

**Bulk Manufacture of Complex Geometry Millirod Implants and Their Degradation and  
Drug Delivery Characteristics**

by

**Melissa Anne Slagle**

A thesis submitted to the graduate faculty  
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Industrial Engineering

Program of Study Committee:

Iris Rivero, Major Professor

Richard Stone

Terri Boylston

Iowa State University

Ames, Iowa

2015

Copyright © Melissa Anne Slagle, 2015. All rights reserved.

## **DEDICATION**

I would like to dedicate this thesis in memory of my grandmother Dorothy Jean Slagle who lost her battle to cancer in 1995. I miss her every day and wish that I had the chance to share this work with her.

## TABLE OF CONTENTS

	Page
LIST OF FIGURES .....	iv
LIST OF TABLES .....	v
NOMENCLATURE .....	vi
ACKNOWLEDGMENTS .....	vii
ABSTRACT.....	viii
CHAPTER 1    General Introduction .....	1
Thesis Organization .....	3
CHAPTER 2    Literature Review.....	
Diabetic Neuropathy .....	4
Current Synthetic Drugs .....	4
Non-Pharmaceutical Alternatives .....	5
Curcumin .....	7
Cryomilling .....	8
Drug Delivery .....	9
Effect of Geometry on Degradation.....	13
Summary of Literature .....	15
CHAPTER 3    Preliminary Work.....	16
Electrospinning of Silk .....	16
Hydrogels for Cell Growth .....	19
CHAPTER 4    Bulk Manufacture of Complex Geometry Millirod Implants and Their Degradation and Drug Delivery Characteristics .....	21
Introduction .....	22
Materials and Methods.....	23
Results .....	27
Discussion .....	32
Conclusions and Future Work .....	35
References .....	36
CHAPTER 5    Conclusions & Future Work .....	39
Conclusions.....	39
Review of Contribution.....	40
Future Work .....	40
REFERENCES .....	41

**LIST OF FIGURES**

	Page
<b>Figure 2.1:</b> Implantable drug delivery devices.....	9
<b>Figure 3.1:</b> Electrospinning setup .....	18
<b>Figure 3.2:</b> SEM images of electrospun silk .....	18
<b>Figure 4.1:</b> XRD profiles of 85% PCL and 15% curcumin in relation to neat PCL and curcumin profiles .....	28
<b>Figure 4.2:</b> XRD profile comparison of solvent and cryomilled mixtures .....	28
<b>Figure 4.3:</b> Cumulative <i>in vitro</i> % mass loss comparison across all groups .....	29
<b>Figure 4.4:</b> Daily average % mass loss trend graph.....	30
<b>Figure 4.5:</b> Average daily release of curcumin loaded cylindrical and threaded implant geometries .....	31
<b>Figure 4.6:</b> SEM images of millirods before and after <i>in vitro</i> degradation .....	32

**LIST OF TABLES**

	Page
<b>Figure 3.1:</b> List of materials used for hydrogel creation.....	20
<b>Figure 4.1:</b> Analysis of variance for average mass loss .....	29

**NOMENCLATURE**

GC-MS	Gas chromatography- mass spectrometry
iMED	Interdisciplinary Manufacturing Engineering and Design
PBS	Phosphate buffer solution
PCL	Poly(caprolactone)
PDN	Painful diabetic neuropathy
PEO	Poly(ethylene oxide)
PGLA	Poly(lactic-co-glycolic acid)
PVA	Polyvinyl alcohol
SEM	Scanning Electron Microscope
TCM	Traditional Chinese medicine
UV	Ultra violet
XRD	X-Ray Diffraction

## ACKNOWLEDGMENTS

I would like to thank my committee chair, Dr. Iris Rivero, and my committee members, Dr. Richard Stone, and Dr. Terri Boylston, for their guidance and support throughout the course of this research. I also would like to thank Kevin Brownfield for creating the gorgeous four part threaded geometry mold that made this research possible in addition to helping me troubleshoot my compression molding missteps. I am indebted to Esra'a Abedell-All for her assistance in looking over my statistical analysis methods and her magical candy supply.

I owe a large thank you to my parents and sister for their support during my graduate studies. It's been a rollercoaster but I think I'm ready to "wrap it up." In addition, I would also like to thank my close friends Brooke Meyer and Bri Nobiling who helped me remain sane since the beginning. Thank you to my colleagues in the iMED research group, and the department faculty and staff for making my time at Iowa State University a wonderful experience.

**ABSTRACT**

This thesis investigated the impact of manufacturing process modifications for the bulk manufacture of curcumin implants for diabetic neuropathy pain relief. A homogeneous mixture of poly(caprolactone) (PCL) and curcumin was blended using cryomilling as an alternative to the solvent mixing method. Cryomilling was selected due to its faster processing time and reduced cost of materials in comparison to the solvent method. X-ray diffraction (XRD) was used to characterize the resulting mixture to determine the efficacy of cryomilling as an option for blending curcumin and PCL powders. In addition to cryomilling, compression molding was selected as the manufacturing method to allow for the creation of implant molds featuring threaded geometry on the millirod surface. Implants were subsequently evaluated *in vitro* for 30 days. Curcumin loaded millirod implants with complex threaded surface geometry were found to have a higher, but not significant, percent mass loss after degradation and average daily curcumin release than the cylindrical implants. It can be concluded that the utilization of cryomilling for the creation of curcumin loaded implants in bulk is an easier to manipulate and more cost effective method of combining PCL and curcumin without sacrificing implant effectiveness.

## CHAPTER I: GENERAL INTRODUCTION

Diabetic neuropathy stems from nerve damage caused by elevated blood sugar levels for a prolonged period of time. Neuropathy in diabetics usually manifests in the legs and feet and the resulting pain is difficult to manage [1-4]. Current accepted anti-inflammatory agents to manage neuropathy pain, such as tricyclic antidepressants, pregabalin, or duloxetine, carry negative side effects including dizziness, weight gain, or arrhythmias [1]. There is a need for an active treatment to help with the management of pain caused by neuropathy that gives patients better pain relief without the negative side effects.

Curcumin is a natural polyphenolic found in turmeric (*Curcuma longa*) that has been shown to disrupt inflammatory pathways in cells [5-7]. Additional studies have linked curcumin to modulation of curcumin to anti-oxidant factors [8]. The ability for modulation of these pathways is increasingly important as diabetes and inflammation are linked [9, 10].

The major challenge facing curcumin as a viable anti-inflammatory agent is its low bioavailability due to poor absorption and rapid metabolism of the compound when ingested [11]. Therefore it is necessary to encapsulate curcumin in order to ensure that the compound maintains its effectiveness as an anti-inflammatory agent. Encapsulation would protect the compound from premature release into the body and allows for continued release over the life of the implant. To increase the drug effectiveness, curcumin should not be introduced to the body orally, but rather implanted locally to the site of discomfort or intraperitoneally [12-14].

Previous work has successfully created cylindrical curcumin implants [14], but little work has been done on the effect of different geometries on drug release rate. Other studies have linked an increased surface area to higher implant degradation and drug release rates

[15]. Bansal et al. did an *in vivo* evaluation of curcumin loaded polymer implants in subcutaneous tissue in rats to confirm its effectiveness as a chemopreventive delivery system [16]. By modifying the shape of the implant to increase surface area, the release rate of the dose can be customized. The proposed implant geometry for this research is a cylinder with a threaded surface feature, allowing for additional surface area while maintaining ease of manufacture.

The rationale for using a threaded implant geometry is that the implant shape increases surface area while providing texture for the implant to anchor to the fascia connective tissues in the body to reduce implant mobility after insertion. Fabrication of this implant was done via compression molding using poly(caprolactone) acid (PCL) incorporated with curcumin.

Curcumin was encapsulated with molded PCL to achieve controlled sustained release at the implant site. PCL is a Federal Drug Administration (FDA) approved polymer for human clinical use due to its proven biocompatibility and low toxicity. This product does not accumulate in vital organs and is created in the process of carbohydrate metabolism. Controlling the surface area of the implant will regulate drug release.

One combination ratio of curcumin and PCL was fabricated: 15% curcumin-85% PCL, in two different geometry types: threaded or cylindrical. The drug load percentage was selected from the previously tested loading range of 2-50%, of which drug loads greater than 10% had constant drug release [14]. In order to have the curcumin suspended in the PCL, samples were cryomilled to reduce particle size and blend the two components. The resulting polymer-curcumin mixture underwent compression molding at 71 °C. Samples were then extracted from the mold and processed to remove any flash or residual material.

Implants were then placed in amber vials in a solution of 10 mLs phosphate buffer solution (pH 7.4) and supplemented with 10% v/v bovine calf serum to simulate the body environment. Vials were incubated at 37° C on a shaker table at 150 rpm. Implant media was changed every 48 hours and the implants were weighed to determine total percent mass loss. Degradation media was analyzed via spectrophotometer to confirm average curcumin release per day of degradation.

XRD analysis of the cryomilled curcumin polymer mix retained a key characteristic peak of curcumin near the 10° mark. This peak was not present in the solvent mixed curcumin and PCL indicating that cryomilling does not mask the major characteristic peak of curcumin to the extent that solvent mixing does. The results from the degradation showed that the degradation of the threaded geometry implant had a significant increase in the percent mass loss and the average daily release of curcumin over cylindrical curcumin loaded implants.

### **Thesis Organization**

The format of this thesis is as follows. Chapter 2 is a literature review encompassing previous research involving curcumin as an anti-inflammatory agent, curcumin based implants, and evaluation of created implants. Chapter 3 covers previous research related to drug delivery systems that led to this project. Chapter 4 contains the manuscript to be submitted to the Journal of Manufacturing Processes and covers the main experiment of this thesis outlining the effect of geometry shape on *in vitro* degradation behavior of curcumin implants. A general conclusion can be found in Chapter 4 in addition to future work.

## **CHAPTER II: LITERATURE REVIEW**

### **2.1 Painful Diabetic Neuropathy**

According to the American Diabetes Association, 30 million Americans have diabetes and 60-70% of diabetics suffer from diabetic neuropathy [17]. Painful diabetic neuropathy (PDN) is associated with numbness in the legs and feet, but can be described as a stabbing or burning sensation in severe cases. Although the pain from PDN is not life threatening, it does impact a diabetic's quality of life as numbness can lead to an increased risk of falling, ulcers or amputation [18, 19].

Neuropathy in diabetics is caused by the increased demand of nerve endings in the body due to elevated glucose levels in the blood for an extended period of time. The excess glucose in the cell system results in additional glycolysis that can overload the electron transport chain resulting in the creation of reactive oxygen species (ROS) [20]. Increased flux through different signaling pathways also result in oxidative stress and inflammatory injury [20, 21].

### **2.2 Synthetic Drugs Used for Painful Diabetic Neuropathy**

Complete relief for diabetic neuropathy pain is difficult to achieve, as there are no treatments currently on the market that relieve associated pain completely. Since PDN is highly correlated to hyperglycemia, strategies to control a patient's glycemic index are generally considered the first step in PDN management [1, 22, 23]. However, certain kinds of antidepressants and antiepileptic drugs have had some effect on reducing pain perception in patients.

Tricyclic antidepressants like amitriptyline work by preventing reuptake of noradrenaline and serotonin in the brain, allowing these neurotransmitters to collect and effectively block pain signals along the spinal cord [24]. Like most antidepressants, determining actual dosage requires trial and error and patients may not see any changes in pain for several weeks after starting the medication. Amitriptyline is not without its side effects which can include dizziness, dry mouth, and weight gain [25-27]. More serious side effects can include seizures, hallucinations, or hypersensitivity syndrome [28, 29].

Antiepileptic drugs are also currently being used to manage neuropathy pain. Some commonly prescribed antiepileptics for PDN include gabapentin and pregabalin. Gabapentin is well tolerated medication with minor side effects such as drowsiness. However a crossover study comparing gabapentin and amitriptyline for PDN pain relief found amitriptyline to provide greater pain relief [30]. Pregabalin is another commonly prescribed medication for the treatment of PDN, and while it is mostly well tolerated, it has rare side effects that include acute renal failure and acute angle glaucoma [1].

As previously mentioned, pain relief for diabetic neuropathy is difficult to manage, even with the introduction of an antidepressant or antiepileptic drug. In many of the studies looking at the efficacy of these drugs for mitigating PDN pain, a drug therapy is considered successful with a 50% reduction in pain [1]. Current offerings leave patients with PDN having to trade off potentially serious side effects for incomplete pain management.

## **2.3 Non-Pharmaceutical Alternatives**

### *2.3.1 Capsaicin*

Several studies have analyzed the efficacy of topical capsaicin creams to help treat PDN symptoms. Capsaicin is responsible for the pungent trigeminal sense of heat during consumption of peppers. Repeated application of capsaicin to skin has been shown to inactivate nociceptors resulting in decreased sensitivity to heat and mechanical stimuli [31]. However, application of creams must be done using gloves to reduce the risk of cross contamination of capsaicin to other parts of the body, such as the face. Introduction of capsaicin to open wounds is not recommended and patients may experience localized burning or stinging at the application site during the first week of treatment [1]. A review of capsaicin efficacy found moderate improvements in treatment of neuropathic pain, but stated that capsaicin creams may be better as an adjunct therapy or sole therapy for patients who are intolerant of other types of pain management [32].

### 2.3.2 *Traditional Chinese Medicine*

Traditional Chinese medicine (TCM) is an ancient form of healthcare in China that utilizes herbal medicines in addition to acupuncture and massage [33]. Over 80 herbal medicines have been characterized with antidiabetic effects, 10 of which are frequently used for diabetes and related complications [34, 35]. The main compound prescribed for diabetes is *Radix astragali*, which is derived from *Astragalus membranaceus* [36]. The active polysaccharides in *R. astragali* are thought to modulate hypoglycemia in patients in addition to having an anti-inflammatory effect by disrupting several different inflammatory pathways [9]. Ginseng (*Radix ginseng*) is another common TCM that is used for management of hyperglycemia through increased production and sensitivity to insulin [37]. However the largest challenge facing the herbal component of TCM is acceptance and quantification of

the effects of herbal remedies. Future use of these homeopathic remedies depends heavily on the validation and analysis of active compounds in TCM through modern evaluation techniques and controlled studies.

### 2.3.3 *Curcumin*

Curcumin is a minor component found in the spice turmeric. Curcuminoids, consisting primarily of curcumin and cyclic curcumin, make up roughly 2%-9% of turmeric and can be extracted in a variety of means [38]. As a drug, curcumin has been shown to be an effective anti-inflammatory agent in previous studies for a variety of diseases including cancer and cardiovascular disease [39-41]. Curcumin has also shown anti hyperglycemic effects in previous studies with type 2 diabetics [42]. As mentioned earlier, control of a patient's glycemic index is a key component to managing PDN.

However, curcumin is rapidly metabolized into glucuronides and curcumin sulfates by the body when orally ingested, requiring large initial doses to be ingested for therapeutic effects to be seen [43]. Cheng et al. reported no dose limiting toxicity at 8,000 mg/day of curcumin, and similar studies confirm no toxicity at dosages as high as 12,000 mg/day [44, 45]. Analysis of serum concentrations in humans taking 8,000 mg doses showed a peak concentration of 3.6  $\mu\text{M}$  of curcumin one hour after oral intake which then held steady at 0.6  $\mu\text{M}$  for the next 20 hours [44]. This facilitates the need to encapsulate curcumin to ensure that the active compounds remain effective systemically. Attachment of curcumin to other components like liposomes, polymers and surfactants have been shown to reduce the degradation of curcumin [46]. An implantation device loaded with curcumin and placed

subcutaneously or intraperitoneally in the body would bypass the digestive system and liver and allow for higher curcumin levels in plasma [47].

## 2.5 Cryomilling

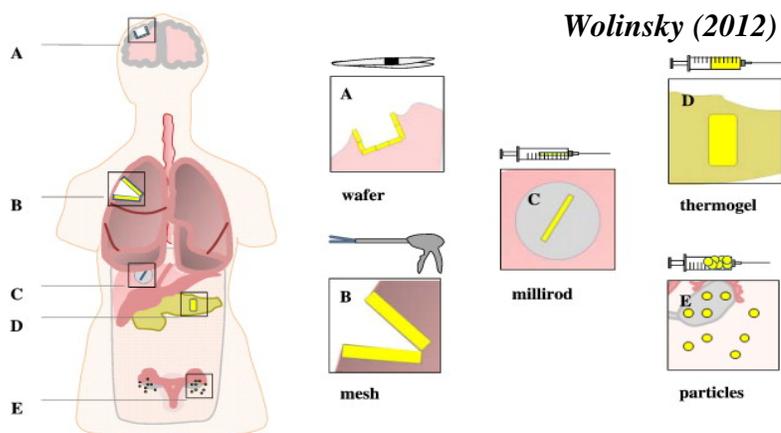
In order to create an effective polymer-drug matrix, previous curcumin implant studies have utilized solvent evaporation to encapsulate the hydrophobic spice [14, 48-50]. This method requires that the curcumin be dissolved in dichloromethane (DCM) while the polymer be dissolved in ethanol. These two solutions would then be mixed together and their solvents would be evaporated off overnight [14].

In order to more rapidly create a polymer-drug matrix, cryomilling can be used to reduce the processing time to under an hour, depending on the parameters set. Cryomilling subjects samples to low temperatures through the use of liquid nitrogen. At this low temperature, materials are beyond their crystalline point making them very brittle. Cryomilling utilizes this key material property change to mix and refine the particle size. One of the large advantages to utilizing cryomilling is that no solvents are required for mixing, especially since both ethanol and DCM can be hazardous when handled [51, 52]. Using a solvent also runs the risk of having residual solvent remain in the PCL and curcumin mixture. Gupta et al. evaluated the residual levels of DCM in solvent mixed PCL and curcumin via GC-MS and found no DCM at a detection level of <1.6 ppm [15]. Estimates of the cost of liquid nitrogen have been previously stated as \$1/L [53]. However, the cost of liquid nitrogen to operate the cryomill from the Iowa State Chemistry Stores is \$0.85/L. In comparison, 200 proof ethanol and dichloromethane currently go for \$100/L and \$58/L respectively when purchased through Sigma Aldrich (does not include cost of shipping). Using the small

benchtop cryomill [SPEX Sample Prep Freezer Mill 6770 (Metuchen, NJ, USA)] 2L of liquid nitrogen can process 5.70 g of PCL and curcumin mixture. This is enough to fabricate 72 threaded geometry implants for a cost of \$0.023 per implant. To solvent mix the same mass of PCL and curcumin, about 12.60 mL of dichloromethane and 6 mL of ethanol are required to dissolve the individual implant components prior to combination. This would produce 72 threaded geometry implants at a cost of \$0.023 per implant but does not include additional waiting costs incurred for solvent evaporation.

Previous work has looked at the efficacy of creating large quantities of curcumin and polymer blends via cryomilling [54]. Based on their findings, Wegiel et al. determined cryomilling was an effective method of creating amorphous curcumin and polymer powders in large quantities. However, no mixing of PCL and curcumin was done in this study.

## 2.6 Drug Delivery Implants



**Figure 2.1:** Types of implantable drug delivery systems, their implantation devices, and example *in vivo* implantation locations [55].

### 2.6.1 *Implants*

The previously mentioned pharmaceutical solutions for PDN pain management are administered in a form that is taken orally. This route normally results in the metabolism of the drug in the liver and is transported through the body via the bloodstream. However, a growing trend in drug delivery systems research is the investigation of drug loaded implantable or injectable self-assembling devices as a method to deliver more effective doses to the area of need. After implantation, the device degrades, simultaneously releasing the loaded drug into the immediate area. An implant specifically designed for diabetic neuropathy pain relief would ideally be designed to be minimally invasive, easy to insert into the target area, and provide effective relief for an extended period of time. There are several different types of implants currently being researched for drug delivery applications that could be used for PDN management including hydrogels, films, wafers and millirods. Some of the drug delivery options, implant locations, and implant devices can be found in Figure 2.1.

### 2.6.2 *Hydrogels*

Hydrogels are composed of hydrophilic polymer chains that can absorb large amounts of water and can be made in stable or degradable forms [56]. There is no set chemical formulation that characterizes a hydrogel, as many different material combinations can be utilized to obtain a hydrogel with the desired liquid loading characteristics [57-59]. These gels are easily mixed with small molecule hydrophilic drugs, and inclusion of hydrophobic domains within a hydrogel can help with the dispersion of hydrophobic drugs [60].

Applications for hydrogels in drug delivery include cancer and treatment of inflammatory bowel disease (IBD). Localized delivery of chemotherapeutics would minimize

damage to healthy cells as some anti-cancer drugs cannot distinguish between cancer cells and normal cell tissue [61]. Guo et al. created a linoleic acid conjugated poloxamer hydrogel for the application of long term delivery of anti-cancer drugs resulting in the disruption of Akt 1 signals [62]. Similarly, chitosan hydrogels loaded with a variety of anti-cancer drugs including paclitaxel and camptothecin have been evaluated as alternative delivery mechanisms in different cancer types with some success *in vitro* and *in vivo* [63, 64]. In another application, a negatively charged ascorbyl palmate hydrogel was created for the delivery of anti-inflammatory drugs for IBD treatment [65]. Evaluation of the hydrogel in mice found a decreased severity of the disease in mice treated with the drug loaded hydrogel. Utilization of the hydrogel also limited the spread of the anti-inflammatory drugs to other parts of the body, as the serum concentration for the hydrogel group was lower than that of the control group using the free form of the drug.

One of the challenges facing hydrogels is during the implantation phase.

Thermosensitive hydrogels remain in an aqueous form at room temperature for easy injection into a target site. Once at body temperature it assembles into a gel [63]. However this solution still runs the risk of clogging the needle or premature gelation in the syringe itself [60].

### 2.6.3 Polymeric Films

Polymeric films are another method of drug delivery implants that have been explored for inclusion into soft tissues [55]. A common method for film creation is casting which involves the introduction of the liquid polymer to a mold and allowing it to cure. Depending on the mold, additional processing to alter the size and shape may be needed [66].

Materials like silk, PLGA, PVA, and PCL have previously been successfully cast into films for drug delivery purposes [67-70].

Electrospinning is another method of manufacturing polymer films through the creation of fibers by applying a voltage to a needle and grounding the system to a collecting plate [71]. The charge difference across the system helps to draw the polymer fibers from the polymer when it is deformed into a Taylor cone [72]. Electrospun mats can be created using a variety of polymers including silk, PLA, and PCL [73-75]. The thread diameter and mat thickness can be controlled through several parameters including time, needle gauge, pumping rate, voltage, and distance between the needle and the collecting plate [76]. Applications for electrospun mats center on active wound healing and infection prevention through the incorporation of drugs [77]. Uses for mats as wound dressings could include application external to the body to assist in burn healing, or internally to the body as a way to assist healing following surgery [78]. In a PDN relief application the mat would need to be implanted to achieve the desired prolonged degradation characteristics.

#### *2.6.4 Wafers*

The Gliadel Wafer is the only FDA approved current market solution for patients requiring brain cancer treatment [79]. The Gliadel Wafer has been used in over 20,000 procedures in the US since its launch in 1997 [80]. The wafer is deposited in a cavity of the brain following removal of the primary tumor [55]. Because implants are placed intracranially, the effective chemotherapeutic can directly target cancerous cells in the immediate surroundings without the challenges of navigating the blood-brain barrier as with systemic chemotherapy options. A recent study by Boateng et al. created freeze dried wafers as drug

delivery devices for wound healing applications [81]. However, due to their physical characteristics, wafers are not able to be implanted via injection and must be placed surgically in the body, or through open wounds [55].

### *2.6.5 Millirods*

Millirods are thin, preformed polymeric cylinders that can serve as extended drug release devices. This type of drug release solution is currently being used in the market for long term contraception. Progestin millirods are injected sub-dermally and can provide three years of contraception protection [82]. Current research is exploring millirod implants as a potential chemopreventive solution. In addition to an easy implantation method, millirods can be made using several different types of manufacturing methods including casting and melt extrusion [55]. At small scales, sacrificial encapsulation of the polymer in silastic tubing is a method of achieving millirods through melt extrusion [14]. For larger bulk scales a sacrificial manufacturing method is not the most efficient or cost effective route of implant creation. Casting, compression molding, or continuous extrusion using a fixed die would be manufacturing methods utilized at a larger scale. Millirods are excellent candidates for bulk manufacture as the main point of customization would be at the point of implantation, allowing for the production of implants in bulk.

## **2.7 Effect of Geometry on Degradation**

Degradation of polymer implants tends to happen in one of two forms: surface or bulk erosion [83-85]. Factors affecting the degradation and erosion of a polymer can include chemical interactions between polymer and drug, pH of the environment, or by the addition

of copolymers [83]. Understanding and modeling erosion behavior of drug delivery devices is complicated by the introduction of water to the polymer implant which may result in swelling and changes in pH [83]. Geometry has only recently gained traction as a way to alter functionality of a drug delivery implant [86]. Findings from a study by Burkersroda et al. measured the degradation of cylindrical polymer matrices and found that polymers were able to undergo both types of degradation profiles depending on geometry and degradation parameters [84]. Klose et al. evaluated PLGA films and microparticles and found that the geometry of the delivery devices to have a large impact on the release profiles for drugs [68].

The focus around geometry in drug delivery systems tends to center around the efficacy of vascular and endothelial carriers [87, 88]. These have typically taken the form of microspheres, but continued research in the kinetics and release properties of different geometry carriers has led to the creation of ellipsoids and disks [88]. Non-spherical delivery devices are thought to have an advantage as vascular delivery systems due to their high surface area to volume characteristics allowing for increased environment interactions and the potential to reach more diverse targets in the body [86]. However not much work has been done specifically on the impact of complex geometry on drug release rates for subcutaneous or intraperitoneally targeted millirod implants beyond changes in millirod diameter [14].

One of the few examples of long term implantable polymer drug delivery devices with complex geometries was a honeycomb structure created by Yang et al [89]. This study was done as a proof of concept for the microfabrication and drug release kinetics. However there was no in-depth discussion relating the surface area to the release rate.

Bansal et al. reported that with an increase in surface area there was an increase in observed drug release [14]. Through the evaluation of cylindrical implants at varying diameters it was also determined that the increased size of an implant also sees an increase in the surface area of the implant resulting in increased drug diffusion into the environment surrounding the implant. Based on this knowledge, our study chose to compare millirod implants with similar cylindrical forms for easy implantation, but with drastically different surface areas to determine the impact on drug release.

## **2.8 Summary of Literature**

Diabetic neuropathy is a fairly common adverse symptom stemming from sustained increased blood glucose levels resulting in pain in the legs and feet that can lead to amputation [2, 18]. Antidepressants and antiepileptic drugs are the current medications used to mitigate the pain of diabetic neuropathy, yet successful treatment under these therapies is only able to achieve a 50% reduction in patient pain [1, 26, 27]. There is a need for a natural, long term treatment for the reduction of painful diabetic neuropathy that utilizes a drug with lower risk of adverse side effects. Curcumin is one such drug that exhibits anti-inflammatory, antioxidant, and anti-hyperglycemic effects with no dose limiting toxicity at high dosages [39, 41, 44]. However encapsulation is required for curcumin to remain bioavailable in the body. By combining curcumin and PCL via cryomilling, the additional time and materials needed for solvent evaporation mixing can be forgone in an effort to streamline the process of manufacturing for scale up. Previous millirod work determined that an increase in surface area saw an increased drug release rate [14].



## CHAPTER III: PRELIMINARY WORK

Prior to investigating the main hypothesis of this thesis, unpublished research relating to drug delivery was explored. Two different types of drug delivery systems, electrospun mats and hydrogels, were initially pursued for applications in alternative chemotherapy delivery and scaffolds for stimulating cell proliferation respectively. Techniques and learnings from these works helped in the development of the thesis research as outlined in Chapter 4.

### 3.1 Electrospinning of Silk

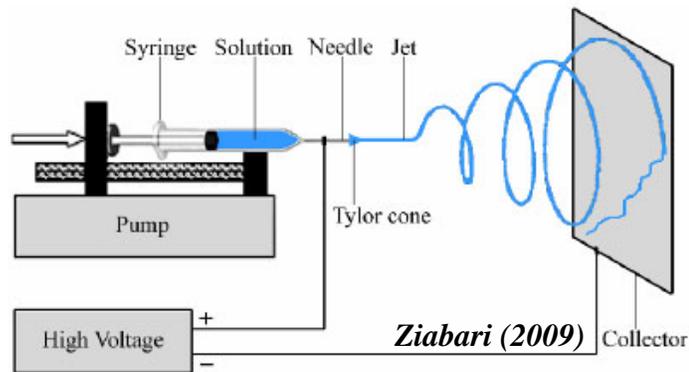
The initial goal of this work was to develop a non-systemic chemotherapy drug delivery device for the treatment of local breast cancer recurrence. Investigation was focused on finding a method of drug delivery that was not utilized for chemotherapy solutions. Selection of materials was based on high performing polymers with low *in vivo* toxicity. The manufacture of this delivery system also needed to be repeatable and scalable for ease of manufacturing purposes.

Ultimately this project continued previous work from the iMED laboratory on the evaluation of electrospun polymer blends, including PCL and polyglycolide, for use as drug delivery systems [90, 91]. As mentioned previously in section 2.6.1, electrospinning is a method of creating micro or nano scale diameter polymer fibers by utilizing an electric field. The experimental setup can be found in Figure 3.1. By applying a high voltage to a polymer loaded syringe needle and grounding the collecting plate, a large charge differential is created. A syringe pump slowly releases polymer from the syringe tip and the voltage

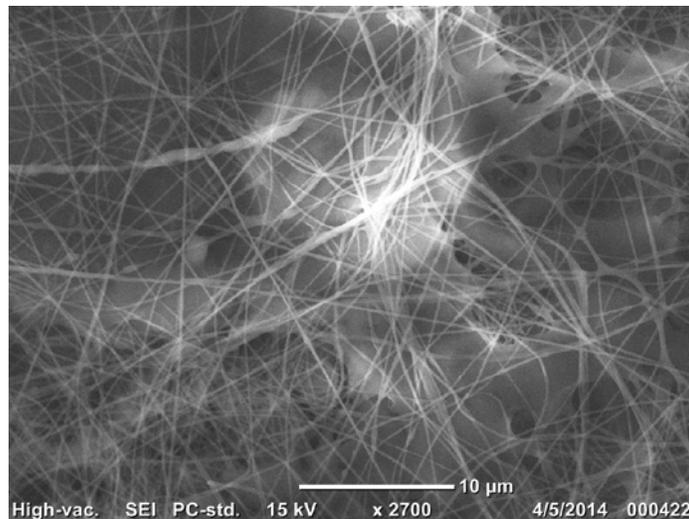
deforms the polymer bead creating a Taylor cone. This results in the release of fine diameter polymer fibers that are collected on the grounded plate. These polymer mats have a high surface area to volume ratio which is beneficial in drug release [75]. In contrast to previous iMED studies silk was selected as the main polymer component for electrospinning due to its beneficial properties as a biomaterial. Silk fibroin extracted from *Bombyx mori* cocoons have been found to have comparable degradation rates to other synthetic polymers, but with a reduced inflammatory response over PLA or collagen films [92]. Silk films have been used in combination with chemotherapeutic drugs like doxorubicin for *in vivo* breast cancer evaluation with positive results. The films showed a decrease in tumor weight and reduced metastatic spread, in addition to outperforming all other tested treatments [67].

Attempts to create silk electrospun mats were based on previous research methodologies [74, 93, 94]. Extraction of silk fibroin followed the protocol set forth by Rockwood et al [94]. Briefly, *B. mori* cocoons were opened and the silkworms were removed before being added to boiling water and sodium bicarbonate to degum the cocoons. After degumming, the remaining silk fibroin was dissolved in lithium bromide and the resulting solution was added to a dialysis cassette. Following dialysis, the resulting fibroin solution was centrifuged. Parameters used for electrospinning the fibroin solution were as follows: Pump rate was 0.02 mL/minute, voltage was set to 15 kV, silk concentration was 8% by weight and was combined with a 5 mL of a 5% PEO solution dissolved in water, and distance from the collecting plate was 10 cm. After electrospinning, PEO was dissolved using 70% vol/vol ethanol and allowed to dry. Spun fibers prior to PEO removal can be seen in Figure 3.2.

Despite successfully electrospinning a small amount of silk fiber on one occasion it was determined that long term storage of fibroin via lyophilization was needed to continue pursuing this material option long term due to the process intensive requirements to prepare the silkworm cocoons for use. Freeze drying the dissolved fibroin concentrates it, but also makes it stable for extended periods of time at room temperature [94]. This is in contrast to the silk fibroin in the solution form, which must be chilled at 4 °C and runs the risk of gelling rendering it useless for an electrospinning solution.



**Figure 3.1:** General electrospinning set up [95].



**Figure 3.2:** SEM image of PEO/silk fibers prior to the removal of PEO.

The ultimate design for this drug delivery system was a two stage drug delivery system. The electrospun silk fiber mat loaded with either a chemotherapeutic or anti-inflammatory drug would be spun first. A second polymer, like PLGA, loaded with a second drug would be spun directly on the silk mat. Testing would have determined the efficacy of the two different release profiles.

### **3.2 Hydrogels for Cell Growth**

A smaller project was done in collaboration with the Sakaguchi Lab at Iowa State University looking at the use of hydrogels as a medium to promote adult hippocampal stem cell proliferation. The main objective of the collaborative research was to have the hydrogel in a pipette tip used for cell culture. This was part of a larger experiment to create complex internal geometry in a cell proliferation scaffold to help guide the movement and growth of stem cells. Table 3.1 shows the materials and quantities used for the hydrogels that were created. Briefly, using a calibrated pipette, each of the materials in Table 3.1 were added to a clean test tube in the order listed. The catalyst, TEMED, is the last to be added, and with its addition the ingredients react and start to foam. The foam was mixed using a scoopula for an additional minute and allowed to sit in a vacuum oven for 20 minutes before the addition of the dehydrating agent.

Two methods of manufacture were attempted. The initial attempt tried to mix the hydrogel directly in the pipette tip. The small end of a 1,000  $\mu\text{L}$  pipette tip was enclosed using Parafilm prior to introducing the chemicals to be mixed. In order to accommodate the smaller container size, the listed ingredient amounts were divided by five. Despite covering the exit hole of the pipette tip, very little hydrogel was formed in the space due to the high

levels of foaming following the addition of the TEMED catalyst. This resulted in very little residual hydrogel left behind in the pipette tip. Future attempts made the hydrogel external to the pipette tip. This was done by creating the hydrogel in a test tube as previously mentioned, but transferring it to an aluminum foil lined petri dish prior to the vacuum drying and dehydration steps. This resulted in a hydrogel disk that allowed for geometry modification of the hydrogel prior to inserting it in the pipette tip. This method also allowed for more direct control on the amount of hydrogel that could be added to the pipette tip.

Following creation, hydrogel samples were sterilized using a 70% ethanol solution before entering the cell culture hood and allowed to dry overnight in the UV hood prior to cell culture [96]. A small sample of the hydrogel was introduced to test plates containing Dulbecco's Modified Eagle Medium (DMEM) and adult hippocampal stem cells were then added to the hydrogel via pipette. DMEM was selected to visually identify the pH changes of the cell medium during proliferation [97]. Evaluation of the culture after 24 hours determined that the hydrogel formulation was not compatible with cell culture as the environment was too basic for sustained cell growth due to the purple/blue color of the DMEM [98].

**Table 3.1:** Chemicals used to create hydrogels for the pipette tip project.

Chemical	Purpose	Amount	Solution
<i>Distilled Water (H<sub>2</sub>O)</i>		460 microL	
<i>Acrylamide (AM)</i>	Monomer	1000 microL	50% solution - 2.5 g AM/5 mL H <sub>2</sub> O
<i>Methylenebisacrylamide (MBA)</i>	Cross Linker	100 microL	2.5% solution- .125 g MBA/ 5mL H <sub>2</sub> O
<i>Pluronic F-127 (F-127)</i>	Foam Stabilizer	100 microL	10% solution- .5g F-127/ 5mL H <sub>2</sub> O
<i>Ammonium Persulfate (APS)</i>	Initiator	40 microL	20% solution- 1 g APS/5 mL H <sub>2</sub> O
<i>Acrylic Acid</i>	Acid	45 microL	99% solution
<i>Sodium Bicarbonate (NaHCO<sub>3</sub>)</i>	Base	900 mg	Powder form
<i>Tetramethylethylenediamine (TEMED)</i>	Catalyst	40 microL	20% solution- 5 mL TEMED/14.25 mL H <sub>2</sub> O
<i>Ethanol</i>	Dehydrator	~200 mL	100% (200 proof)

**CHAPTER IV: BULK MANUFACTURE OF COMPLEX GEOMETRY MILLIROD  
IMPLANTS AND THEIR DEGRADATION AND DRUG DELIVERY  
CHARACTERISTICS**

A paper to be submitted to the proceedings for the 2016 North American Manufacturing  
Research Conference

**Melissa Slagle, Iris V. Rivero**

**ABSTRACT**

This study evaluated the impact of manufacturing process modifications aimed at the bulk manufacture of curcumin implants for diabetic neuropathy pain relief. Poly(caprolactone) (PCL) and curcumin were blended using cryomilling as an alternative to the solvent mixing method which has higher manufacturing and time delay costs. X-ray diffraction (XRD) was used to characterize the resulting mixture to determine the efficacy of cryomilling as an option for blending curcumin and PCL powders. By adopting compression molding as a manufacturing method we were able to create implant molds featuring threaded geometry on the millirod surface. Implants were subsequently evaluated *in vitro* for 30 days. Curcumin loaded millirod implants with a complex threaded surface geometry were found to have a higher, but not significant, percent mass loss after degradation and average daily curcumin release than the cylindrical implants. It can be concluded that the utilization of cryomilling for the creation of curcumin loaded implants in bulk is an easier to manipulate and more cost effective method of combining PCL and curcumin without sacrificing implant effectiveness.

**Keywords:** implant, controlled drug release, curcumin, surface geometry, bulk manufacture

#### 4. 1 INTRODUCTION

Type 2 diabetes is the most prevalent form of diabetes in the United States affecting 9.3% of the total population [1]. This form of diabetes is more commonly associated with obesity or lack of physical activity resulting in reduced insulin production and sensitivity [2]. In addition, type 2 diabetics may also suffer from additional painful or debilitating conditions as a result of diabetes related complications. These can include blindness, amputations, kidney problems, and neuropathy [1]. Of these adverse diabetic complications, 50% of diabetics experience neuropathy in some form, which often manifests in foot and leg pain [3, 4]. A particular form of this condition, painful diabetic neuropathy (PDN), remains difficult to treat, as a 50% reduction in pain is considered a successful treatment [5]. Current solutions for PDN treatment include the use of cyclic antidepressants or antiepileptic drugs, both of which have negative side effects that may include dizziness, blurred vision, or weight gain [5].

Curcumin is a natural anti-inflammatory agent found naturally occurring in the spice turmeric. Many studies have been done to evaluate the impact of curcumin on various diseases including several different types of cancer, asthma, cardiovascular disease, and diabetes [6-13]. Despite the positive effects curcumin exerts as an anti-inflammatory agent, it has been shown that curcumin has poor bioavailability when taken orally [14]. One option to overcome this challenge is through encapsulation of curcumin within a polymer matrix. This type of drug delivery is most notably used for effective birth control through subcutaneous implantation that provides protection for 3 years [15]. This type of solution is ideal for treatment applications that

require sustained treatment over a long period of time. As PDN is a chronic disease currently without a cure, an implantable drug delivery device would provide an alternative method of pain management without jeopardizing patient quality of life.

Previous literature has developed cylindrical millirods impregnated with curcumin as a potential chemopreventive solution [7, 16-18]. Implants were manufactured using the solvent mixing method to create a curcumin polymer matrix. PCL was selected as the polymer as it is a Federal Drug Administration (FDA) approved polymer for human clinical use due to its proven biocompatibility and low toxicity. After removal of the solvents overnight, the molten curcumin and polymer was extruded through silastic tubing and cooled prior to removal from the tube. Based on the findings from Bansal et al. it was confirmed that an increase in implant surface area via changes in the diameter created an increase in drug release rate of curcumin [18]. With that knowledge, we wanted to test the effects of a drastic change in implant geometry on the degradation and drug release rate of curcumin. However, in order to achieve this geometry an alternative method of manufacturing was needed. Through the use of a custom compression mold we developed a threaded cylindrical implant to compare against a traditional cylindrical implant. With the drastic increase in surface area it is expected that the threaded geometry implants will have an increased drug release rate. Additionally, it is expected that the manufacturing modifications will help in scaling up manufacture of implants without compromising quality.

## 4.2 MATERIALS AND METHODS

### 4.2.1 Materials

Poly (caprolactone) 50,000 molecular weight was purchased from (Capa 6506, Perstorp, Sweden). Phosphate-buffered saline powder (pH 7.4) and bovine calf serum (BCS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Additional BCS was purchased from Hyclone (Logan, UT, USA). Curcumin (98% pure) was purchased from Acros Organics (Morris Plains, NJ, USA). No further analysis was done on any of the received materials.

### 4.2.2 Cryomilling

PCL was combined with curcumin at a 15% w/w ratio. Milling was done using a SPEX Sample Prep Freezer Mill 6770 (Metuchen, NJ, USA). PCL and curcumin were combined in a grinding vial and placed in the grinding chamber. The vial was precooled for 15 minutes prior to the start of grinding cycles. The mill was programmed for 5 cycles, a 2 minute cool time, and a 3 minute run time at a rate of 10 cps.

### 4.2.3 XRD Evaluation of Cryomilled PCL and Curcumin

In order to determine if the cryomilling process generated a homogeneous mixture of PCL and curcumin, XRD analysis was done over a full angle spectrum in a Rigaku Miniflex 600 XRD analysis unit (Tokyo, Japan). The voltage and current X-ray generator applied were 40 kV and 15 mA respectively. A scintillation counter (SC-70) was used as the detector. Scan range was from 1.01 to 60 degrees at a step width of 0.02 degrees. The XRD profiles were analyzed using the integrated X-ray powder diffraction software, PDXL, version 2.1.3.4.

#### 4.2.4 Compression Molding

Implants were created using two custom molds. The cylindrical rod mold created implants with a 2 mm diameter, while the threaded rod mold had an implant diameter of 1.5 mm in order to accommodate machining capabilities for the introduction of threads. Molds were loaded into a model 4386 model Carver hydraulic press (Wabash, IN, USA). Blank PCL implants were created using 50,000 molecular weight PCL at a plate temperature of 71° C. Curcumin loaded PCL implants were created using the cryomilled PCL and curcumin at a plate temperature of 71°C and compressed with 1,200 psi. Implants were removed from the molds and separated from the mold stem before evaluation. Implants for both geometries were cut to a length of 1.30 cm. The average threaded implant mass was  $22.0 \pm 0.75$  mg and the average cylindrical implant was  $49.9 \pm 1.04$  mg.

#### 4.2.5 In Vitro Evaluation

Implants were studied *in vitro* by submerging each sample (n=3 for each condition, 12 total implants) in 10 mL of PBS solution (pH 7.4) in 20 mL amber vials. In order to better mimic the *in vivo* environment, the PBS was supplemented with BCS (10% v/v). The sample implants were incubated at 37° C and agitated at 150 rpm on a shaker table (I24 New Brunswick Incubator Shaker, Eppendorf, Germany). Samples were measured at every media change every 48 hours.

#### 4.2.6 Spectrophotometer Characterization

In order to validate curcumin was present in degradation media, 1 mL of ethanol was added to media to solubilize residual curcumin. Media from curcumin samples was collected

every 2 days, n=3. Absorbance of the media was measured spectrophotometrically (DU 720, Beckman Coulter, CA, USA) at 430 nm as previously established [19]. A standard curve was used to determine the quantitative relationship between absorbance values and curcumin concentration present in the degradation media. Curcumin concentration range was from 100 µg-700 µg.

#### 4.2.7 Scanning Electron Microscopy (SEM)

Evaluation of surface area features and the effects of degradation on physical properties of implants was studied using a Jeol JCM-6000 benchtop SEM (Tokyo, Japan). Implants were evaluated prior to *in vitro* degradation and after 30 days of degradation. Samples were mounted using carbon tape and analyzed at 15 kV.

#### 4.2.8 Statistical Analysis

Percent mass loss values were analyzed using a two way ANOVA with significance set at  $p < 0.05$ . ANOVA factors were defined as geometry and curcumin loading. Percent mass loss values were averaged over the 30 day period for a sample size of n=3. Statistical significance for average daily drug release was determined using a standard two tailed t-test. Differences were considered significant for values where  $p < 0.05$ . Normal distribution and equal variances were assumed.

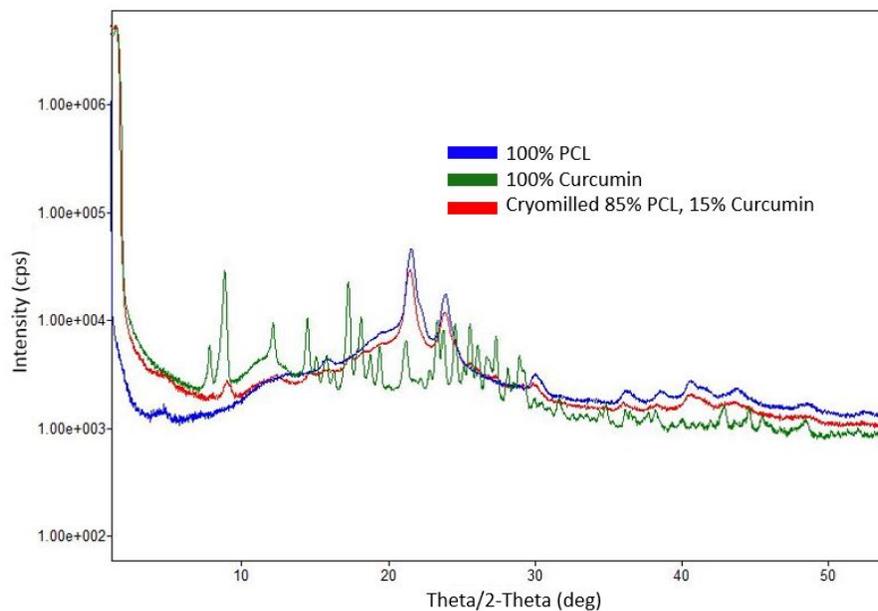
## 4.3 RESULTS

### 4.3.1 XRD Evaluation of Cryomilled PCL and Curcumin

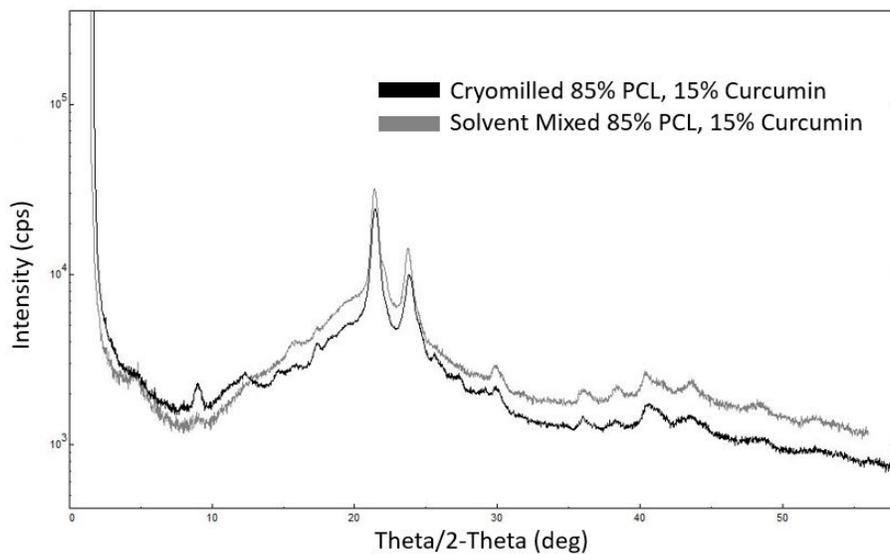
Figure 4.1 shows the XRD profile for the cryomilled PCL and curcumin at an 85% to 15% ratio respectively. Comparison peaks for 100% curcumin and 100% PCL are also shown in Figure 4.1. This result shows that PCL and curcumin mixture has a distinct profile curve unique from its constituent parts. Figure 4.2 shows the XRD analysis of a solvent mixed matrix of PCL and curcumin in the same 85-15% ratio. There was a noticeable difference at the 10 degree point in the XRD characteristic peaks of the cryomilled curcumin and PCL powder in comparison to the solvent mixed PCL and curcumin solid dispersion. The first large characteristic peak for curcumin is found at the 10 degree point, and a reduced version of that peak can be found on the cryomilled XRD profile, but not the solvent mixed profile.

### 4.3.2 In Vitro Results

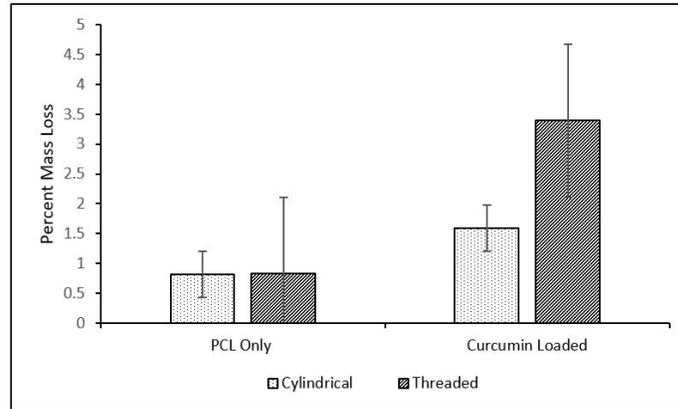
*In vitro* release profiles for both the original cylindrical geometry and the new threaded geometry implants without drug loading can be found in Figure 4.3. Due to drastically different geometries, the starting masses for the threaded and cylindrical implants were not directly comparable by mass loss. Instead, percent mass loss was used to analyze changes in implant weight. There was no significant difference in the average percent weight loss over 30 days of degradation between the two geometries without curcumin loading.



**Figure 4.1:** XRD profiles of 85% PCL and 15% curcumin in relation to neat PCL and curcumin profiles.



**Figure 4.2:** XRD profiles of cryomilled 85% PCL and 15% curcumin in comparison to solvent mixed PCL and curcumin in the same ratio.

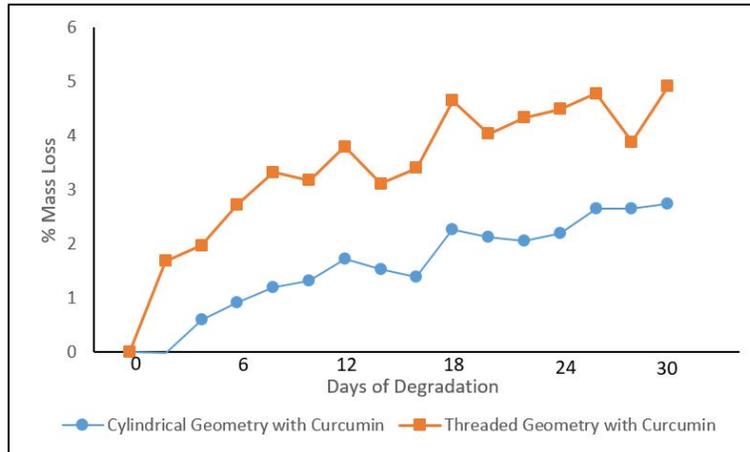


**Figure 4.3:** Graph of percent mass loss findings across all groups.

The percent mass loss of curcumin loaded PCL cylindrical millirods ( $1.59 \pm 0.884$ ) was not significantly different from that of the blank PCL rod mass loss ( $0.813 \pm 0.399$ ). Similarly, a comparison of blank threaded PCL implants ( $0.837 \pm 0.62$ ) to curcumin loaded PCL threaded implants ( $3.39 \pm 1.31$ ) did not have a significantly higher average percent mass loss over the PCL only sample. Lastly, a comparison of average cumulative mass loss for curcumin loaded implants with threaded and cylindrical geometries shows that the curcumin loaded threaded geometry ( $3.39 \pm 1.31$ ) did not have a significantly higher percent mass loss over 30 days than the cylindrical geometry implant with curcumin ( $1.59 \pm 0.884$ ). Table 4.1 shows the ANOVA results for the percent mass loss data. No interactions were found to be significant at a sample size of three. Figure 4.4 shows sample daily percent mass loss trends for the two curcumin loaded implants with different geometries.

**Table 4.1** ANOVA of Cumulative Mass Loss Percentages (n=3)

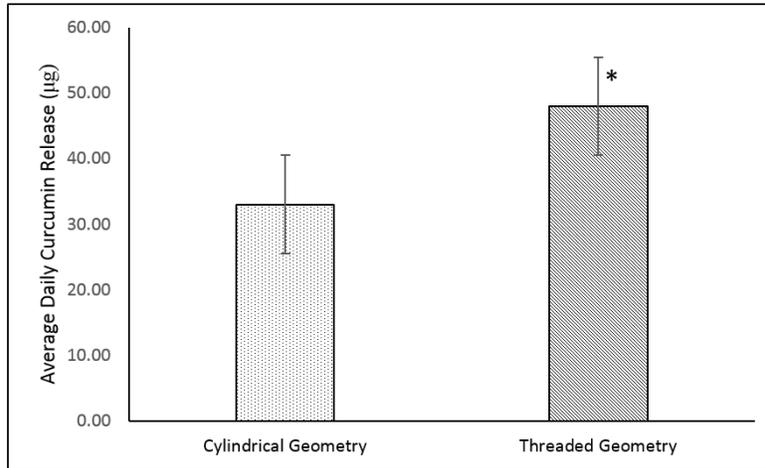
ANOVA							
	Source of Variation	SS	df	MS	F	P-value	F crit
	Geometry	2.417155	1	2.417155181	1.248524	0.296265	5.317655
	Curcumin Loading	8.124152	1	8.124152313	4.196338	0.074682	5.317655
	Interaction	2.470289	1	2.47028925	1.275969	0.291379	5.317655
	Within	15.48808	8	1.936010189			
	Total	28.49968	11				



**Figure 4.4:** *In vitro* release profiles over 30 days of degradation by percent mass loss of 15% curcumin loaded implants between the two different geometry types. There are no significant differences between treatments.

#### 4.3.3 Spectrophotometry Characterization

The results of the spectrophotometry characterization can be found in Figure 4.5. Curcumin concentration was analyzed every 2 days and those values were averaged to determine the average daily release rate over the period of 30 days. Using the standard curve to relate absorbance values to  $\mu\text{g}/\text{day}$  release found that the threaded geometry implant had a significantly higher average daily release than the cylindrical geometry after accounting for mass differences. Threaded geometry implants released an average of  $47.98 \pm 0.11 \mu\text{g}/\text{day}$ , while cylindrical geometry implants released  $33.00 \pm 0.06 \mu\text{g}/\text{day}$ .



**Figure 4.5:** Average daily curcumin release values over the course of 30 days *in vitro* degradation. \* denotes statistical significance.

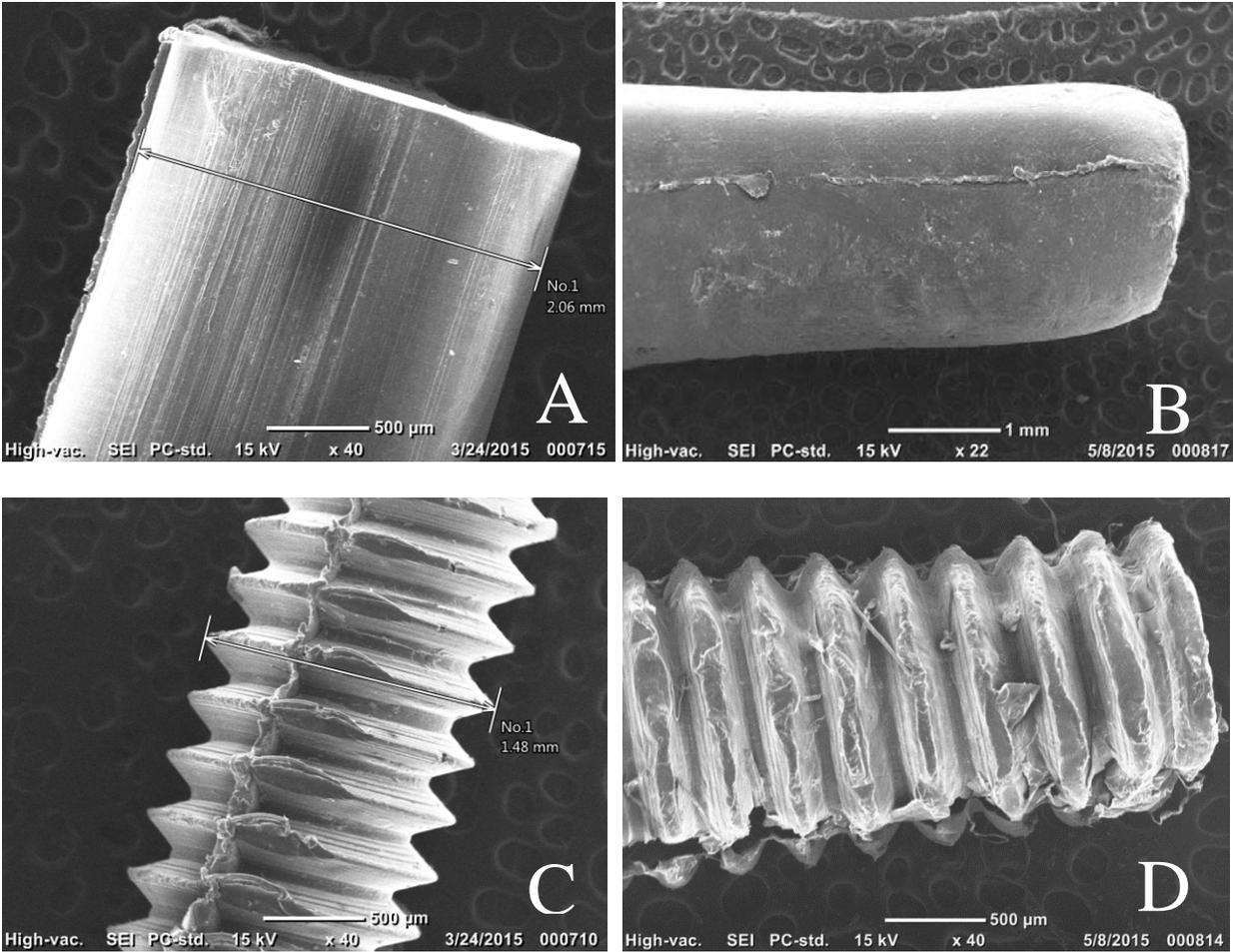
#### 4.3.4 SEM Analysis

Scanning electron microscope analysis of both cylindrical and threaded millirods can be found in Figure 4.6. After *in vitro* degradation a noticeable change in surface morphology was observed in both geometry types. At 30 days of degradation the cylindrical implant began showing signs of small surface holes where there were none previously.

Estimated threaded implant surface area was calculated using the following equation [20]:

$$SA = \pi * \left\{ \left( \frac{b}{2} \right)^2 - \left( \frac{a}{2} \right)^2 \right\} * \frac{d}{c} * 2 * \frac{2}{\sqrt{3}}$$

Where  $a$  is the inner thread diameter,  $b$  is the outer thread diameter,  $c$  is the pitch length, and  $d$  is the length of the implant. Surface area for the threaded geometry implant was found to be 72.4 mm<sup>2</sup>.



**Figure 4.6:** SEM images for sample millirods before and after *in vitro* degradation. (A) Cylindrical curcumin loaded millirod implant before degradation. (B) Cylindrical curcumin loaded millirod after 30 days of *in vitro* degradation. (C) Threaded curcumin loaded implant prior to degradation. (D) Threaded curcumin loaded implant after 30 days of degradation.

#### 4.4 DISCUSSION

Solvent mixing has been the primary method for creating the polymer drug matrix. However, this method requires additional materials in the form of solvents to dissolve the drug and the polymer, as well as time to remove the solvent from the mixture after the drug and polymer were combined. This is usually done overnight in a vacuum environment. The end product is a solid curcumin and polymer mass that must undergo additional processing prior to

being converted into its final form. Removing solvents from the polymer matrix creation would reduce manufacturing material costs in addition to a reduction in material processing time.

Cryomilling can be executed in minutes rather than hours, and the end product is a powder that is easy to introduce into molding or extruding applications. A comparison of the XRD profiles for both solvent mixed and cryomilled PCL and curcumin shows similar overall trends. Peaks indicate diffraction intensity at different angles. These peaks are unique to crystals within a structure and can be used to identify differences in materials present in the sample [21]. Despite maintaining relatively similar peak trends, near the 10 degree mark the cryomilled mixture has a defined peak not present in the solvent mixed sample. This peak is a reduced intensity version of the curcumin characteristic peak that appears at the same degree. This retention of a main characteristic peak for curcumin may point to a better method of mixing without compromising the compound integrity. A study by Wegiel et al. utilized cryomilling as a method to create larger quantities of amorphous curcumin and poly(vinylpyrrolidone) mixtures [22]. From this we can conclude that cryomilling serves as an effective alternative method for creating a homogeneous powder polymer matrix in bulk manufacturing quantities.

Additionally, the use of silastic tubing works well for small scale production of implants with a basic cylindrical geometry. Based on previous literature, modification of surface area is a way to increase drug release rate, but use of tubing limits changes to modifying diameter size and production volume. The use of a mold would allow for production of 8-12 implants at a time in addition to the potential incorporation of new surface geometries with a reduced amount of materials consumed during the process. Both the cylindrical and threaded geometry implants for this study were created through the use of a mold. This allowed for minimal post manufacturing

processing while allowing for the addition of complex surface geometry to the threaded implants that was previously not attainable.

*In vitro* degradation of the cylindrical geometry in comparison to the threaded geometry without drug loading was not statistically significant. By comparing each drug loaded implant geometry type to its respective blank implant counterpart it was determined that increased surface geometry in combination with curcumin loading was not significant for percent mass loss degradation. Both the curcumin loaded cylindrical implant and the curcumin loaded threaded implant showed greater percent mass loss degradation than the blank PCL implants, however, due to the small sample size statistical significance was not obtained.

A comparison of the curcumin loaded cylindrical implant and the curcumin loaded threaded implant showed the threaded geometry had a 46.9% greater percent mass loss over the cylindrical geometry during degradation. However, an analysis of variance was run on the average percent mass loss values and showed no significant values at  $p < 0.05$  indicating that a larger sample size is required to confirm that drug loading is a statistically significant parameter. Analysis of the percent mass loss from Figure 6 indicates a steady decreasing trend over the 30 day period. No burst release kinetics were observed for either implant geometry. SEM analysis of the threaded implants before and after degradation show that the threaded surface geometry was still present and visible after 30 days of degradation. Surface area for the threaded geometry implant was  $72.4 \text{ mm}^2$ , which is a 9.3% increase in surface area over a cylindrical implant of the same diameter.

Spectrophotometer analysis of the degradation serum showed after accounting for differences in implant masses that the threaded geometry implants had a higher average  $\mu\text{g}/\text{day}$  release than the cylindrical geometry. This confirms the hypothesis that an increase in surface

geometry would increase the drug release rate. A 100 mg cylindrical implant with a diameter of 3.22 mm and a 20% drug load created by Gupta et al. had an average daily release of 98  $\mu\text{g}/\text{day}$  and a cumulative release of 9.8% in 20 days [17]. Implants created for this study were not directly comparable to the previous findings due to differences in weight, volume, and drug load. However, extrapolation of mass and drug loading of the cylindrical geometry to better match the previous study gave an average daily release of  $\sim 70 \mu\text{g}/\text{day}$ . Differences in implant length and diameter would create significant differences in implant volume from the comparison implant and may account for discrepancies in average daily release between the two studies.

By using bulk manufacture of implants a cost savings could be imparted on the consumer. However, bulk manufacture limits the amount of customization available for the patient at the point of manufacture. Rather, customization for an individual patient would happen at the point of implantation. Customization could include the number of implants needed, the site of implantation, or post manufacturing modification, such as a change in implant length, to better meet patient dosing needs to manage neuropathy pain. The implant would be best utilized as an adjunct treatment to tricyclic antidepressants and antiepileptic drugs, or as a standalone pain relief option for patients intolerant or unaffected by current drug therapies.

#### **4.5 CONCLUSION**

There is a current need for additional treatment solutions to help manage the symptoms of PDN without the negative side effects of the current off label prescribed drugs. Curcumin presents an attractive anti-inflammatory agent and glycemic regulator, but is plagued by low bioavailability when taken orally. By altering the shape geometry through the introduction of threads, an increase in percent mass loss, although not statistically significant at this sample size,

was observed for curcumin loaded implants. The threaded geometry implants also showed a higher  $\mu\text{g/day}$  release of curcumin *in vitro* than their cylindrical counterparts. By modifying previously used manufacturing methods, this threaded geometry feature for subcutaneous implants can be easily scaled up for larger manufacturing needs.

## REFERENCES

- [1] Center for Disease Control and Prevention, National diabetes statistics report: Estimates of diabetes and its burden in the United States, 2014, Atlanta, GA: US Department of Health and Human Services, (2014).
- [2] L. Rydén, E. Standl, M. Bartnik, G. Van den Berghe, J. Betteridge, M.-J. De Boer, F. Cosentino, B. Jönsson, M. Laakso, K. Malmberg, Guidelines on diabetes, pre-diabetes, and cardiovascular diseases: Executive summary, *European Heart Journal*, 28 (2007) 88-136.
- [3] P. Dyck, K. Kratz, J. Karnes, W. Litchy, R. Klein, J. Pach, D. Wilson, P. O'Brien, L. Melton, The prevalence by staged severity of various types of diabetic neuropathy, retinopathy, and nephropathy in a population-based cohort The Rochester Diabetic Neuropathy Study, *Neurology*, 43 (1993) 817-817.
- [4] A.J. Boulton, A.I. Vinik, J.C. Arezzo, V. Bril, E.L. Feldman, R. Freeman, R.A. Malik, R.E. Maser, J.M. Sosenko, D. Ziegler, Diabetic neuropathies-A statement by the American Diabetes Association, *Diabetes Care*, 28 (2005) 956-962.
- [5] M.M. Huizinga, A. Peltier, Painful diabetic neuropathy: A management-centered review, *Clinical Diabetes*, 25 (2007) 6-15.
- [6] S.S. Bansal, H. Kausar, M.V. Vadhanam, S. Ravoori, J. Pan, S.N. Rai, R.C. Gupta, Curcumin Implants, Not Curcumin Diet, Inhibit Estrogen-Induced Mammary Carcinogenesis in ACI Rats, *Cancer Prevention Research*, 7 (2014) 456-465.
- [7] S.S. Bansal, M. Goel, F. Aqil, M.V. Vadhanam, R.C. Gupta, Advanced drug delivery systems of curcumin for cancer chemoprevention, *Cancer Prevention Research*, 4 (2011) 1158-1171.
- [8] E.-S.M. Ammar, N.M. Gameil, N.M. Shawky, M.A. Nader, Comparative evaluation of anti-inflammatory properties of thymoquinone and curcumin using an asthmatic murine model, *International Immunopharmacology*, 11 (2011) 2232-2236.

- [9] E. Bronte, G. Coppola, R. Di Miceli, V. Sucato, A. Russo, S. Novo, Role of curcumin in idiopathic pulmonary arterial hypertension treatment: A new therapeutic possibility, *Medical Hypotheses*, 81 (2013) 923-926.
- [10] S.P. Weisberg, R. Leibel, D.V. Tortoriello, Dietary curcumin significantly improves obesity-associated inflammation and diabetes in mouse models of diabetes, *Endocrinology*, 149 (2008) 3549-3558.
- [11] M.R. Maradana, R. Thomas, B.J. O'Sullivan, Targeted delivery of curcumin for treating type 2 diabetes, *Molecular Nutrition & Food Research*, 57 (2013) 1550-1556.
- [12] D.-w. Zhang, M. Fu, S.-H. Gao, J.-L. Liu, Curcumin and diabetes: A systematic review, *Evidence-Based Complementary and Alternative Medicine*, 2013 (2013).
- [13] S. Chuengsamarn, S. Rattanamongkolgul, R. Luechapudiporn, C. Phisalaphong, S. Jirawatnotai, Curcumin extract for prevention of type 2 diabetes, *Diabetes Care*, 35 (2012) 2121-2127.
- [14] S. Prasad, A.K. Tyagi, B.B. Aggarwal, Recent developments in delivery, bioavailability, absorption and metabolism of curcumin: The golden pigment from golden spice, *Cancer Research and Treatment: Official Journal of Korean Cancer Association*, 46 (2014) 2.
- [15] A. Stoddard, C. McNicholas, J.F. Peipert, Efficacy and safety of long-acting reversible contraception, *Drugs*, 71 (2011) 969-980.
- [16] S.S. Bansal, H. Kausar, F. Aqil, J. Jeyabalan, M.V. Vadhanam, R.C. Gupta, S. Ravoori, Curcumin implants for continuous systemic delivery: Safety and biocompatibility, *Drug Delivery and Translational Research*, 1 (2011) 332-341.
- [17] R.C. Gupta, S. Bansal, F. Aqil, J. Jeyabalan, P. Cao, H. Kausar, G. Russell, R. Munagala, S. Ravoori, M.V. Vadhanam, Controlled-release systemic delivery—A new concept in cancer chemoprevention, *Carcinogenesis*, (2012) bgs209.
- [18] S.S. Bansal, M.V. Vadhanam, R.C. Gupta, Development and in vitro-in vivo evaluation of polymeric implants for continuous systemic delivery of curcumin, *Pharmaceutical Research*, 28 (2011) 1121-1130.
- [19] K. I. Priyadarsini, Photophysics, photochemistry and photobiology of curcumin: Studies from organic solutions, bio-mimetics and living cells, *Journal of Photochemistry and Photobiology C: Photochemistry Reviews*, vol. 10, pp. 81-95, 2009.
- [20] Sharp Corporation, *Sharp's RoHS Analysis Method Guideline Version 2.2*, 2012, p. 6.

[21] B. Fultz, J. Howe, Diffraction and the X-ray powder diffractometer, *Transmission Electron Microscopy and Diffractometry of Materials* 2013, pp 1-57. Springer Berlin Heidelberg.

[22] L.A. Wegiel, Y. Zhao, L.J. Mauer, K.J. Edgar, L.S. Taylor, Curcumin amorphous solid dispersions: The influence of intra and intermolecular bonding on physical stability, *Pharmaceutical Development and Technology*, 19 (2014) 976-986.

## CHAPTER V: CONCLUSIONS & FUTURE WORK

### 5.1 Conclusions

This thesis has summarized literature on painful diabetic neuropathy and the challenges that surround pain relief for those affected. Current medications in use for treatment do not fully relieve pain and can adversely affect quality of life for those who suffer from PDN. There is a need for the creation of alternative pain relief therapies specifically targeting the treatment of painful diabetic neuropathy.

Effective strategies to control PDN have been to target hyperglycemia overall or block inflammatory and oxidative signals in cells. Curcumin is a natural compound found in the spice turmeric that has been shown to have anti-hyperglycemic, anti-inflammatory, and antioxidant effects in cells. Curcumin has been successfully used for *in vivo* evaluation studies focused on the compound's effectiveness against diabetes. However when taken orally, curcumin has limited bioavailability as the digestive system metabolizes most of the active compounds. In order to increase the effectiveness of the compound, curcumin needs to be encapsulated. The focus of this research was to determine if changes to the manufacturing process for encapsulating curcumin in a polymer matrix would be viable options in a bulk manufacturing setting.

Two primary manufacturing improvements to the creation of curcumin loaded millirod implants were established. The first is the use of cryomilling as a mixing method for the creation of homogeneous PCL and curcumin blends. The second was the incorporation of compression molding to allow for more complex surface geometry inclusions in an implant. These geometry inclusions were found to increase the percent mass loss of the implant in addition to increasing the average daily drug release over a standard cylindrical implant.

## 5.2 Review of Contribution

By utilizing cryomilling as a method of mixing curcumin and PCL compounds, the ease of manufacture of millirod implants in a scaled up manufacturing setting is greatly improved over solvent mixing methods. Introduction of compression molding as the main manufacturing process allows for previously unattainable implant surface geometries that impact implant degradation rate in addition to reducing the amount of materials consumed over the silastic tubing extrusion method. These modifications to the manufacture process are an essential step in moving curcumin implants from benchtop to market.

## 5.3 Future Work

Future studies will look at the impact of increased cryomilling time, and therefore smaller particle size, on average daily release of curcumin and percent mass loss implants. Evaluation of storage capabilities also needs to be done to determine the number of days before a PCL and curcumin matrix becomes crystalline. Crystallinity in curcumin is not ideal as amorphous solid dispersions are more soluble but less stable [54]. This study would be similar to the crystalline stability studies done by Wegiel, et al. [54]. The effective shelf life of the amorphous cryomilled powder would be crucial when considering a bulk manufacturing timeline in addition to the movement of finished implants through the supply chain. Another area of future work will include effective implant locations *in vivo*.

**REFERENCES**

- [1] M. M. Huizinga and A. Peltier, "Painful diabetic neuropathy: a management-centered review," *Clinical Diabetes*, vol. 25, pp. 6-15, 2007.
- [2] A. Veves, M. Backonja, and R. A. Malik, "Painful Diabetic Neuropathy: Epidemiology, Natural History, Early Diagnosis, and Treatment Options," *Pain Medicine*, vol. 9, pp. 660-674, 2008.
- [3] H. Khalil, "Painful diabetic neuropathy management," *International Journal of Evidence-Based Healthcare*, vol. 11, pp. 77-79, 2013.
- [4] V. Bril, J. England, G. M. Franklin, M. Backonja, J. Cohen, D. Del Toro, *et al.*, "Evidence-based guideline: treatment of painful diabetic neuropathy: report of the American Academy of Neurology, the American Association of Neuromuscular and Electrodiagnostic Medicine, and the American Academy of Physical Medicine and Rehabilitation," *PM&R*, vol. 3, pp. 345-352. e21, 2011.
- [5] B. B. Aggarwal and K. B. Harikumar, "Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases," *The International Journal of Biochemistry & Cell Biology*, vol. 41, pp. 40-59, 2009.
- [6] T. Mrudula, P. Suryanarayana, P. Srinivas, and G. B. Reddy, "Effect of curcumin on hyperglycemia-induced vascular endothelial growth factor expression in streptozotocin-induced diabetic rat retina," *Biochemical and Biophysical Research Communications*, vol. 361, pp. 528-532, 2007.
- [7] R. A. Kowluru and M. Kanwar, "Effects of curcumin on retinal oxidative stress and inflammation in diabetes," *Nutr Metab (Lond)*, vol. 4, p. 8, 2007.
- [8] S. K. Gupta, B. Kumar, T. C. Nag, S. S. Agrawal, R. Agrawal, P. Agrawal, *et al.*, "Curcumin prevents experimental diabetic retinopathy in rats through its hypoglycemic, antioxidant, and anti-inflammatory mechanisms," *Journal of Ocular Pharmacology and Therapeutics*, vol. 27, pp. 123-130, 2011.
- [9] W. Xie and L. Du, "Diabetes is an inflammatory disease: evidence from traditional Chinese medicines," *Diabetes, Obesity and Metabolism*, vol. 13, pp. 289-301, 2011.
- [10] D. Zozulinska and B. Wierusz-Wysocka, "Type 2 diabetes mellitus as inflammatory disease," *Diabetes Research and Clinical Practice*, vol. 74, pp. S12-S16, 2006.
- [11] P. Anand, C. Sundaram, S. Jhurani, A. B. Kunnumakkara, and B. B. Aggarwal, "Curcumin and cancer: an "old-age" disease with an "age-old" solution," *Cancer Letters*, vol. 267, pp. 133-164, 2008.

- [12] G. Bajaj and Y. Yeo, "Drug delivery systems for intraperitoneal therapy," *Pharmaceutical Research*, vol. 27, pp. 735-738, 2010.
- [13] B. D. Weinberg, E. Blanco, and J. Gao, "Polymer implants for intratumoral drug delivery and cancer therapy," *Journal of Pharmaceutical Sciences*, vol. 97, pp. 1681-1702, 2008.
- [14] S. S. Bansal, M. V. Vadhanam, and R. C. Gupta, "Development and in vitro-in vivo evaluation of polymeric implants for continuous systemic delivery of curcumin," *Pharmaceutical Research*, vol. 28, pp. 1121-1130, 2011.
- [15] R. C. Gupta, S. Bansal, F. Aqil, J. Jeyabalan, P. Cao, H. Kausar, *et al.*, "Controlled-release systemic delivery—a new concept in cancer chemoprevention," *Carcinogenesis*, p. bgs209, 2012.
- [16] S. S. Bansal, H. Kausar, M. V. Vadhanam, S. Ravoori, J. Pan, S. N. Rai, *et al.*, "Curcumin Implants, Not Curcumin Diet, Inhibit Estrogen-Induced Mammary Carcinogenesis in ACI Rats," *Cancer Prevention Research*, vol. 7, pp. 456-465, 2014.
- [17] A. D. Association, "Fast facts: data and statistics about diabetes," ed, 2013.
- [18] A. Gordois, P. Scuffham, A. Shearer, A. Oglesby, and J. A. Tobian, "The health care costs of diabetic peripheral neuropathy in the US," *Diabetes Care*, vol. 26, pp. 1790-1795, 2003.
- [19] Y. Agrawal, J. P. Carey, C. C. Della Santina, M. C. Schubert, and L. B. Minor, "Diabetes, vestibular dysfunction, and falls: analyses from the National Health and Nutrition Examination Survey," *Otology & Neurotology*, vol. 31, pp. 1445-1450, 2010.
- [20] A. M. Vincent, B. C. Callaghan, A. L. Smith, and E. L. Feldman, "Diabetic neuropathy: cellular mechanisms as therapeutic targets," *Nature Reviews Neurology*, vol. 7, pp. 573-583, 2011.
- [21] I. G. Obrosova, "Increased sorbitol pathway activity generates oxidative stress in tissue sites for diabetic complications," *Antioxidants & Redox Signaling*, vol. 7, pp. 1543-1552, 2005.
- [22] B. C. Callaghan, H. T. Cheng, C. L. Stables, A. L. Smith, and E. L. Feldman, "Diabetic neuropathy: clinical manifestations and current treatments," *The Lancet Neurology*, vol. 11, pp. 521-534, 2012.
- [23] B. C. Callaghan, A. A. Little, E. L. Feldman, and R. A. Hughes, "Enhanced glucose control for preventing and treating diabetic neuropathy," *The Cochrane Library*, 2012.

- [24] W. R. Schafer, "How Do Antidepressants Work? Prospects for Genetic Analysis of Drug Mechanisms," *Cell*, vol. 98, pp. 551-554, 1999.
- [25] E. S. PAYKEL, P. S. MUELLER, and P. De la Vergne, "Amitriptyline, weight gain and carbohydrate craving: a side effect," *The British Journal of Psychiatry*, vol. 123, pp. 501-507, 1973.
- [26] C. Dallocchio, C. Buffa, P. Mazzarello, and S. Chiroli, "Gabapentin vs. amitriptyline in painful diabetic neuropathy: an open-label pilot study," *Journal of Pain and Symptom Management*, vol. 20, pp. 280-285, 2000.
- [27] M. B. Max, S. A. Lynch, J. Muir, S. E. Shoaf, B. Smoller, and R. Dubner, "Effects of desipramine, amitriptyline, and fluoxetine on pain in diabetic neuropathy," *New England Journal of Medicine*, vol. 326, pp. 1250-1256, 1992.
- [28] H. J. Milionis, A. Skopelitou, and M. S. Elisaf, "Hypersensitivity syndrome caused by amitriptyline administration," *Postgraduate Medical Journal*, vol. 76, pp. 361-363, 2000.
- [29] J. C. Markowitz and R. P. Brown, "Seizures with neuroleptics and antidepressants," *General hospital psychiatry*, vol. 9, pp. 135-141, 1987.
- [30] C. M. Morello, S. G. Leckband, C. P. Stoner, D. F. Moorhouse, and G. A. Sahagian, "Randomized double-blind study comparing the efficacy of gabapentin with amitriptyline on diabetic peripheral neuropathy pain," *Archives of Internal Medicine*, vol. 159, pp. 1931-1937, 1999.
- [31] M. Nolano, D. A. Simone, G. Wendelschafer-Crabb, T. Johnson, E. Hazen, and W. R. Kennedy, "Topical capsaicin in humans: parallel loss of epidermal nerve fibers and pain sensation," *Pain*, vol. 81, pp. 135-145, 1999.
- [32] L. Mason, R. A. Moore, S. Derry, J. E. Edwards, and H. J. McQuay, "Systematic review of topical capsaicin for the treatment of chronic pain," *BMJ*, vol. 328, p. 991, 2004.
- [33] J. Xu and Y. Yang, "Traditional Chinese medicine in the Chinese health care system," *Health Policy*, vol. 90, pp. 133-139, 2009.
- [34] W. Li, H. Zheng, J. Bukuru, and N. De Kimpe, "Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus," *Journal of Ethnopharmacology*, vol. 92, pp. 1-21, 2004.
- [35] L.-W. Qi, E.-H. Liu, C. Chu, Y.-B. Peng, H.-X. Cai, and P. Li, "Anti-diabetic agents from natural products—an update from 2004 to 2009," *Current Topics in Medicinal Chemistry*, vol. 10, pp. 434-457, 2010.

- [36] Z. Sang, L. Zhou, X. Fan, and R. J. McCrimmon, "Radix astragali (huangqi) as a treatment for defective hypoglycemia counterregulation in diabetes," *The American Journal of Chinese Medicine*, vol. 38, pp. 1027-1038, 2010.
- [37] J. Yin, H. Zhang, and J. Ye, "Traditional Chinese medicine in treatment of metabolic syndrome," *Endocrine, Metabolic & Immune Disorders Drug Targets*, vol. 8, p. 99, 2008.
- [38] K. I. Priyadarsini, "The Chemistry of Curcumin: From Extraction to Therapeutic Agent," *Molecules*, vol. 19, pp. 20091-20112, 2014.
- [39] A. Shehzad, G. Rehman, and Y. S. Lee, "Curcumin in inflammatory diseases," *Biofactors*, vol. 39, pp. 69-77, 2013.
- [40] S. P. Weisberg, R. Leibel, and D. V. Tortoriello, "Dietary curcumin significantly improves obesity-associated inflammation and diabetes in mouse models of diabetes," *Endocrinology*, vol. 149, pp. 3549-3558, 2008.
- [41] Z. M. Shao, Z. Z. Shen, C. H. Liu, M. R. Sartippour, V. L. Go, D. Heber, *et al.*, "Curcumin exerts multiple suppressive effects on human breast carcinoma cells," *International Journal of Cancer*, vol. 98, pp. 234-240, 2002.
- [42] M. Srinivasan, "Effect of curcumin on blood sugar as seen in a diabetic subject," *Indian Journal of Medical Sciences*, vol. 26, p. 269, 1972.
- [43] M. R. Maradana, R. Thomas, and B. J. O'Sullivan, "Targeted delivery of curcumin for treating type 2 diabetes," *Molecular Nutrition & Food Research*, vol. 57, pp. 1550-1556, 2013.
- [44] A.-L. Cheng, C.-H. Hsu, J.-K. Lin, M.-M. Hsu, Y.-F. Ho, T.-S. Shen, *et al.*, "Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions," *Anticancer Research*, vol. 21, pp. 2895-2900, 2000.
- [45] C. D. Lao, M. T. Ruffin, D. Normolle, D. D. Heath, S. I. Murray, J. M. Bailey, *et al.*, "Dose escalation of a curcuminoid formulation," *BMC Complementary and Alternative Medicine*, vol. 6, p. 10, 2006.
- [46] K. I. Priyadarsini, "Photophysics, photochemistry and photobiology of curcumin: Studies from organic solutions, bio-mimetics and living cells," *Journal of Photochemistry and Photobiology C: Photochemistry Reviews*, vol. 10, pp. 81-95, 2009.
- [47] P. Anand, A. B. Kunnumakkara, R. A. Newman, and B. B. Aggarwal, "Bioavailability of curcumin: problems and promises," *Molecular Pharmaceutics*, vol. 4, pp. 807-818, 2007.

- [48] S. S. Bansal, M. Goel, F. Aqil, M. V. Vadhanam, and R. C. Gupta, "Advanced drug delivery systems of curcumin for cancer chemoprevention," *Cancer Prevention Research*, vol. 4, pp. 1158-1171, 2011.
- [49] S. S. Bansal, H. Kausar, F. Aqil, J. Jeyabalan, M. V. Vadhanam, R. C. Gupta, *et al.*, "Curcumin implants for continuous systemic delivery: safety and biocompatibility," *Drug Delivery And Translational Research*, vol. 1, pp. 332-341, 2011.
- [50] S. S. Bansal, H. Kausar, M. V. Vadhanam, S. Ravoori, and R. C. Gupta, "Controlled systemic delivery by polymeric implants enhances tissue and plasma curcumin levels compared with oral administration," *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 80, pp. 571-577, 2012.
- [51] S. Aldrich, "Dicholormethane- MSDS 270997," ed. Saint Louis, 2015.
- [52] S. Aldrich, "Ethyl Alcohol, Pure- MSDS E7023," ed. Saint Louis, 2015.
- [53] C. R. Garcia, F. Manzi, F. Tediosi, S. L. Hoffman, and E. R. James, "Comparative cost models of a liquid nitrogen vapor phase (LNVP) cold chain-distributed cryopreserved malaria vaccine vs. a conventional vaccine," *Vaccine*, vol. 31, pp. 380-386, 2013.
- [54] L. A. Wegiel, Y. Zhao, L. J. Mauer, K. J. Edgar, and L. S. Taylor, "Curcumin amorphous solid dispersions: the influence of intra and intermolecular bonding on physical stability," *Pharmaceutical Development and Technology*, vol. 19, pp. 976-986, 2014.
- [55] J. B. Wolinsky, Y. L. Colson, and M. W. Grinstaff, "Local drug delivery strategies for cancer treatment: gels, nanoparticles, polymeric films, rods, and wafers," *Journal of Controlled Release*, vol. 159, pp. 14-26, 2012.
- [56] A. S. Hoffman, "Hydrogels for biomedical applications," *Advanced Drug Delivery Reviews*, vol. 64, pp. 18-23, 2012.
- [57] H. Chen, X. Chang, D. Du, J. Li, H. Xu, and X. Yang, "Microemulsion-based hydrogel formulation of ibuprofen for topical delivery," *International Journal of Pharmaceutics*, vol. 315, pp. 52-58, 2006.
- [58] M. Shibayama and T. Sakai, "CHAPTER 2 Fabrication, Structure, Mechanical Properties, and Applications of Tetra-PEG Hydrogels," in *Polymeric and Self Assembled Hydrogels: From Fundamental Understanding to Applications*, ed: The Royal Society of Chemistry, 2013, pp. 7-38.
- [59] D. Seliktar, "Designing Cell-Compatible Hydrogels for Biomedical Applications," *Science*, vol. 336, pp. 1124-1128, 2012.

- [60] T. R. Hoare and D. S. Kohane, "Hydrogels in drug delivery: progress and challenges," *Polymer*, vol. 49, pp. 1993-2007, 2008.
- [61] H. T. Ta, C. R. Dass, and D. E. Dunstan, "Injectable chitosan hydrogels for localised cancer therapy," *Journal of Controlled Release*, vol. 126, pp. 205-216, 2008.
- [62] D.-D. Guo, S.-H. Hong, H.-L. Jiang, J.-H. Kim, A. Minai-Tehrani, J.-E. Kim, *et al.*, "Synergistic effects of Akt1 shRNA and paclitaxel-incorporated conjugated linoleic acid-coupled poloxamer thermosensitive hydrogel on breast cancer," *Biomaterials*, vol. 33, pp. 2272-2281, 2012.
- [63] E. Ruel-Gariépy, M. Shive, A. Bichara, M. Berrada, D. Le Garrec, A. Chenite, *et al.*, "A thermosensitive chitosan-based hydrogel for the local delivery of paclitaxel," *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 57, pp. 53-63, 2004.
- [64] M. Berrada, A. Serreqi, F. Dabbarh, A. Owusu, A. Gupta, and S. Lehnert, "A novel non-toxic camptothecin formulation for cancer chemotherapy," *Biomaterials*, vol. 26, pp. 2115-2120, 2005.
- [65] S. Zhang, J. Ermann, M. D. Succi, A. Zhou, M. J. Hamilton, B. Cao, *et al.*, "An inflammation-targeting hydrogel for local drug delivery in inflammatory bowel disease," *Science Translational Medicine*, vol. 7, pp. 300ra128-300ra128, 2015.
- [66] R. M. Machado, A. Palmeira-De-Oliveira, J. Martinez-De-Oliveira, and R. Palmeira-De-Oliveira, "Vaginal films for drug delivery," *Journal of Pharmaceutical Sciences*, vol. 102, pp. 2069-2081, 2013.
- [67] F. P. Seib and D. L. Kaplan, "Doxorubicin-loaded silk films: drug-silk interactions and in vivo performance in human orthotopic breast cancer," *Biomaterials*, vol. 33, pp. 8442-8450, 2012.
- [68] D. Klose, F. Siepman, K. Elkharraz, and J. Siepman, "PLGA-based drug delivery systems: importance of the type of drug and device geometry," *International Journal of Pharmaceutics*, vol. 354, pp. 95-103, 2008.
- [69] C. G. Pitt, M. M. Gratzl, A. R. Jeffcoat, R. Zweidinger, and A. Schindler, "Sustained drug delivery systems II: Factors affecting release rates from poly ( $\epsilon$ -caprolactone) and related biodegradable polyesters," *Journal of Pharmaceutical Sciences*, vol. 68, pp. 1534-1538, 1979.
- [70] X. Wang, T. Yucel, Q. Lu, X. Hu, and D. L. Kaplan, "Silk nanospheres and microspheres from silk/PVA blend films for drug delivery," *Biomaterials*, vol. 31, pp. 1025-1035, 2010.

- [71] Z.-M. Huang, Y.-Z. Zhang, M. Kotaki, and S. Ramakrishna, "A review on polymer nanofibers by electrospinning and their applications in nanocomposites," *Composites Science and Technology*, vol. 63, pp. 2223-2253, 2003.
- [72] J. Zeng, X. Xu, X. Chen, Q. Liang, X. Bian, L. Yang, *et al.*, "Biodegradable electrospun fibers for drug delivery," *Journal of Controlled Release*, vol. 92, pp. 227-231, 2003.
- [73] J. B. Chiu, Y. K. Luu, D. Fang, B. S. Hsiao, B. Chu, and M. Hadjiargyrou, "Electrospun nanofibrous scaffolds for biomedical applications," *Journal of Biomedical Nanotechnology*, vol. 1, pp. 115-132, 2005.
- [74] H. Wang, H. Shao, and X. Hu, "Structure of silk fibroin fibers made by an electrospinning process from a silk fibroin aqueous solution," *Journal Of Applied Polymer Science*, vol. 101, pp. 961-968, 2006.
- [75] X. Zhang, M. R. Reagan, and D. L. Kaplan, "Electrospun silk biomaterial scaffolds for regenerative medicine," *Advanced Drug Delivery Reviews*, vol. 61, pp. 988-1006, 2009.
- [76] V. Jacobs, R. D. Anandjiwala, and M. Maaza, "The influence of electrospinning parameters on the structural morphology and diameter of electrospun nanofibers," *Journal Of Applied Polymer Science*, vol. 115, pp. 3130-3136, 2010.
- [77] P. Zahedi, I. Rezaeian, S. O. Ranaei-Siadat, S. H. Jafari, and P. Supaphol, "A review on wound dressings with an emphasis on electrospun nanofibrous polymeric bandages," *Polymers for Advanced Technologies*, vol. 21, pp. 77-95, 2010.
- [78] J. S. Boateng, K. H. Matthews, H. N. Stevens, and G. M. Eccleston, "Wound healing dressings and drug delivery systems: a review," *Journal Of Pharmaceutical Sciences*, vol. 97, pp. 2892-2923, 2008.
- [79] M. Gibaldi, M. Lee, A. Desai, and A. S. o. H.-S. Pharmacists, *Gibaldi's Drug Delivery Systems in Pharmaceutical Care: American Society of Health-System Pharmacists*, 2007.
- [80] M. Panigrahi, P. Das, and P. Parikh, "Brain tumor and Gliadel wafer treatment," *Indian Journal Of Cancer*, vol. 48, p. 11, 2011.
- [81] J. S. Boateng, A. D. Auffret, K. H. Matthews, M. J. Humphrey, H. N. Stevens, and G. M. Eccleston, "Characterisation of freeze-dried wafers and solvent evaporated films as potential drug delivery systems to mucosal surfaces," *International Journal Of Pharmaceutics*, vol. 389, pp. 24-31, 2010.
- [82] A. Stoddard, C. McNicholas, and J. F. Peipert, "Efficacy and safety of long-acting reversible contraception," *Drugs*, vol. 71, pp. 969-980, 2011.

- [83] A. Göpferich, "Mechanisms of polymer degradation and erosion," *Biomaterials*, vol. 17, pp. 103-114, 1996.
- [84] F. von Burkersroda, L. Schedl, and A. Göpferich, "Why degradable polymers undergo surface erosion or bulk erosion," *Biomaterials*, vol. 23, pp. 4221-4231, 2002.
- [85] J. Siepmann and A. Göpferich, "Mathematical modeling of bioerodible, polymeric drug delivery systems," *Advanced Drug Delivery Reviews*, vol. 48, pp. 229-247, 2001.
- [86] E. A. Simone, T. D. Dziubla, and V. R. Muzykantov, "Polymeric carriers: role of geometry in drug delivery," 2008.
- [87] J. D. Pillai, S. S. Dunn, M. E. Napier, and J. M. DeSimone, "Novel platforms for vascular carriers with controlled geometry," *IUBMB Life*, vol. 63, pp. 596-606, 2011.
- [88] S. Muro, C. Garnacho, J. A. Champion, J. Leferovich, C. Gajewski, E. H. Schuchman, *et al.*, "Control of endothelial targeting and intracellular delivery of therapeutic enzymes by modulating the size and shape of ICAM-1-targeted carriers," *Molecular Therapy*, vol. 16, pp. 1450-1458, 2008.
- [89] R. Yang, T. Chen, H. Chen, and W. Wang, "Microfabrication of biodegradable (PLGA) honeycomb-structures and potential applications in implantable drug delivery," *Sensors and Actuators B: Chemical*, vol. 106, pp. 506-511, 2005.
- [90] S. Das, A. S. Wajid, S. K. Bhattacharia, M. D. Wilting, I. V. Rivero, and M. J. Green, "Electrospinning of polymer nanofibers loaded with noncovalently functionalized graphene," *Journal of Applied Polymer Science*, vol. 128, pp. 4040-4046, 2013.
- [91] S. S. Spearman, I. V. Rivero, and N. Abidi, "Influence of polycaprolactone/polyglycolide blended electrospun fibers on the morphology and mechanical properties of polycaprolactone," *Journal of Applied Polymer Science*, vol. 131, 2014.
- [92] C. Vepari and D. L. Kaplan, "Silk as a biomaterial," *Progress In Polymer Science*, vol. 32, pp. 991-1007, 2007.
- [93] G. Li, H. Liu, T. Li, and J. Wang, "Surface modification and functionalization of silk fibroin fibers/fabric toward high performance applications," *Materials Science and Engineering: C*, vol. 32, pp. 627-636, 2012.
- [94] D. N. Rockwood, R. C. Preda, T. Yücel, X. Wang, M. L. Lovett, and D. L. Kaplan, "Materials fabrication from *Bombyx mori* silk fibroin," *Nature Protocols*, vol. 6, pp. 1612-1631, 2011.

- [95] M. Ziabari, V. Mottaghitalab, and A. Haghi, "Application of direct tracking method for measuring electrospun nanofiber diameter," *Brazilian Journal of Chemical Engineering*, vol. 26, pp. 53-62, 2009.
- [96] T. F. S. Inc, "Gibco Cell Culture Basics," ed, 2015, p. 12.
- [97] I. Harry, "Nutrition Needs of Mammalian Cells in Tissue Culture," 1955.
- [98] Y. Reinwald and A. El Haj, "Evaluation of the Performance of a Novel Hydrostatic Bioreactor," in *World Congress on Medical Physics and Biomedical Engineering May 26-31, 2012, Beijing, China*, 2013, pp. 1969-1972.