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Studies on Nanocomposite Coating Produced by Laser-assisted process to Prevent Silicone Hydrogels from Bio-fouling

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Abstract

In this thesis, silver nanoparticles incorporated into polyvinylpyrrolidone (PVP) were deposited on silicone hydrogel to improve the hydrophilicity of the silicone hydrogel and prevent the growth of bacteria. Two different processes were employed to produce Ag nanoparticles: (1) Process-A is a photochemical reduction; (2) Process-B is laser ablation in liquid. Following that, MAPLE process was employed to deposit the Ag-PVP nanocomposites on the surface of silicone hydrogel. A solid-state pulsed laser (Nd:YAG) with a wavelength of 532 nm at a fluence of 50.4 mJ/cm² was used in the MAPLE system to deposit Ag-PVP nanocomposite coating. Our results indicate that adsorption of bovine serum albumin (BSA) protein on silicone hydrogels with Ag-PVP nanocomposite coating produced by Process-A followed by MAPLE and Process-B followed by MAPLE were found to be 14.11 μg/cm² and 13.73 μg/cm², respectively. In addition, the relative viability of bacterial colonies on Ag-PVP coated silicone hydrogel declines to 44% and 26% in Process-A followed by MAPLE and Process-B followed by MAPLE respectively, after 6 hours. The value of Young’s Modulus of bare silicone hydrogel, Ag-PVP coated silicone hydrogel prepared by process-A and process-B followed by 2 hours of MAPLE deposition were found to be 0.57 MPa, 0.62 MPa and 0.66 MPa respectively. The value of toughness of bare silicone hydrogel, Ag-PVP coated silicone hydrogel prepared by process-A and process-B followed by 2 hours of MAPLE deposition were found to be 15.14 MJ/m³, 21.54 MJ/m³ and 22.01 MJ/m³ respectively, under uniaxial mechanical test. The mechanical properties were studied under biaxial test as well by using the constitutive model proposed by Humphrey et al.

Keywords
Silicone hydrogel; silver nanoparticles; polyvinylpyrrolidone (PVP); nanocomposite; surface coating; Matrix assisted pulsed laser evaporation (MAPLE); protein adsorption; antibacterial property; mechanical properties.
Co-Authorship Statement

Chapter 1 (Introduction), Chapter 2 (Background and literature review) and Chapter 3 (Experiment Procedures) were written by Vishnuvardhana Wuppaladhodi with some suggestions given by Dr. Jin Zhang. Chapter 4 and Chapter 5 includes the research studies and mechanical testing of nanocomposite coated silicone hydrogel, which have been published or under preparation. Other invaluable help for my thesis are named in the acknowledge section.
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<th>Full Form</th>
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<tr>
<td>POOPT</td>
<td>Poly[3-(4-octyloxyphenyl)thiophene]</td>
</tr>
<tr>
<td>MEH-CN-PPV</td>
<td>poly[2-methoxy-5-(2-ethylhexyloxy)-1,4-(1-cyanovinylene) phenylene]</td>
</tr>
<tr>
<td>MEH-PPV</td>
<td>poly[2-methoxy-5-(2-ethylhexyloxy)-1,4-phenylene vinylene]</td>
</tr>
<tr>
<td>PFO</td>
<td>Poly(9,9-dioctylfluorene)</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>PMMA</td>
<td>Polymethylmethacrylate</td>
</tr>
<tr>
<td>PLA-PVA</td>
<td>Polylactic acid – Polyvinyl Alcohol</td>
</tr>
<tr>
<td>PVP</td>
<td>Polyvinylpyrrolidone</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl Sulfoxide</td>
</tr>
<tr>
<td>P3HT</td>
<td>Poly(3-hexylthiophene)</td>
</tr>
<tr>
<td>PCBM</td>
<td>[6,6]-Phenyl-C_{61}-butyric acid- methyl-ester</td>
</tr>
<tr>
<td>SXFA</td>
<td>Fluoro alcohol Polysiloxane</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum from albumin</td>
</tr>
<tr>
<td>HRP</td>
<td>Horse Radish Peroxides</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffer Solution</td>
</tr>
<tr>
<td>LDH-PEG</td>
<td>Layered Double Hydroxide – Polyethylene Glycol</td>
</tr>
<tr>
<td>LDH-EG</td>
<td>Layered Double Hydroxide – Ethylene Glycol</td>
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<tr>
<td>InAcAc</td>
<td>Indium Acetylacetone</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>NiPc</td>
<td>Nickel Pthalocyanine</td>
</tr>
<tr>
<td>PVA-COOH</td>
<td>Poly Vinyl Alcohol functionalized with carboxylic acid</td>
</tr>
<tr>
<td>RR-P3HT</td>
<td>Regio Regular Poly(3-hexyl-thiophene)</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethyl Formide</td>
</tr>
<tr>
<td>SWN</td>
<td>Single Walled Nanotube</td>
</tr>
<tr>
<td>PS</td>
<td>Polystyrene</td>
</tr>
<tr>
<td>Cfu</td>
<td>Colony forming unit</td>
</tr>
<tr>
<td>E.coli</td>
<td>Escherichia Coli</td>
</tr>
<tr>
<td>LB agar</td>
<td>Liquid Broth agar medium</td>
</tr>
<tr>
<td>MAPLE</td>
<td>Matrix Assisted Pulsed Laser Evaporation</td>
</tr>
<tr>
<td>Nd:YAG</td>
<td>Neodymium-doped Yttrium Aluminium Garnet</td>
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Chapter 1

1 Introduction

1.1 Biocompatible hydrogels

Hydrogels possess a three dimensional polymeric network, and can hold up to over one hundred times more water compared to their dry weight. Due to their unique physical properties, these networks can be shaped or casted into various sizes and shapes. Hydrogels have been extensively used in biomedical applications for quite some time due to their good biocompatibility, high water retaining capacity, flexible methods of synthesis, transparency and desirable physical characteristics [1, 2]. Hydrogels are network of interconnected chains of polymers which are formed from soluble monomers and/or multifunctional polymers that are connected together by cross-linkers. Hydrogels are hydrophilic in nature and are capable of containing over 90% water in between their polymeric networks. In hydrogel, the oxygen permeability through the polymer phase is lower than that of the water phase. As a result, oxygen is transported mainly through the water phase. Among various biomaterials, hydrogels are most commonly employed in biomedical applications as they can mimic the physical, chemical, electrical, and biological properties of most of the biological tissues [2, 3]. Hydrogels have been extensively used in tissue engineering [4], targeted drug delivery [5], chemical and biological sensors [6] and contact lenses [7].

1.2 Silicone Hydrogel

Silicone hydrogels have been specifically developed in order to improve the oxygen transport [8-10] four to six times than that available with traditional hydrogels. Silicone hydrogels, with the incorporation of silicone units which are oxygen permeable, oxygen is transported through the least resistant path which is through the siloxane phase rather than the water phase [11], allowing more oxygen to permeate through the silicone hydrogel. Moreover, silicone hydrogels show excellent properties, such as good biocompatibility, oxygen permeability, transparency, stable chemical structure, good mechanical strength, thermal and oxidative stability, which makes it a better candidate in biomedical application.
1.3 Challenges faced by silicone hydrogels in the field of biomedical applications

Silicone hydrogels are networks of polymers containing lots of siloxane groups (silicon-oxygen bond), which can lead to higher oxygen permeability than other traditional hydrogels [7], which is relatively hydrophobic in nature. Hydrophobic surface has a tendency to cause irreversible protein adsorption to form protein film over the surface of silicone hydrogel, which in turn enhances the microbial colonization and subsequent biofilm formation [12-14]. Biomaterials which are used in biomedical devices or implants are expected to have a hydrophilic surface to prevent biofouling. Therefore, it is essential to alter the surface properties of silicone hydrogel in order to be used in biomedical devices, especially for implants and contact lenses.

1.4 Nanocomposite hydrogels

Nanocomposite hydrogels are nanomaterial incorporated, hydrated polymeric networks that exhibit good elastic behavior and mechanical strength compared to conventional hydrogels. A range of polymers and inorganic nanoparticles are used to design nanocomposite network. By controlling the interactions between nanoparticles and polymer chains, the physical, chemical and biological properties of nanocomposite hydrogels can be engineered [15]. To mimic the properties of biological tissues, polymeric, ceramic or metallic nanomaterials can be incorporated in the hydrogel to improve the characteristics like optical properties and sensitivity which can potentially be very helpful in the field of biomedical applications, chemical and biological sensors [16].

1.5 Silver nanoparticles

Due to its properties and applications, silver is one of the most studied metals in nanotechnology. Properties like particle size distribution, antimicrobial properties, morphology and surface modification, many researchers have performed detailed study on controlled synthesis of silver nanoparticles [17-19]. There are various studies that have performed research on synthesis of silver nanoparticles which resulted in multiple procedures for synthesis of nanoparticle based on particle size control and surface
modification [18-26]. Various methods have been used to prepare silver nanoparticles: in aqueous and non-aqueous media, thermal decomposition (chemical method), by ionization/atomization or various types of laser irradiation (physical method), electrochemical processes and in emulsion systems [27-31].

1.6 Surface modification technique

The term refers to modifying the surface of a material by altering its surface properties to enhance specific functions while retaining the bulk properties of the desired material. The modification can be done by different methods to alter a wide range of characteristics of the surface, such as: roughness [32], hydrophilicity [33], surface charge[34], surface energy, biocompatibility [33, 35] and reactivity [36].

Surface modification can be classified into physical and chemical methods. Chemical methods include chemical vapor deposition (CVD) [37] and wet chemical methods [38]. CVD is used to increase the hydrophilicity of a surfaces by adding suitable functional groups. Unfortunately, CVD precursors can be highly toxic (Ni(CO)₄), corrosive (SiCl₄) or explosive (B₂H₆), which might damage the structure of the biomaterial [39]. The byproducts of CVD can also be hazardous (CO, HF or H₂). The reactions that occur during wet chemical methods are nonspecific and involves chemical agents, which causes adverse toxic effects. Moreover, chemical methods depend upon the use of surface-specific chemistry, so they cannot be employed to modify a wide range of substrates [40].

Physical methods include spin coating [41], dip coating [42] and physical vapor deposition (PVD) [43]. Spin coating and dip coating are much relatively eco-friendly when compared to chemical method but it is hard to control the thickness of the film deposited over the surface compared to PVD. In PVD the thickness of the film formed can be controlled at atomic level or nanometer level. The solvent contamination in PVD is much lower when compared to spin and dip coating. PVD can be divided into four categories namely; vacuum evaporation, sputtering, arc vapor deposition and ion plating [39]. All these methods have their own advantages and disadvantages, and can be applied only to specific range of materials.
A new deposition technique, known as Matrix assisted pulsed laser evaporation (MAPLE), which was developed at the Naval Research Laboratories for depositing thin and uniform layers of chemo selective polymers [44-46], as well as organic compounds such as simple carbohydrates and their polymers. MAPLE was derived from the conventional pulsed laser deposition (PLD) technique. It provides a gentle mechanism for depositing a uniform film of small or large molecular weight species such as inorganic and polymeric molecules, from the condensed phase into the vapor phase.

In the MAPLE technique, a frozen matrix consisting of a solution of a polymeric compound dissolved in a relatively volatile solvent is used as the target for the laser ablation. The target material is diluted in the solvent with a weight concentration lower than 5%, so that the majority of the laser energy is initially absorbed by the solvent molecules and not by the target molecules. At a molecular level in a photo thermal process, the absorption of photons by the frozen solvent molecules is converted to thermal energy which in turn heats the target molecules and allows the solvent to vaporize [47]. Once the target molecules attain sufficient kinetic energy through collective collisions with the evaporating solvent molecules, the target molecules are transferred into the gas phase. The MAPLE process proceeds layer-by-layer, depleting the target in the same concentration as the starting matrix. The target and the substrate are oriented in such a way that, the lifted target molecules start to form a thin coat over the surface of the substrate as the volatile solvent molecules, which have a low adhesion coefficient, are pumped away from the chamber [48].

1.7 Desired materials to improve the surface property of silicone hydrogel

Hydrophilic surface is required to prevent the irreversible protein adsorption on silicone hydrogel. To change the surface property of silicone hydrogel, hydrophilic polymers like polyvinylpyrrolidone (PVP) [49, 50] or Polyethylene glycol (PEG) [51] could be deposited on the surface of silicone hydrogel to improve its hydrophilicity. Polymers such as PVP and PEG are most commonly used for hydrophilic surface modification of biomaterials due to their good biocompatibility, stable chemical structure and inexpensive additive.
Microbial contamination possesses a high risk in the field of biomedical devices, which causes adverse effects in case of contact lenses and biological implants. Silver has been employed as antibacterial agent for ages. To prevent microbial contamination, silver nanoparticles (Ag NPs) are coated over the surface of biomaterials [51, 52]. During their synthesis, to prevent the Ag NPs from aggregation stabilizers are added. Stabilizers such as PVP which are water soluble helps the nanocomposite to meet various biomedical requirements [53, 54] and maintain hydrophilicity on the surface of silicone hydrogel.

1.8 Thesis objective

In the field of biotechnology and biomedical applications like drug delivery, biomedical devices, contact lenses, tissue engineering, wound dressing and tissue scaffolds, silicone hydrogel is one of the commonly used biomaterials. Properties like biocompatibility, high water retaining capacity and oxygen permeability make silicone hydrogel a frontrunner in these fields. But the hydrophobic surface of silicone hydrogel causes irreversible protein adsorption which is a major drawback. Irreversible protein adsorption is highly undesirable as the protein adsorbed surface enhances the microbial adhesion leading to contamination of the biomaterial. The main objective of this thesis is to provide a surface coating to silicone hydrogel to avoid protein adhesion and microbial contamination. A physical vapor deposition technique called as Matrix Assisted Pulsed Laser Evaporation (MAPLE) is used to produce nanocomposite coating on the surface of silicone hydrogel. To study the mechanical properties of this biomaterial, a mathematical constitutive model proposed by Humphrey et al. is used. The constants of the model were back calculated form the experimental data to form an equation to study the mechanical properties of silicone hydrogel. A brief summary of the thesis objective is listed below.

- To produce Ag nanoparticles by two different processes: (1) Process-A is a photochemical reduction; (2) Process-B is laser ablation in liquid. Following that, MAPLE process is employed to deposit the Ag-PVP nanocomposite coatings on silicon hydrogels.
• To compare the protein adsorption and microbial contamination on nanocomposite coated silicone hydrogel by Process A followed by MAPLE and Process B followed by MAPLE with the bare silicone hydrogel.

• To study the mechanical behaviors of nanocomposite coated silicone hydrogel by using uniaxial mechanical test and biaxial mechanical test.

• To analyze the experimental data and mathematical modeling.

1.9 Thesis overview

An overview of the thesis is presented below:

Chapter 2 Literature review

This chapter reviews the application of silicone hydrogel in the field of biomedical industry. It reviews the advantages of silicone hydrogel such as good biocompatibility, high water retaining capacity, high oxygen permeability and mechanical properties which mimics that of biological tissues. It also reviews the problems encountered by silicone hydrogel such as biofouling, and methods to prevent. Different materials that could prevent biofouling are discussed in this chapter and various surface modification techniques are compared.

Chapter 3 Experimental procedure

This chapter describes the experimental procedure for synthesis of silicone hydrogel by photo-polymerization reaction. It also describes the synthesis of silver nanoparticles (Ag NPs) by two different processes (process-A and process-B) and deposition of Ag-PVP nanocomposites by MAPLE process. The MAPLE parameters used for deposition is also mentioned here. In addition to this, various characterization techniques that are performed in this research have been explained in this chapter along with protein adsorption test, antibacterial drop test and mechanical test.

Chapter 4 Silicone hydrogel after nanocomposite deposition by MAPLE
Thin coating of Ag-PVP nanocomposite have been deposited on the surface of hydrogel by process-A followed by MAPLE and process-B followed by MAPLE. In this chapter various characterization techniques like SEM, TEM, EDAX and UV-Vis spectroscopy were used to confirm the deposition of Ag-PVP nanocomposite on the surface of silicone hydrogel. Results of protein adsorption test and anti-bacterial drop test are discussed. Ag-PVP nanocomposite deposition has improved the antibacterial property and protein resisting characteristic of silicone hydrogel and coatings produced by process-B followed by MAPLE produce better results than process-A followed by MAPLE.

Chapter 5 Mechanical tests

This chapter focuses on performing the uniaxial and biaxial mechanical tests on nanocomposite coated silicone hydrogel. Results of uniaxial mechanical test performed on bare silicone hydrogel, and nanocomposite coated silicone hydrogel produced by process-A followed by MAPLE and process-B followed by MAPLE were compared. The data obtained from biaxial mechanical test were studied with the constitutive model proposed by Humphrey et al. By using the experimental data, the constants in the mathematical model were fixed by using MATLAB software.

Chapter 6 Summary and future work

This chapter gives a detailed summary and conclusions of this research. Future work on MAPLE deposition process and development of a constitutive model to study the mechanical properties of silicone hydrogel are discussed.

1.10 References


Chapter 2

2 Literature review

Silicone hydrogels are transparent soft materials that have been used effectively in biomedical applications, such as medical devices, contact lenses and implantable devices due to their biocompatibility and good mechanical properties. However, such materials still have drawbacks due to various environmental factors. Biofouling is a serious problem faced by biomaterials in biomedical industry which will not only limit the function of biomaterials but also cause adverse clinical problems. For example, non-specific protein adsorption on a biomaterial is the first incident which leads to subsequent events including bacterial infection, foreign body reaction and other undesirable responses [1]. To overcome such disadvantages, we need to modify the surface of silicone hydrogel to avoid unwanted surface reactions. Matrix Assisted Pulsed Laser Evaporation (MAPLE) is used to form thin coat of nanocomposite over the surface of silicone hydrogel. In this research, the polymer nanocomposite was coated over the silicone hydrogel to improve its surface and mechanical properties and biaxial test was performed to show the behavior of silicone hydrogel under various load conditions.

2.1 Silicone hydrogel

Silicone hydrogel has a different oxygen transport route which is a less resistant path and has better oxygen transfer rate than the conventional hydrogels. This new oxygen transport mechanism in silicone hydrogel is due to the inclusion of siloxane group (Si-O-Si) in the polymeric network. A study was carried out which compared the oxygen permeability of conventional hydrogel and silicone hydrogel [2]. The results showed that oxygen permeability of silicone hydrogel that transported oxygen through siloxane phase, increased many folds (more than 10 times) compared to conventional hydrogels that transport the oxygen though water phase. The native hydrophobicity and biofouling tendency of silicone hydrogel have been one of its biggest limitations for biomedical applications. The surface of silicone hydrogel requires surface modification in order to be used in biomedical applications. Hydrophobic surface causes protein or lipid adhesion on the surface, which causes various effects like pH variation, interaction between biological
cells, etc. Studies show that proteins get adsorbed on the hydrophobic surface within seconds of their exposure and will cause adverse side effects like microbial infection [3-5]. Therefore, increasing the hydrophilicity of silicone hydrogel to create protein resistant surface which in turn inhibits the microbial growth, is an important requirement for silicone hydrogel when used in biomedical applications [6].

2.2 Biofouling

Biofouling is the accumulation of proteins, cells or other biological materials on the surface of a biomaterial, in our case silicone hydrogel. It possesses a great challenge in the field of biomedical applications like implant devices and contact lenses [7]. Biofouling is caused by the interaction between the hydrophobic surface of silicone hydrogel and the foulants like protein or any other biological material. Protein and microorganisms like bacteria are the most common foulants, which are extensively researched in biomedical field. The protein adhesion is an irreversible process which further leads to bacterial colonization which will limit the function of numerous biomaterials in biomedical devices and even cause adverse clinical events [8].

2.2.1 Protein fouling

The silicone hydrogel is hydrophobic in nature and enhances the protein adsorption on the surface of silicone hydrogel which when used in the biomedical devices, reduces the efficiency of the biomaterial and causes harmful side effects such as blocking the flow through channels and porous membranes, which further leads to thrombus formation or fibrosis and scar tissue formation [9-11]. Moreover, the protein adsorbed on the surface forms a thin layer and will lead to bacterial colonization and subsequent biofilm formation [12]. The figure 2.1 illustrates the mechanism of protein adsorption and thin film formation which will further cause bacterial adhesion. Therefore, low protein fouling is very essential for proper functioning of biomaterials in biomedical field.
Figure 2.1 Mechanism of biofilm formation due to protein adsorption

Protein adsorption on silicone hydrogel is mainly due to the low hydrophilicity of the material. Protein adsorption involves van der Waals force, hydrogen bonding, hydrophobic and electrostatic interactions which is a complex process and still not clear [13]. The surface property of biomaterial plays an important role in protein adsorption. The surfaces that interact with protein can be divided into two categories. One is hydrophilic surface and the other is hydrophobic surface. Paul Roach et al. [14] analyzed the adsorption behavior of bovine serum albumin (BSA) and fibrinogen on hydrophilic (OH) surface and hydrophobic (CH3) surface separately. The results show that hydrophilic surface absorbs more protein than hydrophobic surface. However, hydrophobic surface causes irreversible protein adsorption, which possesses a great threat when used in biomedical applications.

The non-specific protein adsorption is the main reason for biofouling. When protein is adsorbed onto a surface, the non-polar amino acids will be protected inside of the protein molecule and polar amino acid side chain will be held outside to interact with their environment [15], i.e. the hydrophobic core surrounded by polar hydrophilic amino acid chains. If the surface is hydrophobic, the protein molecules tend to rearrange the structure to reach a lower Gibbs energy [14, 16]. The hydrophobic amino acids inside will interact with the hydrophobic surface of silicone hydrogels, which will lead to the unfolding of the protein structure [15]. The unfolded proteins are also known as denatured protein. If the protein is denatured on the surface, then it is not possible to remove the denatured protein. These denatured proteins will also interact with other proteins, which may cause protein
aggregation and cause adverse clinical events [4, 5]. However, hydrophilic surface will not denature the protein structure. Therefore, hydrophilic surface modification will be an efficient way to prevent irreversible protein adsorption on biomaterials.

2.2.1.1 Solutions to prevent protein fouling

There are two methods to prevent irreversible protein adsorption on biomaterials. The first method is defense method where a protein resistance coating is produced onto the surface initially, and the other method is attack method, where a protein degrading coat is provided over the protein adsorbed surface [12]. Polyethylene glycol (PEG) based polymers, polyvinylpyrrolidone (PVP), carbohydrates and peptide-like polymers are the commonly used polymers to modify the surface of biomaterials to provide a protein resistant surface.

In the attack method, we reduce the irreversible protein adsorption by incorporating proteases into the coating mixture. Proteases are enzymes, which are used to digest long protein chains into shorter fragments by breaking down the peptide bonds that link amino acid residues. Prashanth Asuri et al. [17] incorporated serum protease onto single-walled carbon nanotubes to provide nanotube-enzyme composites film to resist protein adsorption, and the result showed that this film resisted up to 99% nonspecific protein adsorption.

2.2.2 Microbial contamination

Microbial contamination is a serious issue in health care, food industry and many other fields and there have been considerable efforts over past few decades to find a solution [18, 19]. The attachment of bacteria to a surface leads to subsequent colonization resulting in the formation of a biofilm [12]. Biofilms are matrix-enclosed microbial accretions that adhere to biological or non-biological surfaces [20]. Protein adsorbed on the surface causes more bacterial adhesion which leads to bacterial colonization. Two types of interactions contribute to the microbial adhesion onto the surface of biomedical device. The first type is the formation of a protein layer and the other is nonspecific interaction. Biofilm formation on the surface of the implants and subsequent infectious complications are also a frequent failure of many biomedical devices, such as total hip arthroplasties, indwelling voice prostheses, vascular or urinary catheters [21]. The development of antimicrobial
reagents to prevent microbial contamination in such medical devices have been attracting attention in the recent years.

2.2.2.1 Solutions to prevent microbial contamination

Similar to the methods used to prevent protein adsorption, there are two methods to inhibit bacterial growth on the surface of the biomaterials; defense method and attack method. In the defense method a non-foulant coating is produced using materials like PEG, PVP and zwitterionic polymers to resist bacterial adhesion [22]. PEG and PVP are commonly known polymers, which are used to reduce protein adsorption and further prevent biofilm formation. Zwitterionic polymers involve anionic and cationic groups along with their chains, which allocate ultra-hydrophilicity and stay neutrally charged at the same time [23]. Gang Cheng et al. [24] grafted zwitterionic poly (carboxybetaine methacrylate) via atom transfer radical polymerization onto glass surface for long-term bacterial resistance test. The results showed that after more than 100 hours, the bacteria attachment was reduced more than 90% compared to bare glass.

In the attack method an antimicrobial film is coated onto the bacteria colonized surface to kill bacteria. Materials like short peptides, cationic polymers, antibiotics, inorganic nanoparticles, etc. [22] are used to provide a coat to kill the bacteria. Xiang Li et al. [23] immobilized two commercialized peptides (RK1 and RK2) onto the surface of silicone hydrogel, and the peptide-coated surface of silicone hydrogel showed excellent microbial inhibiting activity towards bacteria and fungi in urine and PBS buffer.

2.3 Materials used for deposition

2.3.1 Silver Nanoparticles

Silver nanoparticles (Ag NPs) have been studied for ages [25], due to good physicochemical properties of silver such as surface plasmon resonance, antimicrobial property, high electrical and thermal conductivity and catalytic activity. Due to their antibacterial property, Ag NPs have been used to coat numerous medical instruments and products [26]. There are several methods to synthesize Ag NPs, including chemical, physical and photochemical methods [27]. Different nanostructures can be synthesized by
proper control of the nucleation, subsequent growth stages and stabilizer. Various
nanostructures include sphere, cube, tetrahedron, octahedron, right bipyramid, decahedron,
wire, polygonal plates, branched structures, hollow structures, etc. [28]. We synthesized
Ag NPs by chemical method where we used ethylene glycol as the reducing reagent which
reduces silver nitrate in presence of UV radiation and laser ablation in liquid.

2.3.2 Polyvinylpyrrolidone

Polyvinylpyrrolidone (PVP) is a common water soluble synthetic polymer, which has
properties like good biocompatibility, low toxicity and chemical stability [29], and has
been extensively used in food industry, biomedical applications, etc. [30]. PVP is used to
improve the hydrophilicity in order to make the surface of polymeric biomaterials resistant
to foulants such as proteins and lipids [31, 32]. The direct and indirect contact between
PVP and various types of human cells were tested in a study [30] and the results showed
that PVP is generally tissue-compatible. Currently, commercial PVP is treated as a
prospective hydrophilic surface modification compound next to PEG.

2.4 Surface Modification techniques

Non-specific protein adsorption and bacterial contamination are the two main drawbacks
of the silicone hydrogel due to its hydrophobic surface. The common strategy to solve this
problem is by modifying the surface of silicone hydrogel by making it relatively more
hydrophilic. There are various physical and chemical methods, including plasma treatment,
hydrolysis, covalent conjugation, surface grating layer-by-layer deposition, spin coating,
dip coating, laser assisted coating which have been used to modify the surfaces of
biomaterials like silicone hydrogel.

Plasma treatment is used to create hydrophilic hydrogel surface by oxidation of target
material to add reactive functional groups to surface of the substrate by using various
sources like glow discharges, radio frequencies [33] and gas arcs. Many gases like argon,
oxigen, nitrogen and hydrogen, have been used as plasma sources. The oxygen plasma is
the most popular technique employed in hydrogel surface modification. Zhilian et al. used
oxygen plasma to treat PDMS surface to transfer the methyl group into hydroxyl group,
then hyaluronic acid (HA) and collagen were grafted on PDMS surface by chemical
conjugation method for neuronal cell culture [34]. However, plasma treatment can not only add various functional groups under plasma exposure, but also cause aging problems which do not have long-time stability [35].

Hydrolysis is a type of chemical surface modification. Dilute acid or alkali are used in hydrolysis to break the ester bond on the surface to produce hydroxyl groups and carboxyl groups [36]. In covalent conjugation, different cross linker molecules are used to activate the chemical groups on the surface of substrates and conjugate it to the target molecules at the other end. Molecules containing N-hydroxysuccinimide (NHS) are used to activate amine groups. Such molecules have excellent reactivity at physiological pH and thus have been applied in the amine-coupling chemistry for protein conjugation [37]. The molecules containing thiol-reactive pyridyl disulfide group can react with molecules with sulfhydryl group. For example, a heterobifunctional reagent, SPDP, which contain NHS group on one end and pyridyl disulfide group on the other, can act as a cross-linker for the conjugation between materials have amine and thiol residues [38].

Layer-by-layer deposition is a simple wet chemical process for thin film deposition. The films are formed due to the electrostatic interaction between materials with opposite charges. Wei and Thomas deposited Poly(allylamine hydrochloride) (PAH) and poly(sodium styrenesulfonate) (PSS) on several substrates. The multilayer assemblies showed good mechanical integrity without any failure in the multilayers [39]. One advantage of layer-by-layer deposition is the ability to control the thickness of the multilayers.

Surface grafting is a popular chemical surface modification method. Functionalized polymer chain ends are necessary for grafting the polymer to the surface of solid materials by polymerization [40]. Susan J. Sofia et al. [41] grafted poly(ethylene oxide) (PEO) polymer to silicon with covalent bond. The PEO grafted surface was able to reduce three types of protein (cytochrome-c, albumin, and fibronectin) adsorption. J.J. Wang et al. [42] used PEGMA-poly(ethylene glycol) methyl ether acrylate to modify the surface of silicone hydrogel to reduce protein adsorption. The results showed that the PEGMA grafted silicone hydrogel maintained high oxygen permeability, transparency, mechanical properties, and
also efficiently altered the hydrophobic surface to hydrophilic. Although chemical methods are easy to provide more stable covalent bonds with the substrate, the reaction requires various types of chemicals which are toxic to human cells even at a very low concentration. Presence of active functional groups on the surface of substrate or polymer chains is essential. Therefore, this technique could only modify surfaces with specific active groups.

Spin coating is usually applied to produce a thin film on a flat substrate in the form of plate. After adding the coating material onto the center of the substrate, the substrate is rotated at high speed to form a homogenous film due to centrifugal force [43]. It is a popular physical coating method for deposition of polymer films. Aline F. Dário et al. [44] used spin coating to deposit cellulose acetate butyrate (CAB) and poly (methylmethacrylate) onto Silicon wafers. The results showed that the thickness of the films formed were affected by the concentration of the polymer in the solution, molecular weight of the polymer, spinning time and velocity. Typically, just 2-5% of the material dispersed onto the substrate were efficiently used for spin coating, while the remaining 95-98 % is flung off into a coating bowl and disposed [45]. Therefore, in spin coating a lot of coating material is wasted.

Dip coating technique is very popular due to its simple coating procedure, low cost, and reproducibility [34]. The procedure involves inserting the substrates which needs to be coated into the bath of coating solution, it is then removed and air dried. It is used to coat 3D substrates. James Sibarani et al. [46] applied a simple dip coating method to modify the surface of PDMS- poly(dimethyl siloxane) with hydrophilic polymers such as poly(2-methacryloxyloxyethyl phosphorylcholine(MPC)-co-n-butyl methacrylate) (PMB) and poly(MPC-co-2-ethylhexyl methacrylate-co-2-(N,N-dimethylamino)ethyl methacrylate) (PMED). These polymers increased the hydrophilicity of the surface of PDMS. D. Petti et al. [47] used dip coating technique to functionalize a gold surface with copolymer (copoly(DMA-NAS-MAPS)).

The methods mentioned above have their advantages and drawbacks. However, all the methods would make direct contact with the solvents or other chemicals during modification. In addition, these methods have proved ineffective in coating organic molecules on the surface of the biomaterials. As a result, research is currently being carried
out, to find a new technique which allows us to modify the surface with a wide range of materials.

2.5 Laser assisted surface modification techniques

A new deposition technique, known as Matrix assisted pulsed laser evaporation (MAPLE), was developed at the Naval Research Laboratories for depositing thin and uniform layers of polymers [48, 49], as well as organic compounds such as simple carbohydrates [50]. MAPLE was derived from the pulsed laser deposition (PLD) technique which is the most commonly used laser based deposition technique. High energy laser pulses are focused to strike a target inside a vacuum chamber. The target molecules are lifted from the target (vaporized) and then is deposited as a thin film over the surface of the substrate. This technique is suitable for depositing inorganic materials like semiconductors [51], metals [52] and alloys [53]. Even though the basic setup is simple as other deposition techniques, the physical phenomena of laser-target interaction and film growth are quite complex. When the high energy of laser pulse is absorbed by the target, it causes electronic excitation and then the energy is converted into thermal energy, which result in evaporation, ablation and plasma formation. One drawback of PLD is that it is not suitable for the deposition of soft materials like polymeric or organic materials. The high energy laser pulses produced by PLD may damage the chemical structure of organic molecules [54]. To solve this problem, PLD technique was improvised and a new technique was developed. The new technique is called Matrix Assisted Pulsed Laser Evaporation (MAPLE). The difference between PLD and MAPLE is their target. In case of PLD, the target is a metal, alloy or a semiconductor. In case of MAPLE, the desired target is usually dissolved in a liquid solvent matrix and frozen using liquid nitrogen before laser irradiation.

The frozen target in MAPLE, usually contains a low concentration (<5 wt%) of solute material, i.e., polymer or soft organic molecules to be deposited dissolved in a volatile solvent. As shown in the figure 2.2, each polymer or organic molecule is surrounded and thus shielded by a large amount of solvent matrix. The laser pulse initiates a photo-thermal process, sublimating the frozen solvent, and releasing the coating material into the vacuum chamber [55]. The momentum, resulting from this process, carries the solvent and the coating material to the substrate. As the solvent has a high vapor pressure at room
temperature, it will be removed by a mechanical pump in the MAPLE system. The coating material will then adhere to the substrate to form a thin film. The advantage of the MAPLE process is that the laser interaction occurs mostly with the solvent molecules, thus the coating material will not undergo thermal decomposition [56]. Moreover, MAPLE provides a good control over the properties of the films, in terms of homogeneity and roughness. Especially for controlled drug delivery applications, the film thickness can be accurately controlled (in the nanometer range), by varying the laser fluence or the laser pulse frequency [57, 58]. In MAPLE, although the coating process involves laser radiation, the chemical structure of the polymers can be maintained without any damage, by appropriate selection of the solvent matrix and the solution concentration; as a result, most of the laser energy is absorbed by the solvent molecules (and not by the polymeric target).

Figure 2.2 Schematic of MAPLE
### Table 2.1 Summary of Organic and Inorganic films deposited by MAPLE

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Material</th>
<th>Solvent matrix</th>
<th>Wavelength in nm [laser]</th>
<th>Background Pressure</th>
<th>Fluence</th>
<th>Laser frequency (Hz)</th>
<th>Pulse duration</th>
<th>Concentration (wt%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PS, PMMA, PEG</td>
<td>t-butanol, isopropanol, THF, Toluene</td>
<td>2937 [Er:YAG]</td>
<td>1 Pa</td>
<td>70 - 230 mJ/pulse</td>
<td>10</td>
<td>350 μs</td>
<td>1%</td>
<td>[59]</td>
</tr>
<tr>
<td>2</td>
<td>Lipase from Candice Rugosa(LCR)</td>
<td>water</td>
<td>1064 [Nd:YAG]</td>
<td>10^{-4} Pa</td>
<td>526 mJ/pulse</td>
<td>4</td>
<td>2%</td>
<td></td>
<td>[60]</td>
</tr>
<tr>
<td>3</td>
<td>Fluoropolyol</td>
<td>t-butanol, isopropanol, Methanol</td>
<td>2940 [Er:YAG] 248 [KrF] 193 [ArF]</td>
<td>5 mTorr</td>
<td>430 mJ/pulse</td>
<td>10</td>
<td>400 μs</td>
<td>1-2%</td>
<td>[61]</td>
</tr>
<tr>
<td>4</td>
<td>POOPT</td>
<td>Chloroform</td>
<td>355,532,1064 [Nd:YAG]</td>
<td>2.3*10^{-7} Torr</td>
<td>60 - 230 mJ/pulse</td>
<td>10</td>
<td>6 ns</td>
<td>0.56%</td>
<td>[62]</td>
</tr>
<tr>
<td>5</td>
<td>MEH-PPV</td>
<td>Chloroform, Toluene, Chlorobenzene</td>
<td>2940 [Er:YAG] 193/248 8200 [Nd:YAG]</td>
<td>1 μTorr</td>
<td>2 J/cm²</td>
<td>1-2</td>
<td>12-15 ns</td>
<td>1%</td>
<td>[63]</td>
</tr>
<tr>
<td>6</td>
<td>MEH-PPV</td>
<td>toluene, THF, Chloroform</td>
<td>193/248 [Nd:YAG]</td>
<td>10^{-3} mbar</td>
<td>190 mJ/cm²</td>
<td>30</td>
<td>350 μs</td>
<td>0.3%</td>
<td>[64]</td>
</tr>
<tr>
<td>7</td>
<td>PEG</td>
<td>Deionized Water</td>
<td>355,1064 [Nd:YAG]</td>
<td>10^{-6} mbar</td>
<td>5 - 70 J/cm²</td>
<td>5</td>
<td>6-7 ns</td>
<td>0.1-2%</td>
<td>[65]</td>
</tr>
<tr>
<td>8</td>
<td>PEG(3000)</td>
<td>isopropanol</td>
<td>355,532 [Nd:YAG]</td>
<td>1 μTorr</td>
<td>2 J/cm²</td>
<td>1-2</td>
<td>12-15 ns</td>
<td>1%</td>
<td>[66]</td>
</tr>
<tr>
<td>9</td>
<td>PFO</td>
<td>THF, Toluene-Hexane(85:15)</td>
<td>248 [KrF]</td>
<td>10^{-3} Pa</td>
<td>250 mJ/cm²</td>
<td>10</td>
<td>20 ns</td>
<td>0.5%</td>
<td>[67]</td>
</tr>
<tr>
<td>10</td>
<td>PFO, PMMA, Polyethylene</td>
<td>THF, Toluene, Chloroform</td>
<td>248 [KrF] 193 [ArF]</td>
<td>10^{-3}-10^{-4} Pa</td>
<td>50 - 500 mJ/cm²</td>
<td>10</td>
<td>25</td>
<td>0.05-0.5%</td>
<td>[56]</td>
</tr>
<tr>
<td>11</td>
<td>PLA-PVA (usnic acid)</td>
<td>Hexane</td>
<td>248 [KrF]</td>
<td>1 Pa</td>
<td>100 - 400 mJ/cm²</td>
<td>10</td>
<td>25 ns</td>
<td>1-2(w/v)%</td>
<td>[68]</td>
</tr>
<tr>
<td>S. No.</td>
<td>Material</td>
<td>Solvent matrix</td>
<td>Wavelength in nm [laser]</td>
<td>Backgroun d Pressure</td>
<td>Fluence</td>
<td>Laser frequenc y (Hz)</td>
<td>Pulse duration</td>
<td>Concentratio n (wt%)</td>
<td>Ref.</td>
</tr>
<tr>
<td>-------</td>
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<td>------</td>
</tr>
<tr>
<td>12</td>
<td>PVP, Antibiotics, Quercetin dehydrate</td>
<td>DMSO</td>
<td>248 [KrF]</td>
<td>0.5 Pa</td>
<td>150 - 500 mJ/cm²</td>
<td>10</td>
<td>25 ns</td>
<td>10%</td>
<td>[69]</td>
</tr>
<tr>
<td>13</td>
<td>P3HT, PCBM</td>
<td>Toluene</td>
<td>248 [KrF]</td>
<td>1*10⁶ Torr</td>
<td>250mJ/cm²</td>
<td>10</td>
<td>20 ns</td>
<td>1%</td>
<td>[70]</td>
</tr>
<tr>
<td>14</td>
<td>PEG-Block-copolymer</td>
<td>Chloroform</td>
<td>266 [Nd:YAG]</td>
<td>(2–3)×10⁻³ Pa</td>
<td>0.2- 0.9 J/cm²</td>
<td>10</td>
<td>6ns</td>
<td>0.5-1.5%</td>
<td>[71]</td>
</tr>
<tr>
<td>15</td>
<td>SXFA</td>
<td>t-butanol</td>
<td>248 [KrF]</td>
<td>50 mTorr</td>
<td>0.01- 0.5 mJ/cm²</td>
<td>1-5</td>
<td>30 ns</td>
<td>0.5%</td>
<td>[50]</td>
</tr>
<tr>
<td>16</td>
<td>SXFA</td>
<td>Acetone, THF, Chloroform</td>
<td>266 [Nd:YAG]</td>
<td>50 to 1000 Pa</td>
<td>0.03 - 0.18 J/cm²</td>
<td>10</td>
<td>5-7 ns</td>
<td>0.1-2%</td>
<td>[72]</td>
</tr>
<tr>
<td>17</td>
<td>Collagen</td>
<td>Water</td>
<td>248 [KrF ]</td>
<td>10⁻⁴ Pa</td>
<td>20 - 35 mJ/pulse</td>
<td>3</td>
<td>20 ns</td>
<td>2%</td>
<td>[73]</td>
</tr>
<tr>
<td>18</td>
<td>Mussel adhesive proteins</td>
<td>Water</td>
<td>193 [ArF]</td>
<td>10⁻⁴ Pa</td>
<td>400 - 770 mJ/cm²</td>
<td>2</td>
<td>20 ns</td>
<td>2%</td>
<td>[74]</td>
</tr>
<tr>
<td>19</td>
<td>LDH/PEG LDH/EG</td>
<td>Water</td>
<td>266 [Nd:YAG]</td>
<td>1–2 J/cm²</td>
<td>10</td>
<td>5 ns</td>
<td>10%</td>
<td></td>
<td>[75]</td>
</tr>
<tr>
<td>20</td>
<td>Lactoferrin</td>
<td>DD Water</td>
<td>266 [Nd:YAG]</td>
<td>7<em>10⁻⁵ - 2</em>10⁻⁴ mbar</td>
<td>0.1 - 0.8 J/cm²</td>
<td>10</td>
<td>5 ns</td>
<td>0.1-2%</td>
<td>[76]</td>
</tr>
<tr>
<td>21</td>
<td>Insulin, HRP</td>
<td>PBS</td>
<td>193 [ArF]</td>
<td>10⁻³ Torr</td>
<td>0.2 J/cm²</td>
<td>10</td>
<td>20 ns</td>
<td>0.33%</td>
<td>[77]</td>
</tr>
<tr>
<td>22</td>
<td>PVC, Poly Acrylic Acid Polyanniline</td>
<td>Cyclohexanone Xylene, THF Toluene</td>
<td>266, [Nd:YAG]</td>
<td>10⁻³ – 2* 10⁻⁴ mbar</td>
<td>0.1 - 0.7 J/cm²</td>
<td>10</td>
<td>5-7 ns</td>
<td>0.1-4%</td>
<td>[78]</td>
</tr>
<tr>
<td>23</td>
<td>InAcAc NiPc</td>
<td>Cyclohexanone Xylene, THF, Toluene</td>
<td>248 [KrF ]</td>
<td>10⁻⁴ Pa</td>
<td>50 - 600 mJ/cm²</td>
<td>10</td>
<td>15 ns</td>
<td>0.1-5%</td>
<td>[79]</td>
</tr>
<tr>
<td>24</td>
<td>PVA-COOH</td>
<td>DMSO</td>
<td>248 [KrF ]</td>
<td>10⁻⁴ Pa</td>
<td>200 - 700 mJ/cm²</td>
<td>5</td>
<td>25 ns</td>
<td></td>
<td>[80]</td>
</tr>
<tr>
<td>S. No.</td>
<td>Material</td>
<td>Solvent matrix</td>
<td>Wavelength in nm [laser]</td>
<td>Backgroun d Pressure</td>
<td>Fluence</td>
<td>Laser frequenc y (Hz)</td>
<td>Pulse dura tion</td>
<td>Concentratio n (wt%)</td>
<td>Ref.</td>
</tr>
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<td>------</td>
</tr>
<tr>
<td>25</td>
<td>Fibrinogen</td>
<td>Physiological Serum</td>
<td>248 [KrF ]</td>
<td>10^{-4} Pa</td>
<td>400 - 770 mJ/cm²</td>
<td>2</td>
<td>20 ns</td>
<td>2%</td>
<td>[74]</td>
</tr>
<tr>
<td>26</td>
<td>polyanniline</td>
<td>Xylene, Toluene</td>
<td>266, 355 [Nd:YAG]</td>
<td>(3-9)*10^{-4} mbar</td>
<td>0.1-0.5 J/cm²</td>
<td>10</td>
<td>5-7 ns</td>
<td>3%</td>
<td>[81]</td>
</tr>
<tr>
<td>27</td>
<td>PGLA BSA PGA:PLA (1:1)</td>
<td>Toluene, Chloroform Glycerol/PBS Ethyl Acetate</td>
<td>193 [ArF]</td>
<td>3*10^{4} Pa.</td>
<td>0.01-0.5 mJ/cm²</td>
<td>10-15</td>
<td>20 ns</td>
<td>1%</td>
<td>[82]</td>
</tr>
<tr>
<td>28</td>
<td>Pullalan</td>
<td>DMSO, Water, Ethylene Glycol, Ethanol, t-butanol orthoxylene</td>
<td>248 [KrF ]</td>
<td>(1–2)*10^{-3} Pa</td>
<td>210–570 mJ/cm²</td>
<td>2</td>
<td>20 ns</td>
<td>2%</td>
<td>[83]</td>
</tr>
<tr>
<td>29</td>
<td>RR-P3HT</td>
<td></td>
<td>266 [Nd:YAG]</td>
<td>10^{-6} mbar</td>
<td>0.2 J/cm²</td>
<td>10</td>
<td>5 ns</td>
<td>0.8%</td>
<td>[84]</td>
</tr>
<tr>
<td>30</td>
<td>Carbon Nanopearl</td>
<td>Acetone, DMSO, DMF, Toluene, Ethyl Acetate, Methanol</td>
<td>248 [KrF ]</td>
<td>(3–9)*10^{-4} mbar</td>
<td>500-700 mJ/pulse</td>
<td>1-5</td>
<td></td>
<td>1-6%</td>
<td>[85]</td>
</tr>
<tr>
<td>31</td>
<td>TiO₂ NPs SiO₂ NPs</td>
<td>Water, Toluene</td>
<td>193 [ArF] 248 [KrF ]</td>
<td>5*10^{4} Pa</td>
<td>550 mJ/cm²</td>
<td>10</td>
<td>20 ns</td>
<td>0.2%</td>
<td>[55]</td>
</tr>
<tr>
<td>32</td>
<td>SWN + PS + PEG</td>
<td>Toluene (Trace amt of NaOH &amp; Soot / graphite)</td>
<td>193 [ArF] 248 [KrF ]</td>
<td>10^{-5} Torr</td>
<td>0.15-0.25 J/cm²</td>
<td>1-10</td>
<td></td>
<td>4.4%</td>
<td>[82]</td>
</tr>
</tbody>
</table>
2.6 Our contribution

MAPLE technique has been able to modify the surface of biomaterials by depositing delicate materials like organic molecules for over a decade. Various polymers and nanomaterials were chosen to improve the surface properties of biomaterials to overcome their disadvantage in the field of biomedical applications. Irreversible protein adsorption and bacterial contamination on the surface of biomaterials will cause severe effects on the functioning of these biomaterials. As a result, researchers are trying to prevent these drawbacks by various surface modification methods. However, there are not too many people focusing on surface modification by MAPLE to prevent biofouling, which is a contamination free method, specifically suitable for modifying the surface of biomaterials which are used in biomedical devices.

Previous researches show that water soluble polymers like PVP was able to reduce the irreversible protein adsorption and silver nanoparticles have been used for antibacterial purposes for a long time. In this research we synthesize and deposit Ag-PVP nanocomposites over the surface of silicone hydrogel using MAPLE by two techniques. We produce Ag nanoparticles by two processes; process-A and process-B, followed by Ag-PVP nanocomposite deposition in MAPLE.

Much research has not been done to frame a constitutive model to predict the mechanical behavior of silicone hydrogel. We studied the constitutive model proposed by Humphrey et al. We analyzed the experimental data and calculated the constants of the mathematical model using MATLAB programing to study the mechanical properties of silicone hydrogel.

2.7 Summary

Silicone hydrogel is one of the most commonly used biomaterial in biomedical applications. The advantage of silicone hydrogel over other biomaterials is its excellent biocompatibility and high oxygen permeability. However, silicone hydrogels do have few drawbacks like causing biofouling due to its hydrophobic surface.
So, the surface of silicone hydrogel is modified by various chemical and physical methods. MAPLE technique has been reported as an efficient way to modify the surface of biomaterial without any contamination during the deposition process. MAPLE is effective depositing delicate materials like polymers, biomolecules and other organic thin films without damaging the chemical structure at the same time. There are various parameters that are used to control the thin film formation during MAPLE process, such as laser wavelength, laser fluence, laser pulse frequency, background pressure in the chamber, target temperature, substrate temperature, type of solvent matrix, target concentration, target-substrate distance and deposition time. Silicone hydrogel is the biomaterial used in this work, which has a hydrophobic surface and its surface is modified by depositing thin films of hydrophilic polymer such as PVP to reduce irreversible protein adsorption. Silver nanoparticles have been extensively used as antibacterial reagents for over a century. We employ MAPLE technique to deposit Ag-PVP nanocomposite coating to prevent it from biofouling.

2.8 References


Chapter 3

3 Experimental methods

In this chapter, the experiments performed in this project are described in detail: (1) synthesis of silicone hydrogel, (2) synthesis of Ag nanoparticles by process-A and process-B, (3) deposition of nanocomposites prepared by both the processes in MAPLE, (4) characterization methods, (5) protein adsorption test and (6) antimicrobial test.

3.1 Synthesis of silicone hydrogel

Silicone hydrogel was synthesized by a photo-polymerization reaction [1] by polymerizing the monomer; 3-methacryloxypropy-tris(trimethylsiloxy)silane (TRIS, sigma Aldrich buffer solution pH 8.0) with macromer; bis-alpha, omega-(methacryloxypropyl) polydimethylsiloxane (PDMS, sigma Aldrich:156327-07-0) and a copolymer N,N-Dimethylacrylamide (DMA, sigma Aldrich:2680-03-7) along with a photo-initiator; Diphenyl(2,4,6-trimethylbenzoyl) phosphine oxide (sigma Aldrich:75980-60-8) in presence of UV radiation. Ethylene glycol dimethacrylate (EGDMA – sigma Aldrich:97-90-5) is added to crosslink the polymers and a copolymer1-Vinyl-2-pyrrolidinone (NVP, sigma Aldrich:88-12-0) is added to increase the hydrophilicity of the silicone hydrogel. Ethanol was used as the solvent to mix all these chemicals. Briefly, TRIS, PDMS and DMA were mixed in the ratio of 4:1:2 by volume. 1.72 mL of TRIS, 0.43 mL of PDMS and 0.86 mL of DMA were mixed in a beaker using magnetic stirrer. Then 15µL of EGDMA and 0.18mL of NVP were added to the mixture along with 0.3mL of ethanol. Nitrogen gas was purged for 10 minutes to remove the dissolved oxygen. Then 6-8mg of Photo initiator was added to the mixture and stirred for 5 minutes in the magnetic stirrer. The chemical reactions taking place are depicted in FIG. 3 and FIG. 4. Then the liquid mixture is poured into the desired mold and kept under UV radiation for 20-30 minutes to form complete cross linking. Then 20% ethanol solution was used to wash the hydrogel after the polymerization reaction.
Figure 3.1 Photo initiator forming free radicals when exposed to UV radiation
Figure 3.2 Cross linking of TRIS, DMA and PDMS macromer using EGDMA as cross linker
3.2 Synthesis of silver nanoparticles

3.2.1 Process-A

25 ml ethylene glycol (sigma aldrich:107-21-1) was added to a beaker and oxygen was removed by purging it with nitrogen gas for 10 minutes to prevent oxidation reactions. 0.375 g polyvinylpyrrolidone (PVP, 10000 amu, sigma aldrich:9003-39-8) was added to ethylene glycol and stirred for 30 minutes until completely dissolved. The purpose of adding PVP is to stabilize the silver nanoparticles formed during the reduction reaction. 0.25 g of silver nitrate was added to the solution and the stirring was continued for 30 more minutes. Once the silver nitrate was completely dissolved, the solution was kept in UV environment for 24 hours. The solution was centrifuged with acetone (1:6 by volume) to separate the Ag-PVP nanocomposite from the solution. The Ag-PVP was finally washed with 20% ethanol solution [5, 6].

3.2.2 Process-B

25 ml ethylene glycol was added to a beaker and oxygen was removed by purging it with nitrogen gas for 10 minutes to prevent oxidation reactions. 0.375 g polyvinylpyrrolidone (PVP, 10000 amu) was added to ethylene glycol and stirred for 30 minutes until completely dissolved. Then, 0.25 g of silver nitrate was added to the solution and the stirring was continued for 30 more minutes. After silver nitrate was fully dissolved, the solution was introduced into target holder of MAPLE device. Nd:YAG laser was used in the MAPLE with a wavelength (λ) of 532 nm. The repetition rate of the laser was fixed at 10 Hz and the pulse duration was 320 μs. Unfocused laser radiation at a fluence of 50.4mJ/cm² was passed for 1 hour to reduce the silver nitrate (AgNO₃) to silver nanoparticles which then gets surrounded by PVP to prevent aggregation and forms Ag-PVP nanocomposites.

3.3 MAPLE process

Nanocomposites which were formed by process-A and process-B were dispersed in the target holder of MAPLE device, respectively for deposition. The solution was frozen using liquid nitrogen at -190°C. Nd:YAG laser was used in the MAPLE with a wavelength (λ) of 532 nm. The repetition rate of the laser was fixed at 10 Hz and the pulse duration was 320
μs. The temperature of the substrate was maintained around 25°C during the deposition. The depositions were carried out for 30, 60, 90 and 120 minutes respectively for nanocomposites prepared by both the processes with a laser fluence of 50.4 mJ/cm$^2$ and the area of laser spot on the target was about 0.64 cm$^2$ with a background pressure of 1× 10$^{-5}$ Torr and the distance between target and substrate is 4.5 cm. Figure 3.3 shows the deposition of nanocomposite coating by process-A followed by MAPLE and Figure 3.4 shows the deposition of nanocomposite coating by process-B followed by MAPLE.

**Figure 3.3 (a) Process-A: reduction of target solution (AgNO3, PVP and Ethylene Glycol) by UV radiation (b) deposition of nanocomposites in MAPLE**

**Figure 3.4 (a) Process-B: reduction of liquid solution (AgNO3, PVP and Ethylene Glycol) by laser ablation (b) deposition of nanocomposites in the vacuum chamber**
3.4 Materials characterization

The size and shape of nanocomposites deposited were observed by Transmission Electron Microscope (TEM, Philips CM10). The nanocomposite film depositions were confirmed by UV-Visible Spectroscopy (UV-3600 Shimadzu, Japan), Energy-dispersive X-ray Spectroscopy (EDX, Hitachi 3400s) and Scanning Electron Microscopy (SEM, Hitachi 3400s).

3.4.1 Scanning electron microscopy

Scanning Electron Microscope (SEM) is a type of electron microscope, which generates images of a sample by scanning the surface of the samples with electron beam. When the electron beam interacts with the surface of the sample, the electrons will be scattered and absorbed. The signals generated by the SEM includes secondary electrons and back scattered electrons, which can be detected by specific type of detectors. The specimens must be electrically conductive on the surface and also electrically grounded to prevent the accumulation of electrostatic charge. For metal samples slight treatment is required but for organic samples, conductive materials are coated on the surface. Conductive coating materials that can be employed are Platinum, gold, graphite and tungsten. In this research, bare silicone hydrogels and Ag-PVP deposited silicone hydrogel were coated with gold by Hummer VI Sputter Coater. The surface morphology and Energy-dispersive X-ray spectroscopy (EDX) spectra were measured by SEM (Hitachi 3400s) at 10 kV - 40kV.

3.4.2 Transmission electron microscopy

Transmission electron microscope (TEM) is a type of electron microscope where a beam of electrons is allowed to transmit through a thin specimen, where it interacts with the specimen as it passes through. An image is developed from the interaction and the image is magnified and focused onto an imaging device or detected by a camera. The TEM micrographs of Ag-PVP nanocomposite film were obtained by Phillips CM10 TEM equipment. The TEM samples, where prepared by placing the carbon coated copper grid (200 meshes) on the substrate holder along with silicone hydrogel (substrate) during MAPLE deposition process. After deposition the copper grids were completely dried and
used in the TEM equipment to obtain the micrographs which gives the details like size and shape of the Ag-PVP nanocomposite.

3.4.3 Ultraviolet – Visible spectroscopy

Ultraviolet–Visible spectrophotometry (UV-Vis) refers to absorption spectroscopy and reflectance spectroscopy in the ultraviolet and visible spectral region. It uses light in the visible, near-UV and near-infrared ranges. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions [2]. UV-Vis was carried out to confirm the surface plasmon resonance (SPR) of Ag-PVP Nanocomposites in the solution and on the surface of the silicone hydrogel after deposition. UV-Vis is also used to check the size and the shape of the synthesized Ag-PVP NPs. In the antibacterial test, UV-Vis was used to measure the concentration of E.coli in PBS solution.

3.5 Protein adsorption test

Protein adsorption on the surface of biomaterials leads to biofouling, which causes adverse clinical effects in case biomaterials used in biomedical application, therefore the protein adsorption of silicone hydrogel is an important factor and it was measured. Micro BCA method was used to measure the protein adsorption property of silicone hydrogel. Silicone hydrogel samples (bare silicone and Ag-PVP coated silicone) were cut in square shape with 1cmx1cm dimensions and were immersed in Phosphate Buffered Saline (PBS, sigma aldrich:P4417) for 24 hours. Bovine albumin serum-Phosphate Buffer Solution (BSA-PBS) 0.5mg/mL and Sodium Dodecyl Sulfate (SDS, sigma Aldrich:151-21-3) - Phosphate Buffered Saline (SDS-PBS) 1wt% solution were prepared. The samples were soaked in 0.5mg/mL BSA-PBS solution at 37°C for 3 hours. Then these samples were rinsed 3 times in PBS to remove non adsorbed protein on the surface of the hydrogel. Then the samples were immersed in 1wt% SDS-PBS solution and sonicated for 20 minutes to completely detach adsorbed BSA from the surface of the silicone hydrogel. Finally, the BSA protein assay kit (Micro BCA™ Protein Assay Kit, Thermo Scientific, U.S.A.) was used to determine the protein concentration in SDS-PBS solution with a UV-Vis plate reader at 562 nm.
3.6 Antibacterial drop test

Escherichia coli (E.coli) was used to study the antibacterial property of Ag-PVP nanocomposite deposited silicone hydrogels by the “antibacterial drop-test” [3, 4]. E.coli (strain W3110) was cultured on the medium (LB Boath – sigma Aldrich:L7275) at 37°C for 18 - 24 hours. Cultured bacteria were added to 10 mL PBS solution and the concentration bacterial cells were measured in UV – Vis spectroscopy. After measuring the concentration of bacterial cells in PBS, the solution was diluted to 10^6 CFU/ml for the ‘drop-test’ experiments. The test samples (1 cm x 1 cm) were control plates (glass cover slips), bare silicone hydrogel, Ag-PVP coated silicone hydrogel prepared by both one-step and two-step processes. PBS solution was used to sterilize and wash the samples in presence of UV light. Few sets of samples were then placed into sterilized 90 mm petri dishes. Then 100 μl PBS solution with E.coli at a concentration of 10^6 CFU/ml was gently dropped onto the surface of each sample at ambient temperature. Each set of samples were washed after various period of time (such as 1, 2 and 6 hours). After each time period the drops were washed from the surfaces using 5 ml PBS solution in the sterilized Petri dish. Then 10 μl of each bacterial suspension were spread on the LB agar (sigma Aldrich:L2897) plate. Then these petri dishes were incubated for 24 h at 37°C. After 24 hours, the number of bacterial colonies survived on the petri dishes were counted. The relative number, which is the number of bacterial colonies survived on sample plate divided by the number of bacterial colonies survived on control plate, was used to show the results.

3.7 References


Chapter 4

4 Silicone hydrogel after nanocomposite coating by MAPLE

In this chapter, Ag-PVP nanocomposite synthesized by two different processes were deposited on the surface of silicone hydrogel by MAPLE and the surface morphology of the nanocomposite coating was studied using TEM. EDAX and UV-vis spectroscopy were used to confirm the deposition of silver and PVP. Protein adsorption study and the drop-test results reveal the improved property of nanocomposite coated silicone hydrogel.

4.1 Introduction

Inorganic and polymeric thin films with controlled structure is of great use including drug delivery [1], tissue engineering [2], gas and vapor sensing detectors [3], etc. In chemical industries, thin films are usually deposited by electron beam physical vapor deposition (EBPVD) [4], low-pressure chemical vapor deposition (LPCVD) [5], plasma impulse chemical vapor deposition (PICVD) [6], magnetron sputtering [7] and ion beam sputtering (IBS) [8]. The common feature in these techniques is that the target materials are decomposed to atomic level before they are deposited on the surface of substrates. So it is highly impossible for these deposition techniques to be used in depositing complex materials or delicate compounds like polymers or organic compounds while maintain their structure and functions.

The conventional methods used to deposit complex materials are dip coating, spin coating and some wet chemical methods. Each of these deposition techniques has its own advantages and drawbacks, and are applicable to certain range of organic materials. Pulsed laser deposition (PLD) drew much attention as it could be applied to a wide range of materials, such as metals [9], semiconductors [10] and organic compounds [11]. The ablated target molecules which are emitted from the solid target tend to move towards the substrate with high energy and gets deposited over the surface of the substrate. However, it is not suitable for depositing delicate materials like polymers, proteins and other organic compounds. This is due to the ablation caused by high energy laser pulses, which decomposes the target molecules and affect their function. As an improvisation of PLD
technique, MAPLE technique has been developed to avoid the photo-thermal damage caused by ablating the target directly with high energy laser radiation. The major difference between PLD and MAPLE is the target. PLD uses solid targets like metal or semiconductor, whereas in MAPLE the target is composed of a frozen solvent matrix which has the desired coating material dissolved in it. The high energy of laser radiation is mainly absorbed by the solvent so the desired target material is not affected by photo thermal degradation. The solvent molecules evaporate imparting kinetic energy to the solute which will carry it towards the direction of said substrate. The solvent will be pumped away and the solute will get deposited on the substrate.

4.2 Results

Ag-PVP nanocomposites synthesized by process-A and process-B were used to modify the silicone hydrogel, by forming a thin coating over the surface of silicone hydrogel by MAPLE technique, to reduce protein adsorption and inhibit bacterial growth on the surface of silicone hydrogel. MAPLE depositions were carried out to prevent contamination during the coating process and create a homogenous film on the surface of silicone hydrogel.

4.2.1 TEM observations

In process-A, the synthesis of Ag nanoparticles requires 24 hours of UV irradiation and in process-B it requires 1 hour of laser irradiation for complete reduction of silver nitrate. Once the nanoparticles were synthesized, the solution was suspended in the MAPLE target holder for deposition by laser irradiation. Copper grids (200 meshes) were placed on the substrate to characterize the nanoparticles. The TEM micrographs in the figure 4.1 show the particle size Ag NPs formed by process-A followed by MAPLE for different deposition time and figure 4.2. shows the particle size of Ag NPs prepared by process-B followed by MAPLE process. The inserted small figures show the size distribution of the particles.
Figure 4.1 TEM image of Ag nanoparticles prepared by process-A (photo reduction in UV environment) and deposited in MAPLE for (a) 30-min (b) 60-min (c) 90-min (d) 120-min.

Figure 4.2 TEM image of Ag nanoparticles prepared by process-B (laser ablation in liquid) and deposited in MAPLE for (a) 30-min (b) 60-min (c) 90-min (d) 120-min.
Table 4.1 and 4.2 indicates that as the deposition time is increased, the size of nanoparticles formed decreases, but that did not have much effect after exposing them to laser for more than an hour but the size distribution is quite even as the deposition time increases.

Table 4.1 Size of the particles (prepared by process-A) deposited at various deposition time by MAPLE process.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Laser Deposition Time</th>
<th>Size of particles (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30 minutes</td>
<td>14.1</td>
</tr>
<tr>
<td>2</td>
<td>60 minutes</td>
<td>10.0</td>
</tr>
<tr>
<td>3</td>
<td>90 minutes</td>
<td>9.5</td>
</tr>
<tr>
<td>4</td>
<td>120 minutes</td>
<td>9.9</td>
</tr>
</tbody>
</table>

Table 4.1.2 Size of the particles (prepared by process-B) deposited at various deposition time by MAPLE process.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Laser Deposition Time</th>
<th>Size of particles (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30 minutes</td>
<td>13.6</td>
</tr>
<tr>
<td>2</td>
<td>60 minutes</td>
<td>10.3</td>
</tr>
<tr>
<td>3</td>
<td>90 minutes</td>
<td>9.2</td>
</tr>
<tr>
<td>4</td>
<td>120 minutes</td>
<td>9.1</td>
</tr>
</tbody>
</table>

4.2.2 UV-Visible spectroscopy analysis

Figure 4.3 shows the UV-Visible spectrum of Ag-PVP nanocomposite coated silicone hydrogel which were synthesized by process-A followed by MAPLE, process-B followed by MAPLE as well as bare silicone hydrogel. The adsorption peak which is the surface plasmon resonance (SPR) band was affected by the shape and dielectric environment of nanoparticles. Previous research work states that the Ag NPs will be spherical in shape if the SPR band is around 400 nm [12]. Therefore, the shape of Ag NPs should be spherical as depicted from the result of TEM micrographs. UV-Vis spectrum of bare silicone which does not show any adsorption peak around 400 nm. Figure 4.3 confirms the presence of Ag nanoparticles on the surface of silicone hydrogel.
Figure 4.3 UV-Visible spectroscopy image of Ag-PVP nanocomposites deposited on silicone hydrogel which were prepared by process-A followed by MAPLE, process-B followed by MAPLE as well as bare silicone hydrogel.

4.2.3 EDAX analysis

Figure 4.4 and Figure 4.5 include the EDAX mapping and EDAX spectrum of the elements from Ag-PVP deposited on Silicone hydrogel by MAPLE respectively. The dots in Figure 4.4 and Figure 4.5 are the elements of Si, C, O and Ag from silicone hydrogel and PVP. The presence of silver element is also confirmed by the Ag-peak in the EDAX spectrum. Figure 4.4(d) and 4.5 (d) also indicates that the Ag element on the silicone hydrogel is homogenously distributed.
Figure 4.4 EDAX mapping showing (a) Silicon (b) Carbon (c) Oxygen and (d) Silver 
(e) EDAX spectrum of Ag-PVP coated Silicone hydrogel prepared by process-A 
followed by MAPLE.
4.2.4 Protein adsorption

Non-specific protein adsorption is a major drawback for silicone hydrogel which are used in biomedical devices like implants and contact lens. It reduces the efficiency of the implant and even cause adverse effects on human body [13]. The protein adsorption is influenced by the surface characteristics of hydrogels and the properties of proteins including molecular weight, protein structure, net charge and conformational stability [13, 14]. Additionally, protein adsorption and the following formation of protein films on the
surfaces of implants will lead to microbial colonization and consequent biofilm formation [15]. The protein adsorption property of silicone hydrogel and nanocomposite coated silicone hydrogel were tested by micro BCA method. As shown in the Figure 4.6, BSA protein adsorption of bare silicone, Ag-PVP nanocomposite coated silicone hydrogel prepared by process-A followed by MAPLE and process-B followed by MAPLE are 16.09 ± 0.75μg/cm², 14.11 ± 0.68 μg/cm² and 13.73 ± 0.72 μg/cm² respectively. The BSA adsorbed on the surface of the Ag-PVP thin film decreased by 12.34% in nanocomposite coated silicone hydrogel prepared by process-A followed by MAPLE, when compared to bare silicone hydrogel and 14.71 % in nanocomposite coated silicone hydrogel prepared by process-B followed by MAPLE process when compared to bare silicone hydrogel respectively. It can be inferred that Ag-PVP nanocomposite coating can reduce the adsorption of non-specific protein due to the presence of the polymer (PVP). PVP provides a more hydrophilic surface than bare silicone due to the presence of C=O in the structure. When the BSA interacts with hydrophobic surface, it will denature the structure of the protein in order to reach lower Gibbs free energy. But when BSA interacts with hydrophilic surfaces, it will easily get adsorbed onto the surface without changing the structure of the protein, so it is easy to remove the protein from its surface. Therefore, nanocomposite coated silicone hydrogel will adsorb less protein than the bare silicone hydrogel and Ag-PVP nanocomposite coat prepared by process-B followed by MAPLE adsorbs less protein than the nanocomposite prepared by process-A followed by MAPLE.
Figure 4.6 BSA Protein adsorption on the surface of bare silicone hydrogel, Ag-PVP coated silicone hydrogel prepared by process-A followed by MAPLE and Process-B followed by MAPLE

4.2.5 Antibacterial activity

Bacterial adhesion and colonization onto the surface of silicone hydrogel is a critical problem for many biomaterials [16, 17]. Silicone hydrogel provides a much better oxygen permeability compared to conventional hydrogel but the incorporation of TRIS and other monomers which add siloxane group into the hydrogel network leads to decrease in the hydrophilicity of the biomaterial, which could theoretically enhance the bacterial adhesion [18]. It is a known fact that silver ions and silver nanoparticles have the ability to inhibit bacterial growth, so we deposited silver based nanocomposite (Ag-PVP) onto the surface of silicone hydrogel by process-A followed by MAPLE and process-B followed by MAPLE. The antibacterial effect of Ag-PVP nanocomposite coated Silicone hydrogel against *E. coli* is evaluated by the method of film attachment.

Previous studies have shown the interaction between silver ions and bacterial cells [19]. We coated an ultrathin film on the surface of silicone hydrogel to improve the antibacterial
property of silicone hydrogel. Figure 4.7 shows the survived bacterial colonies on agar plates, which were cultured on control plate, bare silicone hydrogel, and Ag-PVP coated silicone hydrogels prepared by two-step and one-step processes, with different culture time. It is quite clear that the bacteria survived on Ag-PVP coated silicone hydrogel keep decreasing with time. The relative survived number of *E.coli* on the bare silicone stays around 95% when the culture time increases, which means bare silicone hydrogel do not have the ability to inhibit bacteria growth. While the relative number of *E.coli* on Silicone-Ag-PVP keep decreasing as the incubation time increasing from 1 hour to 6 hours. Figure 4.8 shows the After 6 hours, the Ag-PVP nanocomposite coated silicone hydrogel eliminates most of the bacterial colonies; relative number declines to 44% in process-A followed by MAPLE and 26% in process-B followed by MAPLE, when compared to bacterial colonies on bare silicone hydrogel. Therefore, we can infer that, process-B followed by MAPLE is a prospective way to coat a thin film of Ag-PVP nanocomposite to prevent bacterial contamination.

![Figure 4.7 Plate counting of survived bacterial colonies on control plate, bare silicone hydrogel and nanocomposite coated silicone hydrogels produced by Process-A followed by MAPLE and Process-B followed by MAPLE.](image-url)
Figure 4.8 Relative number of bacterial colonies survived on bare silicone hydrogel and nanocomposite coated silicone hydrogels by two step process and single step processes after performing antibacterial test for 1 hour, 2 hours and 6 hours.

4.3 Conclusion

MAPLE technique is suitable for silicone hydrogel surface modification with silver and PVP. The Ag-PVP nanocomposite coating and the size of nanoparticles have been confirmed by TEM, EDAX-mapping and UV-visible spectroscopy. It was found that as the deposition time increases, the size of nanoparticles formed decreases, but that did not have much effect after exposing it to laser more than an hour but the size distribution is quite even as the deposition time increases and the average size of Nanoparticles formed by process-B was found to be smaller than that formed by process-A. The adsorption peak which is the surface plasmon resonance (SPR) band in the UV-Vis spectrum shows that the silver nanoparticles are spherical in shape. The EDAX mapping and EDAX spectrum shows the elements present in the bare and Ag-PVP deposited silicone hydrogel which confirms the deposition of silver NPs. The BSA protein adsorption test shows that the amount of protein adsorbed after Ag-PVP coated silicone hydrogel was lower than that of
protein adsorbed on bare silicone hydrogel. In addition, the Ag-PVP coated silicone hydrogel prepared by process-B followed by MAPLE deposition (14.11μg/cm²) shows significant protein resistant property than that prepared by process-A followed by MAPLE deposition (13.73μg/cm²). Antimicrobial test further demonstrates that Ag-PVP nanocomposite coated silicone hydrogel has the ability to inhibit bacterial growth. After 6 hours of incubation, Ag-PVP thin film can kill most of the bacteria on the hydrogel surface. After 6 hours, the relative number of bacterial colonies survived declines to 44% in process-A followed by MAPLE and 26% in process-B followed by MAPLE when compared to bacterial colonies on bare silicone hydrogel. Therefore, it is expected that Ag-PVP deposited silicone hydrogel produced by process-B followed by MAPLE will be more effective in biomedical applications than the bare silicone hydrogel and Ag-PVP deposited silicone hydrogel produced by process-A followed by MAPLE due to its improved antibacterial and protein resistive property.

4.4 References


Chapter 5

5 Mechanical tests

Uniaxial and biaxial mechanical tests were performed on the nanocomposite coated silicone hydrogel produced by both process-A and process-B followed by MAPLE depositions respectively and bare silicone hydrogel. To study the mechanical properties of silicone hydrogel, the constitutive model provided by Humphrey et al. was analyzed along with experimental data. The constants in the mathematical model were fixed by back calculating it with experimental data.

5.1 Introduction

The use of silicone hydrogel in biomedical devices and tissue engineering has become popular due to their viscoelastic characteristics, biocompatibility, feasibility to cast into desired shapes and oxygen transfer ability. One of the major challenges faced by silicone hydrogels in biomedical applications is the ability to replicate the tissues’ mechanical and viscoelastic behavior. At present there are very few methods available for characterizing the mechanical properties of silicone hydrogel. The tensile test is one of the most common methods of mechanical characterization [1]. A material is deformed at a constant rate of elongation and the force required to maintain that rate of elongation is recorded. The force and material elongation are used to obtain a stress-strain curve from which several mechanical properties such as the Young’s modulus, tensile strength and yield strength can be obtained. There is another method; unconfined compression test [2-4], where the hydrogel is compressed between two plates. The force required to compress the hydrogel and the amount of deformation are used to derive a stress-strain curve from which the compressive modulus and strength can be derived. Confined compression test can also be used to study the mechanical properties of hydrogels [5, 6]. It is different from an unconfined compression test, where the hydrogel is held within a chamber, preventing the hydrogel samples from lateral deformation as it is compressed. Bulge or blister test [7] involves the application of a uniform fluid pressure through a window on the substrate to deform the membrane and measure its displacement as a function of the applied pressure by producing a compliance curve. In the bulge test, the biaxial stress of the deformed
sample is axisymmetric and the gripping problems which occur in the tensile test are very minimum here. These advantages make this method popular in testing soft polymers [8]. Liu & Ju (2001) and Ju & Liu (2002) have developed a new technique which enables us to characterize the viscoelastic properties of the polymer membranes. It involves a central indention of a membrane which is clamped along its circumference using a ball of known weight and the corresponding displacement occurring at the center is measured.

5.2 Mechanical test

Silicone hydrogel samples were cut in the shape of square with dimensions 1cm x 1cm and were mounted on BioTester 5000 test system (CellScale Biomaterials Testing). The samples were stretched uni-axially and bi-axially with a pre-loading of 10mN - 30mN or 10 % - 30% of stretching applied based in its maximum stretching limit. The images of the deformation of the sample hydrogel were captured using a 1280x960 pixel charge coupled device (CCD) camera. The stress and strain were calculated from the data and the Stress-Strain curves of different samples were plotted to determine their Young’s modulus (E). the stretch ratios were calculated and was used in the model equation to form a generalized equation to study the properties of silicone hydrogel.

Figure 5.1 Uniaxial stretching
5.3 Uniaxial test

1cm x 1cm samples of silicone hydrogel were cut and mounted on BioTester 5000 test system. The samples were stretched uniaxially with a pre-loading of 30mN or 30% of stretching applied. The stress and strain were calculated from the data and the Stress-Strain curves of different samples were plotted to determine their Young’s modulus (E). The slope of the Stress-strain is the Young’s modulus (E) of the sample. The equation to determine the Young’s modulus is given below.

\[
E = \frac{\text{Stress}}{\text{Strain}} = \frac{\sigma}{\varepsilon} = \frac{F/A}{\delta L/L_0}
\]

(2)

Where \(E\) is in Pascal (Pa), \(F\) the force applied in Newton (N), \(A\) the area perpendicular to the force vector (m\(^2\)), \(\delta L\) the displacement of the materials (m), and \(L_0\) the original length of the materials (m). The Young’s modulus of bare silicone hydrogel was found to be 0.57±0.13 MPa and the values of Young’s modulus of Ag-PVP coated silicone hydrogel prepared by process-A and process-B followed by 2 hours of MAPLE deposition are shown in Table 5.1.
Table 5.1 Young's modulus for bare silicone hydrogel and nanocomposite coated silicone hydrogel prepared by both process-A and process-B followed by MAPLE depositions

<table>
<thead>
<tr>
<th>S.no</th>
<th>Deposition time</th>
<th>E(Young’s modulus) for Ag-PVP coated silicone hydrogel prepared by process-A followed by MAPLE (MPa)</th>
<th>E(Young’s modulus) for Ag-PVP coated silicone hydrogel prepared by process-B followed by MAPLE (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30 min</td>
<td>0.58 ±0.18</td>
<td>0.58 ±0.11</td>
</tr>
<tr>
<td>2</td>
<td>60 min</td>
<td>0.59 ±0.01</td>
<td>0.61 ± 0.17</td>
</tr>
<tr>
<td>3</td>
<td>90 min</td>
<td>0.60 ± 0.15</td>
<td>0.64 ± 0.10</td>
</tr>
<tr>
<td>4</td>
<td>120 min</td>
<td>0.62 ± 0.14</td>
<td>0.66 ± 0.12</td>
</tr>
</tbody>
</table>

For measuring the toughness of the silicone hydrogel sample, maximum stretching is required. Based on the thickness of the silicone hydrogel, 30% of stretching or more is applied on the hydrogel. It is then calculated from the area under the stress-strain curve. The formula or toughness is given below.

\[
\text{Toughness} = \int \sigma \, d\varepsilon
\]  

(3)

The toughness of bare silicone hydrogel was found to be 15.14 ± 0.41 MJ/m³ and the toughness values of Ag-PVP coated silicone hydrogel prepared by process-A and process-B followed by 2 hours of MAPLE deposition are shown in Table 5.2.
Table 5.2 Toughness for bare silicone hydrogel and nanocomposite coated silicone hydrogel prepared by both process-A and process-B followed by MAPLE depositions

<table>
<thead>
<tr>
<th>S.no</th>
<th>Deposition time</th>
<th>Toughness for Ag-PVP coated silicone hydrogel prepared by Process-A followed by MAPLE(MJ/m³)</th>
<th>Toughness for Ag-PVP coated silicone hydrogel prepared by Process-B followed by MAPLE(MJ/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30 min</td>
<td>16.45 ± 0.50</td>
<td>16.673 ± 0.69</td>
</tr>
<tr>
<td>2</td>
<td>60 min</td>
<td>18.46 ± 0.27</td>
<td>19.564 ± 0.35</td>
</tr>
<tr>
<td>3</td>
<td>90 min</td>
<td>20.54 ± 0.17</td>
<td>21.007 ± 0.14</td>
</tr>
<tr>
<td>4</td>
<td>120 min</td>
<td>21.54 ± 0.21</td>
<td>22.014 ± 0.17</td>
</tr>
</tbody>
</table>

5.4 Constitutive model

The main goal in constitutive modeling is to predict the mechanical behavior of the biomaterial under a generalized loading state. For this, we need to obtain the strain energy function and the values of material constants present in it. For biological tissues and soft biomaterials, there are many medical device applications where constitutive models are required to study its mechanical properties. Since silicone hydrogels are generally considered as non-linear incompressible materials, planar biaxial testing is performed where a two dimensional stress-state is established and it is used to characterize the mechanical properties of silicone hydrogel.

For incompressible materials in thin planar configurations, planar biaxial testing allows for a two dimensional stress-state that is sufficient to develop a constitutive equation. In our case, silicone hydrogel undergoes a finite deformation, Humphrey et al. [16, 17] developed
the following generalized strain energy formulation to characterize pseudo-elastic non-linear behavior of biomaterials:

\[ W(I_1, I_4) = C_1(I_4-1)^2 + C_2(I_4-1)^3 + C_3(I_1-3) + C_4(I_4-1)(I_1-3) + C_5(I_1-3)^2 \]  

(1)

Where,

- \( W \) - Strain energy function,
- \( I_1 \) and \( I_4 \) - Invariants of the right Cauchy deformation tensor,
- \( C_1 - C_5 \) are the experimental coefficients that must be fit.

Biological tissues and biomaterials face many challenges in constitutive modeling due to their complex mechanical behavior like, the orientation of fibrous structures which often exhibit mechanical anisotropy. They also exhibit nonlinear stress vs strain relationship with large deformations and viscoelasticity. In general, soft biomaterial do not obey simple material models.

Mechanical studies have usually been confined to uniaxial studies as multi-dimensional boundary conditions are difficult to control. But due to its mechanical anisotropy, uniaxial data cannot be used to extrapolate to three-dimensional constitutive model equation. There have been investigations using inflation of membranes of circular samples, which can provide the necessary experimental data [9, 10] if it is assumed to have isotropic mechanical characteristics. But in reality, all the tissues are anisotropic in nature, hence this method cannot be used. When we try to determine the material constants for complex models, biaxial tests are required that include complex testing methods that allow large variations in stress and strain states for a complete characterization [11-13]. Biaxial testing of biological tissues or biomaterials were developed from the biaxial mechanical studies performed on elastic rubbers [14, 15]. In 1948, Treloar [15] used tests where he applied two independently varying strains in perpendicular directions by simultaneously measuring the stresses.
5.5 Biaxial test

1 cm x 1 cm samples of silicone hydrogel were cut and mounted on BioTester 5000 test system. The samples were stretched bi-axially with a pre-loading of 30 mN or 30% of stretching applied. As the stretching was applied in both the directions, there will be a shear component of force in each direction which tends to change the shape of the sample.

(a) ![Figure 5.3 Bi-axial test (a) before stretching and (b) after stretching](image)

(b) 

Figure 5.4 Strain Distribution in the silicone hydrogel before the start of biaxial mechanical test
Figure 5.5 Strain Distribution in the silicone hydrogel during 10% of stretching in biaxial mechanical test

Figure 5.6 Strain Distribution in the silicone hydrogel during 30% of stretching in biaxial mechanical test
Let us consider the stress state applied onto the 2-D sample. All loads are applied normal to the specimen edge. Stretch ratios were calculated at each instance of stretching experiment. The stretch ratio is defined ratio of final length to the initial length.

\[ \lambda_1 = \frac{\text{Final length}}{\text{Initial length}} \]  \hspace{1cm} (4)

Humphrey et al. proposed the following equations to calculate experimental stress value by considering the specimen as a pseudo-elastic non-linear material. The stress values were calculated for the silicone hydrogel sample which was subjected to a bi-axial stretching by using the following formulae

\[ \sigma_{11}^{\text{Exp}} = \frac{\lambda_1 P_{11}}{(l_2' t')} \]  \hspace{1cm} (5)

\[ \sigma_{22}^{\text{Exp}} = \frac{\lambda_2 P_{22}}{(l_1' t')} \]  \hspace{1cm} (6)

Where,

\[ \sigma_{11}^{\text{Exp}} \] – Experimental Stress in the 1 direction

\[ \sigma_{22}^{\text{Exp}} \] - Experimental Stress in the 2 direction

\[ P_{11} \] - Normal load applied in the 1 direction

\[ P_{22} \] - Normal load applied in the 2 direction
\( \lambda_1 \) – Stretch ratio in the 1 direction

\( \lambda_2 \) – Stretch ratio in the 2 direction

\( l_1', l_2', t' \) – Initial length, breadth and width of the sample

From the strain energy function given in equation (1) the following model equations were derived for the sample which undergoes biaxial stretching. The samples were assumed to be a non-linear incompressible material. The proposed constitutive model is given below.

\[
\sigma_{11}^\text{Model} = 2 \lambda_1 C_1 (\lambda_1 - 1) + 3 \lambda_1 C_2 (\lambda_1 - 1)^2 + \lambda_1 C_4 (\lambda_1^2 + \lambda_2^2 + \lambda_3^2) + 2 (\lambda_1^2 - \lambda_3^2)[C_3 + C_4 (\lambda_1 - 1) + 2 C_5 (\lambda_1^2 + \lambda_2^2 + \lambda_3^2 - 3)] \tag{7}
\]

\[
\sigma_{22}^\text{Model} = 2 (\lambda_2^2 - \lambda_3^2)[C_3 + C_4 (\lambda_1 - 1) + 2 C_5 (\lambda_1^2 + \lambda_2^2 + \lambda_3^2 - 3)] \tag{8}
\]

Where,

\( \sigma_{11}^\text{Model} \) – Model Stress in the 1 direction

\( \sigma_{22}^\text{Model} \) – Model Stress in the 2 direction

\( \lambda_1, \lambda_2, \lambda_3 \) – Stretch ratios in directions 1, 2 and 3

\( C_1, C_2, C_3, C_4, C_5 \) – Experimental coefficients that must be fit to the model
After performing the experiments, the values of experimental stresses $\sigma_{11}^{\text{Exp}}$ and $\sigma_{22}^{\text{Exp}}$ were calculated along with stretch ratios. The main aim of the experiment is to calculate the material constants. These constants depend upon various material properties such as elasticity, toughness, fibrous matter alignment, etc. Therefore, each material has certain unique, specific range for these constants. Experimental stress values from the experiment
is used in the model equation to back calculate the values of C1-C5. A set of these values were then optimized in the MATLAB to fix a particular value for these five constants. The values of these constants are mentioned in Table 5.3.

Table 5.3 C values that fit the model equation

<table>
<thead>
<tr>
<th>C_1</th>
<th>C_2</th>
<th>C_3</th>
<th>C_4</th>
<th>C_5</th>
</tr>
</thead>
<tbody>
<tr>
<td>7375.72</td>
<td>-1983.56</td>
<td>-541.38</td>
<td>2383.96</td>
<td>-1138.62</td>
</tr>
</tbody>
</table>

After calculating the values of the constants, the constants were fixed in the mathematical model. Now the experimental stress values and stress values from the mathematical model in directions 1 and 2 were compared to check how well the experimental data fits the mathematical model.

![Figure 5.10 Comparing the experimental stress values with the stress values from the model in the direction 1, after fixing the constants in the mathematical model.](image)

Figure 5.10 Comparing the experimental stress values with the stress values from the model in the direction 1, after fixing the constants in the mathematical model.
Ultimate tensile strength is the capacity of a material to withstand loads tending to elongate, as opposed to compressive strength, which withstands loads tending to reduce the size. The ultimate tensile strength of bare silicone hydrogel, Ag-PVP coated silicone hydrogel produced by both process-A and process-B followed by MAPLE at various deposition time. The ultimate tensile strength value of bare silicone hydrogel was found to be 346.54 KPa in direction 1 and 311.86 KPa in direction 2. The tensile strength values of Ag-PVP coated silicone hydrogel produced by process-A followed by MAPLE process at various deposition times are shown in Table 5.4 and Ag-PVP coated silicone hydrogel produced by process-B followed by MAPLE process at various deposition times are shown in Table 5.5. The results show that, the values of ultimate tensile strength of Ag-PVP coated silicone hydrogel increased by 14.86% in direction 1 and 14.95% in direction 2 for Process-A followed by 2 hours of MAPLE deposition and 19.10 % in direction 1 and 19.29% in direction 2 for Process-B followed by 2 hours of MAPLE deposition.
Table 5.4 Ultimate tensile strength for Ag-PVP coated silicone hydrogel produced by process-A followed by MAPLE

<table>
<thead>
<tr>
<th>S. no</th>
<th>Deposition time</th>
<th>Maximum Stretch ratio</th>
<th>Model ultimate tensile strength (KPa) in direction 1</th>
<th>Experimental ultimate tensile strength (KPa) in direction 1</th>
<th>Model ultimate tensile strength (KPa) in direction 2</th>
<th>Experimental ultimate tensile strength (KPa) in direction 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30 min</td>
<td>1.086</td>
<td>366.96</td>
<td>365.45 ± 1.21</td>
<td>328.74</td>
<td>326.75 ± 1.47</td>
</tr>
<tr>
<td>2</td>
<td>60 min</td>
<td>1.087</td>
<td>376.98</td>
<td>375.15 ± 0.80</td>
<td>338.02</td>
<td>336.13 ± 1.05</td>
</tr>
<tr>
<td>3</td>
<td>90 min</td>
<td>1.087</td>
<td>388.35</td>
<td>386.42 ± 1.19</td>
<td>351.28</td>
<td>349.56 ± 0.79</td>
</tr>
<tr>
<td>4</td>
<td>120 min</td>
<td>1.086</td>
<td>399.86</td>
<td>398.15 ± 1.65</td>
<td>360.98</td>
<td>358.51 ± 1.15</td>
</tr>
</tbody>
</table>

Table 5.5 Ultimate tensile strength for Ag-PVP coated silicone hydrogel produced by process-B followed by MAPLE

<table>
<thead>
<tr>
<th>S. no</th>
<th>Deposition time</th>
<th>Maximum Stretch ratio</th>
<th>Model ultimate tensile strength (KPa) in direction 1</th>
<th>Experimental ultimate tensile strength (KPa) in direction 1</th>
<th>Model ultimate tensile strength (KPa) in direction 2</th>
<th>Experimental ultimate tensile strength (KPa) in direction 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30 min</td>
<td>1.099</td>
<td>381.05</td>
<td>379.15 ± 1.14</td>
<td>350.56</td>
<td>348.96 ± 1.68</td>
</tr>
<tr>
<td>2</td>
<td>60 min</td>
<td>1.099</td>
<td>388.10</td>
<td>386.57 ± 1.68</td>
<td>357.10</td>
<td>355.65 ± 1.07</td>
</tr>
<tr>
<td>3</td>
<td>90 min</td>
<td>1.099</td>
<td>401.95</td>
<td>400.66 ± 1.10</td>
<td>367.89</td>
<td>366.42 ± 0.94</td>
</tr>
<tr>
<td>4</td>
<td>120 min</td>
<td>1.099</td>
<td>414.56</td>
<td>412.75 ± 0.96</td>
<td>373.76</td>
<td>372.03 ± 1.09</td>
</tr>
</tbody>
</table>

5.6 Conclusion

The values of the Young’s modulus (E) were obtained from the slope of Stress-Strain curve using uniaxial mechanical stretching experiment. The value of Young’s Modulus of bare
silicone hydrogel is 0.57 ± 0.13 MPa, Ag-PVP coated silicone hydrogel prepared by process-A followed by 2 hours of deposition is 0.62 ± 0.14 MPa and Ag-PVP coated silicone hydrogel prepared by process-B followed by 2 hours of deposition is 0.66 ± 0.12 MPa. The values of toughness were obtained from the area under the Stress-Strain curve. The value of toughness was found to be 15.137 ± 0.412 MJ/m³ for bare silicone, 21.54 ± 0.21 MJ/m³ and 22.01 ± 0.18 MJ/m³ for Ag-PVP coated silicone hydrogel prepared by process-A and process-B followed by 2 hours of MAPLE deposition respectively under uniaxial mechanical test. The mechanical properties were studied under biaxial test as well by using the constitutive model proposed by Humphrey et al. The values of the constants in the model were back calculate using MATLAB software and these constants were fixed in the model for silicone hydrogel. Under biaxial mechanical test, the values of ultimate tensile strength of Ag-PVP coated silicone hydrogel increased by 14.86% in direction 1 and 14.95% in direction 2 for Process-A followed by 2 hours of MAPLE deposition and 19.10 % in direction 1 and 19.29% in direction 2 for Process-B followed by 2 hours of MAPLE deposition.

5.7 References


Chapter 6

6 Summary and future work

6.1 Summary

Silicone hydrogel is one of the most common biomaterial used in the field of biomedical applications, but due to some of its poor physical and chemical properties, there are certain limitations. One of such properties is the hydrophobic nature of its surface which causes non-specific protein adsorption which in turn leads to subsequent bacterial colonization resulting in biofilm formation. In this research, our goal was to improve the surface properties of silicone hydrogel, by coating nanocomposites using matrix assisted pulsed laser evaporation (MAPLE) technique which forms a protein resistant surface. Hydrophilic polymer polyvinylpyrrolidone (PVP) is used to increase the hydrophilicity of the surface of silicone hydrogel to reduce protein fouling and silver nanoparticles have been used to inhibit the bacterial growth. So Ag-PVP nanocomposite has been chosen as the desired target material to be deposited onto the surface of silicone hydrogel. Two processes have been developed to synthesize Ag-PVP nanocomposites. Process-A uses ultraviolet radiation to reduce silver nitrate to silver nanoparticles and Process-B is laser ablation in liquid to reduce silver nitrate to silver nanoparticles. The surface of silicone hydrogel has been modified by coating it with Ag-PVP nanocomposite prepared by these two processes, by carrying out the deposition for 30, 60, 90 and 120 minutes in MAPLE.

BSA protein adsorption of bare silicone hydrogel and Ag-PVP coated silicone hydrogel prepared by Process-A and Process-B followed by 2 hours of MAPLE process are 16.09 ± 0.75 μg/cm², 14.11 ± 0.68 μg/cm² and 13.73 ± 0.72 μg/cm² respectively. The BSA adsorbed on the surface of the Ag-PVP thin film decreased by 12.34% in Ag-PVP coated silicone hydrogel prepared by Process-A followed by 2 hours of MAPLE, when compared to bare silicone hydrogel and 14.71% in Ag-PVP coated silicone hydrogel prepared by Process-B followed by 2 hours of MAPLE, when compared to bare silicone hydrogel. The values of the Young’s modulus (E) were obtained from the slope of Stress-Strain curve using uniaxial mechanical stretching experiment. The value of Young’s Modulus of bare silicone hydrogel is 0.57 ± 0.13 MPa, Ag-PVP coated silicone hydrogel prepared by Process-A
followed by 2 hours of MAPLE is $0.62 \pm 0.14$ MPa and Ag-PVP coated silicone hydrogel prepared by Process-B followed by 2 hours of MAPLE is $0.66 \pm 0.12$ MPa. The values of toughness were obtained from the area under the Stress-Strain curve. The value of toughness was found to be $15.14 \pm 0.41$ MJ/m$^3$ for bare silicone, $21.54 \pm 0.20$ MJ/m$^3$ and $22.01 \pm 0.18$ MJ/m$^3$ for Ag-PVP coated silicone hydrogel prepared by Process-A and Process-B followed by 2 hours of MAPLE process, respectively under uniaxial mechanical test. The mechanical properties were studied under biaxial test as well by using the constitutive model proposed by Humphrey et al. The values of the constants in the model were back calculate using MATLAB software and these constants were fixed in the model for silicone hydrogel. Under biaxial test, the values of ultimate tensile strength of Ag-PVP coated silicone hydrogel increased by 14.86% in direction 1 and 14.95% in direction 2 for Process-A followed by 2 hours of MAPLE deposition and 19.10 % in direction 1 and 19.29% in direction 2 for Process-B followed by 2 hours of MAPLE deposition.

### 6.2 Future work

One of the disadvantage of PVP coating is related to the oxidative degradation at certain temperature. Studies have shown that PVP coatings fail to stay protein resistance over a long period of time. To achieve long term protein resistance, a combination of different molecules to form hybrid nanocomposite, such as using different surface coating molecules with long term protein resistance, may be developed.

Our MAPLE system has Optical Parametric Oscillator (OPO) installed in it, which will allow us to change the wavelength to the Near-IR or IR range. Further studies will be needed to find out the effects of wavelength on different biomolecule and nanocomposite depositions.
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