2015

An implantable sustained-release chemotherapy delivery system for the treatment of breast cancer

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An implantable sustained-release chemotherapy delivery system for the treatment of breast cancer

by

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A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Industrial Engineering

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Ames, Iowa

2015

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NOMENCLATURE

PLGA	Poly (Lactic-co-Glycolic Acid)
PTX	Paclitaxel
CHI	Chitosan
DCM	Methylene chloride
PBS	Phosphate buffered saline
PCL	Polycaprolactone
PGA	Poly (glycolic acid)
PLA	Poly (lactic acid)

ACKNOWLEDGMENTS

I would like to take this opportunity to express my thanks to those who helped me with various aspects of conducting research and the writing of this thesis. First and foremost, I would like to thank Professor Iris V. Rivero for her constant guidance, patience, and support throughout this research and the writing of this thesis. Her insights and words of encouragement have often inspired me and renewed my hopes for completing my graduate education. I also appreciate my committee members for their efforts and contributions to this work: Professor John Jackman and Professor Kaitlin Bratlie. Last but not the least, I am grateful to our senior ERD machinist, Mr. Kevin Brownfield, who helped design and build the injection molding device for this project, and provided me with much assistance to fabricate the samples.

ABSTRACT

The influence of chitosan on the degradation characteristics of paclitaxel-loaded Poly (Lactic-co-Glycolic Acid) (PLGA) rod-shaped implants was investigated and modeled. The implant was designed for sustained release of the hydrophobic chemotherapeutic paclitaxel (PTX) intramuscularly or subcutaneously. In this study, integration of PTX and PLGA was achieved via a solvent evaporation method resulting in a solid dispersion of the substances. To customize degradation of the implants and secure delivery of high doses of PTX, chitosan and the PLGA-PTX blend were mixed in a 30:70 mass ratio. Cryomilling was utilized to create the chitosan-PLGA-PTX mixture, due to its proven effectiveness in producing homogenous blends. Implants were then fabricated into rods by injection molding and characterized in terms of content uniformity, morphology, and thermal property. The integrity of the blends was ascertained via x-ray diffraction, while miscibility between the drug and excipients was established by differential scanning calorimetry. *In vitro* drug release was studied in a phosphate buffer of *p*H 7.4 and measured by ultraviolet-visible spectrophotometry (UV-Vis). Meanwhile, the degradation rate was determined by quantifying mass loss at various points in 30 days. This study revealed the chitosan blended PTX-loaded PLGA implant possesses a longer, yet steadier, sustained drug release behavior than the PTX-loaded PLGA implant. The results suggest that introducing chitosan into PLGA implants through this fabrication method could be integrated to regulate and control the degradation rate of PLGA implants.

CHAPTER 1

GENERAL INTRODUCTION

Paclitaxel is an antimicrotubule agent approved for the treatment of various cancers, including breast, ovarian, head, and neck cancers [1-3]. Although traditional, infusion-based, paclitaxel therapy is effective, there are problems associated with the systemic delivery of high doses of paclitaxel that fail to provide a desired drug concentration to localized tumors. For instance, due to paclitaxel's hydrophobic nature, the current commercial aqueous solution is composed of Cremophor EL and ethanol, which may trigger or aggravate side effects, such as nephrotoxicity, induce hypersensitivity reaction, and neurotoxicity [4, 5]. Hence, a more appropriate drug delivery system is needed to not only achieve lower toxicity chemotherapy in comparison to conventional infusion methods, but also to provide a sustained-release of chemotherapeutics to the human body.

Incorporating drugs into biodegradable polymers for their delivery is one of the most widely used methods in current pharmaceutical science research [6]. The basic concept of this method is to maintain the drug’s concentration in body fluids over a long time by releasing the drug from the degraded polymers. Moreover, as a promising approach for delivery of most drugs, polymeric drug delivery systems have the following advantages: (1) there are abundant biocompatible polymers available, not to mention a plethora of modified or hybrid biopolymers and synthetic polymers; and (2) various forms of drug carriers made by polymers are available for processing, such as nano/micro particles, hydrogels, micelles, films, wafers, and foams.

So far, various polymeric drug delivery systems have been demonstrated to considerably enhance the therapeutic efficacy of paclitaxel according to previous research [5, 7-12]. However, there are some limitations and drawbacks associated with these methods. First, for certain

applications, the safety of the materials used during the complicated preparations, such as organic solvents and crosslinking agents [13], needs verification because traces of these substances may remain in the final products, and could be harmful to the human body. Second, the initial burst effect is a typical issue for most micro/nano particle systems [5] and micelles [9]. Lastly, for bulky implants, e.g., films, tablets, and wafers, surgical implantation is inevitable.

To overcome or avoid these common problems, this study proposed a different approachable polymeric drug delivery system for paclitaxel, a biodegradable polymer-based, rod-shaped implant. The rod-shape geometry of the proposed implant provides the advantage of allowing the implant to be easily placed into the human body subcutaneously or intramuscularly by insertion or surgical incision. In comparison, Norplant, also a rod-shaped implant has been marketed in the United States since 1991 and proven effective as an approach for contraception [14]. Another benefit of the rod-shaped design is that it can be fabricated using a common manufacturing method—injection molding—that can be more adequate for bulk fabrication. Therefore, in this study, biodegradable polymers were chosen to constitute the rod-shaped implant to provide sustained drug release.

PLGA, a synthetic polymer composed by poly lactic acid (PLA) and poly glycolic acid (PGA) [15], is one of the FDA-approved biodegradable polymers. It has been extensively used in biomaterials applications, especially in drug delivery systems [16]. Many studies have indicated PLGA has great biocompatibility and biodegradability as a drug delivery carrier for paclitaxel in the form of micro/nano particles [4, 7, 17, 18], foams [12], films [19], electrospun fibers [20], and hydrogels [21]. But, limited work has been completed on PLGA-based, rod-shaped implants for sustained release of paclitaxel. Preferably, the low glass transition temperature of PLGA makes it easy to process through injection molding. However, there are some minor problems

associated with the degradation of polymers composed of PGA or/and PLA in the human body [22, 23] These problems include (1) the degradation mechanism of PLGA, which degrades by bulk erosion, and it is not ideal for drug release since the erosion rate is difficult to control. (2) PLGA degradation yields acidic products, lactic acid and glycolic acid, which may increase the risk of inflammation at the implant site [22, 23].

To circumvent these issues, chitosan was selected to be added to the implant. Chitosan is one of the most abundant natural polymers in the world, and its characteristic biocompatibility and biodegradability makes it very attractive and practical in their use for biomedical applications [6, 23-34], including drug delivery systems [35], wound dressings [27], and artificial tissue/organ implants [26, 36]. In fact, several biomaterial applications composed of PLGA and chitosan have been reported previously. Chakravarthi and Robinson confirmed chitosan-PLGA particles are able to enhance the cellular association and cytotoxicity of paclitaxel [37]. However, blending Chitosan with PLGA to produce a bulk implant for delivery of paclitaxel has not been reported previously. According to the results from the work achieved by Wang et al. [30], the hybrid matrix composed by 70%w/w PGA and 30%w/w Chitosan was established as a new biomaterial with good mechanical properties and degradability. Therefore, a 30% weight ratio of chitosan to the whole implant was chosen for this study. Comparisons were made among PLGA rods, 10%w/w PTX loaded PLGA rods, 30%w/w chitosan loaded PLGA rods, and 10%w/w PTX - 30%w/w Chitosan loaded PLGA rods with regards to degradability on *in vitro* studies.

In this work, limited by the nature of chitosan—e.g., insoluble in most common organic solvents and water, rigid, brittle, and thermal sensitive—the method to blend chitosan with paclitaxel loaded PLGA was narrowed to cryogenic milling. Cryomilling, a variation of

mechanical milling under cryogenic temperatures, has become an effective technique to pulverize and blend substances, including polymers and metals [38]. Several articles have focused on the use of cryomilling to fabricate biodegradable scaffolds comprised of immiscible polymers to avoid undesirable chemical reactions, unstable morphologies, and unwanted polymer degradation caused by melt blending or use of harmful solvents [39, 40]. Followed by cryomilling, injection molding was applied in this work to process the mixed powders into the final products—rod-shaped implants.

The integrity of the blends was ascertained via x-ray diffraction. The miscibility between the drug and excipients was evaluated via differential scanning calorimeter. An *in vitro* experiment was performed to investigate the influence of chitosan on implant degradation behavior. Since erosion is the main mechanism that releases paclitaxel from biodegradable implants [5], the mass loss profile was utilized to determine the implant drug release rate. Meanwhile, the *p*H of the degradation medium was measured at fixed time points to investigate the differences in degradation among the implants.

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CHAPTER 2

LITERATURE REVIEW

This review of literature is divided into four parts: (1) chemotherapeutics, (2) drug delivery systems, (3) biomaterials, (4) manufacturing processes for drug delivery systems.

2.1 Chemotherapeutics

According to the 2015 report Cancer Statistics, breast cancer is identified as one of the three most commonly diagnosed cancers in women, estimated to be as high as 29% of all new cancers diagnosed in women [1]. There are many chemotherapy drugs used for breast cancer treatment, including alkylating agents (such as cyclophosphamide), anthracyclines (such as epirubicin), antimetabolites (such as methotrexate), anti-mitotics (such as vinorelbine), and taxanes (such as paclitaxel), to name a few. Although cyclophosphamide is used to promptly control cancers, its rapid replacement with less toxic drugs is highly recommended as once it is in use it causes severe, life-threatening, adverse effects, such as permanent infertility and acute myeloid leukemia [2, 3]. Other chemotherapy drugs suffer from yielding comparable severe side effects. For example, the antineoplastic agent epirubicin may increase the risk of developing leukemia [4] or left ventricular dysfunction [5]. While vinorelbine, a semi-synthetic vinca alkaloid, is mostly employed as salvage therapy because it has more severe side effects than the standard lines of therapy [6].

Compared with the above mentioned drugs, paclitaxel has many advantages. First, obtained from the pacific yew trees [7], it is environmentally safe and renewable. Second, as an antimicrotubule agent, it has been proven to be highly effective against breast, ovarian, head, and

neck cancers [8-10]. Most importantly, paclitaxel has a rare feature as a chemotherapeutic, which is effective for both reducing tumor size and terminating cancer cells. The primary mechanism related to the antitumor action of paclitaxel is that it promotes microtubule assembly, and induces the formation of nonfunctioning microtubules to prevent the formation and function of normal mitotic spindles, resulting in inhibition of cellular division [10]. Paclitaxel is extremely protein bound (88-98%) [11] and highly lipophilic [12]. However, for paclitaxel to be administrated intravenously an aqueous solution composed by Cremophor EL and ethanol is required, which can cause side effects, such as nephrotoxicity, hypersensitivity, and neurotoxicity [13, 14]. Moreover, due to paclitaxel's undesired nature of hydrophobicity and fast plasma clearance when administered by infusion, its application is limited in oncology [15].

2.2 Drug Delivery Systems

Drug delivery systems are developed to optimize the therapeutic properties of drugs and improve their safety and efficacy. They aim to [16]: (1) reduce the frequency of dosing to improve patient compliance, (2) enhance the bioavailability of the drug to maximize its utilization, and (3) prevent the drug's adverse side effects. So far, polymeric drug delivery systems have been proven effective when it comes to sustaining drug release and maintaining drug concentrations within therapeutic levels [17]. In general, biodegradable polymers are more commonly used than non-degradable polymers for drug delivery because of no necessity to recover the polymer after drug release. The drug's release rate can be determined by the drug's diffusion behavior and/or the degradation mechanism of the polymer system [18]. The following drug delivery systems have been studied extensively: micro/nanoparticles, hydrogels, and implants.

2.2.1 Micro/nanoparticles

Generally speaking, this type of drug carrier aims to entrap, encapsulate, or attach the drug to a particle size of polymer matrix, and release the drug by diffusion and/or polymer erosion once it comes in contact with aqueous body fluids. Nanoparticles can be prepared through two major methods [19]: (1) dispersion of the polymer particles formed by emulsification, coacervation, or supercritical fluid technology; and (2) polymerization of monomers. Microparticles can be achieved through the above mentioned methods by adjusting the process parameters. Early work by Fonseca et al. focused on the development of paclitaxel-loaded poly (lactic-co-glycolic acid) (PLGA) nanoparticles, which demonstrated a strong enhancement on anti-tumoral efficacy in comparison to the free drug Taxol [20]. Natural biodegradable polymers, such as chitin, can also be used to process into micro particles for localized delivery of paclitaxel in solid tumors, as reported by Nsereko and Amiji [21]. Although micro/nanoparticles help increase the stability of drugs and possess useful control release properties, the challenges of burst release and harmful chemical usage during fabrication still remain [19, 22].

2.2.2 Hydrogels

Hydrogel based systems have also attracted increasing interest over the past two decades. The drug release is controlled by the hydrogel's swelling property, as well as its stimuli-responsive structure changes [23]. Lin et al. developed a novel thermo-sensitive paclitaxel-nanocrystals (NCs)-Gel (Pluronic®F127) system, which were characterized by high drug-loading and extended drug retention inside the tumor, due to its sustained, stable drug release [24]. In the work by Shim et al. [25], a PTX-loaded *p*H/T-sensitive block copolymer (OSM–

PCLA–PEG–PCLA–OSM) solution showed the sustained release profile of paclitaxel for one month without burst release. However, the inherent difficulties in stabilization of emulsions and satisfactory drug loading existed in hydrogels based systems [26-28].

2.2.3 Implants

Polymer implants can be fabricated to various shapes and sizes by biodegradable polymers [18], such as pellets, tablets, films, foams, and rods. The implantation methods include subcutaneously insertion and surgical placement [29]. This system is useful and effective for drugs with poor water solubility and short half-lives. A successful, commercial, polymer-based implant, Norplant, has been marketed in the United States since 1991, and has proven to be an effective approach for contraception [29]. By implanting the levonorgestrel-contained tubes in the upper arm, the Norplant system can successfully provide a therapeutical level of levonorgestrel for as long as 5 years. Ong et al. completed a more recent study [15]. They presented potential implants for post-surgical chemotherapy against malignant glioma, a paclitaxel-loaded, PLGA micro-porous foam fabricated by supercritical CO_2 gas foaming. An *in vitro* evaluation showed the foam was able to provide a sustained paclitaxel release with minimum burst release. Another group of scholars found hybrid films from blends of Chitosan and egg phosphatidylcholine (ePC) can provide a sustained release of paclitaxel over a 4-month period in an *in vitro* study [30].

2.3 Biomaterials

Materials (synthetic and natural) used in contact with biological systems are commonly called biomaterials [31]. Biomaterials have four major categories: polymers (the largest

category), metals, ceramics and natural materials. Polymers, including synthetic polymers and natural occurring biopolymers, are widely used in numerous medical devices, such as drug delivery systems, tissue engineering applications, dental, orthopedic, and so on [31, 32].

2.3.1 Synthetic polymers

Synthetic polymers are synthesized through polymerization (addition and condensation) processes. Biodegradable and biocompatible synthetic polymers have been employed widely in drug delivery systems. The following synthetic polymers are commonly used for biomedical applications [31]: polycaprolactone (PCL), polyanhydrides, poly (glycolic acid) (PGA), poly (lactic acid) (PLA), and poly (lactic-co-glycolic acid) (PLGA).

2.3.1.1 Polycaprolactone (PCL)

PCL is a semi-crystalline biodegradable polymer. It has acceptable mechanical properties and a slow rate of hydrolysis, which makes it suitable for long-term (over 1 year) drug delivery carriers, orthopedic applications and stents. The use of PCL in drug delivery applications has been well established [31, 33]. However, because of its hydrophobic nature, semi-crystalline structure, and slow hydrolysis degradation in human body, its clinical application has been limited [34].

2.3.1.2 Polyanhydrides

Polyanhydrides are well-known synthetic biodegradable polymers used mostly in drug delivery systems owing to their unique benefits. For instance, they are easily prepared from economical resources in one-step synthesis without a purification process. Moreover, with certain manipulation, polyanhydrides can release drugs at a predictable rate for weeks to months [35]. One polyanhydride wafer containing chemotherapeutics, Gliade, has been in clinical use for

brain tumor chemotherapy [36, 37]. However, polyanhydrides' hydrolytic instability [31, 35] and high chemical reactivity [31] limit their use.

2.3.1.3 Poly (glycolic acid) (PGA) and poly (lactic acid) (PLA)

PGA and PLA are both linear aliphatic polyesters [31]. Both degrade in the human body by hydrolysis of their ester backbone to non-toxic compounds discharged metabolically. PGA is more hydrophilic, while PLA is more hydrophobic. PGA is a rigid thermoplastic material with high crystallinity, and can be fabricated by polycodensation or ring-opening polymerization, while PLA is a semi-crystalline solid that can be synthesized from low-cost natural raw materials, such as corn. In addition, they are both non-immunogenic and biodegradable polymers with acceptable mechanical properties. Hence, PGA and PLA have become the most widely studied polymers in medical applications and tissue engineering [38, 39], e.g. absorbable sutures [40, 41]. However, two major challenges exist with the use of PGA. One is PGA's high sensitivity to hydrolytic degradation requires devising precise processing [40]. The other challenge is due to a lack of functional diversity in the backbone, where the modulation of its degradation rate and chemical properties are inhibited [41]. As for PLA, hydrophobicity and acid degradation products are the main weaknesses.

2.3.1.4 Poly (lactic-co-glycolic acid) (PLGA)

Poly (lactic-co-glycolic acid) (PLGA) is a synthetic copolymer composed by poly lactic acid (PLA) and poly glycolic acid (PGA) [42]. Among the various polymers used in drug delivery systems, PLGA has gained a lot of popularity due to its biodegradability, biocompatibility, ease of processing and excellent mechanical properties [43]. This polymer degrades to non-toxic, water-soluble products—lactic acid and glycolic acid—through hydrolysis in the human body fluid environment. Owning to its excellent biocompatibility, biodegradability,

and compatibility with drugs, PLGA has become one of the FDA-approved, biodegradable polymers [44]. According to the review conducted by Makadia and Siege [42], PLGA possesses great mechanical ability allowing it be processed into almost any shape and size. The review also pointed out it can be fabricated to micro/nanoparticles [13, 20, 45, 46] by solvent evaporation, phase separation, and spray drying, or processed into implants by solvent-casting, compression molding, and extrusion. PLGA foams [15], films [47], electrospun fibers [48], and hydrogels [25] also have been successfully produced for drug delivery applications and tissue engineering.

Similar to PGA and PLA, PLGA still has drawbacks which hinder its use as a drug carrier. One of the most common problems is the accumulation of acidic degradation products during the degradation of matrix systems, which may trigger a nonbacterial inflammation at the implant site [49, 50]. Also, because PLGA degrades through bulk erosion, the required drug release profile is difficult to obtain.

2.3.2 Natural polymers

Natural polymers (biopolymers) refer to naturally occurring polymers, such as polymers derived from animal or plant sources [31]. These types of polymers have been under extensive investigation for use in drug delivery systems due to their biodegradability, biocompatibility, and renewable nature. Two major representative degradable biopolymers, collagen and polysaccharides (e.g. chitosan), are discussed.

2.3.2.1 Collagen

Collagen is derived from animal sources (e.g., bovine, porcine, etc.) and possesses an excellent biocompatibility because it widely exists in bones, muscles, skin and tendons [51]. It can be used for a variety of medical devices due to its versatile functionality and absorption

features [52], such as artificial skins [53], orthopedic applications [54], soft tissue augmentation [55], dental applications [56] and drug delivery systems [57]. In an early study of collagen-based drug delivery systems for protein drugs (e.g. human serum albumin), Maeda et al. [58] suggested that after a relatively small initial burst release, human serum albumin was released from collagen minipellets mainly by diffusion. Although collagen has outstanding functionality and mechanical properties as a potential biomaterial, its development in clinical use is inhibited due to the imperative request for methodologies to purify and sterilize it [31].

2.3.2.2 Chitosan

Chitosan is one of the most abundant, natural polymers in the world. Its characteristic biocompatibility and biodegradability makes it very attractive and practical for its use in biomedical applications [38, 50, 59-69], including drug delivery systems, wound dressings, and artificial tissue/organ implants [62, 70]. Chitosan does not induce an immune response in the human body. It decomposes into harmless products (amino sugars) by hydrolysis and enzymatic degradation by lysozyme [71]. Bhattarai et al. investigated the newest developments in chitosan hydrogel preparation and concluded chitosan hydrogel can be utilized in the context of localized drug delivery and controlled release to its target [72].

Distinguished from many other natural polymers, chitosan is a cationic polysaccharide [71] composed of randomly distributed β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine units [72, 73]. Hence, adding chitosan to a PLGA implant may provide some advantages in comparison to using PLGA alone, such as neutralizing the acidity at the implant site. In fact, the biomaterial applications involving composites of PLGA and chitosan have been reported previously. Chakravarthi and Robinson confirmed chitosan-PLGA particles are able to enhance cellular association and cytotoxicity of paclitaxel [74]. Other similar studies on the use

of chitosan with biodegradable polymers have been conducted as well. Zhu et al. reported a composite scaffold made from chitosan microspheres and synthetic poly (L-lactic) (PLLA) is applicable for tissue engineering because of its adjustable degradation property [50]. A scaffold composed by Polyglycolide (PGA) and chitosan also has been proposed [38], and the study showed the PGA-chitosan (containing 30%w/w chitosan) hybrid matrix is a promising biomaterial for a variety of artificial tissue systems, due to its high porosity and good mechanical properties. However, blending Chitosan with PLGA to make a bulk implant for delivery of paclitaxel has not been reported previously.

Most of previous studies employed emulsification, solvent evaporation, or chemical conjugation to incorporate into or onto PLGA [74]. However, the substance produced by these methods is unsuitable for injection molding.

2.4 Manufacturing Processes for Drug Delivery Systems

Polymeric drug delivery systems can be processed and fabricated in various forms and sizes by certain manufacturing methods. Most nano/micro particle sized drug delivery systems are prepared through emulsification or polymerization of monomers [19]. For those macroscopic millimeter size drug implants, solvent casting, compression molding, extrusion, or injection molding are primarily employed [42]. In addition, more techniques have been explored to manufacture drug delivery systems, such as cryomilling, a novel method to grind and blend materials under cryogenic condition [75].

2.4.1 Emulsification

Emulsification is the most commonly used technique for fabrication of drug incorporated particles [19]. The process involves two or more liquids usually immiscible, e.g., water and oil. By dispersing one liquid in the other through stirring or sonicating, an emulsion can be achieved, such as oil-in-water emulsion or water-in-oil emulsion. To stabilize the emulsion, surfactants are often used while preparing and storing the emulsion. Mu and Feng [46] proposed a novel formulation containing vitamin E TPGS as an emulsifier or a component of the polymer matrix to fabricate paclitaxel loaded nanoparticles. Although this technique is effective, there still remain some drawbacks. For instance, emulsification requires precise control of processing parameters for consistency of the final product and efficiency of drug encapsulation. Moreover, the need for a large quantity of organic solvent makes the process unrealistic for industrial scalable production [19, 42] and the use of surfactants may cause side effects.

2.4.2 Solvent casting

In the solvent casting method, polymers and drugs are dissolved in a common solvent at ambient temperature, and then the solution is cast into a Teflon plate or glass dish [76]. After evaporation of the solvent, drug-loaded polymer films are obtained. This method is proven to achieve homogenous dispersion of drugs in polymer matrices [77]. However, Makadia and Siegel [42] argued that solvent casting is not ideal for industrial scale-up, due to the use of large amounts of organic solvents and the possibility of causing polymer denaturation.

2.4.3 Extrusion

Extrusion is a continuous process to draw melted materials through a die to create objects of a fixed cross-sectional profile [31]. Polymers and drugs mixtures can be extruded into tubes or fibers. During the process, polymers and drugs mixtures are heated to a semi-liquid state in the chamber, and then pushed by the screw through the die [78]. The extrudate is cooled and solidified before trimming to expected lengths. Since this process requires heat treatment to melt the materials, it could lead to polymer and drug denaturation [42]. Hence, the melting point and thermal stability of drugs, along with the chemical interactions between the used polymers and drugs should be carefully evaluated before adopting this manufacturing method.

2.4.4 Injection molding

Injection molding has been employed widely for the production of biomedical devices in previous research studies [79-85]. This is a common manufacturing technique more appropriate for large-scale, industrial production [78] and available for making various shapes of implants [78, 86]. Briefly, the process melts the thermoplastic or thermoset materials, and injects them into a closed mold to obtain the desired shape and size. Wu et al. [83] proposed a "room-temperature" injection molding combined with the particulate leaching method to fabricate three-dimensional, porous scaffolds by using a "wet" composite of particulate/polymer/solvent in the process of injection molding to avoid the inconvenience of handling high viscosity substances and thermal degradation of polymers. They found the approach might be promising in tissue engineering and other application fields.

2.4.5 Cryomilling

Cryomilling is a technique used to reduce the agglomeration of the substance by crushing it under cryogenic temperatures using a solenoid to move the grinding media back and forth inside the vial. This has become an effective technique to pulverize and blend substances, including polymers and metals [87, 88]. Several articles focus on using cryomilling to fabricate biodegradable scaffolds comprised of immiscible polymers to avoid undesirable chemical reactions, unstable morphologies, and unwanted polymer degradation caused by melt blending or the use of harmful solvents [88, 89]. Lim et al. used cryomilling to achieve micrometer-sized distribution of polycaprolactone (PCL) and tricalcium phosphate (TCP), and then processed them with compaction and melting to obtain composite scaffolds [89]. Their work demonstrated an easy access, polymer powder processing technique to overcome limitations on distribution and loading of biologically active molecules. In conclusion, to achieve a homogeneous blend consisting of highly viscous polymers (e.g., PLGA) and thermal sensitive materials (e.g. chitosan), cryomilling could be an appropriate manufacturing method to not only secures the integrity of the materials, but also to enhance the degree of dispersion.

2.5 Summary of Literature

This literature review aims to demonstrate the need for a more effective drug delivery system for hydrophobic chemotherapeutics. (1) A more appropriate drug delivery system is needed for paclitaxel, since the commercial method has severe side effects and the current novel methods still have challenges to overcome. (2) The proposed PLGA rod-shaped polymer system can be produced and implanted easily into the human body via insertion, and blending chitosan in the PLGA implant could alter the degradation rate to achieve prolonged drug release. (3) No

previous study was presented in the literature for fabrication of chitosan-blended PLGA implant for delivery of anti-tumor agents through cryomilling and injection molding.

2.6 Thesis Organization

Chapter 1 presents the general introduction of this work. Chapter 2 provides a literature review of the state of delivery systems for paclitaxel and possible strategies for improving the drug's release by employing polymers as delivery mechanisms. This review demonstrates the need for a more effective drug delivery method for chemotherapeutics (i.e., paclitaxel) to solve the problems of low efficiency of the systemic delivery of high doses of toxic drugs for localized tumors. To fill this void, Chapter 3 presents original work in the format of paper for peer review publication titled an implantable sustained-release chemotherapy delivery system for the treatment of breast cancer. Chapter 4 of this thesis provides general conclusions and an outline for future research directions.

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CHAPTER 3

AN IMPLANTABLE SUSTAINED-RELEASE CHEMOTHERAPY DELIVERY SYSTEM FOR THE TREATMENT OF BREAST CANCER

Manuscript to be submitted to the Journal of Medical Devices

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Abstract

A Poly (Lactic-co-Glycolic Acid) (PLGA) and chitosan based biodegradable implant was designed for the sustained release of the hydrophobic chemotherapeutic Paclitaxel (PTX) intramuscularly or subcutaneously. PLGA-PTX blends were fabricated via solvent method then mixed with chitosan in a 30:70 mass ratio by cryomilling. The powder mixture was then molded to rods by injection molding. The content uniformity, morphology, and thermal properties were investigated by x-ray diffraction, scanning electron microscope and differential scanning calorimetry, respectively. The drug loading content (LC), encapsulation efficiency (EE) and the drug release were measured by ultraviolet-visible spectrophotometry (UV-Vis). *In vitro* study revealed that the chitosan blended PTX-PLGA implant possesses a prolonged drug release. Chitosan showed a potential to regulate and control PLGA implant degradation.

Key words: Poly (Lactic-co-Glycolic Acid) (PLGA), chitosan, breast cancer, drug delivery, cryomilling

3.1 Introduction

Since chemotherapeutics usually cause a risk of local toxicity in the gastrointestinal track [1], they are commonly administered intravenously instead of orally. In spite of the effectiveness of traditional infusion-based chemotherapy, there are problems associated with the systemic delivery of high doses of toxic drugs that fail to provide a desired drug concentration to localized tumors [2]. For instance, the solubilizer, Cremophor EL, use to form the clinical liquid solution for chemotherapeutic Paclitaxel is found to cause severe side effects [3, 4]. Thus, drug delivery devices have been explored extensively for the purpose of maximization of the anticancer agent effects and minimization of the side effects on healthy cells and tissues [4-7].

Biodegradable polymer drug delivery systems are commonly used since there is no necessity to recover the polymer after drug release, and because they have been proven to be effective for sustained drug release while maintaining drug concentrations within therapeutic levels [8]. Moreover, local administration of chemotherapeutics via implantation of a drug-incorporated polymer device could be a practical way to reduce systemic exposure, increase the dosage of drugs at specific area, [9] and optimize the drugs therapeutic effects [9]. An example of a commercially successful polymer-based drug delivery system is Norplant, which has been marketed in the United States since 1991, and has proven to be an effective approach for contraception [10]. Several forms of drug delivery systems have been made with biodegradable polymers, i.e., micro/nano particles, micelles, hydrogels, and implantable solid dispersions, wafers, films, and rods [8, 11]. Unfortunately, each of these drug delivery systems presents unique shortcomings. For instance, although micro/nano particles have proven to improve treatment efficacy while reducing toxicity, the challenges of low drug loading and high burst release still remain [12, 13]. While with micelles and hydrogels, there are concerns over their use

due to residuals of potentially toxic crosslinking agents [14] or solvents used for their fabrication, and more relevant due to the technical obstacle of colloid stabilization [6, 14, 15]. To circumvent these issues, we present an injectable rod-shaped polymeric implant for the delivery of chemotherapy drugs.

Paclitaxel (PTX) is a highly toxic chemotherapeutic [16]. It is widely used as an antimicrotubule agent for treating a variety of cancers, including breast, ovarian, head, and neck [17]. However, due to its undesired nature of hydrophobicity and fast plasma clearance when administered by infusion, its application is limited in oncology [18]. Various studies have focused on employing polymers to act as drug vehicles for PTX, such as Poly (Lactic-co-Glycolic Acid) (PLGA) [19], Polycaprolactone (PCL) [20] and Poly (lactic acid) (PLA) [21]. Among the available biomaterials, PLGA is the most popular biodegradable polymer, due to its favorable degradation characteristics and immense potential for sustained drug delivery [5].

PLGA is an FDA-approved biocompatible and biodegradable synthetic polymer. Various studies reporting on the use of PLGA as carrier for paclitaxel have been published where the use of PLGA nanoparticles as effective drug carriers in cancer therapy is explored [3, 18, 22-24]. Additionally, other studies emphasizing the use of PLGA as drug delivery implants indicated that besides nanoparticles, PTX-loaded PLGA foams [18], compressed microsphere sheets [25], and microfiber discs [26] can also provide a sustained PTX release with minimum initial burst. However, limited work has been reported on the use of PLGA as injectable solid implants for delivery of PTX, which could be easily inserted and provide a sustained drug release at a tumor site. Therefore this study, introduces the design of injectable PTX solid implants containing the biodegradable cationic polymer, chitosan [27], to reduce the risk of local acidosis resulting from

the accumulation of PLGA hydrolysis products, lactic acid and glycolic acid, at the implantation location [27].

Chitosan is a naturally-occurring polysaccharide [28] composed of randomly distributed β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine units [16, 28]. It has been used in tissue engineering and pharmaceutical fields due to its versatile biological characteristics [27]. However, because chitosan is non-soluble in organic solvents or water [29], the incorporation of PTX cannot be achieved by the solvent method. Meanwhile, to avoid the use of high-heat methods (grinding, melting, etc.), which could result in decomposition of the biopolymer, cryomilling [30-32] was employed to homogeneously blend chitosan with PTX and PLGA [30-32].

In this work, a fabrication process without using solvent to integrate chitosan with PLGA has been developed and investigated. The influence of chitosan on the PTX release of PTX-loaded PLGA rod-shaped implants, and the resulting *p*H levels in the local medium was investigated through *in vitro* release studies. Overall fabrication of implants included a solvent method followed by cryomilling and injection molding. The integrity of the blends was ascertained via X-ray diffraction (XRD). The miscibility between the drug and excipients was evaluated via Differential Scanning Calorimeter (DSC). *In vitro* release studies were performed in a phosphate buffer solution *p*H 7.4.

3.2 Materials and Methods

PLGA 50:50 (PDLG 5002) was purchased from Purac Biomaterials (Gorinchem, Netherlands). Paclitaxel was purchased from TSZCHEM (Framingham, Massachusetts, USA).

Chitosan (medium molecular weight), phosphate buffered saline (PBS) and methylene chloride (DCM) were acquired from Sigma-Aldrich Co. LLC (Missouri, United States).

3.2.1 Preparation of polymer implants

3.2.1.1 Polymer implants formulations

Table 3.1 lists the detailed formulations used in this work. In this study, 10%w/w of PTX drug loading was used [18]. In order to investigate the effect of 30% w/w chitosan (CHI) on 10% w/w PTX drug loaded PLGA implants drug release, PLGA-PTX implants, and PLGA-PTX-CHI implants were compared [33] in *in vitro* release studies. Meanwhile, the polymer erosion of different formulations was monitored by mass loss.

Table 3.1 Polymer implant formulations

Drug Composition	Feed Weight Ratio		
	PLGA	PTX	Chitosan
PLGA	100	0	0
PLGA-PTX	90	10	0
PLGA-CHI	70	0	30
PLGA-PTX-CHI	60	10	30

3.2.1.2 Fabrication protocol for polymer implants

The fabrication protocol of the implants consists of the following three steps: (1) incorporation of PTX into the PLGA polymer matrix by solvent method, (2) blending of chitosan and PTX-loaded PLGA by cryomilling, and (3) molding into rod-shaped implants via a lab-scale injection molding device.

Specifically, first PLGA and PTX were dissolved in the organic solvent methylene chloride (DCM), and magnetic stirred at room temperature for 1 hour. DCM was evaporated in a fume hood for 12 hours followed by drying at 25℃ for 6 hours in a vacuum oven. The solid

dispersion was collected then. Chitosan was introduced to the PLGA PTX-loaded PLGA dispersion via cryomilling (SPEX SamplePrep 6770 Freezer/Mill, United States). The cryomilling protocol is depicted as follows: pre-cool material for 10 minutes, grinding for 6 minutes at a rate of 15 cycles per second (CPS) 3 times, cool down for 2 minutes between each operation.

Implants (1mm of diameter × 15mm of length) were finally molded in a lab-scale injection molding device. Mold was pre-heated to about 70°C [5]. Then, 0.8 g of the prepared powder mixture was charged into the mold. By applying pressure onto the guide rod (piston), the melt polymer mixture was injected through a 5 mm diameter nozzle into the mold cavity and formed into rod-shaped implants. After cooling down the mold, the implants were removed from the mold and stored in a freezer to preserve the integrity of PLGA and PTX.

3.2.2 Polymer implants characterization.

The integrity of the drug-polymers blends was ascertained via x-ray diffraction. Meanwhile, x-ray diffraction was able to investigate the crystallinity of blended polymers, which has direct influence on the mechanical strength, capacity to undergo hydrolysis, and subsequently, biodegradation rate. The x-ray diffraction (XRD) studies were conducted on each formulation sample prior to *in vitro* release studies. Samples were mounted into glass slides and scanned over a 2-theta range of 1-60 ° at a rate of 5 °/min. All XRD patterns were obtained using an X-ray diffractometer (MiniFlex 600, Rigaku, Japan) equipped with Cu K-alpha radiation source (40kV, 15mA). The homogeneity of PLGA-PTX-CHI rod was evaluated through Energy Dispersive X-ray Spectroscopy (EDS). Since PTX and chitosan both have nitrogen element, EDS experiment was conducted to compare nitrogen content in different section of a rod.

The morphology of the surface and cross-section of the implants were observed by scanning electron microscope (SEM) (JCM-6000 NeoScope Benchtop SEM, JEOL Ltd., Japan).

To investigate the miscibility between the drug and excipients, the glass transition temperatures (Tg) of implants were determined by differential scanning calorimetry (DSC 204 F1 Phoenix, NETZSCH, Germany). Additionally, the implants underwent *in vitro* release studies in different periods of time were also examined by DSC to capture changes in glass transition temperatures. All specimens were weighed to approximately 5-10 mg and analyzed by using a heat/cool/heat mode to eliminate their thermal history. A blank aluminum pan (pierced, 40mg) was applied as reference in all analyses. Each run consisted of initially heating up the samples to 250.0 ℃ at a heating rate of 20.0 K/min, followed by a second heating cycle to 250.0 ℃ again at the same heating rate after cooling down to -40.0 ℃ at 20.0 K/min [34]. The Tg of the polymeric rods were analyzed from the second heating cycle.

3.2.3 Drug Loading Content (LC) and Drug Encapsulation Efficiency (EE).

In this study, an established quantitative analysis of PTX [35] through UV-Vis (LAMBDA 750 UV/Vis/NIR Spectrophotometer, PerkinElmer, U.S.) was employed. In this method, methanol and PBS (*p*H 7.4) in a ratio of 30:70 [35] was used as solvent, and PTX absorbance was measured at λ_{max} (230nm) against blank solvent. The standard calibration curve in the concentration range of 1-20 μg/ml was determined as follow:

$$Y = 0.0348X+0.0036 \ (R^2= 0.998) \quad (1)$$

where Y is the absorbency of PTX at 230nm, X is the concentration of PTX and R^2 is the regression coefficient. The percentage recovery of PTX was found to be 98.30% to 102.55%.

The drug loading content (LC) and the drug encapsulation efficiency (EE) were determined in triplicate using UV-Vis assay described below. Accurately known weights (~5mg) of rods were dissolved in 2ml DCM first, and then were added 2ml methanol to the residue after DCM had evaporated. The solution was centrifuged at 6500rpm for 15min after being agitated overnight, then the supernatant was extracted. PBS and methanol was added into the supernatant to adjust the finial solvent made up with 30:70 methanol: PBS. The corresponding concentration was determined by the equation (1). The drug loading content (LC) and the drug encapsulation efficiency (EE) were obtained through the following formula [36]:

$$\text{LC\%} = \frac{m_{PTX}}{M_{implant}} \times 100 \quad (2)$$

$$\text{EE\%} = \frac{m_{PTX}}{M_{PTX}} \times 100, \quad (3)$$

where m_{PTX} is the mass of PTX loaded in implants, and $M_{implant}$ is the mass of implants, M_{PTX} is the mass of PTX added initially during the fabrication.

3.2.4 *In vitro* Release Studies

3.2.4.1 PTX Release from Implants.

The *in vitro* PTX release studies for the implants PLGA-PTX and PLGA-PTX-CHI were carried out in 15mL of PBS (*p*H 7.4) in triplicate. All tubes were capped, sealed, and placed in an incubator at 37 ℃ at 60 rpm [37]. Because human body is a huge buffer system, the study was using PBS (*p*H 7.4) to simulate human body environment. Each day 5ml of buffer was removed and replaced with same amount fresh PBS to maintain sink conditions throughout release. Meanwhile, PTX in the removed release medium was measured to determine the total release amount of PTX. The steps were described below: first, to extract PTX by adding 2ml DCM and allowing mass transfer for one day [18]; then, after DCM had evaporated, methanol was added to

the extracted PTX followed by PBS to adjust the finial solvent concentration to 30:70 methanol: PBS. The corresponding concentration was calculated by the equation (1). To correct the detected data, the extraction efficiency had to be analyzed [18, 38]. Hence, a known amount of PTX was dissolved into PBS solution and was later extracted using the same procedure. The extraction efficiency was found to be 76.4%, which indicated that after the extraction procedure, the obtained solution only contained 76.4% of PTX in the original solution.

3.2.4.2 Mass Loss of Implants.

Previous studies have shown that the drug release kinetics of drug delivery systems made from biodegradable polymers are controlled by diffusion and/or erosion [39]. Polymer erosion refers to depletion and physical changes of polymer which caused by degradation, swelling, dissolution and diffusion of oligomers and monomers [40]. Hence, to investigate the impact of the polymer erosion on drug release, mass loss of the implants with respect to time was taken as a measure of erosion in *in vitro* release studies. Individual implants were weighed initially as $m_{initial}$. At fixed time intervals of two days until day 30 was reached, specimens (n=3) were taken out from the tube and washed with distilled water, then dried by vacuum oven at 20℃ for 24 hours and weighted as m_{XDD}. The mass loss was defined as follows [41, 42]:

$$\%\text{Mass loss} = (m_{initial} - m_{XDD})/m_{initial} \times 100 \quad (4)$$

where, m_{XDD} is the weight of specimen after X Days of Degradation, and $m_{initial}$ is the initial weight of the dry implants before *in vitro* release studies.

3.2.5 *p*H change in release medium

To investigate the *p*H change in the medium during *in vitro* release, triplicate samples of the implants from each drug formulation were placed individually in 15ml of PBS (*p*H 7.4) in an

incubator with the same condition of *in vitro* release studies (37 ℃ and at 60 rpm, refreshing 5ml PBS every day). Meanwhile, to eliminate the influence of refreshing PBS, *p*H change studies without refreshing PBS were conducted as well to investigate the effect of chitosan on release medium *p*H change. At various time intervals, *p*H measurements were performed with the Sartorius Basic Meter PB-11.

3.3 Statistical Analysis

The drug loading content (LC), encapsulation efficiency (EE) of PLGA-PTX and PLGA-PTX-CHI implants were statistically analyzed by Student's t-test. Results of the mass loss in *in vitro* drug release studies, and *p*H change studies are presented as mean ± standard deviation (S.D.), and analyzed by one-way analysis of variance (ANOVA) with Tukey's post hoc analysis. Statistical difference was accepted when $p \leq 0.05$ (95% confidence interval).

3.4 Results and Discussion

3.4.1 Polymer implants characterization

The XRD patterns of pure chitosan, pure PTX and the four formulations of implants are shown in Figure 3.1 arranged in descending order by the intensity of the peak at 2-theta ≈ 20°. As a crystalline polymer, pure chitosan exhibits two clear characteristic peaks at 2-theta - 10.4° and 19.6° in the diffractogram, which is in accordance with the results found by Kumar and Koh [38]. Unlike chitosan's high intensity narrower peaks, a broader peak in the 18-25° is shown in the spectra for the implants PLGA-CHI and PLGA-PTX-CHI, which may have been contributed by the high percentage of the amorphous polymer PLGA. The phenomenon of broadened peak, that is the decreased intensity was due to the reduction in crystallite size. Implants that did not

contain chitosan, PLGA and PLGA-PTX implants, displayed a broad peak in the diffractogram due to the large proportion of amorphous polymer PLGA. Also, no characteristic crystalline peaks were observed for PTX in the spectra suggesting that PTX was amorphous in the implants.

EDS analysis indicated that the concentrations of element nitrogen at different locations of PLGA-PTX-CHI rod are almost the same (1.98 ± 0.13%). Although element nitrogen is present on both PTX and CHI, EDS analysis of PLGA-PTX implants did not detect nitrogen, which probably due to the extremely low concentration of nitrogen in PLGA-PTX implants (the theoretical value: 0.16%) which is beyond the sensitivity range of the EDS. The results showed in Figure 3.2 confirmed that a homogenous composition throughout the rod.

The cross section and surface morphologies of each formulation implant before and after *in vitro* were examined by SEM and presented in Figure 3.3-3.6, respectively. For the PLGA and PLGA-PTX implants, a coarse surface and an interconnected porosity was observed, which could be attributed to the bulk erosion of the PLGA matrix. But, fewer irregular cracks existed on the PLGA-PTX implants than in the PLGA implants, which might be attributed to the hydrophobic PTX being dispersed in the PLGA matrix, and assumed to keep the implants from collapsing and disintegrating. For the PLGA-CHI and PLGA-PTX-CHI implants, although erosion occurred on the surface of the implants, the cross section images implied that there was no sign of water penetration in the interior of the implants after 30 days. This phenomenon might be due to the process of PLGA hydrolysis being suppressed to some degree as a result of chitosan protonation, which is the adsorption of hydrogen ions, leading to an alkaline medium.

The glass transition temperatures (Tg) of as-manufactured samples and *in vitro* tested samples were determined by DSC, results for four formulations implants are presented in Figure 3.7-3.10. It appears that the Tg of PLGA and PLGA-PTX sample implants, gradually decreased

with longer *in vitro* duration. This result corresponds to the PLGA erosion mechanism, bulk erosion, which leads to Tg decrease as PLGA degradation proceeds [44]. Specifically, during the degradation process, the polymer chains split into random segments first, which leads to decrease of the molecular weight of PLGA without an appreciable mass loss; then due to the formation of soluble monomers and oligomers, the mass of PLGA drops rapidly. However, the DSC thermograms for the PLGA-PTX-CHI and PLGA-CHI implants (before and after 30 days of *in vitro* release studies) did not exhibit such change in Tg as the PLGA and PLGA-PTX implants, even though morphological changes were observed on the surface of the implants.

Considering the observations from SEM, it appears that the determined Tgs of PLGA-CHI and PLGA-PTX-CHI implants represented mostly the inner part of the implants, which may have not encountered any degradation or erosion. This observation demonstrates that the degradation mechanism of chitosan incorporated implants may be in line with surface erosion instead of bulk erosion.

According to a prior study conducted by Averineni et al. [3], the characteristic endothermic peak of pure PTX is at around 220°C on the DSC thermogram. However, from DSC results derived for the PLGA-PTX and PLGA-PTX-CHI implants no melting phenomenon was observed. Such behavior may be due to the conversion of PTX from a crystalline to an amorphous state and/or it being dissolved in the polymer matrix. The reason that the DSC curve does not detect the Tg of chitosan may be associated with the thermal decomposition that occurs during the glass transition of chitosan due its semi-rigid molecular properties.

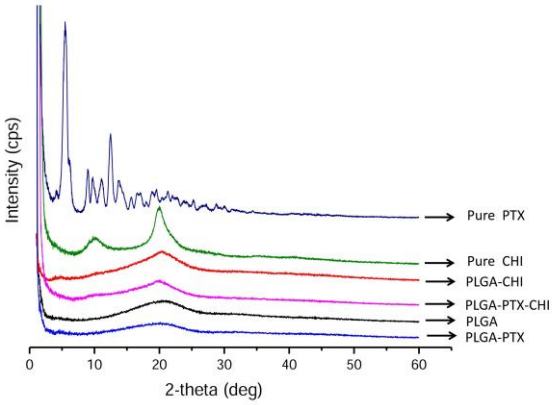
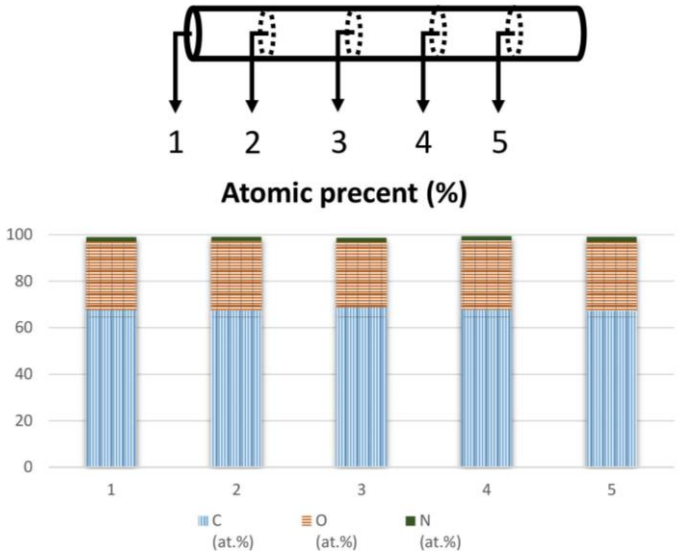


Figure 3.1 XRD diagram for pure CHI, pure PTX and the four formulations of implants



Location	C (at.%)	O (at.%)	N (at.%)
1	67.6	29.4	2.0
2	67.5	29.7	1.9
3	68.9	27.9	1.9
4	67.9	29.6	1.9
5	67.2	29.7	2.2

Figure 3.2 EDS analyses of PLGA-PTX-CHI implant at different locations as labelled by the numbers

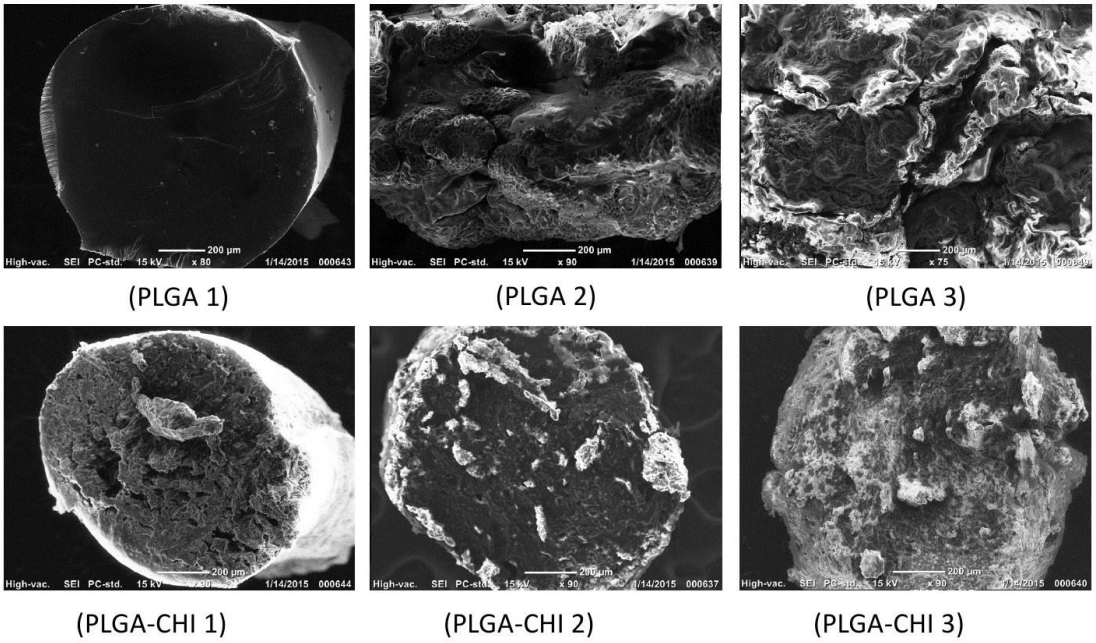


Figure 3.3 SEM images of cross section morphologies of (PLGA 1) PLGA implant specimen before degradation experiment, (PLGA 2) PLGA implant specimen after 14 days degradation, (PLGA 3) PLGA implant specimen after 30 days degradation, (PLGA-CHI 1) PLGA-CHI implant specimen before degradation experiment, (PLGA-CHI 2) PLGA-CHI implant specimen after 14 days degradation, (PLGA-CHI 3) PLGA-CHI implant specimen after 30 days degradation

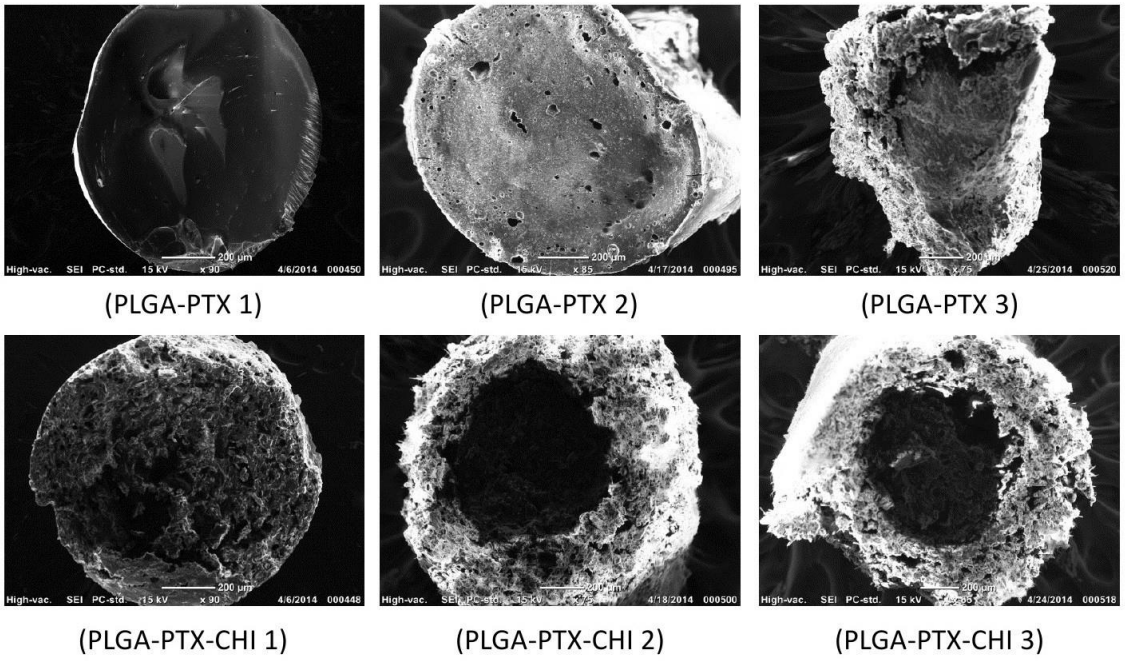


Figure 3.4 SEM images of cross section morphologies of (PLGA-PTX 1) PLGA-PTX implant specimen before degradation experiment, (PLGA-PTX 2) PLGA-PTX implant specimen after 14 days degradation, (PLGA-PTX 3) PLGA-PTX implant specimen after 30 days degradation, (PLGA-PTX-CHI 1) PLGA-PTX-CHI implant specimen before degradation experiment, (PLGA-PTX-CHI 2) PLGA-PTX-CHI implant specimen after 14 days degradation, (PLGA-PTX-CHI 3) PLGA-PTX-CHI implant specimen after 30 days degradation

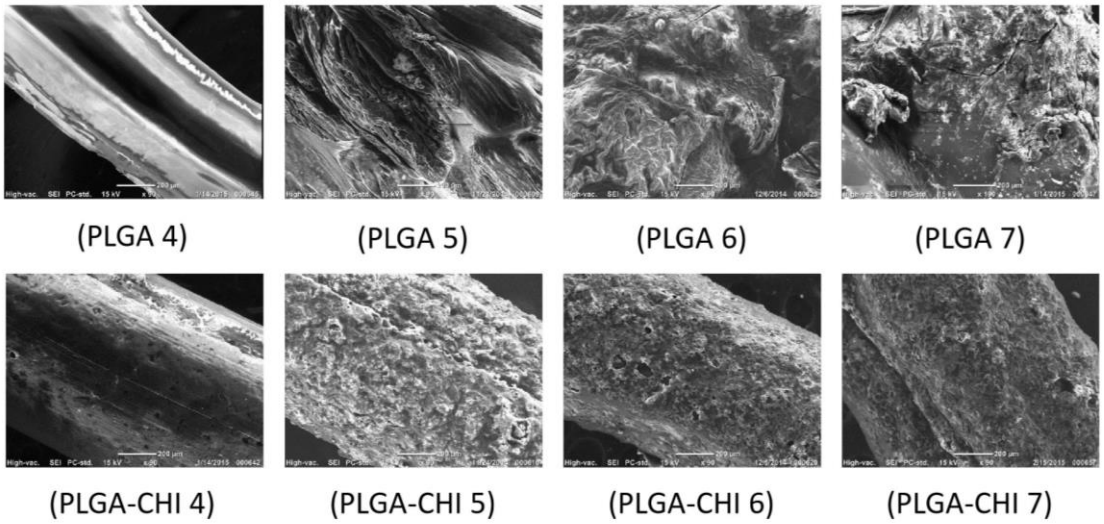


Figure 3.5 SEM images of surface morphologies of (PLGA 4) PLGA implant specimen before degradation experiment, (PLGA 5) PLGA implant specimen after 14 days degradation, (PLGA 6) PLGA implant specimen after 24 days degradation, (PLGA 7) PLGA implant specimen after 30 days degradation, (PLGA-CHI 4) PLGA-CHI implant specimen before degradation experiment, (PLGA-CHI 5) PLGA-CHI implant specimen after 14 days degradation, (PLGA-CHI 6) PLGA-CHI implant specimen after 24 days degradation, (PLGA-CHI 7) PLGA-CHI implant specimen after 30 days degradation

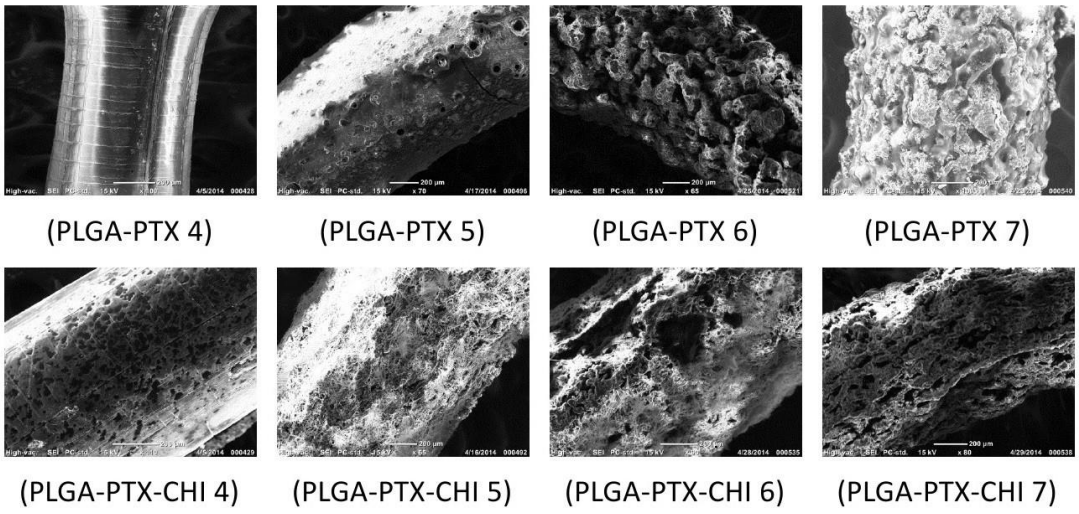


Figure 3.6 SEM images of surface morphologies of (PLGA-PTX 4) PLGA-PTX implant specimen before degradation experiment, (PLGA-PTX 5) PLGA-PTX implant specimen after 14 days degradation, (PLGA-PTX 6) PLGA-PTX implant specimen after 24 days degradation, (PLGA-PTX 7) PLGA-PTX implant specimen after 30 days degradation, (PLGA-PTX-CHI 4) PLGA-PTX-CHI implant specimen before degradation experiment, (PLGA-PTX-CHI 5) PLGA-PTX-CHI implant specimen after 14 days degradation, (PLGA-PTX-CHI 6) PLGA-PTX-CHI implant specimen after 24 days degradation, (PLGA-PTX-CHI 7) PLGA-PTX-CHI implant specimen after 30 days degradation

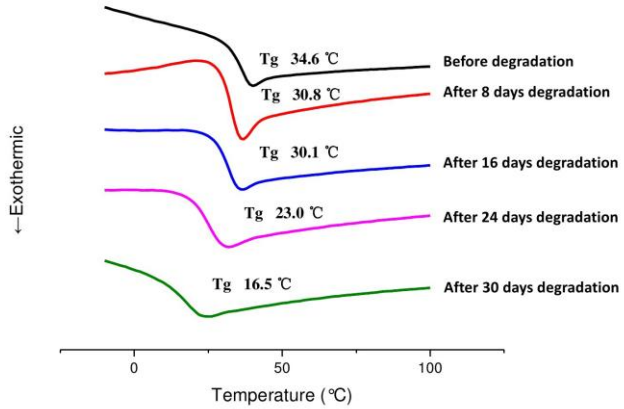


Figure 3.7 DSC thermogram of PLGA implant specimen before degradation experiment, after 8 days degradation, after 16 days degradation, after 24 days degradation and after 30 days degradation

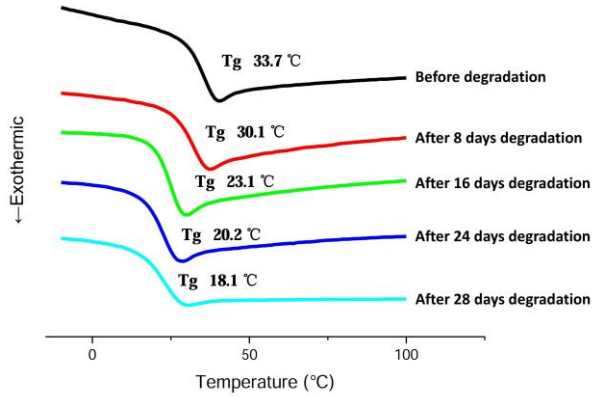


Figure 3.8 DSC thermogram of PLGA-PTX implant specimen before degradation experiment, after 8 days degradation, after 16 days degradation and after 24 days degradation

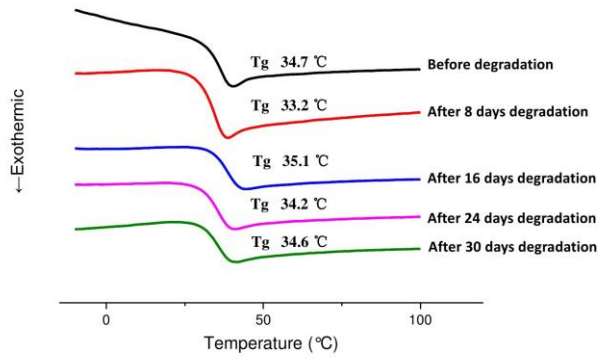


Figure 3.9 DSC thermogram of PLGA-CHI implant specimen before degradation experiment, after 8 days degradation, after 16 days degradation, after 24 days degradation and after 30 days degradation

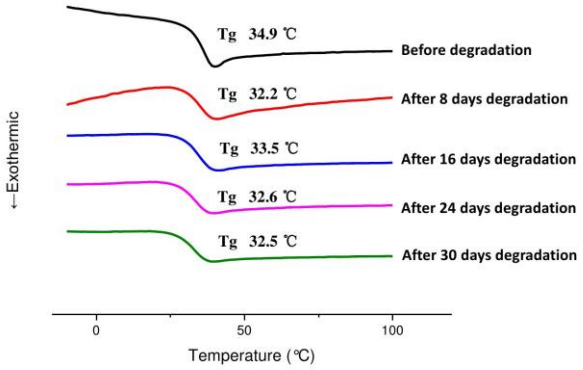


Figure 3.10 DSC thermogram of PLGA-PTX-CHI implant specimen before degradation experiment, after 8 days degradation, after 16 days degradation, after 24 days degradation and after 30 days degradation

3.4.2 Drug Loading Content (LC) and Drug Encapsulation Efficiency (EE)

Table 3.2 summarized the drug loading content (LC) and drug encapsulation efficiency (EE) for PLGA-PTX and PLGA-PTX-CHI implants. No significant differences ($p>0.05$) were observed between two formulations. Drug loading content for PLGA-PTX and PLGA-PTX-CHI implants were 9.81 ± 0.05% and 9.73 ± 0.20% (w/w), respectively, which were close to the feed weight ratio (Table 3.1) of PTX to polymers (10%). During fabrication, some of drugs and polymers could remained in the vial of cryomilling and injection molding mold, giving rise to an encapsulation efficiency of ~ 80%.

Table 3.2 Drug loading content (LC) and drug encapsulation efficiency (EE) of PLGA-PTX and PLGA-PTX-CHI implants

Drug Composition	LC (%)	EE(wt%)
PLGA-PTX	**9.81 ± 0.05**	**86.08 ± 0.40**
PLGA-PTX-CHI	**9.73 ± 0.20**	**84.85 ± 0.63**

The data are expressed as mean ± S.D. (n=3)
Student's t-test, $p > 0.05$

3.4.3 *In Vitro* Release Studies

3.4.3.1 PTX Release from Implant.

The *in vitro* release curves of PTX from PLGA-PTX and PLGA-PTX-CHI implants were shown in Figure 3.11. The release pattern of PTX from PLGA-PTX implants demonstrated a three-phase release profile: a very slow release during first 7 days, followed by a rapid release from day 8 to day 34, then another sustained and slow release appeared in the last 4 days. However, PLGA-PTX-CHI implants began to release PTX after 16 days in a slow and steady rate which was around 5 μg/day. There is no appreciable burst release observed during the study, which probably resulted from the smaller surface to volume ration than micro-/nano- particles.

Additionally, PLGA-PTX-CHI implant contained chitosan in the implant, resulting a steadier and slower drug release and a stable *p*H medium, which inhibited PLGA autocatalytic degradation.

3.4.3.2 Mass Loss of Implants.

PLGA is hydrolytically degraded into smaller chain acids, and its final degradation products are lactic acid and glycolic acid. However, PLGA erosion is more complex than its degradation, since it not only depends on polymer degradation, but also involved in many physical change processes, including dissolution and diffusion of PLGA degradation products and swelling [45]. The drug compound in the polymer matrix may be released by some combination of diffusion through the polymer matrix, diffusion through pores, and dissolution coincident with polymer dissolution. To further understand the influence of PLGA erosion on PTX release, the mass loss was measured as an indicator of erosion.

As shown in Figure 3.12, different drug formulations implants (PLGA implants and PLGA-CHI implants were studied as controls) display different mass loss profiles, which could be characterized by the mass loss rate (slope) and accumulated mass loss in 30 days. Nonlinear regression analysis of mass loss rate for each formulation of the implants is shown in Table 3.3 (coefficient of determination R^2 was comprised between 0.82 and 0.99) [46]. It is evident that the initial mass loss of PLGA-PTX and PLGA-PTX-CHI implants occurred after a lag phase (about 2 weeks), and subsequently a rapid erosion process occurred in PLGA-PTX implants, while a slow depletion occurred in PLGA-PTX-CHI implants. Clearly, there was a significant difference in mass loss rate among the four formulations of the implants from day 18 to day 30 of *in vitro* release studies, where it is observed that PLGA and PLGA-PTX implants possess a much higher mass loss rate than both PLGA-CHI and PLGA-PTX-CHI implants, and that the PLGA-PTX-

CHI implants displayed the lowest mass loss rate. Meanwhile, there was a significant effect of implant formulation on total mass loss percentage in 30 days at $p < 0.05$ level for four implants. Post hoc comparisons by Tukey HSD test indicated that the mean of total mass loss percentage for PLGA-PTX (Mean = 77.24, SD = 3.51) and PLGA (Mean = 75.80, SD = 3.89) implants were significantly different than the PLGA-CHI (Mean = 7.12, SD = 1.61) and PLGA-PTX-CHI (Mean = 4.14, SD = 0.33) implants. However, PLGA-CHI total mass loss percentage did not significantly differ from PLGA-PTX-CHI total mass loss percentage, and PLGA-PTX total mass loss percentage did not significantly differ from PLGA total mass loss percentage. These results suggest that introducing CHI into PLGA rod implants do cause an effect on polymer erosion. But loading PTX into the PLGA rod implants does not appear to significantly change the erosion rate.

For PLGA-PTX implants, the erosion of PLGA had limited impact on the whole drug release behavior because the drug release occurred earlier than pronounced erosion. However, it seemed that drug release and mass loss for PLGA-PTX-CH happened almost simultaneously, and the release pattern was in good overall agreement with the mass loss curve. This result indicated that the PTX release from PLGA-PTX-CHI implant was mostly dominated by erosion.

Hence, the released PTX in the medium of PLGA-PTX implants increased speedily, while it increased gradually in the medium of PLGA-PTX-CHI implants. Combined with the results from SEM morphological observations and the DSC thermographs, the PLGA and PLGA-PTX implants showed a typical bulk erosion degradation mechanism in *in vitro*. However, PLGA-CHI and PLGA-PTX-CHI implants exhibited that they degraded in a similar manner to surface erosion. A previous study about effect of different drugs on the degradation rate of PLGA matrices concluded that the drugs could affect the rate and the mechanism of

polymer implant degradation [47]. In our case, chitosan had an impact on the degradation profile of the PLGA implant compared with the control rods (PLGA and PLGA-PTX implant). According to Kiortsis et al. [48], it is reasonable that hydrophobic ingredients with low water solubility dispersed in PLGA will inhibit water penetration and diffusion into the matrix, consequently leading to a slow degradation and surface erosion.

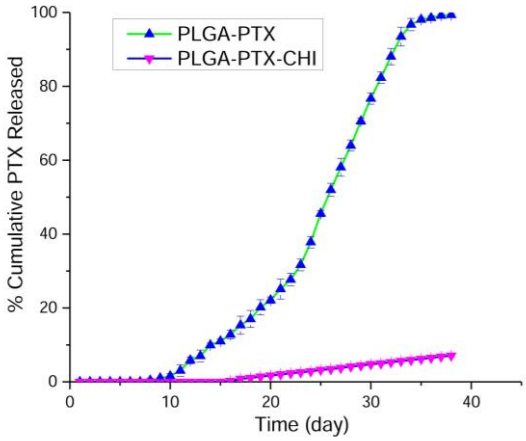


Figure 3.11 Plot of the percentage of accumulated PTX released of PLGA-PTX and PLGA-PTX-CHI implants in *in vitro* release experiment. The data are expressed as mean ± S.D. (n=3)

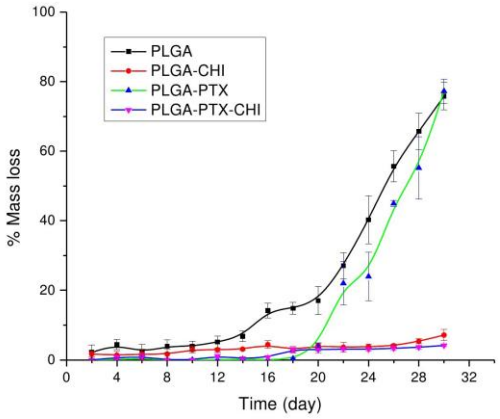


Figure 3.12 Plot of the accumulated mass loss percent of each formulation implant in *in vitro* degradation experiment. The data are expressed as mean ± S.D. (n=3)

Table 3.3 Nonlinear Regression Analysis of each formulation implant mass loss between 18th and 30th day

Drug composition	Slope (Mass loss rate, % day^{-1})		
		95% Confidence Interval	
	Estimate (Mean)	Lower	Upper
Slope of PLGA	5.26	4.32	6.20
Slope of PLGA-CHI	0.28	0.11	0.45
Slope of PLGA-PTX	6.25	4.65	7.86
Slope of PLGA-PTX-CHI	0.09	0.04	0.15

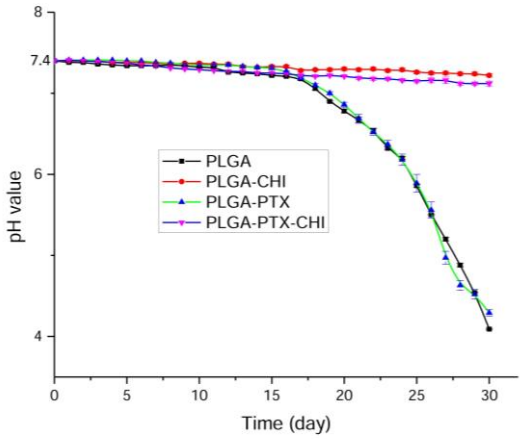
3.4.4 *p*H change in degradation medium

Since changes in *p*H levels have impact on human body functionality, such as immunological reactions [49], it is important to evaluate the *p*H level changes during implant degradation [49, 50]. As Liu et al. [51] presented in their work, accumulation of acidic implant degradation products may increase the risk of inflammation at the implant site. Hence, *p*H studies were conducted to examine the influence of chitosan on *p*H neutralization during drug release. The buffer *p*H was measured at each time point to profile the acidity changes in *in vitro* conditions of each formulation implants in 30 days. The studies were carried out in two slightly different situations, besides the same incubator settings, one was taken the procedures exactly same with *in vitro* drug release study, and the other was immersing the implants in 15ml PBS (*p*H 7.4) without refreshing PBS every day. Data were collected on a daily basis from triplicates of each formulation implant from day 0 to day 30 and plotted in Figure 3.13. Results from non-refreshing PBS study revealed that the buffer *p*H value over the course of the study ranged from a *p*H 7.4 initially to an approximate *p*H 4.1 on day 30 for the PLGA implants, *p*H 4.3 for the PLGA-PTX implants, *p*H 7.1 for the PLGA-CHI implants, and *p*H 7.2 for the PLGA-PTX-CHI

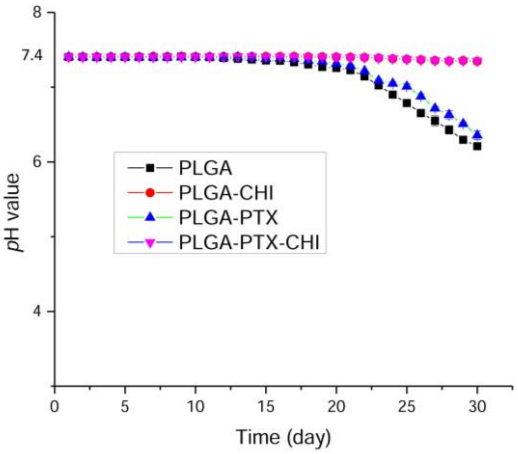
implants, respectively. The decreased *pH* value occurred when too much acid was added to the buffer that they become the excess reactant. In this work, due to the accumulation of acidic degradation products of PLGA, the *p*H value of each implant degradation medium has shown different degrees of decline. According a one-way ANOVA results, it was found that the implant formulation had a significant effect on medium *p*H change. Post hoc comparisons by Tukey HSD test revealed that after 30 days, the mean of medium *p*H value for PLGA-PTX (Mean = 4.29, SD = 0.04) and PLGA (Mean = 4.09, SD = 0.01) implants were significantly different than the PLGA-CHI (Mean = 7.22, SD = 0.02) and PLGA-PTX-CHI (Mean = 7.12, SD = 0.03) implants.

Due to the addition of buffer, the refreshing PBS study showed each formulation had a more stable curve for *p*H changes. PLGA and PLGA-PTX implants only dropped the medium *p*H value to 6.2 and 6.3, respectively, after 30 days. However, PLGA-CHI and PLGA-PTX-CHI implants both kept their medium *p*H value between 7.4 and 7.3. A one-way ANOVA on medium *p*H (value of *p*H over 30 days) from all formulations yielded significant difference ($p < 0.05$). Tukey post hoc analysis showed that PLGA implant was significantly different from the other three implants, so was PLGA-PTX implant; PLGA-CHI and PLGA-PTX-CHI implants did not differ significantly at $p < 0.05$.

In general, from these *p*H studies, the *p*H value of release medium for PLGA and PLGA-PTX implants started to decrease on day 17, and declined rapidly over the next 13 days, while the *p*H value of release medium for PLGA-CHI and PLGA-PTX-CHI implants both remained in a more stable range from 7.4 to 7.1. These findings were compatible with the mass loss results, which were that the PLGA and PLGA-PTX implants began to have apparent mass loss after day 18 as a result of the formation of numerous soluble monomers and oligomers with acidic end-groups yielding to a decrease in *p*H.



(a)



(b)

Figure 3.13 Plot of the *p*H change in PBS (*p*H 7.4) solvent. (a) *p*H change studies without refreshing medium in 30 days. (b) *p*H change studies with refreshing medium every day. The data are expressed as mean ± S.D. (n=3)

3.5 Conclusions

Through this study, a novel method for fabricating PTX-loaded rod shape polymeric implants using cyromilling and injection molding was developed. This proposed drug delivery implant has been integrated PLGA with chitosan without any solvent by cryomilling, and proven

to provide a sustainable drug delivery with no burst release. The results obtained from *in vitro* release studies of four different formulation of implants showed that there was a decrease in the drug release rate of the chitosan blended formulations implants (PLGA-CHI and PLGA-PTX-CHI) in PBS with *p*H of 7.4 in comparison with the formulations made of only PLGA as polymer matrix (PLGA and PLGA-PTX). Meanwhile, the *p*H change studies indicate that introducing chitosan in the PLGA implants could provide a relatively neutral and stable *p*H level during *in vitro* which could benefit the drug release. There is no doubt that by blending chitosan and PLGA to form a drug release matrix, a prolonged release of paclitaxel can be achieved. Nevertheless, further investigation of implants containing PLGA, chitosan and PTX on expanded *in vitro* studies with in vivo studies on rats with induced breast cancer is warranted.

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CHAPTER 4
GENERAL CONCLUSIONS

4.1 Conclusions

A review of literature on drug delivery systems for chemotherapy reveals that PLGA as a polymeric drug carrier for PTX has been extensively studied and its effectiveness proven. In this work, a process for fabrication of rod-shaped implants for delivery of PTX was well established. By cryomilling in a solid dispersion a homogenous blend of the natural polymer chitosan with PTX-loaded PLGA was obtained. The rod-shaped implants were then successfully made through injection molding. *In vitro* degradation studies showed that chitosan based PLGA drug implants could prolong the degradation and keep the degradation environment in a stable and neutral *p*H level. However, to fully understand the degradation profile of chitosan based rod-shaped implants, it is indispensable to conduct a longer *in vitro* experiment as well as *in vivo* experiment.

4.2 Review of Contribution

This thesis provides a unique and feasible method for fabricating an injectable rod-shaped implant composed of two immiscible polymers. Through cryomilling, chitosan could be homogenously blended in PLGA, resulting in a powder mixture, which can be easily molded to a rod shape by injection molding. This implant is intended to be placed in the human body subcutaneously or intramuscularly to provide a continuous release of drug over long periods of time. Hence, comparing to IV bag, it does not require repeated insertion of needles, which increases patient compliance. Introducing chitosan into PTX loaded PLGA implants can be used to regulate and adjust the degradation behavior of PLGA implants, thus the drug release could be controlled.

4.3 Future Perspectives

In this study, the chitosan based implants yielded considerably slow drug release *in vitro*. However, further evaluation for their pharmacokinetics, degradation profile and influence on local *p*H in vivo is still needed because chitosan degradation mainly occurs by enzymatic depolymerization [1]. Also, different proportion of chitosan may result in different implant erosion and degradation speed, thus a customized drug release could be achieved depending on the ratio of ingredients. Besides, this drug delivery system could also be employed for the sustained release of other drugs and bioactive factors.

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