ORGANIC-INORGANIC COATINGS FOR BIOMEDICAL APPLICATIONS

FABRICATION OF ADVANCED ORGANIC-INORGANIC COATINGS USING BIOMIMETIC COLLOIDAL TECHNIQUES

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TITLE: Fabrication of advanced organic-inorganic coatings using biomimetic colloidal techniques

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Lay Abstract

Biomedical devices have various properties they must possess to perform their function within the body without harming the patient. Coatings applied to these devices can mitigate the body's response by reducing corrosion, preventing wear, and promoting bond formation. This increases the lifespan of the device and prevents invasive revision surgeries. Advances in materials engineering and colloidal sciences can help achieve these goals.

Materials selection for novel coatings can be inspired by the composition of real bone - consisting of a polymer matrix with embedded inorganic nanomaterials. Additionally, manufacturing techniques that avoid high temperatures are desirable. Therefore, advances in colloidal sciences which enable coatings to be fabricated by a simple and inexpensive method known as dip coating is of paramount importance. This work used natural biosurfactants bile acids to aid in fabrication of coatings for biomedical devices using advanced polymer poly(ethyl methacrylate) and functional inorganic materials.

Abstract

Surface modifications of bone-interfacing biomedical devices can increase their longevity by promoting bond formation and new bone growth, while reducing the toxic effects of corrosion and wear particles. Coatings which contain biocompatible polymers, bioceramics, drugs, and functional molecules are one route to achieve this. Here, a biomimetic approach is developed for the fabrication of poly(ethyl methacrylate) (PEMA) coatings. For the first time it is shown that PEMA can be solubilized in non-toxic solvents by naturally occurring bile acids. Their unique chemical structure and amphiphilicity allows for efficient solubilization of polymer macromolecules.

Advancements in colloidal sciences enable a facile deposition method termed "dip coating" to be utilized. The feasibility of highly concentrated solutions of high molecular mass PEMA was a key factor for film deposition by dip coating. Singular layers or multilayered PEMA films could be deposited. Heat-treated PEMA films provided corrosion protection to stainless steels. This inexpensive and simple technique can be up scaled to larger manufacturing levels, leading to mass production and clinical development of novel coatings for biomedical applications.

Additional challenges in the fabrication of composite coatings by dip coating were successfully addressed using bile acids. To produce high quality composite coatings by dip coating, a stable suspension is required. Particle aggregation leads to uneven coatings, poor adhesion, and weakened mechanical properties. It was shown that bile acids could act as dispersing agents to mediate this. PEMA coatings containing inorganic materials hydroxyapatite, silica, titania, and diamond were fabricated. The inorganic component of the films could be increased to 50 wt.%. Model drugs tetracycline and ibuprofen were used for the creation of drug-loaded PEMA

coatings. Lastly, composite coatings containing functional molecules including heparin and nanocellulose were created.

Overall, these coatings provide corrosion resistance to metallic orthopedic implants, while enhancing potential biocompatibility of the device. The biomimetic approach developed in this investigation was motivated by the role of bile acids and bile salts as solubilizers of cholesterol and other molecules within the digestive system of mammals. A solubilization mechanism has been proposed. This work paves the way for the fabrication of future composite coatings containing other high molecular mass polymers, inorganic nanomaterials, and functional materials or drugs.

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List of Abbreviations and Symbols

µm	Micrometre (10^{-6} m)
Al_2O_3	Alumina
AmB	Amphotericin B
BA	Bile Acid
BS	Bile Salt
C=O	Carbonyl group
CA	Cholic Acid
CFR-PEEK	Carbon fibre reinforced polyetheretherketone
CH_2CH_3	Ethyl group
CH_3	Methyl group
CNC	Cellulose nanocrystals
CoCr	Cobalt Chromium
CP-Ti	Commercially Pure Titanium
Da	Daltons
ECM	Extracellular Matrix
EMA	Ethyl methacrylate
EPD	Electrophoretic Deposition
FTIR	Fourier Transform Infrared Spectroscopy
GPa	Gigapascals (10^9 Pa)
h	Hours
HA	Hydroxyapatite
HDCA	Hyodeoxycholic Acid
JCPDS	Joint Committee on Powder Diffraction Standards
LCA	Lithocholic Acid
MD	Microdiamond
MMA	Methyl methacrylate
MPa	Megapascals (10^6 Pa)
MSN	Mesoporous silica nanoparticle
MW	Molecular Weight

ND	Nanodiamond
NH_3	Ammonia
nm	Nanometre (10^{-9} m)
NP	Nanoparticle
OH	Hydroxyl group
PEEK	Polyetheretherketone
PEG	Polyethylene glycol
PEMA	Poly(ethyl methacrylate)
PET	Polyethylene terephthalate
PLA	Poly lactic acid
PMMA	Poly(methyl methacrylate)
PPC	Poly(propylene carbonate)
PTX	Paclitaxel
QD	Quantum dot
s	Seconds
SBF	Simulated Body Fluid
SCE	Saturated calomel electrode
SEM	Scanning electron microscope
SiO_2	Silica
TGA	Thermal Gravimetric Analysis
THFMA	Tetrahydrofurfuryl methacrylate
TiO_2	Titania
UDCA	Ursodeoxycholic Acid
UTS	Ultimate Tensile Strength
WHO	World Health Organization
XRD	X-ray Diffraction
ZrO_2	Zirconia

Declaration of Academic Achievement

This thesis was written to fulfill requirements of the M.A.Sc. degree in the Department of Materials Science and Engineering at McMaster University. All work described was undertaken from May 2021 to April 2022.

The experiments described in the following written document were conceived and conducted by the author of this thesis, in consultation with the supervisor, Dr. Igor Zhitomirsky. Exceptions are as follows:

- All X-ray Diffraction (XRD) experiments were carried out by Victoria Jarvis (M.A.Sc), a XRD2/XRD3 specialist in the MAX Diffraction Facility at McMaster University (Figure 3-11, 4-10, and 5-6). Sample preparation was carried out by the author of this thesis.
- All Thermal Gravimetric Analysis (TGA) experiments were carried out by Dr. Paul Dube, the manager of research facilities at the Brockhouse Institute for Materials Research, McMaster University (Figure 4-6). Sample preparation was carried out by the author of this thesis.

Chapter 1: Introduction

1.1 Overall Context

The study of biomaterials involves the fields of medicine, biology, physics, chemistry, and engineering. In the context of materials engineering, a biomaterial is defined as "any substance that has been engineered to interact with components of a living system to direct the course of any therapeutic or diagnostic procedure" [1]. The prefix "bio" does not refer to "biological", but rather "biocompatible" [1]. Thus, the material can be either synthetic or naturally occurring, but must be biocompatible. The requirements of being biocompatible are complex and vary for different applications. Therefore, it can be defined as the ability of a material to perform its desired function while eliciting appropriate host responses. The appropriate response may be different for an orthopedic application than for cardiovascular applications for example [1]. A biomaterial may make up an entire, or parts of, a biomedical device. A biomedical device is simply any device intended for medical purposes. Examples of biomedical devices include bone plates, rods, screws, total joint replacements, vascular stents, grafts, ocular lenses, dental implants, sutures, pacemakers, biosensors, artificial organs, catheters, and more examples which are provided in Figure 1-1 below.

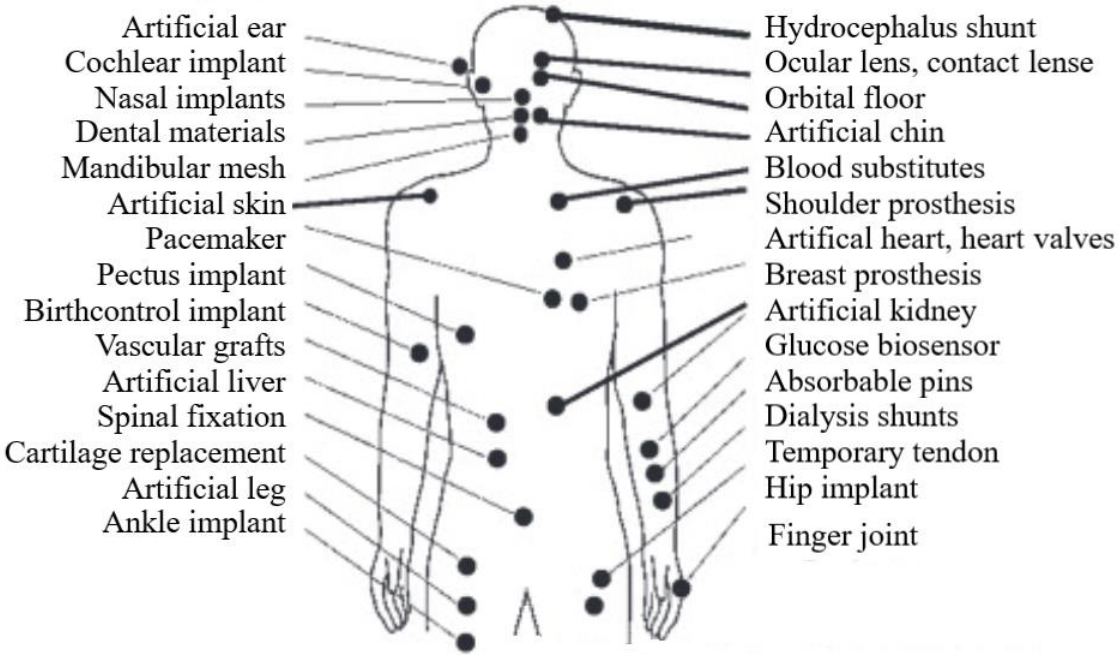

Figure 1-1: Overview of biomedical devices implanted within the body [2].

The common feature amongst all these devices is that they are placed in direct contact with living tissue in the human body. They serve many purposes, with the overall goals of restoring function to injured/degenerated tissues, stimulating healing processes, and increasing the quality of life for the patient. The market value of biomaterials is estimated to be over 100 billion USD globally as of 2019 [3], and over 10,000 forms of standardized devices are approved worldwide by the World Health Organization (WHO). The demand specifically for orthopedic implants is rapidly increasing due to the prevalence of chronic musculoskeletal conditions, osteoarthritis, and osteoporosis because of the rising geriatric population [3]. Therefore, the need for technological advancements in the fabrication of biomaterials for biomedical devices is ever growing.

In addition to a steep increase in demand for orthopedic devices, there is also a need to improve patient outcomes. The success of an orthopedic implant is dependent upon not only mechanical properties, but also on the biological response of the surrounding tissue. Ideally, the implant should form strong bonds at the bone-implant interface to secure the implant in place and

begin promoting new bone growth – known as osseointegration. Vascularization and remodeling of surrounding tissue will occur if the implant provokes a strong immune response which results in a fibrotic encapsulation of the implant [4]. If this occurs, then no further interactions between the implant and the surrounding tissue can take place [4]. At this point, the functionality of the implant is hindered, and a revision surgery is required. In order to avoid such circumstances, surface characteristics of the implant can be modified on the micro- or nanoscale, such as optimizing the roughness, porosity, wettability, composition, charge, and more [4]. A popular method to alter the surface characteristics of a biomedical implant is by depositing a coating on a substate.

The production of new biomaterials is constrained by requirements of compatibility, toxicity, and the invoked inflammatory and immune responses, which can be mitigated by specific surface coatings. However, there is also cost and feasibility to consider for mass production. Large and expensive equipment may be required for the fabrication of currently approved biomedical devices which require constant maintenance and repair. Additionally, during the fabrication of biomedical devices, traditional high-temperature processing techniques can damage organic components of the material. Advancements in materials engineering and colloidal science can address these limitations and provide new strategies for facile material development. For example, the sol-gel method is very versatile and can be used to offer control of a coatings' chemical compositions, micro- or nano- topography, thickness, and uniformity [5]. It is a low-temperature and inexpensive method of film preparation. Despite these advantages, this method has very few applications in biomedical research [5]. The applicability of this method depends on the stability of the prepared colloidal solutions. Therefore, novel strategies to form stable colloidal solutions

for the fabrication of biomedical devices by sol-gel methods would provide a route for low temperature, inexpensive, facile fabrication of new biomedical devices.

It is evident that there is a strong demand for orthopedic implants with sufficient mechanical properties to address the rise in prevalence of bone defects. As well, there is a need to ensure proper integration of such implants so they can function and increase the quality of life of the patient, without the need for a revision surgery due to implant failure. This integration can be achieved by controlling surface features of the implant by applying a coating. Lastly, the production of such functional biomedical devices would benefit from being simple, low temperature, and inexpensive such that no large equipment is required. Therefore, this work aims to provide a solution to the above problems. This work demonstrates the successful creation of organic-inorganic composite coatings onto stainless steel substrates with enhanced functionality for biomedical applications. The method of film deposition was a technique known as dip coating, which was successful due to the application of naturally occurring bile acids as solubilizing and dispersing agents. Overall, this work provides innovative results in the field of biomaterials and colloidal science that addresses needs of our current society.

1.2 Thesis Overview

This piece of presented work is a sandwich thesis, meaning that after a review of relevant literature, three prepared peer-reviewed articles are provided. It may be found that material in the introductions or experimental procedures is slightly repetitive, but each individual article provides unique results with variations in the materials and characterization methods used. The articles are differentiated below.

Chapter two of this thesis provides a review of relevant literature highlighting advanced materials for biomedical devices. This includes metals and alloys, naturally occurring and synthetic polymers, and bioceramics. The properties and applications of these materials are covered, specifically relating to corrosion and wear resistance, mechanical performance, biocompatibility, bioactivity, and more. Additionally, coating fabrication techniques are reviewed while considering the benefits of bio-inspired materials design, such as mimicking the structure of bone and the use of naturally occurring surfactants. At the end of this chapter, the research objectives are outlined.

Chapter three presents a biomimetic approach for the fabrication of poly(ethyl methacrylate) (PEMA) and PEMA-composite coatings, using the applications of bile acids, such as cholic acid (CA) and lithocholic acid (LCA), by a facile dip coating procedure. The use of bile acids was motivated by the analysis of literature on their solubilization of various biomolecules within the human body. Here it is demonstrated for the first time that PEMA can be solubilized in non-toxic solvent isopropanol by bile acids instead of typical toxic solvents. Bile acids can also serve multifunctionality by acting as dispersing agents for the fabrication of organic-inorganic composite coatings containing various bioceramics, drugs, and functional molecules. Such coatings can be applied as monolayers or as multilayers with controlled coating thickness. The fabricated coatings are characterized by scanning electron microscopy, Fourier-transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD). Lastly, after a heat treatment of 180 °C for 1h, PEMA coatings were shown to provide corrosion protection to stainless steels.

Chapter four presents a versatile strategy for the fabrication of PEMA and PEMA composite coatings. Here, PEMA was solubilized in ethanol, another non-toxic solvent, by bile acid lithocholic acid (LCA). A solubilization mechanism for PEMA by LCA is developed in this

chapter. It is hypothesized to be due to polymer chain disentanglement at moderately increased temperature which allowed LCA adsorption onto PEMA surface through hydrophobic interactions. Upon cooling, LCA's negative charge provided electrostatic dispersion due to the formation of charged polymer-surfactant complexes. In this work, the concentration of inorganic materials was increased such that coatings of approximately 50 wt.% organic material and 50 wt.% inorganic material were fabricated. Lastly, composite films containing drugs and other biologically functional materials were fabricated. All coatings were characterized appropriately.

Chapter five outlines the results achieved using two different bile acids ursodeoxycholic acid (UDCA) and hyodeoxycholic acid (HDCA) as solubilizing agents for PEMA in isopropanol. PEMA films were deposited by dip coating as singular layers or multilayers. Furthermore, composite PEMA-diamond films were successfully deposited by dip coating. Both micro- and nanosized diamond particles were used. Diamond films are increasingly popular for use in biomedical applications. However, diamond is one of the most chemically inert materials, and thus its dispersion by adsorbed dispersants is extremely challenging. This exemplifies the stabilization power of bile aids. Moreover, the films could be deposited as alternating layers of PEMA-diamond and PEMA. The morphology and composition of the novel coatings could be controlled by altering the diamond size and concentration.

Finally, chapter six summarizes the significance of the results of this work and demonstrates their contribution to the field of materials engineering, as well as future work.

1.3 References

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Chapter 2: Literature Review

2.1 Advanced metals and alloys for orthopedic implant applications

Bone defects arise due to a variety of factors such as old age, disease, and trauma. Surgical interventions are used to place many orthopedic devices with the hopes of restoring function. Such devices can be classified as temporary devices such as bone plates, pins, and screws, or permanent devices such as total joint replacements [1]. In fact, artificial bone is one of the most common implanted tissues [2]. Many commercially available metals and metal alloys are thoroughly investigated for use in orthopedic and dental applications which are biocompatible and capable of yielding successful long-term results. The three main groups of advanced metals and alloys for orthopedic implants include stainless steels, titanium-based alloys, and cobalt chromium (CoCr) based alloys. These materials have a long-standing history as orthopedic implant materials. CoCr metal alloys were first investigated for biomedical purposes in 1923, followed by stainless steels in 1942, and titanium alloys in 1951, and are all now approved by the United States Food and Drug Administration, available for routine use in orthopedic practice [1,2]. Since then, there have been innumerable advances in their performance related to surrounding host tissue reaction. However, it is imperative to continue monitoring their results in clinical applications related to fatigue resistance, wear resistance, osseointegration, and systemic toxicity of released metal ions during corrosion.

A metal or alloy may be presumed as corrosion resistant due to its formation of a thin layer of metal oxide by passivation. However, an orthopedic implant is likely to be constantly subjected to multiple different types of stresses. Therefore, it is likely this oxide layer will become damaged. Also in this context, re-passivation is hindered, and corrosion can rapidly occur [3]. Corrosion of such metallic implants is detrimental to their performance. It can result in the release of various

metal ions into the body which imposes adverse health effects to the patient, such as localized cell and tissue death (necrosis) leading to implant loosening and deterioration of the mechanical integrity of the implant resulting in premature failure. In brief, the overall extent of tissue reaction is directly proportional to the constituent element release due to corrosion of the metal/alloy [3]. For these reasons, corrosion control of orthopedic implants is extremely important.

The earlier stated three popular alloys for orthopedic applications are generally regarded as corrosion resistant, with low overall bulk corrosion rates. However, orthopedic implants are at risk of many different modes of corrosion. This is because the environmental conditions within the body are unique. A material that performs well in ambient air conditions may corrode severely within the body, or moreover, a metallic implant that performs well in one part of the body may also corrode severely in a different location [1]. Various locations within the body have different oxygen levels and pH values [1]. Many bodily fluids at normal conditions contain roughly 0.9 % saline (Na^+ and Cl^-), as well as other trace ions, amino acids, and proteins. The nominal pH of a healthy body is maintained around a value of 7, however at sites of inflammation due to injury or surgical intervention, the pH may fall as low as 3 [1]. The corrosion behavior of any metallic implant should maintain minimal metal ion release even in the harshest conditions.

Other than being corrosion resistant, these implant materials also must be designed to withstand significant fatigue and wear. Cyclic loading promotes fatigue failure faster than static loading. Orthopedic implants experience cyclic loading as a person walks - an average load of 50 MPa is applied to each leg after a single step, and over the course of a day the total number of steps can range roughly from 2,000 to 10,000 [1]. Additionally, dental implants undergo cyclic loading during chewing [1]. Wear damage can also cause several adverse reactions, such as loosening. The

gradual release of wear particles will lead the immune system to recognize the implant and released particles as a threat which results in significant erosion of the implant and surrounding bone [1].

Oftentimes there is insufficient compatibility between the mechanical properties of the metallic implant material and host bone tissue. The mechanical properties to consider include Young's modulus, ultimate tensile strength (UTS), and toughness [1]. Stainless steels, titanium-based alloys, and CoCr-based alloys are chosen for these orthopedic applications due to their ability to withstand great loads and deform plastically before failure. However, when the implant material has a Young's modulus much higher than that of the surrounding bone (e.g. over 100 GPa for stainless steel and 10-30 GPa for bone), a phenomenon called stress shielding occurs, where the implant bears the majority of the load. When the surrounding bone continually bears less and less of the load, this results in undesirable effects such as atrophy [1]. Therefore, it is a goal to have an implant material with a Young's modulus similar to that of bone.

In summary, in order for an implant to perform its intended function for the desired time frame, it must have high fatigue strength, excellent corrosion resistance in the body, and wear resistance. These properties will aid to increase the longevity of the implant, reduce toxicity to the patient, and prevent unnecessary revision surgeries. Many attempts to address this problem have been made. Common routes are surface modifications of these materials such as coatings. Coatings of bioceramics or polymers are popular (refer to chapter 2.2 and 2.3). These coatings act as a physical barrier between the metallic implant and the environment of which it is placed. On top of this isolation of the metallic implant, coatings serve additional purposes, such as increasing cell adhesion and growth, antibacterial properties, or antithrombogenic properties. Additionally, some applications can utilize polymeric materials (both natural and synthetic), to replace metallic implants, which will be discussed more in upcoming chapters.

2.1.1 Stainless Steels

"Stainless steel" is a name for iron-based alloys with a chromium content of 11-30 wt.%. One stainless steel which shows good biocompatibility is 316L stainless steel. Surgical grade 316L stainless steel boasts many impressive properties, such as cost effectiveness and ease of processing [4]. However, its poor corrosion rate and unsatisfactory fatigue resistance heavily restrict its applications today [1]. Failure analysis reports of these alloys after being implanted in the body show that their failure can be attributed to fatigue cracking which initiated at sites of localized corrosion (Figure 2-1) [1].

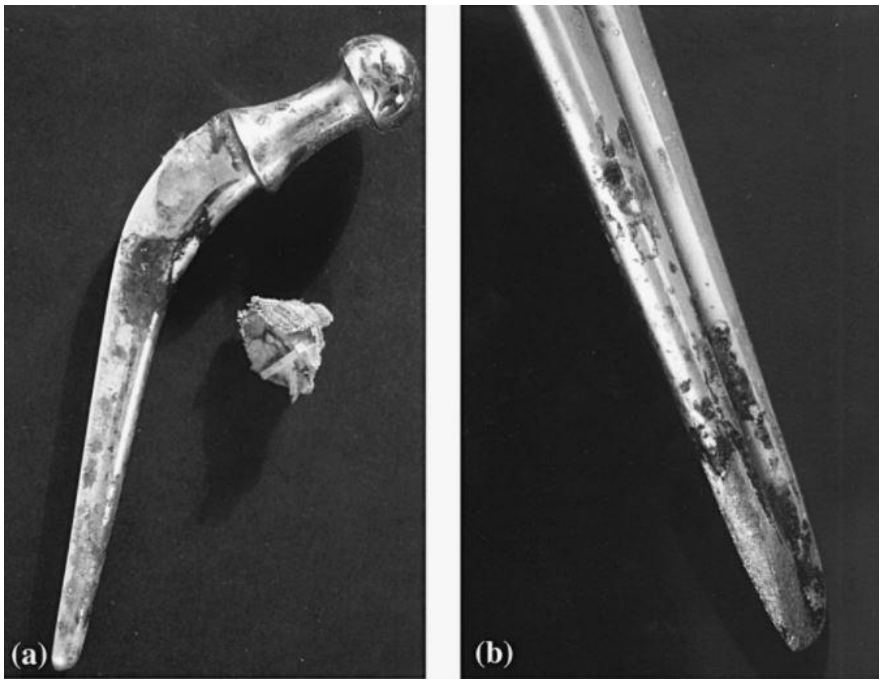

Figure 2-1: Stainless steel hip replacement prosthesis revealing surface corrosion on the stem and head (a), and pitting corrosion on the stem (b). Rapid failure occurred after 9 years [1].

An additional concern is the release of other alloying elements such as nickel and chromium [1]. Released nickel ions cause tissue necrosis nearby or may travel to distant organs such as the liver, kidney, and spleen [1]. The toxicity of released Cr depends on its valence state. Cr (III) can exit the body via urine, but in high concentrations it can damage DNA by releasing

free radicals, which causes breakage within DNA strands [3]. However, hexavalent chromium, Cr (VI), is the primary chromium ion released during corrosion of stainless steels and is classified as group 1 carcinogen [3]. These ions can also affect remote tissues by traveling via lymph and blood to various areas including bone marrow, spleen, liver, and heart [3]. With poor wear resistance, wear debris can cause allergic reactions and inflammatory reactions in surrounding tissues [1]. For these reasons, 316L stainless steels are no longer used for permanent implant devices, but due to their low cost they are still commonly opted for use in temporary devices [1]. Some applications still using 316L stainless steels today include bone screws, plates, and pins, staple prostheses, aneurysm clips, balloon-expandable stents, wire meshes, and rods and hooks for scoliosis treatment [1].

2.1.2 Cobalt-chromium based alloys

Corrosion resistance of cobalt-chromium (CoCr) based alloys is an entire order of magnitude higher than stainless steels [1]. Additionally, CoCr alloys have superior mechanical properties due to the crystallographic nature of the primary element cobalt [1]. With further solute strengthening effects of chromium and other alloying elements such as molybdenum and tungsten, they also possess a high fatigue resistance. For these reasons, these alloys are generally regarded as safe for permanent leg and arm implant applications [1]. However, these alloys are difficult to machine and cost significantly more than stainless steels, so a trade-off exists between cost and desired properties [1]. There are also undesirable biological responses evoked by CoCr alloys, including cytotoxicity and inflammation. Unfortunately, the alloys' innate fatigue resistance is shown to decrease in simulated body fluid (SBF), and they are susceptible to corrosion fatigue [1]. As previously mentioned, the release of Cr metal ions can damage DNA and negatively affect

other organ systems [3]. Release of metallic cobalt ions can cause both severe tissue damage at the location of the implant, as well as lead to various systemic and neurological symptoms, such as muscle cramping, shortness of breath, decline in motor skills, decline in cognitive function and memory, and severe headaches [1]. Therefore, there is a low success rate for these alloys after a 20-year lifespan. However, wrought CoCrMo alloys can provide more than 20 years of service safely, and thus they are the number one choice currently for permanent load-bearing implants such as total knee or ankle replacements [1]. Overall, this alloy is more expensive than stainless steels and still poses the risk of toxicity due to corrosion fatigue release of Co and Cr ions.

2.1.3 Titanium based alloys

While stress shielding is a concern for stainless steels and CoCr based alloys, this issue can be addressed by using titanium alloys. Titanium alloys have a lower Young's modulus, as well as suitable biocompatibility and enhanced corrosion resistance [1]. Stainless steels and CoCr alloys rely on the alloying of Cr for corrosion resistance, whereas titanium itself as a matrix element has excellent corrosion resistance [1]. Due to this improved corrosion resistance, titanium alloys are generally considered more biocompatible than stainless steels and CoCr alloys [1]. In fact, both commercially pure titanium (CP-Ti) and its alloys (e.g. Ti-6Al-4V) are widely used in various orthopedic and dental applications. CP-Ti applications include housing for pacemaker devices, dental implants, maxillofacial and craniofacial implants, and screws or staples for spinal surgery [1]. Ti-6Al-4V applications include dental implants, knee-, hip-, shoulder-, spine-, elbow-, and wrist-replacement parts, and bone screws and nails [1]. However, pure titanium in-vitro has been shown to cause genetic alterations and have adverse effects on white blood cells [1]. Al and V ion release from Ti alloys are shown to cause long-term drastic neurological deficits, such as Alzheimer's disease [1]. This is a considerable risk because it has been shown that titanium's

passive oxide layer can be easily broken down by applied stress, and due to continual applied stress, the passive layer is unable to heal immediately. This leads to local corrosion and material loss [1].

2.2 Advanced biopolymers for biomedical applications

"Biopolymer" is a term to describe polymers which are naturally occurring in living organisms, compared to synthetic polymers which are made by scientists and engineers. Biopolymers have chemical structures which closely resemble the structure of many native macromolecules within the extracellular matrix (ECM). This similarity means that the majority of biopolymers are generally more compatible within the human body than synthetic polymers [5]. Other properties common amongst biopolymers include being hydrophilic, non-immunogenic, non-toxic, and enhanced cell adhesion [5]. Three popular biopolymers will be highlighted in this chapter, including collagen, chitosan, and alginate.

2.2.1 Collagen properties and biomedical applications

Collagen is naturally occurring and can be found in various tissues such as bone, tendons, ligaments, cartilage, skin, blood vessels, and vitreous tissue (in the eye) [6]. This naturally occurring macromolecule has a very organized, network-like, structural arrangement (Figure 2-2) [6]. Thus, collagen has many amazing properties, such as biocompatibility, biodegradability, high availability, and versatility [6], which leads to numerous biomedical applications such as wound healing, treatment for ophthalmic defects, drug delivery systems, tissue engineering, and orthopedic implant material [6]. For use in these many applications, collagen is isolated from the skin, bone, or tendon of bovine, porcine, chicken, or marine organisms (Figure 2-2) [6].

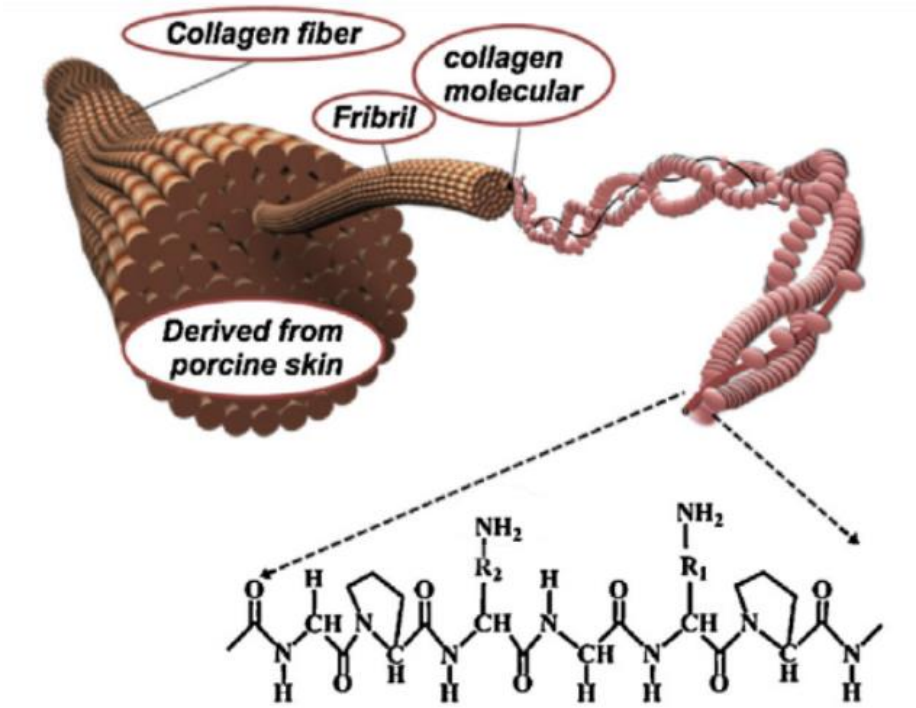

Figure 2-2: Schematic showing collagen chain structure isolated from porcine skin [7].

Due to the functional groups such as amino acids and carboxylic acids found in collagen, it can be easily modified for different applications [6]. For example, wound healing works to repair damaged skin by shrinking the wounded area. Collagenous membranes can be produced in different shapes or forms such as gels, films, or sponges, and then can be impregnated with live cells such as keratinocytes or fibroblasts which signal cell proliferation. Overall, this facilitates wound closure and leads to skin regeneration [8]. Furthermore, collagen-based materials are extremely promising for tissue engineering applications due to the native collagen already present within the body [6]. One study combined collagen with naturally derived alginate dialdehyde and saw enhanced thermostability, as well as increased hydrophilicity leading to better cell attachment and proliferation for applications in tissue engineering [7]. Collagen is not always suitable for further biomedical applications due to its poor mechanical strength, thermal instability, and enzymatic breakdown susceptibility [9]. Crosslinked collagen materials can begin to provide a

solution to the poor mechanical strength, but still no standard crosslinking procedure exists that can maintain a balance between collagen's natural functionality and mechanical stability [9]. Additionally, cross-linking can increase the stiffness, tensile strength, and compressive strength of the polymer, but destroys the unique self-assembled 3D structure of native collagen [9].

2.2.2 Chitosan properties and biomedical applications

Chitosan is the second most abundant naturally occurring polymer on earth, following just cellulose [10]. It is a strongly cationic, linear polysaccharide with a high molecular mass. It is derived from shells of crustaceans such as crabs, shrimps, and lobsters. First the protein and calcium salts from the exoskeletons are eliminated by alkali or acid treatment. This results in chitin, which is a hard, white, nitrogenous polysaccharide that is structurally identical to cellulose [11]. From here, deacetylation is performed by sodium hydroxide at high temperatures, forming chitosan. Chitosan has many promising properties for use in biomedical applications such as ease of gelling, swelling, and film formation, and it is non-toxic, anti-microbial, biocompatible, and biodegradable [11]. Chitosan is insoluble in water and organic solvents, but soluble in strong acids. The amine groups of chitosan become protonated at acidic pHs, imparting a positive charge to the polymer chains [11]. As most cell surfaces are anionic, chitosan shows strong adherence to tissues through electrostatic interactions [11]. The major biomedical applications of chitosan include bone, tendon, and ligament regeneration, tissue engineering, and wound healing [12]. One study used chitosan to coat a coronary stent, and after implantation in an artery, the coating was shown to enhance reendothelialization and hemocompatibility compared to a bare stent [13]. Another study showed chitosan films can be used for contact lens material and other ophthalmic applications due to biodegradability, hydrophilicity and water permeability, and suitable

mechanical properties [14]. Lastly, chitosan has been combined with other biopolymers (gelatin and hyaluronic acid) to form a polymer scaffold for tissue engineering, specifically as artificial skin material [15]. The porosity, elasticity, and cell compatibility showed excellent results for skin regeneration with no cytotoxic effects [15]. Unfortunately, chitosan alone has poor mechanical properties and limited functionality. Therefore, it is quite common for chitosan to be made into blends with other polymers, grafting to create co-polymers, or other composite materials to yield results required for specific biomedical applications. This can make the procedure quite complicated as well as increases cost.

2.2.3 Alginate properties and biomedical applications

Alginates are naturally occurring anionic, hydrophilic, heteropolysaccharides which are extracted from brown seaweed, kelp, and some bacteria [5]. Alginates can differ in monomer composition, sequence, molecular weight, and functional groups depending on the source and location of which it was derived from [5]. For example, alginates derived from seaweeds possess sulfate groups, whereas alginates derived from bacteria (e.g. Azotobacter vinelandii) possess acetyl groups [5]. Major important properties of alginates to note are biodegradability, mucoadhesiveness, biocompatibility, anti-inflammatory, and hemocompatibility [5]. Due to these properties, alginates have many applications in the biomedical field, such as tissue engineering, drug delivery, wound dressing, and more [5]. For example, alginate nanofibers are extremely sought out for tissue engineering scaffold material which can bear a structural, chemical, and mechanical resemblance to a structural component of the ECM and promote superior cell attachment [16]. Furthermore, alginate is increasingly used for drug encapsulation and delivery. One study loaded alginate nanoparticles with paclitaxel (PTX). PTX is an excellent

chemotherapeutic agent which unfortunately has a very short half-life. Therefore, its use is decreasing due to fast elimination in the body [17]. These alginate drug-loaded nanoparticles were stable, non-cytotoxic, and able to continuously show a targeted effect on cancer cells - better than PTX alone [17]. Lastly, alginate based wound dressing materials are renowned for retaining moisture and excellent anti-inflammatory properties. One study combined alginate microfibers with silver nanoparticles to treat second-degree thermal burns [18]. The alginate-silver nanoparticle wound dressing showed faster healing, reduced inflammation, and improved cellular organization and proliferation [18]. However, alginate's potential in biomedical applications still requires further investigation. Work is needed to increase alginate's purity by industrial preparation methods, to investigate the accumulation of alginate within the body, to identify any adverse health effects, and to better understand how monomer composition determines the physicochemical properties [5].

2.3 Synthetic polymers for biomedical applications

While biopolymers have the advantage of being sourced from naturally occurring materials, synthetic polymers also have many attractive properties for biomedical applications. In fact, synthetic polymers can provide many beneficial properties that biopolymers may lack, and thus are widely used - as long as they do not evoke any kind of toxic or immune response [19]. To start, their mechanical strength as well as chemical and thermal resistance directly address weaknesses of many biopolymers. Synthetic polymers also have low costs, ease of processing, tunable mechanical properties, and batch-to-batch consistency which make them suitable for mass-production of biomedical devices [20]. Other properties such as surface chemistry, porosity, and roughness on the micro- and nanoscale can be controlled in such a way that promotes cell adhesion

for applications of tissue engineering and tissue regeneration [20]. Applications of synthetic polymers in the biomedical field include stent and implant coatings, drug delivery, orthopedic fixation devices (rods, plates, screws), load bearing orthopedic devices, tissue engineering scaffolds, biosensors, and more [19]. In this chapter, three popular synthetic polymers for biomedical applications are highlighted including polyetheretherketone, poly(methyl methacrylate), and poly(ethyl methacrylate).

2.3.1 Polyetheretherketone properties and biomedical applications

Polyetheretherketone (PEEK) is a semi-crystalline thermoplastic. PEEK had many applications such as airplane and turbine engines in the 1980's [21]. Then, later in the 1990's it became a popular choice to replace metallic implants, specifically for load bearing orthopedic applications [21]. The young's modulus of PEEK is around 3.6 GPa and can be increased to near 20 GPa with fiber reinforcement [21]. This is very attractive for orthopedic implant applications because it will significantly decrease the stress shielding effect observed for conventional metallic implants (previously described in chapter 2.1) [21]. Additionally, PEEK is biocompatible, with no toxic effects and insignificant inflammatory reactions [21]. However, PEEK's applications in the biomedical field are rather limited because it is classified as bioinert. This means while being safe to be placed in the body, it does not elicit any biological responses that can form bonds or promote the growth of new tissues. Typically, in preparation for its use in biomedical applications, PEEK undergoes surface modification to increase its bioactivity or osteoconductivity. This has proven to be rather challenging because PEEK is a very high-performance polymer with extremely high physical and chemical stability [21]. For example, potassium titanate whiskers are a common filler for PEEK. This conventional PEEK composite has increased strength and stiffness [21]. In

addition, this composite leads to better wear resistance, specifically in water-lubricated conditions, which makes it very promising for dental implant applications with increasing life spans [21]. Furthermore, carbon fiber reinforced PEEK (CFR-PEEK) has many applications as various orthopedic implants, such as nails, disks, cages, and plates which had greater wear resistance compared to commercially available titanium alternatives [22]. Hydroxyapatite (HA) is commonly used to enhance PEEK's bioactivity (explained further in chapter 2.4.1). However, the tensile strength and strain to failure of a PEEK-HA implant is lowered due to the brittle structure and poor mechanical integrity of HA [23]. Moreover, functional groups applied to PEEK's surface can increase cell attachment and growth [21]. A common method to achieve this is plasma-treatment. One study used a NH_3 plasma treatment to induce amino groups onto PEEK's surface. This resulted in excellent cell adhesion and increased hydrophilicity - thus overall acceptance of the implant within the body [24].

2.3.2 Poly(methyl methacrylate) properties and biomedical applications

Poly(methyl methacrylate) (PMMA) is a transparent, amorphous thermoplastic. The presence of an adjacent methyl group (CH_3) prevents the polymer chains from packing closely in a crystalline manner [25]. PMMA has high impact strength, shatter resistance, and is lightweight. Therefore, it is a favorable substitute material for glass [25]. PMMA also has a high Young's modulus and low elongation at failure. It is also one of the hardest thermoplastics, with impressive scratch and weather resistance [25]. It is very resistant to chemicals as well [25]. PMMA also has many properties that make it suitable for a variety of biomedical applications, such as non-toxicity, biocompatibility, low cost, ease of processing, minimal inflammatory response, and fracture resistance [25]. These applications include bone cements, drug delivery systems, craniofacial

implants, other orthopedic implant materials, and more [25]. For example, one study using PMMA as a bone cement showed that antibiotic drug release can be increased by increasing the porosity of the bone cement [25]. Additionally, drug loaded PMMA nanoparticles for topical ophthalmic application to treat elevated intraocular pressure showed better results than conventional eye drops [25]. Lastly, PMMA's ease of processing is beneficial for creating uniquely shaped implants for cranioplasty (Figure 2-3). In fact, PMMA is the most commonly used material for cranioplasty [26]. Certain PMMA implants have shown increased osteoconductivity by mixing HA and PMMA. Studies showed new bone growing on the exposed HA particles [26].

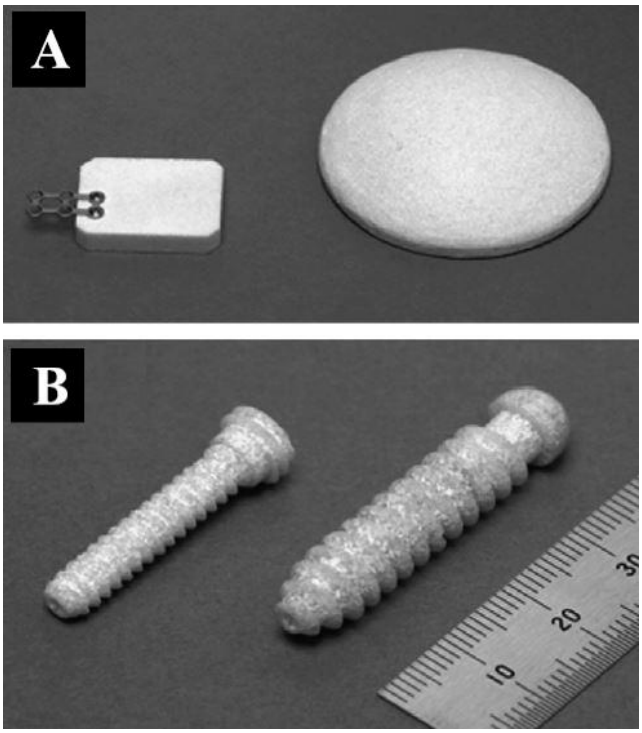

Figure 2-3: PMMA-HA cranial plates (A), and cranial screws (B). This composite is easily processed and can be shaped freely [26].

Despite PMMA's extensive use in biomedical applications, there are still existing issues related to orthopedic implant failure rates of approximately 5% within the first 10 years [27]. Additionally, around 80% of implants fixed with PMMA bone cement experience loosening [27].

These problems arise due to unreacted methyl methacrylate (MMA) monomer being leached into surrounding tissue causing necrosis [27]. PMMA also raises concerns regarding low toughness and low creep resistance resulting in crack formation [27].

2.3.3 Poly(ethyl methacrylate) properties and biomedical applications

Poly(ethyl methacrylate) (PEMA) is another synthetic polymer, which is similar to PMMA but has some notable differences. PEMA is a transparent thermoplastic and is semi-crystalline. It is also lightweight and shatter resistant making it another feasible option for replacing glass. PEMA displays excellent chemical and thermal resistance, ease of processing, and low cost [28]. In addition to shatter resistance, PEMA has other impressive mechanical properties such as high Young's modulus and high toughness [29]. PEMA has many biomedical applications due to its suitable mechanical properties and biocompatibility. To start, PEMA is also a common material for bone cements. It is a popular choice because its monomers, ethyl methacrylate (EMA), are less toxic than MMA monomers, so the possibility of necrosis is decreased [27]. Additionally, PEMA blends such as PEMA and tetrahydrofurfuryl methacrylate (PEMA/THFMA) polymer systems have shown promise for use in cartilage repair [30]. These PEMA-blends promoted cell attachment, growth, and differentiation, resulting in new, dense cartilage tissue growing in the treated defects [30]. PEMA foams are also structurally suitable for tissue engineering scaffolds due to controllable pore size and interconnectivity, and desirable mechanical properties [30]. PEMA's success in orthopedic and dental applications has been enhanced by immobilizing eugenol derivatives onto the surface. Eugenol and its derivatives are analgesic, anti-inflammatory, and antimicrobial, thus enhancing the bioactivity of PEMA based cements [31]. However, the use of PEMA in biomedical applications is constrained due to its chemical resistance resulting in poor

solubility in safe, non-toxic, solvents such as water and alcohols. PEMA is soluble in toxic and carcinogenic solvents such as ketones, benzene, and toluene. In order for the fabricated PEMA-based devices to be safe to be implanted within the body, the use of these solvents must be avoided in all processing steps. Due to the long list of impressive properties of PEMA, it has many other industrial applications as packaging material, thermal energy storage material, and super absorbent material [28]. However, to see more widespread use of PEMA in biomedical applications, its poor solubility remains a challenge.

2.4 Bioceramics for biomedical applications

"Bioceramics" is a term to describe ceramic materials that are biocompatible [2]. They have many uses in biomedical applications in both their crystalline and amorphous forms [2]. Two distinct classifications of bioceramics can be made. First, is bioinert bioceramics which tend to have high chemical and mechanical stability, but when placed alongside natural bone, they do not form any bonds and generate very limited tissue responses [2]. Some examples of these bioceramics include metal oxides such as titania (TiO_2), alumina (Al_2O_3), and zirconia (ZrO_2). The second class of bioceramics are bioactive bioceramics, such as hydroxyapatite, other calcium phosphates, and bioactive glasses [2]. These materials can create bonds with surrounding bone tissues, though their mechanical strength is often lower than that of bioinert bioceramics [2]. The formation of new bone is promoted due to their similarity to inorganic components of real bone, which increases cell attachment, proliferation, and differentiation [2]. Overall, bioceramics are important for the design of new implant materials with better biocompatibility, stability, and longevity.

2.4.1 Metal oxides properties and biomedical applications

The first type of bioceramic to highlight are metal oxides, a bioinert class of bioceramics. These materials have high chemical stability and strength for implant applications [2]. Some examples of these materials include alumina (Al_2O_3), silica (SiO_2), and zirconia (ZrO_2). The body's response to alumina implanted in bone has been thoroughly studied. One way to control alumina's mechanical properties is by preparing porous alumina structures. While alumina is bioinert, by creating certain geometries that mimic structures in the ECM, cell attachment can be significantly enhanced by controlling porosity and roughness [32]. In fact, the most common biomedical application of alumina is surface nano-structuring agent for orthopedic and dental implants [32]. For example, a thin (1-5 μm), porous layer (~160 nm diameter) of anodized alumina was used to coat titanium alloys by electron beam evaporation [33]. These coated substrates showed promising results such as increased osteoblast proliferation and high tensile strength (Figure 2-4).

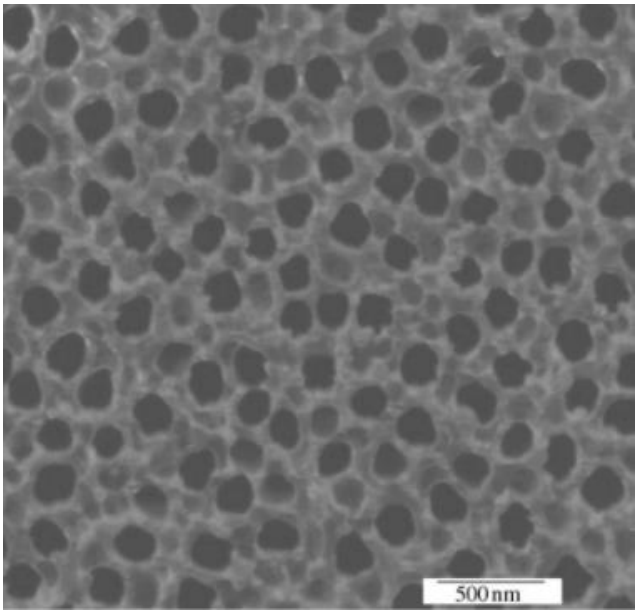

Figure 2-4: SEM micrograph of anodized alumina membrane used for bone cell culturing tests [33].

Next, silica bioceramics can take on many forms to fulfill many different requirements for a long list of biomedical applications, including colloidal silica, silica gel/aerogel, porous scaffolds, or nanoparticles. To be specific, mesoporous silica nanoparticles (MSNs) have gained a lot of attention for drug delivery systems due to high surface area and tailorability for site-specific delivery [34]. For example, one study loaded ibuprofen into MSNs with two different pore arrangements. Upon studying the drug release behavior in SBF, it was found that hexagonal pores released larger amounts of ibuprofen over an extended period of time [34]. Another advantage of silica-based materials for drug delivery is the ability to form core-shell composite materials. One report coated Fe_3O_4 nanoparticles in a layer of mesoporous silica, and then proceeded to load ibuprofen into the silica outer layer. These hybrid nanoparticles were able to release ibuprofen in SBF for over 70 hours [34]. Lastly, zirconia bioceramics are a favorable material for many dental implant applications because its mechanical properties (and color) are very similar to that of teeth [35]. Commercially available zirconia implants show a 1-2 year mean survival rate and have less reported bone losses compared to titanium-based implants [36]. However, more long-term studies are required to improve upon the existing short-term outcomes [36].

2.4.2 Hydroxyapatite properties and biomedical applications

Hydroxyapatite (HA) ($Ca_{10}(PO_4)_6(OH)_2$) has chemical, structural, and biological properties very similar to human bone. In fact, 70% of human bone is hydroxyapatite nanocrystals (rod-like shapes, 25-50 nm in length) which are embedded in a collagen matrix [2]. Thus, HA boasts impressive biocompatibility, bioactivity, and osteoconductivity [23]. In more detail, this means it can form bonds with surrounding natural bone and promote the growth of new tissue by signaling for the right cells. Overall, this leads to implants having more secure integration, increased

longevity, and increased stimulation of new bone growth [23]. Hydroxyapatite itself is brittle, stiff, and has low strength [2]. Therefore, it is very popular to deposit hydroxyapatite within a polymer matrix to augment both the mechanical properties of hydroxyapatite and the bioactivity of the polymer [2]. For example, one study counteracted the poor bioactivity of poly-lactic acid (PLA) by fabricating a HA-collagen-PLA composite scaffold for bone tissue engineering [37]. The poor compressive strength and elastic modulus of HA was increased with increasing PLA content and within a week, osteoblasts were shown to have adhered, spread, and proliferated throughout the scaffold. Within twelve weeks, the model bone defects were integrated with the scaffold, and some scaffold material was replaced with new bone tissue [37]. Additionally, studies have shown that the pH in areas surrounding polyester implants drops due to release of acidic degradation products [38]. The presence of HA particles in a composite polyester material will offset the acidification from the alkaline calcium phosphate [38]. Lastly, it has been shown that the presence of nanosized HA particles increases osteoblast adhesion and amount of new bone formation compared to micron-sized HA materials [38]. Even further, when there is a homogeneous distribution of such nano-scaled HA, the bioactivity of the material is enhanced [38]. This is shown in one study, where nano-scaled HA particles were covalently linked to a silk fibroin substrate [39]. SEM images revealed particles to be separated evenly or in aggregates of only a couple nanocrystals. It was then seen that a greater amount of fibroblast cells adhered to the silk fibroin substrates with HA particles compared to those without [39]. Cells were able to integrate into the gaps of the composite scaffold and grow in a three-dimensional manner [39]. However, it is more challenging to enhance the bioactivity of metallic substrates through a coating of HA due to HA having poor adhesion to metals [40]. In these cases, an additional step of surface preparation is necessary, such as sand/grit blasting, acid/alkali treatment, or deposition of an intermediate layer between the metallic substrate

and the HA coating [40]. An alternate way to increase HA adhesion to metallic substrates is to use temperature driven processes, including plasma spray, thermal spray, or pulse laser deposition [40]. Unfortunately, at the high temperatures required for these processes, HA has been reported to decompose to CaO and $Ca_3PO_4^-$ which are no longer biocompatible [40]. One study avoided these temperatures by opting for a functionally graded multilayer coating which was deposited by electrophoretic deposition onto a Ti-6Al-4V substrate [40]. The first layer deposited was TiO_2, then the next layers were deposited from TiO_2-HA suspensions with increasing HA concentration with each additional layer. The functionally graded coating had superior adhesion (31 MPa) compared to a single HA coating (11 MPa) or a single TiO_2-HA coating (24 MPa) [40].

2.4.3 Bioglass properties and biomedical applications

Bioactive glasses, termed as "Bioglass" with the chemical formula Na_2O-CaO-SiO_2-P_2O_5 are shown to form instantaneous bonds with natural bone [4]. This ability has been shown in several investigations of bioglass immersion in simulated body fluid (SBF) resulting in the formation of a new apatite bone-like layer [4]. However, like other bioactive bioceramics, bioglass lacks mechanical strength to be used as bone replacement material. Thus, it is often used as a coating on metallic implants or added in a composite material. One example used sol-gel techniques to coat a 316L stainless steel substrate with Sr/Mg-substituted 58S bioglass [4]. This study highlighted the ability of bioglass to impart not only bioactivity to the stainless steel substrate, but also enhanced the corrosion resistance properties of the substrate, resulting in a material better suited for orthopedic implant applications [4]. Additionally, both HA and bioglass were used to coat a polyethylene terephthalate (PET) artificial ligament graft (Figure 2-5) by plasma spraying [41]. The ligament grafts were then implanted into rabbits to study the mechanical

properties and osseointegration of the composite. The findings showed that the composite coating resulted in increased bone formation at the graft-bone interface, as well as increased the load-to-failure of the graft, when compared to uncoated PET grafts [41].

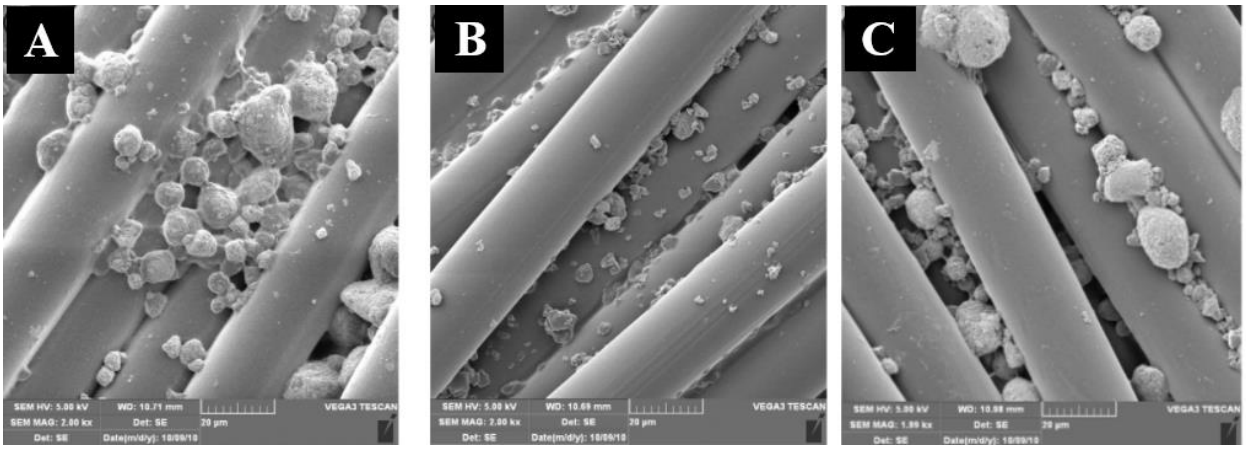

Figure 2-5: SEM micrograph of the PET grafts with HA coating (A), with BG coating (B), and with HB composite coating (C) [41].

Another study used bioglass coatings to improve the bioactivity of 316L stainless steel lattice structures [42]. The bioglass coating was applied by immersing the substrate in a bioglass suspension for 15 minutes followed by a heat treatment at 450 °C for three hours. In this case, a silica layer was used as a pretreatment to increase adhesion of the bioglass to the substrate [42]. After a 7-day immersion in SBF, the silica pre-treated bioglass-coated substrates showed formation of a new apatite layer, with growth increasing with increasing bioglass concentration, thus proving enhanced bioactivity [42]. Lastly, bioglass can also be used to improve upon many polymers' bioactivity and mechanical properties such as stiffness. A study on biodegradable poly(propylene carbonate) (PPC)−starch blends for bone screw applications saw increased bioactivity and mechanical performance upon the addition of bioglass microparticles within the blend [43]. This was shown by increased osteoblast differentiation and higher stiffness [43].

2.5 Bio-inspired composite materials and processes

2.5.1 Bio-inspired composite materials

Inspiration for the design of novel materials and processes can be derived from nature. Naturally occurring materials and processes can be mimicked in a laboratory to achieve superior results. For example, many composite materials are designed to match the microstructure of human bone. Human bone is composed of inorganic and organic materials, specifically a collagen fibril network (~ 30% mass) with hydroxyapatite nanorods (~ 70% mass) embedded within the collagen matrix [2]. This naturally occurring composite design has appropriate physical, chemical, and biological properties to serve its function in the body - such as high strength for structural support and protection. New biomedical devices can mimic this composite design for enhanced bioactivity and regenerative properties [2]. Thus, the desire to develop biocompatible composites similar to the composition of bone is steadily increasing in interest. Many other biocompatible polymers and inorganic materials can be used to achieve specific properties for several applications, all with the same goal in mind. These materials are often lightweight and the combination of nano- and micro-features is advantageous for higher strength and toughness that exceeds the singular materials making up the composite, often by several orders of magnitude [44]. Other than bone, there are several composite materials in nature with hierarchical architectures with superior properties that are worth investigating. Marine shells, nacre, dactyl clubs of shrimp and crabs, and teeth are some more examples of naturally existing composite materials with hierarchical structure and noteworthy properties [44].

2.5.2 Bio-inspired processes

Another remarkable feature about materials in nature is that they are often assembled in a "bottom-up" strategy within their physiological environment, usually at ambient temperatures and pressures. Several bio-inspired fabrication methods also exist for the development of advanced materials for biomedical applications. For example, the chemical mechanism responsible for mussel's strong adhesion to multiple different surfaces in saline can be mimicked to provide excellent dispersion of multiple materials, such as metal oxide nanoparticles, carbon nanotubes, or conductive polymers, through strong interfacial adsorption [45]. Mussel's fast and strong adhesion mechanism involves the complexation of metal atoms on such surfaces by hydroxyl groups of catechol ligands (Figure 2-6A) [45]. This led to a well-developed group of dispersing agents from the catechol family to produce stable suspensions. Molecules within the catechol family that were investigated include DOPA, dopamine, caffeic acid, gallic acid, and more (Figure 2-6B). These naturally occurring molecules are all well-performing dispersing agents and eliminate the need for inorganic acids, alkalis, or iodine as additives for many colloidal processing techniques [45].

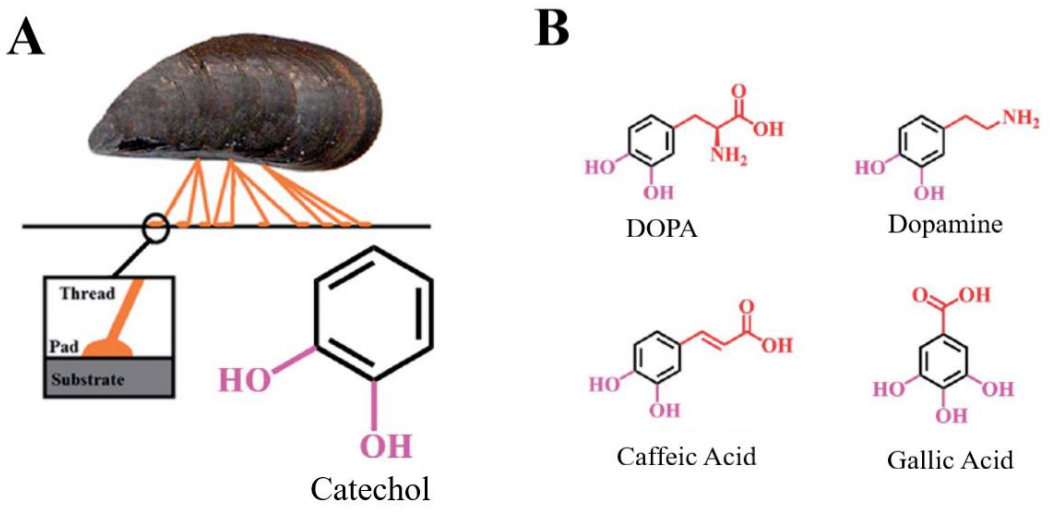

Figure 2-6: Schematic showing mussel adsorption due to catechol group (A), and chemical structure of other molecules within the catechol family (B) [45].

2.5.2.1 Bile Acids and Salts

Another family of naturally occurring molecules which are attractive for synthesis of advanced materials are bile acids (BAs) and bile salts (BSs). They are synthesized in the livers of mammals and circulate the digestive system. These are physiologically important biosurfactants, as their role is to solubilize cholesterol, lipids, proteins, vitamins, proteins, and fatty acids within the body [46]. In fact, the solubility of cholesterol in water increases one million-fold in the presence of bile salt sodium cholate [46]. Their ability to strongly solubilize these small water insoluble molecules is attributed to their unique amphiphilic chemical structure. Their structure is different than that of other commercial surfactants, which normally consist of a polar head group and non-polar hydrocarbon tail. Whereas bile acids/salts have a rigid steroid core, a concave face which is hydrophilic due to OH groups, and a convex face which is hydrophobic due to CH_3 groups [46]. Bile acids contain a carboxylic acid end group, and bile salts (ionized form of bile acids) contain a COONa end group (Figure 2-7), which both dissociate in solutions to COO^- and impart a negative charge to the molecules for further electrostatic stabilization [46]. Another important distinction is that bile salts are well soluble in water and insoluble in organic solvents, whereas bile acids are oppositely insoluble in water but well soluble in organic solvents.

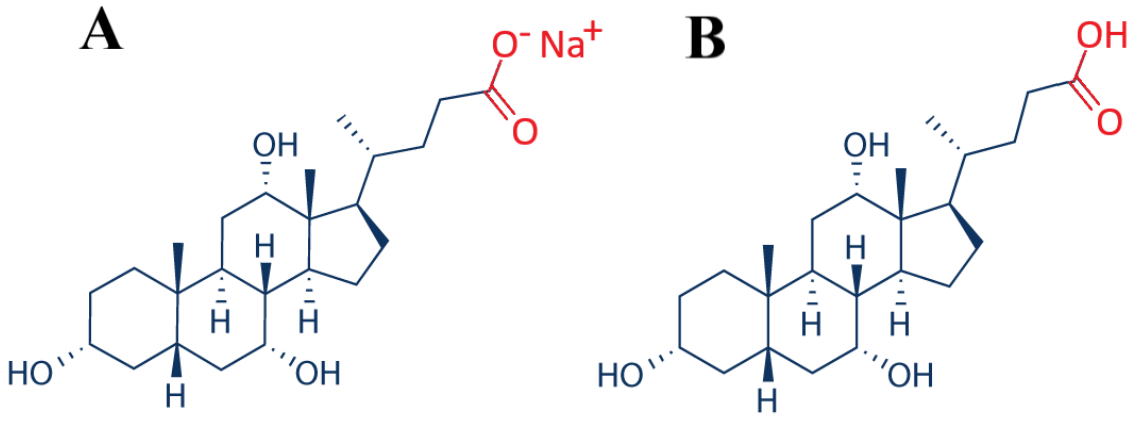

Figure 2-7: Chemical structure of bile salt sodium cholate (A), and bile acid cholic acid (B).

Owing to their natural origin, these molecules are very biocompatible. Therefore, they are commonly sought out for the fabrication of many biomedical devices. Their role in the body as solubilizing agents is re-produced for the synthesis of composite materials containing drugs, carbon nanotubes, diamonds, metal nanoparticles, and other advanced materials [47]. Bile salts are very popular for the design of drug delivery systems through their micelle formation in aqueous solutions. For example, antibiotics such as amphotericin B (AmB) are poorly soluble in aqueous solutions, therefore bile salt sodium deoxycholate sulfate was applied as a drug carrier to produce homogeneous stable solutions for oral administration [48]. Cytotoxicity studies showed that this bile salt drug carrier is biocompatible and safe for drug delivery [48]. In addition, other drugs with poor water solubility such as ibuprofen and tetracycline were solubilized in aqueous solutions by sodium cholate and sodium deoxycholate [49]. Also, due to the imparted negative charge of the dissociated bile salts, the drug-containing BS micelles were able to be deposited as films by electrophoretic deposition [49]. Bile acids also have many different applications in the synthesis of advanced materials, starting with cholic acid, which can act as a reducing and capping agent in the synthesis of silver nanoparticles in organic solvents at room temperature [50]. The formed nanoparticles were stabilized by cholic acid and did not require any additional surfactants [50]. Additionally, deoxycholic acid-conjugated heparin micelles in aqueous solutions were able to be loaded with hydrophobic fluorescent quantum dots (QDs) [51]. The resultant nanoparticles were used for non-invasive imaging applications. They were adsorbed easily into the small intestine, owing to the natural source of deoxycholic acid and the solubilization of the hydrophobic QDs [51]. The applications of BA/BSs for solubilization and dispersion of many small molecules are well documented in the literature. However, the use of bile acids to solubilize large polymer molecules remains a gap in this field.

2.6 Advanced coating techniques for biomedical applications

As described thoroughly above, many different biomedical devices require a coating to provide surface properties rendering the material more suitable for placement within the body, such as corrosion and wear resistance or increased biocompatibility and bioactivity. Many different coating techniques are utilized to create advanced coatings for biomedical applications. Starting with plasma spraying, this technique has become popular for the fabrication of coatings for biomedical applications because it is reliable, low cost, highly efficient, and offers control of the coating thickness [52]. However, complicated equipment is required such as an electrical power source, gas flow control, power feeder, and a water-cooling system [52]. These coatings would also benefit from an additional heat treatment after deposition [52]. The plasma spraying method is very popular for the deposition of ceramic coatings. For example, multilayered coatings containing alumina, titania, and zirconia were successfully deposited on a titanium alloy substrate which exhibited strong adhesion, low porosity, and excellent corrosion and wear resistance [52]. Next, several other spray techniques also widely used to create metal, polymer, or ceramic coatings for biomedical applications [53]. More specifically, air sprayed coatings can be achieved by adjusting air flow pressure and temperature. Magnesium alloys have been coated in HA for increased biocompatibility using a preheated HA powder sprayed through a nozzle kept at a high pressure and temperature [53]. An additional spray technique is thermal spray, which requires equipment capable of reaching extremely elevated temperatures and powerful vacuums [53]. Electrophoretic deposition (EPD) has also been successful in the creation of many coatings with varying thicknesses and morphology which are suitable for biomedical applications. For example, HA has been deposited onto titanium alloy substrates, with applied voltages ranging from 20 to 80 V [40]. The resultant HA coatings are very porous, which is desirable to promote biological

interactions. However, the adhesion is poor and can be improved by a post-deposition sintering step at elevated temperatures [23, 35]. The conditions required for electrophoretic deposition can be harsh, such as one study which coated stainless steel in bioglass by EPD requiring a voltage of 100 V and a post-deposition heat treatment at 800 °C for 2 hours [4]. Additionally, for successful film formation by EPD, the suspensions must be very stable and oftentimes a dispersing agent is required. In the case of electrically neutral materials, a charging agent is also required to adsorb onto the particles to impart a charge and provide electrophoretic motion towards the electrode. Alternatively, instead of coating a metallic substrate, many coating techniques are used to add a surface coating to polymers. To alter the properties of the polymer used for biomedical applications, such as increasing hydrophilicity or biocompatibility, many different techniques can be used such as photo-, chemical-, or radiation grafting, electron beam deposition, gas phase deposition, or laser ablation among others [2].

2.6.1 Dip Coating

A simple method to obtain a continuous coating on a variety of substrates is dip coating. Simply put, a substrate is lowered into a solution, held for a desired amount of time, then slowly withdrawn. Due to surface tension and gravity, some of the solution will remain on the substrate after it is removed and eventually all the solvent will evaporate, leaving behind a solid film of the material which was dissolved in solution (Figure 2-8).

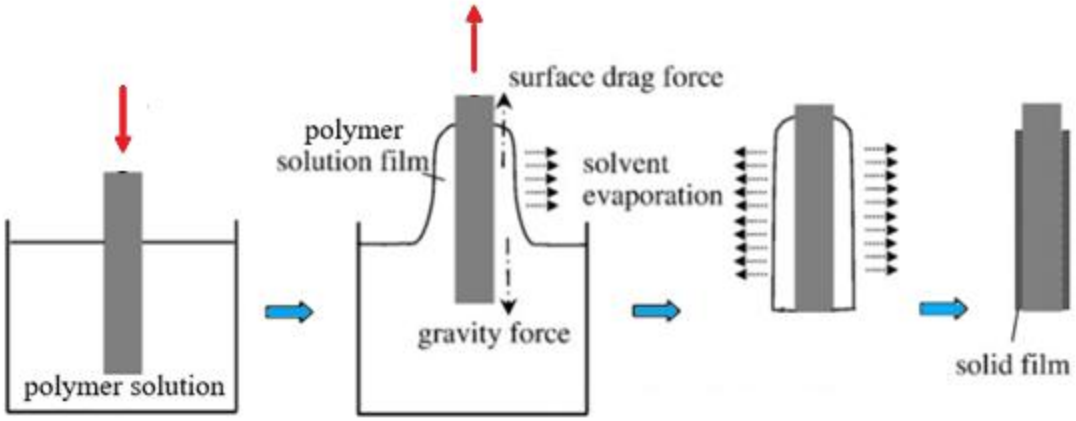

Figure 2-8: Schematic of dip-coating process [54].

Different solvents and polymer concentrations in solution can be used to achieve different morphologies. However, in order to create high quality uniform films, there is a baseline requirement of polymer concentration and molecular weight. It has been stated that a polymer concentration in solution of at least 10 gL^{-1} provides continuous, high quality, uniform films in comparison to films made from solutions of lower concentrations [55]. Additionally, low molecular weight polymers do not have sufficient film forming properties, therefore polymers with a molecular weight above 100,000 Da are desirable for dip coating applications [55]. Furthermore, studies have shown that by repeating the dip coating process, the thickness of the coating can be increased [56]. Dip coating can be used to coat substrates of many shapes such as fibers and wires, as well as to produce patterned microstructures [56, 57]. Lastly, composite coatings are obtainable by dip coating. For example, organic-inorganic composite films of polyethylene glycol (PEG) containing silica in a nitric acid and ethanol solution were used to coat a titanium substrate by dip coating [58]. Different polymer to silica ratios achieved different morphologies and different bioactivity results. Higher PEG content resulted in less crack formation and increased bioactivity of the coatings [58]. This method is desirable for many reasons, such as the ability to be performed

at room temperature, no expensive laboratory equipment required, and it is easily scalable to larger manufacturing levels for mass production. However, another requirement of this process is that the particles in solution must be very well dispersed and stable. Particle aggregation will cause decreased adhesion to the substrate, as well as reduce the mechanical integrity of the coating by acting as crack nucleation sites after drying. Lastly, it is important to consider the solvent used for this deposition method. Even after the coating has dried and the solvent has evaporated, there will still be solvent molecules which remain on the substrate. This can be detrimental if cytotoxic or carcinogenic solvents are used in the creation of coatings for biomedical devices. If this process can be carried out from a stable and well dispersed solution in a non-toxic solvent, with biocompatible materials and surfactants, then novel coatings for biomedical applications can be fabricated at room temperature without the need of expensive equipment, post-processing steps, or adhesion interlayers.

2.7 Research Objectives

Above is a thorough review of literature about various materials for biomedical devices. A focus was placed on orthopedic implant applications due to a prevalence in bone defects and an aging population. A wide range of materials are suitable for this application, including metals and alloys, natural or synthetic polymers, and bioceramics. However, materials for these applications have many constraints of the properties they must possess, such as mechanical properties similar bone, fatigue resistance, wear resistance, and corrosion resistance. The material must also cause no harm to the surrounding native tissue - such as stress shielding, tissue necrosis, allergic reactions, inflammation, infection, toxic debris particles, or any other adverse events. The desired outcome is for the implant material to form bonds with surrounding tissue for secure integration

and to promote growth of new tissue for better healing. This challenge can be addressed by modifying surface properties for improved biocompatibility or bioactivity through the deposition of a coating. With several coating materials and methods covered from literature, the objectives of this thesis can now be stated. The primary objective is as follows:

- Develop new colloidal techniques for the facile fabrication of advanced organic-inorganic composite coatings for biomedical applications

With this, comes multiple short-term objectives:

- Utilize synthetic polymers in the fabrication of coatings for stainless steel substrates. Stainless steels are the most cost-effective metallic implant material; however, their use is limited due to poor corrosion and fatigue resistance. This can be addressed by synthetic polymer coatings for corrosion resistance which also provide ease of processing, consistency, and mechanical integrity.
- Develop a solubilization mechanism for synthetic polymer poly(ethyl methacrylate) (PEMA) to be dissolved in non-toxic solvents for this application. PEMA has many attractive properties, but its biomedical applications are limited due to solubility in traditionally toxic solvents only.
- Take advantage of dip coating which is a simple, cost effective, and scalable film deposition technique. This can also be carried out at room temperature. To achieve this, a high molecular weight polymer (> 100,000 Da) must be dissolved in solution at high concentrations (> 10 gL^{-1}).
- Create biomimetic composite coatings containing organic and inorganic components similar to the composition of natural bone. These composite coatings should also possess

dual scale morphological features (micro- and nanoscale). Select materials specifically for orthopedic implant applications that can enhance biocompatibility and bioactivity.

2.8 References

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Chapter 3: Biomimetic approach to poly(ethyl methacrylate) solubilization, deposition and coating loading with functional biomaterials

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3.1 Abstract

A biomimetic approach is described for the fabrication of poly(ethyl methacrylate) (PEMA) coatings by a dip coating method. In the proposed approach, bile acids, such as cholic acid and lithocholic acid, are used as solubilizing agents for PEMA in isopropanol. For the first time PEMA coatings are fabricated from solutions in isopropanol avoiding the use of traditional toxic solvents. The feasibility of fabricating concentrated solutions of high molecular mass PEMA is a key factor for the deposition. The coatings can be obtained as monolayers or multilayers of controlled coating mass. The analysis of deposition yield data and chemical structures of the bile acids provides an insight into the deposition mechanism. The PEMA coatings provide corrosion protection of stainless steel. Building on advanced properties of bile acids, as natural dispersants, composite coatings containing various bioceramics, such as hydroxyapatite, silica, and titania in the PEMA matrix are obtained. Tetracycline is used as a model drug for the fabrication of drug-containing PEMA coatings. It is found that other functional molecules, such as heparin can be incorporated into the PEMA matrix. The approach developed in this investigation is promising for the fabrication of coatings for biomedical implants and devices using natural surfactants and avoiding the use of toxic solvents. Such coatings can be deposited as laminates of different layers, functionalized with bioceramics, drugs, and various biomolecules.

3.2 Introduction

Poly(ethyl methacrylate) (PEMA) is a thermoplastic polymer with advanced mechanical, thermal, and chemical resistance properties that lend itself to a variety of applications [1, 2]. PEMA has many industrial applications due to its low price and ease of processability. The functional properties of PEMA are usually enhanced by the development of composites, containing different inorganic and organic additives [1, 3]. Pure PEMA is transparent, lightweight, and shatter-resistant material and is therefore often opted as an alternative material for glass [4]. PEMA has a long history being used for corrosion protection of steels, specifically in the canning industry [5]. The thermal stability of this polymer also lends itself to usage as packaging material [6, 7]. PEMA has also been frequently used in the synthesis of superabsorbent materials [1].

PEMA is frequently used for various energy storage applications. PEMA films are highly desirable for creating dielectric layers for high energy storing, low loss capacitors [8]. Thermal energy storage is another application where PEMA has excelled due to its high thermal stability [2]. PEMA-fatty acid composites were used as phase change materials for thermal energy storage [4]. In addition, there is increasing demand in PEMA films for lithium-ion technology with the intention of creating more compact electronic devices [9]. PEMA is widely chosen as a polymer electrolyte film in rechargeable batteries due to its high ionic conductivity at room temperature and suitable mechanical properties [9, 10]. PEMA was combined with different additives such as ionic liquids or other polymers to further increase the ionic conductivity for battery applications [10, 11]. PEMA is an especially attractive polymer for this application due to its high flexibility [10].

A large interest exists for various biomedical applications of PEMA and its composites [12]. For example, PEMA is an attractive material for bone cements, shown to increase

proliferation and osteoblast adhesion while also providing impressive mechanical properties [12, 13]. The water uptake properties of this polymer facilitate the cell attachment and bone repair abilities in vivo [14, 15]. Shape memory materials based on PEMA are promising for medical devices [3]. Foamed PEMA and PEMA blends possess a certain surface chemistry, such as the wettability, that is favourable to support cell adhesion, differentiation, and growth, resulting in dense cartilaginous tissue filling the pores [14, 16]. These properties also make PEMA gels, cements, and films suitable for dental applications, craniofacial implants, and a variety of other orthopedic applications.

The interest in PEMA and composite films for various energy storage [8, 9], biomedical [12], corrosion protection [5], packaging [6, 7] and other applications has generated the need in the development of novel film deposition techniques. PEMA has properties ideal for biomedical applications, however it primarily shows solubility in highly toxic and carcinogenic solvents, such as methyl ethyl ketone, toluene, benzene, and others. This is a strong obstacle for the application of PEMA in the biomedical field. This is because after the solvent has evaporated leaving behind a solid polymer film, solvent molecules will still be present in the bulk or on the surface. Another difficulty is related to the fabrication of PEMA composites, containing functional inorganic or organic materials, which must be well dispersed in the PEMA matrix. Many commercial dispersants are toxic and cannot be used for biomedical applications. Isopropanol was used in many investigations for the fabrication of films and coatings for biomedical applications and offered benefits of reduced cytotoxicity, compared to other organic solvents [17-21]. Good cell attachment and proliferation were observed on the surfaces of the coatings prepared using isopropanol [22-24].

The goal of this investigation was the fabrication of PEMA and PEMA-composite films for biomedical applications by a simple dip coating method using isopropanol solvent and natural bile acids as biosurfactants and solubilizing or dispersing agents. Isopropanol is regarded as a non-solvent for PEMA. One of the key findings of this work was the solubilization of PEMA in isopropanol in the presence of bile acids. This finding opens a new and unexplored route in polymer processing. However, to be successful in the employment of the dip coating technique, the polymer must be at a significantly high molecular mass and high concentration. Therefore, of particular importance was the feasibility of dissolution of high molecular mass PEMA and fabrication of concentrated solutions, which were major factors for the successful PEMA coating deposition by a dip coating method. The analysis of deposition yields achieved using different bile acids provided an insight into the solubilization and deposition mechanisms. We demonstrated that obtained coatings can be used for corrosion protection of metals and alloys. Building on the new PEMA deposition method, composite coatings, containing bioactive ceramics, such as hydroxyapatite, silica, and titania were obtained. The deposition method paved the way for the fabrication of composite coatings containing other organic functional materials for biomedical applications, such as heparin and tetracycline drug molecules. The colloidal strategies developed in this investigation opened new avenues for the fabrication of advanced coatings for biomedical and other applications.

3.3 Experimental Procedures

Poly(ethyl methacrylate) (PEMA, average molecular weight ~ 515,000 Da), cholic acid (CA) and lithocholic acid (LCA), TiO_2 (average particle size ~20 nm), heparin and tetracycline were purchased from Millipore Sigma. High purity silica powder (SiO_2, average diameter ~ 1 μm)

was purchased from PCR Incorporated. Hydroxyapatite nanorods (HAP, average diameter ~ 20 nm, average length ~ 150 nm) were prepared as described previously [25, 26].

CA and LCA were dissolved in isopropanol at a concentration of 1 gL^{-1}. Following this, PEMA was added at a concentration of 10 gL^{-1} to both the CA and LCA solutions and for comparison in pure isopropanol without bile acids (BAs). Upon heating to 55°C, the PEMA suspensions, containing dissolved BAs, turned from an opaque cloudy white to clear and transparent solutions, signaling complete dissolution, whereas the suspension prepared without BAs remained opaque cloudy white. After cooling to room temperature, the PEMA solutions containing BAs, were stable for more than 7 days (Figure 3-1). This indicated the successful dissolution of PEMA in isopropanol facilitated by the small addition of the naturally occurring BAs.

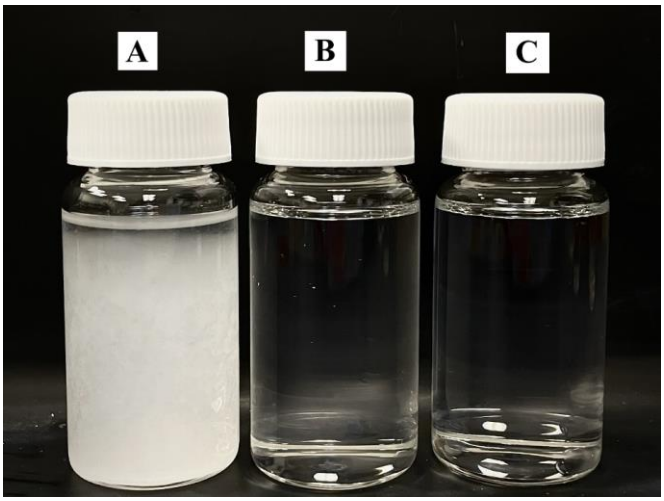

Figure 3-1: Effect of bile acids on the dissolution of PEMA shown by 10 gL^{-1} PEMA in (a) isopropanol, (b) isopropanol with 1 gL^{-1} CA, and (c) 1 gL^{-1} LCA all at room temperature after heating to 55 °C.

At room temperature the PEMA-CA and PEMA-LCA solution were used to coat a 304 stainless steel substrate (thickness ~ 0.1 mm) by the dip coating method. The substrate was cut to 2.5 cm x 5 cm, cleaned with ethanol, manually lowered into the solution for 20 seconds, and left to dry vertically. In order to increase coating thickness, this dipping procedure was repeated after the substrate had dried. To further assess the accumulation of coating on a substrate after repeating the deposition process, the mass normalized by area was recorded for a substrate containing 1 to 5 layers of PEMA. After the successful creation of PEMA coatings, HAP, SiO_2 and TiO_2 were individually added to a PEMA-CA solution at a concentration of 3 gL^{-1}. To facilitate their dispersion, the solutions were stirred and heated to 50 °C, followed by treatment in an ultrasonication bath for 10 minutes. Using the same deposition method as previously described, organic-inorganic composite coatings of PEMA-HAP, PEMA-SiO_2 and PEMA-TiO_2 were fabricated. Deposition was also performed from PEMA solutions, containing heparin and tetracycline.

All produced coatings were characterized by scanning electron microscopy (SEM, JEOL 7000F). The coating microstructure was assessed as deposited, and after being subjected to a heat treatment at 180 °C for one hour. The adhesion of PEMA coatings annealed at 180°C was tested according to ASTM standard D3359-17. Additionally, electrochemical testing was performed on the heat-treated PEMA-CA coating by a PARSTAT 2273 potentiostat. Testing was performed in a NaCl (3 %) solution, with a 3-electrode cell consisting of the coated stainless steel substrate, a saturated calomel electrode (SCE) as reference, and a Pt counter electrode. A scan rate of 1 mV s^{-1} was used for potentiodynamic polarization tests. A Bruker Smart 6000 X-ray diffractometer (XRD, CuKα radiation) was utilized for the analysis of coating composition. Fourier Transform Infrared Spectroscopy (FTIR) studies were performed using a Bruker Vertex 70 spectrometer.

3.4 Results and Discussions

In this investigation a novel method was developed for the solubilization of high molecular mass PEMA in isopropanol, a known non-solvent for the polymer. Sufficiently high concentrations of high molecular mass PEMA were achieved that allowed the facile deposition technique known as dip coating to be performed. Low molecular weight polymers have poor film forming abilities. The concentration of the solution influences the thickness and uniformity of the coatings, such that a low concentrated polymer solution produces thin and uneven coatings. Therefore, the use of concentrated solutions of high molecular mass PEMA was critical for the successful development of the dip coating method. However, the solubilization mechanism is not well understood. Figure 3-2 (a) shows the chemical structure of PEMA. As shown in Figure 3-2 (a), PEMA contains a hydrophobic ethyl group (CH_2CH_3) and a hydrophilic carbonyl group (C=O). Isopropanol can solvate the hydrophobic ethyl group, but isopropanol alone is a non-solvent for PEMA.

BAs have a unique amphiphilic structure that varies from other surfactant molecules. Typical surfactant molecules contain a hydrophilic head group and a hydrophobic hydrocarbon tail. BAs, however, contain a convex face which is hydrophobic due to the presence of methyl groups (CH_3) and a concave face which is hydrophilic due to the presence of hydroxyl groups [27]. The number and positions of hydroxyl groups vary among different Bas [27]. Figure 3-2 (b) shows the chemical structure of CA, which contains three hydroxyl groups. Figure 3-2 (c) shows the chemical structure of LCA comparatively which only has one hydroxyl group. The anionic properties of CA and LCA are related to their COOH groups.

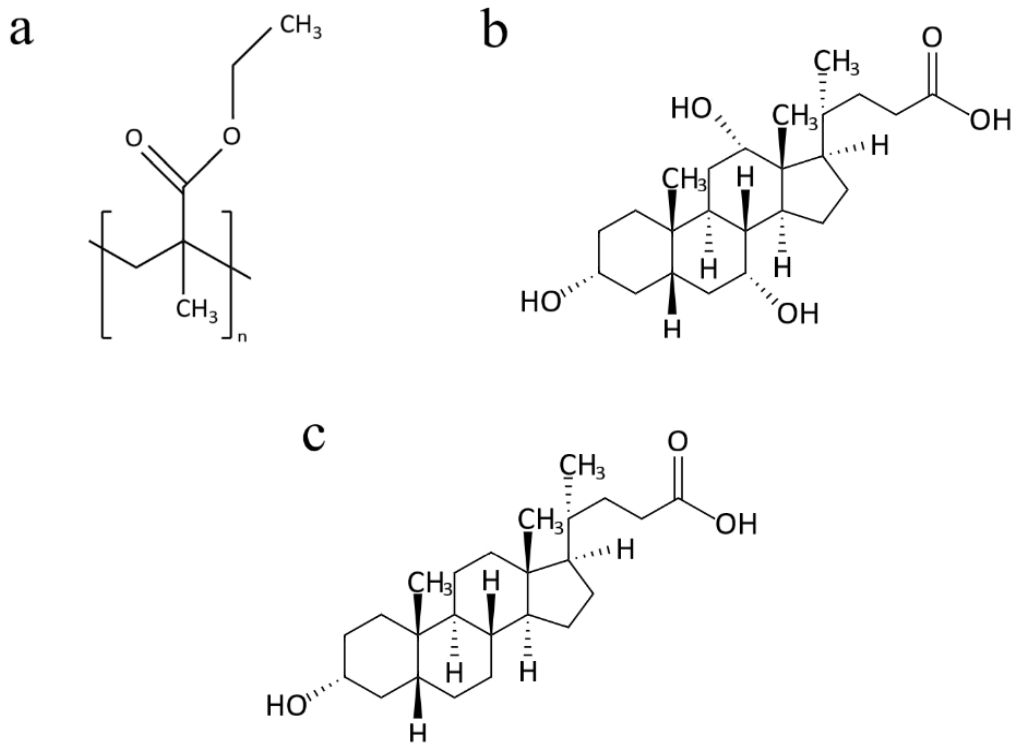

Figure 3-2: Chemical structures of (a) poly(ethyl methacrylate) (PEMA), (b) cholic acid (CA), and (c) lithocholic acid (LCA).

The analysis of literature demonstrated outstanding solubilization power of bile acid salts (BASs) in aqueous solutions [27, 28]. BASs are synthesized in the livers of mammals. They aid in the solubilization of lipids, proteins, vitamins, and other molecules within the body. The cholesterol solubility increased by a factor of 10^6 in the presence of BASs [29]. The impressive solubilization ability of BASs is due to their unique amphiphilic structure. BASs solubilize different organic molecules in water by a mechanism, which involves hydrophobic interactions of the hydrophobic side of the amphiphilic BASs with organic molecules. The hydrophilic side of the BASs molecules, containing OH and charged COO^- groups facilitate solubilization of hydrophobic molecules in the aqueous medium. It is important to note that BASs are highly soluble in water,

whereas BAs, such as CA and LCA, show poor solubility in water, but good solubility in isopropanol.

Previous investigations focused on the solubilization of different materials in water in the presence of BASs [27]. In this investigation, we demonstrate for the first time that BAs can solubilize polymer molecules in organic solvents, such as isopropanol. The comparison of PEMA behavior in isopropanol, with and without BAs, indicates that solubilization of PEMA in isopropanol involved interactions of CA and LCA with PEMA macromolecules. It is hypothesized that such interactions can be based on the hydrophobic interactions of CA and LCA with PEMA. Turning again to the chemical structures of PEMA and BAs (Figure 3-2), we can also suggest another interaction mechanism, involving hydrogen bonding of OH groups of the BAs and the carbonyl group of PEMA. It is known that such a mechanism can result in the formation of polymer-surfactant complexes in solutions [30]. However, CA and LCA molecules, containing different numbers of OH groups showed a similar effect on the PEMA solubilization. Moreover, deposition from PEMA solutions, containing CA and LCA showed comparable deposition yields (Figure 3-3). As shown in Figure 3-3, the coating mass can be steadily increased with each new layer deposited. This exemplified the advantages of using dip coating for the deposition of PEMA coatings. Not only is this method simple, but it also allows for control of the mass and therefore the thickness of the coating as desired for many applications. It will be demonstrated below that dip coating can be used for the fabrication of PEMA composites, containing bioceramics.

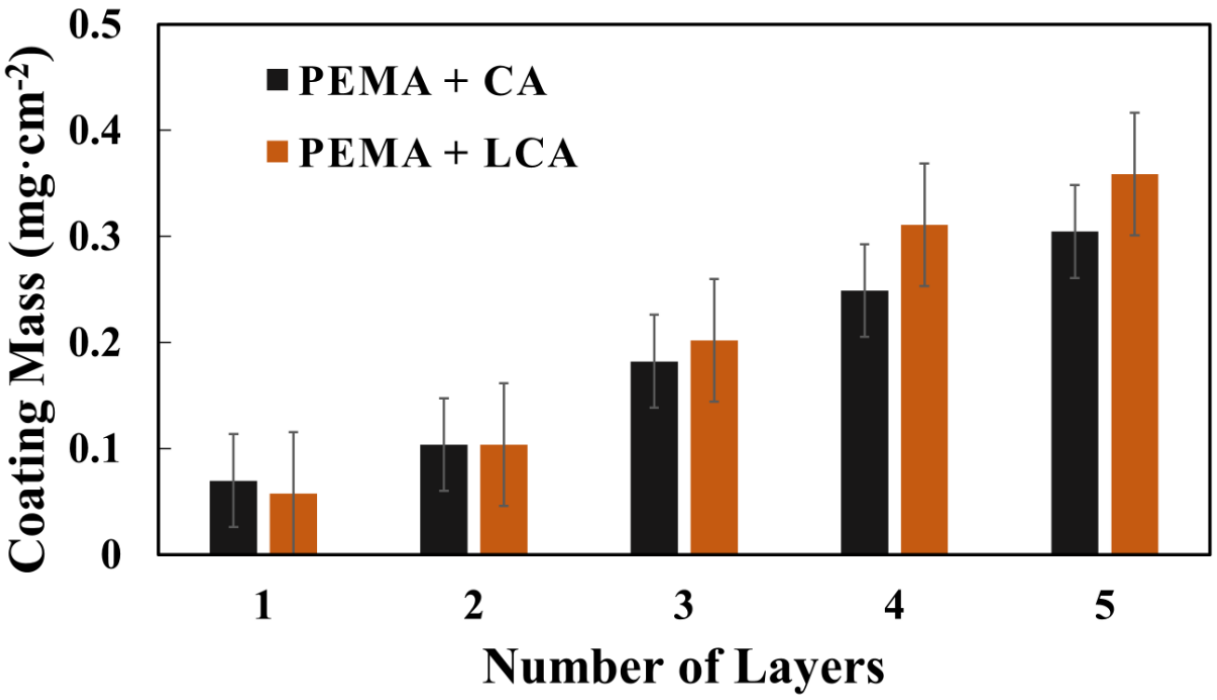

Figure 3-3: Mass of the coatings prepared from 10 gL^{-1} PEMA solutions with 1 gL^{-1} CA or 1 gL^{-1} LCA after depositing one to five layers.

Previous studies [27] revealed the beneficial effect of OH groups of BASs on their adsorption on inorganic particles in water and particle dispersion. In this investigation it was also found that suspensions of HAP, silica, and titania in isopropanol prepared using CA as a dispersant showed enhanced stability, compared to the suspensions of the same materials prepared using LCA. Therefore, the larger number of OH groups in the CA structure was beneficial for its adsorption on the inorganic particles. Therefore, further investigation was focused on the fabrication of PEMA and composite coatings using CA as a solubilizing and dispersing agent.

The as-deposited PEMA prepared by a single dipping procedure contained a porous polymer network on the top of a relatively dense bottom layer (Figure 3-4A). The annealing of such coating at 180°C resulted in formation of relatively dense layers (Figure 3-4B). The adhesion of the annealed coatings tested according to ASTM standard D3359-17 corresponded to 5B

classification. The formation of relatively dense layers was also confirmed by the results of potentiodynamic studies in the 3% NaCl solutions, which indicated that such layers acted as a barrier preventing electrolyte access to the substrate surface and providing corrosion protection of the substrates.

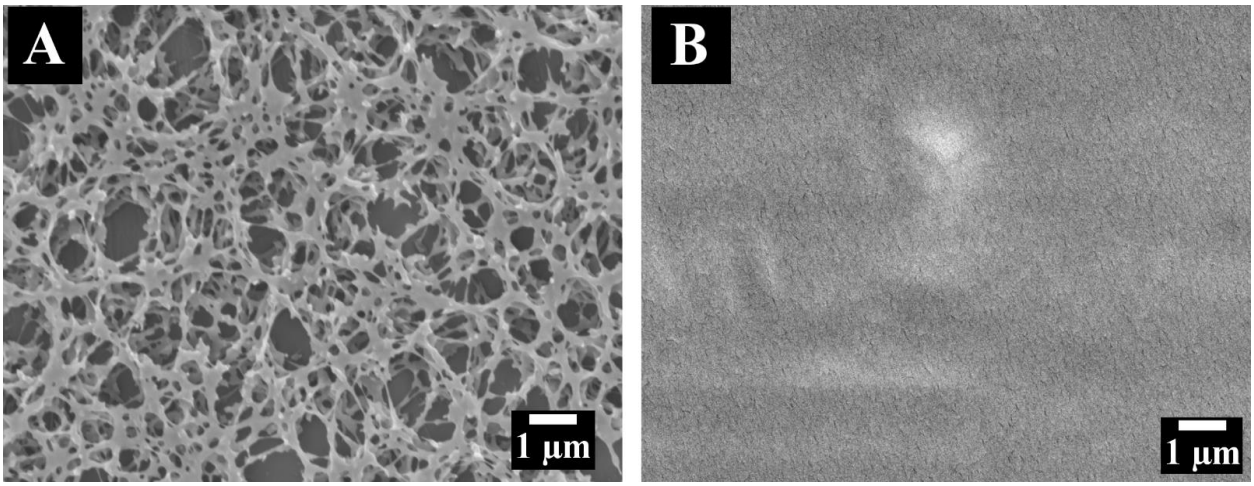

Figure 3-4: SEM micrographs for coatings prepared from 10 gL^{-1} PEMA with 1 gL^{-1} CA: (A) as-deposited and (B) after heat treatment at 180°C for one hour.

Figure 3-5 provides Tafel plots of the uncoated stainless steel and annealed PEMA- coated stainless steel. A significant increase in corrosion potential and decrease in anodic current can be observed for the coated sample compared to the uncoated stainless steel. The corrosion currents for coated and uncoated substrates were 0.053 and 2.63 μA cm^{-2}, respectively. As previously mentioned in the Introduction, PEMA has long been used for corrosion protection of steels. Now with the use of non-toxic solvents and bio-surfactants, these films are promising for use in many biomedical applications where reduced corrosion rates are extremely sought after.

The ability to successfully create PEMA coatings with BAs as a solubilizing agent opened the possibility of creating composite films. CA was further employed as a dispersing agent for titania nanoparticles, HAP nanorods and silica microspheres. Again, the additives must be well dispersed and stable to produce uniform coatings by dip coating. The use of these materials is

advantageous for creating coatings for biomedical applications. Silica and titania increase the biocompatibility of the coatings, have notable bone-forming properties (osteogenesis) and can inhibit inflammatory responses [31-34]. Next, 70 % of natural bone is made of nanostructured HAP. Synthetic HAP is therefore very bioactive and excellent in promoting new bone growth, popular for use in dental and orthopedic implants. Overall, organic-inorganic composite coatings, specifically a polymeric matrix with nano- or micro-sized constituents are extremely desirable for biomedical applications.

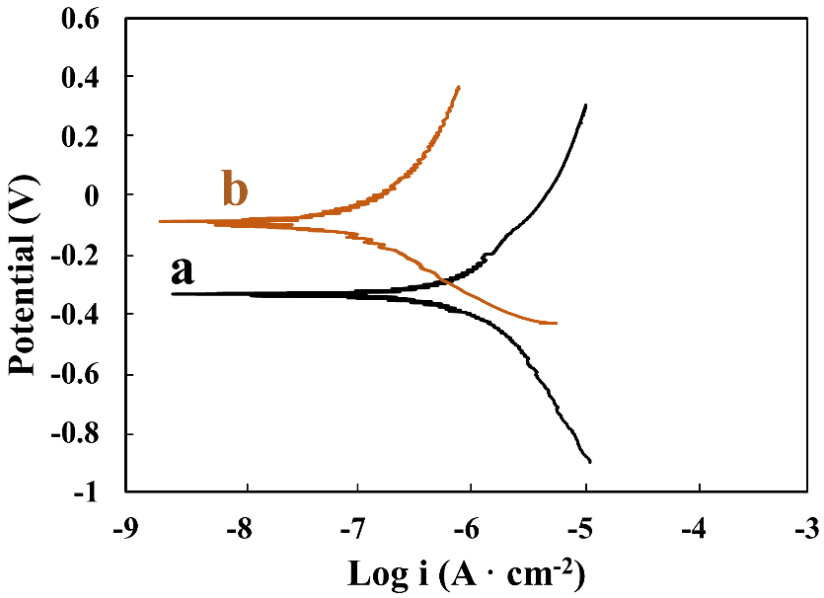

Figure 3-5: Tafel plots for (a) uncoated stainless steel and (b) coated stainless steel sample prepared from a 10 gL^{-1} PEMA solution containing 1 gL^{-1} CA in isopropanol and heat treated at 180°C for one hour.

Figures 3-6 (A, B) show as-deposited coatings where the silica particles are located within the porous network of PEMA. After annealing (Figure 3-6C, D), the PEMA has melted to form a dense layer around the silica spheres.

Figures 3-7 (A, B) provide the SEM images of the HA nanorods dispersed within a PEMA coating. The nanorods are located on top of the as-deposited PEMA and within the pores, likely

due to their small size. Once the film was annealed the HA nanorods were embedded in the relatively dense PEMA layer, seen in Figure 3-7 (C, D). As-deposited PEMA-TiO_2 films showed a porous surface layer (Figure 3-8A, B), containing TiO_2 nanoparticles. Annealing facilitated the formation of relatively dense films, containing TiO_2 nanoparticles (Figure 3-8C, D). The analysis of all SEM images has shown the successful ability of CA to both solubilize PEMA in isopropanol, as well as aid in the dispersion and co-deposition of inorganic, biocompatible materials HA, SiO_2 and TiO_2. PEMA and PEMA-composite coatings were easily created using dip coating due to the high concentration and high molecular mass of PEMA. The biomimetic strategy based on the use of CA as a natural solubilizing and dispersing agent, is a versatile strategy, which allows incorporation of bioceramics for possible applications for surface modification of biomedical implants.

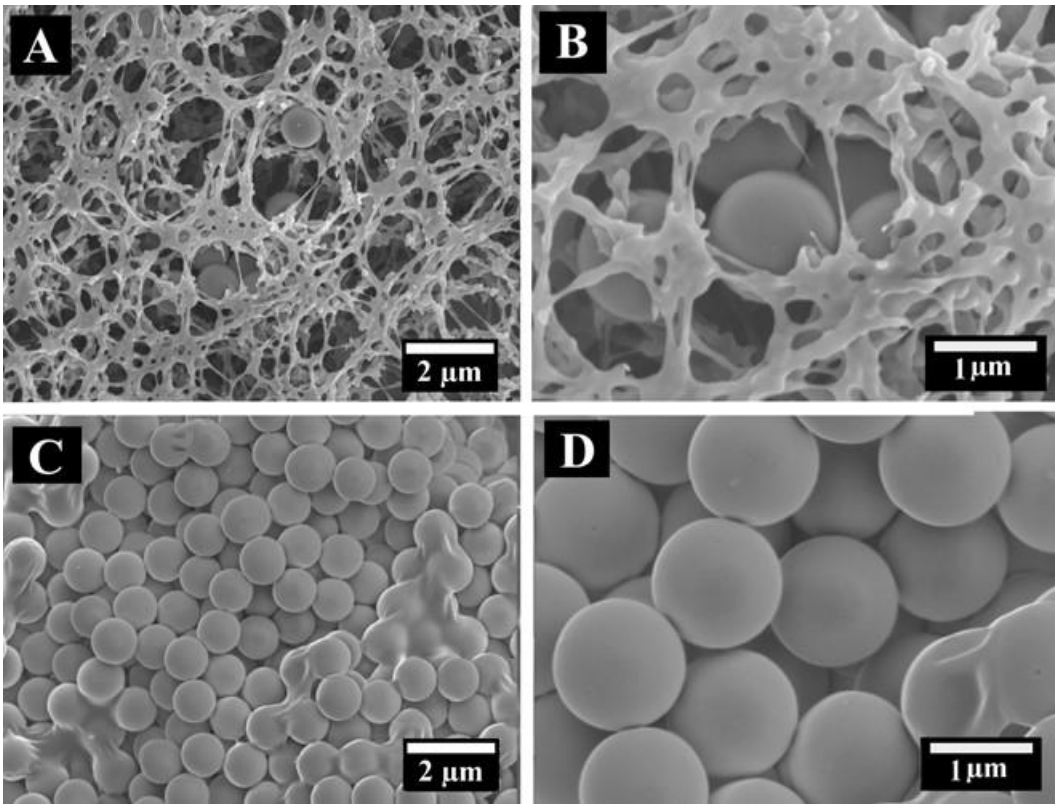

Figure 3-6: SEM micrographs at different magnifications of coatings deposited from 3 gL^{-1} SiO_2 suspensions in solutions of 10 gL^{-1} PEMA with 1 gL^{-1} CA: (A, B) as-deposited (C, D) after heat treatment at 180 °C for one hour.

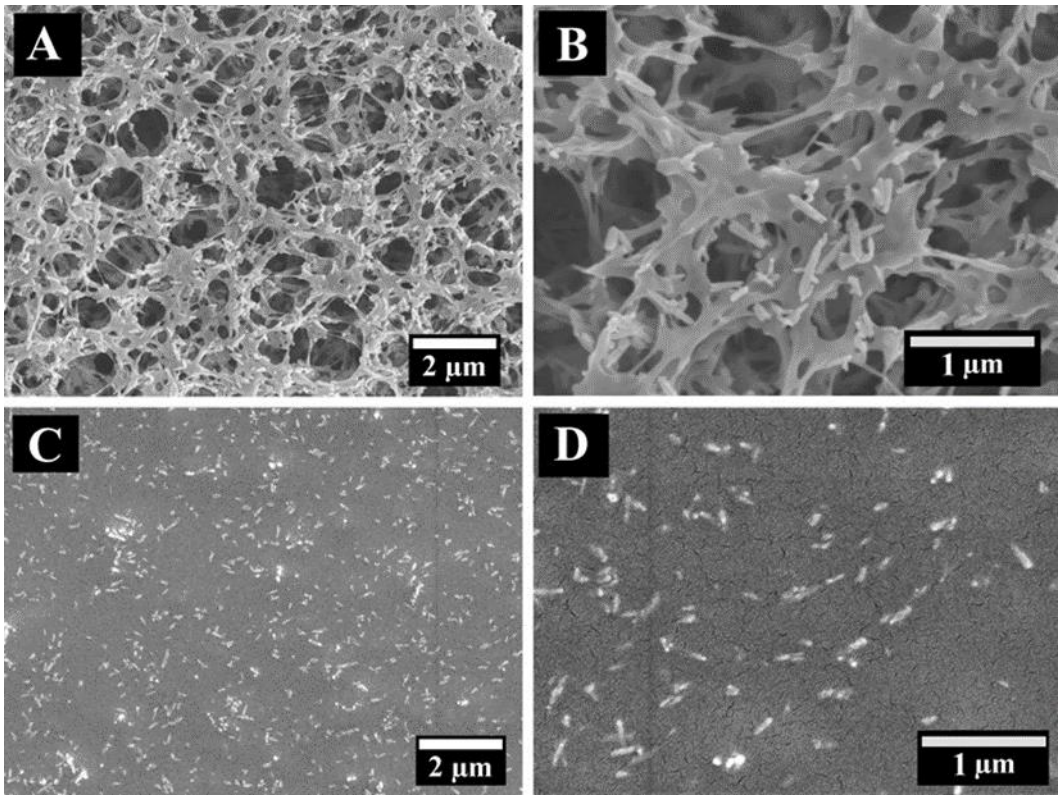

Figure 3-7: SEM micrographs at different magnifications of coatings deposited from 3 gL^{-1} HA suspensions in solutions of 10 gL^{-1} PEMA with 1 gL^{-1} CA: (A, B) as-deposited and (C, D) annealed at 180 °C for one hour.

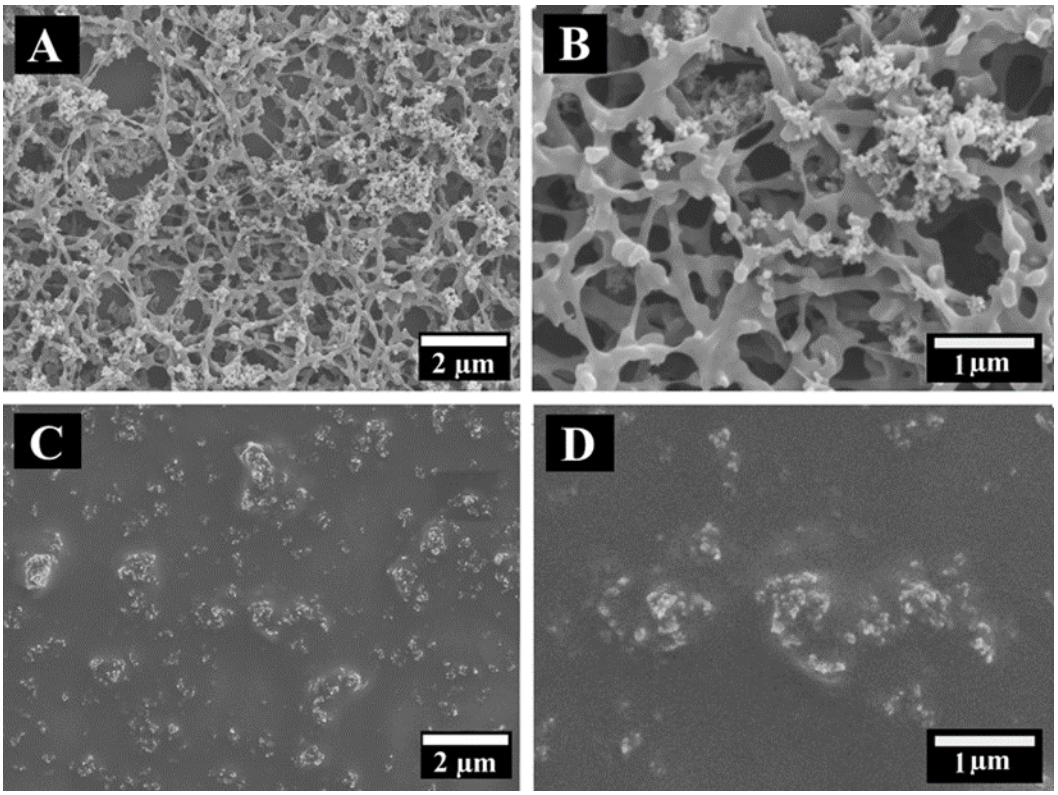

Figure 3-8: SEM micrographs at different magnifications of coatings deposited from 3 gL^{-1} TiO2 suspension in 10 gL^{-1} PEMA solutions with 1 gL^{-1} CA (A, B): as-deposited and (C, D) annealed at 180 °C for one hour.

The approach developed in this investigation allowed the incorporation of other functional materials into the PEMA coatings for biomedical applications. Heparin is an important material for implant applications due to its thrombin inhibition properties [35]. Tetracycline was used as a model drug for the development of PEMA coatings for drug delivery. Tetracycline and heparin are well soluble in isopropanol. Moreover, isopropanol was used [36] as an additive to prevent heparin precipitation in ethanol. Figure 3-9 shows morphology of PEMA-heparin and PEMA-tetracycline coatings. Similar to pure PEMA coatings, the composite coatings contained a porous surface layer.

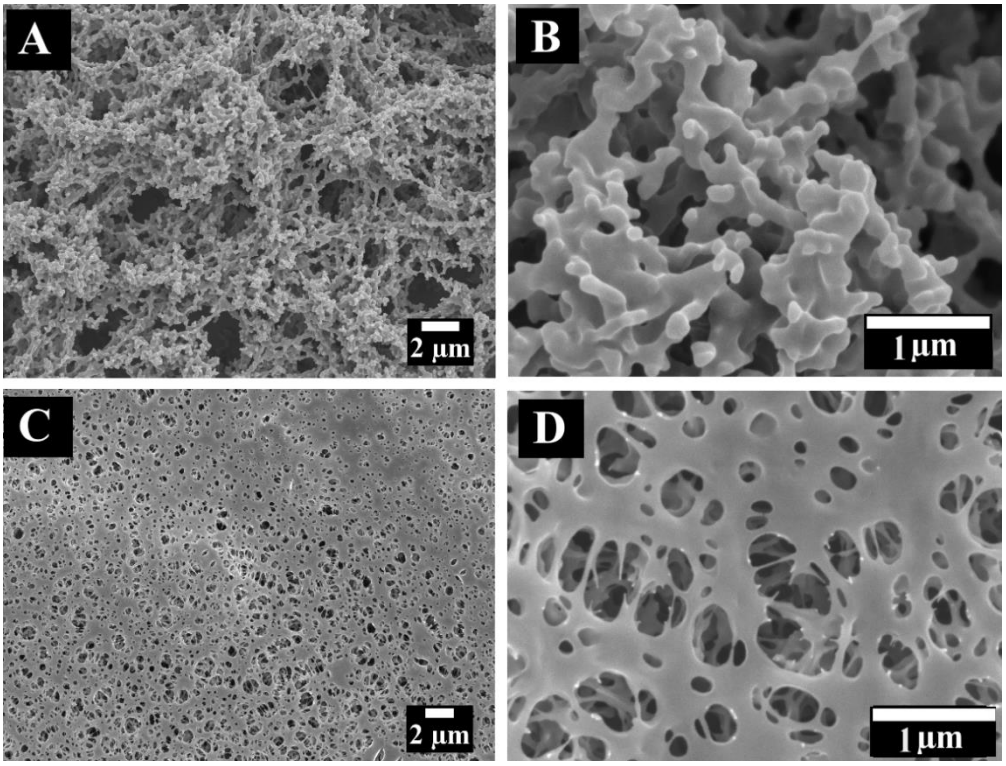

Figure 3-9: SEM micrographs of coatings deposited from10 gL^{-1} PEMA with 1 gL^{-1} CA solutions, containing (A, B) 3 gL^{-1} heparin and (C, D) 3 gL^{-1} tetracycline.

PEMA-heparin coating showed a fibrous porous surface layer, whereas PEMA-tetracycline showed reduced porosity. The incorporation of heparin into the composite coating was confirmed by FTIR analysis (Figure 3-10). The FTIR spectrum of PEMA showed absorptions at 1720, 1140 and 1024 cm^{-1} related to C═O stretching, C-O-C symmetric stretching and C-H bending, respectively [37, 38]. The spectrum of heparin showed peaks at 1602, 1215 and 990 cm^{-1} due to COO^- asymmetric, SO_3^- asymmetric and symmetric stretchings, respectively [39-42]. The deposited material contained peaks of PEMA and heparin, indicating the incorporation of heparin in the PEMA coating.

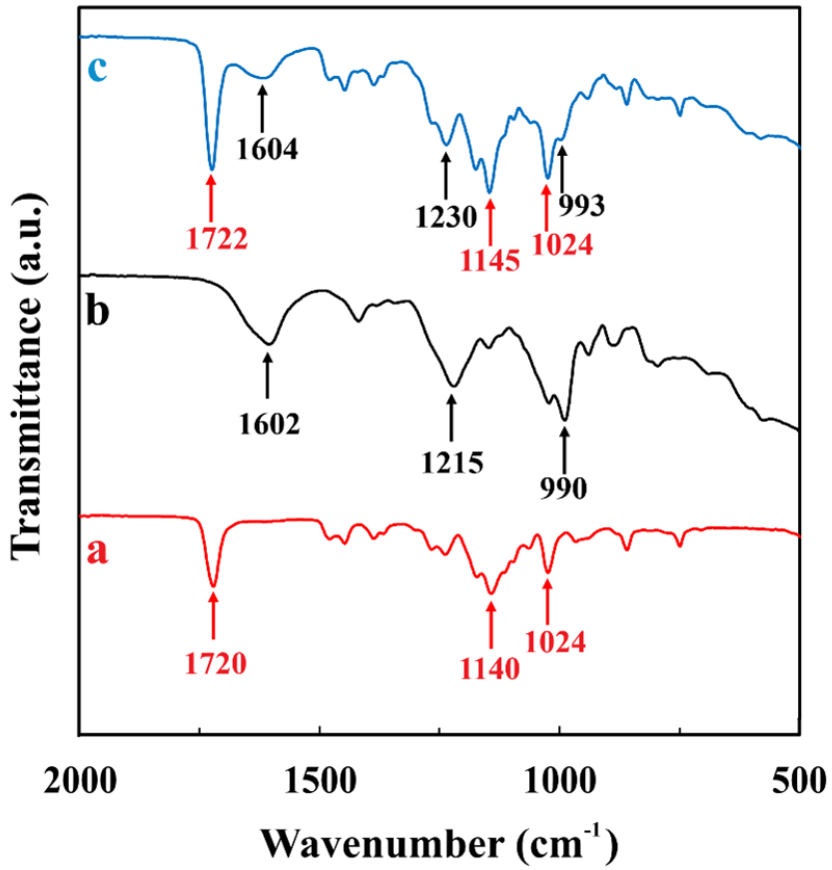

Figure 3-10: FTIR spectra of (a) PEMA, (b) heparin and (c) PEMA-heparin coating.

Tetracycline is a crystalline material (Figure 3-11a) and XRD method was used for the analysis of composite coatings. The X-ray diffraction of the composite showed peaks of tetracycline and confirmed the incorporation of tetracycline into the PEMA coating (Figure 3-11 b). It is expected that other drugs and various functional biomolecules can be co-deposited with PEMA. The ability to deposit multiple layers can potentially be used for the fabrication of graded composition coatings and multilayers, containing different individual functional layers. Such coatings offer benefits of enhanced biocompatibility and improved control of drug release [43].

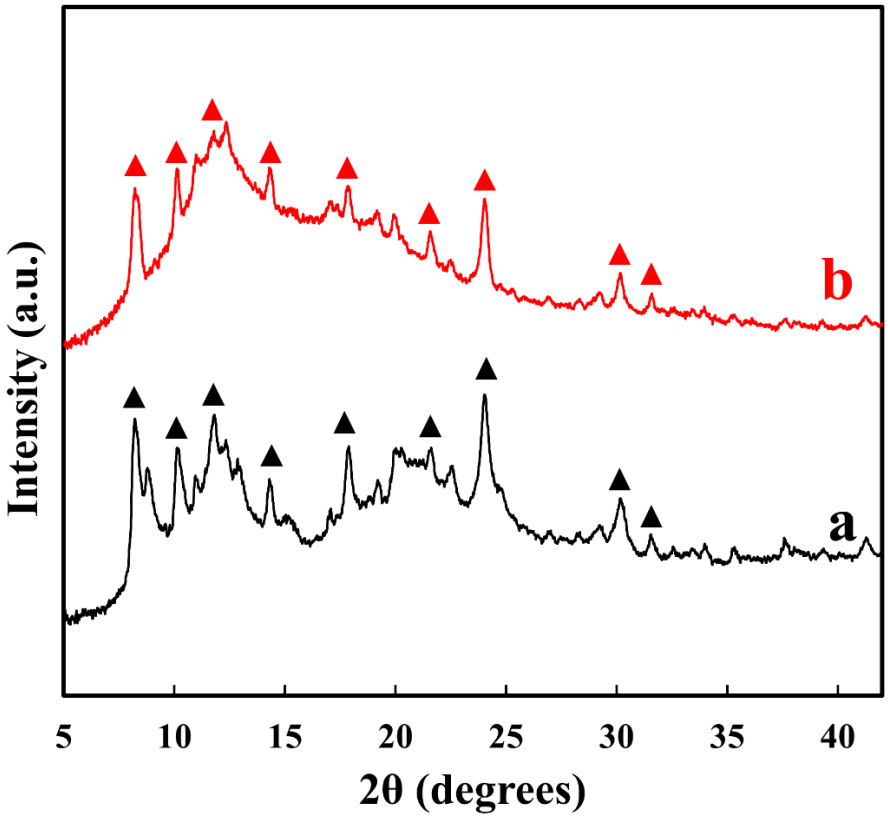

Figure 3-11: X-ray diffraction patterns of (a) as-received tetracycline and (b) PEMA- tetracycline deposit (▲- peaks corresponding to JCPDS file 039-1985).

3.5 Conclusions

The results of this investigation showed the feasibility of dissolution of PEMA in isopropanol using CA and LCA as solubilizing agents. Previous investigations were limited to the use of BASs for solubilization of various biomolecules in aqueous solutions. The use of BAs for solubilization of PEMA in isopropanol opens a new and unexplored route for the processing of polymers and other functional organic materials. The ability to avoid the use of toxic solvents for PEMA dissolution is especially attractive for biomedical applications. The important feature of the proposed approach is the facile solubilization of high molecular mass PEMA in highly concentrated solutions. These are key factors for the fabrication of coatings by a dip coating

method. The coatings can be obtained as monolayers or multilayers of controlled total mass. The analysis of deposition yield data indicated that the solubilization mechanism involved the hydrophobic interactions of BAs and PEMA. It is expected that further exploration of the solubilization power of BAs and related solubilization mechanisms will result in the development of advanced films and coating of other polymers and their composites. Building on the dispersion power of BAs, we demonstrated the successful deposition of composite coatings, containing advanced bioceramics, such as HAP, silica and titania. The proposed method is promising for the fabrication of composites, containing various drugs and other functional materials. As a step in this direction, we demonstrated the feasibility of deposition of PEMA coatings, containing tetracycline and heparin. Of particular importance for the fabrication of coating with advanced functionality is the possibility of fabricating multilayer coatings containing PEMA layers loaded with different functional materials.

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Chapter 4: A Versatile Strategy for the Fabrication of Poly(ethyl methacrylate) Composites

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4.1 Abstract

Poly(ethyl methacrylate) (PEMA) is dissolved in ethanol, known to be a non-solvent for PEMA, due to the solubilizing ability of an added bile acid biosurfactant, lithocholic acid (LCA). The ability to avoid traditional toxic and carcinogenic solvents is important for the fabrication of composites for biomedical applications. The formation of concentrated solutions of high molecular weight PEMA is a key factor for the film deposition using the dip coating method. PEMA films provide corrosion protection for stainless steel. Composite films are prepared, containing bioceramics, such as hydroxyapatite and silica, for biomedical applications. LCA facilitates dispersion of hydroxyapatite and silica in suspensions for film deposition. Ibuprofen and tetracycline are used as model drugs for the fabrication of composite films. PEMA-nanocellulose films are successfully prepared using the dip coating method. The microstructure and composition of the films are investigated. The conceptually new approach developed in this investigation represents a versatile strategy for the fabrication of composites for biomedical and other applications, using natural biosurfactants as solubilizing and dispersing agents.

4.2 Introduction

This investigation was motivated by the advanced functional properties of the polymer poly(ethyl methacrylate) (PEMA), which makes it the material of choice for the fabrication of advanced composites. Of particular importance for composite development are the biocompatibility, high chemical stability, flexibility, advanced mechanical properties, and thermal stability of this polymer [1,2]. PEMA is a low-cost polymer, which has generated significant interest for its corrosion protection of metals [3], packaging [4,5], energy storage in capacitors [6] and batteries [7,8], thermal energy storage [9], and other applications. Significant enhancement of its properties and functionality was achieved by combining PEMA with other functional materials and fabrication of composites [8–10].

PEMA composites are especially promising for various biomedical applications [11]. Investigations focused on the development of PEMA composite biocements with enhanced mechanical properties and biocompatibility [11,12], shape memory composites for biomedical devices [13], composites for bone repair [14,15], and other applications in tissue engineering [14,16]. PEMA has promising properties for dental and orthopedic applications, craniofacial implants, and biosensors. However, PEMA is soluble only in highly carcinogenic and toxic solvents, such as benzene, toluene, and methyl ethyl ketone. This limits PEMA applications in various fields of bioengineering. The ability to use non-toxic solvents for dissolution of PEMA and fabrication of composites by colloidal techniques can provide an avenue for the fabrication of many advanced biomedical applications. Various bioactive ceramics such as hydroxyapatite and silica are promising inorganic materials for the fabrication of organic-inorganic composites [17–19]. There is a growing interest in the application of nanocellulose for the development of advanced composites materials for biomedical applications [20,21]. The interest in various

biomedical applications of nanocellulose is related to the biocompatibility and advanced mechanical properties of this material [22,23]. Polymer-nanocellulose composites have many applications for food packaging [24], barrier coatings [25], and supercapacitors [26]. Moreover, nanocellulose is under investigation for various optical applications [27], protective barrier coatings [28], electrochromic thin film devices [29], and solar cells [30]. Various drug materials were incorporated in polymer composites [31]. The need for composite films in various biomedical applications was a motivating factor for the development of new film deposition techniques [31,32].

The goal of this investigation was to fabricate PEMA and composite films using ethanol as a solvent. We found that PEMA can be dissolved in ethanol in the presence of lithocholic acid as a natural dispersant. This process mimics the solubilization of different biomolecules in the human body in the presence of bile salts, such as lithocholic acid sodium salt. However, in contrast to the aqueous processes in the human body, we demonstrated the solubilization power of water-insoluble lithocholic acid in ethanol. An important finding was the solubilization of high molecular mass PEMA in ethanol and the ability to form concentrated solutions, which facilitated film deposition by a simple dip-coating method. The experimental results presented below indicated that PEMA films provided corrosion protection for stainless steel. Following the goal of this investigation, we fabricated PEMA based composites, containing hydroxyapatite, silica, nanocellulose, and drugs. We analyzed the microstructure, and composition of obtained films. The approach developed in this investigation opens a new avenue for the development of composite films for biomedical applications by colloidal techniques using non-toxic solvents.

4.3 Materials and Methods

PEMA (MW = 515,000 Da), ibuprofen, tetracycline and lithocholic acid (LCA) were purchased from Millipore (Sigma, Oakville, ON, Canada). Hydroxyapatite nanorods (150 nm length, 20 nm diameter) were prepared by the method developed in previous investigations [19,33]. Silica (0.5 μm size) was purchased from PCR Incorporated, (Arcade, NY, USA). Cellulose nanocrystals (CNC) were purchased from CelluForce Inc., (Montreal, QC, Canada).

LCA was dissolved in ethanol at a concentration of 1 gL^{-1}. PEMA was added at a concentration of 10 gL^{-1} to the LCA solution and to pure ethanol without LCA. Upon heating to just 35 °C the PEMA suspension containing LCA turned from an opaque cloudy white to a clear and transparent solution, whereas the PEMA suspension without LCA did not turn clear until heating to 55 °C. This indicated the successful dissolution of PEMA in ethanol facilitated by the small addition of the naturally occurring bile acid. After cooling to room temperature while being continuously stirred, the PEMA solutions containing LCA remained stable and the PEMA solutions without LCA showed precipitation of PEMA (Figure 4-1).

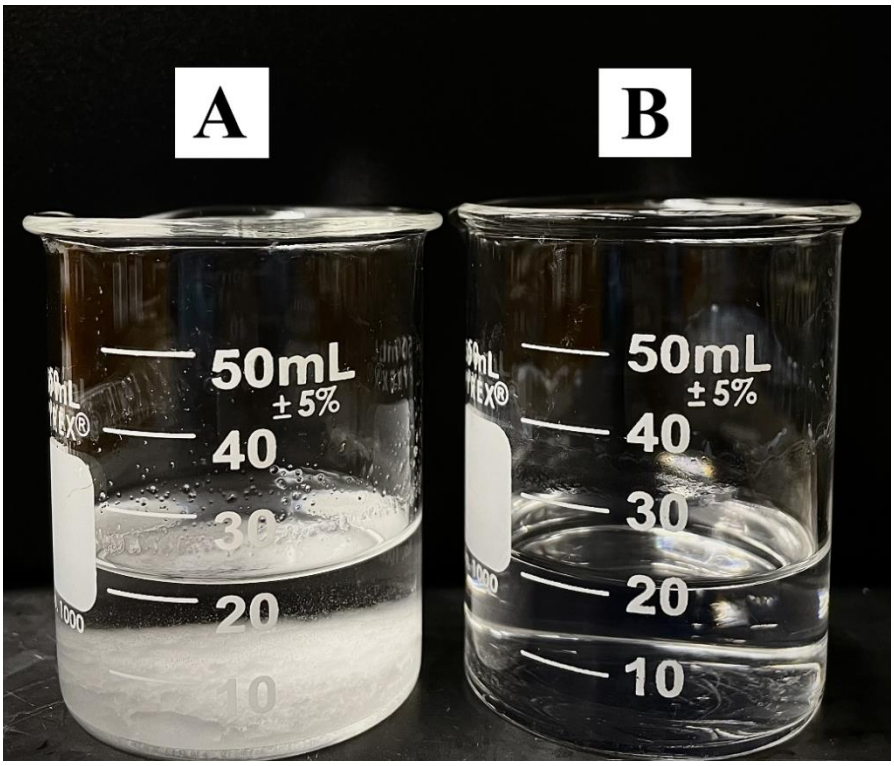

Figure 4-1. Effect of the presence of LCA on the dissolution of PEMA shown by 10 gL^{-1} PEMA in (A) ethanol, (B) ethanol with 1 gL^{-1} LCA.

Substrates of 304 stainless steel (0.1 mm thick, 2.5 cm × 5.0 cm) were coated using the dip coating method from 10 gL^{-1} PEMA solutions in ethanol with 1 gL^{-1} LCA. The samples for electrochemical studies were annealed at 180 °C for one hour. Composite PEMA coatings were subsequently created using the same dip coating procedure with the LCA concentration increased to 3 gL^{-1} to help in its multifunctionality as both a solubilizing agent for PEMA and as a dispersing agent for other components. Bioceramics hydroxyapatite and silica were successfully dispersed at concentrations of 3–10 gL^{-1}.

Composite coatings were also prepared containing drugs, such as tetracycline and ibuprofen, as well as with the biologically functional material nanocellulose crystals. Concentrations of tetracycline, ibuprofen, and nanocellulose were in the range of 3–10 gL^{-1} in the 10 gL^{-1} PEMA solution in ethanol containing 3 gL^{-1} LCA.

Fourier Transform Infrared Spectroscopy (FTIR) studies were performed using a Bruker Vertex 70 spectrometer. A Bruker Smart 6000 X-ray diffractometer (CuKα radiation) was used for X-ray diffraction analysis. All fabricated coatings were characterized by scanning electron microscopy (SEM, JEOL 7000F, Tokyo, Japan). Electrochemical testing was performed by a PARSTAT 2273 potentiostat in a 3% NaCl solution using a 3-electrode cell composed of the coated or uncoated stainless-steel substrate as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a Pt counter electrode. Potentiodynamic polarization tests were performed at a scan rate of 1 mVs^{-1}. The deposits were removed from the stainless-steel substrate for thermogravimetric analysis (TGA), which was performed using a thermoanalyzer (Netzsch STA-409, Exton, PA, USA), operated in air at a heating rate of 10 °C min^{-1}.

4.4 Results and Discussions

The successful application of the dip coating method for the polymer film deposition depends largely on the ability to achieve high polymer concentration in solutions. Another important factor is the molecular weight of a polymer. The use of a high molecular weight polymer is critically important for the film deposition. However, the polymer solubility in a solvent usually decreases with increasing molecular mass of polymer macromolecules due to enhanced interactions of long polymer chains. Ethanol is known as a non-solvent for PEMA. Despite the insolubility of PEMA in ethanol solvent, we dissolved PEMA in ethanol in the presence of LCA as a solubilizing agent. Therefore, the use of toxic and carcinogenic solvents for PEMA deposition can be avoided. Moreover, we achieved solutions of relatively high concentrations using high molecular mass PEMA, which was a key factor of film deposition by a dip-coating method. The use of LCA was motivated by the analysis of literature [34,35] on solubilization of different

biomolecules in a human body by bile salts. The remarkable solubilization power of bile salts in water are related to their electric charge and amphiphilic structure. It should be noted that in contrast to bile salts, which are highly soluble in water, LCA and other bile acids are water insoluble. Bile salts solubilize small lipid molecules, such as cholesterol and fatty acids in water. In contrast we solubilized large PEMA polymer macromolecules in ethanol using bile acid LCA.

The dissolution of polymers in pure solvents usually involves solvent diffusion and polymer chain disentanglement [36]. It is suggested that small LCA molecules in ethanol penetrated between PEMA chains and adsorbed on PEMA. The adsorption mechanism involved hydrophobic interactions of PEMA and LCA. The increase in the solvent temperature to 55 °C facilitated LCA diffusion. The adsorbed charged LCA molecules provided electrostatic repulsion of the PEMA chains and promoted dissolution. After cooling down to room temperature, LCA remained adsorbed on PEMA chains and allowed for the fabrication of stable solutions for the dip coating (Supplementary information, Figure 4-S1).

Figure 4-2A shows an SEM image of a PEMA film. It contains a relatively dense bottom layer and a porous fibrous network on the surface. Annealing resulted in the formation of dense smooth films (Figure 4-2B). The morphological changes in the films during annealing can be attributed to the enhanced mobility of the polymer macromolecules with increased temperature, which can result in the merging of individual particles, reduction or elimination of porosity and formation of dense films [37,38]. The formation of dense films after annealing was critical for their corrosion protection ability. The dense layer acted as a physical barrier, thus preventing electrolyte contact with the stainless-steel substrate. The corrosion protection abilities were verified by potentiodynamic polarization studies in a 3% NaCl solution (Figure 4-3).

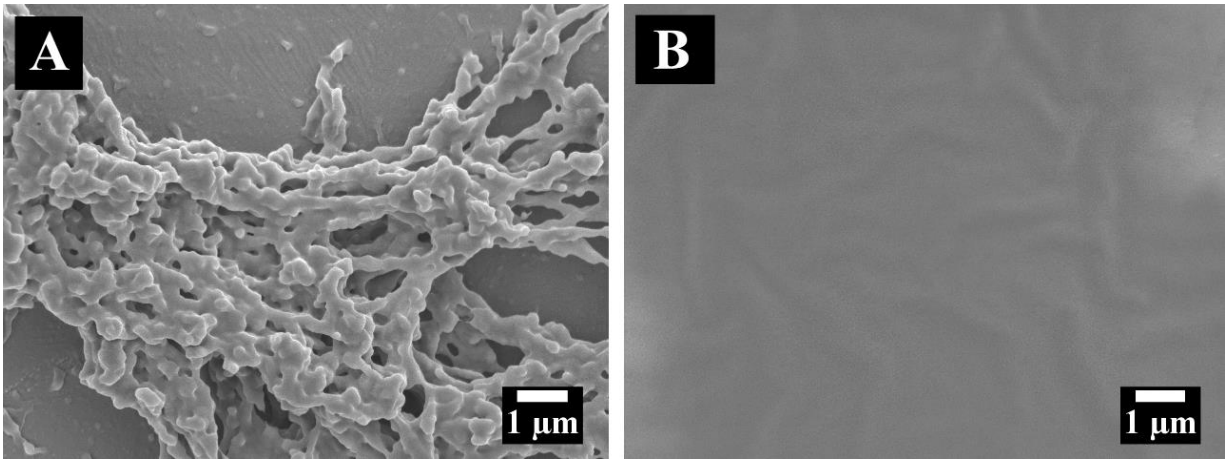

Figure 4-2: SEM micrographs for a film, prepared from 10 gL^{-1} PEMA with 1 gL^{-1} LCA solution: (A) as-deposited and (B) after annealing at 180 °C for one hour.

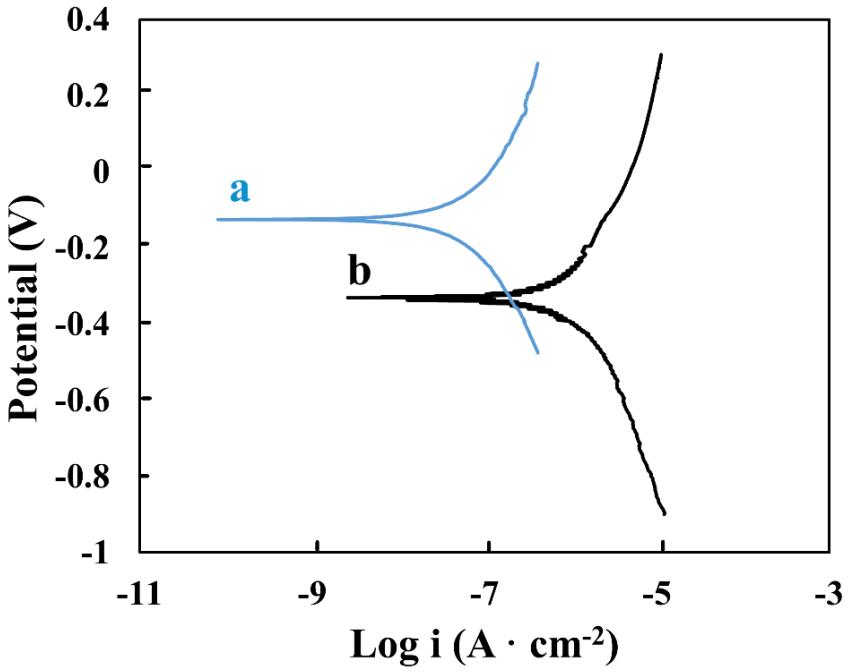

Figure 4-3. Tafel plots for (a) uncoated and (b) coated stainless steel sample prepared from a 10 gL^{-1} PEMA solution containing 1 gL^{-1} LCA in ethanol and annealed at 180 °C for one hour.

The generated Tafel plot (Figure 4-3) compares the uncoated stainless-steel substrate to the substrate containing the annealed PEMA film. A substantial increase in corrosion potential can be

observed for the coated sample relative to the bare substrate, as well as a decrease in anodic current, indicating increased corrosion resistance. In addition to the use of non-toxic and biocompatible solvents and solubilizing agents, the corrosion protection ability of the fabricated film further enhances their potential use in biomedical applications.

The addition of inorganic bioactive materials, such as hydroxyapatite and silica, to polymer films is widely used for the surface modification of biomedical implants [39,40]. These two materials have specific advantageous properties for orthopedic applications. Hydroxyapatite is notably bioactive. Nanostructured hydroxyapatite makes up 70% of natural bone, thus its presence in coatings can contribute to promoting new bone growth.

Silica is biocompatible and its presence in coatings for biomedical applications can help to inhibit inflammatory responses. It can also aid in the formation of new bone material, just as hydroxyapatite [41].

Composite PEMA-hydroxyapatite and PEMA-silica films were prepared by a dip coating method (Supplementary information, Figure 4-S1). In this approach, LCA acted as a solubilizing agent for PEMA and as a dispersant for the ceramic particles, which facilitated the fabrication of stable suspensions of the particles in the PEMA solutions. Figure 4-4 shows SEM images for PEMA-hydroxyapatite films.

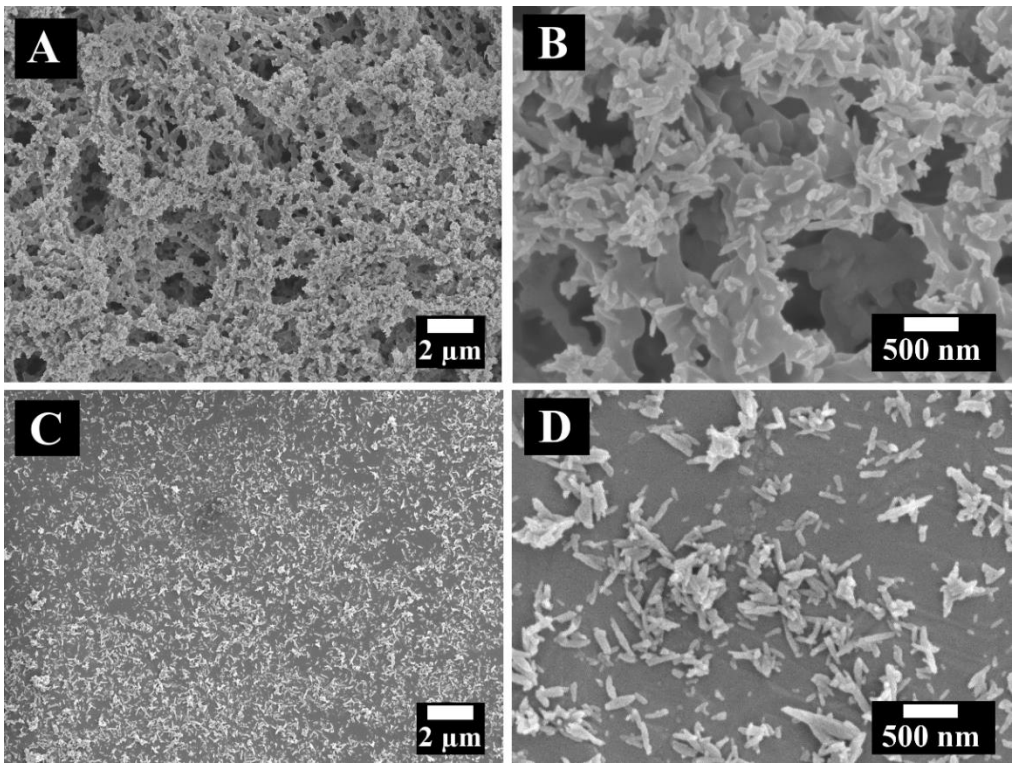

Figure 4-4: SEM micrographs at different magnifications for films prepared from 10 gL^{-1} PEMA solutions in ethanol, containing 3 gL^{-1} LCA and 3 gL^{-1} hydroxyapatite (A, B) as deposited (C, D) annealed at 180 °C for one hour.

The SEM images for as-deposited films showed a porous microstructure; film annealing resulted in the formation of dense layers. High magnification images showed non-agglomerated hydroxyapatite nanorods in the PEMA matrix. Figure 4-5 shows SEM images of PEMA-silica films.

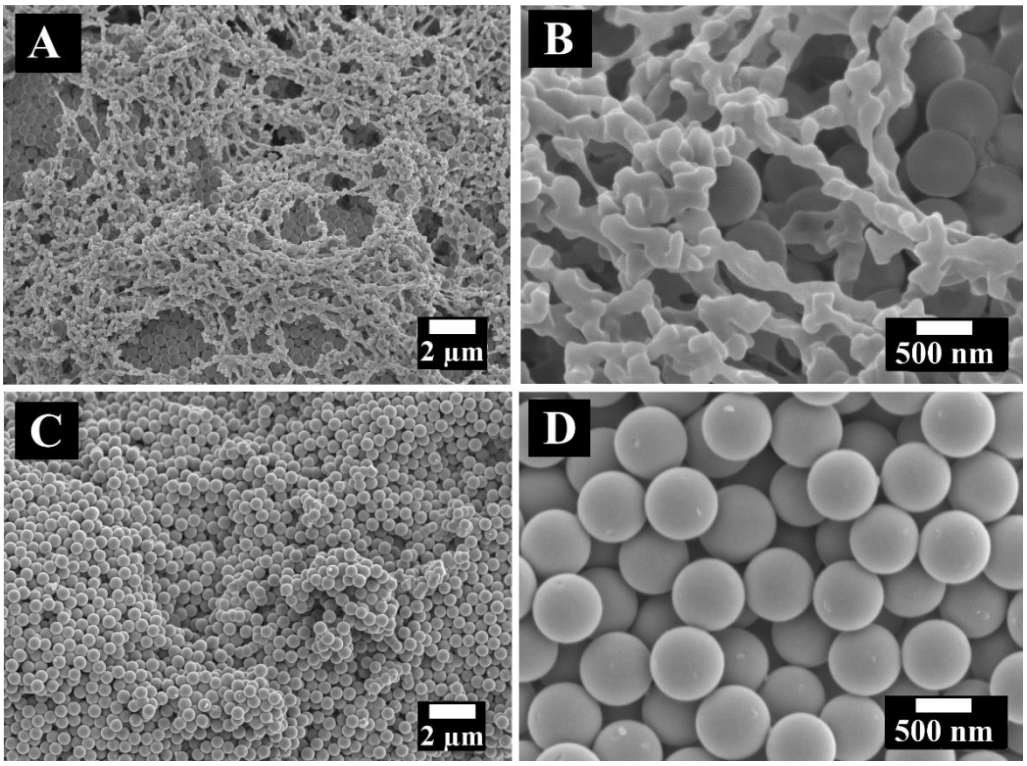

Figure 4-5: SEM micrographs at different magnifications for films prepared from 10 gL^{-1} PEMA solutions in ethanol, containing 3 gL^{-1} LCA and 3 gL^{-1} silica: (A, B) as deposited (C, D) annealed at 180 °C for one hour.

The SEM images of as-deposited PEMA-silica films show a porous microstructure. Annealed films showed spherical silica particles in the PEMA matrix. The silica particles were relatively densely packed and PEMA was distributed between the particles.

Concentrations of the bioceramics were able to be increased from 3 up to 10 gL^{-1} due to the excellent dispersing ability of LCA. In order to quantifiably confirm the increased amount of inorganics within the films, TGA studies were carried out (Figure 4-6). The PEMA-hydroxyapatite and PEMA-silica deposits were analyzed by TGA. Figure 4-6 shows TGA data for films prepared from suspensions with different concentrations of ceramic particles. The observed mass loss was related to the burning out of PEMA and LCA. The total mass loss for PEMA-hydroxyapatite films prepared from 10 gL^{-1} PEMA solutions with 3 gL^{-1} LCA, containing 3 gL^{-1} and 10 gL^{-1}

hydroxyapatite was found to be 82.4 % and 56.4 %, which corresponded to hydroxyapatite content in the composite films of 17.6 %, and 43.6 %, respectively. The total mass loss for PEMA-silica films prepared from 10 gL^{-1} PEMA solutions with 3 gL^{-1} LCA, containing 3 gL^{-1} and 10 gL^{-1} silica was found to be 74.2 % and 50.3 %, which corresponded to silica content in the composite films of 25.8 %, and 49.7 %, respectively. TGA testing results indicated that hydroxyapatite and silica content in the composite films increased with increasing particle concentration in the suspensions and the film composition can be varied.

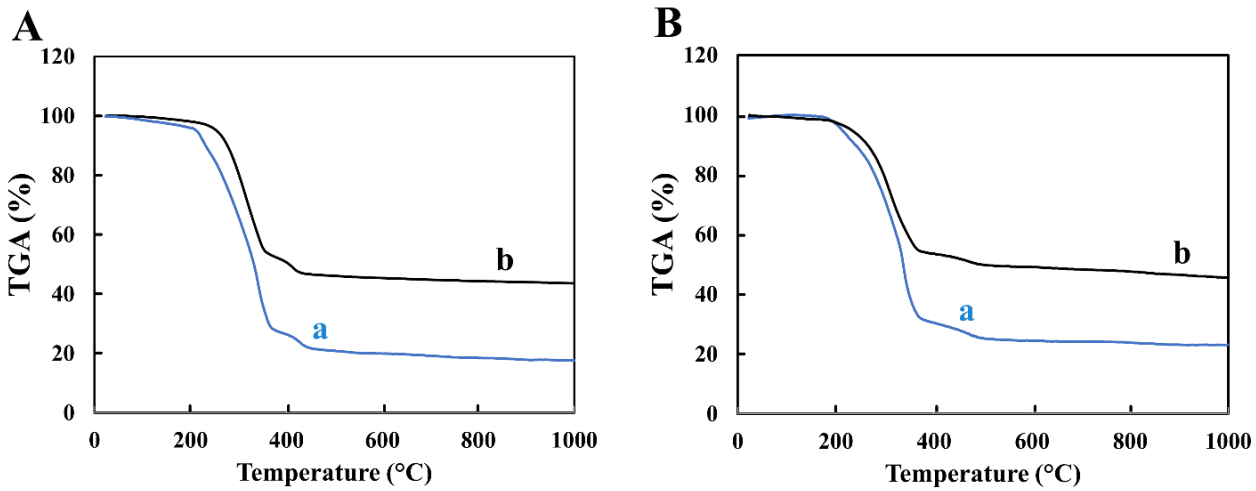

Figure 4-6: TGA data for composites prepared from 10 gL^{-1} PEMA and 3 gL^{-1} LCA solutions in ethanol, containing (A) (a) 3 and (b) 10 gL^{-1} hydroxyapatite, and (B) (a) 3 and (b) 10 gL^{-1} silica.

In this work composite films were obtained from PEMA solutions in ethanol that also contained biologically functional materials. These materials included drugs such as the antibiotic tetracycline and anti-inflammatory ibuprofen, as well as the quickly emerging biomaterial nanocellulose. In this investigation, ibuprofen and tetracycline were used as model drugs for the fabrication of composite films. The analysis of SEM images of as-deposited films at different magnification indicated that such films were porous (Figure 4-7). The SEM images of as-deposited

PEMA-nanocellulose films showed a similar microstructure (Figure 4-8). The composite coatings were studied by XRD and FTIR methods.

In the FTIR spectra of all the starting materials (Figure 4-9A), important characteristic peaks are labelled, and then seen again in the FTIR spectra from deposits of the composite coatings (Figure 4-9B). Ibuprofen's FTIR spectrum has peaks at 779, 933, 1230, 1420 and 1706 cm^{-1} due to CH_2 rocking, CH_3 rocking, C-C stretching, and CH-CO deformation and C=O stretching, respectively [42]. The spectrum of tetracycline has peaks at 1024, 1143, 1236 cm^{-1} related to C-H in plane deformation and 1448 cm^{-1} due to C-H bending [43]. The spectrum of nanocellulose showed absorptions at 1053, 1107 and 1161 cm^{-1}, attributed to the C-O-C pyranose ring vibrations [44,45]. The FTIR spectrum of pure PEMA shows absorptions at 1022, 1140 and 1720 cm^{-1} related to C-H bending, C-O-C symmetric stretching and C=O stretching, respectively [46,47]. The deposited material of all three composite films contained peaks characteristic of PEMA and the specific added functional materials.

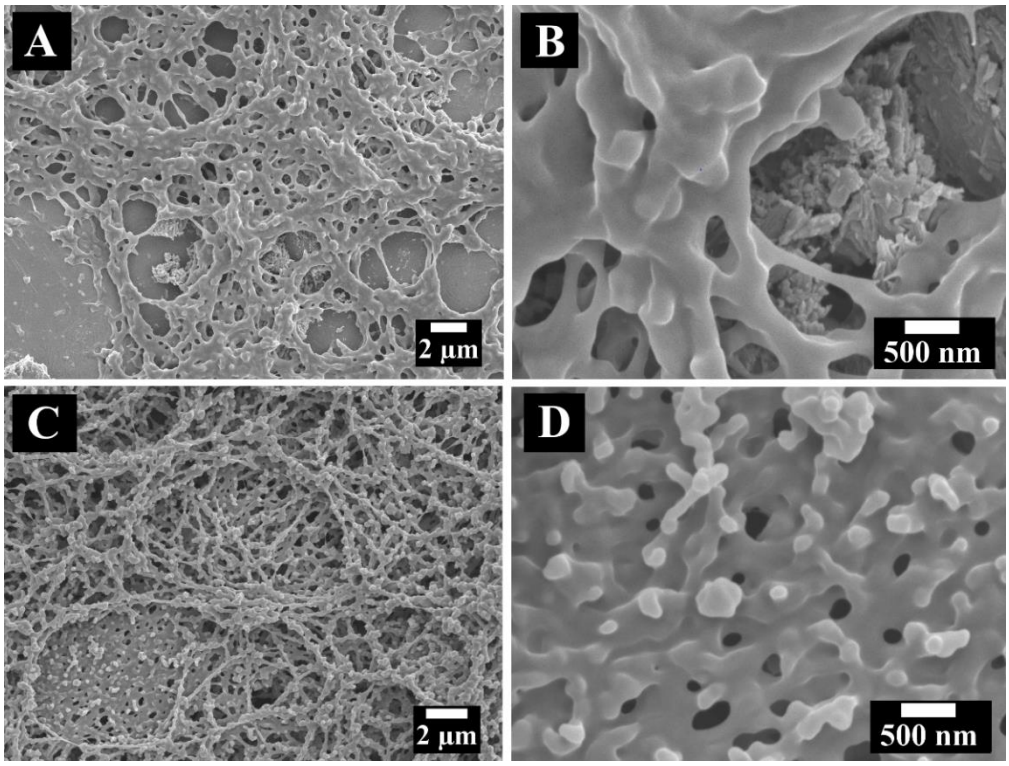

Figure 4-7: SEM micrographs of films at different magnifications for 10 gL^{-1} PEMA solutions, with 3 gL^{-1} LCA and containing (A, B) 3 gL^{-1} ibuprofen and (C, D) 3 gL^{-1} tetracycline.

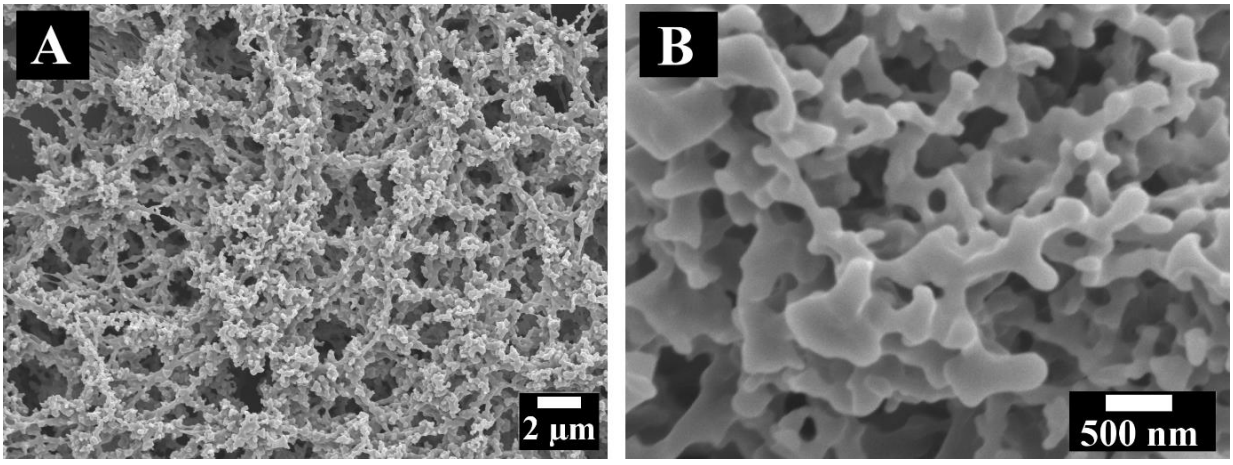

Figure 4-8: SEM micrographs at different magnifications of films, prepared from 10 gL^{-1} PEMA with 3 gL^{-1} LCA and 3 gL^{-1} nanocellulose. ((A) 5000 X), ((B) 30,000 X).

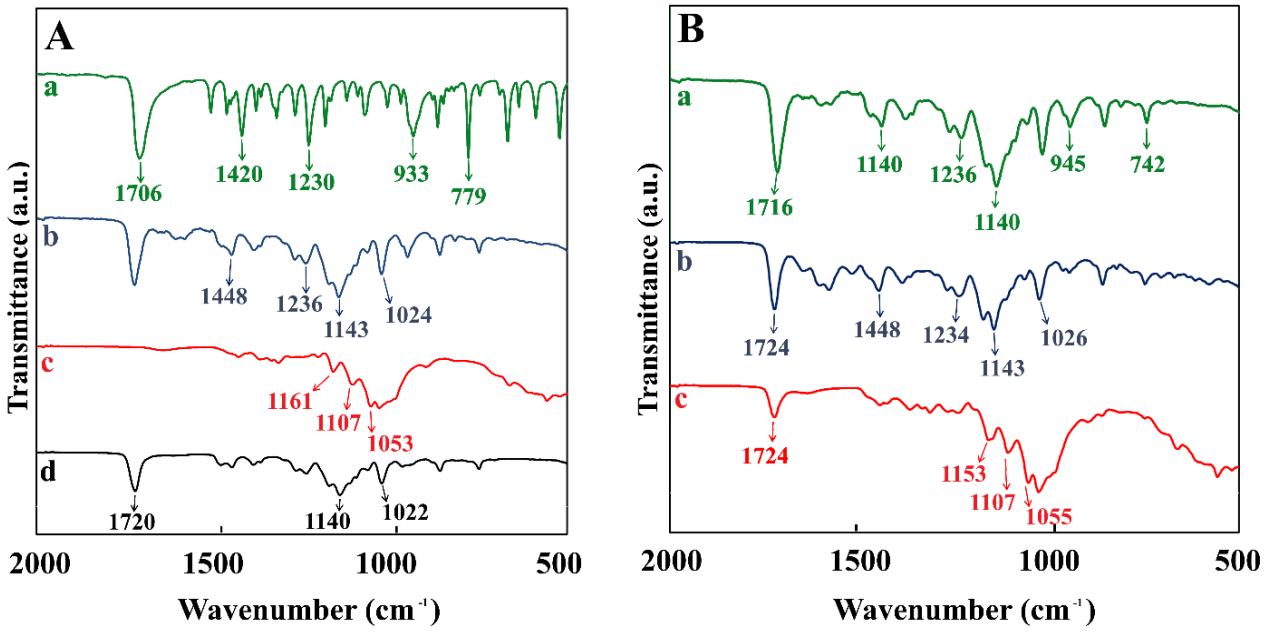

Figure 4-9: FTIR spectra of (A) pure materials: (a) ibuprofen, (b) tetracycline, (c) nanocellulose, and (d) PEMA. (B) composite films: (a) PEMA-ibuprofen, (b) PEMA-tetracycline and (c) PEMA-nanocellulose.

In addition to FTIR characterization, XRD was also used to analyze the composite coatings. The X-ray diffraction patterns for starting materials are shown in Figure 4-10A. PEMA showed very broad peaks in the range of 10-20°. Nanocellulose, tetracycline, and ibuprofen showed peaks corresponding to their JCPDS files 058-1718, 039-1985, and 0032-1723, respectively. The X-ray diffraction patterns of composite films are presented in Figure 4-10B. Composite PEMA-ibuprofen films showed peaks of ibuprofen combined with broad peaks of PEMA. The deposition of tetracycline with PEMA in ethanol led to its amorphization, but the most dominant peaks were still slightly visible. The X-ray diffraction pattern of PEMA-nanocellulose films showed peaks of nanocellulose. It is suggested that broad peaks of PEMA overlapped with nanocellulose peaks and were not observed in the X-ray diffraction pattern of the composite. It is also known that polymer

amorphization can be observed in composites under the influence of another polymer or additive [48–50].

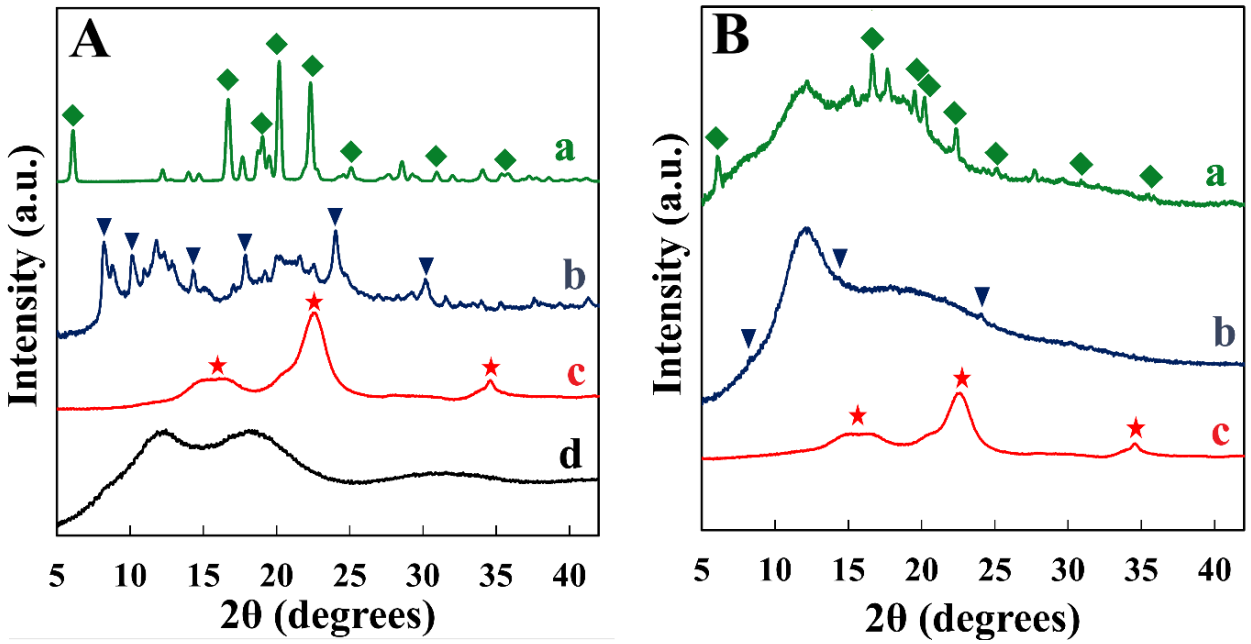

Figure 4-10: XRD patterns of (A) pure materials: (a) ibuprofen, (b) tetracycline, (c) nanocellulose, and (d) PEMA, (B) composite coatings: (a) PEMA-ibuprofen, (b) PEMA-tetracycline and (c) PEMA-nanocellulose (peaks corresponding to JCPDS files: ◆-0032-1723 ▼-039-1985, ★-058-1718).

The XRD studies in addition to the FTIR spectra proved the co-deposition of PEMA with drugs and nanocellulose. The results of this investigation indicated that the dip coating methods presents a versatile strategy for the co-deposition of various functional materials for biomedical applications. Water droplet contact angle measurements showed that as-deposited PEMA films and composite films reduced surface wettability of the substrates (Supplementary information, Figures 4-S2 and 4-S3 and Table 4-S1). We found that the dip coating method can be used for the fabrication of multilayer films, containing individual layers with different functionalities.

4.5 Conclusions

This study showed that PEMA can be dissolved in ethanol in the presence of LCA. The ability to avoid the use of toxic solvents for PEMA opens an avenue for the fabrication of composite films for biomedical and other applications. Of particular importance is the use of a natural bile acid as a solubilizing agent for PEMA and as a dispersing agent for inorganic particles. The formation of concentrated solutions of high molecular mass PEMA was one of the key factors for the successful deposition of PEMA films by a dip-coating method. The annealed PEMA films provided corrosion protection for stainless steel. It was found that LCA acted as a dispersant for the hydroxyapatite and silica and facilitated the fabrication of composite PEMA-hydroxyapatite and PEMA-silica films. The composition of the films can be varied and controlled by the variation in concentration of the inorganic particles in the suspensions. The dip coating method has been used for the fabrication of composite coatings, containing drugs and nanocellulose. Therefore, the proposed method represents a versatile platform for the fabrication of composite films, containing various functional materials.

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4.7 Supplementary Information

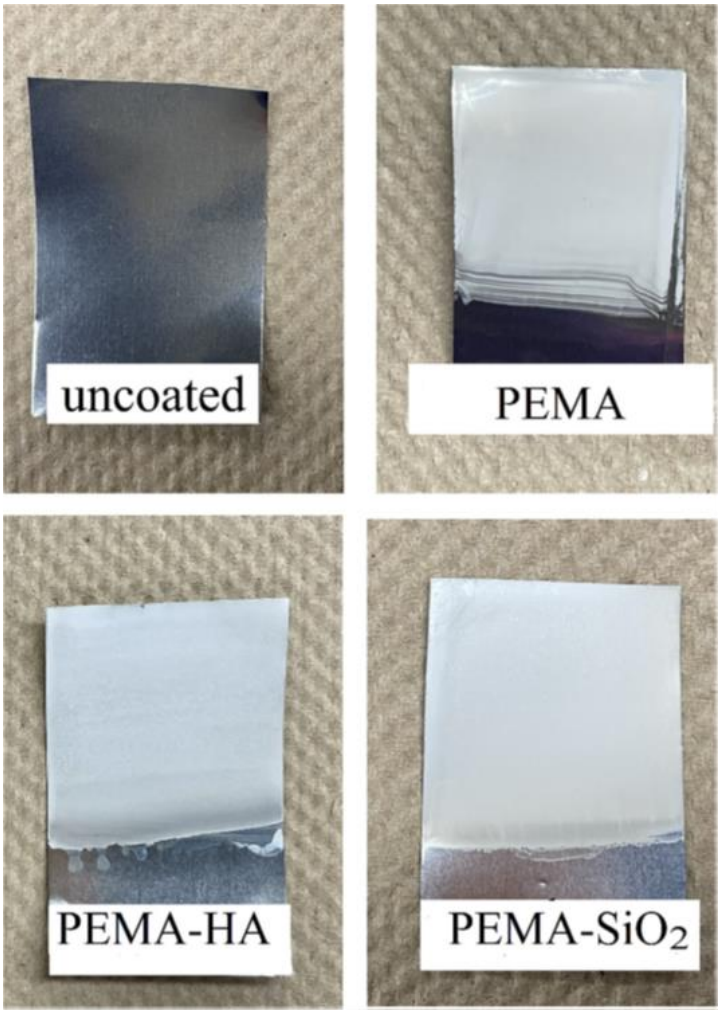

Figure 4-S1: Photographs of uncoated stainless steel and coated with PEMA, PEMA-hydroxyapatite (HA) and PEMA-SiO_2 films. Films were deposited from pure 10 g L^{-1} PEMA solutions with 3gL^{-1} LCA and with 10 g L^{-1} HA or 10 g L^{-1} SiO_2.

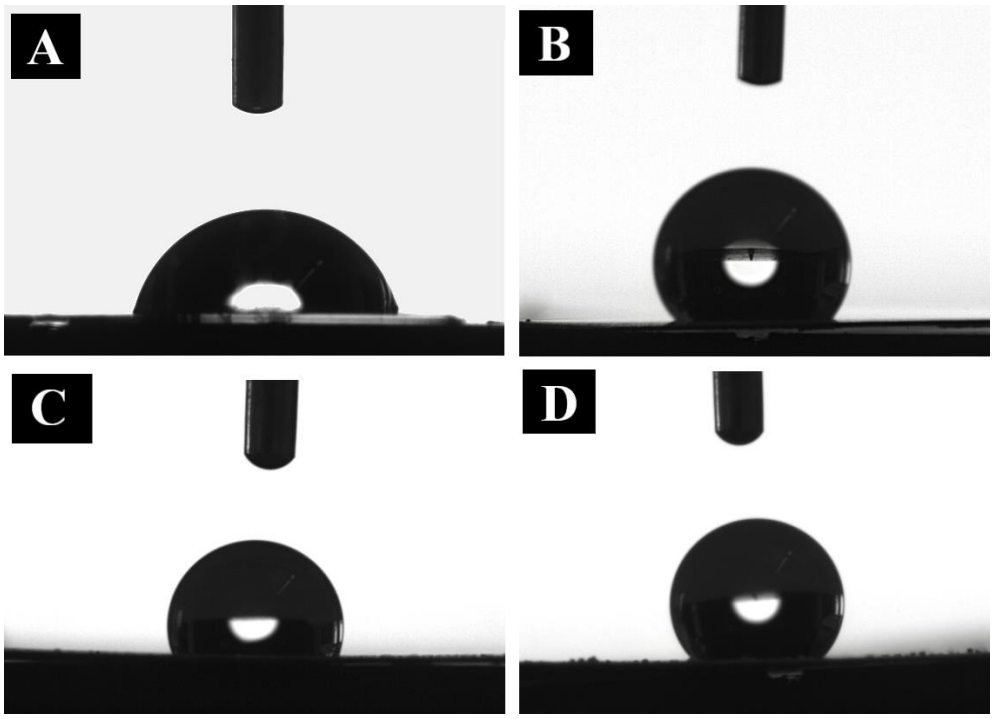

Figure 4-S2: Water droplet contact angle measurements for (A) uncoated stainless steel, and (B-D) containing as-deposited films, prepared from (B) 10 gL^{-1} PEMA solution, (C) 10 gL^{-1} PEMA solution, containing and 10 gL^{-1} HA, and (D) 10 gL^{-1} PEMA solution, containing 10 gL^{-1} SiO_2.

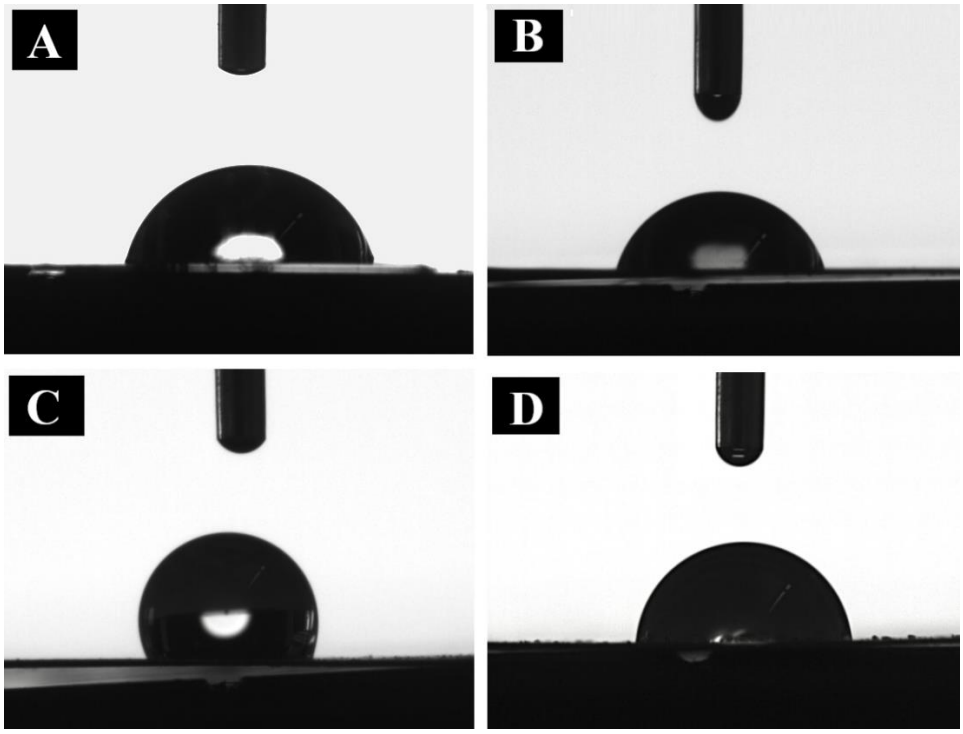

Figure 4-S3: Water droplet contact angle measurements for (A) uncoated stainless steel, and (B-D) containing annealed films, prepared from (B) 10 gL^{-1} PEMA solution, (C) 10 gL^{-1} PEMA solution, containing and 10 gL^{-1} HA, and (D) 10 gL^{-1} PEMA solution, containing 10 gL^{-1} SiO_2.

Table 4-S1: Water droplet contact angle (deg.) data (average for 3 measurements).

Uncoated Stainless Steel	**Coated Stainless Steel**					
	As-Deposited			**Annealed**		
	PEMA	**PEMA-HA**	**PEMA-SiO_2**	**PEMA**	**PEMA-HA**	**PEMA-SiO_2**
75.59	126.85	119.38	129.09	75.85	117.22	86.45

Chapter 5: A biomimetic strategy for the fabrication of micro- and nanodiamond composite films

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Micro

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5.1 Abstract

This investigation is motivated by increasing interest in diamond and composite films for applications in biomedical and electronic devices. A biomimetic strategy is based on the use of commercial bile acids, such as ursodeoxycholic acid (UDCA) and hyodeoxycholic acid (HDCA). Composite films are developed using UDCA and HDCA as solubilizing agents for poly(ethyl methacrylate) (PEMA) in isopropanol and as dispersing agents for micro- and nanodiamonds. In this approach, the use of traditional toxic solvents for PEMA dissolution is avoided. The ability to obtain high concentrations of high molecular mass PEMA and disperse diamond particles in such solutions is a key factor for the development of a dip-coating method. The PEMA dissolution and diamond dispersion mechanisms are discussed. The composition and microstructure of the films can be varied by variation of the diamond particle size and concentration in the suspensions. The films can be obtained as singular layers of different compositions, multilayers of similar composition, or alternating layers of different compositions. The films combine corrosion protection property and biocompatibility of PEMA with advanced functional properties of diamonds.

5.2 Introduction

Diamond particles and films have many commercial uses in electronics, tools, and biomedical devices [1–3]. Due to exceptional biocompatibility, hardness, and wear and corrosion resistance, diamond films have many applications for orthopedic implant devices [1,4]. Diamond coatings enhanced corrosion-resistant properties of TiAlV alloys [5]. Additionally, diamond and related materials can stimulate the formation of new bone, leading to enhanced stability and durability of orthopedic implants [6]. Films of diamond were shown to provide thromboresistance, low protein adsorption, resistance to fracture, and antibacterial properties for biomedical devices [4,7]. Diamond coatings enhanced biocompatibility, and wear and corrosion resistance of stainless steel [8]. Both micro- and nanodiamond materials [9] were used for coatings of biomedical implants, development of composite scaffolds, and cell culture applications. Recently, significant interest has been generated in biomedical applications of nanodiamond for various biomedical devices [10–14]. Nanodiamond coatings on titanium promoted mammalian cell growth and inhibited Staphylococcus aureus colonization [15]. Cell proliferation was enhanced by the nanodiamond coatings [16]. Novel multifunctional diamond-based materials were developed for advanced biomedical applications [17]. Many investigations focused on the development of advanced techniques for the fabrication of diamond-like carbon coatings on biomedical implants for orthopedic applications [18–20]. Such coatings provided enhanced biocompatibility and prevented inflammation [20].

The development of composite films offers benefits of combining functional properties of diamond with properties of polymers [1] and hydroxyapatite [21]. It has been shown that combining various polymers, such as chitosan, polycarbonate, 2-methacryloyloxyethylphosphorylcholine, polytetrafluoroethylene, and silk with diamond allowed

for increased mechanical properties, controlled drug release, improved chemical resistance, decreased biofouling, and increased adhesion to substrates [22,23]. Many strategies included spin coating for polymer deposition followed by an additional deposition step for the diamond deposition using different deposition techniques, such as radio frequency plasma-enhanced chemical vapor deposition, plasma immersion ion implantation, and inductively coupled plasma-assisted sputtering [23,24]. Composite films were also obtained by electrophoretic co-deposition from colloidal suspensions, containing particles of diamonds and submicrometric polymer particles [25] using bile acids as negatively charged dispersants in an ethanol solvent. Colloidal techniques offer many benefits for the fabrication of composites with advanced microstructure and functionality [26–30]. The research progress in this area is largely based on the success in the development of efficient surface functionalization techniques [26,27,30,31]. However, diamond is one of the most chemically inert materials known [9], and its functionalization and dispersion by adsorbed dispersants present difficulties. Moreover, it is challenging to co-disperse large particles of various commercial polymers and diamond particles for colloidal manufacturing of composites.

The goal of this investigation was the deposition of poly(ethyl methacrylate) (PEMA)-diamond composites by a simple dip-coating method. PEMA is an advanced polymer for many applications due to its biocompatibility, high chemical stability, flexibility, good mechanical properties, and thermal stability [32,33]. Biomimetic approaches offer many advantages, such that materials assembled in nature often possess superior properties and they were assembled in their native physiological environment—typically ambient temperature and pressures. Therefore, these strategies can help avoid the need for complicated, high-temperature fabrication processes. For example, the mechanism of mussels' strong adhesion to multiple surfaces can be mimicked for the dispersion of metal oxides [34]. This approach was based on the application of natural bile acid

biosurfactants, such as ursodeoxycholic acid (UDCA) and hyodeoxycholic acid (HDCA), as solubilizing agents for PEMA and dispersants for diamond particles. Bile acids are physiologically important surfactants that aid in the solubilization of lipids, proteins, cholesterol, vitamins, and other molecules within the human body. In this biomimetic approach, the use of traditional toxic solvents for PEMA dissolution was avoided. The high solubilization and dispersion power of the biosurfactants was linked to their unique chemical structure and properties. The results presented below indicate that composite films containing micro- or nanodiamond can be obtained. The method allows for the variation of film composition. The composite films can be prepared as singular layers or laminates, containing layers of different compositions. The composite coatings offer advantages of combining functional properties of PEMA and diamond.

5.3 Materials and Methods

5.3.1 Fabrication of PEMA Solutions and Diamond Suspensions

PEMA (515 kDa), UDCA, HDCA, microdiamond (MD, ≤1 μm), and nanodiamond (ND, <10 nm) were purchased from Millipore Sigma. PEMA was added at a concentration of 10 gL^{-1} to pure isopropanol as well as isopropanol containing dissolved 1 gL^{-1} of either UDCA or HDCA. The PEMA suspensions were stirred while being heated to 45 °C. At this temperature the PEMA suspension changed from opaque white to a transparent solution. In the absence of UDCA and HDCA, PEMA was not solubilized and remained opaque white upon cooling. The UDCA and HDCA containing PEMA solutions were stable and clear at room temperature for over 24 hours. Such solutions were used for the fabrication of PEMA films. MD and ND were added to the PEMA solutions and ultrasonicated in order to achieve stable diamond dispersions in PEMA solutions. In the addition of 1 gL^{-1} of MD or ND, 1 gL^{-1} of HDCA or UDCA was able to successfully disperse

the particles. Whereas, when MD and ND concentrations were increased to 3 gL^{-1}, the concentration of UDCA or HDCA was increased to 3 gL^{-1}.

5.3.2 Film Deposition

PEMA films were fabricated by the dip-coating method on 304 stainless steel substrates from 10 gL^{-1} PEMA solutions. Substrates were cut to 2.5 cm x 5cm and washed with ethanol before film deposition. PEMA and PEMA-diamond films were deposited by manually lowering the substrate into solution for 20 s, followed by gradual removal, and left to dry in air. The films were deposited as singular PEMA layers, PEMA-diamond composite layers (with different diamond concentrations), PEMA multilayers, and alternating layers of PEMA and PEMA-diamond composites.

5.3.3 Film Characterization

The films were observed on a JEOL 7000F scanning electron microscope (SEM). A PARSTAT 2273 potentiostat was used for potentiodynamic polarization tests of the annealed PEMA films in 3 % NaCl electrolyte with a saturated calomel reference electrode (SCE) and a Pt counter electrode. A Bruker Smart 6000 diffractometer (CuKα radiation) was used for X-ray diffraction studies.

5.4 Results and Discussions

Figure 5-1 shows chemical structures of PEMA, UDCA and HDCA. PEMA is a hydrophobic acrylate polymer, which is soluble only in relatively toxic and carcinogenic solvents, such as methyl ethyl ketone, toluene, and benzene. The hydrophobicity of PEMA is attributed to

its hydrocarbon groups. Isopropanol is known as a non-solvent for PEMA. The solubility of PEMA in non-toxic solvents is important for biomedical applications of this polymer and its composites. UDCA and HDCA belong to the bile acid family of organic molecules. It is known that bile acids and bile acid salts are very powerful natural solubilizing and dispersing agents, which outperform various commercial surfactants [35-37].

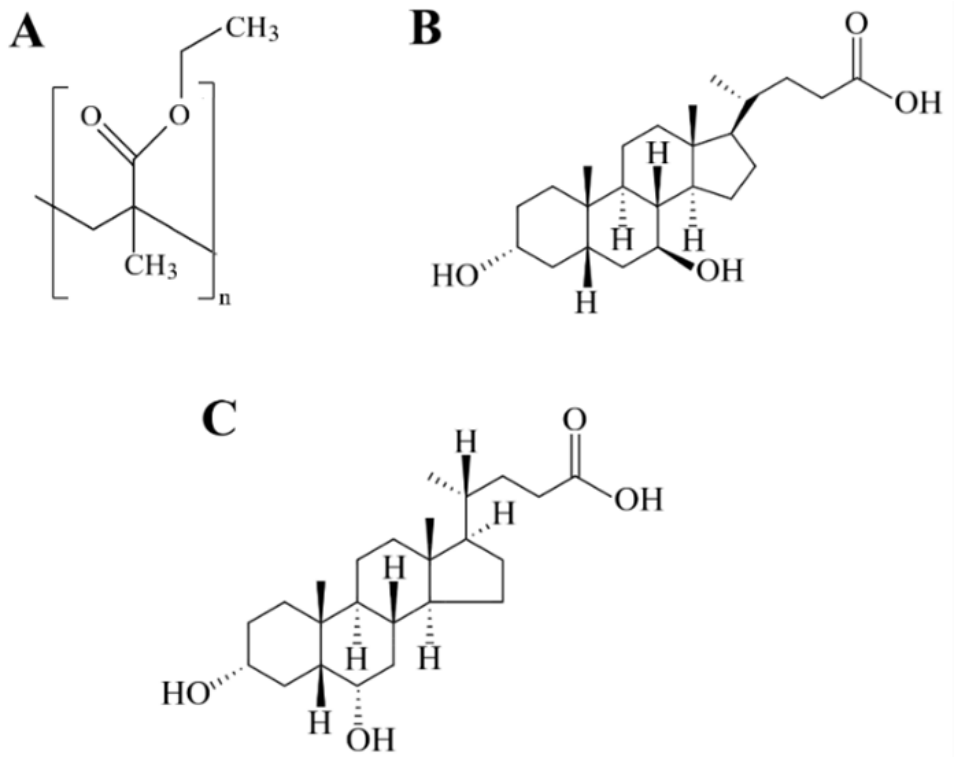

Figure 5-1: Chemical structures of (A) PEMA, (B) UDCA, and (C) HDCA.

UDCA and HDCA are steroid amphiphilic molecules, which have a hydrophobic hydrocarbon convex face and a concave hydrophilic face with a carboxylic and OH groups [36]. The adsorption of bile salts on different hydrophobic surfaces is influenced by hydrophobic interactions of their convex face with hydrophobic surfaces of materials[36]. The electric charge of bile acids and salts is related to their carboxylic groups, which facilitate electrostatic dispersion. Motivated by strong solubilization and dispersion power of bile acids and salts, we developed a biomimetic strategy which involved solubilization of PEMA in isopropanol and dispersion of

diamonds using UDCA and HDCA as solubilizing and dispersing agents, illustrated in Schematic 1 in Supplementary Information.

We demonstrated for the first time that UDCA and HDCA can solubilize PEMA in isopropanol. Figure 5-2A demonstrates solubilization of PEMA in the presence of UDCA and HDCA.

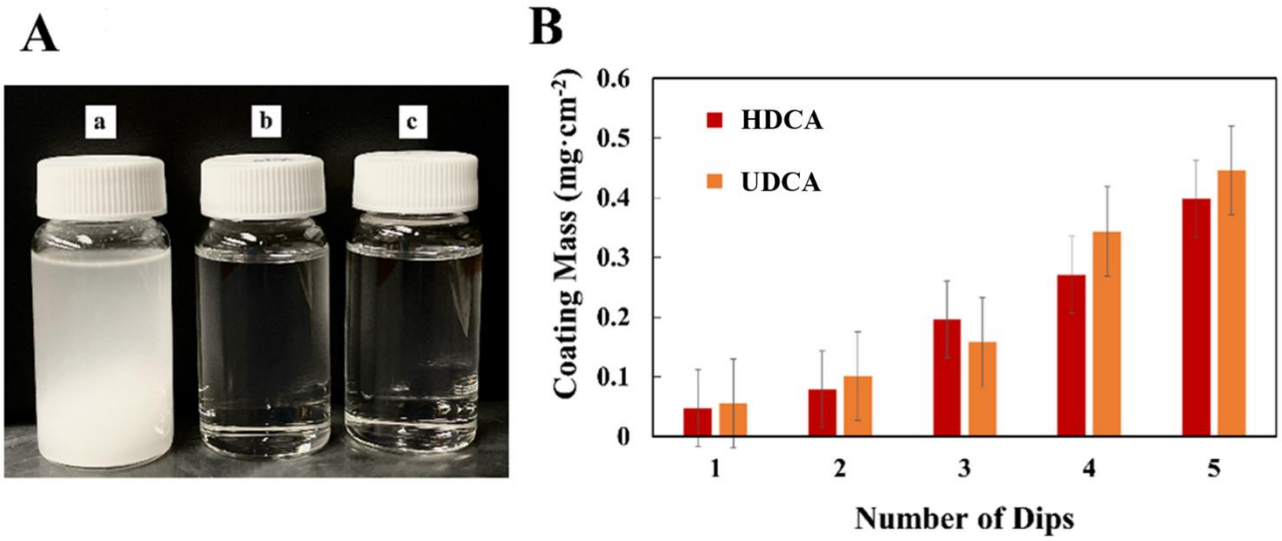

Figure 5-2: (A) 10 gL^{-1} PEMA in isopropanol: (a) without additives, (b) with 1 gL^{-1} HDCA, and (c) with 1 gL^{-1} UDCA at room temperature, (B) deposit mass versus number of dips from 10 gL^{-1} PEMA with 1 gL^{-1} HDCA or UDCA.

It is suggested that hydrophobic interactions of PEMA with bile acids facilitated bile acid adsorption on PEMA, resulting in solubilization. It is in this regard that bile acid salts solubilize different hydrophobic molecules, such as cholesterol, lipids and other molecules and macromolecules in a human body. Of particular importance is the ability to form concentrated solutions of high molecular mass PEMA. This was a key factor for the dip-coating deposition of PEMA and composites. Low molecular mass polymers exhibit poor film-forming and binding properties. However, the solubility of polymers usually decreases with increasing their molecular mass. Relatively high polymer concentrations are necessary for film formation by dip-coating. The

solubilization of polymer molecules in solvents usually involves polymer chain disentanglement [38], which can be enhanced in the presence of adsorbed anionic UDCA and HDCA molecules. It is hypothesized that heating the PEMA suspensions in isopropanol facilitated penetration of small HDCA and UDCA between the PEMA chains and facilitated dissolution.

In this investigation the use of traditional toxic solvents for PEMA solubilization was avoided and PEMA films were successfully deposited on stainless steel substrates from concentrated high molecular mass PEMA solutions in isopropanol. Figure 5-2B shows that PEMA films can be deposited as singular layers or multilayers. Each dip-coat procedure results in the formation of one layer. Thus, by repeating the number of dips, the number of layers increases. Therefore, it is shown that the film mass can be increased with increasing number of the deposited layers. Film mass was averaged over six different samples of the same size. Previous investigations [25] of electrophoretic deposition of materials using bile acids as dispersants showed that deposition yield is influenced by the positions of OH groups in their chemical structure. However, similar deposition yields were obtained by dip-coating method using HDCA and UDCA with different OH group positions in their structure. As-deposited films were porous (Figure 5-3), their annealing at 180 °C for 1 h resulted in the formation of dense films. The changes in film morphology after annealing has been attributed to increased polymer mobility at elevated temperatures above the polymer's glass transition temperature [39]. This results in elimination of pores and the formation of a featureless, dense film.

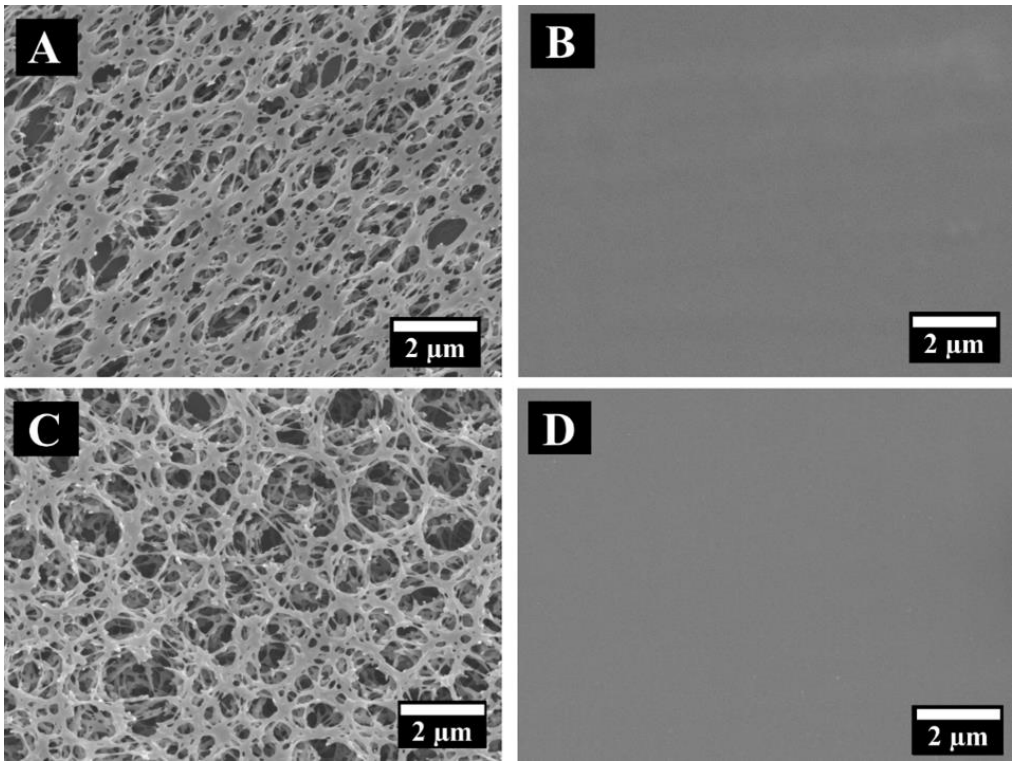

Figure 5-3: SEM micrographs of films, prepared from 10 g L^{-1} PEMA solutions in isopropanol containing (A, B) 1 g L^{-1} UDCA and (C, D) 1 g L^{-1} HDCA: (A, C) as-deposited and (B, D) annealed at 180 °C for 1 h.

Annealed PEMA films provided corrosion protection of stainless steel substrates (Figure 5-4). The corrosion current of the bare substrate of 3.24 µA cm^{-2} was decreased to 0.34 µA cm^{-2} and 0.72 µA cm^{-2} for the films obtained from 1 gL^{-1} HDCA and UDCA-containing PEMA solutions, respectively. The Tafel plots also show the increase in the corrosion potential of the coated substrates. The corrosion current obtained by annealed PEMA films which were solubilized in ethanol by lithocholic acid, from our previous work [39], was 0.15 µA cm^{-1}. We believe the corrosion current presented here is slightly higher due to small differences in thicknesses of the films between the two works.

HDCA and UDCA were also used as dispersants for the dispersion of ND or MD in isopropanol. Figure 5-5 shows sedimentation tests, which indicated that HDCA and UDCA can be used as dispersing agents for ND and MD.

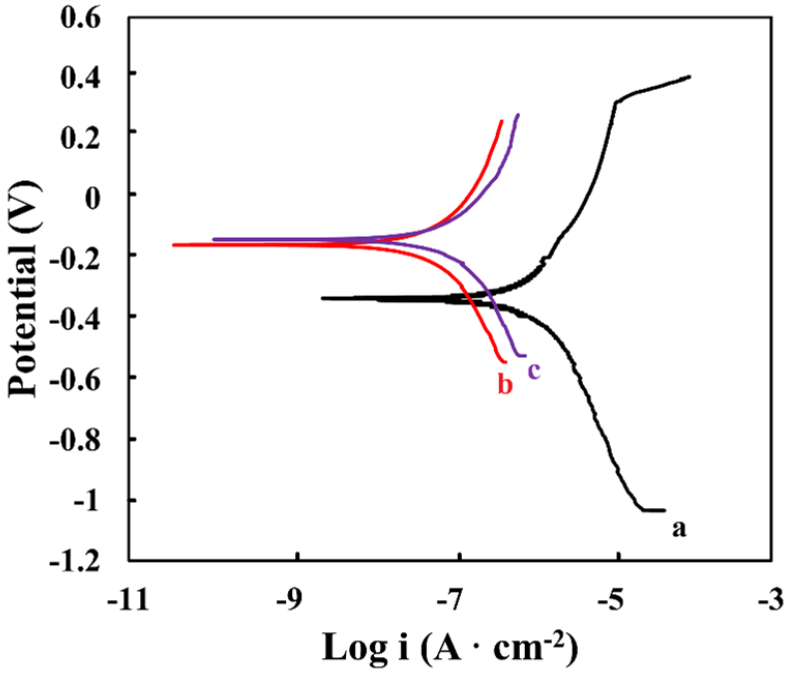

Figure 5-4: Tafel plots of (a) bare stainless steel substrate, (b, c) coated with PEMA using (b) 1 gL^{-1} HDCA and (c) 1 gL^{-1} UDCA.

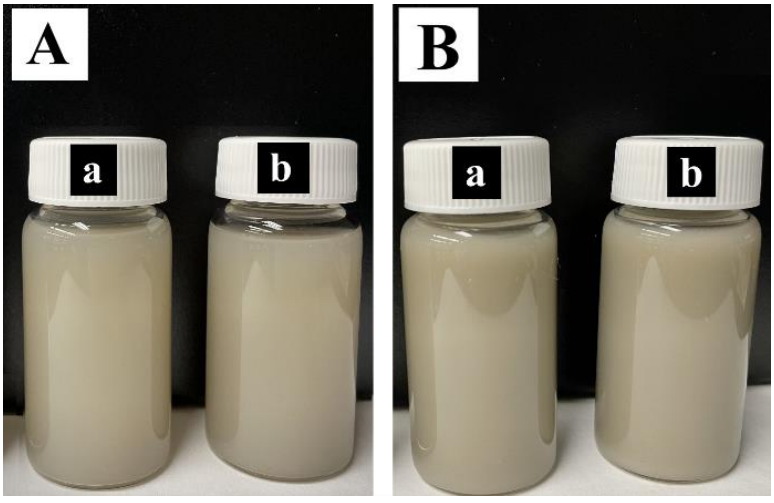

Figure 5-5: Diamond suspensions obtained using (A) HDCA and (B) UDCA, containing either (a) ND, (b) MD.

It is hypothesized that adsorption of anionic HDCA and UDCA molecules on diamond surfaces allowed for electrostatic dispersion of the diamond particles. As pointed out above, diamond is one of the most chemically inert materials known [9]. Therefore, it is challenging to achieve diamond dispersion by adsorbed dispersants. This problem was successfully addressed using HDCA and UDCA molecules as dispersants.

Composite PEMA-diamond films were obtained using HDCA and UDCA as solubilizing agents for PEMA and dispersing agents for diamonds. The incorporation of diamonds in the composite films was confirmed by XRD and SEM. Figure 5-6 shows XRD patterns of the composite films. The XRD patterns showed diamond peaks, corresponding to JCPDS file 6-675. Figure 5-7 presents SEM images of films, prepared from 10 g L^{-1} PEMA solutions, containing 1 g L^{-1} MD or ND, prepared using HDCA or UDCA. The corresponding images at a low magnification are presented in Supplementary in-formation, Figure 5-S1. The SEM images indicated that diamond particles were co-deposited with PEMA. In contrast to as-deposited PEMA films (Figure 5-3C, D), which were porous, the incorporation of diamonds resulted in the formation of dense films (Figures 5-7 and 5-S1). This change in morphology suggested interactions between the PEMA molecules and diamond particles in suspension. The amount of MD and ND in the films can be varied. Figure 5-8 and Figure 5-S2 show SEM images at different magnifications for the films prepared from 10 g L^{-1} PEMA solutions, containing 3 g L^{-1} MD or ND, using HDCA and UDCA. The increase in the MD and ND concentration in solutions resulted in the larger diamond content in the films.

The dip-coating method represents a versatile strategy for the deposition of films with different microstructures. The composite films can also be deposited as laminates, containing layers of different composition. Figure 5-9 shows that the sequential deposition from pure PEMA

solutions and PEMA solutions, containing diamonds resulted in the growth of the total film mass, confirming the deposition of alternating layers of different composition.

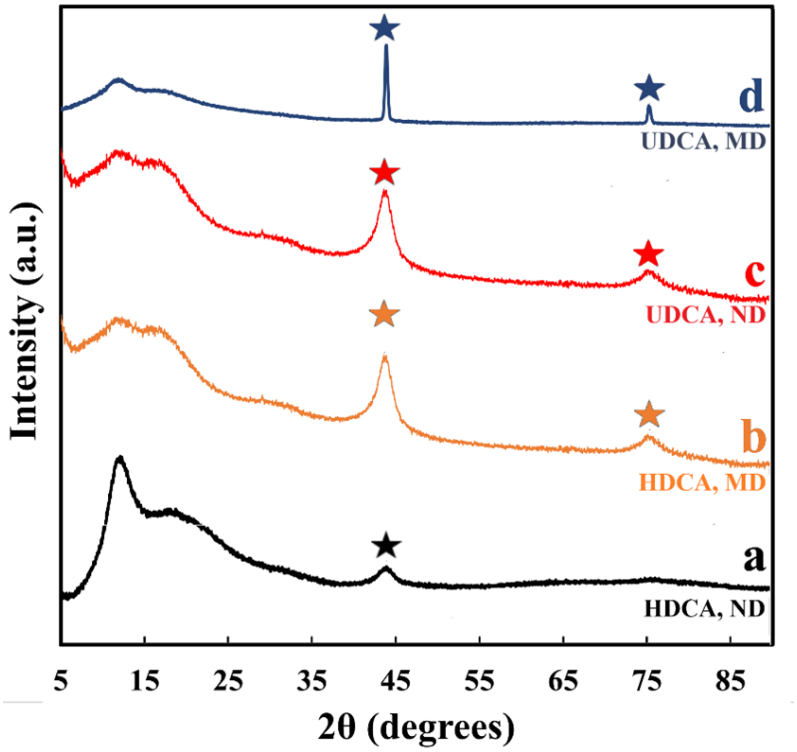

Figure 5-6: X-ray diffraction patterns of composite films, prepared from 10 gL^{-1} PEMA solutions, containing (a) 3 gL^{-1} HDCA with 3 gL^{-1} ND, (b) 3 gL^{-1} HDCA with 3 gL^{-1} MD, (c) 3 g L^{-1} UDCA with 3 gL^{-1} ND, and (d) 3 g L^{-1} UDCA with 3 gL^{-1} MD (★-peaks of diamond).

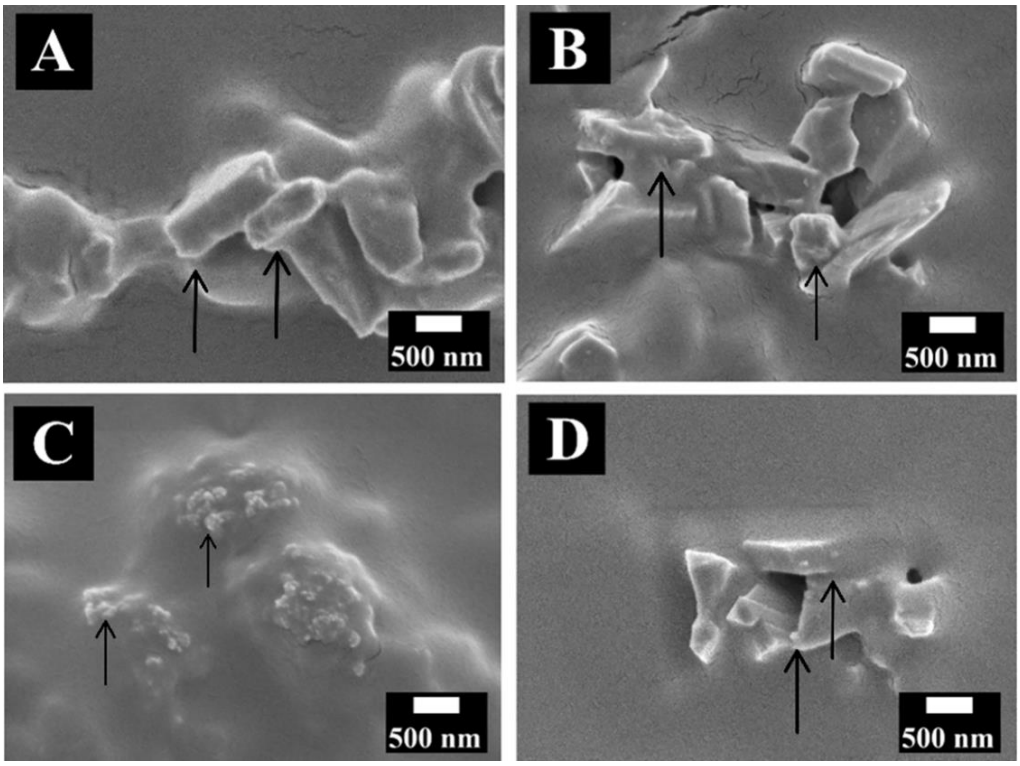

Figure 5-7: SEM micrographs of films prepared from 10 gL^{-1} PEMA solutions containing (A) 3 gL^{-1} UDCA with 1 gL^{-1} MD, (B) 3 gL^{-1} HDCA with 1 gL^{-1} MD, (C) 3 gL^{-1} UDCA with 1 gL^{-1} ND, and (D) 3 gL^{-1} HDCA with 1 gL^{-1} ND. Two arrows per image shown to indicate locations of diamond particles within the film.

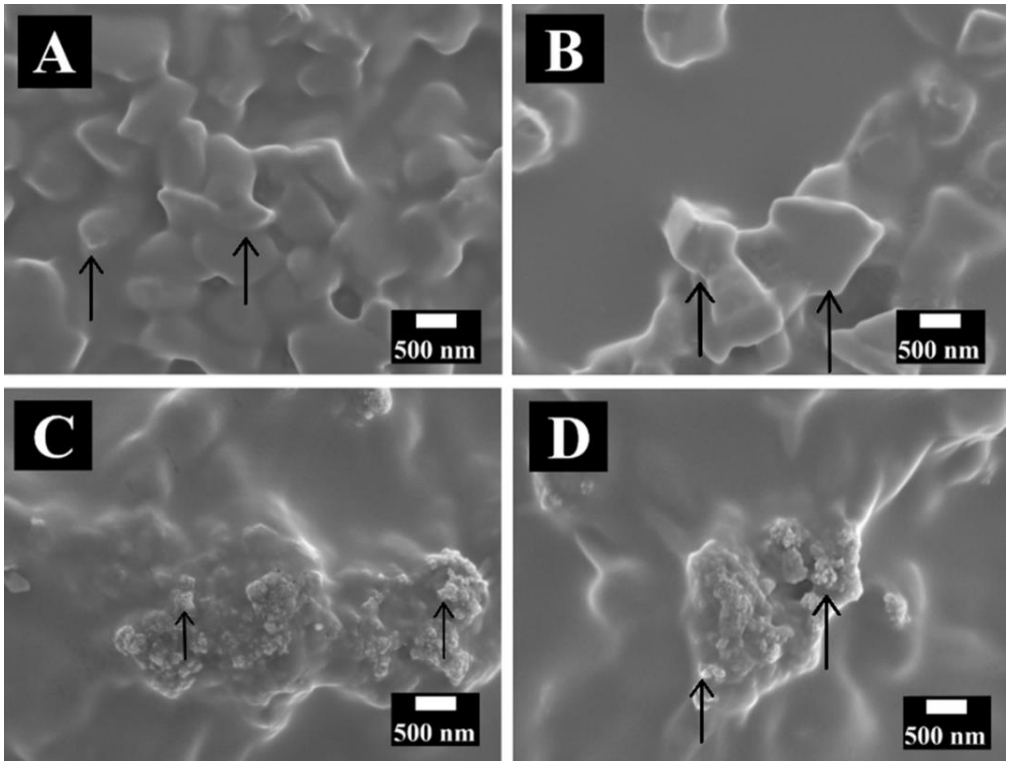

Figure 5-8: SEM micrographs of films prepared from 10 gL^{-1} PEMA solutions containing (A) 3 gL^{-1} UDCA with 3 gL^{-1} MD, (B) 3 gL^{-1} HDCA with 3 gL^{-1} MD, (C) 3 gL^{-1} UDCA with 3 gL^{-1} ND, and (D) 3 gL^{-1} HDCA with 3 gL^{-1} ND. Two arrows per image shown to indicate locations of diamond particles within the film.

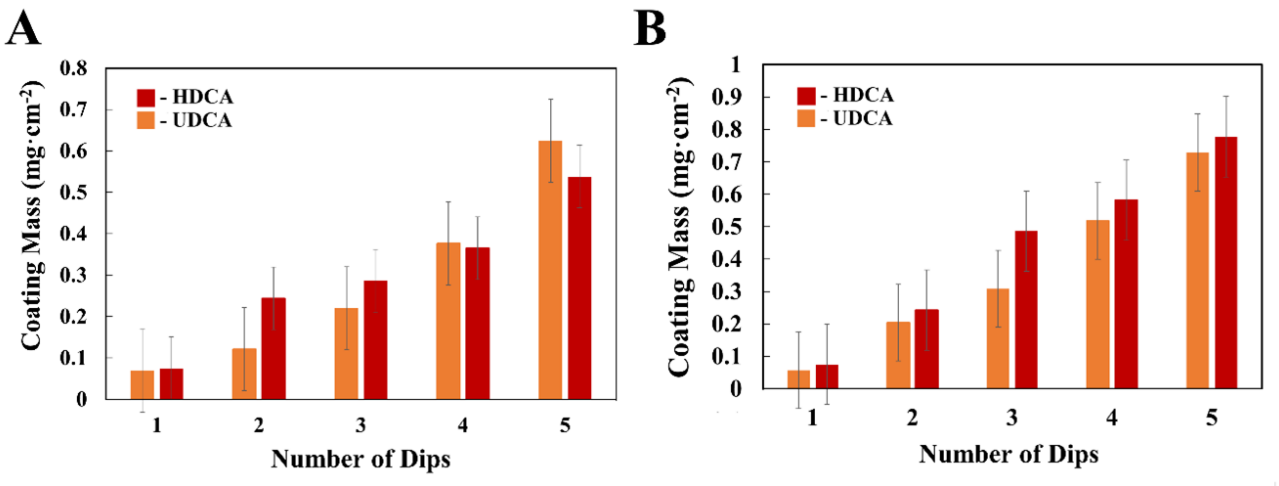

Figure 5-9: Deposit mass versus number of dips of alternating layers of PEMA and PEMA-diamond using HDCA or UDCA. Layers 1, 3 and 5 were deposited from 10 g L^{-1} PEMA solutions,

layers 2 and 4 were deposited from 10 g L^{-1} PEMA solutions, containing (A) 3 g L^{-1} MD and (B) 3 g L^{-1} ND.

5.5 Conclusions

A biomimetic strategy has been developed for deposition of PEMA, PEMA-MD, and PEMA-ND films using HDCA and UDCA bile acids as solubilizing agents for PEMA and dispersing agents for ND and MD. PEMA was successfully dissolved in isopropanol, which is known as a non-solvent for PEMA. The use of traditional toxic solvents can be avoided. The solubilization properties of HDCA and UDCA allowed the fabrication of concentrated high molecular mass PEMA solutions, which played a crucial role in the application of a dip-coating technique for pure polymer and polymer-diamond films. The composite films can combine corrosion protection and other functional properties of PEMA with functional properties of MD and ND. The diamond content in the films can be varied. The films can be deposited as single layers or multilayers, containing alternating layers of different composition.

5.6 References

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5.7 Supplementary Information

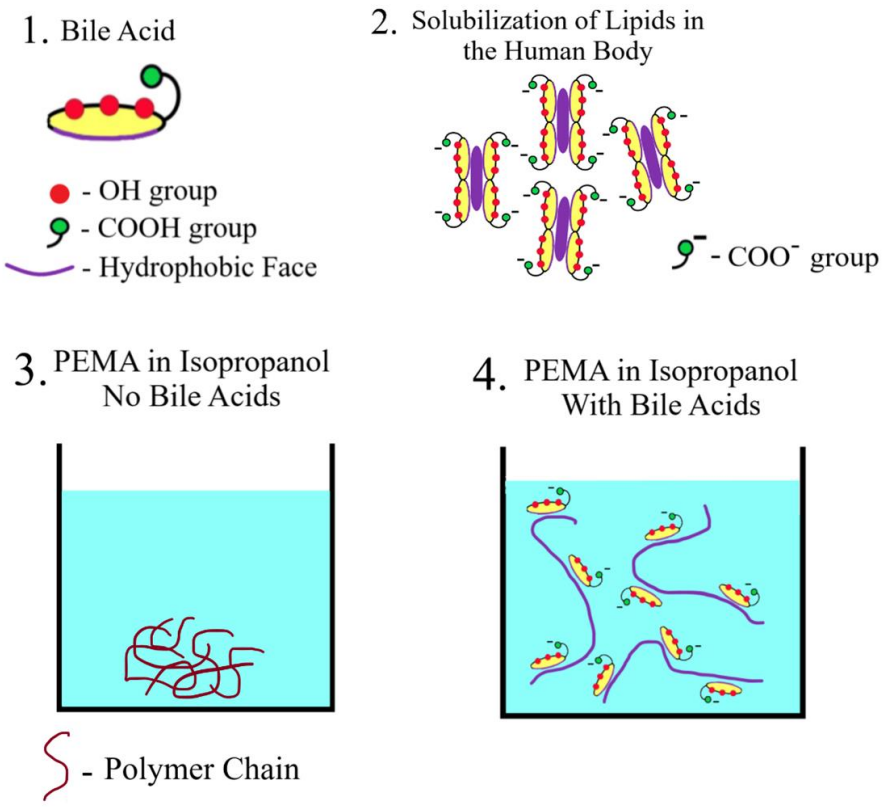

Figure 5-S1: Illustration of the general chemical structure of a bile acid (1), bile acids solubilization of lipids within the human body (2), PEMA insoluble in isopropanol (3), and PEMA solubilized in isopropanol by the addition of bile acids (4).

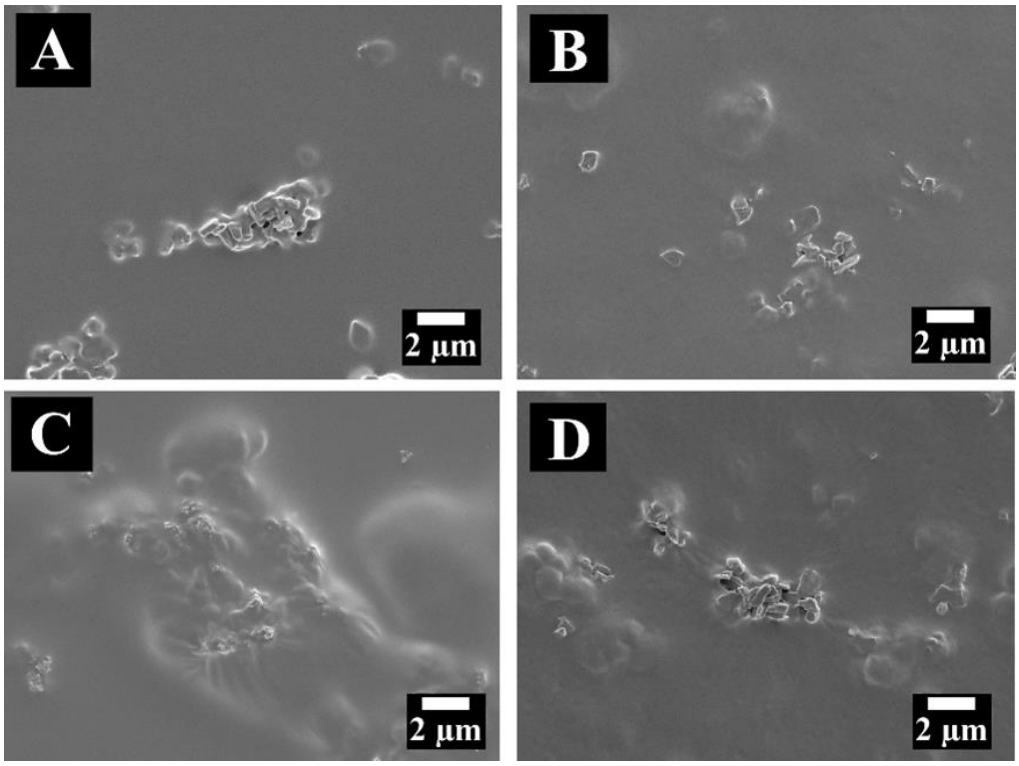

Figure 5-S2: Low magnification SEM micrographs of films prepared from 10 gL^{-1} PEMA solutions containing (A, B) 1 gL^{-1} MD and (C, D) 1 gL^{-1} ND (A) with (A, C) 3 gL^{-1} UDCA (B.D) 3 gL^{-1} HDCA.

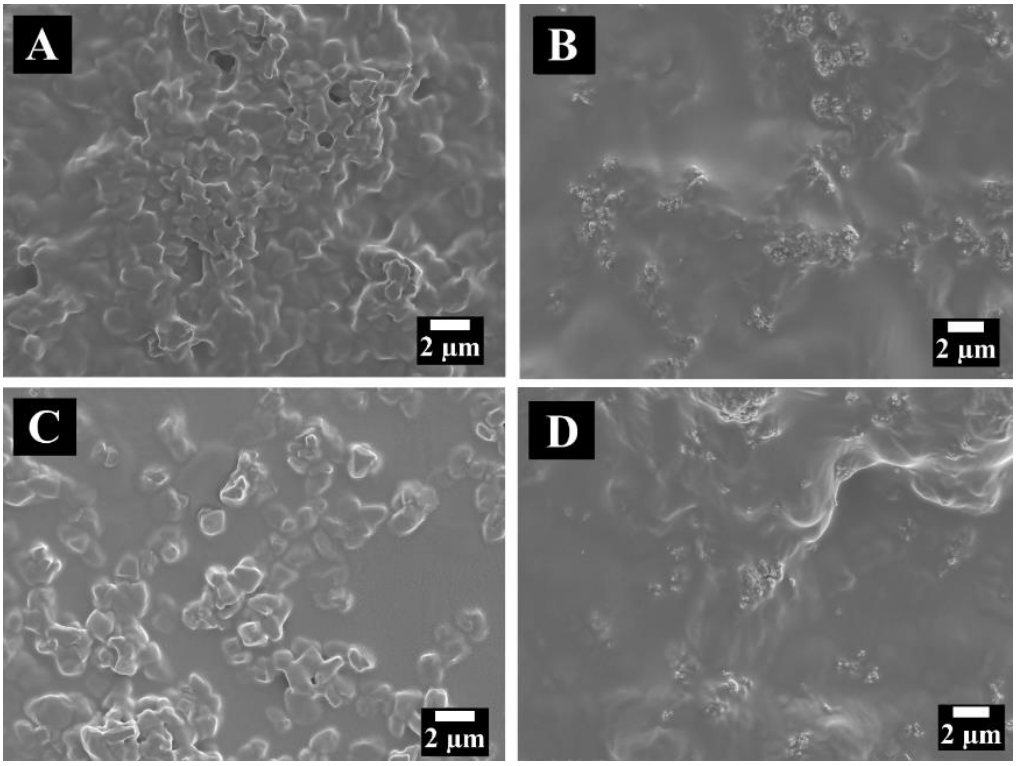

Figure 5-S3: Low magnification SEM micrographs of films prepared from 10 gL^{-1} PEMA solutions containing (A, B) 3 gL^{-1} MD and (C, D) 3 gL^{-1} ND (A) with (A,C) 3 gL^{-1} UDCA (B.D) 3 gL^{-1} HDCA.

Chapter 6: Conclusions and Future Work

6.1 Summary of Conclusions

In summary, the collection of works presented in this sandwich thesis provided a novel biomimetic approach for the facile fabrication of composite coatings primarily for orthopedic implant applications, and potentially a variety of other biomedical devices. As a result of the new fabrication process, four different bile acids (CA, LCA, UDCA, and HDCA) were utilized as solubilizing agents for PEMA in two different solvents (isopropanol and ethanol). Films of PEMA were fabricated at room temperature. Film morphology was varied depending on post-deposition heat treatments, and the heat-treated PEMA films provided corrosion protection to stainless steel. Additionally, several PEMA-composite films were created, including various inorganic materials such as HA, SiO_2, TiO_2, microdiamond, and nanodiamond. Furthermore, coatings were successfully loaded with drugs or other functional materials such as tetracycline, ibuprofen, heparin, and nanocellulose. Specific major findings from each contribution can be summarized as follows:

- Chapter 3: PEMA was successfully solubilized in isopropanol, a non-solvent of the polymer. This work utilized bile acids, motivated by the numerous previous investigations of bile salts for the solubilization of various molecules in aqueous solutions. For the first time it was shown that bile acids cholic acid and lithocholic acid can successfully solubilize polymer macromolecules in organic solvents such as isopropanol. An important result of this solubilization was the high concentration of high molecular weight polymer which was achieved. This allowed for the use of a simple dip coating method for the deposition of polymer films on stainless steel substrates at room temperature. Additionally, the films can be deposited as monolayers or multilayers with increasing coating mass. Here it was

suggested for the first time that the solubilization mechanism involved hydrophobic interactions between the bile acids and PEMA, which will motivate future development of PEMA coatings from other organic solvents. Furthermore, the bile acids were multifunctional, and able to act as solubilizing agents for PEMA and as dispersing agents for the fabrication of organic-inorganic composite coatings containing HA, SiO_2, TiO_2, heparin, and tetracycline. Overall, the proposed method is important for the fabrication of coatings with advanced functionality, avoiding the use of toxic solvents or surfactants, and can be carried out at room temperature.

- Chapter 4: Motivated by the solubilization of PEMA in isopropanol, this work showed that PEMA can also be solubilized in ethanol, another non-solvent of the polymer. Lithocholic acid was successful in solubilizing high concentrations of PEMA which led to the successful employment of a dip coating method for film deposition. The use of lithocholic acid as a dispersing agent for the creation of PEMA-composite coatings required increased LCA concentrations. This provided insights to the solubilization mechanism and revealed the possible formation of charged polymer-bile acid complexes. Additionally, the composition of composite organic-inorganic films can be varied from 100 wt.% organic, up to 50 wt.% inorganic and 50 wt.% organic. Furthermore, composite coatings containing more drugs and functional materials were also fabricated, such as PEMA-ibuprofen, tetracycline, and nanocellulose coatings. Overall, a versatile strategy for composite film fabrication was presented that utilized non-toxic solvents, naturally occurring surfactants, and provided a wide control over film composition.
- Chapter 5: This work used two additional bile acids, hyodeoxycholic acid and ursodeoxycholic acid, to solubilize high concentrations of PEMA in isopropanol. PEMA

films were successfully deposited on stainless steel substrates by dip coating. These films were successfully deposited as monolayers or as multilayers with increasing coating mass. The heat treatment of these pure polymer films showed corrosion protection to stainless steels once again. Additionally, diamond was used to enhance the wear resistance, corrosion resistance, and the potential biocompatibility of the coating. It was shown that both HDCA and UDCA could successfully disperse micro- and nanodiamond particles for the fabrication of PEMA-diamond composite films by dip coating. The morphology of the composite films was vastly different than previous composite PEMA films, which indicated electrostatic interactions of the bile acids with the diamond particles. Furthermore, functionally graded coatings were prepared by depositing alternating layers of pure PEMA and PEMA-diamond films. Lastly, the composition of the PEMA-diamond films could be varied to achieve different diamond content in the films. Overall, this method combined the advantages of PEMA and diamond into a functional composite coating which could be deposited by dip coating at room temperature.

6.2 Future Work and Limitations

Although the composite coatings created throughout thesis were widely characterized with methods such as scanning electron microscopy, Fourier transform infrared spectroscopy, X-ray diffraction, coating mass, potentiodynamic polarization, thermal gravimetric analysis, and contact angle measurements, they would still benefit from further characterization. It would be worthwhile to investigate the difference in mechanical properties between uncoated and coated stainless steel. Furthermore, the biocompatibility of the coating could be quantified by performing *in-vitro* tests such as cell viability and proliferation tests. A step further would be to perform *in-vivo* tests to characterize how host tissues would respond to the various

composite coatings. Lastly, coatings containing drugs would benefit from drug release profile studies. This way, the amount of drug loaded can be optimized to create long lasting drug release at the site of implantation.

For future work, I suggest the investigation of incorporating antibacterial materials into the composite coatings. One issue that was not addressed in this work, regarding orthopedic implants and other biomedical devices, is the risk of infection. This risk could be mitigated by modifying surface characteristics to reduce bacterial adhesion to the surface, or by including materials with a known bactericidal affect. These materials include silver nanoparticles and other metal ions.

Lastly, the new colloidal strategies presented in this thesis open many more avenues for the fabrication of novel advanced films for biomedical and other applications such as optical, automotive, or electronic materials. The conceptually new biomimetic approach developed can lead to the fabrication of coatings made from other polymers and composite materials without the need for toxic solvents. Popular polymers with poor solubility in non-toxic solvents to explore include polycaprolactone or high-density polyethylene. Composite coatings containing different inorganic bioactive materials and drugs are a possibility. The solubilization power of bile acids was introduced and should be explored further to enhance their contributions to colloidal sciences.