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Hydrogel-based Nanocomposites and Laser-assisted Surface Modification for Biomedical Application

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A thesis submitted in partial fulfillment of the requirements for the Master of Engineering Science degree in Chemical and Biochemical Engineering

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by

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Graduate Program in Chemical & Biochemical Engineering

A thesis submitted in partial fulfillment
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Abstract

Hydrogels can be used in contact lens, wound dressing, drug delivery and tissue scaffolds due to their good biocompatibility. However, the poor mechanical properties and non-specific protein adsorption of hydrogels limit their applications. The adverse effects of protein adsorption in hydrogels include biofouling, inflammation, or even body rejection. In this project, two different hydrogel materials, co-polymer 2-hydroxyethyl methacrylate with a low amount of 2-aminoethyl methacrylate, p(HEMA-co-AEMA) and silicone hydrogel were fabricated by photo-polymerization; the former has hydrophilic surface and the latter is hydrophobic. The silica (SiO_2) nanoparticle-loaded hydrogels have been developed by using in situ polymerization. The dispersion of silica nanoparticles in silicone hydrogels is quite homogenous. The Young's modulus of silicone hydrogel-based nanocomposites is improved slightly with the comparison of that of silicone hydrogel. The bovine serum albumin (BSA) adsorption of hydrogels and their nanocomposites was examined by protein assay. It is found that silicone hydrogel and its nanocomposites prefer to adsorb more BSA than p(HEMA-co-AEMA) and its nanocomposites do. Moreover, silica nanoparticles can reduce the protein adsorption of silicone hydrogel. The cytotoxicity of the hydrogels and hydrogel-based nanocomposites has been studied as well.

As the protein adsorption is strongly related to the surface feature of hydrogels, such as electric state, hydrophobicity and steric structure, one of efficient strategies to minimize the protein adsorption is the surface coating with a thin film of protein non-sticking materials. Currently, several surface coating technologies, such as dip coating and spin coating can be used for this purpose. However, the specific surface property requirement in these coating processes limits their applications in biomedical device. The Matrix assisted pulsed laser evaporation (MAPLE) is a new process for organic molecule deposition. This physical vapor deposition is independent on the surface property of target and substrates. It has potential applications in depositing almost every kind of organic molecule. In this project, a solid-state pulsed laser with wavelength at 532nm was used in MAPLE system. The deposited polyethylene glycol (PEG) films as a function of irradiation time have been investigated by Fourier transform infrared spectroscopy (FTIR), and atomic force microscopy (AFM). The results indicate PEG can be successfully deposited through MAPLE system. The thickness of PEG film increases with increasing irradiation time. Finally, the

protein adsorptions before and after PEG deposition using MAPLE have been investigated. It is found that such deposition improved the protein resistance of silicone gels dramatically.

Keywords

P(HEMA-co-AEMA), Silicone, Nanocomposites, Protein adsorption, Surface coating, Polyethylene glycol (PEG), Matrix assisted pulsed laser evaporation (MAPLE).

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List of Abbreviations

AEMA	-	Amino ethyl methacrylate
AFM	-	Atomic force microscopy
APTS	-	3-Aminopropyltriethoxysilane
BCA	-	Bicinchoninic acid
BSA	-	Bovine serum albumin
CTAB	-	Cetrimonium bromide
DMA	-	N,N-Dimethylacrylamide
DMPA	-	2,2-Dimethyl-2-Phenylacetophenone
DMSO	-	Dimethyl sulfoxide
ECM	-	Extracellular matrix
EGDMA	-	Ethylene glycol dimethacrylate
FITC	-	Fluorescein isothiocyanate
FTIR	-	Fourier transform infrared spectroscopy
HEMA	-	2-Hydroxyethyl methacrylate
MAPLE	-	Matrix assisted pulsed laser evaporation
ml	-	Milliliter
mg	-	Milligram
μl	-	Microliter
μm	-	Micrometer
MTT	-	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
nm	-	Nanometer
NPs	-	Nanoparticles
NVP	-	N-vinylpyrolidinone

PBS	-	Phosphate buffered saline
PDMS	-	Polydimethylsiloxane
PEG	-	Polyethylene glycol
PLD	-	Pulsed laser deposition
rpm	-	revolutions per minute
SEM	-	Scanning electron microscope
SPDP	-	N-Succinimidyl 3-[2-pyridyldithio]-propionate
TEOS	-	Tetra ethyl orthosilicate
TPMPH	-	3-(Trihydroxysilyl) propyl methyl phosphonate monosodium
TRIS	-	3-methacryloxypropyl tris (trimethylsiloxy) silane
UV	-	Ultraviolet
XPS	-	X-ray photoelectron spectroscopy

Chapter 1

Background, Hypothesis and Objectives

Hydrogels are interconnected polymer chains which can be formed from soluble monomers and/or multifunctional polymers (macromers) and connected together by crosslinkers. Hydrogels have been widely used as microdevice bases, tissue engineering scaffold, contact lens materials, etc. Two different hydrogels, poly-(2-Hydroxyethyl methacrylate) (pHEMA) and silicone have been applied in different fields due to their proper mechanical strength and stable chemical structures.

1.1 Poly- (2-Hydroxyethyl methacrylate)

PHEMA hydrogel was first developed by Wichterle and Lim in 1960 [1]. After that, pHEMA hydrogel and its derivatives have been widely used in the biomedical field, ranging from production of contact lens [2] and wound dressing [3] to drug delivery devices [4] and surgical prostheses [5].

From the first time pHEMA was developed, some significant properties have been found, such as the high water content, good biocompatibility and the transparency. Good biocompatibility is critical for materials in biomedical application and transparency makes pHEMA hydrogel a potential candidate for contact lens materials. People also make porous pHEMA hydrogel through different processing techniques as cell scaffolds for tissue engineering [6] and drug delivery devices [7]. Moreover, HEMA is a commercially available monomer and can be easily homopolymerized and co-polymerized with the majority of (meth)acrylic co-monomers.

The disadvantage of this material is that it is relatively impermeable to oxygen, which will lead to various hypoxic conditions such as slowing of mitosis, a reduced number of hemidesmosomes, as well as the occurrence of epithelial microcysts [8].

Numerous methods were applied to improve the oxygen permeability of pHEMA, such as adding other monomers to pHEMA to increase the water content and therefore increase the oxygen permeability. N-vinylpyrolidinone (NVP) and methacrylic acid

(MAA) are two hydrophilic monomers which can strongly increase the water content in hydrogel [9].

1.2 Silicone

Silicone has a different oxygen transport mechanism from pHEMA hydrogel. Silicone is made up of siloxane groups which can carry large amount of oxygen. Silicone transports oxygen through the siloxane-phase rather than through the water phase [10]. This new transport mechanism results in a higher oxygen transmissibilities than those encountered with pHEMA.

In addition, silicone shows the similar good properties as pHEMA, such as good biocompatibility, transparency, stable chemical structure and proper mechanical strength, which makes it also a good candidate material for biomedical application.

1.3 Hydrogel based nanocomposites

A nanocomposite is a multiphase solid material where one of the phases has one, two or three dimensions less than 100 nm, or structures having nano-scale repeat distances between the different phases that make up the material [11]. The nanocomposites were developed by mixing or intercalation of nanoparticles, nanotubes or nanosheets with organic monomers, followed by polymerization.

Nanoscale dispersion of filler in the composite can introduce new physical properties and novel behaviors that are absent in the unfilled matrices, or improve some properties which already exist in the unfilled matrices [12]. The advantages include catalytic activity, producing super paramagnetism and others electromagnetic phenomena, reinforced strength and toughness, modified hardness and plasticity. These properties significantly extend their biomedical application such as bone tissue engineering scaffolds, medical devices for releasing therapeutics, biosensors, etc. [13]

1.4 Biofouling

Biofouling is a big challenge in the field of biomaterials science. For example, synthetic materials in the form of prosthetic devices, such as artificial heart valves, coronary stents

and vascular grafts [14], have been used for decades, and have shown acceptable safety. However, they are not truly blood compatible. The risk of thrombotic events (formation of blood clots) is always present and patients need to take anticoagulant drugs continuously after surgery. The side effect of this drug is the increased risk of bleeding [15]. Another application affected by biofouling is contact lenses. The adsorption of non-specific protein may result in protein fouling, patient discomfort, and fouling of microbials may result in the keratitis [16].

The non-specific protein adsorption is the main reason of the biofouling of cells and microbials. The mechanism of protein adsorption is not clear. Two main adsorption models are suggested: one is based on the hydrophilic surface of the substrates; the driven force for the adsorption is electrostatics interaction [17], van der Waals forces and hydrogen bond may also contribute to the adsorption; another model is based on the hydrophobic surface. This model is suitable for the globular protein which has a densely packed hydrophobic core surrounded by a hydrophilic coat of polar amino acids. Entropic gain can be made when the densely packed hydrophobic core reorganized due to the adsorption to drive out the water which was originally in contact with the hydrophobic surface [18]. In this model, the protein is denaturized, which will lead to an irreversible adsorption.

Currently, there are several different molecules used for surface coating to reduce non-specific protein adsorption, such as PEG, zwitterionic materials, carbohydrates, peptides and peptide-like polymers (figure 1.1). PEG molecules are the most commonly used for surface coating due to their high protein resistance and extremely low toxicity [19]. Several factors in combination are responsible for the resistance of PEG. For instance, the electrostatic free state of PEG ensures that no electrostatic attraction of proteins takes place. Furthermore, the structure arrangement of PEG on the surface of substrates shows a strong interaction with surrounding water molecules, which can reduce protein adsorption.

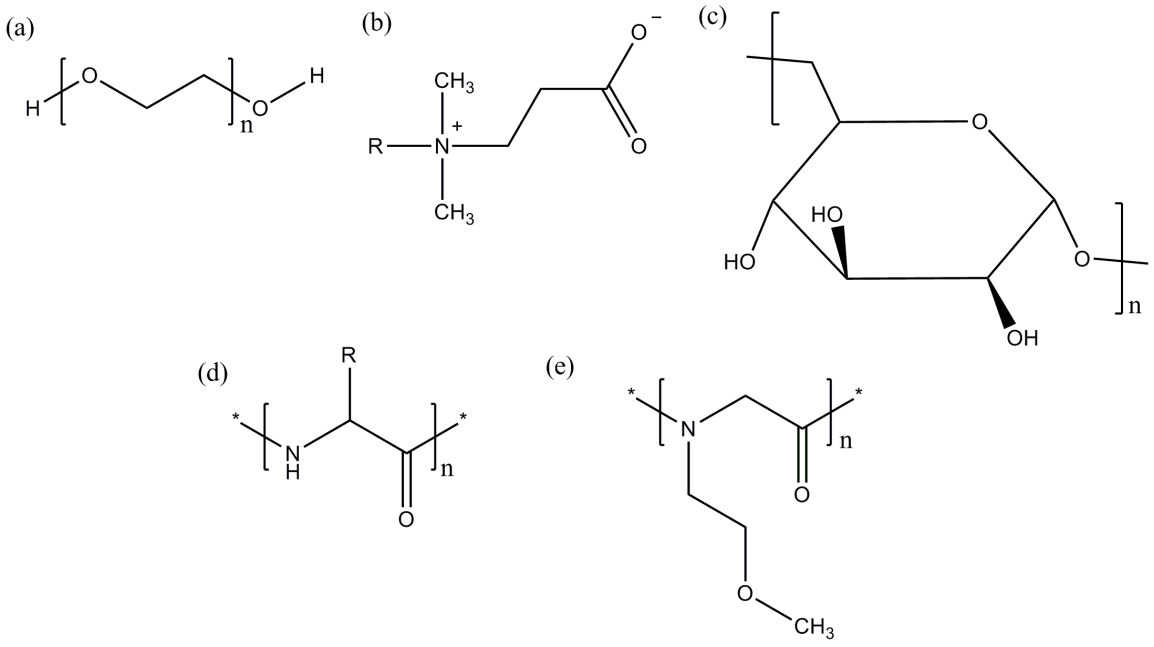

Figure 1.1: Chemical structure of nonfouling molecules for surface coating. (a) PEG, (b) Carboxybetaine, with zwitterionic groups, (c) Dextran, carbohydrate, (d) A normal peptide chain, R indicates the side chain, (e) Peptide-like polymer.

1.5 Laser-assisted coating process for PEG deposition

PEG deposition on substrates was performed by using MAPLE technique [20]. This technique has several advantages, such as accurate thickness control, thin film homogeneity and most of all, it can be applied to most organic molecules if proper deposition parameters are applied. The working mechanism of MAPLE is simple. Briefly, when laser beam strikes a frozen target, which is made up of organic molecules and volatile solvent, most energy is absorbed by the solvent because target materials makes only a small part of the solution (usually lower that 5%). So the thermal damage to organic target is limited. The target materials will be evaporated from the target with high energy and then deposited onto substrate (figure 1.2). Laser wavelength, fluency, target and substrate distance and substrate temperature strongly affect thin film quality and homogeneity, which will be discussed in detail in chapter 2.

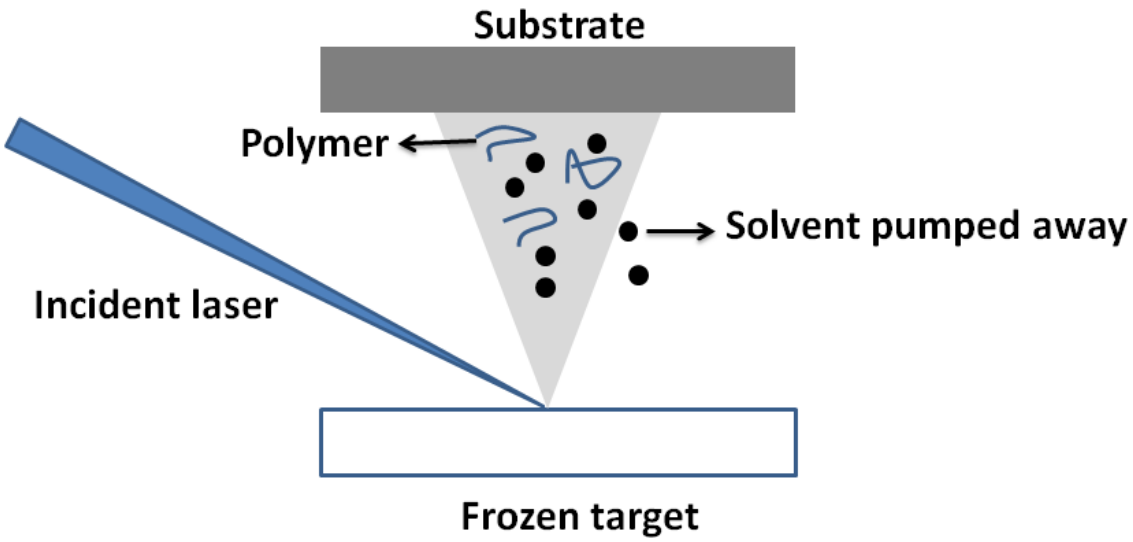

Figure 1.2: Scheme of MAPLE deposition mechanism.

1.6 Objectives

The overall objective of the thesis is to improve the properties of hydrogel materials, particularly, to minimize their protein adsorption. Two strategies will be studied: (1) Addition of silica nanoparticles (SiO_2 NPs) in hydrogel to form nanocomposites. Co-polymer 2-hydroxyethyl methacrylate with a low amount of 2-aminoethyl meth- acrylate p(HEMA-co-AEMA) and silicone hydrogels, as well as their nanocomposites will be synthesized and characterized. (2) Hydrogel surface will be modified by using laser-assisted method. MAPLE deposition technique will be applied for PEG thin film coating to reduce protein adsorption. The step-wise objectives of this thesis are as follows:

- Synthesis p(HEMA-co-AEMA) and silicone hydrogels by using photo-polymerization method. In order to increase the mechanical strength and the potential for future application, hydrogels with silica nanoparticles (SiO_2 NPs) were also developed.

- Characterization and comparison of p(HEMA-co-AEMA) and silicone as well as their nanocomposites for the application as biomaterials.

- The deposition of PEG thin film on the surface of silicone to improve its protein resistance capability.

- Characterization of PEG thin film quality, including its chemical structure, thickness and roughness.

- Comparison of protein resistance of silicone hydrogel before and after PEG film deposition.

1.7 Thesis overview

An overview of each chapter is presented as follows:

Chapter 2 Literature review

This chapter reviews the hydrogel synthesis procedure and the application of hydrogels in biomaterials science, such as contact lens materials and tissue engineering. In the second part, I summarize the common techniques for hydrogel surface modification, especially the technique based on laser.

Chapter 3 Experimental methods

This chapter describes the procedures for synthesizing p(HEMA-co-AEMA), silicone hydrogels, and hydrogel-based nanocomposites. The laser-assisted PEG thin film deposition process and the interaction between pulsed laser and hydrogels are described. The characterization instruments, such as FT-IR, SEM and AFM, are briefly reviewed; finally, the water absorption, protein adsorption and cell viability experiments are also discussed here.

Chapter 4 Synthesis and characterization of co-polymer of pHEMA and silicone hydrogel as well as their nanocomposites

Two types of hydrogels, i.e. copolymerized p(HEMA-co-AEMA) and silicone hydrogel, were synthesized by using photo-polymerization method; the nancomposites were developed by mixing gel monomer with SiO_2 NPs before polymerization. Their

mechanical strength, protein adsorption, swelling ratio and cell viability have been studied.

Chapter 5 PEG thin film deposition and characterization

The PEG thin films were deposited on silicone hydrogels by MAPLE with an Nd: YAG laser at a wavelength of 532 nm. The thin film was characterized by FT-IR for chemical composition and AFM for thickness and surface roughness. It should be noticed that the AFM measurements were conducted on cover glass substrate but not on silicone because the big surface roughness on silicone itself will make big interference to such measurements. The BSA adsorption of silicone before and after PEG film deposition was also examined.

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Chapter 2

Literature on the Interaction between Hydrogel and Laser process

Polymer hydrogels are transparent soft materials that have been applied in medial field, such as contact lens, implantable device and wound dressing because of their biocompatible and proper mechanical properties. However, such materials still have drawbacks because of the strict biomaterial requirements. For example, artificial implants in human body usually adsorb lots of non-specific protein; such protein sticking will induce serious host rejection and finally results in implant failure. To overcome such disadvantages, surface modification is required. Laser is a powerful tool that has been used for surface coating. In this research, the interaction between laser and polymer hydrogels was investigated, and more importantly, laser was employed in hydrogel surface coating with biomolecules to improve their biological effects.

2.1 Introduction of polymer hydrogels and hydrogel-based nanocomposites

Hydrogels are interconnected polymer chains. They can be formed from soluble monomers and/or multifunctional polymers (macromers). Crosslinks are used as junction points to connect the monomers and/or macromers together. Depending on the components and the crosslinking method, hydrogels varied in their morphology, mesh size, degradation behaviors, mechanical strength and biological activities. However, hydrogels also share some features in common. For example, they can absorb water from 10-20% up to thousands of times their dry weight [1]; they have a degree of flexibility similar to natural tissue due to their significant water content; they allow for the free diffusion of gas, nutrients and metabolites due to their porous inside structure.

2.1.1 Hydrogel synthesis

Hydrogels can be divided into physical hydrogel and chemical hydrogel based on their different synthesis methods. For physical hydrogels, the polymer networks were held together by molecular entanglements, and/or secondary forces including hydrogen

bonding, hydrophobic force, ionic interaction or biological recognition parts. For example, Cho et al. used chitosan and glycerol phosphate (GP) disodium salt to form chitosan gel as scaffold for rat bone marrow mesenchymal stem cells. Chitosan is crosslinked based on the ionic interaction between GP and chitosan via the phosphate and ammonium groups, respectively [2]. The physical hydrogels are easy to prepare, and they are actively responsive, but the disadvantages are their low stability and mechanical strength. To resolve the above problems, chemical crosslinking method was introduced during or after physical procedure.

There are several different chemical crosslinking methods based on different crosslinking chemical groups. The most widely used method is the radical polymerization in monomers with vinyl groups [3]. Radical polymerization involves at least three steps: the radicals' generation, then the propagation and finally the termination. The radicals can be generated via the reaction between oxidizing and reducing reagents or photolytic decomposition from a photoinitiator. Once generated, radicals immediately engaged in the subsequent propagation and when the two prolonged monomer chains with free radicals meet together, the reaction is terminated. Other chemical crosslinking methods include carboxylate groups, sulfhydryl groups, etc.

2.1.2 Hydrogel for biomedical application

Hydrogels attract great interest for years because of their biocompatible potential and their flexibility similar to nature soft tissue. Besides, by taking advantage of the specific feature, hydrogel can be used in some specific biomedical applications. In this review, the application in contact lens and tissue engineering will be discussed.

2.1.2.1 Hydrogels for contact lens

Hydrogels are firstly considered in the application of contact lens is due to their soft mechanical strength. However, there are several more important requirements for the contact lens application, such as materials transparency, oxygen transmittance, acceptable biocompatibility and more importantly, protein fouling resistance. It has been reported that protein deposits on contact lens can result in discomfort and keratitis [4]. Poly-2-hydroxyethyl methacrylate (pHEMA) has been used as contact lens materials for more

than 40 years and is still in extensively application today. This material is cheap and very stable. Its water content does not change too much with the temperature or pH [5]. But the disadvantage of this material is it is still relatively impermeable to oxygen, which leads to some harmful hypoxic response such as reduced mitosis and epithelial microcysts [6].

Researchers tried to improve pHEMA oxygen permeability by increasing its water content, since the oxygen transport in pHEMA is through water absorption and releasing. For that, some strong hydrophilic monomers with high water absorption such as N-vinylpyrolidinone (NVP) and methacrylic acid (MAA) were added in the matrix [7]. This method does improve the oxygen permeability, but the more effective way is to develop new materials. The newly developed contact lens materials silicone improved oxygen permeability significantly because silicone transports oxygen through siloxane-phase rather than through the water phase in the conventional pHEMA contact lens [8].

Another factor of contact lens materials which will affect people's wearing experience, especially for continuous wearing experience, is their protein and lipid fouling resistance as such adhesion will induce discomfort and inflammatory responses. However, it is not easy to solve this problem because of the complexity of tear film components and interaction mechanism between tear film proteins and contact lens materials. For example, ionic pHEMA tends to adsorb proteins with opposite charges rather than non-ionic proteins or proteins with the same charges. It is also reported that hydrophilic hydrogels, such as pHEMA, adsorb a larger amount of proteins than hydrophobic gels, such as silicone [9]. But for hydrophobic gels, the pitfall is it will denature the tear film proteins. In usual hydrophilic environment, the hydrophobic amino acids are protected inside the protein, but when exposed to hydrophobic solid surface, such as silicone, proteins tend to rearrange their structure to an unfolded state in order to lower the Gibbs energy [10]. Such denatured proteins are unable to perform their natural tasks, but instead they may interact with other proteins, which may induce aggregation immune reactions [11]. In order to solve this problem, surface modification of silicone is required. Currently there are mainly two methods or in combination to modify the gel surface. One method is converting the polymer chemical group on the surface to more

polar and hydrophilic ones through wet-chemical or plasma oxidation technique [12]. For example, methyl group can be converted into hydroxyl group through plasma oxidation treatment. The major challenge of this process is rendering the products stable for long time [13] and for subsequent manufacturing steps, such as sterilization. The second strategy involves the coating of a new material on the substrate surface [14-16]. There are many chemical and physical techniques available now for surface coating. A detailed discussion will be in section 2.2.

2.1.2.2 Hydrogels for tissue engineering

Tissue engineering is the use of a combination of cell, biomimetic matrices and biology growth factors to improve or replace tissue function. Hydrogels are the most attractive tissue engineering scaffold due to their similar structure and function to extracellular matrices (ECM). However, there are still several basic requirements for tissue engineering hydrogels: (1) they must be biocompatible; (2) they must be nutrients, gas and metabolite permeable; (3) they must provide suitable mechanical support for a prolonged period of time [17][18]. Over the past few decades, hydrogels have been fully developed from the passive support scaffold to an interactive and intelligent matrix which can provide biochemical signals for cell proliferation, migration and differentiation [19][20]. To achieve that, the usual method is incorporating other materials, such as nanoparticles or growth factors, into hydrogels [21][22]. With the development of nanotechnology and biology, more and more materials and technologies have been applied in hydrogel tissue engineering.

Incorporating nanomaterials to enhance mechanical property

Hydrogels, especially the hydrophilic one, have comparatively loose structure and low mechanical strength due to high water content. On the other hand, the tissue scaffold application requires proper mechanical strength. Besides, abundant evidence suggests that mechanical signals provided by cell substrates have effects on cell proliferation, differentiation, apoptosis, migration and gene expression. So it is important to enhance hydrogels with proper mechanical toughness and elasticity to maintain desired cell phenotype and function and provide enough mechanical support *in vivo*. One of the novel

methods to do so is dispersing nanomaterials into hydrogels homogeneously. Such nanoparticle containing hydrogels are called nanocomposites. Kazutoshi et al. found that the mechanical properties greatly increased by adding inorganic nano-clays. In their opinion, the clay sheet acts as a cross-linking agent for the polymer [23]. Xin Zhao et al. also found that the young's modulus of poly(vinyl alcohol) (PVA) hydrogel increased to nearly 10 times with graphene loading of 1.8 vol% [24].

Engineering bioactive hydrogels

In order to mediate cell attachment, proliferation and differentiation, integrin binding sites, such as RGD peptide, as well as cell growth factors, such as TGF (transforming growth factor) and bFGF (Fibroblast Growth Factor basic), need to be incorporated into hydrogels. Such incorporation includes covalent conjugation and physical embedding. One example for covalent conjugation is the immobilization of ECM protein, which can provide cell with anchor points. For example, Maya-Gonen et al. conjugated PEG molecules with collagen, fibrin or albumin proteins. Such PEGylation can provide the anchor points for cell attachment [22]. However, in some cases, the mobility of incorporated molecules is required, such as the growth factors, so the covalent conjugation method is not applicable. So such small molecules were confined in the hydrogel due to the meshes and physical interaction, such as hydrogen bond and Van der Waals force. But this molecule incorporation method is size and chemical feature-dependent. For a very small growth factor, the high permeability of hydrogels cannot provide a long term cell growth stimuli. To overcome this problem, the multiphase loading method was involved. The small molecules were first preloaded into microparticles; the microparticles were then loaded into hydrogels to achieve long term growth factor availability. As an example, TGF-b1 was incorporated into gelatin microparticles, which were then encapsulated in PEG hydrogels to control the *in vitro* releasing rate [25].

2.1.3 Hydrogel-based nanocomposites

Nanocomposites have been defined by Ajayan et al. where they state: "A nanocomposite is as a multiphase solid material where one of the phases has one, two or three

dimensions of less than 100 nm, or structures having nano-scale repeat distances between the different phases that make up the material" [26]. Many methods have been described for the preparation of polymer nanocomposites. The most important ones are i) *In-situ* intercalative polymerization; ii) *In-situ* polymerization; and iii) Sol-gel process. *In-situ* intercalative polymerization involves the encasing of the layered nanosheets within monomer solution, and then the formation of polymer between the intercalated sheets [27]. *In-situ* polymerization involves the dispersion of inorganic particles the polymeric matrix (monomer) and the polymerization of the mixture by addition of an appropriate catalyst [28]. For sol-gel process, organic molecules and monomers are firstly embedded on sol-gel matrices, then the sol-gel reaction will form an inorganic component, and the organic reaction will form an organic polymer network [29].

Hydrogel-based nanocomposites have a lot of advantages compared to hydrogels, such as catalytic activity, producing super paramagnetism and others electromagnetic phenomena, reinforced strength and toughness, modified hardness and plasticity [30]. These advantages give hydrogel-based nanocomposites extended application in biomedical application. For example, Sitharaman et al. designed ultra-short single-walled carbon nanotubes (US-tubes)/ propylene fumarate diacrylate nanocomposites as bone tissue engineering scaffolds. US-tubes were used to reinforce the polymer scaffold. The scaffold exhibited favorable *in vivo* biocompatibility in a rabbit model [31].

2.2 Surface treatment

As mentioned above, non-specific protein adsorption of hydrogel is a big problem for its biomedical application. The common strategy to solve this problem is based on surface engineering techniques. Different physical and chemical surface modification techniques, including plasma treatment, wet chemical methods, laser assisted surface coating, etc, have been applied in biomedical hydrogels. In this section, several surface modification techniques will be reviewed, and their merits and pitfalls will be discussed.

2.2.1 Plasma treatment

The plasma treatment technique is a gas-phase processing method used to create hydrophilic hydrogel surface by oxidation. Plasma is a mixture of electrons, ions and

radicals, which is produced from glow discharges, radio frequencies and gas arcs. Different gases, such as oxygen, nitrogen, hydrogen and argon, have been employed as plasma sources. The oxygen plasma is the most popular 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 [32].

One big problem with oxidation of PDMS or other silicone hydrogel is the hydrophobic recovery [33]. It is believed that it is due to the uncured hydrophobic silicone monomer move from the bulk to the surface [34]. To overcome this problem, Vickers et al. extracted the unreacted monomers in PDMS with a series of solvents before air plasma treatment [35]. Such extracted PDMS shows much longer stable period up to 7 days compared to untreated PDMS only for 3 hours.

2.2.2 Chemical surface modification methods

Hydrolysis, covalent immobilization and wet chemical methods, such as Layer-by-layer (LDL) are three ways to chemically modify a surface. By using dilute acid or alkali in hydrolysis, ester bond on the surface were broken down and produced carboxyl and hydroxyl groups [36]. In covalent conjugation, different cross linker molecules were used to activate the chemical groups on the surface of substrates and conjugated 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 crosslinker for the conjugation between materials have amine and thiol residues [38].

LDL deposition is a simple and cheap wet chemical technique for thin film deposition. The films are formed due to the electrostatic interaction between materials with opposite charges. For example, Wei and Thomas deposited Poly(allylamine hydrochloride) (PAH) and poly(sodium styrenesulfonate) (PSS) on three different

substrates: PET, PET-$CO2^-$, and PET-$NH3^+$. The multilayer assemblies showed good mechanical integrity and no failures were observed in the multilayers [39]. One advantage of LDL deposition is the high degree of thickness control of the multilayers. The growth of the films has linear relation with the number of bilayers.

However, all the chemical methods have their limitations. LDL deposition technique is limited to polyelectrolytes which can form multilayers due to electrostatic interaction. Acidic or alkali hydrolysis is only suitable for materials with ester bond. Covalent conjugation requires specific crosslinkers for activation and conjugation, but sometimes it is not easy or very expensive for activation of specific chemical group. For that, it draws more and more interests for researchers to develop a single technique which can deposit a wild class of materials.

2.2.3 Laser based surface coating

The most commonly used laser based deposition technique is the pulsed laser deposition (PLD). A high power laser beam is focused inside a vacuum chamber to strike a target of the material that is to be deposited. The material is vaporized from the target with high energy and then is deposited as a thin film on the substrate. This technique is suitable for the deposition of inorganic materials like semiconductors [40], metals [41] and alloys [42]. Although the basic setup is simple compared to other deposition techniques, the physical phenomena of laser-target interaction and film growth are quite complex. When the laser beam is absorbed by the target, the energy is first converted to electronic excitation and then into thermal, which result in evaporation, ablation and plasma formation. The ejected materials in vacuum chamber include atoms, molecules, electrons, ions, etc.

One drawback of PLD is that it is not suitable for the deposition of organic materials, because the high power laser beam may break the chemical bond and damage the chemical structure of organic molecules [43]. To solve this problem, PLD technique was modified. The new technique is called Matrix assisted pulsed laser deposition (MAPLE). The biggest difference between PLD and MAPLE is their target. For PLD, the target is semiconductor, metal or alloy. For MAPLE, the target is liquid solution with low

concentration polymer target molecules dissolved in volatile organic solvent. The liquid solution is then freezed by liquid nitrogen. So when the laser beam strikes the target, most energy is absorbed by the solvent because the target material makes only a small part of the solution (usually lower that 5%). Little chemical damage occurred on target molecules during deposition, and still they can be ejected from the target and deposited onto substrate due to solvent evaporation.

Excimer lasers or Nd: YAG lasers with third harmonic at 335 nm are the laser sources mostly used for MAPLE. The infrared laser sources are only utilized in some particular cases [44]. The reported materials which have been deposited as well as the deposition parameters are shown in table 2.1.

2.3 Summary

The review gives a brief description of the synthesis of hydrogel and hydrogel-based nanocomposites and their biomedical application. Since surface property of hydrogel has important effect on the interaction between hydrogels and tissues, several different surface modification techniques, such as oxygen plasma treatment, chemical grafting, layer-by-layer deposition, and laser based surface modification technique were described. Their advantages and disadvantages were also discussed. Finally, the laser based surface deposition technique, MAPLE, were highlighted. The working mechanism was discussed and important parameters for different molecule deposition were listed in table 2.1.

Table 2.1 Summary of organic thin film deposited by MAPLE technique

Materials/Solvent	Fluence, J/cm^2	d_{ts}, cm	Number of pulse ($*10^3$)	Spot, mm^2	Laser freq., Hz	Wave-length, nm	Target den-sity, wt%	Pressure, Pa	Target temp., ^oC	Ref.
BSA/PBS	0.1-0.5	4	1.8-40	20	N/A	248	0.1-1.5	15 N_2	LN	45
Fibrinogen/PBS	0.7	3.5	15	25	15	248	N/A	6.5	LN	46
PEG/propanol/water	4	1	60	2	10	355	4.1	10^{-4}	LN	47
	0.085	1	60	80	10	532	4.1	10^{-4}	LN	
Pullulan/water	0.16/0.24	N/A	7-10.7	1.2	2	248	<2	20 N_2	-196	48
Glucose,sucrose,dextran/H_2O	0.05-0.25	5	N/A	4	2-5	193	5	6.6 Ar	LN	49
Collagen type I/H_2O	0.16-0.6	3	10	18.5	3	248	2	16 N_2	LN	50
Alendronate-hydroxyapatite/H_2O	0.75	4	20	N/A	10	248	~4.7	10	LN	51
M. edulis foot protein-1	0.4-1	7	N/A	3	20	193	2	10^{-4}	-100	52

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Chapter 3

Experimental Methods

In this chapter, the experimental details of this project are described. They include: (1) the synthesis of silica nanoparticles, p(HEMA-co-AEMA) and silicone hydrogel as well as hydrogel nanocomposites; (2) the PEG coating procedure by using MAPLE technique; (3) a brief introduction of materials characterization instruments which were used in this project.

3.1 Synthesis of phosphonate functionalized FITC loaded mesoporous silica nanoparticles (FMSNs)

5.5 mg FITC dissolved in 3 ml ethanol and mixed with 12 μl APTS and stirred under dry nitrogen. After 2 hours, 2 ml TEOS was added and stirred several minutes for homogeneously distribution. Meanwhile, 0.1 g CTAB and 5 ml distilled water were mixed vigorously. After 30 mins, the solution was added to 43 ml water (350 μl of 2M NaOH was added to control pH) and heated to75-80 ^{o}C. After temperature stabilized, 1 ml FITC-APTS solution with TEOS was added slowly (drop by drop) to the aqueous solution. After 15 mins stirring, 127 μl TPMPH was added and stirred continuously for another 2 h. The nanoparticles were harvested and purified with ethanol by centrifuging (8000 rpm, 10 mins) and sonication procedure.

3.2 Hydrogel synthesis

3.2.1 Co-polymer p(HEMA-co-AEMA) synthesis

30 mg AEMA and 15 mg DMPA were dissolved in 100 μl DMSO with vortex, respectively and mixed with 3 ml HEMA and extra 1ml DMSO was added. Then 6 μl Ethylene glycol dimethacrylate (EGDMA) was added into the mixture as the cross-linker. All the substrates above were covered with Aluminum foil to avoid photobleaching. The mixture was bubbled with nitrogen for 10 min to exclude the oxygen. For photo-polymerization, the mixture was drop-wisely added on the surface of cover glasses, which were confined by silicone isolators (Sigma-Aldrich). The cover glasses were

irradiated for 20 min in the UV environment for crosslinking. The products were then soaked in 30% ethanol overnight to remove the chemical residues on the surface. The reaction mechanism was shown in figure 3.1. DMPA produced free radicals under UV radiation, which then initiated the chain reaction between AEMA, HEMA and EGDMA.

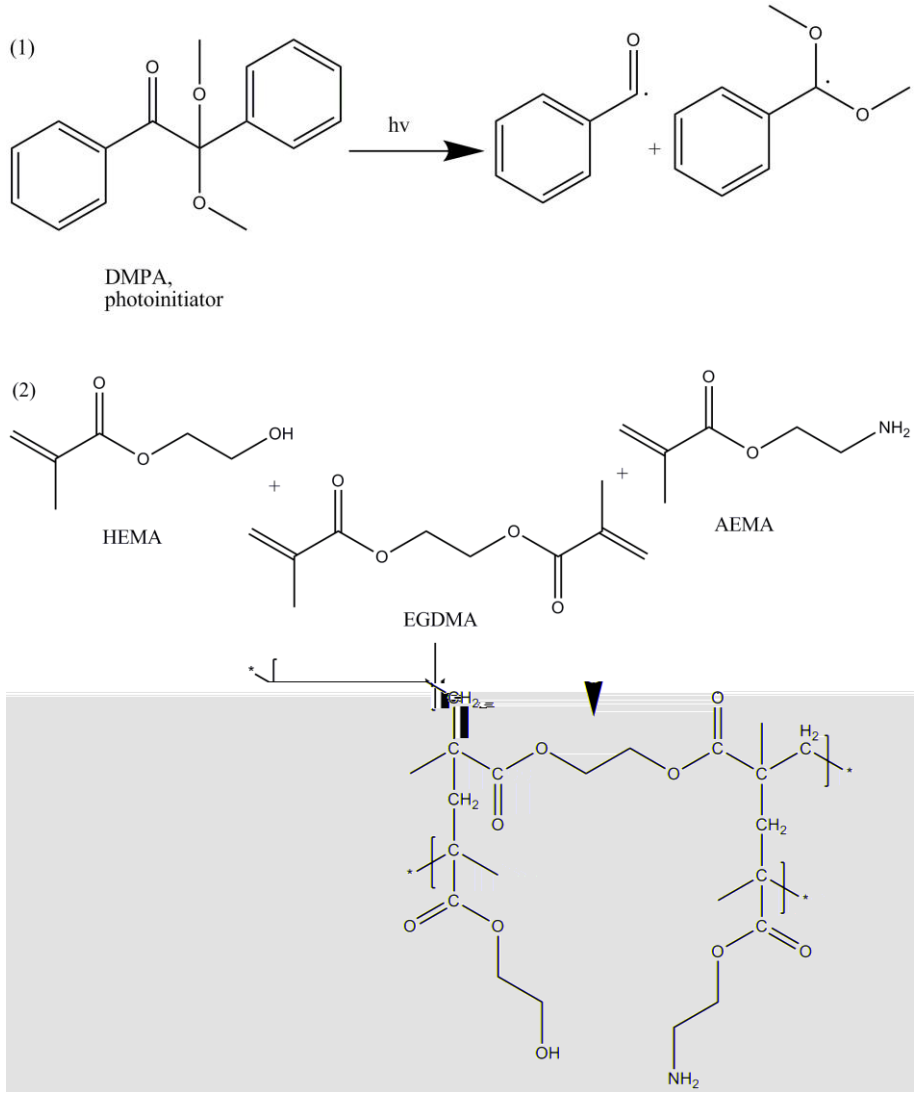

Figure 3.1: Scheme of p(HEMA-co-AEMA) photo initiated crosslinking reaction. Step (1), free radicals produced from photoinitiator under UV irradiation. Step (2), crosslinking reaction happened among HEMA, AEMA and EGDMA. EGDMA employed as a crosslinker.

3.2.2 Silicone synthesis

The silicone hydrogel was produced by following the synthesis procedure developed by Kim et al. [1]. Briefly, 3 ml of mixture of TRIS, bis-alpha,omega-(methacryloxypropyl) polydimethylsiloxane and DMA with the ratio 4:1:2 was combined with 0.18 ml of NVP, 15 ml of EGDMA and 0.3 ml ethanol. Then the mixture was purged with dry nitrogen for 15 min. 8 mg of photoinitiator was then added to the mixture and stirred for 5 min. For photopolymerization, the mixture was also drop-wisely added on the surface of cover glass but was irradiated for 50 min by UV for complete crosslinking. The hydrogel was washed by ethanol and dried in air overnight (figure 3.2).

Both p(HEMA-co-AEMA) and silicone hydrogel were developed by using the same photo-polymerization process through vinyl group crosslinking. First, photo-initiator was irradiated by UV to produce free radicals. Then the free radicals can initiate the crosslinking process by attacking vinyl groups on monomers. The crosslinking process was terminated when two vinyl free radicals reacted to form C-C bond.

3.3 Nanocomposites

To produce NPs-hydrogel nanocomposites, NPs were suspended in hydrogel solution and sonicated to make homogenous distribution. Then the suspensions were purged by nitrogen and prepared for photo-polymerization.

3.4 Laser and hydrogel interaction

3.4.1 PEG coating by MAPLE

Before PEG deposition by MAPLE, the silicone substrate was treated with oxygen plasma to remove extra chemical residues. Silicone hydrogels were etched in STS Reactive Ion Etch with the 13.56 MHz system and the plasma power 90 W for 10 min on each side.

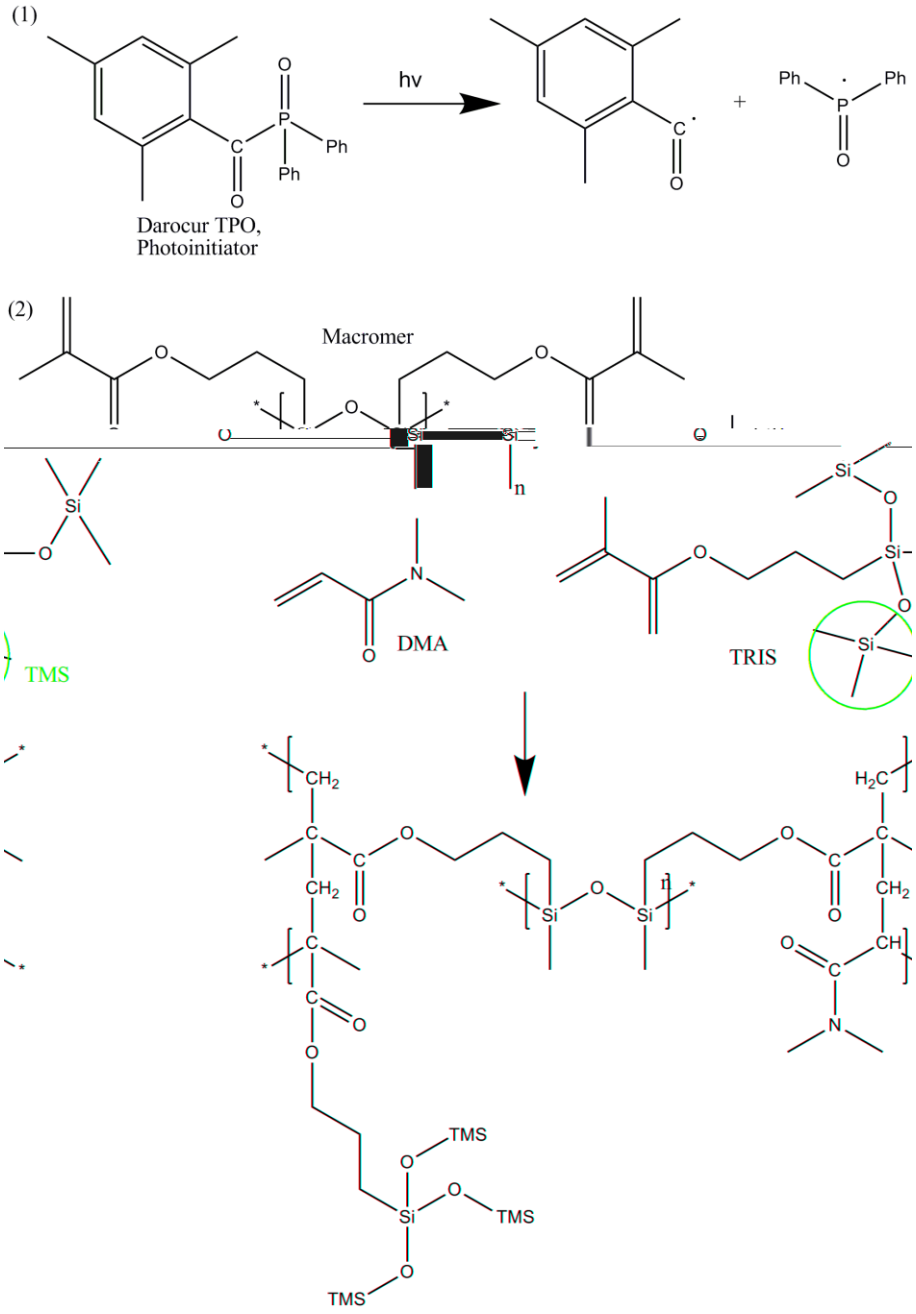

Figure 3.2: Scheme of silicone photo initiated crosslinking reaction. Step (1), free radicals produced from photoinitiator under UV irradiation. Step (2), crosslinking reaction happened among DMA, TRIS and macromere. EGDMA also employed as a crosslinker (not shown in this figure).

For target preparation, 4% wt PEG was dissolved in iso-propanol and then injected into the target holder in the vacuum chamber. The solution was then freezed by liquid nitrogen. After the target solution was totally frozen, the vacuum chamber was pumped out to nearly 10^{-6} Torr. At this time target movement and laser emission started, while the substrate was covered by substrate cover in order to clean the target surface. After 1 min the substrate movement started and the substrate cover removed to initiate the deposition.

The laser used for irradiation has the wavelength of 532 nm and the frequency 10 Hz and the fluency approximately 1 J/cm^2. The laser spot on the target is about 0.15 cm^2 and the distance between target and substrate is 7 cm. The substrate has the temperature of about 32 ^{o}C during the deposition. The pressure inside vacuum chamber increased to about 10^{-5} Torr after 150 min deposition.

3.4.2 Hydrogel laser etching

In order to examine if laser will cause any damage to hydrogels, the chemical groups of hydrogel before and after laser etching were examined by FTIR. The pulsed laser used for hydrogel etching has the wavelength of 532 nm, frequency of 10 Hz and the fluency of about 1 J/cm^2. The etching lasts for 10 min before FTIR analyze.

3.5 Materials characterization

3.5.1 Scanning electron microscopy

SEM is a type of electron microscope that images a sample by scanning the surface with electron beam. The electron beam is supplied by an electron gun and focused by one or two condenser lenses to a spot about 0.4 nm to 5 nm in diameter. When the electron beam interacts with the sample surface, the electron will be scattered and absorbed. The typical signal produced by SEM includes secondary electrons and back-scattered electrons (BSE), and each signal can be detected by specific detector.

For sample preparation, specimens must be electrically conductive on the surface and also electrically grounded to prevent the accumulation of electrostatic charge. Little treatment is required for metal samples, but for organic samples, conductive materials

coating on the surface is needed. Such conductive materials include gold, platinum, tungsten and graphite.

In this research, the hydrogels is not electrically conductive, so metal surface coating is needed. Hydrogels were coated with gold by Hummer VI Sputter Coater and the surface morphology was observed by SEM (Hitachi 3400s) at 10 kV or 20 kV.

3.5.2 Fourier transform infrared spectroscopy

FTIR is a technique which is used to obtain an infrared spectrum of absorption, emission, photoconductivity or Raman scattering of a solid, liquid or gas. This technique is based on the theory that each chemical group has characterized absorption infrared spectrum. The FTIR instrument shines a beam containing many frequencies of light at once, and the adsorption by the sample will be measured. Next, the beam is modified to contain a different combination of frequencies, giving a second data point. This process is repeated many times. Afterwards, a computer takes all these data and works backwards to infer what the absorption is at each wavelength by using a common algorithm called the Fourier transform.

In this project, the chemical groups of p(HEMA-co-AEMA) and silicone hydrogel were characterized by FTIR. This technique was also be used in the comparison of the chemical change of PEG before and after MAPLE deposition.

3.5.3 Atomic force microscopy

AFM is a type of scanning probe microscopy, which can demonstrate the 3D surface topography of a specimen with high resolution up to a nanometer. The most important part of AFM is a cantilever with a probe at its end. The probe has a radius of curvature in nanometers. When probe approaches the specimen surface, forces between probe and specimen may induce a deflection of the cantilever. The forces include van der Waals forces, chemical bonding, electrostatic forces, etc. The deflection of the cantilever can be detected by using a laser spot reflected from the top of cantilever into photodiodes.

In this research, the surface topography of PEG coated cover glass were examined by AFM.

3.6 Mechanical test of hydrogels and nanocomposites

A 8 x 8 mm specimen of nanocomposites and plain p(HEMA-co-AEMA) based hydrogel was mounted in a BioTester 5000 test system (CellScale Biomaterials Testing, Waterloo, Ontario) by using the mounting system. The specimens were stretched uniaxially with a loading of 0.2 mN applied on the tensile test consistently. Meanwhile, the images of the deformation of the specimens were captured using a 1280x960 pixel charge coupled device CCD- camera. The stress and strain produced in order to understand the Stress-Strain curves of different samples and their Young's modulus (E), which is described as the Eq. 3.1 below.

$$E = \frac{Stress}{Strain} = \frac{\sigma}{\varepsilon} = \frac{F}{A} \Big/ \frac{\delta L}{L_0}$$

Eq.3.1

Where E is the Young's modulus in Pascal (Pa), F the force applied in Newton (N), A the original cross-sectional area through which the force is applied in meter square (m^2), δL the displacement of the materials (m), and L_0 the original length of the materials (m). Young's modulus is a measure of the stiffness of a material, i.e., the higher the Young's modulus of a material, the stiffer it is, and the less strain it exhibits for a given stress.

3.7 Swell ratio of hydrogels

For swelling ratio, hydrogels were first freeze dried for 24 h to exclude the water in hydrogel. Swelling experiments were performed in deionized water at room temperature for 20 h in total. The swelling ratio was calculated as below:

$$S = \frac{W_w - W_{dry}}{W_{dry}} \times 100\%$$

Eq. 3.2

W_w and W_{dry} are the weights of water adsorbed in hydrogel and the corresponding dried hydrogel, respectively. W_w were measured at 0.5 h, 1 h, 2 h, 4 h, 8 h and 20 h. Three repetitions were performed for all samples.

3.8 Protein adsorption of hydrogels

Protein adsorption of artificial implants may cause inflammatory response to human body, therefore the protein adsorption of hydrogels were tested. Briefly, the samples were immersed in distilled water overnight, and then soaked in 0.5 mg/ml BSA-PBS solution for 3 h at 37 ^{o}C. After that, samples were rinsed in PBS solution three times to remove the non-adsorbed BSA. The samples were then immersed in 1 wt% SDS-PBS solution and sonicated for 20 min to completely detach BSA from hydrogel surface to the solution. Finally, the BCA protein assay kit (SmartTM micro BCA Protein Assay Kit, intronbio, CAN) was used to determine the protein concentration in SDS-PBS solution with a UV-visible plate reader in 562 nm wavelength.

3.9 Hydrogel biocompatibility test

50,000 3T3 mouse fibroblast cells were seeded into 24 cell culture plate and incubated in 5% CO2 incubator overnight. Samples were chopped into small pieces and incubated with cells for 24 hours with 0.5 g sample per well. The cell viability was accessed by using MTT Assay. Briefly, after remove the samples, the MTT reagent was added to 24-well plate and incubated at 37 ^{o}C for another 4 h, then DMSO was added to dissolve the purple formazan product. The resulting signals were measured at an absorbance of 490 nm.

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Chapter 4

Photopolymerization of Hydrogel-based Nanocomposites

Hydrogel has long been used as drug and cell carriers, and tissue engineering matrices. In this chapter, two different hydrogels, p(HEMA-co-AEMA) and silicone, were synthesized through the UV induced photo-polymerization process. The important properties of hydrogels relevant to their biomedical application, for example, the biocompatibility, the mechanical strength and protein adsorption ability, are also identified.

Objectives:

1. To enhance the mechanical properties of hydrogel materials by mixing with silica nanoparticles.
2. To study the interface between inorganic nanoparticles and hydrogel matrix by using SEM and fluorescent characterization.
3. To study the protein adsorption of hydrogels and their cytotoxicity.

4.1 Introduction

Hydrogels have been believed to be useful as biomedical materials due to their biocompatible potential and the similar mechanical strength as human tissues. Over the past decades, hydrogel with different source (nature or synthetic), components, structure, were developed for different applications. Poly-hydroxyethyl methacrylate (pHEMA) and silicone based hydrogels are two major parts during them. Since pHEMA hydrogel was first produced by Wichterle and Lim in 1960 [1], it has been used as matrix device for controlled drug release [2]. Polydimethyle siloxane (PDMS, one commonly used silicone), has been used as sensor bases for glucose detection [3]. Also, the properties of hydrogels like hydrophilic and permeability can be modified with different strategies. For example, the oxygen permeability of silicone was altered by changing the components ratios [4] and the hydrophilic/hydrophobic of hydrogel can also be changed by adjusting hydrophilic/hydrophobic monomer ratios [5]. Sugiura, S also converted hydrophobic PDMS to hydrophilic with photo-induced surface modification [6]. Such modification created new properties that the original hydrogels do not have, which will extend the

application area of the hydrogels. Park S. et al. used pHEMA combined with collagen and poly(ethylene glycol) (PEG) for the application of artificial cornea [7] and PDMS macromer combined with other monomers were used as contact lens for ocular drug delivery[8].

Hydrogel properties can also be controlled by the development of hydrogel nanocomposites. Hydrogel nanocomposites are multiphase materials which contain nanomaterials or nano-structures in hydrogel. It has been reported that the mechanical property of the composites can be improved significantly [9], and properties such as electrical conductivity, antimicrobial capability will be added to the gel when the gel mixed with metal nanoparticles like gold or silver [10].

Another important property of hydrogel used as implant device or contact lens materials is its protein adsorption. Protein fouling on the surface of implants or contact lens can cause adverse reactions [11]. It is reported that charges and hydrophobicity of the materials influence protein adsorption [12]. Since the surface charge of hydrogel is not easily controllable, more efforts were put into the hydrophobicity control.

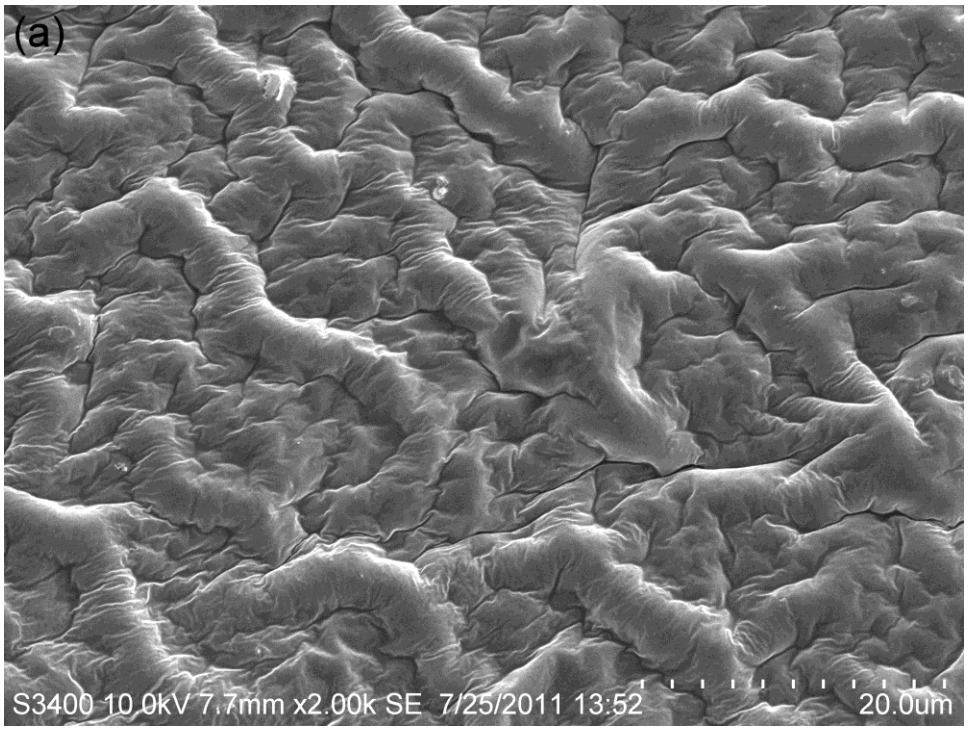
In this study, we synthesized copolymer of p(HEMA-co-AEMA) and silicone hydrogel based on Kim's report [13]. In addition, silica nanoparticles (SiO_2 NPs) were incorporated in both hydrogels to create silica/hydrogel nanocomposites. The chemical groups of hydrogel and nanocomposites were characterized by FTIR. Their tensile modulus, protein adsorption, water absorption and cell toxicity were also measured. The influence of silica nanoparticles in hydrogel was discussed in this chapter.

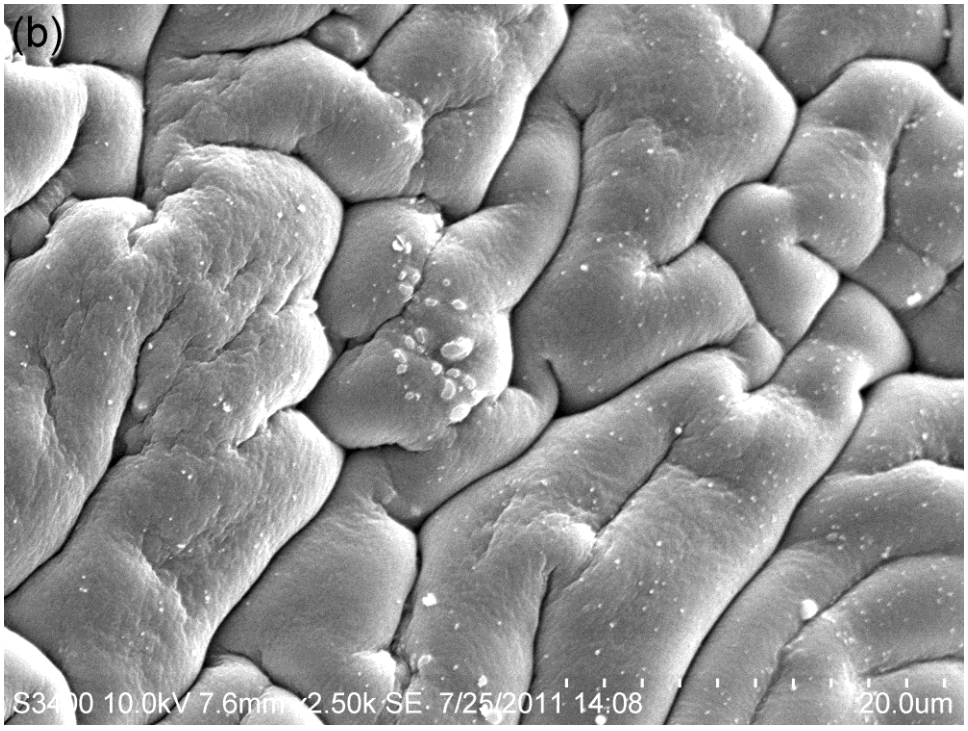
4.2 Results

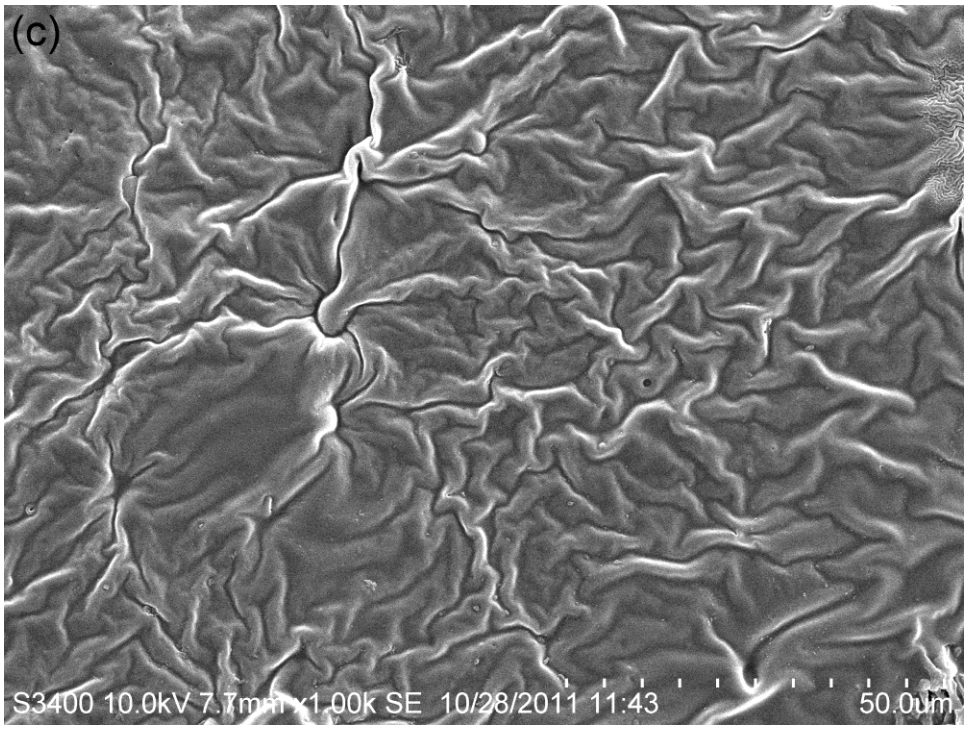
4.2.1 Hydrogel morphology

The surface morphology of p(HEMA-co-AEMA) and silicone as well as their nanocomposites were examined by SEM. It can be found that the polymer fibrils exist on both hydrogels and their nanocomposites due to monomer and macromer crosslinking. SiO_2 NPs can be identified in hydrogel nanocomposites as shown in figure 4.1. The

nanoparticles are dispersed homogeneously in both p(HEMA-co-AEMA) and silicone hydrogels.







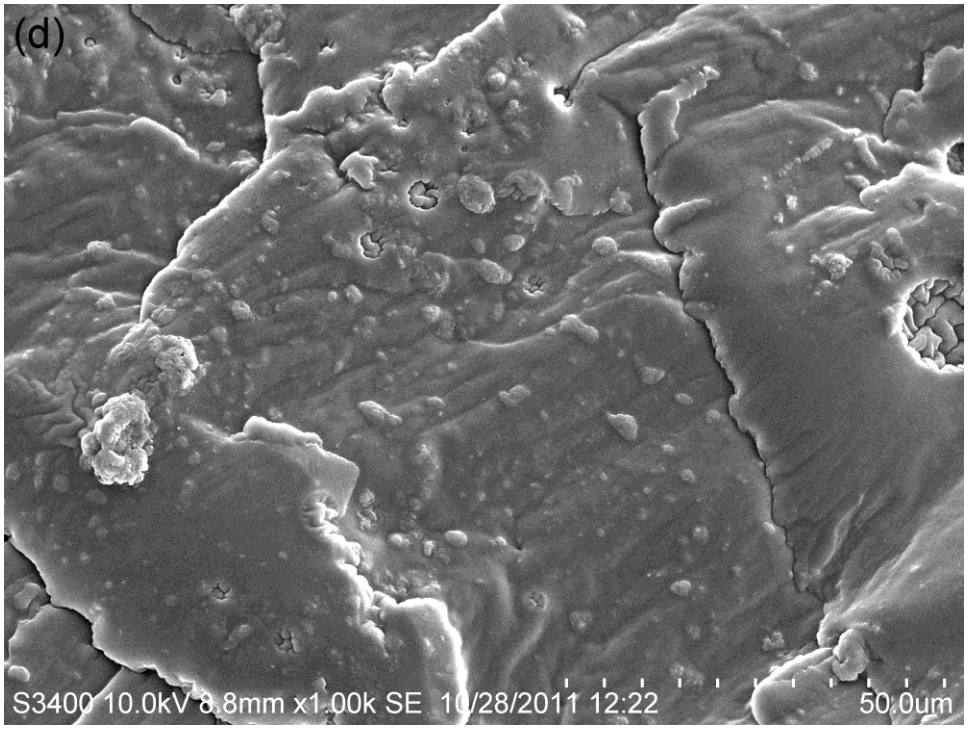


Figure 4.1: SEM image of (a) p(HEMA-co-AEMA) gel, (b) p(HEMA-co-AEMA)-SiO_2 nanocomposites, (c) silicone gel, and (d) silicone-SiO_2 nanocomposites.

4.2.2 Fluorescent characteristic of the nanocomposites

The FITC modified SiO_2nanoparticles were mixed with the hydrogels and the fluorescent spectrum of nanoparticle and the composites were measured. There is 3 nm blue shift of silica nanoparticle after mixing with the hydrogel as shows in figure 4.2 (a). The fluorescent time delay properties of SiO_2 nanoparticles and the nanocomposites were also tested. Figure 4.2 (b) demonstrated that there are no time delay differences between silica nanoparticles and silicone-SiO_2 nanocomposites. This result proves that the incorporation of SiO_2 nanoparticles into hydrogel do not change the time delay property of the nanoparticles.

4.2.3 FTIR analysis

The main chemical groups of silicone and p(HEAM-co-AEMA) and their nanocomposites were examined by FTIR shown in figure 4.3. P(HEAM-co-AEMA) and p(HEAM-co-AEMA)-SiO_2 have –OH stretching frequencies in 3355 nm and $–CH_3$ in 2958 nm. The absorption band at 1706, 1644, 1250, 1163, 1074 and 1022 are all stand for C=O group. The absorption band at 1706 and 1644 come from C=O stretching, while the band 1250, 1163, 1074 and 1022 come from C=O absorption coupling with C-O and C-C stretches. C-N has the absorption band at 1452 nm. Silicone and its silica nanocomposites also share the same functional groups (-OH, C=O and $–CH_3$, except –OH group) with the same absorption band. The significant difference between p(HEAM-co-AEMA) and silicone is that p(HEAM-co-AEMA) has significant –OH absorption band while silicone has $–CH_3$ band. This also proves that p(HEAM-co-AEMA) hydrogel is hydrophilic and silicone is hydrophobic. In the figure it also shows that there is no difference between p(HEAM-co-AEMA) and its composites, nor any difference between silicone and its composites. One reason is that nanoparticle/hydrogel ratio is only 1/200, SiO_2 signal is weak in the spectrum. Another reason is that Si-O band of SiO_2 nanoparticle near 1100 nm is covered by other group bands (figure 4.3).

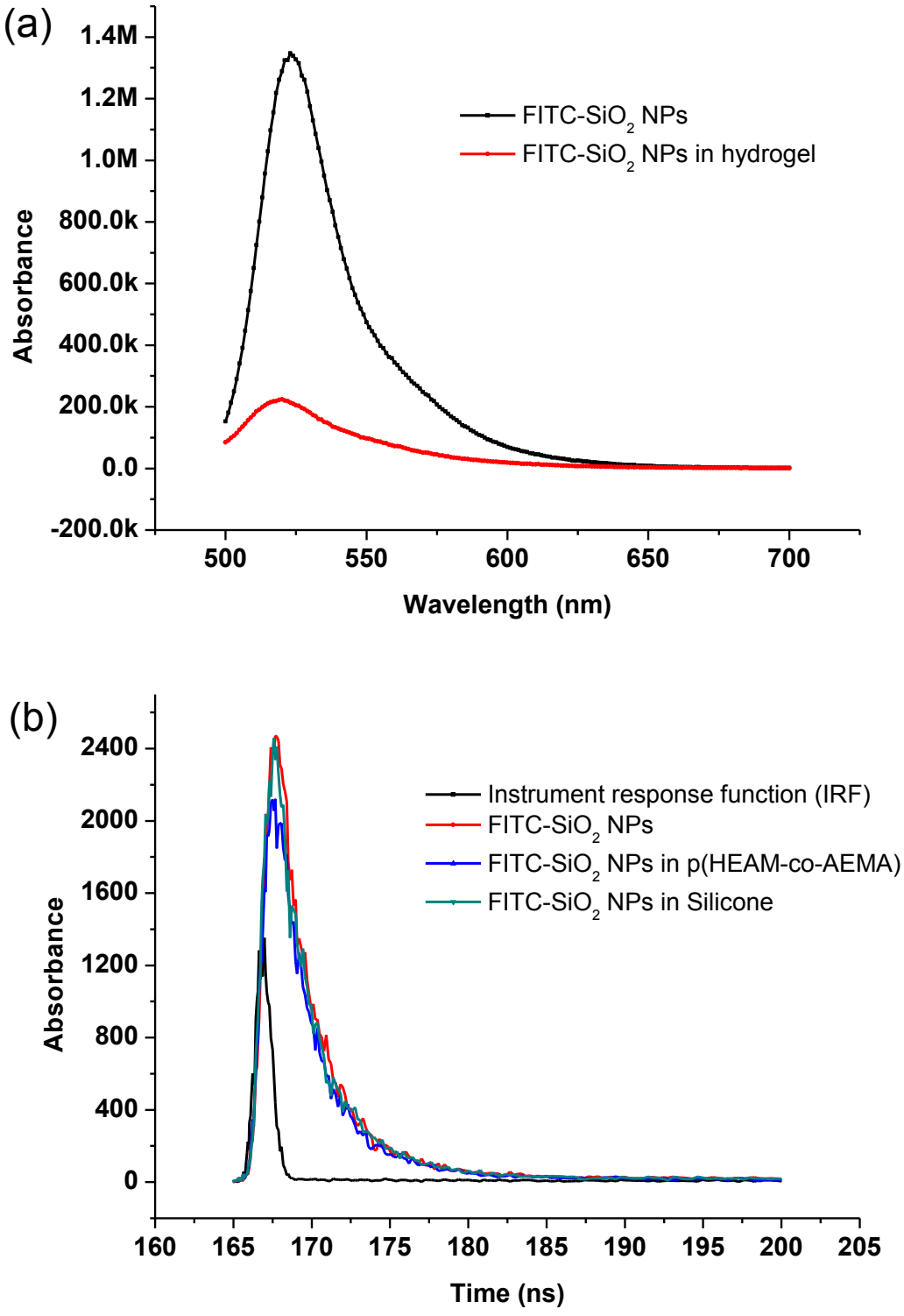


Figure 4.2: The wavelength spectrum of FITC modified silica nanoparticles and nanocomposites (a), the fluorescent time delay of FITC-silica nanoparticle and silicone-FITC-silica nanocomposites (b).

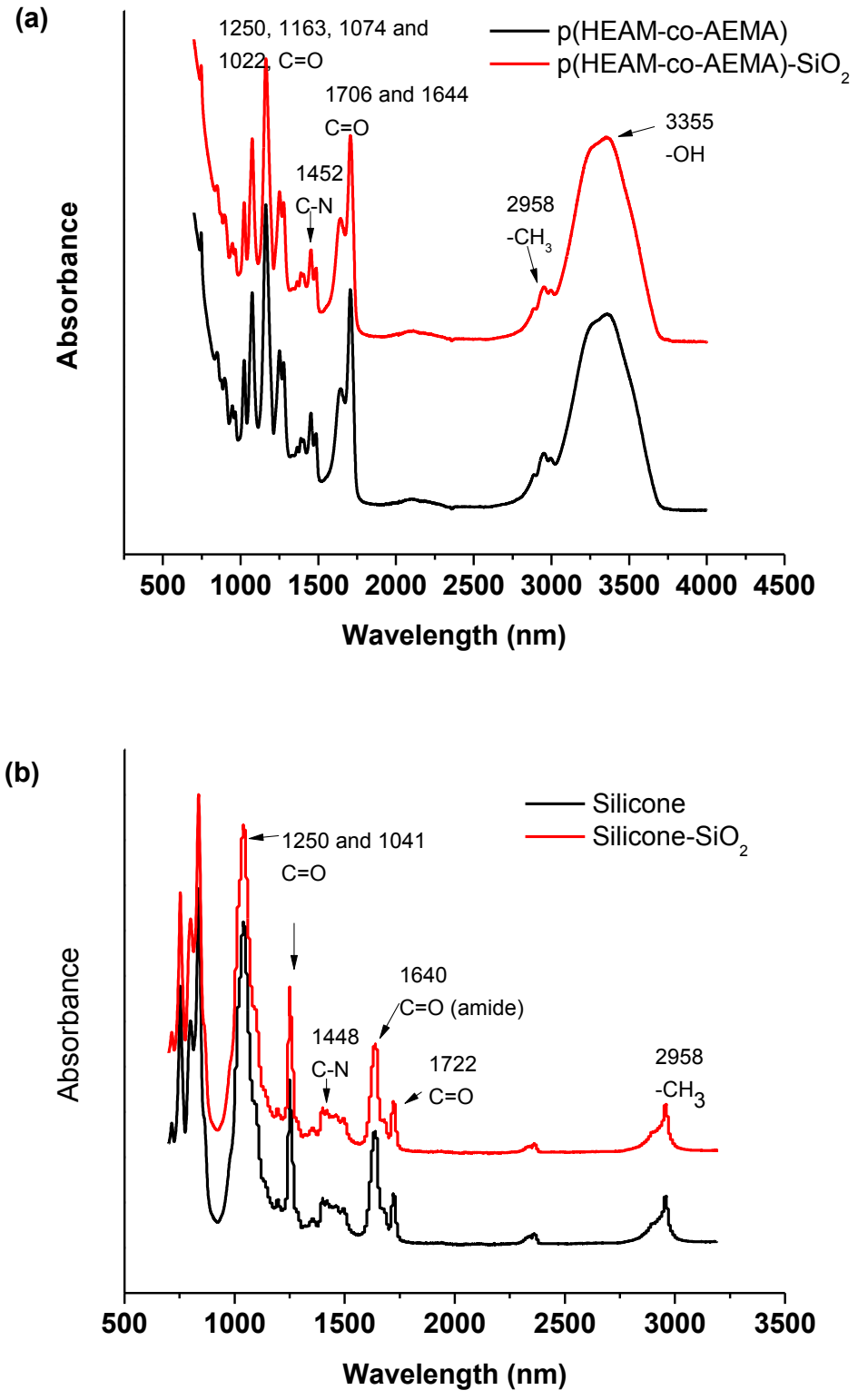


Figure 4.3: FTIR spectrum of p(HEAM-co-AEMA) and its silica nanocomposites (a), and silicone and its silica nanocomposites (b).

4.2.4 Water swelling ratio

The water swell property of silicone and p(HEMA-co-AEMA) were tested and the result is shown in figure 4.4. The water containing in silicone and silicone-SiO_2 composites up to 20 h were 21% and 26%, respectively. The absorbed water ratio was about 70% for both p(HEMA-co-AEMA) and its nanocomposites. Both silicone and p(HEMA-co-AEMA) hydrogel reached a plateau at 8 hours. Comparing the swelling ratio curve, it can be found that p(HEMA-co-AEMA) hydrogels and its composites always have higher water absorption compared to silicone. This may be due to the hydrophilic surface on p(HEMA-co-AEMA), which has higher affinity to water than silicone hydrogels.

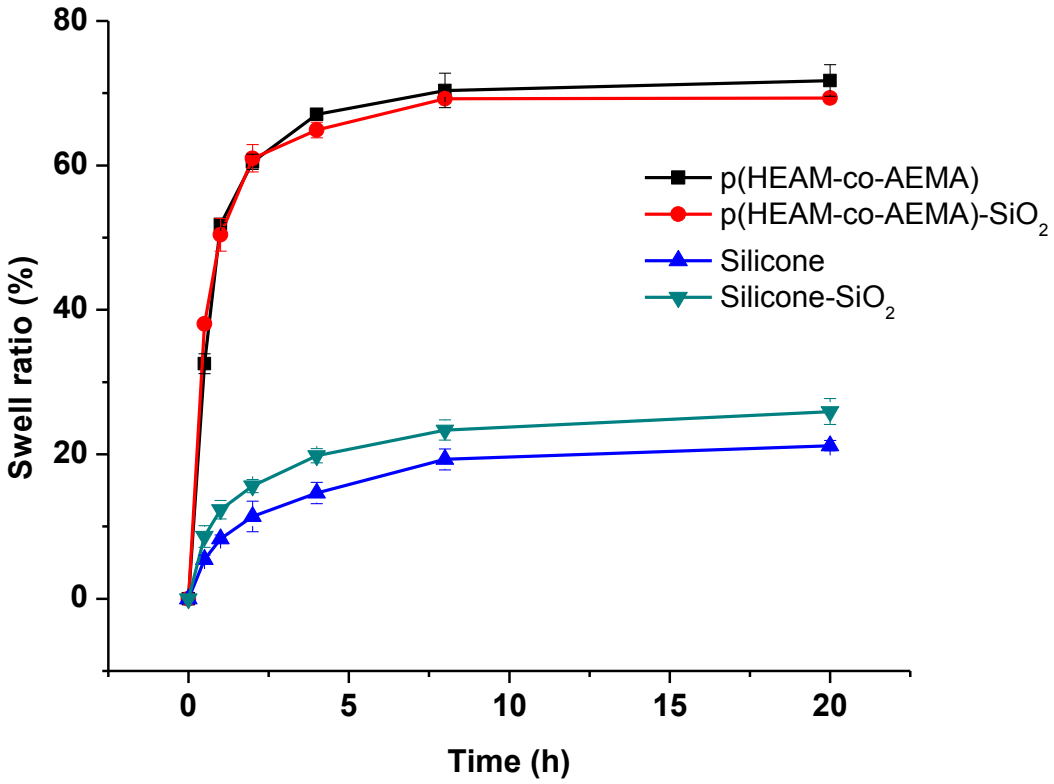


Figure 4.4: Water absorption ratio of p(HEAM-co-AEMA) and silicone hydrogels as well as their nanocomposites.

4.2.5 Mechanical strength of p(HEMA-co-AEMA) and silicone hydrogels

Proper mechanical strength is also a requirement for biomaterials used as body implants. Different body implants require different mechanical strength. For example, bone implants need rigid materials, while skin wound covers need soft materials. Hydrogels, usual are elastic materials, their stiffness were measured by tensile modulus, also known as Young's modulus. The tensile modulus of p(HEAM-co-AEMA) and silicone hydrogels and nanocomposites were tested through the uniaxial tensile test. The applied force and displacement data were collected based on Experimental Details 3.5 and Young's modulus was calculated based on the Equation 3.1. The Young's moduli of different samples were list in table 4.1. It shows that silicone hydrogel and its nanocomposites have higher stiffness than p(HEAM-co-AEMA) gel and its nanocomposites, respectively. The young's modulus was not affected by mixing p(HEAM-co-AEMA) gel with silica nanoparticles. However, such mixing increased the young's modulus slightly in silicone gel, from approximately 0.62 MPa to 0.69 MPa. It is not clear why the nanoparticles addition did not affect the p(HEAM-co-AEMA) stiffness but increased the silicone stiffness. We speculate that this may involve the complex interaction between the polymer molecules and the surface of silica nanoparticles.

Although the hydrogels and composites have different mechanical strength, they can still be useful as body implants or contact lens materials, especially for silicone and its nanocomposites, they have similar young's modulus compared to human skin, which is in the range from 0.42 MPa to 0.85 MPa [14].

Table 4.1: Young's modulus (E) of p(HEMA-co-AEMA) and Silicone hydrogel and their nanocomposites

	p(HEMA-co-AEMA)	**p(HEMA-co-AEMA)-SiO_2**	**Silicone**	**Silicone-SiO_2**
E (MPa)	0.15	0.15	0.62	0.69

4.2.6 Protein adsorption of hydrogels

Uncontrolled protein aggregation is a big hindrance for hydrogels used as implants, for such aggregation may cause adverse human body response. The protein adhesion is influenced on both of the surface characteristics of hydrogels, and the properties of proteins, for example, molecular weight, net charge and conformational stability [15]. The protein sticking property of silicone and p(HEAM-co-AEMA) hydrogels and nanocomposites was assessed by quantifying BSA adsorbed on the surface of samples with micro BCA method. Figure 4.5 show that the BSA adsorption of p(HEAM-co-AEMA) and p(HEAM-co-AEMA)-SiO_2 are 2 and 5 $\mu g/cm^2$, respectively. And silicone hydrogel and its nanocomposites adsorb BSA nearly 20 times higher than p(HEAM-co-AEMA) hydrogels do. We speculate that the higher protein adsorption of silicone hydrogel is due to its hydrophobic components, TRIS and the macromer, bis-alpha, omega-(methacryloxypropyl) polydimethylsiloxane. BSA is a globular protein which can be described as having a densely packed hydrophobic core surrounded by a hydrophilic coat of polar amino acids. Based on previous reports [16, 17], when BSA interacted with hydrophobic surface such as silicone, the hydrophobic core of BSA can become somehow less organized to achieve a more energetically favorable state and an entropy gain can be made. On the other hand, on hydrophilic surfaces such as p(HEAM-co-AEMA), no significant entropic gain can be made during the interaction, because they are already in an energetically favorable condition at the surface. Besides, there is no electrostatic interaction between BSA and p(HEAM-co-AEMA) hydrogel, so p(HEAM-co-AEMA) has much lower BSA adsorption than silicone hydrogel.

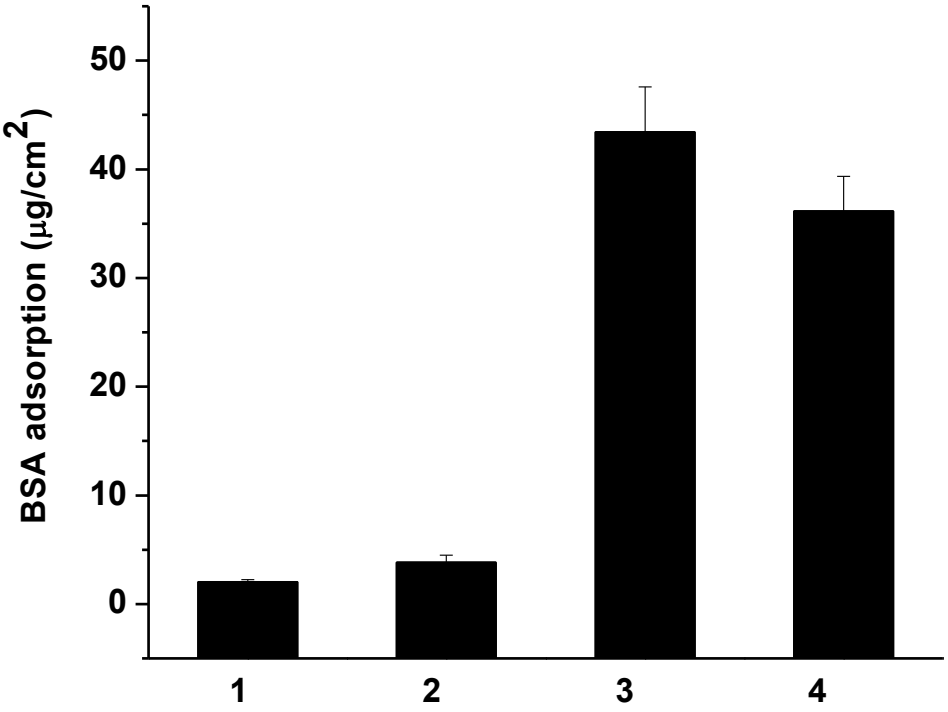


Figure 4.5: BSA adsorption of p(HEMA-co-AEMA) and silicone as well as their silica nanocomposites. 1- p(HEAM-co-AEMA), 2- p(HEAM-co-AEMA)-SiO_2, 3- silicone, and 4- silicone-SiO_2.

4.2.7 Cell viability of hydrogels

The biocompatibility of the hydrogels and nanocomposites were tested because such materials were supposed to contact with different cells as contact lens materials or body implants. NIH/3T3 mouse fibroblast cells were used for cell viability test. Samples were soaked into culture medium and incubated with cells for 24 h. It shows in figure 4.6 that the cell viability results with different materials are all higher than 90% after 24 h, which proves that p(HEAM-co-AEMA) and silicone hydrogels and the hydrogel-SiO_2 nanoparticle nanocomposites have no significant harmful effects to the cells.

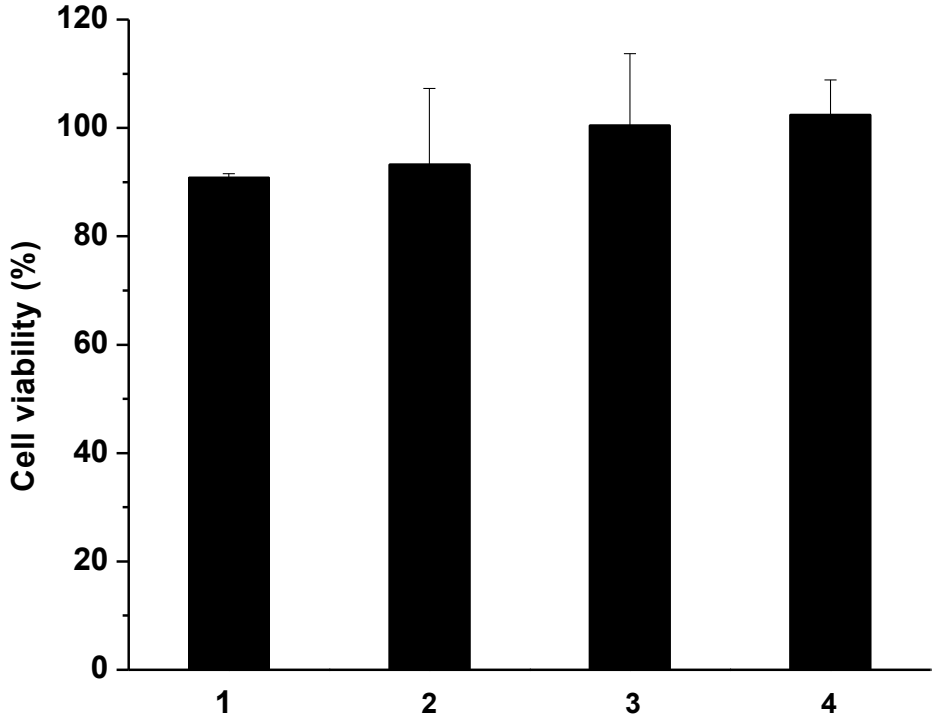


Figure 4.6: Cell viability of p(HEAM-co-AEMA) and silicone hydrogel and their nanocomposites. 1- p(HEAM-co-AEMA), 2- p(HEAM-co-AEMA)-SiO_2, 3-silicone, and 4- silicone-SiO_2.

4.3 Discussion

PHEMA and silicone are both favorable hydrogels for biomedical application such as contact lens, drug delivery substrates and tissue engineering scaffolds. However, they are different in surface properties: p(HEMA-co-AEMA) is hydrophilic and silicone is hydrophobic. This difference has important influence on the interaction between hydrogels and external materials, such as water and protein.

Hydrophilic surface has high affinity to water molecules while hydrophobic surface tends to reject them. In our results, the hydrophilic p(HEMA-co-AEMA) hydrogel absorbed water up to 70% of its dry weight in 20 hours while silicone only up to 20% (figure 4.4).

A more important influence of surface property of hydrogel is the interaction with proteins. This interaction is more complex than the interaction with water because these are many different types of proteins: hydrophilic protein, amphiphilic protein, protein with or without electrostatics, etc. Strong electrostatic interaction happens between surfaces with opposite charges and the interaction between hydrophobic surfaces is largely driven by the entropic gain when water molecules no longer need to be in contact with the hydrophobic surface. In this project, BSA, an amphiphilic globular protein was chosen as a protein model for protein adsorption study. Silicone has nearly 20 time higher protein adsorption than p(HEMA-co-AEMA). This proves that in this case, the hydrophobic interaction between BSA and silicone is stronger than hydrophilic surface interaction between BSA and p(HEMA-co-AEMA), such as van der Waals forces.

Although silicone has higher protein adsorption than p(HEMA-co-AEMA), it is still valuable candidate materials for biomedical application because of other advantages such as higher mechanical strength and higher oxygen transmittance. However, surface modification is needed to improve its protein resistance before silicone can be applied as biomaterials. The ideal non-protein sticking surface should be hydrophilic and electrostatically neutral. So in next chapter, a hydrophilic molecule, PEG, was deposited on the hydrophobic surface of silicone to reduce its protein adsorption.

4.4 Conclusion

P(HEMA-co-AEMA) and silicone hydrogels were produced by photo-polymerization method. The nanocomposites were developed by mixing silica nanoparticles with hydrogels before photo-polymerization. Mixing with nanoparticles induced a 3 nm fluorescent spectrum peak blue shift compared to the free FITC modified nanoparticles, but did not change the time delay property of the nanoparticles. All the materials have good biocompatibility (cell viability > 90%). Pure silicone hydrogel and its nanocomposites have higher tensile modulus than p(HEMA-co-AEMA) hydrogel in wet condition and the tensile strength can be improved slightly by mixing with nanoparticles. We speculated that the lower tensile modulus in p(HEMA-co-AEMA) is due to its higher water absorption. On the other hand, silicone hydrogel and its nanocomposites have higher protein adsorption than p(HEMA-co-AEMA) hydrogels. Both water absorption

and protein adsorption are affected by the hydrophilic or hydrophobic of the hydrogel. For the hydrophilicity in p(HEMA-co-AEMA) reduce the BSA adsorption but induce high water absorption; for silicone, its hydrophobicity increase BSA adsorption but reduce water absorption. Further surface modification is required if we want to apply these hydrogels as implantable or contact lens materials.

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Chapter 5

Interaction between Hydrogel and Laser Process

Polymer and organic thin films are important for a wide range of applications, such as tissue engineering and biosensors. Several physical and chemical technologies, such as gas plasma and layer-by-layer (LDL) deposition, have been used for surface coating of organic molecules, including different proteins and polymers. PEG is a polymer with important application in biomaterial field. In this chapter, a new surface coating technology, MAPLE, has been involved in PEG coating. The chemical components and roughness of the deposited PEG film was characterized by XPS and AFM.

Objectives:

1. To characterize PEG thin film thickness, roughness and chemical groups.
2. To study the BSA adsorption of silicone hydrogel before and after PEG film deposition, discuss the influence of PEG thin film coating on hydrogels protein adsorption.
3. To compare chemical change of hydrogel before and after laser irradiation by using FTIR.

5.1 Introduction

Inorganic, organic and biomolecules thin films with controlled structure is of great use including drug delivery [1], tissue engineering [2], gas and vapor detection [3], etc. For large scale industrial applications, 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 of these techniques is that the target materials are decomposed to atomics before they are deposited on the surface of substrates. So it is impossible for these technologies to be used for complex molecules deposition while maintain their function.

The conventional methods used for complex molecules deposition include dip coating, spin coating and some wet chemical methods. Ghosh et al. used Langmuir–Blodgett dip coating method to deposit functional bio-molecular thin film on self-assembled monolayer [9]. For spin coating, two parameters are very important: viscosity and spin speed [10]. The film thickness can be achieved from 1 to 200 μm. Each of these deposition techniques has its own advantages and drawbacks, and each one allows the treatment of limited organic molecules. So many researches focused on developing techniques which are suitable for a wide range of organic molecule deposition.

Pulsed laser deposition (PLD) draws much attention because it can be applied to a wide range of materials, such as metals [11], semiconductors [12] and compounds [13]. The ablated materials emitted from the target tend to move towards the substrate with high energy and deposited on the surface of substrate. However, it is not suited for the deposition of bio-molecules like polymers and proteins, because the pulsed laser with high energy will break the molecule bond and damage their bio-function.

As an improvement of PLD technique, MAPLE technique has been developed to avoid the photochemical damage. The difference between MAPLE and PLD is the target, for the target in PLD is solid composed of metals or semiconductors, but for MAPLE the target is made of biomolecules as well as the solvents, which then be freezed by liquid nitrogen. The incident laser energy is mainly absorbed by the solvent so the biomolecules will be protected. The solvent will be evaporated and the solute will also be brought out from the target with high energy. The solvent will be pumped away and the solute will be deposited on the substrate. The MAPLE process shows in figure 1.2.

In this chapter, PEG was deposited on the surface of cover glass and silicone substrates. Cover glass is more convenient for AFM characterization. And our final goal is to deposit PEG thin film on silicone hydrogel to improve its protein resistance for potential biomedical application.

5.2 Results

5.2.1 FTIR analysis of PEG deposited on silicone

The deposited PEG on silicone surface was examined by FT-IR in comparison with the blank silicone and silicone with air dried PEG on the surface as well as pure PEG molecule (figure 5.1). Compared to silicone hydrogel, silicone with MAPLE and air dried PEG have more significant band at 3410 nm, which represents the stretching of hydroxyl group in PEG. Both of them also show broad shoulder at band from 2900 nm to 2850 nm compare to the pure silicone. We speculated that this is due to the affection of alkane group stretching in PEG at the band of 2865 nm. This result proves that the PEG molecules have been successfully deposited on the surface of silicone hydrogels without significant chemical structure damage.

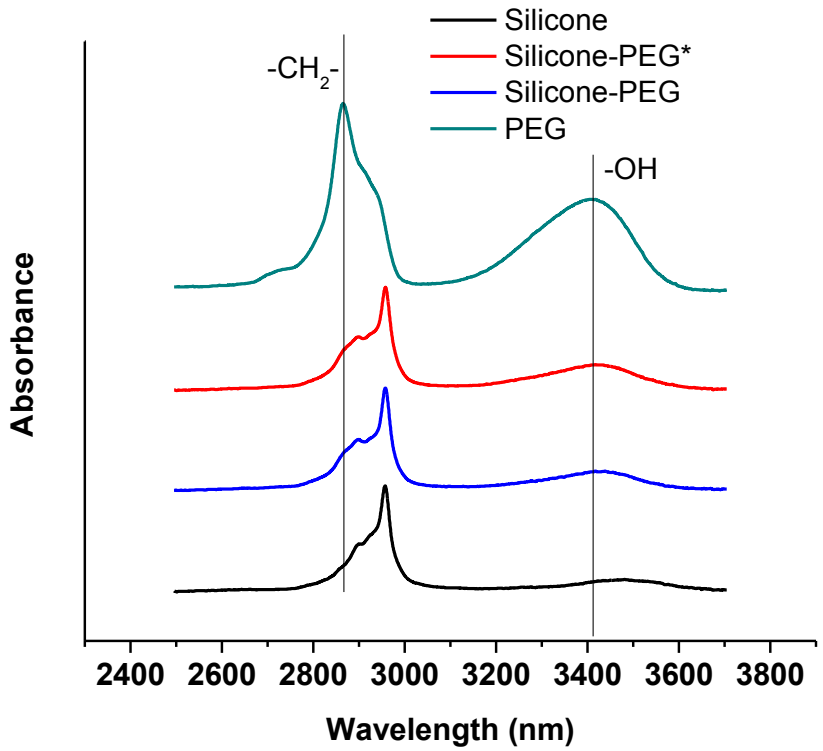

Figure 5.1: FTIR spectrum of PEG deposited silicone hydrogel. * Represents air dried PEG on silicone hydrogel.

5.2.2 AFM images of PEG thin film

AFM was used to measure the roughness and thickness of the PEG thin film on the surface of cover glass. It can be found that PEG molecules form islands rather than films on the cover glasses. The islands density after 2 hours deposition (figure 5.2b) is higher than that after 1 hour deposition (figure 5.2a). The PEG islands on cover glass have the diameter of about 0.7 μm and the thickness of about 33.3 nm after 1 hour deposition, but after 2 hours deposition, the spots grows from 0.7 μm to nearly 3 μm and the maximum thickness to about 357 nm.

It can be observed that the deposited PEG molecules tend to form scattered islands rather than homogeneous thin films on the surface of cover glass. We speculate that the surface property (hydrophilic or hydrophobic) of cover glass may have important efforts for PEG film homogeneity. Surface pre-treatment such as oxygen plasma may be needed before MAPLE deposition. Other parameters, such as the target and substrate distance, deposition time and the substrate temperature, may also affect the film homogeneity.

5.2.3 Protein adsorption of silicone hydrogel with PEG coating

The BSA adsorption of silicone hydrogel before and after PEG deposition by MAPLE technique was measured by using BCA assay. It can be found in figure 5.3 that after PEG deposition, the protein adsorption of silicone decreased to approximately one third compared to pure silicone gel. This result shows that PEG thin film has significant effect to enhance BSA resistance of the substrates. Also, it is noticed that the oxygen treated silicone has similar protein adsorption with the original one. One reason is that the oxygen plasma may not have enough power to change the surface hydrophobicity of silicone. It requires nearly 400 W of plasma to modify silicone surface based on previous report [14] but in this research silicone gel was treated with 90 W oxygen plasma.

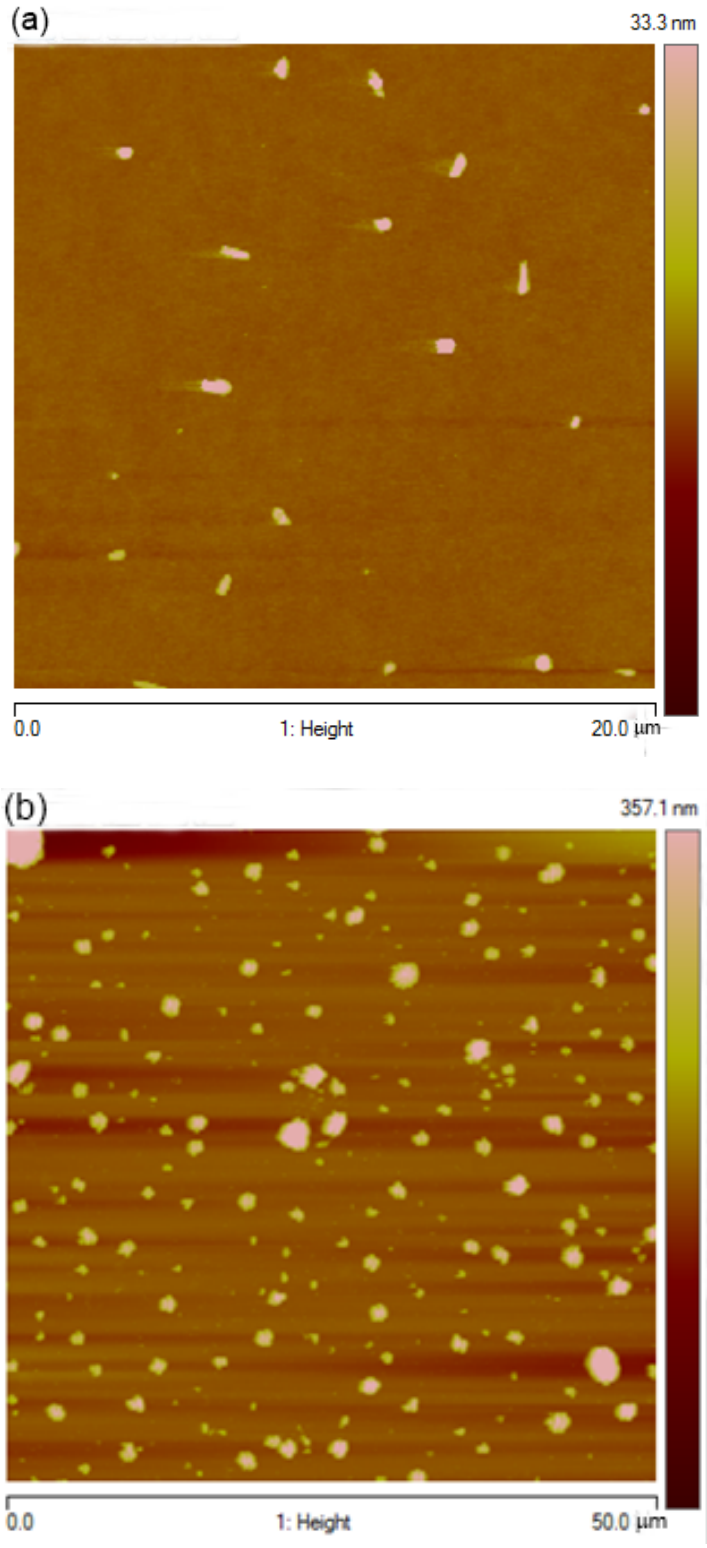

Figure 5.2: PEG film on the surface of cover glass measured by AFM after 1 hour deposition (a) and 2 hours deposition (b).

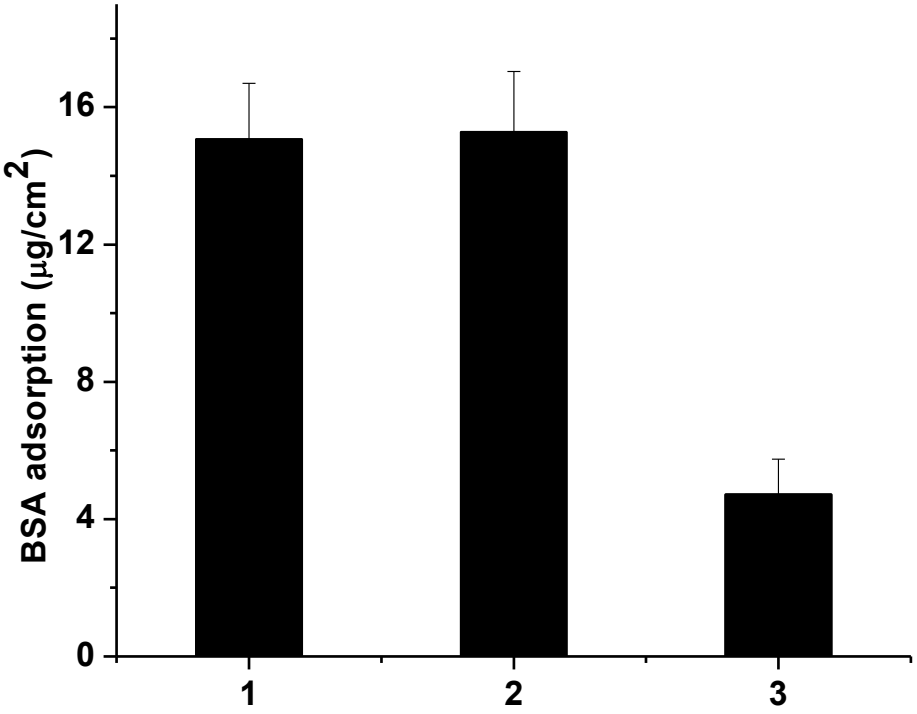

Figure 5.3: BSA adsorption of silicone before and after PEG deposition by MAPLE. 1-silicone; 2- oxygen plasma treated silicone; 3- PEG deposited on oxygen plasma treated silicone.

5.2.4 FT-IR analysis for laser etching

Laser direct etching with hydrogel may be a simpler and more efficient way for surface modification rather than MAPLE technique. Laser etching and ablation have been applied to different kind of materials such as metals [15,16], semiconductors like silicon [17] and polymers [18]. So in this project the laser etching effect on p(HEMA-co-AEMA) and silicone hydrogels was also evaluated. In this chapter, the interaction between laser and hydrogels were tested and the laser etched hydrogels were analyzed by FTIR to determine if the laser etching process can induce any chemical change to the hydrogel samples. In figure 5.4 it can be seen that all the main chemical group bands were present

in the FTIR spectrum before and after laser etching, such as hydroxyl group at 3355 nm, C-N bond and C=O bond in p(HEMA-co-AEMA) and methyl group at 2958 nm in silicone. No new band was observed after laser etching, nor any bands vanished or shifts. This result shows that the laser with 532 nm wavelength did not induce any chemical change to the hydrogels after the etching process. P(HEMA-co-AEMA) and silicone hydrogels are not the laser absorbents in this wavelength.

5.3 Discussion

In order to reduce protein adsorption of silicone hydrogel, PEG molecules were coated on its surface to achieve a hydrophilic and electrostatically neutral state. FTIR spectrum shows that the main chemical groups of PEG were reserved well after MAPLE deposition (figure 5.1). In AFM images (figure 5.2), it can be seen that the deposited PEG molecules formed islands rather than films on cover glasses after 1 and 2 hours deposition, but the spots after 2 hours grow bigger and thicker than that after 1 hour. A longer time deposition may achieve a homogeneous films rather than scattered islands. In PEG deposition on silicone, oxygen plasma was applied to the substrate first to clean its surface and make it is more adhesive to PEG molecules. The deposition process lasted for 4 hours to assure a higher PEG molecule coverage percent. Result in figure 5.3 shows a reduced BSA adsorption on silicone hydrogel with PEG coating.

However, it should be noticed that the protein resistance effect of PEG coated silicone *in vivo* is still unpredictable since complex biofluids contain proteins with widely varying charges and structures, it is significantly more difficult to design a surface which can reject all proteins. Another disadvantage of PEG coating is the poor stability of PEG. This polymer readily undergoes oxidative degradation and a range of bacteria can also metabolize PEG chains. So the development of substitutes for silicone as well as PEG is still needed to achieve a surface that will resist adsorption of all proteins during exposure to complex biological fluids for extended time periods.

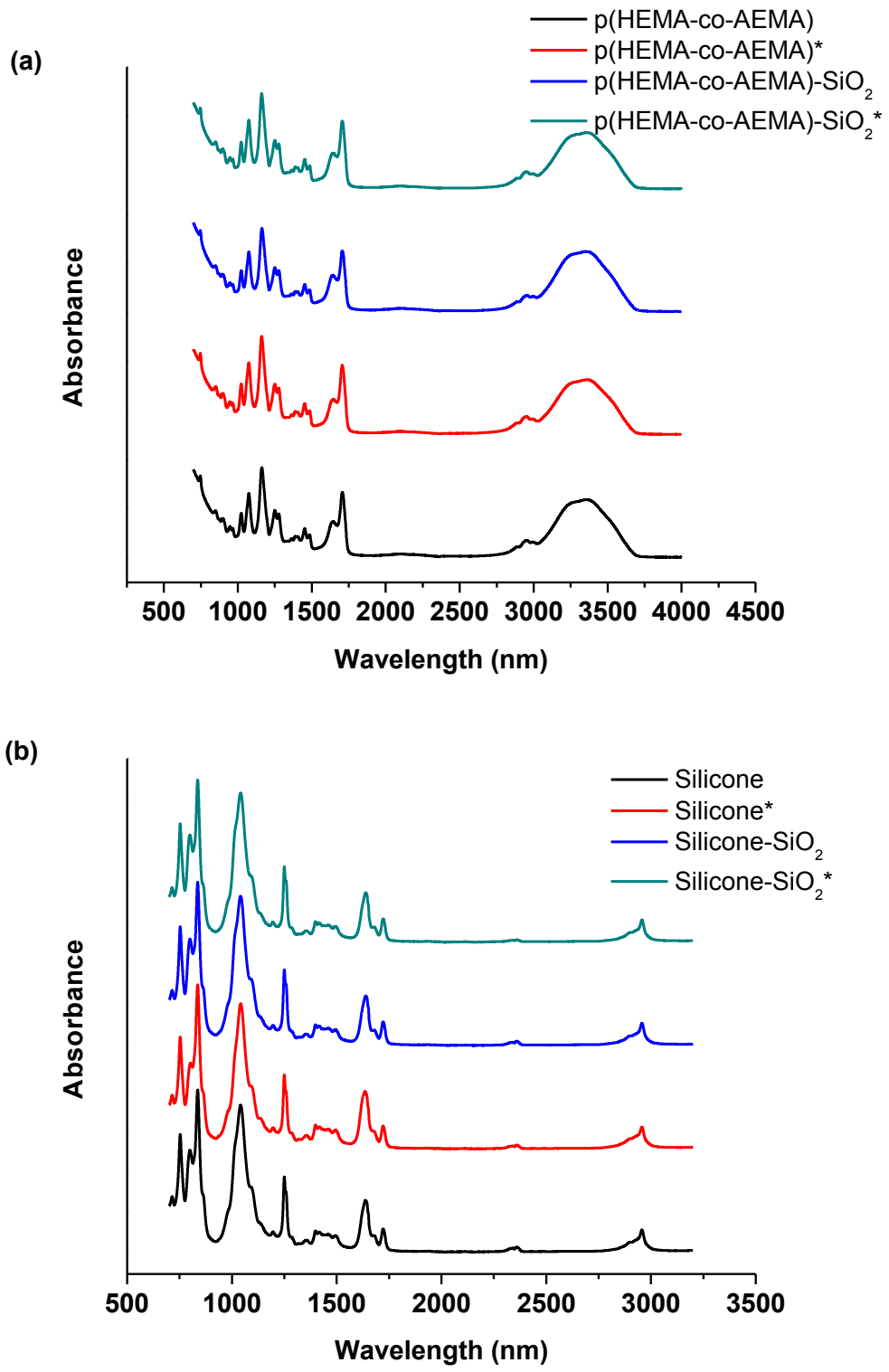

Figure 5.4: FTIR spectrum of p(HEMA-co-AEMA) hydrogel and its nanocomposites (a), and silicone and its nanocomposites (b) beforeand after laser etching. * represents gels after laser etching.

5.4 Conclusion

PEG thin films were deposited by using MAPLE technique with a laser source of 532 nm wavelength. FTIR shows that PEG deposited silicone has the similar IR absorbance spectrum with silicone gel which has air dried PEG on its surface. Both of them have more significant PEG hydroxyl group band at 3410 nm and alkane stretching band at 2865 nm. This result proves that PEG molecules can be deposited by MAPLE technique without any significant structure damage. AFM image shows that after 2 h deposition, PEG on cover glass forms islands with the diameter from 0.6 to 3 μm and the maximum thickness to about 357 nm. The PEG coated silicone gel has reduced BSA adsorption. This result shows that MAPLE technique is a potential powerful surface modification choice for biomaterials development besides chemical methods. On the other hand, we have investigated the laser direct etching on hydrogel for surface modification in this chapter. The Nd: YAG laser with a wavelength of 532 nm did not induce any chemical change during the etching process. P(HEMA-co-AEMA) and silicone are not the laser absorbents in this wavelength. The lasers in UV or IR range with different fluency may be further studied to better understand the interaction between laser and hydrogels.

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Chapter 6

6.1 Summary and Conclusion

Poor chemical and physical properties of polymer hydrogels limit their applications in biomedical devices. In this research project, our overall goal is to improve the properties of hydrogel materials, particularly, to develop materials with fluorescent property, to improve hydrogels' mechanical strength and to develop protein-non-sticking hydrogel materials. Two strategies have been studied: (1) Addition of silica nanoparticles (SiO_2 NPs) or fluorescent molecule labeled SiO_2 NPs in hydrogel to form nanocomposites. (2) Modification of hydrogel surface with PEG using MAPLE deposition technique.

Two hydrogels with different surface properties were fabricated through photo-polymerization method: one is p(HEMA-co-AEMA) with hydrophilic surface, the other one is silicone with hydrophobic surface. Hydroxyl group in p(HEMA-co-AEMA) and methyl group in silicone were confirmed by FTIR. The mechanical strength, swelling ratio, biocompatibility and protein adsorption of hydrogels and their nanocomposites were characterized. All materials have good biocompatibility (cell viability > 90%) after 24 hours incubation with cells. The mechanical strength of silicone and its nanocomposites are approximately 4 times higher than p(HEMA-co-AEMA) and its nanocomposites. The Young's modulus of silicone nanocomposites is 11.3% higher than that of silicone. P(HEMA-co-AEMA) and its nanocomposites have higher water absorption and lower protein adsorption than silicone and its nanocomposites due to their different surface properties. The high water absorption and low protein adsorption of p(HEMA-co-AEMA) is related to its hydrophilic surface. Silicone, on the contrary, can reject water molecules and denature globular protein BSA for higher BSA adsorption due to its hydrophobic surface.

Another focus in this project is to improve protein resistance of silicone. Our strategy is to coating PEG thin film on silicone through MAPLE process to reduce BSA adsorption. PEG has shown very high resistance to protein adsorption. MAPLE technology has advantages such as accurate thickness control and can be applied to most organic molecule targets. PEG molecules were deposited on silicone by using an Nd:

YAG laser source with wavelength of 532 nm. There is no significant chemical change of PEG after laser irradiation by FTIR characterization. AFM images show that the PEG molecules form islands on cover glasses with diameters between 0.6 and 3 μm. The thickness of PEG increased from 33.3 nm to 357 nm when irradiation time increased from 1 to 2 hours. BCA assay of BSA shows that BSA adsorption of PEG deposited silicone decreases to one third compared to pure silicone.

6.2 Future work

One disadvantage of PEG is related to the oxidative degradation at evaluated temperature. A range of bacteria can also metabolize PEG chains. Long-term studies have shown that PEG coatings fail to stay protein resistance over extended periods of time. To achieve long term protein resistance, a combination of different strategies, such as using different silicone components with high protein resistance, or different surface coating molecules with long term protein resistance, may be developed.

On the other hand, as a powerful technique, MAPLE can be applied to almost every organic molecule target as long as we can find out the proper deposition parameters. The surface coating with different molecules may extend the application of silicone hydrogel in biomaterials science. For example, silicone hydrogel with the deposition of specific protein or nanoparticles can be used as biosensors for cancer cells detection.

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