Published in final edited form as:

J Appl Polym Sci. 2018 April ; 135(14): . doi:10.1002/app.46068.

Biodegradable Polymeric Injectable Implants for Long-Term Delivery of Contraceptive Drugs

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Abstract

Development of injectable, long-lasting, contraceptive drug delivery formulations and implants are highly desired to avoid unplanned pregnancies while improving patient compliance and reducing adverse side effects and treatment costs. The present study reports on the fabrication and characterization of two levonorgestrel (LNG) microsphere injectable formulations. Poly(ϵ -caprolactone) (PCL) with 12.5% and 24% (w/w) LNG were fabricated into microspheres, measuring 300 ± 125 μm , *via* the oil-in-water (o/w) emulsion solvent evaporation technique. Formulations showed sustained drug release up to 120 days. FTIR, XRD, DSC, and TGA confirmed the absence of LNG chemical interaction with PCL as well as its molecular level distribution. The *in vitro* release of LNG was calculated to be Fickian diffusion controlled and properly characterized. The inclusion of multiple elevated release temperatures allowed for the application of the Arrhenius model to calculate drug release constants and representative sampling intervals, demonstrating the use of elevated temperatures for accelerated-time drug release studies.

Graphical Abstract

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INTRODUCTION

Hormonal contraceptives have been used to prevent or delay childbearing for more than four decades ¹. The most common contraceptive method remains the combined oral contraceptive (COC) or the progestogen-only pill (POP), which contain combined estrogen and progestogen, and progestogen hormone only, respectively ². Although both have shown >99% effectiveness with correct and consistent use, they require daily oral ingestion of pill at the same time each day, thus dropping effectiveness to roughly 92% and 90–97%, as commonly used, for COCs and POPs respectively ³. There are various alternative forms of contraception namely, male and female condoms, injectables, subdermal implants, vaginal rings, intrauterine devices (IUDs) ^{3,4}. Typical contraceptive implants and injectables, using similar mechanisms, overcome the shortcomings of daily use by providing daily therapeutic release for a defined period of time, have shown >99% effectiveness ³. However, such implants must be inserted and removed by health-care providers via surgical procedure every few months, depending on the product, and are not economically efficient. IUDs which can provide contraceptive therapy for greater than one year are extremely expensive and can cause discomfort to patients. Levonorgestrel (LNG) is a popular, FDA approved progestin hormone that elicits changes in the uterine lining to prevent ovulation. LNG has been used in numerous birth control systems such as the emergency contraceptive Plan B[®] (Teva Pharmaceuticals, North Wales, PA, USA) and long-term intrauterine devices including Kyleena[®], Mirena[®], and Skyla[®] (Bayer, Whippany, NJ, USA). It is a standard for reliable, consistent, and effective contraception.

Degradable polymeric drug delivery devices based on polyesters, poly(ortho esters), poly(amides), poly(anhydrides) poly(ester amides), poly(alkyl cyanoacrylates), and several polysaccharides have been approved by the U.S. Food and Drug Administration (FDA) for clinical use ⁵. Polymer selection, size and shape of the implants greatly depend on the target application, desired drug release concentration and time. For instance, injectable polysaccharide hydrogel, poly(lactic acid), or poly(lactic acid-co-glycolic acid) (PLGA)

formulations have been popularly used for drug delivery in pain management and in tissue engineering^{6–8}. These diffusion- and bulk erosion-controlled formulations of PLGA polymers can deliver drugs up to several weeks, but clearly lack abilities to deliver drugs for an extended period of time due to their hydrophilic nature and fast degradation rates (9). Several microsphere formulations have been explored for delivering contraceptive drugs and release profiles were altered by changing particle size and drug content in the formulation^{9–12}.

Poly(ϵ -caprolactone) (PCL) is an FDA approved, biodegradable polymer which has been extensively investigated for use as implantable biomaterials and injectable implants for controlled release drug delivery systems^{13–20}. PCL has been used in the design and development of long-acting drug-eluting implants due to its hydrophobic and slow eroding nature^{13–17}. LNG is a hydrophobic drug which follows a zero-order release mechanism from PCL matrices, where drug release time can be extended by altering polymer molecular weight, particle size and drug concentration^{12,21,22}. In the present study, we fabricate PCL microspheres with two loading concentrations of LNG for long-term contraceptive drug delivery. The selection of the PCL molecular weight, drug loading concentration, and particle sizes were based on literature reports and pilot studies designed to obtain therapeutic doses of LNG²². The system has many potential advantages over commercially available products which often do not offer long-term sustained release of drug in addition to biodegradability. This ensures a consistent, prolonged contraceptive therapy combined with steady degradation over the course of the therapy, without fragmenting, overcoming the need for secondary surgery. Microsphere formulations were characterized for particle size, possible drug-matrix interaction, drug distribution and drug release kinetics and parameters. Release studies were carried out at three different temperatures namely 37, 42, and 47°C to calculate the rate of drug diffusion through Arrhenius equation²³. Studying elevated temperature release allowed for the use of the Arrhenius model, which is used in the drug industry to model accelerated time drug release²³. Release data was also fitted to several popular release models applying initial time approximations to calculate the nature of drug release. These drug release parameters are essential in the design and development of injectable implants for the delivery LNG. Intramuscular injection of such implants may provide therapeutic doses of contraceptive drug for a prolonged period and degrade *in vivo*^{12,24}. The simplicity of microsphere fabrication makes it an attractive approach, as it easy and affordable to mass-produce and meet commercial demand. Long-term contraceptive delivery has several advantages, namely, that it provides the patient with a single, minimally invasive treatment which remains therapeutically effective for an extended period of time. This is in contrast to daily COCs or POPs which are inconvenient and run the risk of the patient missing a dose, and to expensive, complicated IUD systems which demand regular medical attention.

MATERIALS AND METHODS

Materials

Poly(ϵ -Caprolactone) (PCL) (MW 80 kDa), polyvinyl alcohol (PVA) (MW 30–70 kDa), and levonorgestrel (LNG) (United States Pharmacopeia Reference Standard) were obtained from

Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade methanol, chloroform, isopropyl alcohol as well as regenerated cellulose dialysis tubing (MWCO 10–12 KDa) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). All other reagents used in this study were of HPLC-grade and used without further purification. Ultrapure deionized water (18.2 MΩ-cm at 25 °C) was used for every experiment by the Milli-Q plus system from EMD Millipore (Billerica, MA, USA).

Preparation of Levonorgestrel Loaded Poly(ϵ -Caprolactone) Microspheres

LNG-loaded PCL microspheres were fabricated using an oil-in-water (o/w) emulsion, solvent evaporation method²². In brief, a 13% (w/v) PCL solution was prepared in chloroform and 12.5% or 24% (w/w) LNG was added and stirred to dissolve the drug. This oil phase was dispersed in 500 mL of DI water with 3% (w/v) PVA as a surfactant with constant stirring at 500 RPM using an overhead rotor. The stirring was continued overnight and hardened microspheres were collected by filtration and washed repeatedly. Microspheres were vacuum dried and kept desiccated until further use. Control PCL microspheres without LNG were prepared in an identical manner.

Determination of Drug Encapsulation Efficiency

Two milligrams of PCL-LNG microspheres were dissolved in 5 mL of dichloromethane. Once dissolved, 15 mL of methanol was added to the solution to precipitate out PCL which was subsequently removed by centrifugation. The supernatant was analyzed for LNG using UV-Vis spectroscopy (λ_{max} 240 nm) (Genesys 10S, Thermo Fisher Scientific, Waltham, MA, USA) and converted to a drug concentration using a standard curve previously created for LNG in methanol. Encapsulation efficiency was calculated as a percentage using Equation 1.

$$\text{Encapsulation Efficiency (\%)} = \frac{\text{Amount of drug in microspheres}}{\text{Amount of drug added to formulation}} * 100 \quad \text{Equation 1}$$

Scanning Electron Microscopy

The surface morphology of PCL microsphere formulations were visualized using scanning electron microscopy (SEM) (JEOL JSM-6335F, JEOL USA, Inc., MA, USA). Microspheres were coated with Au/Pd using a Polaron E5100 sputtering system (Quorum Technologies, East Sussex, UK) prior to imaging. Images were captured at various magnifications and ImageJ (NIH, Rockville, MD) was used to measure the particle size.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy was used to characterize the possible interaction between LNG and PCL. Samples were recorded in the range of 400–4000 cm⁻¹ and analyses were made using the Nicolet OMNIC Sepcta Software (Thermo Fisher Scientific, Waltham, MA, USA).

X-Ray Diffraction

To understand the nature of drug distribution within polymer matrix X-ray diffraction (XRD) studies were performed using a Bruker D2 Phaser (Bruker AXS, Madison, WI, USA). XRD pattern for PCL microspheres, LNG powder, and PCL-LNG microspheres were recorded from 5–60° (2 θ) at a scanning speed of 0.2 deg/s. Cu-K α (λ 1.54056 Å) radiation at 40 kV and 30 mA was used as the X-ray source.

Differential Scanning Calorimetry (DSC)

DSC was used to characterize the nature and possible interactions between LNG and PCL. DSC thermograms were obtained for PCL microspheres, LNG powder, and PCL-LNG microspheres (DSC Q100, TA Instruments, New Castle, DE, USA). A ~ 10 mg of each sample in an aluminum pan was heated from 0–300 °C at a rate of 10 °C/min under 20 mL/min nitrogen purge. The DSC data was analyzed using the associated TA Instrument's Universal Analysis software package.

Thermal Gravimetric Analysis (TGA)

TGA was used to characterize possible interactions between LNG and PCL (TGA Q-500, TA Instruments, New Castle, DE, USA). A ~ 10 mg sample of PCL microsphere, LNG powder, and PCL-LNG microsphere in an aluminum pan was heated from 0–700 °C at a rate of 10 °C/min under nitrogen purge. Thermograms were recorded to capture weight loss as a function of increasing temperature.

In Vitro Drug Release

Dissolution was carried out at 37, 42, and 47°C in a 10mL mixture of 75% (v/v) phosphate buffered saline (PBS) at pH 7.4 and 25% (v/v) isopropyl alcohol (IPA) in temperature controlled shaker water baths. Selection of the dissolution media was based on previous literature discussing solubility of LNG²⁵. Approximately 50 mg of microspheres were taken in dialysis bags to which 1mL of above referred dissolution media was added. Each bag was suspended into 15 mL polypropylene centrifuge tubes and filled with 9 mL of dissolution media as release media. For each formulation, a sample size n=5 was used for each temperature. At various, predetermined time intervals, the two milliliters of release media were removed and replaced by fresh media. The concentration of LNG in the release media was determined by UV-Vis spectroscopy (Genesys 10S, Thermo Fisher Scientific, Waltham, MA, USA) at a λ_{max} of 240 nm using a standard curve previously created for LNG in dissolution media. One set of samples were also characterized for daily LNG release using a similar release setup. In brief, the entire 9mL dissolution media of 50mg formulations containing 12.5 and 24 wt. % LNG were collected every 24 hours for 60 days for the analysis.

Prototype Sustained Release Injectable Implant

Figure 1 summarizes the fabrication and sintering process of the injectable implant where drug loaded microspheres were fused into a cylindrical structure using a solvent approach²⁶. Briefly, PCL-LNG microspheres were filled into a stainless-steel mold and a small quantity of methylene chloride was added to wet the microsphere surfaces fusing them into the

desired shape. This fused microsphere structure was coated with an elastomer. The flexible elastomer acts as a rate control membrane (RCM) and also keeps the biodegradable implant intact at the site of injection, allowing it to be removed in one piece if desired during treatment.

RESULTS AND DISCUSSION

Polymer Characterization

Drug elution from an implant is dependent on several physicochemical properties of the drug and the excipients utilized in fabricating it. Several biodegradable LNG injectable and implant formulations have been prepared using PLA, PLGA, and PCL with varied drug release profiles^{10,11,22,25}. The primary objective of the current study was to identify the drug diffusion constants of PCL-LNG formulations at two different drug loadings. These parameters and studies conducted here lay the foundation for the design and characterization of the proposed injectable device in Figure 1. Polymeric microparticles have been the primary choice for encapsulating hydrophobic drugs due to their high encapsulation efficiencies, controlled process, and reproducibility. The chosen polymer concentration and stirring speed resulted in spherical microspheres with ~90 % yield. Surface morphology, spherical shape, and their size distribution is presented in Figure 2. SEM of sintered PCL microspheres in rod-shape show morphological features attributed to sintering, namely, fused edges and inter-particle porosity (Figure 2C). The particles appear to be free of major surface-bound crystalline drug particles and impurities. However, a large distribution of particle size was observed for both PCL and PCL-LNG microspheres. A total of 100 particles from 3 different SEM images were measured using ImageJ (NIH, Rockville, MD) to measure the particle sizes. Both microspheres with and without drugs were found to be in the size range of $300 \pm 125 \mu\text{m}$. For injectable, sustained drug-delivery formulations, a heterogeneous size distribution of particles allows for a gradient drug release effect of both fast, burst release and slower, controlled release from smaller and larger size microspheres, respectively.

Various analytical techniques employed to detect and understand any potential interaction between drug and polymer indicated no such interactions. FTIR spectra of neat PCL microspheres, LNG powder, and PCL-LNG microspheres showed discrete peaks attributed to carbonyl and hydroxyl groups (Figure 3). Characteristic stretching bands for carbonyl, $-\text{CH}_2$ and OH in LNG were found at 1653 , 2932 and 3347 cm^{-1} . The characteristic carbonyl stretching for PCL was found at 1729 cm^{-1} . After LNG incorporation into PCL there were no changes in the characteristic band frequencies of the drug and polymer indicating the absence of potential drug matrix interaction. These findings are consistent with earlier reports using either PLGA or PCL matrices for LNG formulations^{10,11,22,25}.

A typical XRD pattern for drug, polymer and formulations presented in Figure 4 shows the variation in their relative crystallinity (Figure 4). The XRD pattern of PCL showed two peaks at 21° and 23° related crystals in PCL. Similarly, multiple peaks at 14° , 16° , 17° , 19° , 21° , and 23° of LNG did not alter following its encapsulation in PCL. For instance, the major LNG peaks at 14° , 21° , and 23° remained unaffected in PCL matrix indicative of unaltered crystallinity and no adverse drug-matrix interaction. The hydrophobic nature of

PCL and absence of functional groups on its backbone reduces the potential drug-matrix interaction in the dry state ²².

DSC thermograms presented in Figure 5 shows melting points for PCL and LNG at 63.4°C and 239°C respectively. These characteristic melting points remained unchanged in PCL-LNG formulation indicating lack of drug-matrix interaction. TGA thermograms presented in Figure 6 for PCL, LNG, and PCL-LNG three-phase degradation. The PCL sample showed a significant water loss starting at 330°C with an additional inflection at 408°C and a slight inflection at 450°C. The LNG sample shows an initial weight loss starting at 200°C and a secondary loss starting at 350°C, with the third inflection coming at 500°C. Similar degradation pattern was observed for PCL-LNG where weight loss was observed at 205°, 350° and 450°C. These observations along with FTIR and XRD analysis collectively support the conclusion of an absence of drug-matrix interactions. Based on the lack of drug-matrix interaction as evidenced through various analytical techniques, drug release may be diffusion controlled as seen with identical formulations ^{10,22,24}.

Drug Release

The encapsulation efficiency for 12.5 wt% PCL-LNG microspheres was measured to be $78.5 \pm 2.3\%$. These microsphere formulations were able to release LNG up to 56 days at 37°C (Figure 7A). However, the same formulations at 42 and 47°C released all the drug in 42 and 28 days respectively. The cumulative release of LNG at three temperatures namely 37, 42, and 47°C was found to be ~64% (2.53 mg), ~68 % (2.69 mg), and ~66 % (2.66 mg) respectively. A burst release was observed in the first 7 days for release studies at 37, 42 and 47°C and this was accounted at ~34, ~55, and ~58% respectively. The encapsulation efficiency for 24 wt% PCL-LNG microspheres was found to be $75.3 \pm 1.8\%$. Higher drug loading resulted in sustained release of LNG over 120 days at 37°C which almost double the time of 12.5% loading (Figure 7B). Release of LNG for 24% loading at temperatures 42 and 47°C remained to through 56 and 49 days, respectively. The cumulative release of LNG at three temperatures namely 37, 42 and 47°C was found to be ~74% (6.19 mg), ~63% (5.49 mg), and ~67% (5.83 mg) respectively. An identical burst release pattern to 12.5% was also observed for 24% in the first 7 days at 37, 42 and 47°C and this was accounted at ~24, ~36, and ~40% respectively. Release pattern for LNG followed a linear trend at all release temperatures 37, 42 and 47°C that was found to be 7–56, 7–49 and 7–42 days respectively.

Figure 8 summarizes the daily release of LNG for 12.5% and 24% LNG-loaded PCL microspheres. The total LNG release was dependent on the amount of drug encapsulated. For instance, at day 1 a total of 6.2 and 4.3 µg of LNG was release from 12.5 and 24% loadings respectively. The same trend continued for 6, 8 and 12 days where 6.7, 6.2 and 6.5 µg for 24% loading and 4.8, 4.2, and 4.1 µg for 12.5% loading was observed. At endpoint, day 56, LNG release from 24% was found to be 3.8 µg while 0.08 µg for 12.5%. Both formulations showed an identical release pattern that was dependent on initial drug loading. Roughly 5 µg of LNG was released each day for the 24% drug loading up to 56 days.

The drug release of LNG from PCL microspheres was shown to follow a typical pattern of diffusion from a solid polymeric matrix where water permeation allows for the diffusion of drug. A characteristic burst release can be seen in the first week of the study, accounting for

upwards of 55% of cumulative drug release. The smaller microparticles, drug on the surface and water uptake may be the primary causes of the initial burst release²⁷. Similar observations were made by many researchers when microparticulate systems had wide variation in particle size^{4,6,10,12}. If properly controlled, this burst release can be effectively used to achieve therapeutic levels of drug in a short, efficient manner. Selection of uniform sized microspheres and extensive washing of the formulation to remove drugs on the surface may be used to avoid initial burst. The PCL matrix hydrophobicity and its slow degradation results in a controlled release of drug for a prolonged period as compared to hydrophilic matrices²⁸. The secondary phase, immediately following the initial burst release, shows a zero-order release profile and drug release principally diffusion controlled²⁹⁻³¹. For hydrophobic drugs, such as LNG, this secondary phase is typically zero-order³⁰. The drug diffusion was concentration dependent and showed two different levels of drug release rates based on the LNG loading. Drug diffusion rates were higher at elevated temperature such as 42 and 47°C as compared to 37°C.

Drug Release Kinetics

The drug release kinetics and mechanism of release from PCL-LNG microspheres were calculated for both drug loadings at 37°C. The release data was fitted with standard Higuchi and Korsmeyer-Peppas models. The Higuchi model (Equation 2) describes the release of dispersed drug from a homogeneous matrix using the pseudo steady-state approximation³². Where Q is the concentration of drug in the drug matrix at time t and k is the Higuchi dissolution constant^{33,34}. The values of correlation coefficient (r^2) and rate constants (k) are tabulated in Table 1. For Higuchi plot r^2 values were found to be 0.926 and 0.996 for 12.5 and 24 % drug loadings, respectively.

$$Q=kt^{1/2} \quad \text{Equation 2}$$

The drug release data from PCL-LNG microspheres was also fitted by the Korsmeyer-Peppas model to determine the mechanism of drug release (Equation 3)³⁵⁻³⁷:

$$\frac{M_t}{M_\infty}=kt^n \quad \text{Equation 3}$$

Where M_t and M_∞ are the drug concentrations released at time t and the total drug content in the matrix. Drug release rate constant is k and n is the diffusional exponent^{33,34}. The fractional release (M_t/M_∞) up to 60% of LNG at time t are fitted to the above equation, and the values of k and n have been calculated by the least squares method. These values are presented in Table 1. In the present systems, the n -values vary between 0.466 and 0.493, with $r^2 > 0.95$, indicating the release mechanism follows Fickian transport for both the formulations.

Upon fitting drug release data to the Higuchi and Korsmeyer-Peppas models, the results are in agreement and conclusively show that LNG release from the PCL microspheres was

governed by Fickian diffusion. A strong correlation observed between the drug release and square root of time for both the formulations indicated Fickian mode of diffusion according to Higuchi model. An n -value of 0.5, according to the Korsmeyer-Peppas equation, also suggested Fickian diffusion for both 12.5% and 24% drug loadings^{34,38}. Fickian diffusion occurs due to the random molecular motion of drugs where there is a net movement from a region of high concentration to a region of lower concentration³⁹. This, along with a decrease in drug release rate over time, evident from the plots of cumulative LNG release (Figure 7), can be attributed to the depletion of drug in the matrix over time, where the distance for diffusion (diffusion path length) becomes increasingly greater as the boundary between the drug matrix and the drug-depleted matrix recedes with time⁴⁰.

Arrhenius Model

Elevated temperatures showed an increase in drug release from polymer microspheres. Due to the Fickian diffusion-controlled mechanism of drug release, this is an expected result. If one were to consider the microsphere drug delivery system, temperature increases cause increased energy within the contained system⁴¹. This increase in energy results in faster molecular motion, and thus, faster diffusion of drug molecules from the polymer matrix. The inclusion of increased temperatures in our study was to enable the investigation of modelling accelerated time drug release using the Arrhenius model. A zero-order reaction is one in which the reaction rate is independent of the concentration of the drug and remains constant. The rate equation for the change in drug concentration for zero-order reactions is shown in Equation 4 where C_t is the drug concentration at time t , C_0 is the initial drug concentration, and k_0 is the rate constant for a zero-order reaction.

$$C_t = C_0 - k_0 t \quad \text{Equation 4}$$

Near zero-order release constants were calculated for the 24% LNG-loaded PCL microspheres at each temperature from cumulative drug release versus time graphs using Equation 4. Initial burst release up to 5 days was excluded in the linear regression calculations. The near zero-order release constants were found to be 5.39, 7.19 and 8.11 μg for 37, 42, and 47°C respectively. The effect of temperature on a zero-order rate constant is given by the Arrhenius equation (Equation 5), where E_A is the activation energy, R is the universal gas constant, A is the pre-exponential factor, and T is absolute temperature.

$$k = A \cdot e^{\frac{-E_A}{RT}} \quad \text{Equation 5}$$

An Arrhenius plot of $\log k$ versus the reciprocal of absolute temperature yields a straight line from which the slope can be used to calculate E_A (Equation 7).

$$\log k = \log A - \frac{E_A}{2.303RT} \quad \text{Equation 6}$$

$$\text{Slope} = -E_A / (2.303R) \quad \text{Equation 7}$$

Drug release from solids in which the mechanism of release at different temperatures is primarily a diffusion controlled process are often found to be well predicted by the Arrhenius equation⁴². As such, the zero-order release constants calculated above were plotted against the inverse of absolute temperature (Figure 9), according to the Arrhenius equation (Equation 5). The Arrhenius plot showed strong correlation between release constant and temperature ($r^2=0.988$) which validates the assumption that temperature is an effective parameter to accelerate drug release from the tested system and verifies that the release mechanism is not affected by these accelerated test conditions.

In order to accurately model accelerated time release conditions using temperature variations, a correlation must be made between accelerated and real-time release, based off of the Arrhenius equation. In other words, a relationship must be established to relate sampling intervals at elevated temperatures to a 24-hour real-time sampling at 37 °C. With proper correlations, and provided that the release mechanism is unchanged, elevated temperature drug release studies with appropriately modified sampling intervals would reflect 24-hour real-time release and grant the possibility to measure the changes in daily release profiles under accelerated test conditions. Manipulation and simplification of the Arrhenius equation (Equation 5) yields an equation from which the sampling interval (timepoint t) for any temperature can be calculated⁴³.

$$t(h)_{T\uparrow} = e^{\frac{E_A}{R} \left(\frac{1}{T\uparrow} - \frac{1}{T_{37^\circ\text{C}}} \right)} \cdot 24 \text{ h} \quad \text{Equation 8}$$

The corresponding sampling intervals of ~16 and ~11 hours were calculated using Error! Reference source not found. for 42°C and 47°C, respectively, which are representative of sampling every 24 hours at 37°C.

Sustained Release Injectable Implant

In an effort to create an implant for sustained, controlled release of LNG, the PCL-LNG microspheres were sintered to create a cylindrical implant rod measuring 0.2 cm in diameter and 2.0 centimeters in length (Figure 1)⁴⁴. The implant can be injected intramuscularly using a standard, sterile hypodermic needle and syringe. In order to create a more prolonged release of LNG, for proposed future works, the cylindrical sintered microsphere implant will be surrounded by a novel formulation of PCL-based elastomer, on which we have previously published⁴⁵. The elastomer is formulated using various ratios of PCL-triol, succinic acid, and pentaerythritol, all of which are FDA approved, biocompatible, and widely used in the

medical, food, and beverage industries. The elastomer shell surrounding the sintered microsphere rod will serve as a barrier membrane, but will also be homogeneously loaded with LNG, to provide a greater, more sustained and long-lasting release.

The advantages of using a dual-layer (microsphere rod center and elastomer shell) technology for a contraceptive implant are trifold. First, the implant is able to provide a sustained, prolonged release of LNG contraceptive therapy, with drug eluting from the elastomeric shell as well as from the PCL microspheres. Second, due to its nature, the elastomeric shell has the tendency to swell when placed in media, thus is capable of releasing drug from the matrix at a quicker rate allowing for threshold therapeutic levels to be reached more rapidly^{46,47}. Third, the presence of the elastomer shell grants the implant unique degradation properties, enabling it to degrade intact, as a whole, without troublesome fragmenting. Such degradation can be extremely advantageous should the patient wish to terminate contraceptive therapy for medical or personal reasons.

The proposed contraceptive implant system, consisting of the PCL-LNG microspheres investigated in this study and a proposed novel elastomeric, drug eluting shell, represents a significant step forward in terms of efficiency, cost, and ease of use, as compared with existing contraceptive methods. Major shortcomings of current contraceptives include their lack of biodegradation, significant cost, and duration of therapy^{2,48,49}. The PCL-based implant system being designed is capable of providing long term contraceptive therapy while maintaining a low profile (0.2 cm in diameter, 2.0 cm in length) and fully degrading into non-toxic components, post-treatment. The slim design of the implant allows for it to be implanted intramuscularly into the deltoid of the monodominant arm via a traditional, sterile, hypodermic needle and syringe. This allows for extreme cost effectiveness, essentially limited only by the cost of the LNG contraceptive drug.

CONCLUSION

Prolonged, controlled release of contraceptive steroids in injectable and implant form are highly desirable for sustained therapy, patient compliance and convenience, medical cost savings, and reduced invasive surgical operations. In the present study, LNG was successfully encapsulated in a PCL microspheric matrix using the o/w emulsion technique for an injectable contraceptive formulation. The sustained, prolonged release of LNG was shown at 12.5 and 24% drug loading conditions and three incubation temperatures, establishing proper characterizations of drug release. Characterization techniques including FTIR, XRD, DSC, and TGA confirmed the absence of adverse chemical interactions between the drug and the polymer, as well as its molecular level distribution.

The release of LNG from PCL microspheres was calculated and characterized to be Fickian diffusion controlled using both Higuchi and Korsmeyer-Peppas release models. The drug release pattern was observed to be biphasic for both the formulations, characterized by a short-burst release followed by a zero-order release. The inclusion of multiple elevated temperatures allowed for the use of the Arrhenius model to demonstrate the use of elevated temperatures for accelerated time release studies. The numerous advantages of polymeric microsphere drug delivery vehicles make this a feasible and efficient system for the

controlled and sustained delivery of contraceptive therapeutics in an implantable and injectable formulation. Future studies will focus on the development and subsequent testing of an injectable, fully biodegradable, contraceptive implant system composed of sintered PCL-LNG microspheres surrounded by an LNG-loaded PCL-based elastomeric shell.

Acknowledgments

Authors acknowledge funding support from the National Institute of Biomedical Imaging and Bioengineering of the National Institutes of Health (award number R01EB020640), Connecticut Regenerative Medicine Research Fund (15-RMB-UHC-08) and the National Science Foundation Graduate Research Fellowship award in support of this work.

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FIGURE 1.

(A) Simplified schematic of microsphere implant fabrication shows microsphere o/w emulsion technique, followed by chemical sintering of loose particles into rod-shaped injectable implant structure. (B) From left to right: LNG-loaded PCL microspheres shown dyed blue for visualization. Following sintering, the final dimensions of the cylindrical implant measure roughly 0.2 cm in diameter and 2.0 cm in length. A larger sintered implant prototype is shown in the undyed, natural color. A prototype of the rod sintered microsphere implant surrounded by a PCL-based elastomer shell, which serves as a barrier membrane and is also homogeneously loaded with LNG, is shown on the far right.

FIGURE 2.

Scanning electron microscopy (SEM) images of (A) neat PCL microspheres; (B) PCL-LNG microspheres; and (C) SEM of sintered PCL microspheres. Scale bar = 100 μm . Both LNG-loaded and neat PCL microspheres show spherical shapes with an average particle size of $300 \pm 125 \mu\text{m}$. Particle sizes were comparable in both samples and there is an absence of surface-attached drug and/or impurities. Sintered microspheres show consistent fusion of particle edges with inter-particle porosity.

FIGURE 3.

Attenuated total reflection (ATR) Fourier transform infrared (FTIR) spectroscopy of PCL microspheres, LNG powder, and PCL-LNG microspheres. Characteristic stretching bands for carbonyl, -CH₂, and -OH in LNG were found at 1653, 2932, and 3347 cm⁻¹. The characteristic carbonyl stretching for PCL was found at 1729 cm⁻¹. The LNG-loaded PCL samples (shown in blue) show characteristics of both neat LNG and neat PCL samples indicating no chemical changes.

FIGURE 4.

X-ray diffraction (XRD) spectra of PCL microspheres, LNG powder, and PCL-LNG microspheres. XRD was recorded from 5–60° (2 θ) at a scanning speed of 0.2 deg/s. Cu-K α radiation at 40 kV and 30 mA was used as the X-ray source. PCL-LNG samples showed the major neat PCL peak 21° and showed that major LNG peaks at 14°, 21°, and 23° remained unaffected in PCL-LNG samples, indicative of absence of drug-matrix interaction.

FIGURE 5.

Differential scanning calorimetry (DSC) spectra of PCL microspheres, LNG powder, and PCL-LNG microspheres. Samples weighed $10 \text{ mg} \pm 1 \text{ mg}$ and were heated from 0–300 °C at a rate of 10 °C/min under 20 mL/min nitrogen purge. Melting points for PCL and LNG were shown to be at 63 °C and 239 °C respectively. These characteristic melting points did not alter in PCL-LNG formulation indicating lack of drug matrix interaction.

FIGURE 6.

Thermal gravimetric analysis (TGA) of PCL microspheres, LNG powder, and PCL-LNG microspheres. Samples weighed $10 \text{ mg} \pm 1 \text{ mg}$ and were heated from 0–700 °C at a rate of 10 °C/min under nitrogen purge. A triphasic degradation is shown for PCL, LNG, and PCL-LNG samples. PCL showed a significant water loss starting at 330°C with an additional inflection at 408°C and a slight inflection at 450°C. LNG showed an initial weight loss starting at 200°C and a secondary loss starting at 350°C, with the third inflection coming at 500°C. Similar degradation pattern was observed for PCL-LNG where weight loss was observed at 205°, 350° and 450°C. These observations confirm lack of drug-polymer interaction.

FIGURE 7.

In vitro drug release curves showing percent release of LNG from (A) 12.5 wt% LNG-loaded PCL microspheres and (B) 24 wt% LNG-loaded PCL microspheres, at 37 °C, 42 °C, and 47°C. A controlled release of drug for a prolonged period is shown for both drug loading formulations. An initial burst release is observed within the first 7 days, followed by a zero-order release profile, controlled by Fickian diffusion. The drug diffusion was concentration dependent and showed two different levels of drug release rates based on the LNG loading. Drug diffusion rates were higher at elevated temperature such as 42 and 47°C as compared to 37°C.

FIGURE 8.

Daily release of LNG (µg) from 12.5 wt% LNG-loaded PCL microspheres and 24 wt% LNG-loaded PCL microspheres. Both 12.5% and 24% formulations showed identical release patterns, that were dependent on initial drug loading. Roughly 5 µg of LNG was released each day for the 24% drug loading up to 56 days.

FIGURE 9.

Arrhenius model fitted to experimental zero order rate constants of drug release from 24 wt % LNG-loaded PCL microspheres at 37 °C, 42 °C, and 47°C. Natural log of release constant is plotted against inverse temperature in Kelvin. The red diamond represents the predicted value of the 37 °C rate constant which is in agreement with the experimental value at 37 °C and the linear regression of the rate constants of drug release. The Arrhenius plot showed strong correlation between release constant and temperature ($r^2=0.988$) which validates the assumption that temperature is an effective parameter to accelerate drug release from the tested system and verifies that the release mechanism is not affected by these accelerated test conditions.

TABLE I

Higuchi and Korsmeyer-Peppas drug release models fitted to 12.5 wt% LNG-loaded PCL microspheres and 24 wt% LNG-loaded PCL microspheres at 37 °C. Values of correlation coefficient (r^2) and rate constants (k) are tabulated, along with the diffusion exponent (n) which characterizes mechanism of drug release. An n value approximately 0.5 indicates a purely Fickian diffusion-mediated mechanism of drug release. Higuchi models for both drug loading formulations show r^2 values near unity ($r^2=0.926$ and $r^2=0.996$, respectively) indicative of strong correlation to diffusion controlled release mechanisms. Similarly, the diffusion coefficients ($n=0.466$ and $n=0.493$, respectively) calculated using the Korsmeyer-Peppas model show strong indications of Fickian diffusion-controlled drug release.

Kinetic Model	Parameter	12.5 wt% LNG	24 wt% LNG
Higuchi	r^2	0.926	0.996
	K_H	1.403	1.397
Korsmeyer-Peppas	r^2	0.957	0.983
	n	0.466	0.493
	K_{HP}	0.023	0.019