomic % for ppAAc coating measured following an Auger survey scan, high resolution scan and 30s ion bombardment on the same analysis spot.

<table>
<thead>
<tr>
<th>Type of Scan/Treatment</th>
<th>Atomic %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Survey</td>
<td>95.5</td>
</tr>
<tr>
<td>High resolution</td>
<td>96.6</td>
</tr>
<tr>
<td>Ion bombardment</td>
<td>98.7</td>
</tr>
</tbody>
</table>

Overall these findings showed that AES could identify atomic species at the surface of the plasma polymers with overall higher carbon content for ppAAc and the presence of nitrogen in the ppAAm coating. Whilst these differences could be used to potentially locate different regions of the plasma polymers on a patterned surface, the technique was not suitable for quantitative measurements of atomic % of the plasma coated surfaces.

Preliminary attempts using AES spatial mapping were not successful in generating images of the patterned surface using atomic composition as a means of contrast between the ppAAm and ppAAc regions (data not shown). Furthermore, the extensive analysis times compounded by the oxygen depletion issues previously described made the Auger mapping technique impractical. As an alternative, line scan analyses were conducted on ppAAm and ppAAc patterned surfaces. Figure 7.18 shows how Auger line scans identified ppAAc lines of a particular size patterned onto ppAAm. An SEM of the surface following the line scan is shown in Figure 7.18A. The location of the scan is clearly visible from damage of the surface due to electron bombardment. The ppAAc lines can be clearly identified by a peak in carbon and an associated dip in nitrogen content (Figure 7.18B). The peaks and dips are separated by regular intervals of approximately 5 µm as designed. Eight lines were detected within the line scan however the 9th line designed to be 200 nm, which is clearly visible on the SEM image was not detected. Although oxygen would be expected to peak at the location of the ppAAc lines depletion meant only low intensities were detected.
Figure 7.18 Auger line scan analysis of a 5 minute ppAAc coating patterned onto ppAAm. A) SEM image of the sample following Auger line scan analysis with numbers indicating the designed line width and B) Auger line scan data showing the relative concentrations of carbon, nitrogen and oxygen along the scan line.
A second sample was prepared, this time with a thicker ppAAc coating deposited for 10 minutes patterned onto ppAAm (Figure 7.19). Once more, the smallest lines were not detected by the line scan. As shown in Figure 7.19B the line designed to be 200 nm in width was barely visible and for all lines, the change in nitrogen was clearer than the change in carbon across the patterned features.

Figure 7.19 Auger line scan analysis of a 10 minute ppAAc coating patterned onto ppAAm. A) SEM image of the sample following two Auger line scans (yellow lines) with numbers indicating the designed line width and B) Auger line scan data for line 1 and line 2 showing the relative concentrations of carbon and nitrogen.

In an attempt to investigate differences in carbon and nitrogen for the sub 100 nm line widths, a higher magnification was used to focus on the 50, 40 and 30 nm lines (Figure 7.20A). An Auger line scan across the pattern did not reveal any noticeable differences between carbon and nitrogen content across the patterned lines (Figure 7.20B).
An inverse pattern of ppAAm patterned onto ppAAc was fabricated and Auger line scans were conducted for carbon and nitrogen (Figure 7.21). As expected the line scans for carbon and nitrogen were inverted compared with the previous samples. The ppAAm lines were identified by a dip in carbon signal and a simultaneous peak in nitrogen signal. The sub 100 nm lines were not identified by the line scans however the 200 and 100 nm lines did show small changes in the carbon and nitrogen signal (Figure 7.21B).
7.2.4.2 ToF-SIMS

ToF-SIMS analysis was used to obtain more information about the chemical composition of the patterned surfaces and explore the limits of the technique for imaging sub-micron features. When imaging with ToF-SIMS there is a trade-off between achieving greater spatial resolution and decreased mass resolution. This is demonstrated in Figure 7.22A where two different areas of a sample (each containing ppAAC patterned features on ppAAM) have been imaged using spectrometry (higher mass resolution) and ultimate imaging (higher spatial resolution) modes of the instrument. The broadening of the peaks demonstrates the loss of mass resolution. Figure 7.22B shows how spectrometry mode enabled the secondary ion species to be identified as C$_3$H$_4$O$^+$ (55.0178 u) and C$_4$H$_7^+$ (55.0542 u) and if required, images could...
be reconstructed from these individual fragments accordingly. However, at higher spatial resolution, mass resolution was lost meaning only a single peak at a nominal mass of 55 u (rounded to the nearest integer mass) was present, containing signal from both species. A reconstructed image from this data at 55 u would therefore contain signal contributions from more than one chemical species.

Figure 7.22 ToF-SIMS spectra from two different regions of a 10 minute ppAAc coating patterned onto ppAAm collected using spectrometry and ultimate imaging modes.
In order to overcome this limitation and identify secondary ion species unique to the ppAAm and ppAAc nanopatterned plasma polymers, multivariate analysis was first conducted on an image collected in spectrometry mode (with higher mass resolution) of a larger feature patterned close to the nanopatterned lines. Figure 7.23 shows a patterned ppAAc ‘2’ on ppAAm. The negative loading plot indicated several dominant secondary ion fragments in the region of the patterned ppAAc including Na$^+$, C$_2$H$_3^+$, C$_3$H$_3^+$, C$_2$H$_3$O$^+$ and C$_3$H$_3$O$^+$ species which correlated well with the multivariate analysis from the ppAAc micropatterns on ppAAm (Chapter 6). This was in contrast to the positive loadings plot, which revealed unexpected species at the surface of the ppAAm plasma polymer. Multivariate analysis of ppAAc micropatterns on ppAAm had previously identified a variety of nitrogen containing secondary ions at the surface of the ppAAm (Chapter 6) however analysis of this sample revealed dominant CH$_3^+$, C$_3$H$_5^+$, C$_2$H$_3$O$_2^+$ and C$_4$H$_5$O$^+$ species.

![Figure 7.23 PCA analysis of a 10 minute ppAAc film patterned on ppAAm.](image)

To understand which materials these secondary ion species (identified in the principle component analysis (PCA)) were derived from, selected peaks from the spectra collected from the patterned surface were compared with the same peaks from high
mass resolution spectra collected from control (unpatterned) ppAAm, ppAAc and PMMA coatings. All the spectra were normalised for total ion intensity to enable direct comparison. As shown in Figure 7.24B while PMMA showed the highest relative intensity of C$_3$H$_5^+$, this species was also present in the other materials. This is represented in the reconstructed image where the patterned feature is not visible, making it an ineffective peak for differentiating between the different chemistries. The CH$_3^+$ species (Figure 7.24A) was also present in all the materials however it was most intense on the PMMA coating. As shown on the reconstructed image the signal from this fragment was more intense on the background than in the pattern itself, suggesting that there may be PMMA on this region of the surface. C$_4$H$_5$O$^+$ was also particularly dominant in the PMMA spectra with a less intense peak present in the spectra from ppAAc and minimal signal from the ppAAm spectra (Figure 7.24D). The fabrication of the pattern meant that the background around the ‘2’ feature should be ppAAm, but as shown in Figure 7.24C the C$_2$H$_3$O$_2^+$ peak associated with PMMA was clearly present as a dominant background ion signal. These results clearly indicated the presence of PMMA residue on the ppAAm coated substrates.

The dominant species derived from the ppAAc film were also investigated (Figure 7.25). The contaminant species Na$^+$ and K$^+$ were only associated with the ppAAc regions of the patterned surface and provided good pattern contrast in the reconstructed images (Figure 7.25A and C). C$_2$H$_3^+$ and C$_3$H$_3^+$ species were present in both PMMA and ppAAm therefore they did little to distinguish between the materials (Figure 7.25B and C). C$_2$H$_3$O$^-$ and C$_3$H$_3$O$^+$ were dominant species in the ppAAc film, a finding consistent with Mishra et al.\cite{3} and provided good contrast within the reconstructed images (Figure 7.25D and E).

Further analysis was undertaken to investigate if a uniform PMMA coating covered the background ppAAm plasma polymer or if only fragments had been left behind. To do this, some of the nitrogen-containing species associated with ppAAm (as identified from multivariate analysis of the ppAAm/ppAAc micropatterns in Chapter 6) were selected. The spectra from the plain ppAAm, ppAAc and PMMA samples were then compared at these nominal mass values. As shown in Figure 7.26 the overall signal intensity for the nitrogen-containing species was low indicating low yield of these species in the positive ion spectra. However, it was clear that these nitrogen containing
species, whilst present in both the ppAAm and patterned surfaces, were not present in PMMA. Several of the species were also present in ppAAc however $C_3H_4N^+$ provided a clear peak associated strongly with the ppAAm film. Despite this, the pattern image could not be reconstructed using these species suggesting that residual PMMA may be masking the underlying ppAAm layer. Given the 2 nm depth sensitivity of the ToF-SIMS technique even a small amount of PMMA residue could mask signal from the underlying ppAAm film.
Figure 7.24 Comparing ToF-SIMS spectra collected from plain PMMA, ppAAc and ppAAm samples with the spectra collected from a 10 minute ppAAc film patterned on ppAAm at the dominant peaks from the positive loadings plot. Images were reconstructed from signal from A) CH$_3^+$, B) C$_3$H$_5^+$, C) C$_2$H$_3$O$_2^+$ and D) C$_4$H$_5$O$^+$ secondary ion fragments. (Spectrometry mode, 74.48 x 71.48 µm, 128 x 128 pixel)
Figure 7.25 Comparing ToF-SIMS spectra collected from plain PMMA, ppAAc and ppAAm samples with the spectra collected from a 10 minute ppAAc film patterned on ppAAm at the dominant peaks from the negative loadings plot. Images have been reconstructed from signal derived from A) Na$^+$, B) C$_2$H$_3^+$, C) C$_3$H$_3^+$ and K$^+$, D) C$_2$H$_3$O$^-$, and E) C$_3$H$_3$O$^-$ secondary ion fragments. (Spectrometry mode, 74.48 x 71.48 µm, 128 x 128 pixel)
Figure 7.26 Comparing ToF-SIMS spectra collected from plain PMMA, ppAAc and ppAAm samples with the spectra collected from a 10 minute ppAAc film patterned on ppAAm at selected nitrogen-containing peaks. Images have been reconstructed from signal derived from A) \(\text{CH}_2\text{N}^+\) and \(\text{C}_2\text{H}_4^+\), B) \(\text{CH}_3\text{N}^+\), C) \(\text{C}_2\text{H}_4\text{N}^+\) and \(\text{C}_2\text{H}_2\text{O}^+\), D) \(\text{C}_3\text{H}_4\text{N}^+\) and E) \(\text{C}_3\text{H}_6\text{N}^+\) and \(\text{C}_3\text{H}_4\text{O}^+\) secondary ion fragments. (Spectrometry mode, 74.48 x 71.48 μm, 128 x 128 pixel)
A high spatial resolution image of ppAAc patterned lines on the same sample was then acquired in ultimate imaging mode. Due to the low yield of the nitrogen-containing species, oxygen-containing species associated with ppAAc (C$_2$H$_3$O$^+$ and C$_3$H$_7$O$^+$) were used to reconstruct images of the patterns (Figure 7.27). Due to peak broadening effects and loss of mass resolution it is important to note that at a nominal mass of 43 u, signal from both C$_2$H$_3$O$^+$ and C$_3$H$_7$O$^+$ species are present in the image. However, as identified in Figure 7.25 the C$_2$H$_3$O$^+$ peak derived from the ppAAc film is the dominant species. In a similar manner at a nominal mass of 55 u both C$_3$H$_5$O$^+$ and C$_4$H$_7^+$ would be present however once again C$_3$H$_5$O$^+$, derived from the ppAAc film, is the dominant species. To provide a clearer image of the pattern the contributions from these two nominal masses were then combined.

![Images of ppAAc patterns](image)

Figure 7.27 ToF-SIMS images of a 10 minute ppAAc film patterned onto ppAAm. Images have been reconstructed from secondary ion fragments originating from the same nominal mass (rounded to the nearest integer mass) at 43 u and 55 u. Signal from the two images was added to form a combined image. (Ultimate imaging mode, 70.21 x 70.21 μm, 256 x 256 pixel)
A similar sample was prepared with a 5 minute ppAAc film patterned onto ppAAm. Images were constructed from signal derived from peaks at 43 u and 55 u and then combined to from a clear image of the pattern (Figure 7.28). Designed line widths down to the 30 nm line (~60 nm in width as measured by SEM and AFM) can be seen.

Figure 7.28 ToF-SIMS images of a 5 minute ppAAc film patterned onto ppAAm. Images have been reconstructed from secondary ion fragments originating from the same nominal mass (to the nearest integer mass) at 43 u and 55 u. Signal from the two images was added to form a combined image. (Fast imaging mode, 75 x 75 µm, 512 x 512 pixel)
C₃H₅⁺ and C₂H₃O₂⁺ contaminant species derived from PMMA at 15 u and 59 u were clearly associated with the underlying ppAAm background layer and not ppAAc as shown in Figure 7.29. As previously observed Na⁺ contamination was associated with ppAAc.

Contaminant species

**PMMA**

15 u

![Image of ToF-SIMS image at 15 u with MC: 12; TC: 5.808e+005]

59 u

![Image of ToF-SIMS image at 59 u with MC: 14; TC: 6.705e+005]

23 u

![Image of ToF-SIMS image at 23 u with MC: 8; TC: 7.705e+004]

Figure 7.29 ToF-SIMS images of a 5 minute ppAAc film patterned onto ppAAm. Images have been reconstructed from secondary ion fragments originating from the same nominal mass (rounded to the nearest integer mass) at 15 u, 59 u and 23 u. (Fast imaging mode, 75 x 75 µm, 512 x 512 pixel)
The inverse pattern combination of ppAAm patterned onto ppAAc was fabricated and images were reconstructed from the 43 u and 55 u peaks associated with ppAAc (Figure 7.30). This combination yielded more edge effects and residual ppAAm material on the pattern, which is clearly shown by the images.

Figure 7.30 ToF-SIMS images of a 10 minute ppAAm film patterned onto ppAAc. Images have been reconstructed from secondary ion fragments originating from the same nominal mass (rounded to the nearest integer mass) at 43 u and 55 u. (Fast imaging mode, 74.48 x 74.48 μm, 512 x 512 pixel)
Interestingly, as shown in Figure 7.31 species associated with PMMA contamination were observed on the ppAAc background film and not ppAAm (as previously observed, Figure 7.29). This result suggested that the PMMA contamination was independent of plasma polymer chemistry and affected only the base plasma polymer masked by the PMMA.

**Contaminant species**

PMMA

![ToF-SIMS images of a 10 minute ppAAm film patterned onto ppAAc. Images have been reconstructed from secondary ion fragments originating from the same nominal mass (rounded to the nearest integer mass) at 15 u, 59 u and 23 u. (Fast imaging mode, 74.48 x 74.48 µm, 512 x 512 pixel)](image)

**Na⁺**

23
7.3 Discussion

The work presented in this chapter has demonstrated the successful fabrication of sub 100 nm dual plasma polymer patterns using EBL lift-off methods. The smallest complete line features patterned were ~50-60 nm in width as confirmed by SEM and AFM analysis. ppAAc patterned onto ppAAm produced enhanced pattern quality with reduced edge effects when compared with the reverse combination of chemistries. This was evident from the SEM data (Figure 7.9 and Figure 7.10) showing greater edge effects from residual ppAAm material. ToF-SIMS images (Figure 7.30) also showed regions of ppAAm material remaining on the surface following the lift-off process. These results are consistent with those discussed in Chapter 6 where ppAAc was removed more readily from the surface during the lift-off process. Whilst this resulted in less uniform features during micropatterning, the effect generated reduced edge effects and less residual material when patterning using EBL techniques at the sub-micron scale. This effect is likely related to the structure and mechanical properties of the plasma polymer films. As revealed by electrochemical impedance spectroscopy (EIS) in Chapter 4 the ppAAc film had a dual layer structure with possible differences in porosity when hydrated. These layers together with a nanoporous network \[4\] could facilitate penetration of acetone. This, together with weaker mechanical properties of the film would act to facilitate the lift-off process. In the case of incomplete ppAAm lift-off, depositing a thinner film (5 minutes instead of 10 minutes) did not improve lift-off suggesting the possibility of a chemical interaction between the ppAAm and the PMMA during the deposition process. It is not clear if the ppAAm is bound directly to the ppAAc layer or if PMMA remains sandwiched between the layers (Figure 7.30). ToF-SIMS also revealed PMMA contamination was only present at the surface of both ppAAc and ppAAm films when they formed the base layer of the pattern. Contamination was therefore incorporated during the masking process (involving spin coating, baking, EBL patterning and development) and not the acetone sonication lift-off process. In the case of ppAAc as the base layer, ToF-SIMS identified secondary ion fragments associated with ppAAc and associated contaminant \[Na^+\] suggesting the PMMA was not a uniform coating across the surface of the plasma polymer (Figure 7.30 and Figure 7.31). For ppAAm as the base layer it was more difficult to ascertain if PMMA residue was greater when compared to ppAAc due to the low yield of the nitrogen-containing species in the positive ion spectra (Figure 7.26). XPS or ToF-SIMS
chemical depth profiling and investigation of the negative ion spectra could be used in future studies to investigate these phenomena further.

For both pattern combinations, the patterned line width was always greater than the designed line width. As measured using AFM, the developed PMMA features were in good agreement with the design (Table 7.2), suggesting the widening of feature size was due to etching of the PMMA mask during the second plasma polymer deposition and/or edge effects from the lift-off process. In the former case oxygen plasma has been used previously to etch PMMA\(^5\) however this process would be highly dependent on the preparation of the PMMA and the plasma reactor set up and parameters. It is unclear to what extent the reactive oxygen species present at the electrode in the setup used in this work would contribute to etching of the PMMA layer. Interestingly, the presence of residual plasma polymer material was minimised as the line width was reduced for both pattern chemistries. This suggested a link between mechanical properties of the film and the surface area of the patterned film adhered to the base plasma polymer. In order to create higher resolution sub ~50 nm patterns further optimisation of the lift-process would be required. One approach would be to decrease the thickness of the PMMA resist which in this work was measured at ~60 nm. This would act to reduce the effects of electron beam broadening caused by low energy elastic collisions as electrons enter the resist.\(^1\) A thinner resist layer would also reduce the profile of the PMMA above the base plasma polymer layer thus minimising the residual plasma polymer material associated with deposition onto the sides or ‘wall’ of the PMMA features. It is important to note that it was unclear if the EBL patterning process had successfully produced a 20 nm line feature in PMMA (Figure 7.12). The profile of the feature was not 60 nm in depth, suggesting either unsuccessful exposure/lift-off of the PMMA or an artefact from the AFM tip during imaging of the smallest feature. Tip convolution artefacts are common in AFM and occur when the dimensions of the feature being imaged are similar to that of the probe.\(^6\) This makes accurate measurement of the smallest lines fabricated here difficult, where FWHM approximations become inappropriate. It is not clear if the absence or incomplete patterning of the plasma polymer lines at this resolution was caused by ineffective PMMA patterning or that a limit for patterning of the film has been reached at that particular line width. To investigate this further, optimisation of the PMMA mask patterning at high resolution would be required together with optimisation of the AFM imaging methodology for
accurate measurement of the smallest features. The use of metallic film deposition over the PMMA pattern followed by lift-off could also be used for measurement of feature width using SEM and/or AFM.\textsuperscript{[1]} A second approach to improve pattern resolution would be to reduce deposition times of the plasma polymers. This would minimise any effects related to etching of the PMMA mask during deposition of the second plasma polymer layer. Thinner films could also facilitate lift-off by changing mechanical properties of the films enabling cleaner tearing of the upper film. Optimisation of plasma polymer deposition parameters such as power would also be an option for changing film characteristics.

This work also highlighted the challenges involved in chemical characterisation of plasma polymer nanopatterns. AES revealed atomic differences, with respect to carbon and nitrogen between the ppAAm and ppAAc films, however, damage to the surface from the electron beam meant the technique could not be used to accurately quantify atomic composition. This effect was especially acute for oxygen. Auger line scan data showed differences between nitrogen and carbon content across the surface of the patterns however as the line features became smaller $< \sim 150 \text{ nm}$ the features were difficult to distinguish even at a higher magnification (Figure 7.20 and Figure 7.21). Electrical charging of the sample was also observed when attempting to image nanopatterns on the glass coverslip/ITO substrate, this was minimised by using a silicon wafer substrate. This effect together with the oxygen depletion observed was unsurprising given that insulating and organic materials are more susceptible to electron beam damage due to their low electrical and thermal conductivities.\textsuperscript{[2]} ToF-SIMS provided greater mass resolution and differences in chemistry for lines down to $\sim 60 \text{ nm}$ in width could be observed. This was especially the case for the C$_2$H$_3$O$^+$ and C$_3$H$_5$O$^+$ species associated with ppAAc (Figure 7.28). The trade-off between spatial and mass resolution was an important consideration when reconstructing the images. A reduction in the beam diameter reduces the amount of material for analysis within each pixel, limiting the number of secondary ions generated and reducing both sensitivity and dynamic range.\textsuperscript{[7]} Whilst spectroscopy mode was suitable for the micropatterned surfaces in Chapter 6 the nominal beam size of over one micron deemed it unsuitable for the submicron patterns. Higher resolution features demanded the use of ‘fast’ and ‘ultimate’ imaging modes providing nominal beam diameters of 150 nm and 80 nm, respectively, but at the cost of mass resolution. The restriction on beam size means
effective measurement of feature dimensions may not be possible especially if the beam size is larger than the measured feature, however, AFM and ToF-SIMS can be employed to measure both feature size and chemistry respectively. Due to the low yield of nitrogen-containing species in the positive ion spectra, it was difficult to form an image of the ppAAm patterns with sufficient contrast. For ppAAc patterns, it is likely that the presence of sodium salts acted to enhance the probability of ionisation thus increasing the yield of the oxygen-containing and hydrocarbon species present. A further consideration was the effect of different surface areas of the two plasma polymers on the ToF-SIMS spectra. On the nanopatterns there is a considerable difference between the surface area coated with the base plasma polymer material and the patterned lines. As a result, when spectra were reconstructed by summing data from across the whole surface (for example over a 100 x 100 μm scan area) the background chemistry would be expected to dominate in the resultant spectra.

The results of this study demonstrate the utility and challenges of ToF-SIMS in the chemical analysis of nanoscale surface chemistry. ToF-SIMS is a highly versatile technique and has been used previously to image a variety of organic samples at the nanoscale. Gunnarsson et al. used ToF-SIMS for imaging of individual adsorbed vesicles ~300 nm in diameter and demonstrated a lateral resolution of 60 nm when imaging a lipid bilayer edge. The technique has also been used to investigate the nanoscale phase separation within polymer light emitting diodes in 3D. Nanoscale patterns of plasma polymerised octadiene films (ppOct) with SiO₂ and Au have also been characterised using ToF-SIMS. Unlike some other sub 100 nm characterisation methods the technique is commercially available and provides label-free molecular and elemental information for both conducting and non-conducting samples. In this work we have demonstrated the use of ToF-SIMS for imaging and chemical characterisation of high resolution dual plasma polymer nanopatterns however characterisation of dual plasma polymer patterns at the sub 100 nm scale remains challenging.

Previous literature exploring nanoscale patterning of plasma polymers using EBL has involved direct exposure of the films to the electron beam. Whilst this method holds great promise for the development of new photoresists and 3D nanolithography, it requires precise optimisation of the plasma polymer layer. Standardisation of such a layer may be difficult given the vast array of reactor configurations and film properties.
produced in different laboratories.[15] Alternative physical masking techniques for plasma polymer nanopatterning such as colloidal lithography[11, 16], whilst more cost effective, do not enable for precise control of arrays and non-repeating features. This work demonstrates that the fabrication of high resolution sub 100 nm dual plasma polymer chemistries is possible using a standard PMMA resist.

7.4 Conclusion
This chapter has successfully demonstrated the fabrication of spatially controlled plasma polymer chemistries down to ~50-60 nm in size using standard EBL lift-off techniques. AFM is a well-established technique for physical characterisation of patterns at the nanoscale however chemical characterisation of organic patterns at this scale is challenging. ToF-SIMS provided an effective technique for chemical imaging of the dual plasma polymer patterns with lines down to ~60 nm in width (as measured by AFM) visible. This work pushes the resolution boundaries of dual plasma polymer patterning by standard EBL lift-off methods. Further work is required to understand and optimise the lift-off process to minimise incomplete lift-off and PMMA contamination effects for certain pattern combinations. As the resolution of such patterns increases the greatest challenge will be in the chemical imaging of these organic patterns with high lateral resolution.
7.5 References


8 Conclusion

This thesis sets the foundations for the use of plasma polymers in the development of increasingly complex cell membrane model platforms. By using plasma polymers of different surface chemistry, combined with changes in buffer pH, lipid vesicle interactions were controlled. This approach was used on patterned plasma polymer surfaces to spatially control location and mobility of lipids on a single substrate.

In order to understand the mechanism of lipid vesicle interactions with plasma polymers it was first necessary to characterise the physiochemical properties of the films. The effect of substrate position (in the plasma reactor) on the resulting film properties was also investigated. It was further necessary to explore the behaviour of the plasma polymers in aqueous conditions, given the application of the films to membrane model systems requiring physiological-like conditions. For all monomers investigated, thinner films were deposited further away from the electrode which can be related to the energy distribution within the plasma glow and the position of the monomer inlet and electrode. These parameters resulted in higher fragmentation and deposition rates close to the electrode. Fluctuations in film thickness occurred between runs at this position and further work is required to investigate the energy distribution and plasma glow characteristics for the reactor configuration used.

ppOct films provided a hydrocarbon-rich, hydrophobic environment and ppAAlc and ppAAm films provided moderately hydrophilic surfaces with alcohol/ether and amine functionality, respectively. For these films, reactor position had little, if any, effect on surface chemistry. This was reflected in the QCM-D response upon exposure to PBS solution, which was similar or identical to that of a rigid gold control surface. XPS revealed that chloride ions from the PBS solution had been retained at the surface of the ppAAm films (even after rinsing in Milli-Q water), irrespective of substrate position, due to the positive charge of the films.

In contrast, the effect of reactor position on ppAAc film chemistry, swelling and stability was significant. XPS derivatization showed the carboxylic acid content of the films increased further way from the electrode and closer to the monomer inlet. Increased COOH content resulted in a greater degree of both swelling and thinning of the films in PBS. XPS also showed that sodium ions were retained in the film more
readily with increasing COOH content. Some individual samples exhibited uneven and localised thinning and even changes in surface chemistry across the surface. The thinning and loss of material from the films was indicated by XPS and not QCM-D, where the changes in response were likely dominated by mass uptake due to swelling. However, XPS can only indicate that the film is approaching the sampling depth of the technique (~ 10 nm) or that delamination has occurred. Further analysis, using a technique such as ellipsometry, is required to quantify film thickness changes occurring during exposure to aqueous conditions.

As monitored by QCM-D, swelling of ppAAm and ppAAc films deposited close to the electrode was dependent on pH, related to the different isoelectric points of the two chemistries. ppAAm swelled dramatically at pH 4, when increasingly positively charged, and considerably less at pH 7, when the positive charge at the surface was reduced. In contrast, ppAAc showed minimal swelling at pH 4 (when neutral) and increased swelling activity at pH 7 when negatively charged. These changes were attributed to the increased association of ions and water into the film when in a charged state. This was confirmed by XPS which showed counterions from the buffer solution had entered and been retained at the film surface for the two plasma polymers.

Further investigation of ppAAm and ppAAc films deposited close to the electrode was conducted using EIS to monitor the response of the plasma polymers to changes in pH and ionic strength. Modelling of EIS data revealed that in all conditions investigated ppAAc exhibited a dual layer structure. Each of the layers had different properties and responses to changes in aqueous conditions, which was related to differences in both physical and chemical composition. In contrast, ppAAm was typically modelled as a single insulating layer, however, upon exposure to pH 10 conditions, a second layer emerged, which was reversible when cycling back to pH 7. QCM-D experiments revealed that mass had been irreversibly lost from the film during the pH 10/7 rinse cycles, attributed to loss of low MW material. Surprisingly, XPS showed that no significant changes in film surface chemistry had occurred. These results demonstrated EIS as an important tool to observe not only water/ion influx or film thickness changes but also, crucially, film structure. Further work is required to investigate both the physical and chemical nature of the dual layered structures of ppAAc and ppAAm using techniques such as AFM, ellipsometry and chemical depth profiling by XPS and/or
ToF-SIMS to obtain a deeper understanding of film properties and behaviour in aqueous conditions.

The interaction of DOPC lipid vesicles with plasma polymers was highly dependent on the surface chemistry of the films and buffer pH. This was unsurprising given the importance of electrostatic interactions in vesicle adsorption and collapse processes. On ppAAc, limited vesicle interaction was observed at pH 7 when the film was negatively charged, as observed using QCM-D. Lowering the pH to 4 enabled vesicle adsorption by bringing the surface into a neutral charge regime and reducing electrostatic repulsion between the vesicles and surface. However, lowering the pH alone was not sufficient to enable vesicle rupture on ppAAc. Rinsing of the vesicular layer on ppAAc at pH 7 had a dramatic effect at the surface causing a distinctive spike in dissipation followed by a large reduction in both mass and dissipation at the surface. The spike was attributed to the electrostatic repulsion between the vesicles, causing deformation and pore formation of the vesicle walls. The pores would enable swelling of the vesicles (due to the osmotic difference across the vesicle walls) and finally, rupture. As confirmed by QCM-D and FRAP, immobile lipid remained at the ppAAc surface, however, it was unclear in what form. Interpretation of the QCM-D data was difficult due to the frequency and dissipation contributions from the underlying plasma polymer layer. This would require advanced modelling and analysis of the data together with complementary techniques, such as ellipsometry and AFM, to confirm the structure of the lipid at the surface. The pH-controlled rupture of vesicles adsorbed to ppAAc could also be pursued as the basis of an encapsulation and release platform.

On ppAAm, DOPC vesicles adsorbed to the surface in all pH conditions investigated and swelling of the film at pH did not deter vesicle adsorption. This was due to favourable electrostatic interactions between the positively charged surface and slightly negatively charged vesicles. Despite these attractive interactions and the tight packing of vesicles at the surface, vesicle rupture was not observed on plain ppAAm films and the vesicle layers remained stable when rinsing with buffers of different pH. This makes ppAAm a suitable surface for use in applications requiring stable vesicular layers such as vesicular cell membrane mimics and drug/compound encapsulation and release systems.
The importance of electrostatic interactions gives rise to variety of other avenues for investigation. The effect of using a negatively charged lipid with the positively charged ppAAm or positively charged lipid and ppAAc would be of particular interest. The use of divalent ions such as calcium to bridge interactions between a negatively charged lipid and negatively charged ppAAc surface would be another approach for promotion of vesicle adsorption and collapse on plasma polymers.

The effect of pH and buffer composition on the interaction of DOPC lipid vesicles with commercially available gold QCM-D sensors was also investigated. Gold is a useful surface for sensing applications and enables the use of other characterisation techniques, such as SPR. This work highlighted both the complexity and variability of the interactions occurring at the gold surface even in seemingly identical conditions. Previous work in the literature had demonstrated that flame annealing of the gold surface and removal of chloride ions from solution was required to enable vesicle collapse on gold surfaces.\(^1\) In this work it was proposed that buffer chemistry also plays an important role. Chloride-free HEPES buffer enabled vesicle adsorption, whilst chloride-free Tris buffer prevented vesicle interactions with the surface. This is an unexpected result and requires further investigation due to the complex interactions involved. The role of pH is also important but the results observed by QCM-D when using a pH 4 to 7 transition were highly variable. AFM revealed a structural change at the gold surface from an adsorbed vesicular layer at pH 4, to a flattening of the structure at pH 7. Further AFM analysis of both the topographical and nanomechanical properties of the lipid structure is required to understand the pH-induced change occurring at the surface.

Control of lipid vesicle interactions, using plasma polymer surface chemistry and pH, was used on ppAAm/ppAAc micropatterns to create regions of different lipid mobility and structure on a single substrate. What was most surprising about this work was that lipid material on the ppAAm regions was mobile, suggesting the presence of an SLB. Previous QCM-D and FRAP experiments on plain ppAAm films showed the formation of an immobile vesicular layers over a wide range of pH. At pH 7, vesicles would not adsorb to the ppAAc feature edges, making it unlikely that vesicle rupture was caused by edge effects. It was therefore hypothesised that electrostatic repulsion at the negatively charged ppAAc edge caused deformation of nearby vesicles adsorbed to
ppAAm. This, together with the presence of a tightly packed vesicular layer on the ppAAm, electrostatic attraction at the surface and the osmotic stress across the wall of the vesicles, all contributed to further vesicle deformation. This would create interbilayer stresses sufficient enough to create a rupture cascade radiating out from the ppAAc pattern edges. The lipid on ppAAm remained mobile throughout the experiment when rinsing at pH 4 and again at pH 7. This is an important result because it suggests that by forming a patterned surface with areas of electrostatic repulsion and attraction, vesicle rupture can be initiated on surfaces which typically do not support SLB formation. Further work is required to investigate if the same effect takes place on a pattern of inverted chemistry or patterns of different dimensions. This technique may also be useful for enabling collapse of vesicles with more complex lipid composition.

By using pH and the patterned surface chemistry it was also shown that two different fluorescently doped vesicles could be deposited onto spatially controlled areas depending on surface chemistry. Immobile lipid islands could be created on ppAAc which were surrounded by mobile lipid areas on ppAAm. This is highly significant for the development of increasingly complex cell membrane model platforms requiring precise control of lipid position and mobility for the recreation of membrane architectures such as rafts. More work is required to fully investigate the lipid structures forming on the patterned surfaces using techniques such as QCM-D and AFM.

The successful fabrication of dual plasma polymer chemistries at the nanoscale, using standard EBL techniques, is significant for a number of applications including the creation of nm sized membrane architectures. An important aspect of future work will be to investigate how lipid vesicles interact with the nanoscale patterns and if they will enable spatial control of lipid structure and mobility, as observed on the ppAAm/ppAAc micropatterns. Further optimisation of the patterning process, for both micro- and nanopatterning, is also required to improve lift-off for certain chemical combinations and to minimise edge effects due to tearing.

This work further highlighted the challenges of characterising chemical patterns at the sub 100 nm scale. This task becomes increasingly difficult when attempting to characterise lipid vesicle interactions and mobility at this scale. Many high resolution techniques operate in vacuum conditions which is not suitable for aqueous environments. Other high resolution techniques may rely on fluorescent tagging of the
lipid, however, the autofluorescence of the plasma polymers will become an important consideration when attempting to achieve sufficient signal-to-noise ratio at the nanoscale.

In summary, this thesis has demonstrated the suitability of plasma polymers, with different surface chemistries, as substrates for cell membrane model systems. In particular, ppAAc and ppAAm have emerged as versatile substrates due to their charge behaviour at different pH, which can be used to control electrostatic interactions with lipid vesicles. The plasma polymer films can be patterned on both a micro- and nanoscale, providing a wealth of opportunities for the development of increasingly complex cell membrane platforms, which can spatially control both lipid location and mobility using surface chemistry.

8.1 References

Presentations


