Development of New Silicone-based Biomaterials

A thesis submitted to the University of Manchester for the degree of

PhD

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Ghislaine Robert-Nicoud

School of Medicine
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Abstract

In the present thesis, we propose a modification of silicone surfaces using the controlled deposition of amphiphilic block copolymers from aqueous colloidal dispersions. The surface modifiers are based on poly(dimethylsiloxane) (PDMS) as the hydrophobic part, in order to allow a good compatibility with PDMS artefacts, and poly(glycerol monomethacrylate) (PGMMA) as the hydrophilic block, since this polymer has demonstrated good biocompatibility and low cell attachment. The hydroxyl groups present on PGMMA offer the possibility of further surface functionalization.

We have demonstrated the convenience of preparing well-defined amphiphilic block copolymers of PDMS and PGMMA (which we refer to as Sil-GMMA polymers) via atom transfer radical polymerization using a protection/deprotection route (i.e. the silylation of GMMA alcohols groups). Depending on the ratio between hydrophobic and hydrophilic blocks, Sil-GMMA copolymers can self-assemble into micellar and other colloidal structures. Diffusion ordered nuclear magnetic resonance experiments have shown that those micelles did not interact with albumin, suggesting a “stealth” behaviour.

Once a library of Sil-GMMA polymers with various block ratio was prepared, the adsorption of Sil-GMMA colloidal dispersions in water/ethanol on PDMS surfaces by simple physisorption was studied. As expected, high PDMS content favoured Sil-GMMA adsorption on silicone surfaces. The presence of our surface modifiers on silicone surfaces was confirmed by a decrease in water contact angle and spectroscopy techniques. We have shown that the surface coatings were stable upon storage in water. Additionally, fibrinogen adsorption was decreased by Sil-GMMA adsorption while albumin adsorption appeared to increase. The preparation of surfaces repellent to fibrinogen and interacting with a “passivating” protein such as albumin is promising.

At the same time, this thesis also reports preliminary investigations on the use of enzymes in order to incorporate new functionality to GMMA containing polymers. Although enzymatic activity was observed when using PGMMA instead of glycerol with two different enzymes (glycerol kinase and glycerol dehydrogenase), PGMMA conversions were always low (< 2%).
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Dedication

To my family
Acknowledgments

I take this opportunity to express my deepest appreciation and gratitude to Professor Nicola Tirelli for offering me this PhD-Position and guiding me through this journey. I would like to sincerely thank him for all the time he has spent in teaching me and guiding me through this research and for the positive and encouraging attitude he always promoted.

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I would like to thank all the friends I have met in Manchester, without them the amazing “Manchester adventure” that I have lived for the last four years would not have been possible.

Last but not least, my thanks go to my family and Chris, for their love and their support in any occasion.
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<th>Description</th>
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<tr>
<td>$\delta_s$ and $\delta_{as}$</td>
<td>symmetric and asymmetric deformation vibration</td>
</tr>
<tr>
<td>$\nu_s$ and $\nu_{as}$</td>
<td>symmetric and asymmetric stretching vibration</td>
</tr>
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<td>$\rho$</td>
<td>rocking vibration</td>
</tr>
<tr>
<td>cm</td>
<td>$10^{-2}$ meter</td>
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<td>$\mu$m</td>
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<td>Adenosine diphosphate</td>
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<td>AFM</td>
<td>Atomic Force Microscopy</td>
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<td>AIBN</td>
<td>Azobisisobutyronitrile</td>
</tr>
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<td>ARGET</td>
<td>Activators Regenerated by Electron Transfer</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>ATRP</td>
<td>Atom Transfer Radical Polymerisation</td>
</tr>
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<td>BCA</td>
<td>Bicinchoninic acid</td>
</tr>
<tr>
<td>BHT</td>
<td>2, 6-di-tert-butyl-4-methylphenol</td>
</tr>
<tr>
<td>BPF</td>
<td>Bovine Plasma Fibrinogen</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine Serum Albumin</td>
</tr>
<tr>
<td>CAC</td>
<td>Critical Aggregation Concentration</td>
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<tr>
<td>CDCl$_3$</td>
<td>Deuterated chloroform</td>
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<td>CH$_3$COOH</td>
<td>Acetic acid</td>
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<tr>
<td>CPP</td>
<td>Critical Packing Parameter</td>
</tr>
<tr>
<td>CRP</td>
<td>Controlled (living) Radical Polymerisation</td>
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<tr>
<td>CTA</td>
<td>Chain Transfer Agent</td>
</tr>
<tr>
<td>DLS</td>
<td>Dynamic Light Scattering</td>
</tr>
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<td>DMMA</td>
<td>2,2-dimethyl-1,3-dioxolane methyl methacrylate</td>
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<tr>
<td>DMS</td>
<td>Dimethylsiloxane</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>DOSY</td>
<td>Diffusion Ordered Spectroscopy</td>
</tr>
<tr>
<td>DP</td>
<td>Degree of Polymerisation</td>
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<td>DMSO(-d6)</td>
<td>(Deuterated) Dimethylsulfoxide</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular Matrix</td>
</tr>
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<td>EPS</td>
<td>Extracellular Polymeric Substances</td>
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<td>Ethanol</td>
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<td>FT-IR</td>
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<td>GDH</td>
<td>Glycerol dehydrogenase</td>
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<td>GMMA</td>
<td>Glycerol monomethacrylate</td>
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<td>GPC</td>
<td>Gel Permeation Chromatography</td>
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<td>HLB</td>
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<td>HMTETA</td>
<td>Hexamethyltriethyltetraamine</td>
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<td>$^1$H NMR</td>
<td>Proton Nuclear Magnetic Resonance</td>
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<td>ICAR</td>
<td>Initiators for Continuous Activator Regeneration</td>
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<tr>
<td>IPN</td>
<td>Interpenetrating Polymer Network</td>
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<tr>
<td>IV&lt;sub&gt;n&lt;/sub&gt;</td>
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<tr>
<td>LDH</td>
<td>Lactate dehydrogenase</td>
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<td>LbL</td>
<td>Layer by Layer</td>
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<td>Number Average Molecular Weight</td>
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<tr>
<td>MW</td>
<td>Molecular Weight</td>
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<tr>
<td>NAD(H)</td>
<td>Nicotinamide Adenine Dinucleotide (reduced form)</td>
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<tr>
<td>NMP</td>
<td>Nitroxide Mediated Living Radical Polymerisation</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>PEG</td>
<td>Poly(ethylene glycol)</td>
</tr>
<tr>
<td>PD</td>
<td>Polydispersity Index</td>
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<td>PDMMA</td>
<td>Poly(2,2-dimethyl-1,3-dioxolane methyl methacrylate)</td>
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<td>Poly(dimethylsiloxane)</td>
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<tr>
<td>$^{31}$P-NMR</td>
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<tr>
<td>PEP</td>
<td>Phosphoenolpyruvate</td>
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<td>PGMMA</td>
<td>Poly(glycerol methacrylate)</td>
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<td>PHEMA</td>
<td>Poly(2-hydroxyethyl methacrylate)</td>
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<td>PK</td>
<td>Pyruvate Kinase</td>
</tr>
<tr>
<td>PMPC</td>
<td>Poly(2-methacryloyloxyethyl phosphorylcholine)</td>
</tr>
<tr>
<td>PPO</td>
<td>Poly(propylene oxide)</td>
</tr>
<tr>
<td>PRE</td>
<td>Persistent Radical Effect</td>
</tr>
<tr>
<td>PSS</td>
<td>Poly(styrene sulfonate)</td>
</tr>
<tr>
<td>RAFT</td>
<td>Reversible Addition-Fragmentation Chain Transfer</td>
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<td>RMS</td>
<td>Root mean square roughness</td>
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<td>S10, S20, S50</td>
<td>Silicone elastomers (Sylgard 184) prepared with “base” to “cross-linker” ratio of respectively 10:1, 20:1 and 50:1</td>
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<td>SDS</td>
<td>Sodium Dodecyl Sulphate</td>
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<td>SEC</td>
<td>Size Exclusion Chromatography</td>
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<td>SGMMA</td>
<td>Bis-trimethylsilyl glycerol monomethacrylate</td>
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<td>SI ATRP</td>
<td>Surface-initiated ATRP</td>
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<td>Sil-DMMA</td>
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<td>Sil-SGMMA</td>
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<td>THF</td>
<td>Tetrahydrofuran</td>
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<td>United States of America</td>
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<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>XPS</td>
<td>X-ray Photoelectron Spectroscopy</td>
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1 Introduction, background and scope of the thesis

1.1 Silicone biomaterials

Silicones are polymers formed of siloxane units of the general formula \([R_2SiO]_n\). The most common member of this family is the linear poly(dimethylsiloxane) (PDMS); its very low glass transition temperature (-123 °C)\(^1\) means that at room temperature and for relatively low molecular weight values (≤ ~10000 g/mol) it behaves as a viscous oil. However, by varying the -Si-O- chain length, the nature of the side groups and adding the possibility of chemical crosslinking, silicone materials can be synthesized with a wide variety of properties and compositions.

1.1.1 Synthesis and cross-linking of silicone polymers

The main steps involved in the synthesis of silicone polymers are described in Scheme 1-1. Short cyclic and linear polysiloxanes are usually derived from chlorosilanes and longer polymer chains are prepared from condensations or ring opening polymerization reactions catalyzed by strong acids or bases. The products are mixtures of cyclic and linear polymers of various chain lengths where the ratio between the different components can be controlled varying the side groups and the reaction conditions (temperature, solvent, catalyst, water content) while the chain length can be varied using different amounts of chain end “blockers”. Stable linear polymers can be obtained after neutralization or elimination of the catalyst and removal of the cyclics under vacuum at elevated temperature. Alternatively, linear polymers with predetermined molecular weights, narrow molecular weight distributions and specific chain end functionalities can be obtained by anionic ring opening polymerization of 3 or 4 membered siloxane rings under appropriate conditions.

Hydrosilylation (the platinum-catalyzed reaction between a silane and an unsaturated bond) is the most popular reaction for functionalizing polysiloxanes; however the use of various chain end-groups and substituents also allows the preparation of functionalized silicones\(^2\).

The three main mechanisms of cross-linking reactions used to prepare silicone elastomers (“curing”) are radical, condensation and addition (Scheme 1-1).
Introduction, background and scope of the thesis

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<th>Polymer synthesis</th>
<th>Elastomer cross-linking</th>
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<tr>
<td><strong>1. Silica reduction to silicon</strong></td>
<td><strong>1. Radical</strong></td>
</tr>
<tr>
<td>( \text{Si}_2\text{O}_2 + 2\text{C} \rightarrow \text{Si} + 2\text{CO} )</td>
<td>a) Initiation</td>
</tr>
<tr>
<td><strong>2. Chlorosilanes synthesis</strong></td>
<td>b) Propagation</td>
</tr>
<tr>
<td>( \text{Si} + 2\text{CH}_3\text{Cl} \rightarrow (\text{CH}_3)_2\text{SiCl}_2 + \text{CH}_3\text{SiCl}_3 + (\text{CH}_3)_2\text{SiCl} + \text{CH}_3\text{HSiCl}_2 + ... )</td>
<td>( \text{R}^+ + \frac{\text{Si-O}_n}{\text{Si-O}_n} \rightarrow \frac{\text{R-Si-O}_n}{\text{R-Si-O}_n} )</td>
</tr>
<tr>
<td><strong>3. Chlorosilanes hydrolysis</strong></td>
<td>c) Termination</td>
</tr>
<tr>
<td>( \text{Cl-Si-Cl} + 2\text{H}_2\text{O} \rightarrow \text{H}_2\text{Si-O}_x + \text{Si-O}_3,4,5 + \text{HCl} )</td>
<td>( 2\text{P}^+ \rightarrow \text{P-P} ) with ( \text{P}^+ = \frac{\text{Si-O}_n}{\text{Si-O}_n} )</td>
</tr>
<tr>
<td><strong>4. Polymerization</strong></td>
<td><strong>2. Condensation</strong></td>
</tr>
<tr>
<td>a) Ring opening</td>
<td>4 ( \text{HO-Si-O}_n \rightarrow \text{RO-Si-OR} ) Sn catalyst - 4 ROH</td>
</tr>
<tr>
<td>( \text{cyclic oligomers} )</td>
<td>( \text{Sn catalyst} )</td>
</tr>
<tr>
<td>b) Condensation</td>
<td>( \text{polymer} ) with ( \text{P} = \frac{\text{Si-O}_n}{\text{Si-O}_n} )</td>
</tr>
<tr>
<td>( \text{linear oligomers} )</td>
<td><strong>3. Addition</strong></td>
</tr>
<tr>
<td><strong>5. Functionalization</strong></td>
<td>( \text{Pt catalyst} )</td>
</tr>
<tr>
<td>1) &quot;Reequilibration&quot;</td>
<td>( \text{H-at-C} \rightarrow \text{H-at-Si} ) Pt catalyst</td>
</tr>
<tr>
<td>( \text{oligomers or polymers} )</td>
<td>( \text{CH}_3\text{CH}_3\text{R} \rightarrow \text{CH}_3\text{CH}_2\text{R} )</td>
</tr>
<tr>
<td>2) Hydrosilylation</td>
<td>( \text{Pt catalyst} )</td>
</tr>
<tr>
<td>( \text{H-at-Si} \rightarrow \text{H-at-C} ) Pt catalyst</td>
<td></td>
</tr>
</tbody>
</table>

**Scheme 1-1.** The basic steps in silicone polymers synthesis and examples illustrating functionalization and cross-linking reactions used in silicone industry. Modified from 3
Efficient radical cross-linking requires the introduction of vinyl groups on the polymer chains and organic peroxides are usually used to initiate the radical reactions which are performed at elevated temperatures.

A condensation reaction can also be used to create three dimensional networks. Usually, hydroxyl-terminated silicones are reacted with silicic acid esters in the presence of a tin catalyst at room temperature.

In addition processes, cross-linking is achieved by hydrosilylation, reacting polymers or oligomers containing several Si-H groups with multi-vinyl species. Depending on the catalytic system, the reaction can occur at room or elevated temperatures. The advantage of this process is that no by-products are created (unlike the condensation cure producing alcohol), avoiding shrinking during cross-linking. However addition cures are not compatible with substances containing amines and sulphurs because these functions easily bind to the Pt-complex, “poisoning” its catalytic activity.

Besides cross-linking, the majority of silicone elastomers require the incorporation of “fillers” (introduced in the material before curing) in order to improve the mechanical strength (principally the tensile strength and elongation capacity) of the materials. “Fumed” silica, agglomerates of amorphous silica nanoparticles, is usually used as filler.

1.1.2 Pros and cons of silicones as biomaterials

Applications. PDMS and its derivatives have been widely employed as biomaterials, for example in the production of medical devices, such as intraocular lenses, urinary catheters and breast implants. In particular, silicone mammary prostheses have been widely used for more than 50 years in reconstructive and aesthetic surgery. The properties that make silicones particularly appropriate for these applications include chemical and thermal stability, providing the possibility of autoclave sterilization and minimal deterioration with time, making silicone materials suitable for long term applications. Silicones are easily molded in almost any shape while their hydrophobicity insures the absence of swelling in physiological environment. Their transparency and high permeability to gases, in particular oxygen, is very important for ophthalmologic applications. The softness, elasticity and flexibility of silicones implanted materials, together with low surface tension, ensure low
mechanical damages, friction and abrasion with adjacent tissues. And last but not least, the low toxicity of PDMS and its derivatives is a major advantage for biomedical applications. However, there are also counterindications. Although the number of breast augmentations performed every year (above 8000 for UK and 300,000 for USA in 2007) is constantly increasing, controversies about safety and efficacy of silicone implants increase too: complications like breast implant rupture and capsular contracture are common; even if their incidence is actually decreasing, they are a magnet for media attention.

Complications. Most commonly, complications with biomaterials, including silicone breast implants and catheters, are related to protein adsorption and cell attachment. After the implantation of any biomaterial, the surface is coated with proteins and the structure, thickness and composition of the adsorbed layer determines the possible biological reactions. When the surface is hydrophobic, the driving force of protein adsorption is the minimization of the energetically unfavourable situations of the water molecules exposed to the surface (hydrophobic effect); proteins can undergo conformational changes from a globular conformation in solution to a multi-point, irreversibly adsorbed state presenting hydrophobic residues on the polymer surface and hydrophilic ones to water. The presence of these denatured proteins may then allow the human body to recognize the presence of a foreign object, in this case triggering an immune reaction. Most often, this takes the form of a fibrous inflammatory reaction (fibrosis) with the development of a capsule made of strong connective tissue that surrounds the material from the biological environment. The capsule will generally minimize the mobility of the artefact, “glueing” it to surrounding tissues, and will also stop chemical and biological direct communication. Depending on the extent of the inflammation, the fibrous capsule can contract resulting in excessive firmness and pain. Capsular contracture can even cause further complications such as distortion of the implanted breast appearance. Correction of capsular contracture may require the removal (and possibly the replacement) of the implant itself; these surgical procedures have both financial and psychological costs for the patients.

Another major complication affecting silicone biomaterials is bacterial attachment which can lead to encrustation and/or biofilm formation, as particularly observed in the case of catheters. Biofilms help to trap nutrients for growth of the enclosed cells and prevent the
detachment of cells on the silicone surface in flowing systems. Furthermore, biofilms act as a barrier and protect bacteria from host defense mechanisms and antimicrobial activity, therefore promoting chronicization of infections. Biofilms are formed after free bacteria cells irreversibly attach to a surface. Bacterial cells are held together in a biofilm by a matrix composed of polysaccharides, protein and nucleic acids known as extracellular polymeric substances (EPS). Studies have indicated that all patients develop bacterial infections within 30 days of catheterization and 40% of hospital acquired infections are a result of catheterization. \(^5\) \(^1\) \(^3\) \(P.\) \(mirabilis\), \(E.\) \(faecalis\), \(K.\) \(pneumoniae\), \(E.\) \(coli\) and \(S.\) \(epidermis\) are micro organisms associated with catheter related infection.\(^1\) \(^5\)

**Strategy.** Although irreversible on hydrophobic surfaces, protein adsorption is usually reversible on neutral, hydrophilic ones. As a result, the modification of polymer surfaces with highly hydrophilic materials is often used to improve biocorntact properties. The performance of these coatings is based on their high surface coverage and hydrophilicity: in order to adsorb on the coated surface, a protein must displace a high amount of water interacting with the hydrophilic polymer and this is not energetically favorable (“water barrier layer”). Obviously, the absence of interactions with biomolecules is also necessary to resist protein adsorption, this is the reason why neutral hydrophilic polymers are favoured: proteins can interact with charged surfaces due to electrostatic forces. Moreover, steric interference (due to the chain flexibility) is one of the key factors in the mechanism of protein resistance from polymeric hydrophilic coatings: compression of the polymer chain upon approach of the protein is also energetically unfavourable.

Surfaces resistant to protein adsorption and cell adhesion are so called “non” or “anti-fouling”, in parallel to “stealth” nano-sized materials. The development of anti-fouling surfaces is not only important for silicone biomaterials, but also essential for many technologies ranging from ship hulls coatings to biosensors and carriers for targeted drug delivery.\(^1\)\(^9\)

Modifications of silicone biomaterials with hydrophilic polymers are discussed later in this introduction (see section 1.2.3). Related background knowledge in surface modification techniques is necessary and is first introduced in the following section. Additional information related to amphiphilic block copolymers (see section 1.3), polymer synthesis
Introduction, background and scope of the thesis

(see section 1.4) and analytical techniques (see section 1.5) are also provided at the end of this introduction.

1.2 **Surface modification of organic (polymer) materials**

1.2.1 **Physical techniques (Physisorption)**

1.2.1.1 **Hydrophobically driven adsorption**

Physisorption on a solid surface is usually achieved using block copolymers\textsuperscript{20-22}. The adsorption can either be driven by selective solvation or selective adsorption. In the case of selective solvation an ideal solvent is a precipitant for one block which forms an “anchor” layer on the surface and a good solvent for the other block(s) which form(s) a polymer film in the solution. The properties of the modified surface depend on the selectivity of the medium, the nature of the copolymers, the architecture of copolymers, the length of each block and the interactions between blocks and surface\textsuperscript{23}. In general, the anchoring block should be long enough to cause stable immobilization on the surface and in most cases, amphiphilic block copolymers are used and the selective solvent is water (for background on amphiphiles self-assembly, see section 1.3.1). The physisorption approach has the advantage to be an easy process: no laborious chemistry is used, no pretreatment of the surface is necessary and it is applicable to substrates of various shapes. However, the polymer films formed by physisorption are not stable towards treatment with a desorption solvent.

The conformation of tethered chains and the thickness of surface grafted polymer films can be controlled by systematic variation of grafting density and degree of polymerization (DP) of the grafted polymers. If the grafting density is high enough, steric repulsion between the different polymer chains leads to chain stretching and a brush-type conformation of the surface tethered-chain is obtained (Figure 1-1)\textsuperscript{23,24}. On the contrary, when the grafting density is low enough, the surface anchored chains adopt more relaxed conformations, such as the so-called polymeric “mushroom”\textsuperscript{25}. “Mushroom” type of films are usually obtained when modifying a surface by physisorption as it becomes increasingly harder for new
chains to diffuse to the surface and adsorb on it when the surface grafting density increases. On the other hand, amphiphilic molecules can rearrange on a surface in an attempt to maximize packing on the surface.

Figure 1-1. Various morphologies of polymer grafted on a surface, depending on the ratio between the size of the polymeric chain and the grafting density.

**1.2.1.2 Electrostatic adsorption**

When using electrostatic adsorption to modify surfaces, charge bearing polymer chains are adsorbed on oppositely charged surfaces. The adsorbing polymers usually bear many charged repeating units (polyelectrolytes). The adsorption process results from a subtle balance between electrostatic repulsion between repeating units of the same charge and electrostatic attraction between the substrate and the polymer chain usually resulting in a “flattened” adsorbed polymer on the surface forming a dense adsorption layer. An even denser film coating can easily be obtained by the sequential alternate adsorption of polycation and polyanion layers, via the so called layer-by-layer deposition (LbL). Films comprising up to 100 layers were prepared by LbL and this technique was used for the preparation of thin films coatings for different applications ranging from biosensors to coatings for medical implants. The tuning of cell adhesion and proliferation surface properties, either favouring or hindering cell adhesion, for example through the addition of antifouling end layers such as poly(ethylene glycol) (PEG), is possible.

Many research groups have reported the formation of LbL films on PDMS elastomers without surface pretreatment using poly(styrene sulfonate) (PSS) as the first anchoring layer. Since silicone is hydrophobic and does not present any surface charge, the adsorption of the first polyelectrolyte layer is not electrostatically-driven, but is probably hydrophobically driven since PSS is amphiphilic. Other research groups have pretreated silicone surfaces with plasma (introducing charges through oxidized functions) before LbL modification.
It is possible to stabilize LbL films by cross-linking them, avoiding the structural changes sometimes observed after exposure to a different solvent or changes in pH and ionic strength\textsuperscript{35}.

1.2.2 Chemical techniques

1.2.2.1 Derivatization of functional groups (chemisorptions)

The majority of chemical derivatizations on surfaces are conducted via the so called “self assembled monolayers” (SAMs) technique\textsuperscript{36,37}. Silane compounds are used to form SAMs on the surfaces of silicon oxides\textsuperscript{38} or –OH containing polymers such as cellulose\textsuperscript{39}. However, the use of the silane chemistry on the surface of inert polymers such as PDMS requires the introduction of a surface reactive group\textsuperscript{40}. Si-OH surface groups can be obtained through UV/ozone\textsuperscript{41}, plasma\textsuperscript{42} or acidic (H\textsubscript{2}SO\textsubscript{4} or HF)\textsuperscript{43} pretreatments.

“Grafting to” of polymers

In a “grafting to” approach, preformed end-functionalized polymer molecules are coupled with an appropriate reactive group on the surface.\textsuperscript{44-46} Except for the attaching end, the polymer is not particularly attracted to (or even repelled from) the grafting surface. Hence, the grafted chains adopt random-coil structures (“mushroom” conformation), or if sufficiently high grafting densities are achieved, brush-type conformations. The covalent bond formed between surface and polymer chain makes the polymeric layer robust and resistant to common chemical environmental conditions.

This approach requires the introduction of surface reactive groups on the polymeric surface in most of the cases. On the other hand, this technique has the advantage of allowing the grafting of well-characterized polymers that can be prepared by controlled polymerization techniques (for background on controlled polymerizations, see section 1.4). As for the physisorption strategy, one disadvantage of the “grafting to” approach is the limited diffusion of polymeric chain to the surface leading to low grafting densities. Furthermore, film thickness is also limited by the molecular weight of the polymer in solution.
1.2.2.2 “Grafting from” (Surface initiated polymerizations)

In a “grafting from” approach, a polymer chain is grown from an initiator immobilized on the surface. As already mentioned, the conformation of tethered chains and the thickness of surface grafted polymer films are controlled by the grafting density and the DP of the grafted polymers. Because there is no significant diffusion barrier in surface initiated polymerizations (as only low molecular weight compounds – the monomers - have to reach the growing chain end during film formation), long polymer chains at high grafting density can be prepared via the “grafting from” approach, allowing the formation of polymers brushes.

The grafting density (related to the surface initiator density) depends on the method of initiator attachment to the surface. SAMs\(^{47}\) have been widely used to attach polymerization initiators on surfaces: they allow a good control over initiator density on the surface, using, for example different ratio of “active” and “inactive” initiator as self-assembling molecules\(^{48,49}\). It is also possible to create patterns of covalently attached initiators by microcontact printing and therefore patterned polymer layers can be grown from surfaces\(^{50}\). SAMs can be formed on various surfaces, as long as the anchor functionality is chosen right (for example, thiols have been used to form initiators on gold surfaces\(^{48}\), titanium substrates have been coated with polymer brushes using catechol modified initiators\(^{51}\) and silanes have been used to modify hydroxylated surfaces such as glass\(^{52}\) and plasma oxidized polymers\(^{53}\)). However, surface initiated polymerization on polymeric substrates using SAMs type initiators is often challenging because of the substrate incompatibility with organic solvents as well as the toxicity of the reagents generally used for initiator attachment. Moreover, the sequential processing scheme generally used (introduction of surface groups and then attachment of initiators) can be of limited practicability.

An alternative approach developed for polymeric materials consists of the use of substrates that have a homogeneous distribution of initiator groups (the initiators are not just on the surface). Depending on the polarity of substrate and solvent, discrimination between surface and bulk polymerization can be achieved. It has been demonstrated that using this
method for surface initiated controlled polymerization allows the growth of very thick polymeric coatings and block copolymers\textsuperscript{54,55}.

Alternatively, physisorbed (mono)layers of macroinitiators have been used to initiate surface polymerization, the driving force of the adsorption being either the hydrophobic effect\textsuperscript{56} or electrostatic, using polyelectrolytic macroinitiators on charged substrates\textsuperscript{57-60}. Anionic macronitiators have been adsorbed on positively charged silica particles\textsuperscript{59} or cationic (aminated) planar substrates\textsuperscript{57} and positively charged macroinitiators have been adsorbed on oxidized polystyrene substrates.\textsuperscript{60} The advantages of this process are various: it is fast and efficient, it can be achieved at room temperature in aqueous solution and the surface initiator density can be enhanced using a layer by layer deposition of oppositely charged polyelectrolytic macroinitiators\textsuperscript{58}.

Polymer films can be formed on surfaces using free radical polymerization,\textsuperscript{61,62} however the use of living techniques for surface initiated polymerization is particularly advantageous as precise control over the molecular weight is accessible (for background knowledge on controlled polymerization techniques, see Section 1.4). Furthermore, the properties of polymer films can be tuned by the consequent polymerization of different monomers (preparation of block copolymers): varying the composition and the size of each polymer segment directly affects the morphology and the behavior of the polymeric layer. Living polymerisation methods that have been used to synthesize polymer brushes include cationic,\textsuperscript{63,64} anionic,\textsuperscript{63,65} ring-opening metathesis,\textsuperscript{66} and controlled radical polymerization techniques (CRP) such as nitroxide mediated living radical polymerizations (NMP),\textsuperscript{67} reversible addition-fragmentation chain transfer (RAFT) polymerizations\textsuperscript{68,69} and atom transfer radical polymerization (ATRP)\textsuperscript{70,71} techniques. Among them, surface-initiated CRP\textsuperscript{72} techniques have attracted particular attention due to the mild conditions required and the large range of monomers polymerizable by this method.

**Surface-initiated controlled radical polymerization**

In an early study, Wirth et al. reported the growth of polyacrylamide from functionalized silica particles using ATRP with minimized termination reactions.\textsuperscript{73} No information was given on the control over film thickness, however the authors demonstrated later that film thickness could be controlled by the surface-initiated acrylamide polymerisation on silicon
Since then, many reports of surface initiated CRPs have been reported and it has been demonstrated that surface-initiated CRP can be successfully performed maintaining the living character of the chain end and with a high degree of control during the polymerization despite a deviation from theoretical molecular weights.

Surface CRP presents additional difficulties compared to CRP in solution. A consideration in using multifunctional initiators is that the impact of termination reactions may be more significant than for linear polymers as termination can occur by coupling and chain transfer with the neighboring surface-immobilized chains.

Concerning ATRP from surfaces, the main challenge may be the fact that the concentration of the persistent radical (deactivator) is too low to reversibly trap propagating radicals required to minimize side and termination reactions. This can lead to uncontrolled chain growth or loss of the living character. In ATRP catalyzed by Cu complexes in solution, the reaction of Cu(I) with initiator produces the deactivating Cu(II) complex (see section 1.4.2.2 for an introduction on ATRP mechanism). In the case of polymerization from a surface, however, the concentration of the Cu(II) complex is too low to control the reaction because of the small concentration of initiators at the substrate. Several research groups have added free initiator to the polymerization solution in order to overcome this problem. The “free” (untethered) chains terminate in solution, thereby forming a sufficient amount of the deactivator. Additionally, assuming similar kinetics for both surface and solution initiated polymerizations, the final DP of tethered chains can be tuned by the ratio between the concentration of monomer and the concentration of initiator (which is approximatively the concentration of sacrificial initiator added at the initial stages of the polymerization, the concentration of the initiator attached on the surface being very low). However the correlation between surface initiated and solution polymerization should be treated with precaution: the surface polymerization rate may be slower than the one in solution due to the low concentration of initiating groups. A study revealed differences between the polymer layer thicknesses expected for fully extended chains of the average size reached at the particular moment in solution and the observed thicknesses of polymer layers formed by surface initiated ATRP. In spite of its advantages, the use of “sacrificial” initiator wastes monomer which is in some cases expensive and the separation of polymer...
formed in solution from grafted substrate may present a problem. An alternative way to ensure a sufficient concentration of deactivating Cu(II) species without initiating polymerization in solution is the addition of the persistent radical to the solution prior to the reaction\textsuperscript{75}.

Another difficulty associated with surface polymerization is the determination of the products molecular weight and molecular weight distribution. Direct measurements on polymers formed by surface initiated polymerization, for example by nuclear magnetic resonance (NMR) or size exclusion chromatography (SEC), is not possible. In order to perform NMR or SEC, the polymer brushes have to be detached from the surface\textsuperscript{45,74}. Although this method gives access to important information, a cleavage process without altering the formed polymers is not accessible in most of the cases. Furthermore, the amount of polymer prepared by surface-initiated polymerization is sometimes too low to be analyzed. The increase in layer thickness is usually the main parameter used to follow surface-initiated polymerizations. Polymer layer thickness can be characterized by ellipsometry\textsuperscript{57,74} or microscopy techniques such as scanning electron microscopy and atomic force microscopy (AFM) from which additional information about the polymeric layer structure can also be obtained (see Section 1.5.4)\textsuperscript{54,60}.

### 1.2.3 Silicone surface modification

As previously discussed, the preparation of hydrophilic polymeric coatings on silicone surfaces, providing them with protein resistant properties, is attractive. Some examples of the different techniques and polymers that have been used to modify silicone substrates are presented in Table 1-1.

Most studies aiming to improve the biocompatibility of silicone materials are using PEG\textsuperscript{49,76-79}. However, the use of other biocompatible materials such as poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC)\textsuperscript{80-84} and poly(2-hydroxyethyl methacrylate) (PHEMA)\textsuperscript{73,85} has also been researched for modifying silicone surfaces.
Table 1-1. Examples of silicone surface modification with hydrophilic polymers

<table>
<thead>
<tr>
<th>Grafting technique</th>
<th>Activation protocol</th>
<th>Surface reactive site</th>
<th>Anchoring group (and initiator)</th>
<th>Anchored polymer</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physisorption</td>
<td>No activation</td>
<td>-</td>
<td>PPO</td>
<td>PEG</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MTS</td>
<td>PMPC</td>
<td>81</td>
</tr>
<tr>
<td>Swelling-deswelling</td>
<td>No activation</td>
<td>-</td>
<td>PDMS</td>
<td>PEG</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PMPC</td>
<td>80</td>
</tr>
<tr>
<td>Grafting to</td>
<td>Acid catalyzed equilibration with Si-H containing siloxane</td>
<td>Si-H</td>
<td>allyl</td>
<td>PEG</td>
<td>76</td>
</tr>
<tr>
<td>Grafting from</td>
<td>1) free radical polymerization</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>UV + benzophenone</td>
<td>Si-CH$_2$ radical</td>
<td>-</td>
<td>PMPC</td>
<td>82,83</td>
</tr>
<tr>
<td></td>
<td>Ozone</td>
<td>-OOH</td>
<td>-</td>
<td>PMPC</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>HCl</td>
<td>-OH</td>
<td></td>
<td>PHEMA</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>O$_2$ plasma</td>
<td>-OH</td>
<td></td>
<td>poly(2-(methacryloyloxy)ethyl trimethylammonium chloride)</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>2) ATRP</td>
<td>-OH</td>
<td></td>
<td>Poly(dendronizedPEG monomethacrylate)</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>H$_2$O$_2$/H$_2$SO$_4$</td>
<td>-OH</td>
<td></td>
<td>Poly(acrylamide)</td>
<td>87-89</td>
</tr>
<tr>
<td></td>
<td>UV/ozone</td>
<td>-OOH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IPN layer of initiator formed by vapor deposition and hydrolysis</td>
<td>-</td>
<td></td>
<td>Poly(PEG monomethacrylate)</td>
<td>49</td>
</tr>
</tbody>
</table>
In the present project, we have focused our attention on a different polymer, poly(2,3-dihydroxypropyl methacrylate), more commonly referred to as poly(glycerol monomethacrylate) (PGMMA). PGMMA was selected as a suitable coating for possible biomedical applications due to its bis(hydroxyl) group present on each repeating unit providing high hydrophilicity and ease of further functionalization. PGMMA based materials have been shown to be nontoxic, protein-repellent, non cell-adhesive\textsuperscript{60,90-94} and our group has previously used PGMMA for antifouling surface preparation\textsuperscript{60}.

The selection of a modifying method also needs consideration. The majority of the silicone surface modification reported in the literature is using surface initiated polymerization: the possibility to achieve high surface coverage and the wide range of polymer that can be prepared by this technique are attractive\textsuperscript{49,73,79,82-89}. However due to the inertness of silicones, the surface needs to be activated either via UV irradiation\textsuperscript{82,83} or ozone treatment\textsuperscript{84} in order to initiate free radical polymerization or via oxidizing treatments in order to introduce $-\text{OH}$ surface groups that can react with silane ATRP initiators\textsuperscript{73,86-89}.

Alternatively, Tugulu et al. used vapor deposition/hydrolysis cycles to generate a surface-confined ATRP-initiator functionalized interpenetrating polymer network (IPN) layer\textsuperscript{49}.

The grafting to approach also needs the introduction of surface reactive groups. For example, Si-H groups were introduced in silicone elastomers by acid-catalysed equilibration in presence of poly(hydromethylsiloxane) allowing the grafting of allyl-terminated PEG\textsuperscript{76} and PMPC\textsuperscript{95} derivatives via platinum-catalyzed hydrosilylations.

Another method used to introduce PEG or PMPC on silicone surfaces is the so-called “swelling-deswelling” method. Silicone elastomers were swollen in an organic solution of surface modifiers (which were amphiphilic block copolymers comprising PDMS segments). The amphiphilic copolymers (that penetrated into the silicone network) rearranged at the surface upon exposure to water\textsuperscript{80,94}. Although this method has advantages, e.g no harsh chemicals were used and reasonably high surface coverages were obtained, the swelling-deswelling process induces topological changes and is not suitable for various applications.

PEG was also grafted onto PDMS substrates by simple physisorption of Pluronic\textsuperscript{®}. However, an amphiphilic copolymer modifier is not easily immobilized on PDMS surfaces,
even in aqueous media, due to the high molecular movement of PDMS macromolecules (reflected by its low glass transition temperature) and poly(propylene oxide), having no favorable interactions with PDMS, is not a good anchor group. Therefore, an amphiphilic copolymer containing dimethylsiloxane (DMS) units as the stabilizing parts should provide greater stability to the surface modification (DMS units maximize the interaction with the PDMS elastomer). However, Seo et al. have shown that amphiphilic block copolymers of PMPC and PDMS do not provide a stable silicone surface coating. On the contrary, the authors found that the adsorption of random copolymers of (2-methacryloyloxyethyl phosphorylcholine) (MPC) and 3-(methacryloyloxy)propyl-tris(trimethylsilyloxy)silane (MTS) with the appropriate units ratio, were better suited for the stable modification of silicones.

In spite of the concerns about the stability of polymeric films formed by physisorption, we chose to use physisorption to modify silicone surfaces because it is an easy process, it is shape-independent and it does not require toxic chemicals or a pre-treatment step to modify silicone elastomers.

1.3 In-depth A: Amphiphilic block copolymers

1.3.1 Amphiphiles and self-assembly

Amphiphilic molecules consist of a water-seeking (hydrophilic part) and a water-excluding (hydrophobic) one. As such, they are not completely water-soluble but also not totally water-insoluble and they tend to self-assemble (generally above a certain critical concentration) to avoid the presentation of hydrophobic residues to water.

The form and order of the self-assembled state depends on the ratio between the hydrophilic and the hydrophobic part, which can be expressed through quantitative scales such as the Hydrophilic Lipophilic Balance, HLB, or the Critical Packing Parameter, CPP and other factors, such as the temperature and the concentration of the amphiphile in water.

The HLB estimates the capability of an amphiphile molecule to form aggregates in water. The number indicates the polarity of the molecule in an arbitrary range of 1-20. There are
several ways to calculate HLB, one of the most common described by Griffin\textsuperscript{96} is 
\[ \text{HLB} = \frac{(E + P)}{5} \]
where E is the % wt. of the hydrophilic part and P is the % wt. of the hydrophobe.

The CPP is defined by the formula 
\[ \text{CPP} = \frac{V}{a_0 l_c} \]
where \( V \) is the partial molar volume of the hydrophobe, \( a_0 \) is the surface area of the hydrophile and \( l_c \) is the effective length of the hydrophobic part. CPP therefore has values ranging between 0 and \( \infty \).

If the amphiphilic molecules have equally large hydrophilic and hydrophobic components (when typical values for CCP and HLB are \( \approx 1 \) and \( \approx 7-9 \) respectively), they form planar structures, e.g. bilayers. In order to protect the bilayer edge (where hydrophobic chains are in contact with water), the bilayer can fold in on itself providing a spherical geometry such as vesicles.

When CPP > 1 and HLB < 6, the hydrophilic part of the amphiphile is smaller in size than the hydrophobic one and the creation of a flat hydrophobic-hydrophilic interface is not possible. The interface becomes curved, with the hydrophobic part on the concave side, and the formation of inverse aggregates where water is the dispersed phase (such as inverse micelles) is typically observed. If CPP < 1 and HLB > 10, the reverse applies and regular aggregates, such as micelles, are formed where water is the dispersing phase (Figure 1-2).

\[ \text{Figure 1-2.} \text{ Schematic representation of common morphologies adopted by an amphiphile molecule in water} \]
\[ \text{for the purpose of simplification, cubic phases were omitted).} \]
1.3.2 Amphiphilic block copolymers for biomedical applications

Block copolymers are macromolecules composed of linear or nonlinear arrangements of polymeric chains, each chain (block) being composed of a chemically different polymer. Different block copolymer architectures are possible: linear, star, graft, myktoarmed, etc.

Similarly to amphiphilic compounds in water, the presence of incompatible blocks in a polymeric structure (also in the absence of a solvent) determines a microphase separation into a number of different morphologies,\(^97,98\) which depends on block length and interfacial tensions.

It is therefore not surprising that amphiphilic block copolymers, combining an amphiphilic nature and the presence of mutually incompatible blocks, have the ability to self-assemble into a rich variety of self-assembled structures (such as the ones presented in Figure 1-2) at appropriate concentrations and temperatures in an aqueous environment. Amphiphilic block copolymers usually have clear advantages over low molecular weight amphiphiles (surfactants and lipids): a) easy modulation of the HLB by simply changing the weight fraction of the blocks and b) very low values for critical aggregation concentrations (CAC), increasing the stability of self-assembled structures against dilution.

Self-assembled structures of amphiphilic copolymers have many characteristics that make them ideal for potential biomedical applications, such as drug delivery, the preparation of hydrogels as injectable controlled release systems\(^99\) or as functional scaffolds for tissue engineering\(^100\) and non-fouling material preparation\(^21\). The use of amphiphilic block copolymers to prepare non-fouling surfaces is discussed in another section of this introduction (see section 1.2.1).

Concerning drug delivery applications, the use of amphiphilic copolymers for solubilizing poorly water soluble drugs in the local hydrophobic cavities offered by micelles (in their hydrophobic core) and vesicles (in their bilayer) in a water environment has received a lot of attention\(^101,102\). Poor absorption and bioavailability upon oral administration are generally associated with the low solubility of many drugs in water while aggregates formed by insoluble drugs administered intravenously could cause embolization of blood vessels\(^103\). In addition, drug delivery applications can be expanded to hydrophilic therapeutic agents by using vesicles as they are able to enclose aqueous volumes within...
their structure, allowing encapsulation of hydrophilic drugs. The main drawback of using self-assembled structures for drug solubilization is the stability against dilution: below their CAC, aggregates would disappear (and therefore their hydrophobic cavities and/or enclosed aqueous volumes too) and the payload may be released too quickly or precipitates (hydrophobic drugs). However, as already mentioned, the CAC of assembly formed by amphiphilic copolymers is generally very low and resistance against dilution can be obtained by cross-linking the aggregates\textsuperscript{104-106}. Another important advantage when using copolymers in drug delivery applications is the slow chain exchange dynamics between molecules in the assembled structure and molecules in solution\textsuperscript{107}. Low concentrations of molecularly dissolved amphiphilic copolymers in solution prevent cytotoxic interactions with biological phospholipid membranes. Moreover, the nanosized dimensions of block copolymer micelles (diameters in the range of 10-100 nm\textsuperscript{21}) and of some vesicles (diameters in the range of 50 nm – 10 μm\textsuperscript{108}) provide the advantage of allowing application to the body via direct injection into the blood circulation: nanocarriers smaller than ~200 nm in diameter are less susceptible to opsonization and subsequent phagocytosis by the cells of the immune system\textsuperscript{109} while objects with a diameter > ~5 nm avoid excretion via the kidneys\textsuperscript{110}. At the same time, the possibility of renal excretion of the dissolved macromolecules reduces concerns about their long-term permanence\textsuperscript{111,112}.

Among the most popular amphiphilic systems for the solubilization of drugs, poly(ethylene glycol) (PEG) based copolymers, in particular the commercially available PEG/poly(propylene glycol) di- and tri-block (Pluronics or Poloxamers) copolymers, occupy a position of preeminence\textsuperscript{113,114}. The “stealth” character of PEG based materials have been widely described: its neutral hydophilic corona prevents plasma protein adsorption and recognition by the reticuloendothelial system, therefore prolonging the circulation of the PEG based nanocarriers in vivo\textsuperscript{115} However, other biocompatible neutral hydrophilic polymers, such as PMPC\textsuperscript{116,117} and PGMA\textsuperscript{118} can also be used to prepare drug carriers.

The pharmaceutical efficiency of a micelle or vesicle encapsulated drug can be further increased by targeting approaches, either via conjugation of a recognition protein or peptide on the hydrophilic part\textsuperscript{119} and/or a release specifically triggered at the site of interest.
Targeted release can be triggered by a stimulus associated with certain pathological processes (e.g. a local increase in temperature, acidity and/or oxidative environment) using temperature\textsuperscript{120}, pH\textsuperscript{121} or oxidation\textsuperscript{122} sensitive amphiphilic copolymers.

1.4 \textit{In-depth B: Living/controlled polymerization}

1.4.1 General definitions

The mechanism of chain-growth polymerisation, employed for instance in free radical, anionic and cationic polymerisation, presents three distinguished elementary steps: a) initiation, b) propagation and, c) termination (e.g. through coupling, disproportionation or spontaneous deactivation). Spontaneous and uncontrolled termination reactions or side reactions such as chain transfers often hinder the preparation of polymers with narrow molecular weight distributions and precisely controlled architecture (poor control over terminal groups). On the contrary, in living polymerizations, undesired termination or transfer reactions on growing polymer chains have been removed. As a consequence, an active reactive group remains located at the end of the polymer chain, thus the polymer is “living”; it is able to grow whenever additional monomer is provided.

If additionally the initiation is faster than, or at least comparable in rate to propagation, polymers with very narrow molecular weight distributions can be obtained. Although such polymerizations allowing a precise control over molecular weight should be more correctly called “controlled”, the terms “living” and “controlled” are commonly interchanged and used in an indifferent way. The distribution of molecular weights is often measured by the polydispersity index (PD) which is the ratio between the weight average molecular weight ($M_w$) and the number average molecular weight ($M_n$) (PD = $M_w/M_n$). A polymer with a perfectly uniform chain length would have a polydispersity index equal to unity. Polymers with narrow weight distribution (1.0 < PD < 1.5) can be obtained with controlled polymerization techniques.\textsuperscript{123} The general behavior of a controlled polymerization mechanism is illustrated in Figure 1-3: a) the molecular weight (expressed as the number average one) increases linearly with monomer conversion due to the living character of the
terminal groups, b) the polydispersity decreases with increasing conversion, since, besides polymerization mechanism-specific effects (which take place e.g. in ATRP), small differences in chain length have a relatively larger importance at low average MW values rather than at high ones.

![Figure 1-3](image)

**Figure 1-3.** Schematic representation of the evolution of the molecular weights and polydispersities with conversion during a controlled polymerization.

Living polymerization mechanisms have played a major role in the synthesis of block copolymers, since they allow polymers to be prepared in stages, each stage corresponding to a different block and therefore to a different monomer composition. Additional advantages of living polymerizations are a) the ability to pre-determine molar mass and b) the possibility to synthesize end-functional polymers by using appropriate end-cappers.

In 1953, Flory described the properties associated with living polymerization, he noted that since all of the chain ends grow at the same rate, the molecular weight is determined by the amount of initiator used versus monomer (Equation 1-1).

**Equation 1-1**  
\[ DP = \text{Degree of polymerization} = ([\text{monomer}]_0/[\text{initiator}]_0) \times \text{conv.} \]

with \( \text{conv.} = \text{conversion}, 0 \leq \text{conv.} \leq 1 \)

The name "living" was introduced later by M. Szwarc in 1956 about the anionic polymerization of styrene with an alkali metal/naphthalene system in tetrahydrofuran because the chain ends remained active until killed. His work was the first report of living polymerization and has opened the way of a major development in polymer synthesis.
Living polymerization processes include anionic living polymerization\textsuperscript{128,129}, cationic living polymerization\textsuperscript{130,131}, ring opening metathesis polymerization\textsuperscript{132,133}, group transfer polymerization\textsuperscript{134,135} and free radical living polymerization\textsuperscript{136}.

1.4.2 Living Radical Polymerization

Living Radical polymerization, or controlled radical polymerization (CRP), is one of the most rapidly developing areas of synthetic chemistry. There are several reasons why CRP techniques have been investigated with particular interest in recent years. The radical process is the leading industrial method to produce polymers due to its tolerance to impurities and different functional groups as well as the mild conditions used in radical polymerizations. With CRP, advantages of radical polymerization are combined with the precise control over polymeric structure associated with living polymerization, a great variety of materials can therefore be prepared: by varying the monomers one can get access to various functionalities and compositions while the use of multifunctional initiators gives rise to a large possibility of topologies including graft, star and hyperbranched polymers\textsuperscript{136}.

The process of radical living polymerization is based on establishing a rapid dynamic equilibration between a small amount of growing free radicals (active species) and a large majority of dormant species (Scheme 1-2).

\[ \text{Scheme 1-2. Scheme of dynamic equilibria in CRP. Equilibria are between propagating radicals (P}_n^* \text{ or P}_m^* \text{) and various dormant species. Radicals may either be reversibly trapped in a deactivation/activation process (NMP and ATRP) (a), or they can be involved in a “reversible transfer”, degenerative exchange process (RAFT) (b).} \]

For this equilibrium to be effective in controlling a radical polymerization, there are two necessary conditions: first, the equilibrium between dormant and active (free radical)
species must lie strongly to the side of the dormant species to ensure that the overall concentration of radicals will remain very low and that the rate of irreversible termination will be negligible relative to the apparent rate of polymerization; second, the rate of exchange between dormant and active species must be faster than the rate of propagation to ensure that all polymer chains have an equal probability of adding monomer. These properties, associated with a fast initiation (all polymer chains start to grow at the same time), leads to nearly uniform chain length.

It has to be mentioned that irreversible terminations are only minimized in these polymerizations and not excluded from the mechanism, therefore, above some molecular weight values characteristic for each monomer, all living radical polymerizations can no longer be considered controlled because the effects of termination and transfer reactions become significant.

The main CRP techniques are nitroxide mediated living radical polymerisations (NMP), reversible addition-fragmentation chain transfer (RAFT) polymerization and atom transfer radical polymerization (ATRP). However, RAFT and ATRP techniques are the two most studied CRP techniques.

In the following sections, RAFT techniques are briefly presented while ATRP mechanisms and components are discussed in more details due to their relevance for the synthesis described in this thesis.

1.4.2.1 Reversible addition-fragmentation chain transfer (RAFT)

RAFT polymerization operates on the principle of degenerative chain transfer and as such differs fundamentally from other CRP techniques.

RAFT was first reported in 1998 and is compatible with most monomer types (including functional monomers containing, for example, acid, acid salt, hydroxyl or tertiary amino groups) and with a very wide range of reaction conditions. An essential feature of the RAFT polymerization technique is the addition of a chain transfer agent (CTA) or RAFT agent in a conventional free radical polymerization to control the active species concentration. A general mechanism for RAFT polymerization is shown in Scheme 1-3.
(i) Initiation

```
Initiator → $I^\bullet \xrightarrow{M} P_n^\bullet$
```

(ii) Reversible chain transfer

```
P_n^\bullet + X\xrightarrow{kp+M} Z\xrightarrow{k_p+M} P_n-X\xrightarrow{X-R} X-R\xrightarrow{P_n-X-X+R^\bullet}
```

(iii) Reinitiation

```
R^\bullet \xrightarrow{M} R-M^\bullet \xrightarrow{M} P_m^\bullet
```

(iv) Chain transfer equilibration

```
P_m^\bullet + P_n-X\xrightarrow{k_{tr}} P_m-X\xrightarrow{P_n+P_n^\bullet}
```

Scheme 1-3. General mechanism for RAFT polymerization. Reproduced from 139

Traditional methods for radical generation (azo compounds, photoinitiators or gamma irradiation) are used to generate the primary radical. This radical may add to the monomer starting a short polymer chain ($P_n^\bullet$) (Scheme 1-3, step (i) initiation). However, it is unlikely that an important quantity of monomers will add to the generated radical before adding to the RAFT agent (1) which is generally present at a higher concentration than the initiator. When using the correct monomer/RAFT agent combination, the fragmentation of 2 forms preferentially the compound 3 and the radical fragment R’ (Scheme 1-3 step (ii) reversible chain transfer). If the fragment R’ is a good reinitiating species; it will then add to the monomer, initiating a new polymeric chain (Scheme 1-3 step (iii) reinitiation). The net result of this sequence of forward reactions is the generation of a new polymer (oligomer)
chain, derived from the R fragment of the initial RAFT agent and the formation of a new thiocarbonylthio compound (3) which is itself capable of acting as a RAFT agent.

The first part of the overall RAFT mechanism is complex; many of these steps are reversible and various reaction pathways are available to the generated radical species (they are not all represented in Scheme 1-3 in a purpose of clarity). In summary, the result of this sequence of reactions, (steps (i) to (iii)), often referred to as the RAFT pre-equilibrium, is the activation of all RAFT agent molecules (1) to new polymeric (oligomeric)-type RAFT agents (3).

Once the pre-equilibrium is complete, the main RAFT equilibrium (Scheme 1-3 step (iv) chain transfer equilibration) predominates the RAFT polymerization. Most of the chain growth (the monomer conversion) occurs during this stage. The control over the radical species concentration involves the degenerative chain transfer of the thiocarbonylthio species between different growing polymer chains (active and dormant chains) via the radical intermediate species 4. An essential feature of the RAFT process is that the product of chain transfer is also a chain transfer agent with similar activity to the precursor transfer agent.

Although exchange reactions in RAFT processes are very fast, minimizing termination reactions, the formation of “dead chains” (inactive species) cannot be completely prevented. It is, in principle, possible for any of the radical species to participate in a number of undesirable, non-RAFT radical reactions including combination, disproportionation and conventional chain transfer.

In an ideal RAFT process, the degree of polymerization is defined by the ratio of concentrations of consumed monomer to that of the initial transfer agent (assuming the concentration of transfer agent is much higher than that of the initiator) (Equation 1-2).

\[
\overline{M}_n \approx m_M ([M]_0 - [M]_t)/[CTA] \\
\text{where } m_M = \text{monomer MW}
\]

The careful choice of a RAFT agent as a function of the monomer and reaction conditions is essential to perform polymerization with appropriate control. Chain transfer agents are
generally thiocarbonylthio compounds. The key structural features of RAFT agents are the so-called Z and R groups (general structure of commons RAFT agents is presented in Scheme 1-4).

\[ S - S - R \]
\[ Z = \text{aryl, alkyl} \quad \text{dithioesters} \]
\[ Z = \text{O-Y} \quad \text{xanthates} \]
\[ Y = \text{aryl, alkyl} \]
\[ Z = \text{N-YY} \quad \text{dithiocarbamates} \]
\[ Z = \text{S-R'} \text{ or } \text{S-R} \quad \text{trithiocarbonates} \]

Scheme 1-4. General structure of commons thiocarbonylthio RAFT agents

The Z group determines the general reactivity of the C=S bond towards radical addition. Stabilizing Z groups such as aryl groups increase the rate of addition and decrease the rate of fragmentation in the main RAFT equilibrium. These RAFT agents are efficient in styrenes and methacrylates polymerization, but they retard polymerization of acrylates and inhibit polymerization of vinyl esters. On the other hand, radical species react less readily with the C=S bond of xanthates (-OY Z group where Y=aryl or alkyl) and dithiocarbamates (-NY\textsubscript{2} Z group) due to the lower double bond character of the C=S bond (zwitterionic structures are contributive to the overall resonance hybrid of these compounds). Therefore their use as RAFT agents is less efficient for monomers such as styrenes but is good for vinyl esters. However, substituents facilitating the delocalization of the lone pair(s) of electrons on N or O can render xanthates and dithiocarbamates effective RAFT agents for a wider range of monomer classes\textsuperscript{142}.

The R group must be a good free radical (homolytic) leaving group, and the radical that is generated from the homolytic dissociation must be able to reinitiate polymerization. Steric and electronic effects are important factors on the R group leaving ability.

The advantages of RAFT over others CRP techniques include the fact that this process works with a wider range of monomers than NMP or ATRP and the products do not contain residual metal. However, a limitation of this technique is the stability of many transfer agents. Additionally, the removal of dithioester and some other end groups is often required due to their colour, toxicity and potential odour. Therefore, for some synthetic purposes, other CRP techniques such as ATRP may be better suited.
1.4.2.2 Atom transfer radical polymerization (ATRP)

**ATRP mechanism and kinetics**

Since the first reports on ATRP in 1995 by Sawamoto and co-workers\(^\text{143}\) and Matyjaszewski and co-workers,\(^\text{144}\) the development and understanding of this new area of chemistry has evolved rapidly. A general mechanism for ATRP is shown in Scheme 1-5. The dormant chains are alkyl halides (R-X). The radicals, or the active species, are generated through a reversible redox process catalyzed by a transition metal complex \((\text{M}_{t}^{n-}Y/\text{Ligand})\), where Y may be another ligand or the counterion) which undergoes an electron oxidation with abstraction of a (pseudo)halogen atom, X, from the dormant species, R-X. This process occurs with a rate constant of activation, \(k_{\text{act}}\), and deactivation \(k_{\text{deact}}\). Polymer chains grow by the addition of the intermediate radicals to monomers with the rate constant of propagation \(k_p\). Termination reactions \((k_i)\) also occur in ATRP, mainly through radical coupling or disproportion; however, in a well-controlled ATRP, no more than a few percent of the polymer chains undergo termination\(^\text{123}\).

\[
\begin{align*}
\text{R}^+X^-\text{M}_{t}^{n+}Y/\text{Ligand} & \quad \xrightarrow{k_{\text{act}}} \quad \text{R}^-X^-\text{M}_{t}^{n+}Y/\text{Ligand} \\
\xrightarrow{k_{\text{deact}}} \quad \text{R}^+X^-\text{M}_{t}^{n+}Y/\text{Ligand} & \quad \xrightarrow{k_p} \quad \text{R}^+X^-\text{M}_{t}^{n+}Y/\text{Ligand}
\end{align*}
\]

**Scheme 1-5.** General mechanism for transition-metal-catalyzed ATRP. Reproduced from\(^\text{141}\)

As in other CRP methods, rapid reversible deactivation \((k_{\text{deact}} > k_{\text{act}})\) and fast initiation are required to achieve the uniform growth of all the chains. The apparent rate of initiation has to be comparable to the apparent propagation rate: if the initiation is too quick, an excessive number of radicals are produced and a significant number of radical terminations occur; but if the initiation is too slow, the chains do not start to grow at the same time and polymers with higher polydispersities than the theoretical ones are yielded.

A self-regulating effect is provided in ATRP by the “persistent radical effect” (PRE). This kinetic feature was discovered in reactions involving both transient and persistent radical intermediates when a highly dominant and unusually selective formation of the cross-reaction products of these radicals was observed\(^\text{145-147}\). Qualitatively, PRE can be explained by a simple principle. Persistent radicals cannot terminate with each other but only cross-
couple with the transient radicals. On the other hand, the transient radicals do not only react with the persistent species but also with themselves leading to self-termination.

\[
\begin{align*}
\text{Termination} & \quad R^+X^- + R^+ \xrightarrow{k_t} \text{termination products} \\
\text{Cross-coupling} & \quad \xrightarrow{k_{deact}} \quad \text{transient radical} + \text{persistent species} \\
\text{Propagation} & \quad k_p \quad + \quad M \xrightarrow{k_{act}} \quad \text{transient radical} + \text{Cu(II) complex}
\end{align*}
\]

**Scheme 1-6.** Persistent radical effect in Cu-catalyzed ATRP

In the case of Cu-catalyzed ATRP (Scheme 1-4), persistent “radicals” are Cu(II) complexes and transient radicals are the active species (growing chains). Every act of transient radical–radical termination (coupling or disproportion between the active species) is accompanied by the irreversible accumulation of the Cu(II) species. Thus Cu(II) concentration progressively increases with time. Consequently, the concentration of active species decrease with time. The active species then predominantly react with the persistent radicals, which are present at higher concentration, rather than with themselves. This means that the active radicals are rapidly trapped in the deactivation process by Cu(II) complexes and the probability of radical termination is minimized with time. The growing centers are then rapidly reformed by the activation of the dormant chain\textsuperscript{148}. As a consequence, termination reactions become insignificant.

The rate law for the ATRP mechanism can be derived assuming a fast and complete initiation, using a fast equilibrium approximation and omitting the termination reactions (Equation 1-3):

\[
R_p = k_p[M][R^+] = k_p[M] K_{eq} [RX]_0 [Cu^I] / [Cu^{II}X]
\]

where \([M]\) is the monomer concentration, \([R^+]\) is the radical species concentration, \(k_p\) is the propagation constant, \([RX]_0\) is the initial initiator concentration which is equal to the number of dormant chains, \([Cu^I]\) is the copper(I) concentration, \([Cu^{II}X]\) is the copper(II) complex concentration and \(K_{eq} = k_{act} / k_{deact} = [R^+] [Cu^{II}X] / [Cu^I] [RX]_0\).
From the previous equation, a first order kinetic equation with respect to the monomer concentration can be deduced (Equation 1-4).

\[
\text{Equation 1-4} \quad \ln\left(\frac{[M]_0}{[M]}\right) = (k_p K_{eq} [RX]_0 [Cu^I] / [Cu^{II}X]) t = k_{obs} t
\]

In ATRP, as in other controlled polymerizations, the average molecular weight increases in a predictable and linear fashion with the conversion and is controlled by the initial stoechiometry of the reagents, allowing the preparation of polymers with predetermined final molecular weight (Equation 1-1).

As demonstrated by Equations 1-4 and 1-1, the “living” character of ATRP can be assessed by the linearity of both plots \(\ln([M]_0/[M])\) versus time and \(M_n\) versus monomer conversion. If termination reactions occur, the increase in Cu(II) concentration would lead to a curved instead of linear plot of \(\ln[M]_0/[M]\) versus time.

In absence of termination and transfer reactions and assuming a complete initiation, the polydispersity index can be described by Equation 1-5:

\[
\text{Equation 1-5} \quad M_w/M_n = 1 + ([RX]_0 k_p/k_{deact}[Cu^{II}X]) (2/p – 1))
\]

where \(p = \) conversion

Therefore the polydispersity decreases:

1) increasing the conversion

2) if the catalyst rapidly deactivates the growing chains, decreasing the ratio \(k_p/k_{deact}\).

3) if the deactivator concentration is increased, adding for example at the beginning of the polymerization a certain amount of Cu(II) halide. We can also derive from Equation 1-5 that highest polydispersities are usually obtained for low molecular weight polymers, since generally high concentrations of initiator are used, or when the propagation rate is particularly large.

It is worth mentioning that although the ATRP system is tolerant to water, it is oxygen sensitive: even if ATRP will proceed with small oxygen traces (the oxygen can be scavenged by the catalyst, which is present at much higher concentration than the polymeric radicals), the oxidation of the catalyst reduces the metal complex concentration,
potentially forming a substantial excess of deactivator and thus reducing the rate of polymerization.

**ATRP components and conditions**

The ATRP is a multicomponent system composed of the monomer, an initiator with a transferable (pseudo)halogen, a catalyst (composed of a transition metal species with any suitable ligand) and one must choose carefully every component when designing an ATRP experiment. Others factors, such as solvent, temperature and reaction time also have an important influence on the ATRP process.

**Monomer.** The choice of monomer determines the choice of all the other polymerization parameters; each monomer possesses an intrinsic radical propagation rate, so the concentration of propagating radicals and the rate of radical deactivation may need to be adjusted to maintain polymerization control. Typical monomers polymerized by ATRP contain constituents that can stabilize the propagating radicals. They include styrenes, (meth)acrylates, (meth)acrylamides and acrylonitrile. The ATRP of monomers containing a carboxylic group is challenging due to the acidic function that can “poison” the catalyst by coordinating the metal centre.

**Initiator.** Alkyl halides are generally used as initiators in ATRP. The main role of the alkyl halide (RX) species is to determine the number of initiated chains, although in some cases the introduction of a specific function using a functional initiator is equally important. The halide group has to rapidly and selectively migrate between the growing chain and the transition metal complex and halogens like bromine or chlorine are generally used; however some pseudohalogenes such as thiocyanates and thiocarbamates have also been used. A good initiator should react fast and quantitatively. Aryl, carbonyl or allyl groups can be used as activating subsistuents on the \( \alpha \)-carbon of the initiator.

**Catalyst.** The ATRP catalyst, depending upon the nature of the metal and ligands, determines the dynamics of exchange between the dormant and active species. There are several requirements for an effective ATRP catalyst: first, the metal complex must have an accessible one-electron redox couple to promote atom transfer and its metal center should have reasonable affinity toward the halide group. Secondly, the coordination number of the
metal center must increase by one in order to accommodate a new ligand (e.g. a transition from a tetracoordinate state in the lower oxidation state of the metal to a pentacoordinate state in the higher oxidation state). An efficient catalyst must permit a fast and quantitative initiation so that all the chains start to grow simultaneously and it has to quickly shift the equilibrium towards the dormant species in order to have a low radical concentration and minimize the termination reactions.

Copper systems are most extensively employed as ATRP catalysts: copper-based ATRP systems have been successfully adapted for the controlled polymerization of styrenes, acrylates, methacrylates, acrylonitrile, and other monomers\textsuperscript{141} however many studies investigated the catalytic activity of various transition metals such as ruthenium or iron for ATRP\textsuperscript{123,141}.

The ligand has to solubilize the transition metal salt in the media and adjust the redox potential of the metal center for appropriate reactivity and dynamics during the atom transfer. In general, electron donating ligands stabilize the higher oxidation state of the metal, accelerating the polymerization. The most effective ligands with copper catalytic systems are usually nitrogen-based ligands such as derivatives of 2,2'-bipyridine and aliphatic polyamines (structures presented in Scheme 1-7)\textsuperscript{141,149}.

The main disadvantage of ATRP is the removal of the transition metal complex from the product. The presence of residual amounts of metal catalyst in polymers often raises concern, in particular for biomaterial applications. This is the reason why new ATRP techniques, known as “initiators for continuous activator regeneration” (ICAR) and “activators regenerated by electron transfer” (ARGET) ATRPs\textsuperscript{150-151} requiring lower concentrations of metal catalyst (in the range of ppm) have been developed by Matyjaszewski et al.

\textbf{Scheme 1-7.} Structures of ligands frequently employed for Cu based catalysts in ATRP. a) bipyridine and its 2-2’-substituted derivatives; b) aliphatic polyamines
Temperature. The choice of temperature depends mostly on the monomer, the catalyst and the targeted molecular weight. The rate of polymerization in ATRP increases with increasing temperature due to the increase of both the radical propagation rate constant and the atom transfer equilibrium constant and better control may be observed due to higher activation energy for the radical propagation than for the radical termination. However, chain transfer and other side reactions also become more pronounced at elevated temperatures.\textsuperscript{141}

Reaction time. The most important effect of reaction time in ATRP is observed at high monomer conversions at which the rate of propagation slows down considerably while the rate of side reactions does not change significantly, as most of them are monomer concentration independent. Therefore prolonged reaction times lead to loss of end groups and conversion should not exceed 95\% in order to obtain polymers with high end functionality or to subsequently synthesize block copolymers.

In light of these considerations, the choice of each component of the ATRP system must be considered carefully for the polymerization of each particular monomer in order to obtain well-defined polymers.

1.5 \textit{In-depth C: Techniques for surface characterization}

Spectroscopic techniques such as infrared (IR), X-ray photoelectron spectroscopy (XPS) and secondary ion mass spectrometry (SIMS) are valuable tools for the characterization of the chemical composition of a surface. Although a simpler technique, the measurement of contact angle with water remains an important tool in the surface evaluation of hydrophlicity.\textsuperscript{152}

Information about the surface topography at the microscopic scale is obtained via techniques such as scanning electron microscopy (SEM) and atomic force microscopy (AFM).

Other techniques include ellipsometry which allows the characterization of the optical constants and thickness of thin films (it can be used to non-destructively monitor the
growth and evolution of thin films *in situ* and quartz crystal microbalance, which can be also used to monitor the kinetics of film formation or protein adsorption.

The choice of a surface characterization method depends on the type of measurement needed and the required precision and accuracy, however many other factors (the sample preparation, the possible undesirable effects of the analysis on the surface of interest etc.) also influence the selection of a surface characterization method. In our surface modification study, the choice of different surface analysis methods was based on the complementarity of the techniques: water contact angle measurements provided information about the surface hydrophilicity, the chemical composition was investigated using FTIR and XPS while topographic information, physical and mechanical properties were obtained using AFM.

### 1.5.1 Contact angle

Contact angle measurement is a very popular technique for the quick estimation of surface energies.\(^ {152} \) This technique is based on the measurement of the shape of a liquid drop (typically water) deposited on the surface being investigated (static contact angle) or the shape of the meniscus along the wetting line during dipping or withdrawal of the substrate in liquid (dynamic contact angle). Dynamic contact angle is a useful tool to study surfaces that reorient upon exposure to different fluids. Alternatively, the “captive bubble” technique (where the contact angle between an air bubble and an immersed surface is measured) can be used to obtain information about hydrated surfaces.

Contact angle is a result of a balance between the cohesive energy of the test liquid and the adhesive forces between the solid substrate and the liquid. Contact angle is very sensitive to the chemical composition and reactivity of the surface, and changes widely from close to zero for highly hydrophilic surfaces (silicon, mica) to 110-120° for hydrophobic ones (methyl and fluoro groups). The contact angle also depends greatly on the micro and nano-structure of the surface, as demonstrated in the preparation of super-hydrophobic surfaces\(^ {153} \). The surface roughness, heterogeneity and level of positional and orientational ordering of terminal groups are all influencing the contact angle.
Typically, no special sample procedures are required for contact angle analysis. However, the surfaces should be clean and not swell or dissolve in water.

1.5.2 Fourier transform-infrared spectroscopy

Infrared spectroscopy (IR) exploits the fact that chemical bonds absorb specific frequencies that are characteristic of their vibrational modes. The infrared spectrum of a sample is recorded by passing a beam of infrared light through a sample and measuring the amount of transmitted light. In order to eliminate the instrument influence on the measurement, both the sample and a reference are measured, usually simultaneously using a beam splitter in the apparatus. Examination of the absorbed frequencies can reveal the nature of chemical bonds present in the sample. An infrared spectrum can be obtained using a monochromator to scan the infrared wavelength range, however the interferometric approach, combined with Fourier transform algorithms, provides several distinct advantages including higher resolution and higher energy throughput.

Fourier transform-infrared spectroscopy (FT-IR) is based upon splitting the polychromatic source radiation into two equal beams of approximately equal intensity. The radiation from each path is reflected back by two mirrors onto the beam splitter and the recombined beam is analyzed. The recombined two beams may either interact constructively or destructively depending upon the phase difference of the two optical paths. This constructive or destructive interference will vary as the path length in one of the arms is varied (by moving one of the mirrors reflecting the light back). The resulting pattern forms an interferogram that represents the relationship between the light energy and the path difference in the two arms of the interferometer. Interferograms are converted into a spectrum by using a Fourier transform algorithm.

FT-IR it is not strictly a surface analytical tool, however spectra of thin films can be acquired in attenuated total reflection (ATR) mode where the sample outer 1-7 μm are analyzed.

IR spectroscopy presents various advantages: sample preparation is minimal, instrumentation is relatively inexpensive, and the resulting spectra are rich with chemical bonding information. However IR usually provides only qualitative information about
surface compositions. Therefore a complementary spectroscopy method, for example XPS, can be used when quantitative information on the surface composition is needed.

1.5.3 X-ray photoelectron spectroscopy

In X-ray photoelectron spectroscopy (XPS),\(^{155}\) a sample is irradiated with a source of monochromatic X-rays in an ultra high vacuum. Due to the impact of the beam, the atoms in the sample are ionised and electrons are ejected from the surface, either as a result of the photoemission process (XPS), or of the radiationless de-excitation in the Auger electron emission process. The kinetic energy (\(E_K\)) of these electrons is then detected in a spectrometer and is related to the binding energy (\(E_B\)) through the equation:

\[
E_B = h\nu - E_K - W
\]

where \(h\nu\) is the photon energy (depending on the X-ray source) and \(W\) is the “work function” which can be approximated as the difference between the energy of the Fermi level \(E_F\) and the energy of the vacuum level \(E_V\) and is determined by a calibration of the spectrometer.

The electronic energy levels of each element are characteristic, and as a consequence each binding energy peak is characteristic of one element. Sensitivities of about 0.1 atom% can be obtained with XPS; however hydrogen and helium cannot be detected.

Using appropriate atomic sensitivity factors, atomic percentages of the surface composition can be obtained from the relative signal intensities.

Electronic states are modified by the molecular environment of the atom and therefore additional structural information can be obtained from the chemical shift of binding energies: the photoelectrons from atoms in a more electropositive state are shifted to lower \(K_E\) (higher \(K_B\)) and the opposite is true for atoms in a more electronegative state.

Due to the high probability of electron energy losses, only electrons emitted within a few atomic layers in the surface region have a significant chance of leaving the solid and reaching the detector and XPS usually does not probe deeper than 10 nm.
1.5.4 Atomic force microscopy

The atomic force microscope (AFM) was initially invented as an imaging tool for determining surface topographies at sub-nanometer resolution. In recent years, AFM has also been developed as a technique for micro/nano-manipulation and force spectroscopy of materials at the sub-micron scale\textsuperscript{156}.

The general principle of AFM is to scan a sharp tip over the surface of a sample while sensing the so-called near-field physical interactions between the tip and the sample (mechanical contact force, van der Waals forces, chemical bonding, electrostatic forces, magnetic forces, etc.). The sample is mounted on a piezoelectric scanner which ensures three-dimensional positioning with high accuracy. While the tip (or sample) is being scanned, the force interacting between tip and sample is monitored with picoNewton sensitivity. This force is measured by the deflection of a soft cantilever, typically made of silicon or silicon nitride with a tip radius of curvature on the order of nanometers. The cantilever deflection is detected by a laser beam focused on the end of the cantilever and reflected into a photodiode (Figure 1-4).

The AFM can be operated in a number of modes, depending on the application. In general, imaging modes are divided into the so called contact mode, non-contact modes and tapping modes.

In contact mode, the force between the tip and the surface is kept constant; the static tip deflection is therefore used as a feedback signal. However, close to the surface of the sample, attractive forces can cause the tip to “snap-in” to the surface. Therefore contact mode AFM is almost always done where the overall force is repulsive.
In the non contact modes, the cantilever is externally oscillated at or close to its fundamental resonance frequency (or a harmonic). The oscillation amplitude, phase and resonance frequency are modified by tip-sample interaction forces and it is these changes in oscillation which provide information about the sample's characteristics. Non contact modes can either be frequency modulated or amplitude modulated.

In frequency modulation, changes in the oscillation frequency provide information about tip-sample interactions.

In amplitude modulation, changes in the oscillation amplitude or phase provide the feedback signal for imaging: the system maintains a constant oscillation amplitude by adjusting the average tip-to-sample distance when van der Waals, or any other long range force, acts on the resonance frequency of the cantilever.

Non-contact mode AFM prevents tip or sample degradation effects that are sometimes observed when using contact AFM. This makes non-contact AFM preferable to contact AFM for measuring soft materials.

In tapping mode, the cantilever is driven to oscillate up and down at near its resonance frequency by a small piezoelectric element mounted in the AFM tip holder. The amplitude of this oscillation is greater than the one used in non-contact mode. Due to the interaction of forces acting on the cantilever when the tip comes close to the surface, the amplitude of...
this oscillation decreases as the tip gets closer to the sample. The resulting tapping mode image is therefore produced by imaging the force of the oscillating contacts of the tip with the sample surface. This is an improvement on conventional contact AFM, in which the cantilever just drags across the surface at constant force and can result in surface damage. Besides, if the sample surface is not homogeneous, near-field interactions cause a shift not only in the amplitude of the cantilever oscillation but in its phase as well; distribution of the phase shift under scanning over the sample surface reflects distribution of the surface characteristics. Such mode of operation (Phase Imaging mode) allows the creation of map variations in surface properties such as chemical composition, adhesion, friction or viscoelasticity in comparison with surface topography.

Another major application of AFM is force spectroscopy, the direct measurement of tip-sample interaction forces as a function of the distance between the tip and sample: the cantilever deflection is recorded as a function of the vertical displacement of the piezoelectric scanner, i.e. as the sample is pushed towards the tip and retracted (nanoindentation test\textsuperscript{161}). This results in a cantilever deflection ($d$) versus scanner displacement ($z$) curve, which can be transformed into a force–distance curve by converting the cantilever deflection into a force ($F$) using Hooke’s law ($F = -k \times d$, where $k$ is the cantilever spring constant) and subtracting the deflection from the scanner displacement to obtain the distance ($z-d$). Spatially resolved maps of sample properties and molecular interactions can be produced. However, for quantitative force measurements, calibration of the actual spring constants of the cantilevers is necessary\textsuperscript{157}.

Many researchers have used AFM to determine sample mechanical properties\textsuperscript{158-161} such as elasticity (Young’s modulus), hardness, adhesion forces, and surface charge densities.

AFM has several advantages over SEM. Unlike the electron microscope which provides a two-dimensional projection or a two-dimensional image of a sample, AFM provides a true three-dimensional surface profile.

Additionally, samples viewed by AFM do not require any special treatments (such as metal/carbon coatings) that would irreversibly change or damage the sample. While an electron microscope needs an expensive vacuum environment for proper operation, most
AFM modes can work perfectly well in ambient air or even a liquid environment. This makes it possible to study biological macromolecules and even living organisms.

1.6 In-depth D: Techniques for colloidal characterization

1.6.1 Dynamic light scattering

Dynamic Light Scattering (DLS), also called Photon Correlation Spectroscopy or Quasi-Elastic Light Scattering, is a technique estimating the size (typically in the sub-micron region) of particles in solution via the measurement of Brownian motion\textsuperscript{163}.

The Brownian motion is the random movement of particles caused by collisions with the solvent molecules that surround them. DLS works on the principle that the Brownian motion of larger particles is slower than that of smaller particles (an intuitive way to understand it is to think that the small particles are “kicked” further by the solvent molecules). The velocity of the Brownian motion is defined by a property known as the translational diffusion coefficient (D) and is related to the size of a spherical particle by the Stokes-Einstein equation:

\begin{equation}
\label{eq:1-7}
 d_h = \frac{(kT)}{(3 \pi \eta D)}
\end{equation}

where $d_h$ is hydrodynamic diameter, $k$ the Boltzmann’s constant, $T$ the absolute temperature and $\eta$ the viscosity.

As the diameter obtained from DLS refers to how a particle diffuses within a fluid it is thus referred to as hydrodynamic diameter ($d_h$) and depends not only on the size of the particle “core”, but also on any surface structure, as well as the type of medium and its ionic strength.

In order to derive $d_h$ from a DLS measurement, an accurate knowledge of the temperature and viscosity (which is also temperature dependent) is necessary (Equation 1-7). A stable temperature throughout the measurement is also essential in order to avoid non-random movements caused by convection currents.
The diffusion speed of the particles is detected by measuring the rate at which the intensity of the scattered light fluctuates when illuminating the sample with a laser. The rate at which intensity fluctuations occur will depend on the size of the particles: small particles cause the intensity to fluctuate more rapidly than the large ones. A signal comparator, generally referred to as digital autocorrelator, is usually used to analyze the scattered light signals. A digital autocorrelator is designed to measure the degree of similarity between two signals, or one signal with itself at varying time intervals. If the intensity of a signal is compared with itself at a particular point in time and a time much later (usually in the order of a millisecond or tens of milliseconds), there will be no correlation between the two signals, but if the intensity of signal at time = t is compared to the intensity a very small time later (t+δt) (usually nanoseconds or microseconds), there will be a strong relationship (or correlation) between the intensities of two signals. If the signal, derived from a random process such as Brownian motion, at time t is compared to the signal at t+2δt, there will still be a reasonable comparison or correlation between the two signals, but it will not be as good as the comparison between times t and t+δt because the correlation is decreasing with time. If the particles are large the signal will be changing slowly and the correlation will persist for a long time. If the particles are small, they move rapidly and the correlation will decrease more rapidly. The digital autocorrelator will thus construct the correlation function G(τ) of the scattered intensity:

Equation 1-8 \[ G(\tau) = < I(t) I(t + \tau) > \]

In Equation 1-8, \( \tau = \) is the time difference (the sample time) of the correlator. For a large number of monodisperse particles in Brownian motion, the correlation function is an exponential decaying function:

Equation 1-9 \[ G(\tau) = A[1 + B \exp(-2\Gamma\tau)] \]

where A is the baseline of the correlation function, B is the intercept of the correlation function, and

Equation 1-10 \[ \Gamma = Dq^2 \]
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where D is the translational diffusion coefficient and \( q = (4\pi n/\lambda_o)\sin(\theta/2) \) with \( n \) = refractive index of dispersant, \( \lambda_o \) = wavelength of the laser and \( \theta \) = scattering angle. For polydisperse samples, the equation can be written as:

Equation 1-11

\[
G(\tau) = A[1 + B \, g_1(\tau)^2]
\]

where \( g_1(\tau) = \) is the sum of all the exponential decays contained in the correlation function.

Size is obtained from the correlation function by using various algorithms. When the correlation function is fitted with a single exponential (assuming a single spherical particle size), a mean size (z-average diameter) and an estimate of the width of the distribution (polydispersity index) are obtained. When algorithms such as Non-negative least squares (NNLS) or CONTIN are used, the correlation is fitted with multiple exponentials and distribution of particle sizes (still assumed to be spherical) are obtained. The size distribution obtained from DLS is a plot of the relative intensity of light scattered by particles in various size ranges and is therefore known as an intensity size distribution. Because bigger particles scatter light more than smaller ones (intensity of scattering is proportional to \( d^6 \) from Rayleigh’s approximation), intensity size distributions are not representatives of the number of particles for each class size. A more realistic view of the sizes distributions can be obtained through the conversion to a volume distribution if the sample refractive index is known.

1.6.2 Diffusion ordered NMR

Diffusion-ordered 2D NMR (DOSY NMR) experiments are a noninvasive analytical method that can identify molecular components of mixtures and simultaneously characterize the sizes of aggregates and other structures present. These experiments yield conventional chemical shift spectra in one dimension and spectra of diffusion coefficients in the other. As for DLS, the size of a theoretical spherical particle having the same diffusion coefficient than the one measured can be derived using the Stokes-Einstein equation (Equation 1-7).

Basically, DOSY NMR is based on diffusion-related echo attenuation. Spectra are obtained with the pulsed field gradient method, and data sets are collected as the gradient pulse areas
are incremented. At each point on the chemical shift axis, a set of intensities is obtained as a function of the square of the area of the gradient pulse $K$:

$$I(K,v) = \Sigma A_n(v)exp[-D_n(\Delta - 3\delta)K^2]$$

with $K = yg\delta$ where $y$ is the magnetogyric ratio, $g$ is the gradient pulse amplitude and $\delta$ is the gradient pulse duration. $D_n$ is the diffusion coefficient of the $n^{th}$ diffusing species and $A_n(v)$ is the 1D NMR spectrum of the $n^{th}$ diffusing species in the limit that $g = 0$.

The important point is that each frequency $v$ in the NMR spectrum is associated with a 1D data set that is described by a sum of exponential components with $K^2$ as the independent variable. Thus, each frequency $v$ in the NMR spectrum is associated with a 1D data set and can be described by:

$$G(s) = \int g(\lambda)exp(-\lambda s)d\lambda \quad \text{where} \quad \lambda = D(\Delta - 3\delta) \text{ and } s = K^2$$

Thus at each chemical shift, the NMR signal as a function of $K^2$ is a sum of decaying exponentials. Nonlinear least squares analyses have been used to transform Equation 1-13 and obtain a spectrum of diffusion rates. This is quite successful for species with isolated resonance lines (single exponential decays) and for species at the same chemical shift but having widely separated diffusion coefficients. This approach is not suitable for polydisperse samples. As for DLS analysis, the use of CONTIN algorithms appears to be more suited to polydisperse samples and is also applicable to discrete datasets.

1.7 Scope of the thesis

The development of controlled radical polymerization during the last two decades has allowed polymer chemists to prepare macromolecules with predetermined molecular weight and low polydispersity and to synthesize complex architecture copolymers. This project reports on the preparation of new amphiphilic block copolymers of PDMS synthesized using a “living” radical polymerizations, specifically ATRP, that allow the formation of various self-assembled structures in water.

The new silicone-based materials prepared in this thesis are intended for biomedical applications. When used in vivo, biomaterials should be protein repellent in order to avoid
recognition and clearance by phagocytic cells (in the case of nano carriers) or an excessive inflammation reaction (in the case of implanted materials). This “stealth” or “anti-fouling” character can be achieved by the presence of hydrophilic and protein-repellent polymers at their surface, which can eventually be functionalized to obtain more sophisticated biologically responses. Poly(glycerol monomethacrylate) (PGMMA) was selected as the hydrophilic polymer providing protein repellent properties to the new silicones materials prepared in this thesis. The presence of two hydroxyl groups on each monomer unit makes PGMMA highly hydrophilic and offers potential for further functionalization.

The scope of this thesis is therefore the preparation of well-defined amphiphilic block copolymers of PDMS and PGMMA and the evaluation of the “stealth” or “anti-fouling” character of their self-assembled structures, in colloidal form and adsorbed on silicone surfaces (Figure 1-5).

Figure 1-5. Scheme of the self-assembly of amphiphilic copolymers of PDMS and PGMMA in aqueous environment. Depending on the block ratio, various nanosized objects can be formed and their PGMMA corona provides them “stealth” properties. Alternatively, amphiphilic copolymers can adsorb on silicone surfaces and alter their protein adsorption properties.
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In particular, the synthesis of PDMS-PGMMA block copolymers and the study of their colloidal assemblies formed in water are presented in Chapter 2 while the use of those block copolymers for silicone surface modification is reported in Chapter 3. In addition, Chapter 4 presents initial experiments investigating the possibility of using enzymatic reactions to incorporate specific functionality on the diol groups of GMMA containing polymers.

1.8 Authors contribution

Christopher Cadman contributed to about 2 % of the thesis by acquiring TEM images (see Chapter 2). Dr. Robert Evans contributed to about 3 % of the work presented here by performing DOSY experiments (see Chapter 2). Roberto Donno contributed to about 3 % by performing AFM analysis of silicone surfaces (see Chapter 3).

The main author of this thesis, Ghislaine Robert-Nicoud (GRN), prepared all the samples for the analysis mentioned here and performed the rest of the work presented in this thesis. GRN contributed to about 92 % of the thesis.

1.9 References

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2 Poly(glycerol methacrylate)/silicone macro-amphiphiles: synthesis, aggregation in water and protein adsorption

Abstract

This study has focused on the optimization of preparative details for the synthesis of amphiphilic block copolymers containing poly(glycerol monomethacrylate) (PGMMA), showing the advantages of a protection/deprotection strategy based on silyl groups.

In this example, PGMMA blocks were synthesized via ATRP started by a doubly functional poly(dimethyl siloxane) (PDMS) macrorinitiator.

Above a certain length of GMMA blocks (DP≈30) the resulting macroamphiphiles exhibited a virtually indistinguishable critical aggregation concentration in water, which was therefore primarily dependent on the features of the PDMS block. For GMMA blocks with DP>50, 35-50 nm spherical micelles were the predominant colloidal species.

In preliminary experiments, diffusion NMR has shown that the tethering of PGMMA chains on micelles did not dramatically alter their absence of significant interactions with albumin in solution, which was used as a model protein for an assessment of the “stealth” character of the colloids.
2.1 Introduction

The self-assembly of amphiphilic block copolymers in a water environment allows the preparation of a variety of nanostructures, such as spherical or worm-like micelles, vesicles, which can be used as delivery or imaging agents\(^1\,^2\). Key points for the biological use of these systems is the chemical and biological inertia (or responsiveness) of the hydrophobic part and the “stealth” character and chemical functionality of the hydrophilic one.

As chemically inert building blocks, silicones are almost unrivalled. The factors that have popularized their use for provisional or permanent implants are: high chemical stability and oxygen permeability (to avoid the development of hypoxic areas), the low values of glass transition temperature and surface tension (essential to provide high compliance) and the overall low toxicity. In particular, poly(dimethylsiloxane) (PDMS) has been employed for decades in the production of a variety of medical devices, such as intraocular lenses\(^3\), urinary catheters\(^4\) and breast implants\(^5\), and has also been extensively investigated for other applications, specifically in microfluidic analytical devices\(^6\). In all cases, a major target of current research is the reduction of the unspecific protein adsorption on PDMS surfaces upon exposure to biological fluids; this phenomenon can trigger inflammatory reactions, e.g. activation of leukocytes\(^7\), in silicone implants, and can seriously affect the determination of analytes in body fluids with silicone-based sensors\(^8\).

A number of techniques have been used to modify silicone surfaces to render them less prone to protein adsorption. The most common strategy involves the oxidation with UV radiation and/or ozone\(^9\,\,^{11}\) plasma or, under milder conditions, with oxidizing solutions\(^{12\,\,14}\), generally followed by functionalization through silanization reactions\(^12\), polyelectrolyte adsorption\(^13\) or surface-initiated polymerization\(^{11\,\,14}\); in alternative approaches, grafting polymerization induced by UV irradiation\(^15\) or plasma\(^9\) has also been used. Physisorption of (macro)amphiphiles on the hydrophobic PDMS is a less investigated solution: the advantage of the higher simplicity is counterbalanced by the generally lower stability of the surface; few examples can be found, with the use of Pluronic F108\(^{16\,\,17}\), low MW surfactants such as aliphatic maltosides\(^17\) and biosurfactants\(^18\). A main drawback of this approach is the absence of amphiphiles
specifically designed for the purpose of the adsorption on PDMS; for this purpose we have tackled the design of PDMS-containing macroamphiphiles.

PDMS-containing-block copolymers have been widely studied, predominantly with the aim to complement silicone properties with e.g. enhanced mechanical performance or functional behaviour. Most commonly, the synthesis of these copolymers has employed functional PDMS as macroinitiators for atom transfer radical polymerization (ATRP), e.g. of styrenes\textsuperscript{19,20} and methacrylates\textsuperscript{21,22}, ring-opening polymerization\textsuperscript{23,24}, or both\textsuperscript{25}. However, to date only a relatively small number of amphiphilic PDMS-based block copolymeric structures have been prepared, for example using poly(2-dimethylaminoethyl methacrylate)\textsuperscript{26}, poly(2-methacryloyloylethyl phosphorylcholine)\textsuperscript{27}, poly(N-vinylpyrrolidone)\textsuperscript{28} or poly(2-methyloxazoline)\textsuperscript{29} as hydrophilic blocks. As hydrophilic component, we are specifically interested in the use of poly(2,3-dihydroxypropyl methacrylate), more commonly referred to as poly(glycerol monomethacrylate) (PGMMA). The diol present on each repeating unit provides high hydrophilicity and ease of further functionalization, while the PGMMA structure has been shown to be sufficiently “stealth” to be used in drug delivery systems\textsuperscript{30-32} or hydrogels\textsuperscript{33-36} for diverse applications including coatings for implantable glucose sensors\textsuperscript{37} and preparation of surfaces with reduced cell adhesion\textsuperscript{38}.

This study has focused on the feasibility of the preparation of PDMS/PGMMA block copolymeric structures (Scheme 2-1).

The high polarity of PGMMA makes it possible only in few cases to find a common solvent for GMMA and other monomers/initiators\textsuperscript{39}. Most commonly PGMMA blocks are prepared by polymerizing organosoluble precursor structures, where vicinal diol units are introduced (osmylation of poly(allyl methacrylate)\textsuperscript{40}, hydrolysis of the poly(glycidyl methacrylate)\textsuperscript{41,42} or deprotected (from isopropylidene (2,2-dimethyl-1,3-dioxolane) derivatives\textsuperscript{43-48} or from bis(trimethylsilyl) ones\textsuperscript{49}) after polymerization. We have chosen the latter strategy (Scheme 2-1), using a PDMS macroinitiator to perform Atom Transfer Radical Polymerization (ATRP) on isopropylidene or bis(trimethyl silyl) protected GMMA derivatives.

The aggregation behaviour of the resulting PDMS/PGMMA block copolymers (Sil-GMMA, Scheme 2-1) was then studied in a water environment, also starting a preliminary evaluation of the interactions of these structures with protein in solution;
the latter may help foreseeing the protein adsorption behaviour of these polymers on a PDMS surface.

Scheme 2-1. A macroinitiator based on the bis(hydroxy-terminated) PDMS was used to polymerize DMMA and SGMMA, which were respectively obtained through the acrylation of protected glycerol and through the silylation of GMMA. The resulting Sil-DMMA and Sil-SGMMA were deprotected to yield GMMA-PDMS-GMMA block copolymers (Sil-GMMA).

2.2 Experimental section

2.2.1 Materials

Hydroxyethoxypropyl-terminated poly(dimethylsiloxane) (PDMS) was purchased from ABCR (Karlsruhe, Germany). 2-bromoisobutiryl bromide, 2,2-dimethyl-1,3-dioxolane-4-methanol, 2,6-di-tert-butyl-4-methylphenol (BHT), acetic acid, chlorotrimethylsilane, copper chloride, dioxane, ethyl 2-bromoisobutryl, hexamethyl triethylenetramine (HMTETA), triethylamine (TEA), were purchased from Aldrich (Gillingham, UK). Methacryloyl chloride was purchased from Alfa Aesar (Lancashire, UK). 1-Glycerol
monomethacrylate (GMMA) was purchased from Cognis (Southampton, UK). Dichloromethane, dimethyl sulfoxide, methanol, tetrahydrofuran (THF) were purchased from Fisher Scientific, (Loughborough, UK). Anhydrous sodium sulphate and sodium carbonate were purchased from Fluka (Gillingham, UK). Deuterated chloroform and deuterated dimethyl sulfoxide were purchased from Goss Scientific (Nantwich, UK). All materials were used as received from the supplier. GMMA contained about 10\% mol of 2-glycerol monomethacrylate, which was not separated.

2.2.2 Physico-chemical characterization

Molecular characterization

Spectroscopy: $^1$H NMR spectra were recorded on 1 wt.% polymer solutions in deuterated chloroform or dimethyl sulfoxide using a 300 MHz Bruker NMR spectrometer. FT-IR spectra were recorded in ATR mode on a Tensor 27 Bruker spectrometer.

Gel Permeation Chromatography (GPC) was performed on a Polymer Laboratories PL-GPC50 featuring two 5 µm (30 cm) mixed C columns, was operated in THF containing 250 ppm BHT, at 30 °C and with a flow rate of 1.0 mL min$^{-1}$. Purified polymer samples were analysed using universal calibration (a series of near monodisperse linear polystyrene were used as calibration standards in conjunction with a refractive index and viscometer detectors) for PDMS homopolymers and copolymers with high PDMS content, or triple detection analysis (refractive index, viscometer and dual angle light scattering detector at 15° and 90°) for copolymers with low PDMS content, i.e. Sil-DMMA2 – 4 and Sil-SGMMA 2 – 6. For the study of polymerization kinetics, the concentration and refractive index increment (dn/dc) of the polymers with variable molecular weight are unknown; in these cases the refractive index detector was used in conjunction with a series of near monodisperse linear polystyrene as calibration standard. GMMA homopolymer (synthesized from GMMA in methanol (50wt%) at room temperature with ethyl 2-bromoacetate as an initiator, Cu(I)Cl as catalyst and 2,2’-bipyridine as a ligand in respectively 1 and 2 stoichiometric ratios to the initiator, the copper catalyst was removed by filtration through a silica gel column using methanol as eluant and dialysis in water) was analyzed by GPC in dimethylacetamide and 0.5% LiNO$_3$ using a combination of two 10 µm (30 cm) different columns (PL-gel 500Å and
Mixed-B) at 50 °C and with a flow rate of 1.0 mL min⁻¹. The detection was performed
with a differential refractometer in conjunction with a series of narrowly distributed
polyethylene glycol standards.

**Colloidal characterization**

**Dynamic light scattering (DLS).** Size distribution measurements were performed using
a Zetasizer Nano ZS instrument (Model ZEN2500, Malvern Instruments Ltd., UK) at a
scattering angle of 173° and at a temperature of 25 °C. The values of hydrodynamic
diameter (which corresponds to the weighted Z-average) were obtained from a cumulant
analysis algorithm wherein a single particle size is assumed and a single exponential fit
is applied to the autocorrelation function while the size distributions are derived from a
deconvolution of the measured intensity autocorrelation function of the sample
accomplished using a non-negatively constrained least squares (NNLS) fitting algorithm
(Zetasizer Nano Software, Malvern Instruments Ltd., UK). The sample solutions were
prepared at various concentrations (1-10 mg/mL) in deionized water previously passed
through 0.22 μm filter to remove dust; the colloidal dispersions were not filtered after
preparation.

**Transmission electron microscopy (TEM).** A 2% aqueous phosphotungstic acid solution
(adjusted to pH 7.3 using NaOH 1 M) was used as a contrast enhancing solution. The
grids (mesh 300 Cu, diameter 3.05 mm) were covered with a formvar film and then
coated with carbon (Agar Scientific, Essex, UK). A drop of sample was left for 90
seconds on top of the grid and the excess solution was removed using filter paper. A
drop of contrast solution was then placed on the grid and left for 90 seconds. The excess
solution was removed with filter paper leaving a thin layer of solution and the grid was
allowed to fully dry in air before analysis. A transmission electron microscope Philips
CM30 HRTEM operated at 300 kV was used to obtain images of the samples.

**Critical aggregation concentration (pyrene method).** 10 μL of 2 x 10⁻⁴ M pyrene
solution in DMSO were added to 1 mL of polymer dispersions in deionized water
(concentration ranging between 10 mg/mL and 1 x 10⁻⁶ mg/mL) for a 2 x 10⁻⁶ M final
pyrene concentration. The samples were incubated at room temperature for 4h, then
fluorescence spectra were recorded at room temperature using a PerkinElmer LS55
luminescence spectrophotometer and an excitation wavelength of 335 nm. The emission
intensity was recorded at 375 nm (I₁) and 392 nm (I₃).
Chapter 2

Diffusion ordered NMR spectroscopy (DOSY). All measurements were carried out on a Bruker Avance III 400 MHz spectrometer, without spinning, using a 5 mm insert probe equipped with a gradient coil producing a maximum gradient strength of 1290 G cm\(^{-1}\).

\(^1\)H data were acquired with a simple pulse-acquire sequence. 32 transients were acquired. DOSY data were acquired using Oneshot\(^R\) sequence. Experiments were acquired with 256 transients, a diffusion delay \(\Delta\) of 0.1 s and a total diffusion encoding time \(\delta\) of 0.002 s. 10 gradient levels were acquired from 40 to 200 G cm\(^{-1}\), in equal steps of gradient squared. All experiments were carried out with active temperature regulation at 25.0 °C. Due to the broad nature of the protein spectra, resolution enhancement was not used. DOSY spectra were constructed by fitting to the Stejskal-Tanner equation and measurement of the diffusion coefficient obtained by using the strong peak at 3.1 ppm. This peak had a suitably high level of signal to noise and does not overlap with any of the polymers used in the study.

2.2.3 Preparative procedures

*Synthesis of macroinitiator (2-bromoisobutyrylethoxypropyl terminated PDMS, PDMSbisBr)*

2.8 mL (20 mmol) of triethylamine were added slowly under stirring to a solution of 30.0 g (4 mmol) of hydroxyethoxypropyl-terminated PDMS (PDMSbisOH) in 250 mL of dichloromethane. 2.4 mL (20 mmol) of 2-bromoisobutyryl bromide dissolved in 10 mL of dichloromethane were then added dropwise. The solution was stirred at room temperature under an inert atmosphere overnight. The resulting mixture was extracted with 200 mL of water three times to remove the salts generated by the reaction. The organic phase was then dried over anhydrous sodium sulphate and the product was concentrated by rotary evaporation. The resulting liquid was extracted three times with 200 mL of methanol and dried under reduced pressured, providing 27.3 g of a colourless liquid (yield 91%).

\(^1\)H NMR (CDCl\(_3\)): \(\delta = -0.10\) (broad, 650H, Si(CH\(_3\))\(_2\) in PDMS chain), 0.5 (t, \(J = 8.6\) Hz, 4H, -CH\(_2\)CH\(_2\)[Si(CH\(_3\))\(_2\)O\(_n\)-], 1.6 (m, 4H, -CH\(_2\)CH\(_2\)[Si(CH\(_3\))\(_2\)O\(_n\)-]), 1.9 (s, 12H, -C(CH\(_3\))\(_2\)Br), 3.4 (t, \(J = 6.9\) Hz, 4H, -CH\(_2\)OCH\(_2\)CH\(_2\)O(O)C-), 3.7 (t, \(J = 5.0\) Hz, 4H, -CH\(_2\)OCH\(_2\)CH\(_2\)O(O)C-), 4.3 (t, \(J = 4.9\) Hz, 4H, -CH\(_2\)OCH\(_2\)CH\(_2\)O(O)C-) ppm.
FT-IR (film on ATR crystal): 2963 (νas CH3), 2904 (νs CH3), 1743 (ν C=O), 1407, 1259 (δ, Si-CH3), 1078 (νas Si-O-Si), 1009 (νs Si-O-Si), 864, 787 (ρSi-CH3), 743 (ν Si-C), 693 cm⁻¹.

**Synthesis of Sil-GMMA through dioxolane precursors**

A) Synthesis of 2,2-dimethyl-1,3-dioxolane methyl methacrylate (DMMA). 21.1 g (0.16 mol) of 2,2-dimethyl-1,3-dioxolane-4-methanol and 22 mL (0.16 mol) triethylamine were dissolved in 160 mL dichloromethane under an inert atmosphere. The reaction mixture was stirred at 4 °C using an ice bath for 15 minutes. 13 g (0.13 mol) of methacryloyl chloride diluted in 20 mL of dichloromethane were then added dropwise, stirring then the reaction mixture at 20 °C for 24 hours. The mixture was then filtered to remove the triethylamine hydrochloride salt and the organic phase was washed three times with 200 mL of 0.1 M sodium carbonate and three times with 200 mL of deionized water, finally drying it over anhydrous sodium sulphate. Dichloromethane was removed by rotary evaporator and the product distilled (about 50 ºC / 1 mbar) in the presence of 0.02% wt. of radical inhibitor (BHT). A colorless liquid (17.0 g, yield 57%) was obtained.

1H NMR (CDCl3): δ = 1.35-1.42 (d, J = 18.7 Hz, 6H, -C(CH3)2), 1.94 (s, 3H, =CCCH3), 3.76-4.38 (m, 5H, O-CH2CHCH2-O), 5.58 (s, 1H, -C=CH2 cis), 6.13 (s, 1H, -C=CH2 trans) ppm.

FT-IR (film on ATR crystal): 2987 (νas CH3), 2959 (νas CH2), 2937 (νs CH3), 2885 (νs CH2), 1719 (ν C=O), 1637 (ν C=C), 1454 (δ O-CH2), 1375, 1320, 1396, 1215 (ν C-O-C acetal), 1158 (ν C-O-C ester), 1083, 1055, 939 (δoop =CH2), 846 (νs C-O-C-O), 814, 655 (δoop C=O) cm⁻¹.

B) Synthesis of PDMMA-PDMS-PDMMA (Sil-DMMA). In a typical experiment, monomers and solvent were mixed after separate degassing, in order to better control the volume of solvent used. A mixture of 500 mg of PDMS macroinitiator (≈ 0.1 mmol of bromides), a variable quantity of DMMA (corresponding to 20, 30, 60, 80, 120 equivalents to bromoisobutyryl groups) and 22 mg of HMTETA (0.1 mmol, corresponding to 1 equivalent to bromoisobutyryl groups) were degassed by bubbling N2 gas for 45 minutes followed by the addition of degassed tetrahydrofuran (variable quantity in order to obtain 33 wt.% solutions). 10 mg of copper (I) chloride (0.1 mmol,
corresponding to 1 equivalent to bromoisobutiryl groups) were then added under an inert atmosphere and the solution was stirred at 45 °C until conversion (measured by $^1$H NMR) reached a plateau; this corresponded to 4-8 h polymerization time, depending on the target degree of polymerization (DP). For kinetic studies, samples were collected at regular intervals, exposed to air to quench the reaction and analyzed by GPC in THF and $^1$H NMR in CDCl$_3$. The conversion was calculated using the ratios of integrated signals in NMR and the molecular weight was obtained via GPC. After exposure to air, the reaction mixture was diluted with THF, passed through a silica gel 60 (0.063-0.200 mm) column to remove the catalyst and concentrated at the rotary evaporator. The polymer was finally purified by three precipitations in ice cold methanol from THF. Typical yield after drying under reduced pressure: 67-91%wt.

$^1$H NMR (CDCl$_3$): $\delta = -0.30-0.40$ (s, $-\text{Si(CH}_3)_2$ in PDMS chain), 0.70-1.10 (broad, 3H, $-\text{CH}_2\text{C(CH}_3)_3$- in PDMMA chain), 1.30 and 1.36 (d, $J = 19.0$ Hz, 6H, $-\text{C(CH}_3)_2$ isopropylidene ring), 1.72-2.04 (broad, 2H, $-\text{CH}_2\text{C(CH}_3)_3$- in PDMMA chain), 3.60-4.30 (m broad, 5H, $-\text{CH}_2\text{CH}_2$ isopropylidene ring) ppm.

FT-IR (film on ATR crystal): 2986 ($\nu_{\text{as}}$ CH$_3$), 2961 ($\nu_{\text{as}}$ CH$_2$), 2939 ($\nu_{\text{s}}$ CH$_3$), 2885 ($\nu_{\text{s}}$ CH$_2$), 1728 ($\nu$ C=O ester), 1482, 1453 ($\delta$ O-CH$_2$), 1374, 1257 ($\delta$ Si-CH$_3$), 1214 ($\nu_{\text{as}}$ C-O-C acetal), 1152 ($\nu$ C-O-C ester), 1084 ($\nu$ Si-O-Si), 1052 ($\nu$ Si-O-Si), 978, 931, 842 ($\nu_{\text{s}}$ C-O-C-O), 799 ($\rho$ Si-CH$_3$), 739 ($\nu$ Si-C), 701, 663 cm$^{-1}$.

C) Deprotection. 0.10 g of Sil-DMMA was either dissolved in THF (1 mL), adding 40 µL of an aqueous acidic solution (HCl 1 M, formic acid 44 or 66 %wt.), or exposed directly in heterogeneous conditions to 1 mL of aqueous acidic solutions (HCl 1 M, formic acid 44 or 66 %wt.). The experiments were run at room temperature for 8 hours; the mixtures were then diluted with water to a total volume of 10 mL. Any precipitate formed during the reaction or the dilution was collected, dried under reduced pressure and analyzed by $^1$H NMR spectroscopy. The aqueous solution was purified through dialysis, freeze dried, washed with hexane and dried under reduced pressure, producing very variable amounts of predominantly deprotected polymer: 1-80% of the theoretical weight after deprotection.
**Synthesis of Sil-GMMA through silylated precursors**

A) Synthesis of bis-trimethylsilyl glycerol monomethacrylate (SGMMA). 20.0 g (0.125 mol) of GMMA and 90 mL (0.645 mol) of triethylamine were dissolved in 250 mL of dichloromethane under an inert atmosphere. The solution was cooled to 4 °C using an ice bath and a solution of 50 mL (0.389 mol) trimethylsilyl chloride in 50 mL of dichloromethane was then added dropwise. The reaction mixture was brought to room temperature and further stirred for 4 hours; the solvent was then removed at the rotary evaporator. The product was obtained by distillation (about 70ºC / 1 mbar) in the presence of 0.05% of radical inhibitor (BHT). A colorless liquid (23.0 g, yield 61%) was obtained.

$^1$H NMR (CDCl$_3$): $\delta = 0.08$ (d, $J = 5.8$ Hz, 18H, –Si(CH$_3$)$_3$), 1.92 (s, 3H, CH$_2$C(CH$_3$)$_3$), 3.52 (m, 2H, -CH$_2$CH(OSi(CH$_3$)$_3$)$_2$CH$_2$O- and diastereotopic -CH$_2$CH(OSi(CH$_3$)$_3$)$_2$CH$_2$O-), 3.72 (m, 0.4H, isoglycerol), 3.91 (q, $J = 5.5$ Hz, 1H, diastereotopic -CH$_2$CH(OSi(CH$_3$)$_3$)$_2$CH$_2$O-), 4.05 (m, 1H, diastereotopic -CH$_2$CH(OSi(CH$_3$)$_3$)$_2$CH$_2$O-), 4.89 (m, 0.1H, isoglycerol), 5.53 (s, 1H, cis to methyl CH$_2$C(CH$_3$)-), 6.08 (s, 1H, trans to methyl H$_2$CC(CH$_3$)-) ppm. The signals arising from the 2-glycerol derivative are in italics.

FT-IR (film on ATR crystal): 2957 (v$_\text{as}$ C–H), 2905 (v$_s$ CH$_3$), 2874 (v$_\text{as}$ CH$_2$), 1722 (v C=O ester), 1637 (v C=C), 1454 (δ O-CH$_2$), 1402, 1296 (v$_\text{as}$ C-O-C), 1250 (δ$_s$ Si-CH$_3$), 1165 (s$_s$ C-O-C), 1144 (δ Si-O-CH$_2$), 1099 (v$_\text{as}$ Si-O-C), 1014, 995 (v$_s$ Si-O-C), 939 (δ$_{\text{oop}}$=CH$_2$), 835 (p Si-CH$_3$), 750 (v Si-C), 689, 654 cm$^{-1}$.

B) Synthesis of PSGMMA-PDMS-PSGMMA (Sil-SGMMA). Typical experiments were run as described for the polymerization of DMMA, replacing tetrahydrofuran with dioxane and using a higher temperature (70 ºC) and higher monomer concentrations (50% wt.). The polymers were purified by three precipitations in ice cold methanol.

$^1$H NMR (CDCl$_3$): $\delta = 0.07$ (s, –Si(CH$_3$)$_2$ in PDMS chain), 0.12 (d, $J = 7.4$ Hz, 18H –Si(CH$_3$)$_3$), 0.75-1.20 (broad, 3H, -CH$_2$C(CH$_3$)- in PSGMMA chain), 2.10-1.70 (broad, 2H, -CH$_2$C(CH$_3$)- in PSGMMA chain), 3.40-4.20 (m broad, 5H, -CH$_2$CH(OSi(CH$_3$)$_3$)$_2$CH$_2$O-) ppm.
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FT-IR (film on ATR crystal): 2957 (ν_{as} C-H), 2902 (ν_{s} CH₃), 2872 (ν_{as} CH₂), 1732 (ν C=O ester), 1485, 1448 (δ O-CH₂), 1400, 1250 (δ_s Si-CH₃), 1144 (δ Si-O-C), 1094 (ν_{as} Si-O), 1009 (ν_s Si-O), 864, 835 (ρ Si-CH₃ from PSGMMA), 800 (ρ Si-CH₃ from PDMS), 746 (ν Si-C), 685 cm⁻¹.

C) Deprotection. 0.10 g of Sil-SGMMA were diluted in 1 mL of THF. Glacial acetic acid (0.1 - 0.5 mL) and water (0.1 mL) were added and the solution was stirred for 8h. The precipitate formed during the reaction was collected and dissolved in DMSO (2 mL). Water (10 mL) was slowly added to the polymer solution and the product was then purified through dialysis. After freeze drying a white solid was collected, washed with hexane and dried under reduced pressure (yields: 27-89% of the theoretical weight after deprotection).

¹H NMR (DMSO-d₆): δ = -0.10-0.60 (broad, -Si(CH₃)₂ in PDMS chain protons), 0.70-1.10 (broad, 3H -CH₂C(CH₃)- in PGMMMA chain), 1.72-2.12 (broad, 2H, -CH₂C(CH₃)- in PGMMMA chain), 3.70-4.20 (broad, 5H, -OCH₂CH(OH)CH₂(OH)), 4.76 and 5.01 (d, J = 72.4 Hz, OCH₂CH(OH)CH₂(OH)) ppm.

FT-IR (film on ATR crystal): 3648-3080 (ν OH), 2953 (ν_{as} CH), 2888 (ν_{s} CH), 1718 (ν C=O ester), 1457 (δ O-CH₂), 1395, 1259 (δ_s Si-CH₃), 1156 (ν C-O-C ester), 1094 (ν_{as} Si-O), 1017 (ν_s Si-O), 928, 859, 800 (ρ Si-CH₃), 751 (ν Si-C H₃), 662 cm⁻¹.

Preparation of Sil-GMMA dispersions in water

a) Preparation from hot water. 5 mL of deionized water were added to 5 mg of Sil-GMMA polymers, stirring the resulting dispersion for 6 h at 60°C.

b) Preparation from DMSO. 40 mg of Sil-GMMA polymers were dissolved in 2 mL of DMSO. These solutions were added dropwise to 2 mL of deionized water under stirring and stirred at room temperature for 24 h.

Both kinds of dispersions were dialyzed against water with SpectraPor regenerated cellulose membranes with a 3,500 g/mol molecular weight cut-off; the exact concentrations were determined after freeze drying 1 mL of the solutions.
2.3 Results and discussion

2.3.1 Polymer synthesis

For a synthesis of PDMS/GMMA block copolymers under homogeneous conditions, we have prepared two hydrophobic monomers, which can yield GMMA units upon acidic deprotection. The isopropylidene (acetonide) derivative DMMA was synthesized through the reaction of methacryloyl chloride with 2,2-dimethyl-1,3-dioxolane-4-methanol; the silane derivative SGMMA was prepared reacting GMMA with trimethylsilyl chloride (SGMMA) (see Scheme 2-1, right). The PDMS macroinitiator (PDMSbisBr) was prepared via esterification of a difunctional, commercially available hydroxyethoxypropyl-terminated PDMS (PDMSbisOH, $M_n \approx 8,000$ g/mol) with 2-bromoisoobutyryl bromide. The functionalization did not appreciably affect the average size and polydispersity of the polymer (Table 2-1).

![Figure 2-1](image)

**Figure 2-1.** Evolution of monomer conversion as a function of time (left) and of $M_n$ and polydispersity as a function of monomer conversion (right) for the ATRP of DMMA (open symbols) and SGMMA (closed symbols) initiated by PDMSbisBr. Polymerization conditions: [initiator]:[CuCl]:[HMTETA] :{monomer} = 1:1:1:30, reaction carried out at 45 °C in THF (33 wt.%) for DMMA and at 70 °C in dioxane (50 wt.%) for SGMMA.

The ATRP conditions for DMMA and SGMMA were optimized using a low molecular weight initiator, ethyl 2-bromoisobutyrate. Possibly due to the larger steric hindrance and viscosity, SGMMA required higher concentrations and temperatures than DMMA; operating in dioxane at 70°C, 50%wt. SGMMA showed a comparable, although slightly more rapid, polymerization kinetics than 33 %wt. DMMA in THF at 45°C (see Supplementary Information, Figure 2-1SI). These conditions were applied to the ATRP of DMMA and SGMMA initiated by PDMSbisBr; both processes were characterized by
a similar first-order kinetics (Figure 2-1, left) and linear dependency of the molecular weight on the polymerization conversion (Figure 2-1, right).

**Table 2-1.** Characterization data for Sil-DMMA and Sil-SGMMA block copolymers

<table>
<thead>
<tr>
<th>Sample</th>
<th>Target DP (DMMA or SGMMA)</th>
<th>Conversion b (%)</th>
<th>DP b</th>
<th>DP c</th>
<th>$M_n^d$ (g/mol)</th>
<th>GPC</th>
<th>$M_w^e / M_n^e$</th>
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<tr>
<td>PDMSbisOH</td>
<td>-</td>
<td>-</td>
<td>&lt;94&gt;</td>
<td>7200</td>
<td>8200</td>
<td>1.23</td>
<td></td>
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<tr>
<td>PDMSbisBr</td>
<td>-</td>
<td>-</td>
<td>&lt;110&gt;</td>
<td>8100</td>
<td>8800</td>
<td>1.18</td>
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<tr>
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<td>31</td>
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<td>23900</td>
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<td>51</td>
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<td>56100</td>
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<td>Sil-SGMMA1</td>
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<td>19</td>
<td>22</td>
<td>20800</td>
<td>22500</td>
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<td>29</td>
<td>25000</td>
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<td>41300</td>
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<td>78</td>
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<td>61100</td>
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<td>Sil-SGMMA6</td>
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<td>100</td>
<td>102</td>
<td>69400</td>
<td>73800</td>
<td>1.06</td>
</tr>
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</table>

*a* The degree of polymerization (DP) is here expressed as the monomer/initiator molar ratio, i.e. it refers to the length of each DMMA or SGMMA arm.  
*b* The degree of polymerization of GMMA derivatives was calculated from the ratio of the integral of the $^1$H NMR resonance of PDMMA or PSGMMA chain group (CH$_3$ at 0.7-1.2 ppm) and the integral of the resonance of unreacted monomer (CH$_2$=C- at 5.5 and 6.1 ppm) in the reaction mixture before purification.  
*c* The degree of polymerization of GMMA derivatives was calculated from the ratio of the integral of the $^1$H NMR resonance of PDMMA or PSGMMA chain groups (CH$_3$ at 0.7-1.2 ppm) and the integral of the resonance of PDMS chain (CH$_3$ at 0.07 ppm) in the purified polymer.  
*d* Calculated using the DP of point b.  
*e* Calculated from GPC in THF with triple detection (RI, viscosity and LS detectors) except for PDMSbisBr1, Sil-DMMA1, PDMSbisBr2 and Sil-SGMMA1; for these samples, due to low LS signals, only RI and VIS signals were used to determine the MW using universal calibration.  
*f* Degree of polymerization of PDMS. Calculated from the ratio of the integrals of the $^1$H NMR resonance of PDMS chain groups (CH$_3$ at 0 ppm) and the integral of the resonance of terminal groups (CH$_2$ at 1.6 ppm).

The controlled character of the polymerization was further confirmed by the linear relation between monomer/initiator molar ratio (target degree of polymerization) and the actual DMMA or SGMMA degree of polymerization, which was accompanied by almost quantitative monomer conversions and low polydispersity values (Figure 2-2 and Table 2-1). Discrepancies were recorded only for highest monomer/initiator ratios: Sil-DMMA4 (target DP per arm: 120) featured a significantly higher polydispersity, indicating some loss of control, while for Sil-SGMMA6 (target DP per arm: 200) the
SGMMA polymerization appeared to stop at about 50% conversion. This is possibly due to the rather low concentration of initiator groups that made this polymerization more sensitive to termination reactions.

It is noteworthy that the polydispersity of SGMMA block copolymers was always lower than that of DMMA ones, indicating that the combination of monomer and reaction conditions provided a better overall control on the polymerization.

![Figure 2-2. GPC traces (triple detection) of the ATRP products of DMMA (left) and SGMMA (right) using PDMSbisBr as a macroinitiator. The reactions always provided polymers with monomodal MW distribution and reasonably narrow polydispersity. Please note all polymers are labeled with the theoretical DP of DMMA or SGMMA, while Sil-SGMMA6 is labeled with its actual DP (100).](image)

The deprotection conditions were studied using Sil-DMMA3 and Sil-SGMMA3, i.e. two block copolymers with a comparable number of siloxane and methacrylic units.

The preparation of GMMA-containing polymers through the hydrolysis of DMMA repeating units has been reported using aqueous HCl with\textsuperscript{43,45-47} or without\textsuperscript{44} dilution with organic solvents. However, using aqueous HCl or HCOOH under heterogeneous conditions (large excess acidic water + polymer) or in organic solution (polymer in THF with smaller excess of acidic water), we recorded either incomplete deprotection or significant cleavage of the PDMS/polymethacrylate junction (Figure 2-3): when significant deprotection was recorded, the polymer could be separated in two phases with different water solubility, always with a low PDMS/methacrylate ratio in the water-soluble fraction (see Supplementary Information, Table 2-1SI).
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Figure 2-3. **Left:** $^1$H NMR spectra of Sil-DMMA3 before and after deprotection under heterogeneous conditions (no organic solvent; water-soluble phase) with HCOOH. The spectra after deprotection were recorded in DMSO-d$_6$ while the pristine polymer was analyzed in CDCl$_3$, due to different solubility of the polymers. The use of a 44%-wt. HCOOH water solution maintained the integrity of the polymer chain, but allowed to deprotect 85-90% of the acetonide groups (note the presence of residual resonance at 1.35-1.40 ppm, arrow). 66%-wt. HCOOH cleaved quantitatively both acetonides and the PDMS/methacrylic block junction (note the absence of PDMS resonance in the water-soluble phase, arrow, 0 ppm). **Right:** IR spectra of PDMBSbisBr and of Sil-DMMA3 before and after deprotection (water-soluble phase). The presence of OH groups (peak 1) and the disappearance of typical vibrations of the acetonide group (peaks 3 and 4) witnessed the deprotection after exposure to HCOOH; despite some broadening, the ester absorption (peak 2) was substantially unaltered, suggesting the integrity of the methacrylic esters. However, the Si-CH$_3$ rocking vibration completely disappeared, confirming the $^1$H NMR data.

On the contrary, the removal of the silylated protective groups of Sil-SGMMA copolymers was less challenging. Operating in THF solution (= small stoichiometric excess of acidic water) and using the less aggressive acetic acid, the silylated groups were quantitatively cleaved without detrimental effects on the PDMS/polymethacrylate junction (see Supplementary Information, Table 2-1SI). With any amount of acetic acid (from 1:1 to 1:5 polymer/AcOH weight ratio) the polymer presented a complete or almost complete removal of trimethylsilyl groups (Figure 2-4); with the highest amounts of acetic acid (from 1:3 to 1:5), the polymer precipitated quantitatively during the reaction, which made it easily recoverable from the reaction environment.

The strongly different solubility of the two blocks hindered a reliable GPC analysis virtually in any common solvent: even if able to disperse the polymers, polar solvents such as DMSO and DMF were not able to fully swell the PDMS block, as demonstrated by the broad silane $^1$H NMR resonance (see inset in Figure 2-4, left); further, in DMSO the copolymers with short GMMA blocks (Sil-GMMA1-2) showed significant
broadening also of the GMMA resonances, which partially impaired the quantitative character of NMR analysis too (see Supplementary Information, Table 2-2SI).

However, the deprotection procedure did not affect the PDMS/GMMA ratio for the copolymers with longer GMMA blocks (Sil-GMMA3-6), nor signals associated to ester hydrolysis could be highlighted by IR and NMR; further, negligible material could be extracted with hexane, ensuring the absence of free PDMS blocks. We have therefore assumed the macromolecular structure to have retained its integrity.

For further experiments, we have thus opted for the silylated precursor (SGMMA) route for the preparation of GMMA amphiphilic block copolymers.

**Figure 2-4.** Left: $^1$H NMR spectra of Sil-SGMMA3 before (in CDCl$_3$, bottom) and after deprotection (in DMSO-d$_6$, top) with a close up of the Si-CH$_3$ resonance region. The deprotection was performed with a 5-fold (in weight) acetic acid/polymer ratio. The spectra after deprotection were recorded in DMSO-d$_6$ (broader resonance at 0 ppm, due to poor DMSO solubility of the PDMS block), while the pristine polymer was analyzed in CDCl$_3$, due to different solubility of the polymers. The appearance of the diol resonances (peaks 9 and 10) and the disappearance of the trimethylsilyl ones (peaks 7 and 8) are an indication of the occurrence of hydrolysis Right: IR spectra of PDMSbisBr and of Sil-SGMMA3 before and after deprotection. The presence of an OH stretching absorption and the disappearance of the Si-CH$_3$ rocking vibrations associated to the trimethylsilyl groups (peaks 7 and 8) witness the occurrence of hydrolysis, while the permanence of the PDMS Si-CH$_3$ rocking (peak 1) ensures the integrity of the polymer backbone.
2.3.2 Self-assembly in water

The direct dispersion of Sil-GMMA triblock copolymers in water was not possible for those with shorter GMMA chains, and provided very large aggregates for the other. On the other hand stable dispersions could be obtained using water at 60ºC or by diluting DMSO solutions in water at room temperature, followed by DMSO removal by dialysis. In general the two methods provided colloidal objects of comparable size (Figure 2-5 and Table 2-2), although larger aggregates could still be seen for Sil-GMMA1 and sometimes also for Sil-GMMA2 in hot water. The long-term stability of these dispersions (at least several weeks) suggests that the poor dispersibility in water has a kinetic origin, possibly due to H-bonded entanglements between GMMA blocks.

Table 2-2. Characterization data for the colloidal aggregates of Sil-GMMA block copolymers: Z-average diameters ($d_h$), Width at Half Maximum (WHM) and critical aggregation concentrations.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$d_h$ / WHM (nm)</th>
<th>cac (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sil-GMMA1</td>
<td>170 / 350</td>
<td>0.01</td>
</tr>
<tr>
<td>Sil-GMMA2</td>
<td>100 / 150</td>
<td>0.06</td>
</tr>
<tr>
<td>Sil-GMMA3</td>
<td>70 / 80</td>
<td>0.14</td>
</tr>
<tr>
<td>Sil-GMMA4</td>
<td>55 / 60</td>
<td>0.18</td>
</tr>
<tr>
<td>Sil-GMMA5</td>
<td>65 / 50</td>
<td>0.19</td>
</tr>
<tr>
<td>Sil-GMMA6</td>
<td>45 / 50</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Figure 2-5. Size distributions of 1 mg/mL suspensions of Sil-GMMA triblock copolymers in deionized water prepared in hot water (left) and or by dilution of a DMSO solution (right)
The size of the aggregates decreased with increasing GMMA content, suggesting a transition from lower to higher surface/volume morphologies, e.g. from lamellar or worm-like micellar aggregates to spherical micelles. TEM analysis confirmed this; Sil-GMMA1-3 were shown to exhibit irregular but often elongated morphologies, possibly but not necessarily consistent with worm-like micellar aggregation, while Sil-GMMA4-6 gave rise to spherical aggregates (Figure 2-6). The significant difference in size of the latter in TEM pictures (10-20 nm) and DLS observations (30-40 nm) suggests a strong water swelling of the GMMA blocks in a water environment.

Figure 2-6. TEM images of aggregates formed by Sil-GMMA1 (a), Sil-GMMA3 (b), Sil-GMMA5 (c), Sil-GMMA6 (d).

The concentration dependency of the aggregation of the Sil-GMMA triblock copolymers was studied using the pyrene solubilization technique\textsuperscript{50}. The pyrene fluorescence is sensitive to changes in its microenvironment (Figure 2-7A) which permits monitoring its incorporation into host aggregates when amphiphiles exceed
their critical aggregation concentration (cac). With increasing amphiphile concentration, the increase of $I_1/I_3$ ratio (the ratio between the intensities of the first and the third vibrational band in pyrene emission spectra, respectively at 375 and 392 nm) indicates the localization of the fluorescent probe in a hydrophobic environment. For Sil-GMMA copolymers the $I_1/I_3$ ratio increased from 0.80 to 0.96 (Figure 2-7B) and the cac values were calculated as the polymer concentrations at the inflection points (= zero of the 2$^{nd}$ derivatives) of the curves (Figure 2-7C and Table 2-2).

**Figure 2-7.** A: Emission spectra of pyrene (2 x 10$^{-6}$ M, $\lambda_{exc}$: nm) in water as a function of Sil-GMMA6 concentration. B: $I_1/I_3$ Emission ratio for pyrene as a function of -log(polymer concentration); the polymer water dispersions were obtained from DMSO solution after dialysis of the organic solvent. The lines represent sigmoidal (Boltzman) fits of the experimental points. C: the inflection point of each curve was determined as the zero of the 2$^{nd}$ derivative and was used to calculate the cac (Sil-GMMA1). D: dependency of the cac (black squares, expressed in $\mu$M per liter and obtained using calculated $M_n$ values for the deprotected polymers (NMR data in Table 1 corrected by the different mass of protected and deprotected GMMA) and of the Z-average hydrodynamic radius (empty circles) on the degree of polymerization of the GMMA blocks.

The dependency of the cac on the GMMA block length showed a plateau value of about 8 $\mu$M for Sil-GMMA3-6 (Figure 2-7D); this did not precisely correspond to the behavior of the aggregate size: for example, as supported by the TEM pictures (Figure...
Chapter 2

2-6), Sil-GMMA3 produced a majority of objects sized > 50 nm, although it presented virtually the same molar cac as Sil-GMMA6, which produced only 30-40 nm spherical micelles. The substantial invariance of the molar cac would appear to suggest that above DP(GMMA)≈30, which corresponds to GMMA volume fractions >0.5, the aggregation (micellization) would depend predominantly only on the features of the insoluble block; indeed, similar effects have been reported for Pluronics51,52, while it has been shown that the dependency of the micellization ΔG primarily on the length of the hydrophobe applies to block copolymers too, although with important corrections for polydispersity53.

On the other hand, it is the hydrophile length that then determined morphology and size of the aggregates and thus also the number of polymer chains involved in each of them.

2.3.3 Protein adsorption on Sil-GMMA colloids

If a protein is irreversibly adsorbed on a colloid of comparable or larger dimension, its diffusion coefficient will correspondingly decrease. Our group has previously shown that diffusion NMR (1H DOSY NMR also known as PFG NMR) can be employed to follow the diffusion coefficient of bovine serum albumin (BSA) in the presence of other species, in this way highlighting aggregation phenomena54.

In deuterated water at 25ºC 1H DOSY NMR provides a diffusion coefficient of 0.40 ± 0.02 × 10⁻¹⁰ m² s⁻¹ for BSA; this result is obtained using any of the BSA proton resonances (see Supplementary Information, Figure 2-2SI). Using the Stokes-Einstein equation, this corresponds to a hydrodynamic diameter of about 6.1 nm, which is very comparable to the values obtained through field flow fractionation55.

We have here monitored the diffusion coefficient of BSA in the presence of PGMMA homopolymer (M_n ≈ 14,000 g/mol, M_w/M_n = 1.31) or Sil-GMMA6 (Figure 2-8); the latter is the copolymer with the highest content of PGMMA, and therefore likely the highest stability of its micellar aggregates. The study is currently ongoing with aggregates of the other block copolymers. We have used the signal at δ = 2.92 ppm, where no overlapping with PGMMA resonances complicates the data processing, to calculate the BSA diffusion coefficients (see Supplementary Information, Table 2-3SI).

The presence of PGMMA did not influence the diffusion coefficient of BSA up to concentrations of 0.1 wt.%, above which it appeared to slightly increase it. This result
excludes any significant PGMMA/BSA aggregation; on the contrary, it would imply PGMMA to cause a decrease in BSA dimension, which is possibly explained as a loss of hydration water of the protein in the presence of a large amount of hydrophilic polymer.

Sil-GMMA6 appeared to present a similar absence of aggregation in the presence of BSA; a slight increase in BSA size ($\approx +10\%$ in linear dimension) was recorded with $1\%$ wt. Sil-GMMA6, but this effect may be caused rather by depletion interactions than by adsorption on the Sil-GMMA6 micelles.

![Figure 2-8. Diffusion coefficients of BSA (1 wt.% in D$_2$O) in the presence of different concentrations of PGMMA (solid squares) or Sil-GMMA6 (open squares) divided by that of BSA alone. For ease of reading, the dashed line marks the BSA diffusion coefficient.](image)

**2.4 Conclusions**

We have demonstrated that silylation of GMMA alcohols is the most convenient protection/deprotection route to PGMMA/PDMS block copolymers, which can be prepared with low polydispersity and good control over the length of the hydrophilic block. This allowed a rather precise control over the aggregation behaviour of the resulting Sil-GMMA copolymers in water; while most polymers exhibited an identical critical aggregation concentration in the range of $7-8\ \mu$M, it was shown that $35-40$ nm spherical micellar aggregates were the thermodynamically favoured species for GMMA blocks larger than $40-50$ monomeric units. PGMMA both as soluble polymer and on the surface of such micelles had a negligible influence on the diffusion coefficient of BSA, suggesting a rather “stealth”-like behaviour.
2.5 References

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(22) Miller, P. J.; Matyjaszewski, K. Macromolecules 1999, 32, 8760.
(24) Liu, Q.; Wilson, G. R.; Davis, R. M.; Riffle, J. S. Polymer 1993, 34, 3030.


2.6 Supplementary Information

Figure 2-1SI. Evolution of monomer conversion as a function of time (top) and of $\overline{M}_n$ and polydispersity as a function of monomer conversion (bottom) for the ATRP of DMMA (open symbols) and SGMMA (closed symbols) initiated by ethyl 2-bromoisobutyrate. Polymerization conditions: [initiator]:[CuCl]:[HMTETA] : [monomer] = 1:1:1:30, reaction carried out at 45 °C in THF (33 wt.%) for DMMA and at 70 °C in dioxane (50 wt.%) for SGMMA.
Table 2-1SL. Summary of the deprotection conditions and results for Sil-DMMA3 and Sil-SGMMA3 (reaction time: 8 hours, but negligible differences were recorded after 48 hours). With Sil-DMMA3 two fractions were generally obtained, a water-soluble and a water-insoluble one. The two parameters, PDMS/PGMMA and residual protecting group, respectively provide information about the integrity of the macromolecular structure and the efficiency of deprotection.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Physical state</th>
<th>Aqueous acid (a)</th>
<th>PDMS/PGMMA (b) (mol/mol)</th>
<th>Residual protecting groups (mol %) (c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dispersion</td>
<td>10X, 3.7%HCl</td>
<td>1.1 (water sol.)</td>
<td>100 (water sol.)</td>
</tr>
<tr>
<td></td>
<td>10% THF sol.</td>
<td>0.4X, 3.7% HCl</td>
<td>0.7 (water sol.)</td>
<td>92 (water sol.)</td>
</tr>
<tr>
<td></td>
<td>dispersion</td>
<td>10X, 44% HCOOH</td>
<td>1.2 (water sol.)</td>
<td>11 (water sol.)</td>
</tr>
<tr>
<td></td>
<td>10% THF sol.</td>
<td>0.4X, 44% HCOOH</td>
<td>1.1 (water ins.)</td>
<td>90 (water ins.)</td>
</tr>
<tr>
<td></td>
<td>dispersion</td>
<td>10X, 66% HCOOH</td>
<td>10.6 (water ins.)</td>
<td>0 (water ins.)</td>
</tr>
<tr>
<td></td>
<td>10% THF sol.</td>
<td>0.4X, 66% HCOOH</td>
<td>1.1 (water ins.)</td>
<td>91 (water ins.)</td>
</tr>
<tr>
<td></td>
<td>dispersion</td>
<td>10X, 66% HCOOH</td>
<td>10.3 (water ins.)</td>
<td>1 (water ins.)</td>
</tr>
<tr>
<td></td>
<td>10% THF sol.</td>
<td>1X H(_2)O + 1X AcOH</td>
<td>1.2</td>
<td>2 (water ins.)</td>
</tr>
<tr>
<td></td>
<td>10% THF sol.</td>
<td>1X H(_2)O + 2X AcOH</td>
<td>1.4</td>
<td>1 (water ins.)</td>
</tr>
<tr>
<td></td>
<td>10% THF sol.</td>
<td>1X H(_2)O + 3X AcOH</td>
<td>1.4</td>
<td>0 (water ins.)</td>
</tr>
<tr>
<td></td>
<td>10% THF sol.</td>
<td>1X H(_2)O + 4X AcOH</td>
<td>1.4</td>
<td>1 (water ins.)</td>
</tr>
<tr>
<td></td>
<td>10% THF sol.</td>
<td>1X H(_2)O + 5X AcOH</td>
<td>1.1</td>
<td>2 (water ins.)</td>
</tr>
</tbody>
</table>

\(a\) expressed in relation to the weight of polymer; for example, if 100 mg of polymer, 10X means 1 g of aqueous acidic phase.

\(b\) ratio between the integrals of the peak at 0 ppm (Si-CH\(_3\)) and that of the peak at 0.8-1.2 ppm (polymer chain CH\(_3\)) ±0.1.

\(c\) ratio between the integrals of the peak at 1.3 ppm (C(CH\(_3\))\(_2\)) or of that at 0.05-0.13 ppm (Si-CH\(_3\)) and that of the peak at 0.8-1.2 ppm (polymer chain CH\(_3\)).

Table 2-2SL. Degree of polymerization of the methacrylic chain for the different Sil-SGMMA copolymers before and after deprotection with a 1:4 polymer/AcOH weight ratio.

<table>
<thead>
<tr>
<th>Sample</th>
<th>protected (DP_n^a)</th>
<th>deprotected (DP_n^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sil-(S)GMMA1</td>
<td>22</td>
<td>13</td>
</tr>
<tr>
<td>Sil-(S)GMMA2</td>
<td>29</td>
<td>20</td>
</tr>
<tr>
<td>Sil-(S)GMMA3</td>
<td>44</td>
<td>40</td>
</tr>
<tr>
<td>Sil-(S)GMMA4</td>
<td>53</td>
<td>55</td>
</tr>
<tr>
<td>Sil-(S)GMMA5</td>
<td>78</td>
<td>76</td>
</tr>
<tr>
<td>Sil-(S)GMMA6</td>
<td>102</td>
<td>122</td>
</tr>
</tbody>
</table>

\(a\) Calculated from the ratio of the \(^1\)H NMR resonance of P(S)GMMA chain group (CH\(_3\) at 0.7-1.2 ppm) and the resonance of PDMS chain (CH\(_3\) at 0.07 ppm in CDCl\(_3\) for the polymers before deprotection and at -0.06 ppm in DMSO-d6 for the deprotected polymers)
Figure 2-2SI. DOSY plot for bovine serum albumin (BSA), showing that the same value of diffusion coefficient was obtained for all the proton resonances of BSA.

Table 2-3SI. Diffusion coefficient of BSA in the presence of PGMMA polymers.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Polymer concentration (wt.%)</th>
<th>$10^{10} \cdot D_{BSA}$ $^a$ (m$^2$s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>0.40</td>
</tr>
<tr>
<td>PGMMA</td>
<td>0.01</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>0.47</td>
</tr>
<tr>
<td>Sil-GMMA6</td>
<td>0.01</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>0.36</td>
</tr>
</tbody>
</table>

$^a$ $D_{BSA}$ values present standard errors of 0.02-0.03
3 Surface modification of poly(dimethylsiloxane) substrates with poly(glycerol monomethacrylate) based copolymers

Abstract

This study showed the feasibility of the surface modification of poly(dimethylsiloxane) (PDMS) substrates by physisorption of ABA amphiphilic triblock copolymers comprising PDMS as a central hydrophobic part and poly(glycerol monomethacrylate) (PGMMA) as terminal blocks (Sil-GMMA polymers). The adsorption of macroamphiphiles with high PDMS content (≥ ~80 mol%) on PDMS surface from water/ethanol solutions was confirmed by infrared and X-ray photoelectron spectroscopies, atomic force microscopy and a decrease in water contact angle. It was found that although silicone surfaces coated with Sil-GMMA recovered their hydrophobicity upon prolonged storage in air, the surface hydrophilicity was preserved upon storage in water. The effect of Sil-GMMA surface modification on fibrinogen and albumin adsorption was investigated using two different analytical techniques (bicinchoninic acid and fluorescamine assays). It was found that that the Sil-GMMA adsorption produced a significant switch from a higher affinity for fibrinogen (unmodified PDMS) to higher affinity for albumin (coated PDMS).
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3.1 Introduction

Silicone (poly(dimethylsiloxane), PDMS)-based materials have found wide application as biomaterials due to the combination of a number of favourable properties such as ease of cross-linking, low glass-transition temperature, chemical stability, optical transparency, oxygen permeability and low toxicity. In particular, PDMS has been used for decades in the production of different medical devices\(^1\), e.g. to manufacture intraocular lenses\(^2\), urinary catheters\(^3\) and breast implants\(^4\), and also in microfluidics\(^5\). However, due to its hydrophobic nature, the surface adsorption of proteins on PDMS often leads to undesired reactions such as foreign body reactions and/or platelet adhesions\(^6,7\); PDMS also allows bacterial attachment, leading to the formation of biofilms, which are the first promoters of the chronicization of infections\(^3,8\).

The modification of biomaterial surfaces with hydrophilic polymers is a popular strategy to control biocompatibility and tissue reactions, by favouring or discouraging protein adsorption and cell adhesion. Poly(ethylene glycol) (PEG) is the gold standard for this application and has been often used for the surface modification of PDMS\(^9-13\). Also other biocompatible polymers, such as poly(2-methacryloyloxyethyl phosphorylcholine)\(^14-16\) and poly(2-hydroxyethyl methacrylate)\(^17\), have been researched for the same aim. Here, we have focused on a different hydrophilic, non-ionic structure: poly(2,3-dihydroxypropyl methacrylate), more commonly referred to as poly(glycerol monomethacrylate) (PGMMA). PGMMA features two vicinal alcohols on each repeating unit, which provide high hydrophilicity but also the possibility of intramolecular hydrogen bonding, which suggests a lower nucleophilic potential of this group in comparison to isolated alcohols, and therefore the potential of a “stealth” behaviour. Further, this group provides ease of functionalization, for example with pharmacologically active compounds\(^18\). Indeed, when PGMMA has been used to prepare hydrogels\(^19-24\) and surface layers\(^22\), the resulting materials have been shown to be non-toxic, protein-repellent, and non cell-adhesive.

PGMMA chains on silicone surfaces could be introduced through chemical or physical techniques. The covalent attachment of polymer chains to PDMS could be achieved either by the “grafted to”\(^9\) or the “grafted from” (surface initiated polymerization)\(^10,12,16,17\) approach. However, these techniques are laborious (sequence of introduction of surface
groups and then attachment of preformed polymer chains or their surface-initiated growth) and often make use of toxic reagents. In addition, simple chemical modifications, such as the formation of polar groups by plasma or corona treatments, are marred by the rapid recovery of surface hydrophobicity, due to migration of the polar groups into the PDMS bulk. Purely physical methods offer the significant advantage of milder and less laborious conditions and they are broadly independent on the material shape; the swelling-deswelling method uses amphiphilic PDMS block copolymers that phase segregate on the surface of PDMS networks upon their deswelling. The direct physisorption of amphiphilic copolymers on silicone surfaces is based on a similar concept, i.e. the minimization of PDMS surface energy by the presence of a phase-segregated hydrophilic layer, but it avoids the morphological changes of the swelling-deswelling process. We have chosen this method, designing block copolymers based on PGMMA and PDMS, where the chemical similarity between substrate and hydrophobic block should enhance the stability of the adsorbed polymers.

Specifically, we have employed ABA amphiphilic triblock copolymers with PDMS as a central hydrophobic part and PGMMA terminal blocks, hereafter referred to as Sil-GMMA polymers (Scheme 3-1). ATRP of protected GMMA initiated by a bifunctional PDMS macroinitiator allowed to prepare a family Sil-GMMAs with variable degree of polymerization, with the aim to optimize the polymer structure for the adsorption on PDMS: by influencing the exposure of the hydrophobic block and the curvature of the hydrophobe/water interface, the PDMS/PGMMA balance arguably is a major determination of the efficiency of the physisorption process.
Sil-GMMA triblock copolymers were synthesized in a protected form via ATRP using a PDMS macroinitiator and silylated GMMA as a monomer. The hydrolysis of the trimethylsilyl groups provided the final amphiphilic macromolecules. The table provides the molar ratio between GMMA and DMS units in the polymer, calculated from the ratio of the $^1$H NMR resonance of PGMMA chain group (CH$_3$ at 0.7-1.2 ppm) and the resonance of PDMS chain (CH$_3$ at -0.06 ppm).

### Experimental Section

#### 3.2.1 Materials

A silicone elastomer kit (Sylgard 184, Dow Corning) was purchased from Elsworth Adhesives Ltd (Glasgow, UK). Bovine plasma fibrinogen Type I-S (BPF), bovine serum albumin (BSA), copper (I) chloride, dimethyl sulfoxide (DMSO), fluorescamine, hexamethytrisylxene (HMTETA), sodium dodecyl sulphate (SDS) were purchased from Sigma-Aldrich (Gillingham, UK). Phosphate buffer saline (PBS) was bought from Oxoid (Basingstoke, UK). Acetic acid, dioxane, ethanol, hexane, methanol and tetrahydrofuran were purchased from Fisher Scientific (Loughborough, UK).
Deionized water, ethanol and hexane were passed through a 0.22 μm filter before use. Bis-trimethylsilyl GMMA and 2-bromoisobutirylethoxypropyl terminated PDMS were prepared according to a previously reported procedure (see Chapter 2).

3.2.2 Physico-chemical characterization

**IR spectroscopy.** Fourier transform infrared (FT-IR) spectra were recorded in attenuated total reflectance mode on a Tensor 27 Bruker spectrometer.

**Dynamic light scattering (DLS).** Size measurements were performed using a Zetasizer Nano ZS instrument (Model ZEN2500, Malvern Instruments Ltd., UK) at a scattering angle of 173° and at a temperature of 25 °C. The values of hydrodynamic diameter (which corresponds to the weighted Z-average) were obtained from a cumulant analysis algorithm wherein a single particle size is assumed and a single exponential fit is applied to the autocorrelation (Zetasizer Nano Software, Malvern Instruments Ltd., UK). The colloidal dispersions were prepared at a concentration of 10 mg/mL in filtered water and ethanol and were not filtered after preparation.

**Mechanical characterization.** Silicone substrates were analyzed using a texture analyzer (Model TA-XT plus, Stable Micro System Ltd, UK) in compression mode applying up to 5 kg of force. The elastic modulus was obtained from the slope of the plot of stress (F/A) versus strain (ΔL/L) where F is force, A is the contact area with the probe, L is the height of the sample and ΔL is the change in height. Stress-strain curves were fitted in their linear region, where typical strains were in the range of 0.04-0.4 for stiff elastomers and 0.02-0.2 for soft ones. The data reported here are the mean and standard deviation of at least 3 different substrates.

**Water contact angle.** The sessile drop method was used to measure water contact angles with a Krüss 100-DSA (**drop shape analysis**). All samples were dried in air for 30 min before measurements. The advancing contact angle was measured immediately after deposition of the water drop on the surface while the receding contact angle was measured after the water drop was left to evaporate for 3 minutes. The values reported here are the results of at least 3 drops on each surface.
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X-Ray photoelectron spectroscopy (XPS). Spectra were collected on a Kratos ULTRA-DLD instrument configured with a monochromated Al Kα (1486.6 eV) X-ray source. Measurements were carried out on electron taking-off angles (t.o.a.) from the sample surface of 90° and 10°. Survey spectra and high sensitivity scans of the Br 3d area were obtained at a pass energy of 80 eV. The pass energy was 20 eV for the narrow scans. All data analyses were accomplished using a CasaXPS software. The curve fittings of the high resolution peaks were carried out using a combination of Gaussian and Lorentzian functions (70:30%). The binding energies were referenced to the C 1s binding energy of PDMS CH₃ groups (284.3 eV).²⁷

Atomic Force Microscopy (AFM). All measurements were performed in air at 25°C using a Molecular Force Probe 3D AFM (MFP-3D, Asylum Research, Santa Barbara, CA). The nominal parameters of the silicon cantilevers (model AC160TS, Olympus) used in this study are: spring constant = 42 N/m, tip radius <10 nm, tip height = 7-15 μm, resonance frequency = 300 kHz.

A) Imaging. Tapping mode parameters: scan frequency = 1 Hz (25.70 μm/sec), gain = 10, setpoint values 550 mV - 650 mV, resonance frequency 313 - 344 kHz. Three different 10 μm X 10 μm regions of each sample (unmodified and coated S10, S20) were imaged, calculating also the root mean square (RMS) roughness.

B) Nanoindentation. Measurements on mica surfaces were used to calibrate the deflection sensitivity of the cantilever, which is necessary to convert force-displacement measurements into force-indentation dependences. The spring constant of the cantilevers was calculated with the thermal method²⁸ and ranged between 36.2 N/m and 43.7 N/m. The deflection sensitivity ranged between 65 nm/V and 75 nm/V. Three different 10 μm X 10 μm regions were investigated for each sample obtaining force maps for each region with a spatial resolution of 0.5 μm (20 X 20 curves on an area of 10 μm²). All force curves were acquired with a loading/unloading rate equal to 6 μm/sec (frequency = 1 Hz) and the maximum force applied on the surface of each samples was 500 nN.

Assuming a Hookean behaviour for the silicon cantilever, force-displacement data were converted to force-indentation plots. Igor Pro software (Asylum Research) was used to fit all the force curves in order to calculate the Young’s modulus; the power law exponent (β)
of the data ranged between 1.80 and 2.00, suggesting the use of a Hertz-cone model (punch model: β = 1, Hertz-sphere model: β = 1.5, Hertz-cone model: β = 2. 0.5 ≤ β ≤ 2.5 means that Hertz model cannot be used to fit the data due to excessive adhesion forces), which is described by the following equation:

\[ F = \frac{\pi}{2} E^* h^2 \tan \alpha \]

Where \( F \) is the load, \( h \) the displacement of the specimen surface (indentation), \( \alpha \) is the half opening apex-angle of the tip and \( E^* \) is the combined modulus, defined as follows:

\[ \frac{1}{E^*} = \frac{(1 - \nu_s^2)}{E_s} + \frac{(1 - \nu_t^2)}{E_t} \]

Where \( E_s, \nu_s \) and \( E_t, \nu_t \) are the Young’s moduli and the Poisson’s ratios for the sample and the tip, respectively. \( E_t = 150 \text{ GPa}, \nu_t = 0.17, \alpha = 18^\circ \) and \( \nu_s = 0.5 \).

The adhesion forces between the AFM tip and the sample surface were determined both in loading (indentation) and unloading (retraction) force curves. The contact point was identified as the minimum of the loading curve. Upon retraction, the tip keeps in contact with the surface until the cantilever force overcomes the adhesion force: at this point the force reaches a maximum and then rapidly decreases to 0 (the tip is pulled out of contact with the surface). The difference between the minimum (negative) force and the force felt at a large distance from the surface is the adhesion force.

**Protein adsorption tests.** PDMS substrates were cured as described in the Preparative Procedures in a tissue-culture polystyrene (TCPS) 24-well plate or in a black TCPS 96-well plate (BD Falcon) to perform, respectively bicinchoninic acid (BCA) or fluorescamine assays. 1 mL (BCA) or 0.1 mL (fluorescamine) of protein solution in PBS at pH 7.3 (1 mg/mL BPF/ 1 and 10 mg/mL BSA for BCA; 0.1, 0.5 and 1 mg/mL BPF / 0.1, 1 and 10 mg/mL BSA for fluorescamine) was poured into each well and incubated at 37 °C for 3 h. The protein solution was then removed and the wells were washed with fresh PBS twice.

**BCA:** after adding 0.55 mL of SDS aqueous solution (10 mg/mL), the plate was sonicated for 20 min at room temperature. The protein concentration in the SDS solution was then determined using a QuantiPro™ BCA protein assay kit, reading the absorbance of the BCA-Cu\(^{1}\) complex at 562 nm with a Bio-Tek Synergy 2 multi-mode microplate reader (3 samples, 4 measurements per sample).
Fluorescamine: 0.075 mL of 2 mg/mL fluorescamine solution in DMSO and 0.075 mL of PBS (pH 7.3) were poured into each well. The fluorescence was read on a Bio-Tek Synergy 2 multi-mode microplate reader using filters for excitation at 360 ± 40 nm and for emission at 460 ± 40 nm after 15 min (mean and standard deviation of the results from 12 samples).

3.2.3 Preparative procedures

Substrate preparation. The elastomer kit Sylgard 184 consists of a “base” and “cross-linker”, which are respectively a silane (Si-H) containing poly(dimethyl siloxane) and tetramethyl tetravinyl cyclotetrasiloxane. PDMS substrates with different cross linking densities were prepared by mixing the base and the cross-linker at various weight ratios (5:1, 10:1, 20:1 and 50:1 corresponding to the substrates S5, S10, S20 and S50 respectively), degassed under reduced pressure for 30 min and transferred into TCPS Petri dishes. The PDMS films were cured at room temperature for 2 days and disks with a diameter of 9 mm and a thickness of approximately 2 mm were cut using a cork borer. The substrates were washed with hexane and dried under reduced pressure.

Synthesis of Sil-GMMA copolymers. In a typical synthesis (Sil-GMMA1) a mixture of 500 mg of 2-bromoisobutyrylethoxypropyl terminated PDMS (PDMS macroinitiator, 8,000 g/mol), bis-trimethylsilyl GMMA (751 mg, 2.47 mmol, corresponding to 20 equivalents to bromoisobutyryl groups) and HMTETA (29 mg, 0.12 mmol, corresponding to 1 equivalent to bromoisobutyryl groups) were degassed by bubbling argon for 45 minutes followed by the addition of degassed dioxane (1.3 mL). Copper (I) chloride, (12 mg, 0.12 mmol, corresponding to 1 equivalent to bromoisobutyryl groups) was then added under inert atmosphere. The solution was stirred at 70 °C for 4h. The polymer was purified by three precipitations in ice cold methanol. The bis-trimethylsilylated group was then hydrolyzed by dissolving 100 mg of the polymer in 1 mL of tetrahydrofuran and adding 0.1 mL of water and 0.4 mL of acetic acid. After 4 h, the precipitated polymer was collected, redissolved in DMSO and purified by dialysis (3,500 g/mol molecular weight cut off) in water. After freeze-drying, a white block copolymer was obtained. Different compositions of block copolymer were synthesized by using the same PDMS macroinitiator and varying the feed ratio of bis-trimethylsilyl GMMA monomer.
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Adsorption of block copolymers. Block copolymers with various blocks ratios were dissolved in ethanol at different concentrations (40, 20 and 10 mg/mL) and then diluted to respectively 20, 10 and 5 mg/mL with water. The PMDS elastomer disks were then exposed to 1 mL of each polymer solution for 3 days, washed with fresh water and dried under reduced pressure for 1 h. In order to assess the long term stability of the surface modifications, disks were then stored in air or water for 14 days.

3.3 Results and discussion

3.3.1 Characterization of the uncoated substrates

The mechanical properties of a substrate are known to strongly influence the behavior of cells at its interface, also independently on its composition. It has also been suggested that they may influence the surface adsorption of dispersed/soluble material: for example, polyelectrolytes have been shown to adsorb on silicone surfaces inversely proportionally to its stiffness\(^3^3\), although these differences did not appear to affect the amount of proteins later adsorbed from serum. Here we have produced silicone elastomers using the popular two-component formulation Sylgard 184, composed of a base (mostlyvinylated PDMS, vinlylated or trimethylsilylated silica and a Pt catalyst) and a cross-linker (silane-containing PDMS + vinylated PDMS and vinlylated or trimethylsilylated silica + tetramethyl tetravinyl cyclotetrasiloxane)\(^3^4\). We have used weight ratios comprised between 10:1 (S10) and 50:1 (S50); the correspondingly different cross-link densities resulted in both different mechanical properties and surface properties, which are summarized in Table 3-1.

Morphology: AFM showed that S10 and S20 presented extremely smooth surfaces, with roughness below 1 nm (Figure 3-1, top); the analysis of samples with lower amounts of cross-linker provided unreliable results (S50), possibly due to the incomplete cross-linking leading to adhesion of the material to the AFM tip and its contamination.

Mechanical properties: in macroscopic samples, the elastic modulus (compression) scaled approximately linearly with the amount of cross-linker (∝ cross-link density), varying from \(0.095 \pm 0.016\) (S50) to \(2.90 \pm 0.05\) MPa (S10). Higher cross-link densities did not show appreciably higher modulus (for example a 5:1 ratio gave \(E = 2.74 \pm 0.10\) MPa), while
lower ones resulted in viscous oils. Nanoindentation on S10 and S20 confirmed these results, and also showed the good spatial homogeneity of the modulus, which exhibited variations < 5% in areas of 10*10 μm (Figure 3-1, middle).

Table 3-1. Mechanical and surface properties determined by compression tests, AFM/nanoindentation and water contact angle for PDMS elastomers with different cross-link densities.

<table>
<thead>
<tr>
<th>Sample (base:cross linker wt ratio)</th>
<th>Young's modulus (MPa)</th>
<th>Contact angle (º)</th>
<th>Adhesion force (nN)</th>
<th>Roughness (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>compression(^a)</td>
<td>nanoindent.(^b)</td>
<td>adv.</td>
<td>rec.</td>
</tr>
<tr>
<td>S10 (10 :1)</td>
<td>2.90 ± 0.05</td>
<td>0.930± 0.006</td>
<td>105±1</td>
<td>103±1</td>
</tr>
<tr>
<td>S20 (20 :1)</td>
<td>0.76 ± 0.05</td>
<td>0.426± 0.004</td>
<td>112±2</td>
<td>108±4</td>
</tr>
<tr>
<td>S50 (50 :1)</td>
<td>0.095 ±0.016</td>
<td>=</td>
<td>115±2</td>
<td>111±2</td>
</tr>
</tbody>
</table>

\(^a\) measured on cylindrical samples on an area of approx. 15 mm\(^2\).

\(^b\) measured in air with AFM in nanoindentation mode.

\(^c\) measured as the maximum negative force recorded in the indentation (loading) and in the retraction (unloading) curves. The adhesion force in loading is often referred to as van der Waals component of the total adhesion. See also Supplementary Information, Figure 3-1SI.

Surface properties: the water contact angle of the silicone elastomers increased with decreasing cross-link density; this is a consequence of the slightly higher hydrophilicity of the cross-linker, which has a reduced content of methyl groups in comparison to the macromonomer. Advancing and receding angles showed negligible differences (also upon prolonged storage in water), ensuring the absence of any significant wetting phenomenon in the substrate. The adhesion forces recorded in nanoindentation appeared to indicate higher adhesion to the AFM tip for S20 than for S10; however, rather than implying significant physico-chemical differences between the two materials, we are inclined to ascribe this effect to the larger deformability of S20, possibly determining a larger contact area between tip and material during indentation.
Figure 3.1. Typical maps of S10 and S20 samples obtained via AFM imaging in tapping mode (top), or nanoindentation (middle and bottom). The nanoindentation maps report the Young’s modulus (middle) calculated using a Hertzian model and the adhesion force (bottom) calculated from retraction curves (see Supplementary Information, Figure 3-1SI). In both cases, the maps showed a high mechanical homogeneity of the PDMS surfaces at a resolution of 500 nm (20 points in a 10 µm scale).
3.3.2 Surface modification via colloidal deposition

We have worked under the hypothesis that colloidal deposition is favoured by the presence of a hydrosoluble organic solvent, such as ethanol\textsuperscript{15}; by decreasing the incompatibility between PDMS and dispersing medium, such a solvent would increase both the overall dispersibility of the polymers and accelerate their dynamics (exchanges more likely due to a higher critical aggregation concentration), possibly also increasing the likelihood of interactions with the silicone substrates. We have further hypothesized that colloids showing time-dependent size/morphology in the dispersing medium may show better deposition, due to their increased dynamics; we have therefore investigated the time evolution of the size of the aggregates formed by Sil-GMMA polymers in two water/ethanol mixtures (Figure 3-2). Both 9:1 and 1:1 ethanol/water provided colloids with a larger size with increasing PDMS content, as it happens in water (see Chapter 2); however, in the first mixture the aggregates were larger sized and substantially stable for a period of 4 weeks, while the second one showed a clear time evolution with the colloids eventually presenting sizes similar to those in pure water. We have therefore performed all deposition experiments in 1:1 ethanol/water for a duration of three days, during which it is reasonable to assume to have the most rapid dynamics due to the most significant variations in aggregate size.

![Figure 3-2](image-url)  
*Figure 3-2.* Time dependency of the size of Sil-GMMA aggregates in 10 mg/mL water/ethanol 9:1 (left) and 1:1 (right) mixtures.
**Infrared spectroscopy.** Under these conditions, the deposition of Sil-GMMA1-5 was qualitatively evaluated using ATR-IR spectroscopy (Figure 3-3). The intensity of the C=O stretching peak was clearly inversely proportional to the GMMA content; the copolymers Sil-GMMA4-5 with a lower PDMS content were therefore not employed in the rest of the study.

![ATR-IR spectra of silicone substrates](image)

**Figure 3-3.** ATR-IR spectra of silicone substrates (S10) unmodified and after 3 day exposure to Sil-GMMA5, Sil-GMMA4, Sil-GMMA3, Sil-GMMA2 and SilGMMMA1 dispersions (10 mg/mL in EtOH/H$_2$O 1:1). The insert shows the region of the C=O stretching. The carbonyl absorption has a low intensity, in comparison to that of Si-C stretching from the underlying silicone; this derives from the penetration depth of the IR radiation in the substrate, which is in the range of microns, while the adsorbed films are obviously much thinner.

**Water contact angle.** In order to estimate the efficiency of Sil-GMMA1-3 coating and the possibility of re-organization of the corresponding surface layers, we have measured the advancing and receding water contact angles on freshly modified substrates and after two weeks of storage in air or in water (Figure 3-4). All experiments were typically performed at a concentration of 10 mg/mL; it is noteworthy that, in the range 5-20 mg/mL, the Sil-GMMA concentration water did not significantly affect the contact angle of the coated substrates (see Supplementary Information, Figure 3-2SI), suggesting a rather complete coverage of the silicone surface.
Figure 3-4. Top: advancing (A) and receding (B) contact angles of S10, unmodified and after a 3-day exposure to 10 mg/mL Sil-GMMA1-3 dispersion in 1:1 ethanol/water. The measurements were performed on freshly prepared samples (grey columns) and after storage in air (patterned columns) or water for 14 days (white columns). Bottom: advancing (C) and receding (D) water contact angles of substrates with different cross-link densities, unmodified and after a 3-day exposure to 10 mg/mL Sil-GMMA1 dispersion in 1:1 ethanol/water. The measurements were performed on freshly prepared samples (grey columns) and after storage in water for 14 days (white columns).

Neither the deposition of Sil-GMMA3 (Figure 3-4A and B), nor the use of S50 (Figure 3-4C and D) caused appreciable modification of the contact angles, suggesting small and/or unstable deposition: this is hardly surprising for Sil-GMMA3, which forms more stable and higher curvature aggregates in water and therefore is possibly more prone to desorption. On the contrary, the insignificant effects on S50 may be related to the ease of migration of the polymers from the surface to the bulk of this poorly cross-linked material. Both Sil-GMMA3 and S50 were therefore abandoned, concentrating the study on the remaining two polymers and two substrates.
In all these systems, no significant difference was recorded between Sil-GMMA1 and 2 (Figure 3-4A and B) or between S10 and S20 (Figure 3-4C and D). In general, the surface adsorption lowered more the receding than the advancing contact angles, suggesting significant re-organization of the coated surfaces during wetting/de-wetting cycles. This is further confirmed observing the effect of storage: lower contact angles were recorded immediately after preparation or upon storage in water, but both advancing and receding angles of the surfaces stored in air (Figure 3-4A and B) were statistically indistinguishable from those of the unmodified substrates, which suggests the migration of the polymer deep in the silicone bulk. Such phenomena of hydrophobic recovery are well-known in silicones\textsuperscript{25,26,35}; the high mobility of the PDMS matrix (glass transition temperatures around \(-120^\circ\mathrm{C}\)) allows rather rapid surface-to-bulk migration of hydrophilic groups to occur during prolonged exposure to air.

Last, it is noteworthy that lower contact angles were also characterized by large experimental errors, since different regions of the coated samples appeared to be largely different in surface hydrophilicity; we ascribe this rather to a differential migration of the physisorbed Sil-GMMAs in the silicone bulk (all samples were dried for 30 min prior to the measurements) than to a real patchy nature of the deposition.

**XPS.** The deposition of the two polymers on S10 was further analyzed using XPS (Table 3-2). The composition of the upper layers of unmodified S10 (take-off angle (t.o.a.) = 10°), was considerably different from that expected from pure PDMS, with a significant increase in its oxygen content independent of the storage conditions (air or water); the composition of deeper layers (t.o.a. = 90°) was on the contrary closer to that of PDMS.

This is not surprising: similar results on untreated Sylgard 184 with the same base/crosslinker ratio were obtained by the group of Vancso\textsuperscript{35,36} and were interpreted as a consequence of the presence of silica fillers; this may be due to a partial segregation of the filler during the hydrosilylation-based cross-linking.

In comparison to S10, Sil-GMMAs should increase the carbon content and decrease the silicon one, presenting also traces of bromine. Small differences were recorded between unmodified and coated samples: at a t.o.a. = 10° the silicon content appeared slightly reduced and bromine could be measured, although only on samples stored in water. At a
further analysis, high resolution C 1s spectra clearly showed the presence of carbonyl groups (peak at 288.9 eV, shifted of 3.9 eV relative to saturated hydrocarbon\textsuperscript{27}) after coating and upon storage in water (Figure 3-5B, compared to Figure 3-5A); this was accompanied by a stronger C-O signal and by the presence of Br atoms at a t.o.a of 10° (see Supplementary Information, Figure 3-3SI), while no photoelectrons from Br were detected at a t.o.a = 90° on coated samples or on unmodified samples at t.o.a.=10°.

Table 3-2. Surface composition (% atom) of silicone disks (S10) unmodified and treated with Sil-GMMA1-2 copolymers after storage for 14 days in air or water

<table>
<thead>
<tr>
<th>Sample</th>
<th>C  \textsuperscript{a}</th>
<th>O  \textsuperscript{a}</th>
<th>Si  \textsuperscript{a}</th>
<th>Br  \textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDMS</td>
<td>50</td>
<td>25</td>
<td>25</td>
<td>-</td>
</tr>
<tr>
<td>Sil-GMMA1</td>
<td>Theoretical composition</td>
<td>57</td>
<td>31</td>
<td>12</td>
</tr>
<tr>
<td>Sil-GMMA2</td>
<td></td>
<td>59</td>
<td>32</td>
<td>9</td>
</tr>
<tr>
<td>S10 (unmodified)</td>
<td>47</td>
<td>47</td>
<td>30</td>
<td>26</td>
</tr>
<tr>
<td>Sil-GMMA1</td>
<td>Stored in air</td>
<td>48</td>
<td>49</td>
<td>30</td>
</tr>
<tr>
<td>Sil-GMMA2</td>
<td></td>
<td>49</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td>S10 (unmodified)</td>
<td>49</td>
<td>49</td>
<td>30</td>
<td>24</td>
</tr>
<tr>
<td>Sil-GMMA1</td>
<td>Stored in water</td>
<td>50</td>
<td>49</td>
<td>30</td>
</tr>
<tr>
<td>Sil-GMMA2</td>
<td></td>
<td>50</td>
<td>48</td>
<td>30</td>
</tr>
</tbody>
</table>

\textsuperscript{a} t.o.a. = 10° (left) and 90° (right). Considering that 95% of the observed photoelectron signal derives from a layer thickness $3\lambda \sin(t\text{o.a.})$, with C1s electron mean free path ($\lambda$) = 2.9 nm (value obtained for AlK$\alpha$ X-ray photoemitted electrons\textsuperscript{37}), 10° and 90° should correspond, respectively, to 1.5 and 8.7 nm.

Figure 3-5. XPS C 1s high resolution spectra (t.o.a. 10°) of silicone substrate (S10) unmodified (A) and modified with Sil-GMMA1 (B) after storage in water for 14 days. The presence of the C-C and C-O bonds in the left spectrum is due to the structure of the cross-linkers and silanizing agents used in Sylgard 84. The increased presence of both, in addition to that of carbonyl groups clearly indicates the presence of PGMMA chains.
XPS therefore broadly confirmed the presence of Sil-GMMA1-2 on S10, but in rather small quantities and only in the upper surface layers (first 1 or 2 nanometers) and upon storage in water. Confirming the contact angle data, a hydrophobic recovery appeared to cancel the presence of the Sil-GMMA polymers upon storage in air.

**AFM.** The AFM analysis of PDMS surfaces (S10 and S20) coated with Sil-GMMA1 is currently ongoing. Preliminary data obtained after 24h in air showed the presence of large aggregates on the surface of both S10 and S20 (Figure 3-6). These aggregates were composed by 3D irregular objects with a height up to more than a 100 nm, completely or in part surrounded by a flat, about 20 nm-thick film (Figure 3-6, top). The phase angle was virtually identical for both components, which on the other hand differed much from the PDMS substrates (bottom pictures in Figure 3-6); this indicates that the aggregates are homogeneous in composition and differ both chemically and mechanically from the substrate, therefore it is easy to identify them as Sil-GMMA1. The morphology of the aggregates could stem from a progressive contraction of a Sil-GMMA1 film due to the prolonged exposure to air; due to high energy of the air/GMMA interface, the thin Sil-GMMA1 film may reorganize in 3D domains with inward-facing GMMA blocks. The possible penetration of the aggregates in the PDMS substrate would then further minimize the surface energy by reducing the air/material interface, rationalizing both XPS and contact angle data.

Further AFM investigations (height and phase images, Young’s modulus and adhesion forces measurements on freshly prepared samples and at different time points during drying) are on going, such experiments would provide additional information on the mechanical and physical surface properties of Sil-GMMA1 modified samples.
Figure 3-6. AFM height (top) and phase (bottom) images of Sil-GMMA1-covered S10 (left) and S20 (right) samples dried for 24h in air. In the magnified details of the top pictures, the arrows show the direction of the height scans presented in the graphs. In all cases, a rather homogeneous, 20 nm-thick film can be recognized (white bar in graphs).
3.3.3 Protein adsorption

The adsorption of (plasma) proteins to the surfaces of synthetic materials is widely recognized as one of the most important causes of cell (platelet, leukocyte) adhesion and ultimately foreign body reaction. In general, surface heterogeneities, electrostatic or other polar interactions can considerably affect protein adsorption\textsuperscript{38,39}, but on PDMS materials it is generally accepted that this occurs mainly due to the hydrophobic effect, i.e. the entropy-driven release of ordered water from silicone surfaces. Therefore, the surface hydrophilicity caused by Sil-GMMA adsorption may have a significant influence on the amount and quality of adsorbed proteins.

For this study we have employed bovine serum albumin (BSA) and bovine plasma fibrinogen (BPF), which are used as model for the corresponding human proteins; in humans albumin is the most abundant plasma protein, with a concentration of about 30-50 mg/mL, while that of fibrinogen is 1-3 mg/mL. Albumin is one of the first proteins to adsorb on the surface of implanted biomaterials\textsuperscript{40-42}; its deposition is often considered to act as a sort of surface passivation, since it does not specifically encourage platelet or leukocyte adhesion. On the contrary, the adsorption of fibrinogen is well known to trigger undesired reactions\textsuperscript{6,7}.

Adsorption experiments were performed on S10 freshly coated with Sil-GMMA1-3. The amount of adsorbed protein was first evaluated via the bicinchonic acid (BCA) assay after desorption with sodium dodecyl sulfate (Figure 3-7A and B). Due to the unselective nature of the test, we have separately investigated BSA and BPF, which were used at concentrations similar to those in plasma (resp. 10 and 1 mg/mL, using 1 mg/mL BSA as a control).

The presence of Sil-GMMA2 and 3 did not appreciably affect the adsorption of BPF, but increased that of BSA; on the other hand, Sil-GMMA1 affected both, with a ~30% decrease in BPF and a ~100% increase in BSA. This effect is not new: using polyurethane-PGMMA hydrogels Mequanint et al. observed higher albumin and lower fibrinogen adsorption with increasing PGMMA content\textsuperscript{21}, thus showing PGMMA to have preferential interactions with albumin.
We have then used fluorescamine to detect the presence of primary amino groups directly on Sil-GMMA1-coated vs. unmodified S10, without desorption (Figure 3-7C). Upon reaction with amines, fluorescamine exhibits a maximum of emission at 460 nm when excited at 390 nm\(^4^3\); since we have analyzed only one type of protein at a time, the fluorescence intensity at 460 nm is directly proportional to the amount of protein adsorbed; due to higher sensitivity of the method, it also allowed to extend the study to more diluted protein solutions. Confirming the results of the BCA assays, at all concentrations we have recorded an increase in BSA and a decrease in BPF adsorption.

**Figure 3-5.** A. Amount of protein detached from unmodified and coated S10 and analyzed through the BCA assay; the substrates were incubated for 3 h in different protein solutions (BPF 1 mg/mL, BSA 10 and 1 mg/mL). B. The data of graph A are expressed in terms relative to the unmodified PDMS substrate (S10). C. Results of the fluorescamine assay (fluorescence intensity at 460 nm) on S10 unmodified and modified with Sil-GMMA1 after 3h of incubation in different protein solutions (BPF 1, 0.5 and 0.1 mg/mL, BSA 10, 1 and 0.1 mg/mL). D. Relative comparison of the affinity of unmodified or Sil-GMMA1-coated S10 for the two proteins. The data are obtained dividing the BPF fluorescence intensity (fluorescamine assay) or amount per surface area (BCA assay) by the corresponding figures for BSA.
Figure 3-7D graphically summarizes the comparison of the relative affinity of Sil-GMMA1-coated and unmodified S10 for the two proteins, at a fibrinogen/albumin ratio similar to that in blood (1:10); please note that these data refer to individual adsorption experiments and not to competitive binding. Both analytical techniques showed a clear switch from a BPF-philic to a BSA-philic character upon surface coating, which can be promising for decreasing cell attachment and the likelihood of foreign body reaction in vivo. However it has been shown that adsorbed albumin is easily replaced by proteins like fibrinogen, which usually have higher affinity for surfaces. Obviously, the extent of this effect depends on the type of surfaces. Therefore studies investigating competitive proteins adsorption, or albumin replacement by fibrinogen will be undertaken. A further caveat is that protein adsorption may not provide an unequivocal understanding of the biological performance, since the degree of biological activity of adsorbed proteins depends on their molecular spreading (degree of unfolding) and/or variations in the orientation or accessibility of the cell binding domains, but also on the composition of the adsorbed layer: for example, there is evidence that co-adsorbed albumin influences the “surface biological activity” of fibrinogen.

3.4 Conclusions

We have demonstrated that the physisorption of PDMS-based macroamphiphiles containing hydrophilic PGMMA blocks (Sil-GMMAs) is possible and provides significant surface coverage when polymers with high PDMS content are employed.

The coating procedure determines a rather modest decrease in the contact angle and the coating agents tend to migrate to bulk PDMS upon prolonged exposure to air. Nevertheless, when kept in a water environment to avoid hydrophobic recovery, the surface layers have a profound effect on protein adsorption; the resulting switch from a preferentially fibrinogen-adsorbing (more cell-adhesive) character to a preferentially albumin-adsorbing (less cell-adhesive) can be very beneficial for the modulation of cell adhesion and activation.

Since the simplicity of this technique makes it attractive for the modification of silicone surfaces, these findings suggest that a stabilization of the surface layers e.g. via covalent cross-linking may further improve the performance of the coatings.
3.5 References


(33) Brown, X. Q.; Ookawa, K.; Wong, J. Y. Biomaterials 2005, 26, 3123.
3.6 Supplementary information

Figure 3-1SI. AFM nanoindentation curves for unmodified S10 and S20 samples measured in air.

Figure 3-2SI. Advancing (left) and receding (right) water contact angles of silicone substrates (S10) unmodified and after 3 days in SilGMMA dispersions (various concentrations in EtOH/H₂O 1:1), on freshly prepared samples (grey columns) and after storage in water for 14 days (white columns).
Figure 3-3SI. Left (A): XPS wide scan (t.o.a. 10°) of an unmodified silicone disk (S10) stored for 14 days in water. Right (B): XPS Br 3d scans (t.o.a. 10°, 3 different positions) of silicone substrate (S10) modified with Sil-GMMA1 and stored in water for 14 days.
4 Attempts of enzymatic derivatization of (glycerol monomethacrylate) containing polymers

Abstract

The use of enzymes in synthetic chemistry provides several advantages over “conventional” catalysts: enzymes generally offer high selectivities, they operate under mild conditions and are biocompatible. In this preliminary study, we investigated the possibility of using the structural similarity between polymers of glycerol monomethacrylate (GMMA) and glycerol in order to incorporate new functionality on GMMA containing polymers via enzymatic post-polymerization modifications. Spectrophotometric assays allowed the observation of activity of two different enzymes (glycerol kinase and glycerol dehydrogenase) when using polyGMMA instead of glycerol as substrate, although enzymatic activities were greatly reduced. However substrates conversions were always low and a $^{31}$P NMR experiment confirmed that polyGMMA was not significantly phosphorylated by glycerol kinase.
4.1 Introduction

The use of enzymes is not restricted to their natural substrates and aqueous reaction media; they are known to catalyze many synthetic transformations in organic synthesis\(^1\). Enzymes offer several catalytic advantages such as high chemo-, regio- and stereo-selectivity, the ability to operate under mild conditions, catalyst recyclability and biocompatibility. These advantages have also attracted polymer scientists; several examples of enzymatic polymer synthesis can be found in literature and many of them are discussed in a recent review from Kabayashia and Makino\(^4\). The development of new polymeric materials by enzymatic modification reactions of existing polymers, however, presents far less examples than those of polymer synthesis by enzymatic polymerization reactions. Nevertheless some studies have already investigated the possibility of introducing new functionalities in a specific manner on polymers under mild reaction conditions, and a small number of reviews discuss some examples\(^4,5\).

Amongst the enzymatic modifications of polymers reported in the literature, lipases are the most widely used enzymes. For example, (meth)acrylated polydimethylsiloxanes (PDMSs) and polyethylene glycols (PEGs) were prepared by lipase-catalyzed transesterifications and some products were further modified by Michael-type addition, once again lipase-catalyzed\(^6\).

In this study, we particularly focused our attention on the enzymatic modification of glycerol monomethacrylate (GMMA) polymers. Poly(glycerol monomethacrylate) (PGMMA) was selected as a suitable building block for possible biomedical applications due to its high hydrophilicity and functionality. PGMMA has been used to prepare drug delivery systems\(^7,9\) and a number of hydrogels\(^10-15\) for diverse applications. As it can be seen in Scheme 4-1, the structure of GMMA is differing from that of glycerol by only one substitution. We therefore investigated the possibility of using glycerol specific enzymes to introduce new functionality on PGMMA.

The introduction of an oxidized function on the side chain of PGMMA, offering the possibility of further modifications, was first investigated using glycerol dehydrogenase (GDH). GDH catalyzes the oxidation of glycerol to dihydroxyacetone using the cofactor nicotinamide adenine dinucleotide (NAD) and it has been shown that in addition to glycerol, GDH also accepts a number of substrates having substituents on one hydroxyl group\(^16-18\). The activity of enzymes from the dehydrogenases family with polymers like
PEG has been studied in the context of understanding PEG metabolism and biodegradation\textsuperscript{19,20}, however we did not find previous examples of the use of dehydrogenase with the intention to introduce functionality on a synthetic polymer.

The second enzyme we used was glycerol kinase (GK) which catalyzes the phosphorylation of glycerol to form \((R)\)-glycerol 1-phosphate (or \(L\)-glycerol 3-phosphate) using adenosine triphosphate (ATP). Previous work on the substrate specificity of GK has also shown that it accepts a number of substituents in place of one hydroxyl group of glycerol\textsuperscript{21-24}. As far as we know, the only example of kinase polymer modification reported in the literature is an enzymatic phosphorylation of cellulose which has shown that even with very low phosphorylation conversion (0.03%) the materials properties were improved in term of dyeability and flame resistance. We were particularly interested in observing the effect of introducing a phosphoryl group on GMMA units on a different property: the self-assembly of PGMMA containing amphiphilic block copolymers. It has been previously demonstrated that the block copolymer used in this study (Sil-GMMA6) self-assembled into micelles of a hydrodynamic diameter around 35 nm where polydimethylsiloxane formed a central hydrophobic core and PGMMA a hydrophilic corona in an aqueous environment. On the other hand, block copolymers with the same block composition but containing higher hydrophobic block ratios were shown to self-assemble into worm-like micelles or vesicles (see Chapter 2). Introducing a charged group (phosphate) onto the neutral hydrophilic block of Sil-GMMA copolymers might bring interesting changes to their self-assembled morphologies: by increasing the hydrophilicity of the PGMMA block, one could expect to see a vesicle-micelle type of transition.

Scheme 4-1. Structure of the substrates used in this study (a) and scheme of the reactions catalyzed by GDH (b) and GK (c)
4.2 Experimental section

4.2.1 Materials

All materials were used as received from the supplier. 2,2’-bipyridine, β-nicotinamide adenine dinucleotide (NAD) hydrate, β-nicotinamide adenine dinucleotide reduced (NADH) dipotassium salt, adenosine 5’-triphosphate (ATP) disodium salt hydrate, copper chloride (CuCl), ethyl α-bromoisoobutyrate, ammonium sulfate suspension of glycerol kinase (GK) from *E. Coli*, lyophilized powder of glycerol dehydrogenase (GDH) from *Cellulomonas sp*, glycine, lyophilized powder of lactate dehydrogenase (LDH) from rabbit muscle, magnesium sulfate heptahydrate (MgSO$_4$·7H$_2$O), phospho(enol)pyruvic (PEP) acid monopotassium salt and lyophilized powder of pyruvate kinase (PK) from rabbit muscle were purchased from Aldrich (Gillingham, UK). Glycerol monomethacrylate (GMMA) was purchased from Cognis (Southampton, UK). Diethyl ether and methanol was purchased from Fisher Scientific, (Loughborough, UK). Deuterated water was purchased from Goss Scientific (Nantwich, UK). Phosphate buffer saline (PBS) was bought from Oxoid (Basingstoke, UK).

ABA triblock copolymers of polydimethylsiloxane and PGMMA (Sil-GMMA6) were synthesized according to a previously reported procedure (see Chapter 2).

Dialysis of the polymer solutions was performed with SpectraPor regenerated cellulose membranes with 1,000 or 3,500 g/mol molecular weight cut-off.

4.2.2 Physico-chemical characterisation

*Molecular characterization*

**Spectroscopy:** $^1$H and proton decoupled $^{31}$P NMR spectra were recorded on 1 wt.% polymer solutions in deuterated water using a 300 MHz Bruker NMR spectrometer. FT-IR spectra were recorded in ATR mode on a Tensor 27 Bruker spectrometer.

**Enzymatic assays**

**UV-vis experiments:** Spectra were recorded on a Bio-Tek Synergy 2 multi-mode microplate reader.
GDH assay: Substrate dehydrogenation was directly followed by the appearance of NADH, monitored by spectrophotometry. In a typical experiment, 25 µl of substrate (aqueous solutions of glycerol, GMMA, PGMMMA DP10 and DP50 or SilGMMA6), 20 µl of GDH solution in KH$_2$PO$_4$ buffer at pH 7.5 containing MnCl$_2$ and 155 µl of NAD and NH$_4$(SO$_2$) aqueous solutions were diluted in 50 µl of K$_2$CO$_3$ buffer at pH 10.0 in a 96 well plate prepared at 5°C, so that the final volume in each well was 250 µl, the final pH of the solution was 9.0 and the final concentrations were the following: varying from 0 to 100 mM for the substrates, 0.04 u/ml for GDH, 100 mM for K$_2$CO$_3$, 1.6 mM for KH$_2$PO$_4$, 0.002 mM for MnCl$_2$, 33 mM for (NH$_4$)$_2$SO$_4$ and 5 mM for NAD. The absorbance at 340 nm was recorded at 37°C every minute for 30 min for kinetic measurements. For each time point, the NADH concentration (C) was calculated from the absorbance at 340 nm (A) through the Beer-Lambert law $A = dC$, using $\varepsilon = 6.18$ mM$^{-1}$cm$^{-1}$ as the NADH molar extinction coefficient$^{21}$ and $l = 0.73$ cm as the path length calculated from our calibration. Initial reaction rates were then defined as $v_0 = dC/dt$ calculated from the linear regressions obtained when fitting the plots of NADH concentration in function of time between the time points 5 min (delay for temperature equilibration) and 10 min. In the case of slow reactions, time points up to 15 min were used.

In order to achieve higher dehydrogenation yields, the plate was stored at room temperature for 3 days and the absorbance was regularly measured.

GK assay: Substrate phosphorylation was monitored in a coupled system with PK and LDH by spectrophotometric analysis. In a typical experiment, 10 µl of substrate (aqueous solutions of glycerol, PGMMMA DP10 and DP50 or SilGMMA6), 30 µl of enzyme solution in PBS at pH 7.3 and 40 µl of aqueous reagent solution were diluted in 170 µl of glycine buffer at pH 9.8 or in PBS at pH 7.3 in a 96 well plate prepared at 5°C, so that the final volume in each well was 250 µl, the final pH was at 9.8 or 7.3 and the final concentrations were the following: varying from 0 (used as a blank) to 72 mM for the substrates, 4.3 u/ml for PK, 4.8 u/ml for LDH, 0.2 u/ml for GK, 1.7 mM for PEP, 5.6 mM for MgSO$_4$·7H$_2$O, 0.3 mM for NADH and 1.7 mM for ATP. The absorbance at 340 nm was recorded at 37°C every minute for 30 min for kinetic measurements. The data treatment using the decrease in NADH concentration calculated from $\Delta A = A_{\text{blank}} - A$ was similar to the one performed in the GDH assay.
In order to achieve higher phosphorylation yields, the plate was stored at room temperature for 2 days and the absorbance was measured daily.

For NMR experiments at pH 9.8, the concentrations of the reagents were all increased by 5 fold while the total volume was brought up to 5 ml. After 5 days of reaction, the volume was lowered to approximatively 1 ml under reduced pressure and 50% D$_2$O solutions were analyzed by $^{31}$P NMR. The polymer was purified by dialysis against deionized water. After freeze-drying, the product was analyzed by $^{31}$P and $^1$H NMR analysis in D$_2$O.

4.2.3 Preparative procedures

*Synthesis of poly(glycerol monomethacrylate) (PGMMA)*

PGMMA was synthesized according to a modified literature procedure$^{25}$. In a typical experiment, a mixture of ethyl 2-bromoisobutiryl (150 mg, 0.75 mmol), GMMA (2.24 or 11.19 g, respectively 7.5 and 37.6 mmol, corresponding to 10 and 50 equivalents to bromoisobutiryl) and 2,2'-bipyridine (232 mg, 1.5 mmol, 2 equivalents to bromoisobutiryl) were degassed by bubbling argon gas for 45 minutes followed by the addition of degassed methanol (12 ml, 50 wt.% solutions). Copper (I) chloride, (74 mg, 0.75 mmol, 1 equivalent to bromoisobutiryl) was then added under argon gas to start the polymerisation reaction. When the targeted degree of polymerisation (DP) was 10, the solution was stirred at room temperature for 1h while the reaction was stirred for 4h when DP 50 was targeted. Samples were collected for $^1$H NMR and the conversion was calculated using the ratios of integrated signals (at least 99% for the two polymers). The reaction mixture was then passed through a silica gel column to remove the copper catalyst. After evaporation of the solvent, the polymer with DP 50 was redissolved in methanol and precipitated three times in ice cold diethyl ether. Both polymers were finally purified by dialysis against deionised water. After freeze drying, white solids were collected (yields: 82-89 %).

$^1$H NMR (D$_2$O): δ = 0.8-1.3 (broad, -C(CH$_3$)$_2$CH$_2$-), 1.8-2.2 (broad, -C(CH$_3$)$_3$CH$_2$-), 3.5-4.2 (broad, -CH$_2$CH(OH)CH$_2$OH) ppm.

FT-IR (film on ATR crystal): 3650-3050 (v OH), 2985 (v as CH), 2943 (v CH), 2886 (v s CH), 1715 (v C=O ester), 1477, 1451 (δ O-CH$_2$), 1391, 1322, 1245, 1151 (v C-O-C), 1042, 984, 929, 853, 654 cm$^{-1}$.
4.3 Results and discussion

4.3.1 Glycerol Dehydrogenase (GDH)

Firstly, GDH activity with GMMA and its homopolymer was tested by measuring the initial reaction velocity, which can be monitored by the appearance of NADH absorbance at 340 nm (Scheme 4-2), while varying the substrate concentration. It has been previously reported that the optimum pH when working with GDH is around 9\textsuperscript{16}, all our experiments with GDH were therefore performed at pH 9.0.

\[
\text{GDH} \quad \text{glycerol} + \text{NAD} \rightleftharpoons \text{dihydroxyacetone} + \text{NADH}
\]

Scheme 4-2. Principle of the GDH assay, the reaction velocity or substrate conversion are measured at pH 9.0 by following the appearance of NADH absorbance at 340 nm.

Even though GDH initial reaction velocity was greatly reduced when substituting glycerol with GMMA or PGMMA (for example at 10 mM: from 95 to 97% of reduction compared to glycerol), some NADH production could still be observed (Figure 4-1 left). More importantly, the enzyme activity lasted up to 3 days as can be seen in the continuous increase in NADH production (Figure 4-1 right). Surprisingly, no major difference was observed between GDH activity with a small molecule like GMMA monomer, an oligomer (PGMMA DP 10) and a longer polymer (PGMMA DP 50). The difference in the chemical nature of the substrate, rather than steric interference, was thought to be the reason for the decrease in activity.

However, when taking into account the initial substrate concentrations, the oxidation conversions reached were not satisfactory when using PGMMA as a substrate (≤ 2 mol%). Additionally, a size effect was this time observed: when using similar concentration of GMMA units, but presented in the form of micelles corona (Sil-GMMA6), almost no NADH production was detected (Figure 4-1 right).
Figure 4-1. Left: GDH initial reaction velocities calculated from the increase in NADH absorbance in function of the concentration of glycerol (squares), GMMA (circles) or GMMA units in PGMMA DP10 (up triangles) and PGMMA DP50 (down triangles) at pH 9.0. (n= 6, error bars were smaller than the symbols). Right: Substrate conversions calculated from the increase in NADH absorbance at pH 9.0 using two different substrates: PGMMA DP50 with GMMA units concentration of 10 mM (closed circles) and 5 mM (open circles) or Sil-GMMA6 with GMMA units concentration of 10 mM (closed triangles) and 5 mM (open triangles). The molar conversion of glycerol 5 mM (up to 19.1 mol %) was also determined as positive control; however the data were not shown for clarity of reading (n= 6).

4.3.2 Glycerol Kinase (GK)

Spectrophotometric assay

The activity of a second enzyme, GK, with GMMA containing polymers was also tested by measuring the initial reaction velocity while varying the substrate concentration. The GK assay used in this study was more complicated than the one used for GDH, as the ADP production was indirectly monitored by the disappearance of NADH absorption at 340 nm in a coupled system with pyruvate kinase (PK) and lactate dehydrogenase (LDH) (Scheme 4-3). It has been previously reported that the optimum pH when working with GK is around 9.8. Nevertheless, in order to avoid the doubt that the enzyme activity detected was due to the cleavage of the ester bond present in GMMA, which if complete would form glycerol and poly(methacrylic acid), GK activity was also tested at pH 7.3.

\[
\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \alpha\text{-glycerophosphate} + \text{ADP}
\]

\[
\text{ADP} + \text{PEP} \xrightarrow{\text{PK}} \text{ATP} + \text{pyruvate}
\]

\[
\text{pyruvate} + \text{NAD}^+ + H^+ \xrightarrow{\text{LDH}} \text{lactate} + \text{NADH}
\]

Scheme 4-3. Principle of the GK assay, the decrease in NADH absorbance at 340nm is measured in a coupled system with PK and LDH.
When experiments were performed at pH 9.8, the kinetics results presented similarities with the ones for GDH: even if some NADH consumption could be observed, the system reaction velocity was reduced when substituting glycerol with PGMMA (however less intensively, for example reductions of 79 to 82 % were observed at substrate concentrations of 12 mM when compared to glycerol). On the other hand, decreasing the pH from 9.8 to 7.3 appeared to disfavor the substrate specificity of our system: the reaction velocity appeared to be reduced with glycerol while the one with GMMA and PGMMA, in the form of oligomer (DP10) or polymer chain (DP50), was increased (Figure 4-2). Contrary to GDH results, higher GK activities were obtained when using the monomer GMMA as substrate than when PGMMA was used. It is possible that bulky substrates such as PGMMA cannot easily access the GK active site, even if no major difference was observed between an oligomer (PGMMA DP 10) or a longer polymer chain (PGMMA DP 50).

![Figure 4-2. GK initial reaction velocities calculated from the decrease in NADH absorbance in function of the concentration of glycerol (squares), GMMA (circles) and GMMA units in PGMMA DP10 (up triangles) and PGMMA DP50 (down triangles) at pH 7.3 (left) or pH 9.8 (right). (n= 6, error bars were ommitted for ease of reading)](image)

In order to have a better understanding of the GK system and of the possibilities to use this reaction for post-polymerization of PGMMA on a preparative scale, a longer timescale study was necessary. In this case, using the spectrophotometric assay appeared to be more challenging than when studying GDH. The NADH consumption did not increase (or even appeared to reverse when using glycerol as a substrate) during a 48 h experiment (Figure 4-3). As with the GDH study, the PGMMA phosphorylation conversions obtained were not satisfactory (< 2 mol%) and presenting the GMMA units in the form of micelles (Sil-GMMA6) appeared to inhibit almost all enzyme activity,
probably indicating another effect of steric interference. However an important limitation of the spectrophotometric assay has to be noted: the absorbance of the blank (a solution containing all the enzymes, cofactors and PEP but without glycerol or similar substrate) kept decreasing during the assay probably due to the production of ADP from ATP degradation. Because NADH conversions were calculated using the difference in absorbance between the blank and the assays, conversions might have been underestimated and, in the case of glycerol, this resulted in an apparent “back reaction”: conversions quickly rose due to a fast reaction but instead of following a plateau the calculated glycerol conversion declined due to the decrease in blank absorbance.

![Figure 4-3](image)

**Figure 4-3.** GK substrate conversions calculated from the decrease in NADH absorbance at pH 9.8 using three different substrates at a concentration of 5 mM: glycerol (squares), PGMMA DP 50 (circles) and Sil-GMMA6 (triangles). The absorbance of the blank kept decreasing during the assay, probably due to ATP, PEP or NADH degradation and as a result, substrate conversion might be underestimated and the apparent “back reaction” observed with glycerol after reaching a plateau might be an artefact. (n= 6, when omitted error bars were smaller than the symbols)

**NMR experiments**

In order to confirm that the observation of low conversions in the spectrophotometric assay was not due to the limitations of the method, an additional analytic technique was necessary and nuclear magnetic resonance spectroscopy (NMR) was performed after reacting PGMMA with GK at pH 9.8 for 3 days (Figure 4-4). The PK and LDH system was still used alongside GK as it provides an ATP “recycling service”, avoiding the accumulation of ADP acting as GK inhibitor\(^{27}\). Proton decoupled \(^{31}\)P NMR was first performed on the sample after reaction, and it can be observed that most of the ATP had formed ADP by the disappearance of the ATP peak around -22 ppm while the peaks around -5 and -11 ppm did not disappear. However, this ATP consumption was not
accompanied with substrate phosphorylation: even if a small new peak appeared around 4 ppm, it was not present anymore after dialysis, indicating that it was not the resonance of phosphorylated PGMMA, but most probably of glycerol phosphate. Even if the polymer appeared intact after purification (as it can be seen in the $^1$H NMR, Figure 4-5 right), it is possible that small amounts of GMMA units were hydrolyzed under the basic conditions used in this experiment to form glycerol which then reacted with the enzymatic system to form glycerol phosphate. However the presence of impurities such as unreacted GMMA monomer or an electrostatic repulsion between the negatively charged enzyme (the isoelectric point of GK is around 4.2) and the partially phosphorylated PGMMA could also explain the low or inexistent phosphorylations.

![Figure 4-4.](image)

**Figure 4-4.** Left: Proton decoupled $^{31}$P NMR spectra in D$_2$O of PGMMA phosphorylation (after 3 days of reaction at pH 9.8) non purified (a) and after dialysis (b). The peaks marked were assigned to the following groups: 1: glycerol phosphate, 2: HPO$_4^{-}$ from PBS buffer, 3: PEP, 4: $\gamma$-ATP and $\beta$-ADP, 5: $\alpha$-ATP and $\alpha$-ADP, 6: $\beta$-ATP. Right: $^1$H NMR spectrum of the purified PGMMA in D$_2$O. The peaks marked were assigned to the following groups (with corresponding integrals in brackets): 1: HOCH$_2$C(H)(OH)CH$_2$- from PGMMA side group (5.2), 2: CH$_2$ from the main chain (1.8), 3: CH$_3$ from the main chain (3.0).

In order to have a better understanding of the system, additional NMR studies could also be performed at pH 7.3. Another possibility would be to use a copolymer positively charged under the reaction conditions (such as a copolymer of GMMA and units comprising quaternized amino groups) as a substrate in order to maximize the electrostatic interactions between GK and the polymer. On the other hand, a strong interaction with a polyelectrolyte might inhibit enzyme activity, a precise study of the influence of the positively charged unit composition would therefore be necessary.
4.4 Conclusions

Enzymatic activities using GK and GDH to modify PGMMA polymers was observed using spectrophotometric assays, even if, as expected, the activities were greatly reduced when compared to the ones obtained with the enzymes natural substrate (glycerol). However, when taking into account the relatively high initial substrate concentrations necessary to observe enzyme activity, the oxidation or phosphorylation conversions obtained were low (< 2 mol%). Additionally, the enzymes activity seemed to be inhibited when PGMMA polymers were self-assembled into micellar structures. It therefore appears that the enzymes stereo- and chemoselectivity for glycerol do not easily allow their use with different substrates. In conclusion, the use of GK or GDH to modify PGMMA containing polymers on a preparative scale seems unlikely without further research and important optimization work.

4.5 References

5 Conclusions and Outlook

During the last few decades, silicones have been widely used for the preparation of biomedical devices. Unfortunately, non specific protein adsorption and cell adhesion can trigger undesired reactions. Because it is the surface of any implanted material which interacts first with its environment, biomaterial surface properties determine the first stages of the biological response. Therefore the interest in designing materials for biomedical applications presenting surfaces providing resistance to non-specific interactions but potentially showing specific and desirable ones with their surroundings is increasing.

Surface initiated polymerization techniques are an attractive approach to modify the surface of biomaterials: they allow the preparation of well-defined polymeric layers which have been shown to be stable and, using an appropriate protein repellent polymer, to inhibit non specific protein adsorption. On the other hand their application on a large scale is not easy due to the rather complex and multi-step processes usually involved.

On the other hand, despite its simplicity, the use of physisorption to reduce unspecific protein adsorption on PDMS surfaces upon exposure to biological fluids is generally little investigated, probably due to the difficulty to prepare stable coatings with this method. An “ideal” (simple, stable and efficient) silicone surface modification for biomedical applications is therefore still lacking. In this thesis, the design of PDMS containing amphiphilic copolymers for the modification of silicone surfaces by simple physisorption is presented, and although the surface hydrophobicity is recovered upon storage in air, a stabilization of the surface layers may further improve the performance of the coatings.

In more details, the convenience of a protection/deprotection route using the silylation of GMMA alcohols groups for the preparation of well-defined amphiphilic block copolymers of silicone via a controlled radical polymerization technique was demonstrated in Chapter 2. Those macroamphiphiles self-assembled in water environment to form various nano-structures depending on the ratio between hydrophilic and hydrophobic blocks. Preliminary experiments suggested that well-defined micelles formed by our macroamphiphiles did not interact with albumin, suggesting a “stealth” behaviour. Additional experiments investigating the interaction of albumin with other aggregates formed by those amphiphiles are on-going. The “stealth”
Conclusions and Outlook

behaviour of nanostructures formed by our PDMS-PGMMA block copolymers is also being tested using a different model protein (fibrinogen). However further studies are required to progress this first proof of principle, e.g. by studying the cell uptake of and/or activation of our nanostructures in vitro and in vivo.

In terms of general perspectives, the advantages of using such materials for drug delivery or imaging are multiple: their synthesis via controlled radical polymerization offers the possibility to tune the type of self-assembled nanostructures formed in aqueous environment by controlling the hydrophilic/hydrophobic blocks ratio. The hydrophobic building block (PDMS) is stable and inert while the hydrophilic corona (PGMMA) provides a “stealth” character. Additionally, the diol present on each repeating unit of PGMMA provides ease of further functionalization, for example with a therapeutic agent or a cross-linker in order to improve stability against dilution. Therefore, further studies investigating the drug delivery capacities of PDMS-PGMMA based systems could be interesting. We, on the other hand, focused our attention on another biomedical application: PDMS surface modifications.

In Chapter 3, we have demonstrated the feasibility of adsorbing PDMS containing macroamphiphiles on silicone surfaces by simple physisorption. The presence of our surface modifier on silicone surfaces was confirmed by FT-IR, XPS and a decrease in water contact angle. We have shown that although our surface coatings were not stable when stored in air, they were stable upon storage in aqueous environment. Additional AFM experiments investigating the mechanical (Young’s modulus) and physical (surface tension) changes induced by the adsorption of our surface modifier and its rearranging upon drying are ongoing.

The effect of our surface modification on protein adsorption was also investigated. Fibrinogen adsorption was decreased by the presence of our macroamphiphile with the highest PDMS content on the silicone surface while albumin adsorption appeared to increase. This study demonstrated the necessity to carefully design “protein-repellent” coatings, surfaces repelling one protein might be attracting another one. In our case, the preparation of surfaces repellent to fibrinogen and interacting with a passivating protein such as albumin is appealing. However, other factors, such as the degree of biological activity of adsorbed proteins and the composition of the adsorbed layer need to be considered when trying to control the interactions between a surface and its biological environment. Therefore additional competitive protein adsorption with possibly various
plasma proteins would provide a better understanding of the biological properties of our modified surfaces. Additionally, cell-adhesion studies will be necessary in order to fully evaluate the potential of such surfaces.

At the same time, a preliminary study investigating the possibility of using the structural similarity between PGMMA and glycerol in order to incorporate new functionality on PGMMA containing polymers via enzymatic post-polymerization modifications is presented in Chapter 4. The use of enzymes would provide several advantages over “conventional” catalysts: enzymes generally offer high selectivities, they operate under mild conditions and are biocompatible. Although spectrophotometric assays allowed the observation of enzymatic activity when using PGMMA instead of glycerol with two different enzymes (glycerol kinase and glycerol dehydrogenase), substrates conversions were always low. Additionally, a $^{31}$P NMR experiment confirmed that PGMMA was not significantly phosphorylated by glycerol kinase. On the other hand, we have shown that the substrate specificity of glycerol kinase was modulated by a change in pH. It is therefore possible that a more complete study of the pH dependency of glycerol kinase and dehydrogenase activities with non natural substrates such as PGMMA would provide improvements in substrate conversions. However it is highly possible that the enzymes stereo- and chemoselectivity for glycerol do not easily allow their use with different substrates, especially substrates of large sizes which are likely to not access enzymes “catalytic cavities”. Although attracting, post-polymerization modifications of PGMMA using glycerol kinase or dehydrogenase seem unlikely without significant additional studies and optimization work.