Polymeric Composite Matrix with High Biobased Content as Pharmaceutically Relevant Molecular Encapsulation and Release Platform

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Abstract

Drug delivery systems (DDS) that can temporally control the rate and extent of release of therapeutically active molecules find applications in many clinical settings, ranging from infection control to cancer therapy. With an aim to design a locally implantable, controlled release DDS, we demonstrated the feasibility of using cellulose nanocrystal (CNC)-reinforced poly (l-lactic acid) (PLA) composite beads. Performance of the platform was evaluated using doxorubicin (DOX) as a model drug for applications in triple negative breast cancer. A facile, non-solvent induced phase separation (NIPS) method was adopted to form the composite beads. We observed that CNC-loading within these beads played a critical role in the mechanical stability, porosity, water uptake, diffusion, release, and pharmacological activity of the drug from the delivery system. When loaded with DOX, the composite beads significantly controlled the release of the drug in a pH-dependent pattern. For example, the PLA/CNC beads containing 37.5 wt % of CNCs showed a biphasic release of DOX where 41% and 82% of the loaded drug was released at pH 7.4 and pH 5.5, respectively, over 7 days. Drug release followed Korsmeyer kinetics, indicating that the release mechanism was mostly diffusion and swelling-controlled. We showed that DOX released from the drug-loaded PLA/CNC composite beads locally suppressed the growth and proliferation of triple-negative breast cancer cells, MBA-MB-231, via the apoptotic pathway. The efficacy of the DDS was evaluated in human tissue explants. We envision that such systems will find applications for designing biobased platforms with programmed stability and drug delivery functions.

Introduction

Controlled release of pharmacologically active compounds has remained a major goal of drug delivery research over the past decades. Implantable drug delivery system (DDS) placed within tissues exhibit sustained drug release and have shown success in multiple clinical settings including local delivery of antibiotic agents[1-8], long-term delivery of hormonal drugs[9-13], cardio-protective agents[14], low-dose delivery of non-steroidal or steroidal anti-inflammatory agents[15-21]. Controlled-release DDS have also found significant usage in cancer therapy. In neoplastic diseases, an unmet clinical problem is the post-surgical control of residual, microscopic disease. Cancer tissues tend to be poorly perfused, hypoxic, and acidic, therefore potentially leading to selection of resistant cancer cell populations, leading to recurrence of the disease even after surgical resection. Intelligently programmed, surgically implantable drug delivery beads have been shown to control such re-proliferation of remaining cancer cells [22-25]. Thereby, implanting pre-designed drug carries can facilitate post-surgical outcomes by releasing the drug in a controllable manner under native tissue conditions [22-24, 26, 27]. As such, these engineered delivery systems can elevate local drug concentrations, reduce off-target systemic toxicities, and limit the extent and severity of side effects[28]. For example, the Gliadel® wafer-based technique was commercially adopted as a drug delivery implant for inhibition of recurrent brain tumor cells[29-34]. These biodegradable, poly-anhydride-derived dime-sized wafers are used to treat brain tissue neoplasia directly, avoiding the complications of systematic chemotherapy and drug-diffusion challenges associated with the blood-brain barrier[29, 31, 32, 35] . Besides neuroblastoma, polymeric micro-implants are used in several other types of cancers[23, 27, 36-38]. For instance, Paclimer®, a microparticle based drug delivery system, was recently used to inhibit tumor growth in ovarian region[39, 40]. Polyphosphoester loaded with paclitaxel was reported to inhibit lung

cancer cells[23, 41-43]. These microparticles have also been explored for the treatment of malignant glioma of the brain[43]. Other than cancer, drug-loaded, polymeric matrices and meshes have been used as a surgical intervention strategy or for contraceptive purposes since the middle of the last century[44-46]. Biodegradable polymers have historically been used in the design of the above-mentioned DDS as they are programmed to degrade after completion of the task in a time-controlled fashion. In addition, the degradation products are absorbed or naturally discharged by the body [47, 48]. One of the risks associated with biodegradable polymeric matrices for such local applications is uncontrolled mechanical failures and early degradation of the system under physiological conditions upon prolonged exposure[44, 49]. Such premature instability can potentially result in uncontrolled drug release, local and systemic complications (clot and embolism), and drug- or composite-associated toxicity[50]. For non-biodegradable systems, although such mechanical instability is avoidable, post-application removal might present challenges due to integration of the device with neighboring tissue, and fouling, oftentimes leading to infection or nerve damage[47, 51]. Lactic acid derived biodegradable polymer, poly (l-lactic acid), PLA, has been one of the most explored polymer candidates for fabricating locally implantable systems [52-61,62-65]. When used as a component of DDS, drug release from PLA-rich release devices is generally governed by molecular diffusion, swelling, and erosion of the matrix polymer. These mechanisms are primarily associated with polymer properties and are highly random and uncontrolled in nature, resulting in the need for formulation additives to maintain desired kinetics of drug release [66, 67-71]. Another critical aspect of polyester degradation, such as PLA, is the acidic byproducts of their hydrolytic breakdown. PLA degrades naturally within the body, producing lactic acid and carbon dioxide, which are metabolized intracellularly or discharged through urine and breath[58]. Not only do these acidic byproducts tend to cause inflammatory responses, but they also can trigger an autocatalytic effect on composite degradation[72, 73]. If porosity of the

scaffold is low, trapping of acidic byproducts within the systems creates a hollow core, causing heterogeneous defects to take place within the scaffold[74]. Therefore, reducing and replacing the volume-fraction of PLA with a hydrophilic, multifunctional polymer will improve therapeutic and stability performance of PLA-rich composites by formation of nanoscale, tortuous channels without affecting the composite properties and functions.

Cellulose is the most abundant natural polymer on earth and is a major component of plants and marine animals (e.g., tunicates) [75-78]. Organized structures of cellulose, such as cellulose nanocrystals (CNCs) are separated using mechanical and chemical extraction (acid hydrolysis) processes from bio-source materials such as wood, plants, and living organism (i.e., bacteria)[76-81]. These nanocrystals, which present rigid rod-like particles with a width of 2-5 nm and a length of 200-230 nm[82], possess outstanding mechanical properties, large surface area, high aspect ratio (i.e., length to width ratio), biocompatibility, nontoxicity, and the presence of abundant hydroxyl groups (-OH) available for formation of hydrogen bonding in aqueous environment[82-84]. These cellulosic nanomaterials have been extensively studied for their applications in nanocomposites, flexible optical and electrical devices, biomedical and tissue engineering, coatings, filtration, and separation[78-80, 82, 84-88]. Although use of CNCs as swellable nanoscale scaffolds have been reported earlier [79, 89-93], mechanistic underpinnings of the effect of CNC content on drug release from PLA/CNC composite have been under-investigated. We hypothesize that hydrophilic nano-scaffolds such as CNCs, when present within a polymer matrix, can provide the matrix with mechanical stability, porosity, and water absorption capacity. These properties of CNCs enable the CNC-loaded matrix to transform into its swollen state without scaffold degradation, creating porosity for efficient effluent and by-product removal. In addition, such swelling also contributes to controlled drug release via formation of a gel-like barrier surrounding the matrix [94]. Therefore, we expect to attain more programmable rates of diffusion and dissolution of the

drug loaded within a PLA/CNC composite matrix as a function of CNC content. In addition, porosity is critical for maintaining scaffold integrity via facilitating removal of acidic byproduct resulting from scaffold degradation, especially for scaffolds composed of polyesters[95]. Furthermore, in a cancer setting, the acidic and enzyme-rich cancer microenvironment exerts significant influence on PLA degradation, which acutely affects the rate and extent of drug release from PLA-based matrices [96-100]. Therefore, in this report we have systemically designed PLA/CNC composite matrices in the form of beads of ~1.5 ± 0.3 mm diameter for delivery of a frontline chemotherapeutic agent, doxorubicin (DOX). We used PLA as the biodegradable matrix and CNCs to induce high porosity and swellability within the beads, which facilitated permeation of liquid medium inside the matrix and promoted DOX release from these beads. Our goal is to study the effects of CNCs incorporation within DOX-loaded PLA beads as a function of mechanical stability, porosity, swelling properties, and drug release kinetics under cancer tissue-mimicking pH. As a proof-of concept demonstration, we applied the beads in a simulated, localized cancer therapy using breast cancer cell lines and pancreatic cancer patient-derived tissues. Our long-range goal is to engineer biocompatible composite beads with programmable drug delivery and a stability profile that could be tailored to respond to a particular disease environment.

Experimental Section

Materials. Poly (lactic acid) (M_n ~30 kDa, M_w ~60 kDa), N, N -dimethylformamide (DMF) and doxorubicin hydrochloride (DOX HCl) were purchased from Sigma-Aldrich. Cellulose nanocrystals (CNCs) were procured from the University of Maine, USA. Triple-negative breast cancer MDA-MB-231 and MCF-7 cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA).

Preparation of Beads. Non-solvent Induced Phase Separation (NIPS) technique was used to prepare spherical PLA/CNC beads. A predetermined amount of PLA (**Table 1**) was dissolved in 10 mL DMF at 90˚C by stirring for 3 hours. CNCs were added to the solution and dispersed thoroughly using a high-speed vortex mixer (10 min) followed by magnetic stirring at 1,500 rpm for another 10 min. The temperature was maintained at 70˚C. The mixture was fed into a 15 ml syringe and pumped out through a needle (18G, 1.2mm×40mm) at a 0.3 ml/min flow rate using a syringe pump (Fusion 200, Chemyx Inc.). The mixture was directly dripped into a coagulation bath containing 500 mL of water under continuous stirring, producing PLA/CNCs composite beads (**Figure S1A,** Supporting Information). The beads were separated from water using filter paper and were frozen in -30ºC flowed by lyophilization for 17 h. The composition and sample code for each type of produced beads are summarized in **Table 1**.

Table 1. Compositions of PLA/CNCs beads.

Sample code	PLA content* (mg)	CNCs content* (mg)	CNC/PLA ratio	DOX amount added* (mg)
P1C0	1000	0	0	15
P1C0.2	1000	200	0.2	15
P1C0.33	1000	330	0.33	15
P1C0.5	1000	500	0.5	15
P1C0.6	1000	600	0.6	15
P1C0.7	1000	700	0.7	15
P1C0.8	1000	800	0.8	15
P1C1	1000	1000	1	15

* These amounts were dissolved in 10 mL DMF for all formulations.

Formation of DOX-loaded beads. To prepare drug-loaded beads, 15 mg DOX was dissolved in each PLA/CNCs mixture and beads were prepared following the same procedure

described above (**Figure S1B,** Supporting Information). The beads were stored in a container wrapped with aluminum foil to protect the drug from photo-degradation. Drug loading efficiency was calculated using the following equation (1) [101],

$$\text{Drug loading efficiency } \% = \frac{\text{Mass of DOX loaded onto beads}}{\text{Mass of DOX in solution}} \times 100 \qquad \text{(i)}$$

Rheology test on liquid solution. A rheology test was conducted using TA ARES G2 rheometer (Serial number: 4010-0868) on the PLA/CNC/DMF solution at 70˚C to keep the sample formulation in the liquid phase during the test. The percentage of strain was investigated for the P1C0 formulation in between 25 mm diameter circular parallel plates with a 1 mm gap and rotation of fixed angular frequency of 10 rad/s. Storage modulus, loss modulus, and complex viscosity were measured while maintaining a constant strain and variable angular frequencies of 1-500 rad/s at 70˚C.

Morphology. Morphological characterization of the beads was conducted using a scanning electron microscope (SEM). The outer surfaces and cross sections of the beads were imaged using a JEOL JSM-6490LV SEM (JEOL USA, Inc., Peabody, Massachusetts USA). Beads were cut into half transversely with a new razor blade to expose the interior and attached to cylindrical aluminum mounts with colloidal silver paint (SPI Supplies, West Chester, Pennsylvania, USA). Then they were sputter coated (Cressington 108auto, Ted Pella, Redding, California USA) with a conductive layer of gold. Images were obtained with a SEM at an accelerating voltage of 15 kV. Barrett-Joyner-Halenda (BJH) pore size analysis for mesopore[102, 103] measurement was conducted for P1C0, P1C0.2, P1C0.5, P1C0.8 and P1C1 beads. We selected equivalent spaced CNC-containing bead formulations to follow the effects of CNC-content on pore size. Barrett-Joyner-Halenda (BJH) porosity characterization was done after drying the beads in a lyophilizer for 17 h and degassing afterwards at room temperature for 24 h. Adsorption and desorption of the sample was performed in nitrogen

medium. The beads were also directly imaged using GE Phoenix V/tome/x microfocus X-ray computed tomography (Micro-CT) equipped with a 180 kV nanofocus X-ray tube and a high-contrast GE DXR250RT flat panel detector (GE Sensing & Inspection Technologies GmbH, Germany). Images of 1500 projections were acquired at a voltage of 60 kV and a current of 200 μA using a molybdenum target. Detector timing was 333 msec. Sample magnification was 38.99X with a voxel size of 5.1 μm. The acquired images were reconstructed into a volume data set using GE datos/x 3D computer tomography software version 2.2 (GE Sensing & Inspection Technologies GmbH, Germany). The reconstructed volume was viewed, and porosity analysis was performed using VGStudio Max version 3.2 Software (Volume Graphics, Inc., NC, USA).

Fourier transform infrared spectroscopy (FTIR). To understand the interactions between the components of the beads, the cross-sectional surfaces of the beads were subjected to FTIR (Thermo Scientific Nicolet 8700) examination under the attenuated total reflection (ATR) mode. Spectra of the samples were obtained between 4000-500 cm^{-1} based on 32 repetitive scans.

Differential scanning calorimetry (DSC). Differential scanning calorimetry tests were conducted on the beads between 0˚C and 250˚C using a Seiko DSC 220 at the heating rate of 15˚C min^{-1}. The glass transition (T_g), crystallization, cold crystallization (T_{cc}), and melting temperatures (T_m) of the beads were determined from the thermograms.

Compression test. Mechanical properties of the beads were characterized using a TA Discovery dynamic mechanical analyzer (DMA850) operating in the compression mode. The force-displacement curves were plotted to study the effects of the porosity on the mechanical strength of the beads.

Water uptake. PLA/CNCs beads were weighed and immersed in phosphate buffered saline (PBS) (pH 7.4) at room temperature. At stipulated time points, beads were removed from the solution, lightly dabbed to remove liquid attached to bead surface, and weighed. The process was repeated for 130 h total immersion time. The percentage of water absorption was calculated based on the weights using the following equation (2)[104, 105]:

$$\text{Water Uptake } \% = \frac{W_t - W_0}{W_0} \times 100 \qquad (2)$$

where W_0 and W_t are the initial dry weight of the beads and their weight after being immersed for time t, respectively.

Drug release. DOX-loaded PLA/CNCs beads (n = 5 beads per formulation with 3 replicates for each formulation) were submerged in 1.5 mL pH-7.4 or pH-5.5 buffer in test tubes (pH was maintained by adding 0.1M NaOH or 0.1M HCl solutions in PBS). The samples were agitated continuously using a VWR orbital shaker (Model 1000). After predetermined time intervals, 1 mL sample buffer was collected, and the tubes were replenished immediately with the same volume of fresh buffer. The amount of DOX in the sample was measured using UV-Vis spectroscopy (Varian Cary 5000). DOX concentration was determined based on the intensity of the absorption peak at 480 nm[106-108]. The cumulative release profile was calculated using the following equation (3)[48, 109]:

$$Cumulative\ \%\ release = \frac{volume\ withdrawn}{volume\ of\ bath} \times \%release\ at\ time\ t + \sum \%release\ at\ time\ t-1 \qquad (3)$$

Release kinetics analysis. The Korsmeyer-Peppas model was used to study the kinetics of drug release from the beads[110, 111]. This model is a frequently used mathematical

model to interpret non-linear diffusion-based drug release process. According to the model, the fraction of released drug can be calculated using the following equation (4),

$$\frac{M_t}{M_\infty} = kt^n \qquad (4)$$

where M_t/M_∞ denotes the fractional released drug at time *t*, *k* denotes the release rate constant, and *n* is the release exponent that categorizes a particular release mechanism. For spherical matrices, $n \leq 0.43$ indicates a Fickian (Case I) release; $0.43 < n < 0.89$, non-Fickian (anomalous) release; $n = 0.89$, Case II (zero order) release; and $n > 0.89$, super case II release [112]. Theoretically, Fickian transport occurs when the polymer relaxation time is greater than the solvent diffusion time, whereas non-Fickian diffusion takes place when the relaxation time and diffusion time are equal[68]. Due to the factors including solute concentration gradient, degree of polymer swelling, and diffusion distance, most of the polymeric systems follow the Fickian diffusion mechanism. From the release exponent *n* and release rate constant *k*, mean dissolution time (MDT) was also calculated to investigate the release rate of DOX and the drug sustaining efficacy of the composite beads using the following equation (5) [109, 113],

$$MDT = \left(\frac{n}{n+1}\right) \times k^{\frac{-1}{n}} \qquad (5)$$

where *k* denotes the release rate constant and *n* is the release exponent calculated from the Korsmeyer-Peppas model.

Cellular viability study. Two breast cancer cell lines, MDA-MB-231 and MCF-7 were seeded ($1x10^3$ cells / well) in 48-well cell culture plates and allowed to grow overnight in an incubator (37° C with 5% CO_2) using Dulbecco's Modified Eagle Medium (DMEM) supplemented with 1% v/v Penicillin-Streptomycin (pen-strep) and 10% Fetal Bovine Serum

(FBS). The cells were then treated with DOX-loaded bead formulations (drug concentration was varied by using varying number of beads) along with drug-free beads (P1C0.6 formulation, without DOX) along with a control group (without beads) for 24, 48, and 72 h. Subsequently, the cells were washed with PBS and the cell viability was measured using the Alamar Blue assay. Briefly, DMEM and the Alamar Blue reagent were mixed at a 9:1 ratio and the mixture was incubated for 5 h in a cell culture plate. The fluorescence of the sample was recorded at 560 nm (excitation) and 590 nm (emission) wavelengths using a Synergy H1 microplate reader (BioTek).

Cellular uptake study. The breast cancer cell lines MCF-7 and MDA-MB-231 were cultured in high glucose DMEM media containing 10% v/v FBS and 1% pen-strep. In a 12-well plate, 1 x 10^3 MDA-MB-231 or MCF-7 cells were cultured in each well and incubated at 37° C with 5% CO_2. The DOX-loaded beads were used as the treatment and the DOX-free beads (beads without DOX) were used as the control. For treatment group, 6 beads (64 μg DOX per bead, total 384 μg, quantified via UV-Vis Spectroscopy at λ_{max} of DOX) of a formulation were added to each well and an exposure time of 3 and 6 h was allowed. After the treatment, the cells were washed with PBS, stained with DAPI dye (NucBlue, Invitrogen) for 10 minutes, and washed again with PBS. A Leica fluorescence microscope with a 20x objective lens was used to identify DOX fluorescence (emission wavelength at 595nm). The integral density per unit area of drug uptake was measured using NIH ImageJ software.

Cellular uptake study by FACs. The amount of DOX delivered inside MDA-MB-231 cells was evaluated using flow cytometry. For this experiment, MDA-MB-231 cells were cultured in DMEM complete media and were treated with DOX-loaded beads for 3 h and 6 h. The treated cells were trypsinized and fixed in chilled ethanol. The samples were centrifuged to form a cell pellet, which was then resuspended in sterile PBS prior to cytometry measurement. The samples were examined in a flow cytometer (BD Accuri C6, BD

Bioscience, San Diego, CA, USA) using FlowJo® software to measure cellular uptake at different time points[114].

Detection of mitochondrial membrane potential. Depolarization of mitochondrial membrane potential is one of the most sensitive indicators of apoptosis. The transmembrane electrochemical gradient of mitochondria was determined using the mitochondrial potential sensor JC-1, a lipophilic and cationic cell-permeable dye. In untreated control cells, JC-1 can freely pass through the mitochondrial membrane and generates J-aggregates, which are fluorescent red. In apoptotic cells, the reduction of mitochondrial membrane potential hinders the diffusion of JC-1 and the chemical remains as monomers in the cytosol, which are fluorescent green. The J-aggregates/monomers ratio is a strong indicator of mitochondrial transmembrane potential and helps to distinguish the apoptotic cells from the control cells. For this study, MDA-MB-231 cells (2.5 x 10^5 cells/ml) were incubated with 6 beads (drug release amount equivalent to IC_{50} value of DOX) for 24, 48, and 72 h at 37°C, 5% CO_2. Then the cells were washed with PBS and incubated with JC-1 (7.5 μM in PBS) under dark conditions for 15 min at 20–25°C [115]. The JC-1 treated cells were analyzed by flow cytometry (BD Accuri C6, BD Bioscience, San Diego, CA, USA) and data were analyzed using FlowJo software.

Polymer stability study in plasma. To evaluate the stability of drug delivery beads in plasma, we submerged the PLA/CNC beads (P1C0, P1C0.6 and P1C1 formulations, without DOX) in reconstituted mouse plasma (Sigma Aldrich, P9275) under constant orbital shaking at 37˚C for 5 days. After the stipulated time, beads were recovered and assessed for morphological and microstructural changes using SEM imaging. For evaluating drug release from the beads, we submerged DOX-loaded P1C0.6 formulations in mouse plasma (3 replicate beads), and concentration of DOX in plasma was monitored for 5 days using UV-Vis spectroscopy.

Functional efficacy of released drug on patient-derived xenograft tumors. Human pancreatic ductal adenocarcinoma (PDAC) tumor was obtained from an F10 generation patient-derived xenograft (PDX) NOD scid gamma mouse. The tumor tissue, chopped into 3 mm^3 chunks, was placed in a 24-well plate precoated with Matrigel. High-glucose DMEM containing 10% FBS and 1X antibiotic/antimycotic solution along with 0.01 mg/ml insulin, 0.01 mg/ml hydrocortisone and 5mM GlutaMAX was added to each well[116]. Ex vivo tumors were treated in the presence of either DOX-loaded beads (n=4) or drug-free beads (n=4) or a control group without any beads (n=2). DOX-treated tumors were surrounded by four DOX-loaded beads (15 µg DOX/bead) and control-treated tumors were surrounded by four drug-free beads. The setup was maintained at 37°C with 5% CO_2 for a period of 4 days. Fresh culture media was supplied daily. At the end of the treatment period, the tumor fragments were fixed in formalin and underwent standard histology protocols. The fixed explants were embedded in paraffin wax and 5 µm sections were prepared using a microtome. Tissue slides were stored at 4°C until further use.

Immunohistochemistry. Tissue sections of tumors treated with DOX-loaded or drug-free beads were deparaffinized at 60°C for 2 h and rehydrated using xylene and ethanol. Antigen retrieval was performed at 95°C for 30 minutes using a Tris-EDTA buffer at pH 9.0. Tumor tissue was then stained for Ki-67 (Invitrogen MA5-14520) at a concentration of 1:200 overnight in a moisture chamber at 4°C and detected using a secondary goat anti-rabbit Alexa Fluor 488 (Invitrogen A11034) at a concentration of 1:250 at room temperature. The nucleus was stained for using DAPI for 5 minutes at room temperature and the mounting was done using VECTASHIELD (without DAPI) mounting medium.

Results and Discussion

Effect of CNC content on the rheology of the composite suspension. First, we identified the optimum viscosity of the PLA/CNC/DMF mixture via a rheology test at a fixed angular frequency. We observed that, at an angular frequency of 10 rad/s, the dynamic modulus of the PLA matrix was stable up to 15% strain (**Figure 1A**) from which we selected 5% strain for a dynamic frequency sweep for all subsequent rheology tests [117]. Storage modulus, loss modulus, and complex viscosity were measured at 5% strain and variable angular frequencies of 1-500 rad/s at 70˚C. Storage modulus and loss modulus were found to increase from pristine PLA/DMF solution with increasing CNC content in different formulations (**Figure 1B** and **Figure 1C**). Due to intrinsic rigidity of CNCs, stress is transferred from PLA/DMF to CNCs resulting in such increment of storage modulus [117]. The viscosity profile (**Figure 1D)** gradually increased with increasing CNCs content in PLA/DMF suspensions. The increment of viscosity usually relies on the type, concentration size, shape, and distribution in filler particles within the polymer matrix. In our case, viscosity of the PLA/CNC/DMF mixture influenced the solvent-nonsolvent diffusion during bead formation affecting porosity distribution.

Figure 1: Rheology testing on PLA/CNC/DMF solution at 70˚C to evaluate (A) Storage modulus, loss modulus, and complex viscosity for P1C0 formulation with variable strain percentage and fixed 10 rad/s angular frequency; and (B) Storage modulus, (C) Loss modulus and (D) Complex viscosity for all formulations with respect to variable angular frequency and strain fixed at 5%.

Formation, morphology, and microstructural studies of PLA/CNC composite beads. Physical view of the beads fabricated by facile, non-solvent induced phase separation,

with or without DOX loading, is presented in **Figure S1 (A-B)**. Scanning electron microscopy of these beads showed changes in surface roughness as increasing concentrations of CNCs were incorporated in the formulation as shown in **Figure 2 (A-E)**.

Figure 2. SEM image on surface of PLA/CNC bead of (A) P1C0, (B) P1C0.2, (C) P1C0.5, (D) P1C0.8, and (E) P1C1 formulations, respectively.

The microstructure of beads was studied both qualitatively and quantitatively using SEM, BJH, and micro-CT. **Figure 3** showed the cross-sectional micrographs of the beads of five formulations, i.e., P1C0, P1C0.2, P1C0.5, P1C0.8 and P1C1. The average pore diameter percentage was calculated for the various bead formulations with a diameter size in the range of 0-25 μm using ImageJ software (**Figure 3)**. All five samples showed a typical core-sheath structure of porous polymer materials produced from the coagulation process. The relatively dense sheath was formed through rapid removal of the solvent from the surface layer of the PLA/CNCs solution droplets, whereas the porous core was by diffusional exchange of the solvent and the non-solvent during the phase separation process[118, 119]. The images showed a clear trend that the porosity of the beads increased as the concentration of CNC was increased. **Figure 3** also shows that while the number of pores increased, their diameters decreased with increasing CNC concentrations. The refinement of the porous structure could be due to the increased viscosity of the solution after CNCs were incorporated. This observation also corroborated with our rheology studies, which showed that the viscosity of the bead-forming suspension was elevated as the CNC-content within the matrix increased (**Figure 1**). A high solution viscosity hinders the penetration of the nonsolvent and hence reduced the size and number of large pores[120]. CNCs as nanocrystals could form a spanning fibrous network within the PLA matrix, which could also prevent the formation of large

cavities inside the beads. Indeed, the fiber network structures in the beads were observed via SEM imaging and are shown in **Figure S2** (Supporting Information).

Figure 3. SEM images of the cross sections of the PLA/CNCs composite beads and their diameters. A1-A4: P1C0, B1-B4: P1C0.2, C1-C4: P1C0.5, D1-D4: P1C0.8 and E1-E4: P1C1 at three different magnification levels (i.e., x30, x300, and x1,800), and the average percentages of beads with diameters in the range of 0-25 μm.

The pores on the outer surface/sheath layer of the beads were also studied using SEM. **Figure 4** shows the distribution of uniform micropores on the surface of all beads. The porosity of the surface increased with increasing concentrations of CNCs, in agreement with the porosity trend of the bead core. These uniformly distributed micropores would be favorable for bead performance as controlled-release drug delivery systems as these structural features will facilitate molecular diffusion during the drug release process.

Figure 4. SEM images of the outer surface of the beads. (A) Neat PLA (P1C0), (B) P1C0.33, (C) P1C0.5, and (D) P1C1.

From the BJH adsorption pore distribution analysis, the average internal mesopore width was found to decrease with increasing CNC content (**Figure 5** and **Table 2**). As viscosity increases with increasing content of CNCs, formation of network structure within the PLA matrix is favored that slows down the diffusion of solvent to the bulk nonsolvent

phase, contributing to the formation of smaller pores and reducing the size and number of larger pores [120].

Figure 5: BJH analysis for mesopore size distribution inside beads (P1C0, P1C0.2, P1C0.5, P1C0.8 and P1C1)

Table 2: BJH adsorption and desorption mesopore size distribution

Sample	Adsorption average pore width (nm)	Desorption average pore width (nm)
P1C0	17.988	16.102
P1C0.2	14.087	9.987
P1C0.5	12.787	11.437
P1C0.8	12.480	8.545
P1C1	11.734	8.813

We further examined the internal air and matter percentage of composite beads using Micro-CT characterization. As shown in **Figure 6** and **Table 3**, the air percentage of the beads was found to be directly related to the concentration of CNCs. For example, the air percentages of P1C0, P1C0.2, P1C0.5, P1C0.8 and P1C1 were 64.0, 73.3, 75.9, 78.8 and

82.1%, respectively, confirming the observations from the SEM study. Micro-CT also revealed that the pores are internally connected thereby forming a tortuous construct. These structural features will be critical for extending the diffusion path of small molecules and drugs, which are incorporated within the bead interior, thereby extending the residence time of drug within the beads. The compression tests on the beads showed that P1C0.5 had higher strength than P1C1 because of its lower air percentage (Supporting Information, **Figure S3**). Thus, via incorporation of CNCs, manipulation of bead strength can be achievable to fit to the intended therapeutic applications.

Figure 6. Micro-CT images of A1-A3: P1C0, B1-B3: P1C0.2, C1-C3: P1C0.5, D1-D3: P1C0.8 and E1-E3: P1C1 beads at three different magnification levels (3D view, 162% and 1541% zoom respectively)

Sample	Material Vol	Air Vol	Total Vol	Material %	Air %
P1C0	2.79	4.95	7.74	36.0	64.0
P1C0.2	3.86	10.61	14.47	26.7	73.3
P1C0.5	3.37	10.63	13.99	24.1	75.9
P1C0.8	4.02	14.95	18.97	21.2	78.8
P1C1	3.42	15.66	19.08	17.9	82.1

Table 3: Material and air percentage calculated from Micro-CT analysis on beads

Chemical features of PLA/CNC composite beads. Attenuated Total Reflectance **(**ATR) spectroscopy was conducted on the cross-section of the beads to investigate the interactions among different functional groups of the samples. Illustrated in **Figure 7,** the

strong peak at 1740-1750 cm^{-1} was attributed to the C=O stretching of the aliphatic ester of PLA [121]. The peaks at 2998, 1450, and 1385 cm^{-1} were due to C-H and CH_3 asymmetric stretching and bending of PLA. The peaks at 1180, 1081, 2948, and 1360 cm^{-1} could be attributed to C-O-C symmetric and asymmetric stretching and -CH stretching and bending of PLA, respectively[121, 122]. The broad band at 3200-3600 cm^{-1} on the spectrum of CNCs was attributed to the stretching of the hydroxyl groups of the nanofibers. The intensity of this band for the PLA/CNCs composites increased with the increasing concentration of CNCs. The peak at 1656 cm^{-1} was due to the absorbed water of CNCs [123]. No obvious shift of the peaks could be observed from **Figure 7**, suggesting negligible chemical interactions between PLA and CNCs.

Figure 7. ATR spectra for CNCs, PLA (P1C0), and different formulations of PLA/CNCs beads with increasing CNC content.

Thermal properties of PLA/CNC composite beads. Differential Scanning Calorimetry (DSC) thermograms of neat PLA and PLA/CNCs containing different concentrations of CNCs are presented in **Figure 8**. Thermal properties obtained from this set of samples are summarized in **Table 4** based on these thermograms. All the samples exhibited glass transition (T_g), cold crystallization (T_{cc}), and melting (T_m) behaviors as a function of CNC content (**Figure 8A)**. For example, T_g of the samples decreased slightly with the increasing content of CNCs (**Figure 8B** and **Table 4**). Other groups reported similar phenomena that indicate weak interactions between PLA and CNCs that ultimately affect PLA chain mobility and facilitate the glass transition [124-126]. Similarly, the cold crystallization

temperature (T_{cc}) gradually decreased from 94.39 ˚C for P1C0 to 88.76˚C for P1C1 (**Figure 8C and Table 4**).

Figure 8. DSC thermograms of neat PLA and PLA/CNCs plotted between different temperature ranges. (A) the complete curves showing the glass transition (T_g), cold crystallization (T_{cc}), and melting (T_m) temperature, (B) comparison of glass transition region, (C) comparison of cold crystallization region, and (D) comparison of melting region magnified from (A).

The observed effect of CNCs on thermal behavior of the composite can be attributed to the fundamental feature of CNCs to act as an effective nucleation agent for various polymers, as widely reported in literature[124, 127, 128] . The reduction in T_{cc} can be attributed to the nucleation effect of CNCs that promoted PLA nucleation and, therefore, enabled the polymer to crystalize at lower temperatures. From P1C0 to P1C1, the melting temperature (T_m) of the samples increased with the increasing CNC content; a low-temperature secondary melting peak also became increasingly pronounced (**Figure 8D and Table 4**). This double-peak melting behavior has been reported on PLA blends and composites, and it has been attributed to the melting of small and imperfect PLA crystals at a relatively low temperature, recrystallization, and melting of higher-perfection crystals at a higher temperature[129, 130]. The pronounced low-temperature melting peak of P1C1 is due to the melting of a relatively large number of imperfect PLA crystals that were nucleated by a high content of CNCs. This melting behavior agrees with the strong nucleation effect of CNCs observed from the cold crystallization study.

Table 4: T_g, T_{cc} and T_m of neat PLA and PLA/CNCs.

Sample	T_g (°C)	T_{cc} (°C)	T_m (°C)
P1C0	67.2	94.4	166.8
P1C0.33	66.6	91.6	168.1
P1C0.5	66.2	90.6	168.2
P1C1	65.3	88.8	168.6

Water uptake. One of the major objectives of this work was to enhance liquid diffusion inside PLA/CNCs beads for optimized drug release. The diffusion of PBS buffer (pH 7.4) in the beads can be evaluated by water uptake within the beads. **Figure 9** shows that neat PLA exhibited water uptake (calculated using equation 2) of 37% of the weight of bead at the end of 132-h study period. Within this test period, water uptake within the beads was significantly influenced by CNC content. For instance, P1C0.33, P1C0.5, and P1C1 showed water uptake of 287%, 333%, and 438% of the weight of the bead, respectively. CNCs promote solution diffusion through creating pores in the beads and providing abundant hydrophilic -OH groups within the material. The increase in water uptake of the beads agrees with the porosity trend as well.

Figure 9. Water uptake of the beads as a function of time.

DOX encapsulation efficiency within PLA/CNC composite beads. Encapsulation efficiency of DOX was calculated from equation (1) for each drug-loaded beads and tabulated in **Table 5**. Interestingly, we observed that increasing the CNC concentration within the PLA matrix, increased drug loading efficiency. For example, the P1C0 formulation

showed the lowest encapsulation efficiency for DOX, and most of the drug bound closer to the bead surface was released during preparation of the beads under magnetic stirring condition. On the other hand, formulations containing increasing concentrations of CNCs were able to bind the drug within the polymer matrix due to the higher level of porosity and hydrophilicty contributed from the CNC architecture.

Table 5: Loading efficiencies of neat PLA and PLA/CNCs beads.

Sample	Encapsulation efficiency
P1C0	44.75±5.68
P1C0.2	57.91±5.56
P1C0.33	64.47±4.68
P1C0.5	75.55±3.56
P1C0.6	80.12±5.57
P1C0.7	87.71±4.36
P1C0.8	92.63±3.45
P1C1	98.27±1.47

Drug release study. The most important feature of a drug delivery system lies in its capacity to control the liberation of the active molecule in a temporally controlled pattern. In order to investigate the DOX release capacity from PLA/CNC composite beads, a calibration curve of DOX in PBS was first obtained quantifying the relationship between the UV-vis peak intensity (at λ_{max} = 480 nm) and DOX concentration in the buffer (Supporting Information, **Figure S4**). Cumulative drug release from the beads at predetermined time intervals was calculated from equation (3) based on the calibration curve and the resultant release plots are presented in **Figure 10**. As an example, the UV-vis spectra that were used to calculate the cumulative release for P1C0.6 are shown in **Figure S5** (Supporting Information). We observed that the amount of CNC incorporated within the CNC/PLA composite beads markedly affects DOX release properties from these beads. For example, the P1C0 formulation, which contained no CNCs, showed 8% cumulative release of DOX over

the course of 7 days in pH 7.4, as compared to 22%, 31%, and 41% releases for the P1C0.33, P1C0.5, and P1C0.6 formulations respectively (**Figure 10A**). The positive correlation between the release and the concentration of CNCs within the composite beads can be attributed to the increased porosities and tortuosity formed within the beads at high CNC level, which facilitates the diffusion of buffer into beads, promoting dissolution and subsequent diffusion of the entrapped drug out of the composite[101, 131, 132].

DOX release from the beads was also found to be significantly affected by the pH of the release media. As shown in **Figure 10B**, where the pH of the release media was maintained at 5.5, the cumulative release of DOX for all the formulations were much higher than those incubated at pH 7.4. For example, the cumulative release of DOX from P1C0, P1C0.33, P1C0.5, and P1C0.6 beads incubated in pH 5.5 buffer released 16%, 46%, 67%, and 82%, respectively after 7 days. Protonation of the amine groups in DOX coupled with acidity-induced swelling of cellulose facilitate solvent entry and drug dissolution, triggering faster DOX release from the beads[133, 134]. However, further increases in the CNC contents (P1C0.7, P1C0.8, and P1C1) were found to reduce DOX release for both pH conditions (**Figure 10C and 10D**). The cumulative drug release (pH-7.4, 7 days) for P1C0.7, P1C0.8, and P1C1 were 32%, 21%, and 13%, respectively. This result indicates that excessive swelling due to the incorporation of large quantities of CNCs caused the development of a swollen layer (gel layer) around the bead that slowed down drug release[94, 135]. This is to note that, reduction of pH could increase DOX release from these samples. At pH 5.5, the release of DOX from P1C0.7, P1C0.8, and P1C1 formulations were 51%, 37%, and 27% respectively, after 7-days. This experiment revealed that controllable rates of diffusion and dissolution of the drug can be achieved from the PLA/CNC composite matrix by adjusting the CNC content. The drug-polymer interaction plays an important role in drug release. DOX contains many amine and hydroxyl groups and, therefore, can interact with the hydroxyl groups of CNCs through

hydrogen bonding. DOX's strong binding to CNCs is expected to hinder its dissolution and diffusion and reduce its releases from the matrix. At high CNC concentrations, this hindrance can outweigh the positive porosity effect of CNCs and eventually lead to decreases in DOX release. Moreover, CNCs at high concentrations tend to form a network structure in PLA, which functions as a physical barrier and dampen the release rate of DOX from the beads.

Figure 10. Cumulative DOX release versus time for the P1C0, P1C0.33, P1C0.5, and P1C0.6 beads at (A) pH 7.4, (B) pH 5.5. Cumulative DOX release for P1C0.7, P1C0.8 and P1C1 compared with P1C0.6 release profile at (C) pH 7.4, (D) pH 5.5. (n=4, Two-way ANOVA, Bonferroni post tests, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ or ****$p < 0.0001$)

Release kinetic study. The Korsmeyer-Peppas model (equation 4) was used to study the release kinetics of DOX from the PLA/CNC beads [110]. The release profiles in **Figure 10** were fitted with the model to determine the release exponent *n* and release rate constant *k*. **Figure 11** shows the comparative release exponent and release rate constant values, which were calculated from the linear log-log regression of fractional released drug with time. The release exponent, *n* was found to vary between 0.27 and 0.36 at pH 7.4, which suggests that Fickian diffusion is the principal mechanism of DOX release for all formulations under this pH condition. At pH 5.5, P1C0 still followed Fickian diffusion because their *n* values were lower than 0.43. The *n* values for P1C0.33, P1C0.5, and P1C0.6 were higher than 0.43, indicating non-Fickian release kinetics for these beads. This observation suggests that anomalous diffusion of DOX from P1C0.33, P1C0.5, and P1C0.6 occurred at pH 5.5. This is likely attributed to the combined effects from normal diffusion and swelling behavior of the

matrix. The dependence of *n* on the CNC content and pH of the release media indicates that both hydrophilic components in the polymer matrix, i.e., CNCs and ionizable DOX, contribute substantially to the release behavior of the beads. Beads with elevated concentration of CNCs (from P1C0.6 to P1C1 formulations) showed reduction in pore diameters and strong binding to DOX, which hinder its dissolution and diffusion and reduce the numerical value or release exponent and the rate constant.

Figure 11. (A) Release exponent (n) and (B) release rate constant (k) calculated from regression of the Korsmeyer-Peppas model (R^2>0.96) for P1C0, P1C0.33, P1C0.5, P1C0.6, P10.7, P1C0.8 and P1C1 bead formulations at pH 7.4 and pH 5.5; (n=6, Two-way ANOVA, Bonferroni post tests, *p < 0.05, **p < 0.01, ***p < 0.001 or ****p < 0.0001)

Mean dissolution time (MDT) was calculated from equation (5) using the values of *n* and *k* to determine the rate and mechanism of DOX release from the beads and estimating the sustaining efficacy of the composite structure on DOX release [109, 113, 136] (**Table 6**). A low value of MDT suggests rapid diffusion of the solute through the polymer matrix[109]. Both P1C0.5 and P1C0.6 showed low MDTs at pH 5.5, which indicates rapid diffusion of DOX through these systems. This observation indicates that CNC concentration promotes the diffusion due to enhancement in porosity and channels. This result also demonstrates that low pH media conditions facilitate the rapid dissolution of the DOX. Beads with higher CNC content (from P1C0.6 to P1C1 formulations) showed elevated MDT values with respect to P1C0.5 and P1C0.6 formulations, which is due to reduction in pore diameter and strong binding interactions between the drug and CNCs.

Formulations	**MDT (Days)**	
	pH 7.4	**pH 5.5**

P1C0	$(1.55\pm0.03) \times 10^7$	$(8.49\pm0.01) \times 10^6$
P1C0.33	$(4.19\pm0.21) \times 10^3$	5.72 ± 0.92
P1C0.5	250.38 ± 11.07	1.04 ± 0.04
P1C0.6	50.28 ± 3.31	0.8 ± 0.1
P1C0.7	51.75 ± 1.83	8.75 ± 0.91
P1C0.8	275.88 ± 7.96	23.58 ± 2.58
P1C1	2015.58 ± 13.92	125.73 ± 8.91

Table 6: MDT for different formulations of PLA/CNC composite beads at pH 7.4 and 5.5.

Cell viability assay. Since the PLA/CNC beads were found to be a DOX-releasable system, we set out to determine the cytotoxic effect of the released drug from PLA/CNC beads against two breast cancer cell lines, i.e., MDA-MB-231 and MCF-7. Before doing this experiment, we studied the cytotoxic effect of drug-free beads on these cell lines for all formulations (**Figure S6,** Supporting Information). None of the compositions of drug-free beads triggered cytotoxicity against the selected breast cancer cell lines, indicating the cytocompatibility of the PLA/CNC matrix. For drug-loaded PLA/CNC beads, we selected P1C0.6 for cellular studies due to its optimum drug release kinetics compared to other formulations. First, MDA-MB-231 cells and MCF-7 cells were treated with 1-6 beads of the P1C0.6 formulation (15 µg DOX per bead, 15-90 µg) and control beads (without DOX), for 24, 48, and 72 h treatment. No cytotoxicity was observed after treating the cells with control beads during all treatment time points. We observed that the DOX loaded into the beads was able to suppress cell growth as a function of concentration, number of beads, and exposure

time (**Figure 12 and Figure S7,** Supporting Information). For example, the cell viability among all treatment groups decreased significantly as the exposure time of the cells to the DOX-encapsulated beads was extended from 24 to 72 hours (**Figure 12**). The amount of drug released from 1-6 beads after 24, 48, and 72 h was measured and shown in **Figure S7** (Supporting Information). It was found that the amount of drug released in media from the beads was able to reach an IC_{50} value (6.59 ±0.29 μM) within 72 h, and therefore, for MDA-MB-231 cells, a minimum of 60 μg DOX-loaded P1C0.6 beads (i.e., 4 beads containing 15 μg DOX per beads) was required. Cell viability results for the MDA-MB-231 cell line demonstrated that after treating the cells with 90 μg of DOX-loaded beads (6 beads containing 15 μg DOX per beads), the formulation was able to kill approximately 74.5% of MDA-MB-231 cancer cells after 72 h of treatment. Similarly, for MCF-7 cancer cells, it was found that to reach IC_{50} value (8.29 ±0.56 μM) within 72 h, 75 μg DOX-loaded P1C0.6 bead (5 beads containing 15 μg DOX per beads) was required. We observed that when 90 μg of DOX was loaded into PLA/CNC beads (6 beads containing 15 μg DOX per beads), the formulation was able to kill approximately 60.8% of MCF-7 cells after 72 h compared to untreated control (cells treated with media alone).

Figure 12. Cytotoxicity profile of the DOX-loaded P1C0.6 formulation for a treatment duration of 24 h, 48 h and 72 h on (A) MDA-MB-231 and (B) MCF-7 breast cancer cell lines 1-6: number of beads that were used for the cytotoxicity studies, Plain indicates control beads of the P1C0.6 formulation not loaded with DOX. ($n = 5$, Two-way ANOVA, Bonferroni post-tests, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ or ****$p < 0.0001$)

Cellular uptake study by confocal microscopy. The cellular uptake of a DOX-loaded P1C0.6 formulation of PLA/CNC beads was evaluated in MDA-MB-231 and MCF-7 (**Figure 13 A-B**) using confocal microscopy. Both cell lines were treated with 6 DOX-containing beads (64 µg DOX per bead, 384 µg) to reach the IC_{50} equivalent DOX released from the beads in media after 3 and 6 h. The fluorescence intensity of the images was normalized with respect to the number of cells[137]. After quantifying the fluorescence integral density of the images by Image J software, we observed that the integral density of DOX accumulated within MDA-MB-231 cells after 6 h treatment was 3.8 times higher than those treated for 3 h (**Figure 13 A' and B'**). Such time-dependent cellular uptake of DOX was also observed for MCF-7 cells. This experiment suggests that the composite beads were not only able to release DOX within the cellular environment, but the liberated drug was also able to translocate inside cells to trigger its cognate cytotoxic effects.

Figure 13. Fluorescence microscopic images (A) MDA-MB-231 cells and (B) MCF-7 cells with P1C0.6-DOX loaded beads for 3h and 6h. Quantitative fluorescence integral density for (A') MDA-MB-231 cells and (B') MCF-7 cells ($n = 6$, Two-way ANOVA, Bonferroni post-tests, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ or ****$p < 0.0001$)

Quantification of cellular uptake of CNC beads by FACs. We further quantified the cellular internalization of the beads by flow cytometry in MDA-MB-231 cells. The percentage of cellular uptake was determined in this cell line upon treatment with DOX-loaded PLA/CNC composite beads of the P1C0.6 formulation for 3 h and 6 h. We observed that 75.3% of cells showed DOX uptake after 3 h of incubation, whereas 88.2% of cells

showed drug uptake after 6 h incubation with beads, compared to untreated cells (**Figure 14** and **Figure S8**, Supporting Information).

Figure 14. In vitro cellular uptake study of DOX-loaded PLA/CNC (P1C0.6) composite beads in MDA-MB-231 cells quantified by flow cytometry. The data shown are representative of three individual samples.

Mechanism of cell death by treatment with DOX-loaded PLA/CNC composite beads. DOX-loaded PlC0.6 showed the highest cytotoxicity towards MDA-MB-231 among all the tested bead formulations. It is important to understand the mechanism of cell death. As our earlier experiments for cytotoxicity and cellular uptake studies showed that the liberated DOX indeed translocated and suppressed cell growth, we hypothesized apoptosis to be one of the major pathways for cell death. Since the loss of mitochondrial transmembrane potential is one of the major characteristic features of apoptosis, we set out to measure this potential using cationic, lipophilic, fluorescent dye JC-1. In normal cells JC-1 can easily enter mitochondria and form J-aggregate, which reflect a green fluorescence (590 nm). On the contrary, in apoptotic cells, JC-1 cannot enter mitochondria and remains in the cytosol in its monomeric form that emits red fluorescence (530 nm). The green fluorescence was estimated using Filter 2 (FL2) and the red fluorescence was measured using Filter 1 (FL1) as shown in **Figure 15**. Thus, by measuring the time-dependent change of the fluorescence intensity, we observed a shift in the percentage of normal to apoptotic cells, after 24, 48, and 72 h post-incubation with the DOX-loaded beads, suggesting the induction of apoptosis in MDA-MB-231 cells by a mitochondrial-dependent pathway (**Figure 15**).

Figure 15. Induction of a mitochondrial-dependent pathway of apoptosis by PLA/CNC composite beads in MDA-MB-231 cells. Data are representative of triplicate measurements.

Polymer stability study in plasma. Since the fabricated PLA/CNC beads are intended for post-surgical implantation inside tissue, it is critical to understand the effect of plasma on bead morphology and microstructure. Therefore, we incubated DOX-free beads of P1C0, P1C0.6, and P1C1 formulations for 5 days (120 h) in mouse plasma under constant stirring at 37˚C. Post incubation, SEM studies were conducted on these beads. As shown in **Figure 16 (A1-C3)**, while P1C1 beads demonstrated a slight degree of surface degradation, most likely due to swelling of CNCs, P1C0 and P1C0.6 did not show any signature of surface degradation over time.

Figure 16. SEM images of the outer surface and cross sections of the mouse plasma treated (5 days) PLA/CNCs composite beads (without DOX). A1-A3: P1C0, B1-B3: P1C0.6, C1-C3: P1C1 at three different magnification levels (i.e., x30, x300, and x1,800); (D) Drug release profile of P1C0.6 DOX-loaded bead and free DOX in mouse plasma for 5 days; E1-E2: SEM images of the outer and cross sections surface DOX-loaded P1C0.6 bead after 5 days of drug release in mouse plasma.

We also showed that, DOX-loaded PLA/CNC beads (P1C0.6 formulation) released DOX in mouse plasma in a tightly controlled kinetics (**Figure 16D**). Approximately 37% of loaded DOX was released after 5 days of exposure in mouse plasma. This result demonstrated that the drug release profile was stable and did not show any sudden drug

release due to polymeric breakdown. SEM image on collected DOX-loaded P1C0.6 bead on 5th day showed no sign of surface degradation (**Figure E1-E2**). These experiments indicated that it is possible to design drug-loaded PLA/CNC composite beads with adjustable stability in mouse plasma by optimizing the PLA to CNC ratio for achieving desirable release kinetics of any bead-encapsulated contents.

Functional efficiency of drug-loaded PLA/CNC beads in patient-derived cancer tissue: Formalin-fixed and paraffin-embedded tissue sections of PDX PDAC tumor chunks treated with drug-free (plain) beads (n=4) or DOX-loaded beads (n=4) were stained for the cell proliferation marker, Ki-67. As expected, tumors treated with plain beads had more Ki-67 positive cells (**Figure 17A**; green staining) than those treated with DOX-loaded beads (**Figure 17B**), indicating that the drug-loaded beads were able to inhibit tumor cell proliferation in an ex vivo tissue model. These data suggest DOX was successfully released from the beads over a period of four days and was able to penetrate the 3D tumor tissue to induce cellular DNA damage and reduce cell proliferation. Additionally, **Figure 17C** shows the percentage of Ki-67 positive cells with respect to the total number of DAPI (nucleus)-stained cells in each tissue section using the image processing program, ImageJ. These data, although not statistically significant owing to a smaller sample size, suggest that the DOX-loaded beads are effective at inhibiting cell proliferation in a 3D tumor.

H&E staining was also performed to test the integrity of the tumor architecture after four days of treatment with drug-free or DOX-loaded beads. Representative H&E images from the two treatment groups (**Figure 17D**) show that the tissue sections obtained from tumors treated with plain beads (i) have clusters of pancreatic ductal adenocarcinoma cells. In contrast, the tissue sections obtained from tumors treated with the DOX-loaded beads have a reduced number of cancer cell clusters. These data, along with the Ki-67 images, are an

indication that the beads carrying DOX were able to release the drug in a controlled manner over a period of 4 days and reduce tumor cell proliferation.

Figure 17. Effect of DOX-loaded beads on proliferation and integrity of human PDAC tumors. Immunohistochemistry for Ki-67 (green staining) was performed for 4 replicates each of human PDAC tumor chunks treated with (A) drug-free/plain beads and (B) DOX-loaded beads. DAPI (blue staining) was used to detect the nucleus of each cell and the merged images and (C) quantification of the staining showed greater abundance of Ki-67 (cell proliferation) in control-treated tumors compared to DOX-treated tumors. (D) A greater number of tumor cell clusters (red arrows) were observed in the representative images of the H&E-stained tumor sections for (i) plain beads compared to (ii) DOX-

Conclusion. We demonstrated the feasibility and performance of CNC-reinforced PLA beads as a depot-type controlled release formulation. We provided proof-of-principle for the system using DOX as a model frontline chemotherapy using cancer cell lines and patient-derived tumor tissue. Our study showed that CNCs could be a suitable material to include in a polymer matrix intended for formation of an implantable drug releasing system. Structural analysis of our CNC-containing drug-releasing beads confirmed that combination of porosity and mechanical strength jointly governed drug release kinetics from the system. The amount of CNCs plays a pivotal role in the performance and stability of the beads. The formulations with optimized ratios of PLA and CNCs within the beads can be used for adjusting the release kinetics of bead-entrapped drug molecules. We observed that PLA/CNC beads release drugs in their bioavailable form, which can be internalized by cancer cells. Further, the beads are effective at inhibiting tumor cell proliferation in a 3D tissue model. We are currently working on pharmacokinetic and pharmacodynamic evaluation of these systems under in

vivo conditions. We envision that the PLA/CNC beads can be used for the development of surgically-implantable, prolonged-release drug delivery systems.

References

1. Rand, B. C. C.; Penn-Barwell, J. G.; Wenke, J. C. Combined local and systemic antibiotic delivery improves eradication of wound contamination. *The Bone & Joint Journal* **2015,** 97-B (10), 1423-1427 DOI: 10.1302/0301-620X.97B10.35651.
2. McConoughey, S. J.; Howlin, R. P.; Wiseman, J.; Stoodley, P.; Calhoun, J. H. Comparing PMMA and calcium sulfate as carriers for the local delivery of antibiotics to infected surgical sites. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **2015,** 103 (4), 870-877 DOI: 10.1002/jbm.b.33247.
3. El-Husseiny, M.; Patel, S.; MacFarlane, R. J.; Haddad, F. S. Biodegradable antibiotic delivery systems. *The Journal of Bone and Joint Surgery. British volume* **2011,** 93-B (2), 151-157 DOI: 10.1302/0301-620X.93B2.24933.
4. ter Boo, G.-J. A.; Grijpma, D. W.; Moriarty, T. F.; Richards, R. G.; Eglin, D. Antimicrobial delivery systems for local infection prophylaxis in orthopedic- and trauma surgery. *Biomaterials* **2015,** 52, 113-125 DOI: 10.1016/j.biomaterials.2015.02.020.
5. Logoluso, N.; Drago, L.; Gallazzi, E.; George, D. A.; Morelli, I.; Romanò, C. L. Calcium-Based, Antibiotic-Loaded Bone Substitute as an Implant Coating: A Pilot Clinical Study. *Journal of bone and joint infection* **2016,** 1, 59-64 DOI: 10.7150/jbji.17586.
6. Bloom, H. L.; Constantin, L.; Dan, D.; De Lurgio, D. B.; El-Chami, M.; Ganz, L. I.; Gleed, K. J.; Hackett, F. K.; Kanuru, N. K.; Lerner, D. J.; Rasekh, A.; Simons, G. R.; Sogade, F. O.; Sohail, M. R.; for the, C. I. Implantation Success and Infection in Cardiovascular Implantable Electronic Device Procedures Utilizing an Antibacterial Envelope. *Pacing and Clinical Electrophysiology* **2011,** 34 (2), 133-142 DOI: 10.1111/j.1540-8159.2010.02931.x.
7. Caplin, J. D.; García, A. J. Implantable antimicrobial biomaterials for local drug delivery in bone infection models. *Acta Biomaterialia* **2019,** 93, 2-11 DOI: 10.1016/j.actbio.2019.01.015.
8. Shah, S. R.; Tatara, A. M.; D'Souza, R. N.; Mikos, A. G.; Kasper, F. K. Evolving strategies for preventing biofilm on implantable materials. *Materials Today* **2013,** 16 (5), 177-182 DOI: 10.1016/j.mattod.2013.05.003.
9. X, L.; GJ, P.; LK, M.; PX, M. Pulsatile release of parathyroid hormone from an implantable delivery system. *Biomaterials* **2007,** 28 (28), DOI: 10.1016/j.biomaterials.2007.05.034.
10. Shore, N. Introducing Vantas: The First Once-Yearly Luteinising Hormone-Releasing Hormone Agonist. *European Urology Supplements* **2010,** 9 (8), 701-705 DOI: 10.1016/j.eursup.2010.08.004.
11. Sutradhar, K. B.; Sumi, C. D. Implantable microchip: the futuristic controlled drug delivery system. *Drug Delivery* **2016,** 23 (1), 1-11 DOI: 10.3109/10717544.2014.903579.
12. BR, G.; DR, K. Goserelin acetate implant: a depot luteinizing hormone-releasing hormone analog for advanced prostate cancer. *DICP : the annals of pharmacotherapy* **1991,** 25 (7-8), DOI: 10.1177/106002809102500716.
13. Lee, S. H.; Piao, H.; Cho, Y. C.; Kim, S.-N.; Choi, G.; Kim, C. R.; Ji, H. B.; Park, C. G.; Lee, C.; Shin, C. I.; Koh, W.-G.; Choy, Y. B.; Choy, J.-H. Implantable multireservoir device with stimulus-responsive membrane for on-demand and pulsatile delivery of growth hormone. *Proceedings of the National Academy of Sciences* **2019,** 116 (24), 11664 DOI: 10.1073/pnas.1906931116.
14. Stefanini, G. G.; Holmes, D. R. Drug-Eluting Coronary-Artery Stents. *New England Journal of Medicine* **2013,** 368 (3), 254-265 DOI: 10.1056/nejmra1210816.

15. da Silva, G. F.; Cesário, F.; Garcia, A. M. R.; Weckwerth, P. H.; Duarte, M. A. H.; de Oliveira, R. C.; Vivan, R. R. Effect of association of non-steroidal anti-inflammatory and antibiotic agents with calcium hydroxide pastes on their cytotoxicity and biocompatibility. *Clinical Oral Investigations* **2020,** 24 (2), 757-763 DOI: 10.1007/s00784-019-02923-y.
16. Jayant, R. D.; McShane, M. J.; Srivastava, R. In vitro and in vivo evaluation of anti-inflammatory agents using nanoengineered alginate carriers: Towards localized implant inflammation suppression. *International Journal of Pharmaceutics* **2011,** 403 (1), 268-275 DOI: 10.1016/j.ijpharm.2010.10.035.
17. Luo, J. D.; Miller, C.; Jirjis, T.; Nasir, M.; Sharma, D. The effect of non-steroidal anti-inflammatory drugs on the osteogenic activity in osseointegration: a systematic review. *International Journal of Implant Dentistry* **2018,** 4 (1), 30 DOI: 10.1186/s40729-018-0141-7.
18. Nyangoga, H.; Aguado, E.; Goyenvalle, E.; Baslé, M. F.; Chappard, D. A non-steroidal anti-inflammatory drug (ketoprofen) does not delay β-TCP bone graft healing. *Acta Biomaterialia* **2010,** 6 (8), 3310-3317 DOI: 10.1016/j.actbio.2010.01.042.
19. Nazari, K.; Kontogiannidou, E.; Ahmad, R. H.; Gratsani, A.; Rasekh, M.; Arshad, M. S.; Sunar, B. S.; Armitage, D.; Bouropoulos, N.; Chang, M.-W.; Li, X.; Fatouros, D. G.; Ahmad, Z. Development and characterisation of cellulose based electrospun mats for buccal delivery of non-steroidal anti-inflammatory drug (NSAID). *European Journal of Pharmaceutical Sciences* **2017,** 102, 147-155 DOI: 10.1016/j.ejps.2017.02.033.
20. Lazăr, A. D.; Dinescu, S.; Albu-Kaya, M. G.; Gharbia, S.; Hermenean, A.; Costache, M. Release of the Non-Steroidal Anti-Inflammatory Drug Flufenamic Acid by Multiparticulate Delivery Systems Promotes Adipogenic Differentiation of Adipose-Derived Stem Cells. *Materials* **2020,** 13 (7), 1550 DOI: 10.3390/ma13071550.
21. Lin, P.; Lin, C.-W.; Mansour, R.; Gu, F. Improving biocompatibility by surface modification techniques on implantable bioelectronics. *Biosensors and Bioelectronics* **2013,** 47, 451-460 DOI: 10.1016/j.bios.2013.01.071.
22. Xie, Y.; Bagby, T. R.; Cohen, M.; Forrest, M. L. Drug delivery to the lymphatic system: importance in future cancer diagnosis and therapies. *Expert Opinion on Drug Delivery* **2009,** 6 (8), 785-792 DOI: 10.1517/17425240903085128.
23. Wolinsky, J. B.; Colson, Y. L.; Grinstaff, M. W. Local drug delivery strategies for cancer treatment: Gels, nanoparticles, polymeric films, rods, and wafers. *Journal of Controlled Release* **2012,** 159 (1), 14-26 DOI: 10.1016/j.jconrel.2011.11.031.
24. Lei, N.; Gong, C.; Qian, Z.; Luo, F.; Wang, C.; Wang, H.; Wei, Y. Therapeutic application of injectable thermosensitive hydrogel in preventing local breast cancer recurrence and improving incision wound healing in a mouse model. *Nanoscale* **2012,** 4 (18), 5686 DOI: 10.1039/c2nr30731f.
25. Xue, J.; Zhao, Z.; Zhang, L.; Xue, L.; Shen, S.; Wen, Y.; Wei, Z.; Wang, L.; Kong, L.; Sun, H.; Ping, Q.; Mo, R.; Zhang, C. Neutrophil-mediated anticancer drug delivery for suppression of postoperative malignant glioma recurrence. *Nature Nanotechnology* **2017,** 12 (7), 692-700 DOI: 10.1038/nnano.2017.54.
26. Franchelli, S.; Vassallo, F.; Porzio, C.; Mannucci, M.; Priano, V.; Schenone, E.; Leone, M. S.; Canavese, G.; Santi, P.; De Maria, A. Breast Implant Infections after Surgical Reconstruction in Patients with Breast Cancer: Assessment of Risk Factors and Pathogens over Extended Post-Operative Observation. *Surgical Infections* **2012,** 13 (3), 154-158 DOI: 10.1089/sur.2011.004.
27. Weinberg, B. D.; Blanco, E.; Gao, J. Polymer implants for intratumoral drug delivery and cancer therapy. *Journal of Pharmaceutical Sciences* **2008,** 97 (5), 1681-1702 DOI: 10.1002/jps.21038.
28. Senapati, S.; Mahanta, A. K.; Kumar, S.; Maiti, P. Controlled drug delivery vehicles for cancer treatment and their performance. *Signal Transduction and Targeted Therapy* **2018,** 3 (1), DOI: 10.1038/s41392-017-0004-3.
29. Panigrahi, M.; Das, P. K.; Parikh, P. M. Brain tumor and Gliadel wafer treatment. *Indian Journal of Cancer* **2011,** 48 (1), 11-17 DOI: 10.4103/0019-509X.76623.

30. Brem, H.; Gabikian, P. Biodegradable polymer implants to treat brain tumors. *Journal of Controlled Release* **2001,** 74 (1-3), 63-67 DOI: 10.1016/s0168-3659(01)00311-x.
31. Brem, H.; Piantadosi, S.; Burger, P. C.; Walker, M.; Selker, R.; Vick, N. A.; Black, K.; Sisti, M.; Brem, S.; Mohr, G.; Muller, P.; Morawetz, R.; Schold, S. C. Placebo-controlled trial of safety and efficacy of intraoperative controlled delivery by biodegradable polymers of chemotherapy for recurrent gliomas. *The Lancet* **1995,** 345 (8956), 1008-1012 DOI: 10.1016/S0140-6736(95)90755-6.
32. Westphal, M.; Hilt, D. C.; Bortey, E.; Delavault, P.; Olivares, R.; Warnke, P. C.; Whittle, I. R.; JäÄSkeläInen, J.; Ram, Z. A phase 3 trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (Gliadel wafers) in patients with primary malignant glioma. *Neuro-Oncology* **2003,** 5 (2), 79-88 DOI: 10.1093/neuonc/5.2.79.
33. Chowdhary, S. A.; Ryken, T.; Newton, H. B. Survival outcomes and safety of carmustine wafers in the treatment of high-grade gliomas: a meta-analysis. *Journal of Neuro-Oncology* **2015,** 122 (2), 367-382 DOI: 10.1007/s11060-015-1724-2.
34. Qi, Z.-Y.; Xing, W.; Shao, C.; Yang, C.; Wang, Z. *Drug Design, Development and Therapy* **2015**, 3341 DOI: 10.2147/dddt.s85943.
35. Weber, E. L.; Goebel, E. A. Cerebral edema associated with Gliadel wafers: Two case studies. *Neuro-Oncology* **2005,** 7 (1), 84-89 DOI: 10.1215/s1152851704000614.
36. WM, S.; LK, F. Polymeric implants for cancer chemotherapy. *Advanced drug delivery reviews* **1997,** 26 (2-3), DOI: 10.1016/s0169-409x(97)00036-7.
37. De Souza, R.; Zahedi, P.; Allen, C. J.; Piquette-Miller, M. Polymeric drug delivery systems for localized cancer chemotherapy. *Drug Delivery* **2010,** 17 (6), 365-375 DOI: 10.3109/10717541003762854.
38. Talebian, S.; Foroughi, J.; Wade, S. J.; Vine, K. L.; Dolatshahi-Pirouz, A.; Mehrali, M.; Conde, J.; Wallace, G. G. Biopolymers for Antitumor Implantable Drug Delivery Systems: Recent Advances and Future Outlook. *Advanced Materials* **2018,** 30 (31), 1706665 DOI: 10.1002/adma.201706665.
39. De Clercq, K.; Xie, F.; De Wever, O.; Descamps, B.; Hoorens, A.; Vermeulen, A.; Ceelen, W.; Vervaet, C. Preclinical evaluation of local prolonged release of paclitaxel from gelatin microspheres for the prevention of recurrence of peritoneal carcinomatosis in advanced ovarian cancer. *Scientific Reports* **2019,** 9 (1), 14881 DOI: 10.1038/s41598-019-51419-y.
40. Armstrong, D. K.; Fleming, G. F.; Markman, M.; Bailey, H. H.; A phase I trial of intraperitoneal sustained-release paclitaxel microspheres (Paclimer®) in recurrent ovarian cancer: A Gynecologic Oncology Group study. *Gynecologic Oncology* **2006,** 103 (2), 391-396 DOI: 10.1016/j.ygyno.2006.02.029.
41. Harper, E.; Dang, W.; Lapidus, R. G.; Garver, R. I. Enhanced Efficacy of a Novel Controlled Release Paclitaxel Formulation (PACLIMER Delivery System) for Local-Regional Therapy of Lung Cancer Tumor Nodules in Mice. *Clinical Cancer Research* **1999,** 5 (12), 4242.; ISSN: 1557-3265; PMID: 10632366
42. Lapidus, R. G.; Dang, W.; Rosen, D. M.; Gady, A. M.; Zabelinka, Y.; O'Meally, R.; Deweese, T. L.; Denmeade, S. R. Anti-tumor effect of combination therapy with intratumoral controlled-release paclitaxel (PACLIMER® microspheres) and radiation. *The Prostate* **2004,** 58 (3), 291-298 DOI: 10.1002/pros.10331.
43. Li, K. W.; Dang, W.; Tyler, B. M.; Troiano, G.; Tihan, T.; Brem, H.; Walter, K. A. Polilactofate Microspheres for Paclitaxel Delivery to Central Nervous System Malignancies. *Clinical Cancer Research* **2003,** 9 (9), 3441. ; ISSN: 1557-3265; PMID: 12960135
44. Song, R.; Murphy, M.; Li, C.; Ting, K.; Soo, C.; Zheng, Z. Current development of biodegradable polymeric materials for biomedical applications. *Drug Design, Development and Therapy* **2018,** Volume 12, 3117-3145 DOI: 10.2147/dddt.s165440.
45. Gao, Z.-H.; Shukla, A. J.; Johnson, J. R.; Crowley, W. R. Controlled Release of a Contraceptive Steroid from Biodegradable and Injectable Gel Formulations: In Vitro Evaluation. *Pharmaceutical Research* **1995,** 12 (6), 857-863 DOI: 10.1023/A:1016209020160.

46. Beck, L. R.; Cowsar, D. R.; Lewis, D. H.; Gibson, J. W.; Flowers, C. E. New long-acting injectable microcapsule contraceptive system. *American Journal of Obstetrics and Gynecology* **1979,** 135 (3), 419-426 DOI: 10.1016/0002-9378(79)90717-8.
47. Stewart, S. A.; Domínguez-Robles, J.; Donnelly, R. F.; Larrañeta, E. Implantable Polymeric Drug Delivery Devices: Classification, Manufacture, Materials, and Clinical Applications. *Polymers* **2018,** 10 (12), 1379 DOI: 10.3390/polym10121379.
48. Ulery, B. D.; Nair, L. S.; Laurencin, C. T. Biomedical applications of biodegradable polymers. *Journal of Polymer Science Part B: Polymer Physics* **2011,** 49 (12), 832-864 DOI: 10.1002/polb.22259.
49. Woodard, L. N.; Grunlan, M. A. Hydrolytic Degradation and Erosion of Polyester Biomaterials. *ACS Macro Letters* **2018,** 7 (8), 976-982 DOI: 10.1021/acsmacrolett.8b00424.
50. Maisel, W. H. Unanswered Questions — Drug-Eluting Stents and the Risk of Late Thrombosis. *New England Journal of Medicine* **2007,** 356 (10), 981-984 DOI: 10.1056/nejmp068305.
51. Kumar, A.; Pillai, J., Chapter 13 - Implantable drug delivery systems: An overview. In *Nanostructures for the Engineering of Cells, Tissues and Organs*, Grumezescu, A. M., Ed. William Andrew Publishing: 2018; pp 473-511.; ISBN: 9780128136669
52. Saito, N.; Murakami, N.; Takahashi, J.; Horiuchi, H.; Ota, H.; Kato, H.; Okada, T.; Nozaki, K.; Takaoka, K. Synthetic biodegradable polymers as drug delivery systems for bone morphogenetic proteins. *Advanced Drug Delivery Reviews* **2005,** 57 (7), 1037-1048 DOI: 10.1016/j.addr.2004.12.016.
53. Jain, R.; Shah, N. H.; Malick, A. W.; Rhodes, C. T. Controlled Drug Delivery by Biodegradable Poly(Ester) Devices: Different Preparative Approaches. *Drug Development and Industrial Pharmacy* **1998,** 24 (8), 703-727 DOI: 10.3109/03639049809082719.
54. Jalil, R.-U. Biodegradable Poly(Lactic Acid) and Poly (Lactide-Co-Glycolide) Polymers in Sustained Drug Delivery. *Drug Development and Industrial Pharmacy* **1990,** 16 (16), 2353-2367 DOI: 10.3109/03639049009058535.
55. Jain, R. A. The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. *Biomaterials* **2000,** 21 (23), 2475-2490 DOI: 10.1016/S0142-9612(00)00115-0.
56. Hu, Y.; Wu, X.; JinRui, X. Self-assembled supramolecular hydrogels formed by biodegradable PLA/CS diblock copolymers and β-cyclodextrin for controlled dual drug delivery. *International Journal of Biological Macromolecules* **2018,** 108, 18-23 DOI: 10.1016/j.ijbiomac.2017.11.104.
57. Xie, Z.; Su, Y.; Kim, G. B.; Selvi, E.; Ma, C.; Aragon-Sanabria, V.; Hsieh, J.-T.; Dong, C.; Yang, J. Immune Cell-Mediated Biodegradable Theranostic Nanoparticles for Melanoma Targeting and Drug Delivery. *Small* **2017,** 13 (10), 1603121 DOI: 10.1002/smll.201603121.
58. Da Silva, D.; Kaduri, M.; Poley, M.; Adir, O.; Krinsky, N.; Shainsky-Roitman, J.; Schroeder, A. Biocompatibility, biodegradation and excretion of polylactic acid (PLA) in medical implants and theranostic systems. *Chemical Engineering Journal* **2018,** 340, 9-14 DOI: 10.1016/j.cej.2018.01.010.
59. Ali, W.; Mehboob, A.; Han, M.-G.; Chang, S.-H. Effect of fluoride coating on degradation behaviour of unidirectional Mg/PLA biodegradable composite for load-bearing bone implant application. *Composites Part A: Applied Science and Manufacturing* **2019,** 124, 105464 DOI: 10.1016/j.compositesa.2019.05.032.
60. Lee, B. K.; Yun, Y.; Park, K. PLA micro- and nano-particles. *Advanced Drug Delivery Reviews* **2016,** 107, 176-191 DOI: 10.1016/j.addr.2016.05.020.
61. Tyler, B.; Gullotti, D.; Mangraviti, A.; Utsuki, T.; Brem, H. Polylactic acid (PLA) controlled delivery carriers for biomedical applications. *Advanced Drug Delivery Reviews* **2016,** 107, 163-175 DOI: 10.1016/j.addr.2016.06.018.
62. O'Keefe, B. J.; Hillmyer, M. A.; Tolman, W. B. Polymerization of lactide and related cyclic esters by discrete metal complexes. *Journal of the Chemical Society, Dalton Transactions* **2001,** (15), 2215-2224 DOI: 10.1039/b104197p.
63. Wu, J.; Yu, T.-L.; Chen, C.-T.; Lin, C.-C. Recent developments in main group metal complexes catalyzed/initiated polymerization of lactides and related cyclic esters. *Coordination Chemistry Reviews* **2006,** 250 (5), 602-626 DOI: 10.1016/j.ccr.2005.07.010.

64. Kamber, N. E.; Jeong, W.; Waymouth, R. M.; Pratt, R. C.; Lohmeijer, B. G. G.; Hedrick, J. L. Organocatalytic Ring-Opening Polymerization. *Chemical Reviews* **2007,** 107 (12), 5813-5840 DOI: 10.1021/cr068415b.
65. Stanford, M. J.; Dove, A. P. Stereocontrolled ring-opening polymerisation of lactide. *Chem. Soc. Rev.* **2010,** 39 (2), 486-494 DOI: 10.1039/b815104k.
66. Timmins, P.; Desai, D.; Chen, W.; Wray, P.; Brown, J.; Hanley, S. Advances in mechanistic understanding of release rate control mechanisms of extended-release hydrophilic matrix tablets. *Therapeutic Delivery* **2016,** 7 (8), 553-572 DOI: 10.4155/tde-2016-0026.
67. 4 - Main mechanisms to control the drug release. In *Strategies to Modify the Drug Release from Pharmaceutical Systems*, Bruschi, M. L., Ed. Woodhead Publishing: 2015; pp 37-62.; ISBN: 9780081001127
68. Fu, Y.; Kao, W. J. Drug release kinetics and transport mechanisms of non-degradable and degradable polymeric delivery systems. *Expert opinion on drug delivery* **2010,** 7 (4), 429-444 DOI: 10.1517/17425241003602259.
69. Lu, Z.; Chen, W.; Olivier, E.; Hamman, J. H. Matrix Polymeric Excipients: Comparing a Novel Interpolyelectrolyte Complex with Hydroxypropylmethylcellulose. *Drug Delivery* **2008,** 15 (2), 87-96 DOI: 10.1080/10717540801905009.
70. Yin, X.; Li, H.; Guo, Z.; Wu, L.; Chen, F.; de Matas, M.; Shao, Q.; Xiao, T.; York, P.; He, Y.; Zhang, J. Quantification of swelling and erosion in the controlled release of a poorly water-soluble drug using synchrotron X-ray computed microtomography. *The AAPS journal* **2013,** 15 (4), 1025-1034 DOI: 10.1208/s12248-013-9498-y.
71. Lamberti, G.; Galdi, I.; Barba, A. A. Controlled release from hydrogel-based solid matrices. A model accounting for water up-take, swelling and erosion. *International Journal of Pharmaceutics* **2011,** 407 (1), 78-86 DOI: 10.1016/j.ijpharm.2011.01.023.
72. Pitt, C. G.; Chasalow, F. I.; Hibionada, Y. M.; Klimas, D. M.; Schindler, A. Aliphatic polyesters. I. The degradation of poly(ϵ-caprolactone) in vivo. *Journal of Applied Polymer Science* **1981,** 26 (11), 3779-3787 DOI: 10.1002/app.1981.070261124.
73. Pitt, G.; Gratzl, M.; Kimmel, G.; Surles, J.; Sohindler, A. Aliphatic polyesters II. The degradation of poly (DL-lactide), poly (ε-caprolactone), and their copolymers in vivo. *Biomaterials* **1981,** 2 (4), 215-220 DOI: 10.1016/0142-9612(81)90060-0.
74. Thompson, D. E.; Agrawal, C. M.; Athanasiou, K. The Effects of Dynamic Compressive Loading on Biodegradable Implants of 50–50% Polylactic Acid–Polyglycolic Acid. *Tissue Engineering* **1996,** 2 (1), 61-74 DOI: 10.1089/ten.1996.2.61.
75. Prabhu, D. M.; Li, W. J., The most abundant natural resource: Cellulose and its derivatives and their applications. 2015; pp 3-15.; URI: 8080/jspui/handle/123456789/3517
76. Cudjoe, E.; Hunsen, M.; Xue, Z.; Way, A. E.; Barrios, E.; Olson, R. A.; Hore, M. J. A.; Rowan, S. J. Miscanthus Giganteus: A commercially viable sustainable source of cellulose nanocrystals. *Carbohydrate Polymers* **2017,** 155, 230-241 DOI: 10.1016/j.carbpol.2016.08.049.
77. Yang, H.; Zhang, Y.; Kato, R.; Rowan, S. J. Preparation of cellulose nanofibers from Miscanthus x. Giganteus by ammonium persulfate oxidation. *Carbohydrate Polymers* **2019,** 212, 30-39 DOI: 10.1016/j.carbpol.2019.02.008.
78. Calvino, C.; Macke, N.; Kato, R.; Rowan, S. J. Development, processing and applications of bio-sourced cellulose nanocrystal composites. *Progress in Polymer Science* **2020,** 103, 101221 DOI: 10.1016/j.progpolymsci.2020.101221.
79. George, J.; Sabapathi, S. N. Cellulose nanocrystals: synthesis, functional properties, and applications. *Nanotechnology, science and applications* **2015,** 8, 45-54 DOI: 10.2147/NSA.S64386.
80. Habibi, Y.; Lucia, L. A.; Rojas, O. J. Cellulose Nanocrystals: Chemistry, Self-Assembly, and Applications. *Chemical Reviews* **2010,** 110 (6), 3479-3500 DOI: 10.1021/cr900339w.
81. Bondeson, D.; Mathew, A.; Oksman, K. Optimization of the isolation of nanocrystals from microcrystalline celluloseby acid hydrolysis. *Cellulose* **2006,** 13 (2), 171 DOI: 10.1007/s10570-006-9061-4.

82. Hazra, R. S.; Kale, N.; Aland, G.; Qayyumi, B.; Mitra, D.; Jiang, L.; Bajwa, D.; Khandare, J.; Chaturvedi, P.; Quadir, M. Cellulose Mediated Transferrin Nanocages for Enumeration of Circulating Tumor Cells for Head and Neck Cancer. *Scientific Reports* **2020,** 10 (1), 10010 DOI: 10.1038/s41598-020-66625-2.
83. Mariano, M.; El Kissi, N.; Dufresne, A. Cellulose nanocrystals and related nanocomposites: Review of some properties and challenges. *Journal of Polymer Science Part B: Polymer Physics* **2014,** 52 (12), 791-806 DOI: 10.1002/polb.23490.
84. Madhusudana Rao, K.; Kumar, A.; Han, S. S. Polysaccharide based bionanocomposite hydrogels reinforced with cellulose nanocrystals: Drug release and biocompatibility analyses. *International Journal of Biological Macromolecules* **2017,** 101, 165-171 DOI: 10.1016/j.ijbiomac.2017.03.080.
85. Picheth, G. F.; Pirich, C. L.; Sierakowski, M. R.; Woehl, M. A.; Sakakibara, C. N.; de Souza, C. F.; Martin, A. A.; da Silva, R.; de Freitas, R. A. Bacterial cellulose in biomedical applications: A review. *International Journal of Biological Macromolecules* **2017,** 104, 97-106 DOI: 10.1016/j.ijbiomac.2017.05.171.
86. Czaja, W. K.; Young, D. J.; Kawecki, M.; Brown, R. M. The Future Prospects of Microbial Cellulose in Biomedical Applications. *Biomacromolecules* **2007,** 8 (1), 1-12 DOI: 10.1021/bm060620d.
87. Jorfi, M.; Foster, E. J. Recent advances in nanocellulose for biomedical applications. *Journal of Applied Polymer Science* **2015,** 132 (14), DOI: 10.1002/app.41719.
88. Islam, M. S.; Chen, L.; Sisler, J.; Tam, K. C. Cellulose nanocrystal (CNC)–inorganic hybrid systems: synthesis, properties and applications. *Journal of Materials Chemistry B* **2018,** (6), DOI: 10.1039/C7TB03016A.
89. Shojaeiarani, J.; Bajwa, D.; Shirzadifar, A. A review on cellulose nanocrystals as promising biocompounds for the synthesis of nanocomposite hydrogels. *Carbohydrate Polymers* **2019,** 216, 247-259 DOI: 10.1016/j.carbpol.2019.04.033.
90. Lv, J.; Zhang, G.; Zhang, H.; Zhao, C.; Yang, F. Improvement of antifouling performances for modified PVDF ultrafiltration membrane with hydrophilic cellulose nanocrystal. *Applied Surface Science* **2018,** 440, 1091-1100 DOI: 10.1016/j.apsusc.2018.01.256.
91. Bai, L.; Bossa, N.; Qu, F.; Winglee, J.; Li, G.; Sun, K.; Liang, H.; Wiesner, M. R. Comparison of Hydrophilicity and Mechanical Properties of Nanocomposite Membranes with Cellulose Nanocrystals and Carbon Nanotubes. *Environmental Science & Technology* **2017,** 51 (1), 253-262 DOI: 10.1021/acs.est.6b04280.
92. Jayaramudu, T.; Ko, H.-U.; Kim, H. C.; Kim, J. W.; Kim, J. Swelling Behavior of Polyacrylamide-Cellulose Nanocrystal Hydrogels: Swelling Kinetics, Temperature, and pH Effects. *Materials (Basel, Switzerland)* **2019,** 12 (13), 2080 DOI: 10.3390/ma12132080.
93. Jahan, Z.; Niazi, M. B. K.; Gregersen, Ø. W. Mechanical, thermal and swelling properties of cellulose nanocrystals/PVA nanocomposites membranes. *Journal of Industrial and Engineering Chemistry* **2018,** 57, 113-124 DOI: 10.1016/j.jiec.2017.08.014.
94. Chen, N.; Wang, H.; Ling, C.; Vermerris, W.; Wang, B.; Tong, Z. Cellulose-based injectable hydrogel composite for pH-responsive and controllable drug delivery. *Carbohydrate Polymers* **2019,** 225, 115207 DOI: 10.1016/j.carbpol.2019.115207.
95. Athanasiou, K. A.; Schmitz, J. P.; Agrawal, C. M. The Effects of Porosity on in Vitro Degradation of Polylactic Acid–Polyglycolic Acid Implants Used in Repair of Articular Cartilage. *Tissue Engineering* **1998,** 4 (1), 53-63 DOI: 10.1089/ten.1998.4.53.
96. Golshan, M.; Salami-Kalajahi, M.; Roghani-Mamaqani, H.; Mohammadi, M. Poly(propylene imine) dendrimer-grafted nanocrystalline cellulose: Doxorubicin loading and release behavior. *Polymer* **2017,** 117, 287-294 DOI: 10.1016/j.polymer.2017.04.047.
97. He, L.; Liang, H.; Lin, L.; Shah, B. R.; Li, Y.; Chen, Y.; Li, B. Green-step assembly of low density lipoprotein/sodium carboxymethyl cellulose nanogels for facile loading and pH-dependent release of doxorubicin. *Colloids and Surfaces B: Biointerfaces* **2015,** 126, 288-296 DOI: 10.1016/j.colsurfb.2014.12.024.

98. Hujaya, S. D.; Lorite, G. S.; Vainio, S. J.; Liimatainen, H. Polyion complex hydrogels from chemically modified cellulose nanofibrils: Structure-function relationship and potential for controlled and pH-responsive release of doxorubicin. *Acta Biomaterialia* **2018,** 75, 346-357 DOI: 10.1016/j.actbio.2018.06.013.
99. Omidi, S.; Pirhayati, M.; Kakanejadifard, A. Co-delivery of doxorubicin and curcumin by a pH-sensitive, injectable, and in situ hydrogel composed of chitosan, graphene, and cellulose nanowhisker. *Carbohydrate Polymers* **2020,** 231, 115745 DOI: 10.1016/j.carbpol.2019.115745.
100. Rao, Z.; Ge, H.; Liu, L.; Zhu, C.; Min, L.; Liu, M.; Fan, L.; Li, D. Carboxymethyl cellulose modified graphene oxide as pH-sensitive drug delivery system. *International Journal of Biological Macromolecules* **2018,** 107, 1184-1192 DOI: 10.1016/j.ijbiomac.2017.09.096.
101. Voon, L. K.; Pang, S. C.; Chin, S. F. Porous Cellulose Beads Fabricated from Regenerated Cellulose as Potential Drug Delivery Carriers. *Journal of Chemistry* **2017,** 2017, 1-11 DOI: 10.1155/2017/1943432.
102. Gutschmidt, D.; Hazra, R. S.; Zhou, X.; Xu, X.; Sabzi, M.; Jiang, L. Electrospun, sepiolite-loaded poly(vinyl alcohol)/soy protein isolate nanofibers: Preparation, characterization, and their drug release behavior. *International Journal of Pharmaceutics* **2021,** 594, 120172 DOI: 10.1016/j.ijpharm.2020.120172.
103. Fu, S.; Fang, Q.; Li, A.; Li, Z.; Han, J.; Dang, X.; Han, W. Accurate characterization of full pore size distribution of tight sandstones by low-temperature nitrogen gas adsorption and high-pressure mercury intrusion combination method. *Energy Science & Engineering* **2021,** 9 (1), 80-100 DOI: 10.1002/ese3.817.
104. Naznin, M.; Abedin, M. Z.; Khan, M. A.; Gafur, M. A. Influence of Acacia catechu Extracts and Urea and Gamma Irradiation on the Mechanical Properties of Starch/PVA-Based Material. *ISRN Polymer Science* **2012,** 2012, 348685 DOI: 10.5402/2012/348685.
105. Neher, B.; Bhuiyan, M. M. R.; Kabir, H.; Qadir, M. R.; Gafur, M. A.; Ahmed, F. Study of Mechanical and Physical Properties of Palm Fiber Reinforced Acrylonitrile Butadiene Styrene Composite. *Materials Sciences and Applications* **2014,** 05 (01), 39-45 DOI: 10.4236/msa.2014.51006.
106. Zhao, Y.-Z.; Sun, C.-Z.; Lu, C.-T.; Dai, D.-D.; Lv, H.-F.; Wu, Y.; Wan, C.-W.; Chen, L.-J.; Lin, M.; Li, X.-K. Characterization and anti-tumor activity of chemical conjugation of doxorubicin in polymeric micelles (DOX-P) in vitro. *Cancer Letters* **2011,** 311 (2), 187-194 DOI: 10.1016/j.canlet.2011.07.013.
107. Scheeren, L. E.; Nogueira-Librelotto, D. R.; Fernandes, J. R.; Macedo, L. B.; Marcolino, A. I. P.; Vinardell, M. P.; Rolim, C. M. B. Comparative Study of Reversed-Phase High-Performance Liquid Chromatography and Ultraviolet–Visible Spectrophotometry to Determine Doxorubicin in pH-Sensitive Nanoparticles. *Analytical Letters* **2018,** 51 (10), 1445-1463 DOI: 10.1080/00032719.2017.1380034.
108. Ji, J.; Liu, M.; Meng, Y.; Liu, R.; Yan, Y.; Dong, J.; Guo, Z.; Ye, C. Experimental Study of Magnetic Multi-Walled Carbon Nanotube-Doxorubicin Conjugate in a Lymph Node Metastatic Model of Breast Cancer. **2016,** 22, 2363-2373 DOI: 10.12659/msm.898597.
109. Chitemere, R.; Stafslien, S.; Jiang, L.; Webster, D.; Quadir, M. Soy-Based Soft Matrices for Encapsulation and Delivery of Hydrophilic Compounds. *Polymers* **2018,** 10 (6), 583 DOI: 10.3390/polym10060583.
110. Korsmeyer, R. W.; Gurny, R.; Doelker, E.; Buri, P.; Peppas, N. A. Mechanisms of solute release from porous hydrophilic polymers. *International Journal of Pharmaceutics* **1983,** 15 (1), 25-35 DOI: 10.1016/0378-5173(83)90064-9.
111. S, D.; PN, M.; L, N.; P, C. Kinetic modeling on drug release from controlled drug delivery systems. *Acta poloniae pharmaceutica* **2010,** 67 (3).; ISSN 0001-6837; PMID: 20524422
112. MS, R.; MA, Q.; SS, H. Comparative evaluation of plastic, hydrophobic and hydrophilic polymers as matrices for controlled-release drug delivery. *Journal of pharmacy & pharmaceutical sciences : a publication of the Canadian Society for Pharmaceutical Sciences, Societe canadienne des sciences pharmaceutiques* **2003,** 6 (2). PMID: 12935440

113. Möckel, J. E.; Lippold, B. C. Zero-Order Drug Release from Hydrocolloid Matrices. *Pharmaceutical Research* **1993,** 10 (7), 1066-1070 DOI: doi:10.1023/A:1018931210396.
114. Dutta, D.; Chakraborty, A.; Mukherjee, B.; Gupta, S. Aptamer-Conjugated Apigenin Nanoparticles To Target Colorectal Carcinoma: A Promising Safe Alternative of Colorectal Cancer Chemotherapy. *ACS Applied Bio Materials* **2018,** 1 (5), 1538-1556 DOI: 10.1021/acsabm.8b00441.
115. Dutta, D.; Paul, B.; Mukherjee, B.; Mondal, L.; Sen, S.; Chowdhury, C.; Debnath, M. C. Nanoencapsulated betulinic acid analogue distinctively improves colorectal carcinoma in vitro and in vivo. *Scientific Reports* **2019,** 9 (1), 11506 DOI: 10.1038/s41598-019-47743-y.
116. Kokkinos, J.; Sharbeen, G.; Haghighi, K. S.; Ignacio, R. M. C.; Kopecky, C.; Gonzales-Aloy, E.; Youkhana, J.; Timpson, P.; Pereira, B. A.; Ritchie, S.; Pandzic, E.; Boyer, C.; Davis, T. P.; Butler, L. M.; Goldstein, D.; McCarroll, J. A.; Phillips, P. A. Ex vivo culture of intact human patient derived pancreatic tumour tissue. *Scientific Reports* **2021,** 11 (1), DOI: 10.1038/s41598-021-81299-0.
117. Shumigin, D.; Tarasova, E.; Krumme, A.; Meier, P. Rheological and Mechanical Properties of Poly(lactic) Acid/Cellulose and LDPE/Cellulose Composites. *Materials Science* **1970,** 17 (1), 32-37 DOI: 10.5755/j01.ms.17.1.245.
118. Cohen, C.; Tanny, G. B.; Prager, S. Diffusion-controlled formation of porous structures in ternary polymer systems. *Journal of Polymer Science: Polymer Physics Edition* **1979,** 17 (3), 477-489 DOI: 10.1002/pol.1979.180170312.
119. Paul, D. R. Diffusion during the coagulation step of wet-spinning. *Journal of Applied Polymer Science* **1968,** 12 (3), 383-402 DOI: 10.1002/app.1968.070120301.
120. Marino, T.; Russo, F.; Figoli, A. The Formation of Polyvinylidene Fluoride Membranes with Tailored Properties via Vapour/Non-Solvent Induced Phase Separation. *Membranes* **2018,** 8 (3), 71 DOI: 10.3390/membranes8030071.
121. Furukawa, T.; Sato, H.; Murakami, R.; Zhang, J.; Duan, Y.-X.; Noda, I.; Ochiai, S.; Ozaki, Y. Structure, Dispersibility, and Crystallinity of Poly(hydroxybutyrate)/Poly(l-lactic acid) Blends Studied by FT-IR Microspectroscopy and Differential Scanning Calorimetry. *Macromolecules* **2005,** 38 (15), 6445-6454 DOI: 10.1021/ma0504668.
122. Zhou, L.; He, H.; Li, M.-C.; Huang, S.; Mei, C.; Wu, Q. Enhancing mechanical properties of poly(lactic acid) through its in-situ crosslinking with maleic anhydride-modified cellulose nanocrystals from cottonseed hulls. *Industrial Crops and Products* **2018,** 112, 449-459 DOI: 10.1016/j.indcrop.2017.12.044.
123. Chen, W.; Yu, H.; Liu, Y.; Chen, P.; Zhang, M.; Hai, Y. Individualization of cellulose nanofibers from wood using high-intensity ultrasonication combined with chemical pretreatments. *Carbohydrate Polymers* **2011,** 83 (4), 1804-1811 DOI: 10.1016/j.carbpol.2010.10.040.
124. Sung, S. H.; Chang, Y.; Han, J. Development of polylactic acid nanocomposite films reinforced with cellulose nanocrystals derived from coffee silverskin. *Carbohydrate Polymers* **2017,** 169, 495-503 DOI: 10.1016/j.carbpol.2017.04.037.
125. Sanchez-Garcia, M. D.; Lagaron, J. M. On the use of plant cellulose nanowhiskers to enhance the barrier properties of polylactic acid. *Cellulose* **2010,** 17 (5), 987-1004 DOI: doi:10.1007/s10570-010-9430-x.
126. Fortunati, E.; Luzi, F.; Puglia, D.; Dominici, F.; Santulli, C.; Kenny, J. M.; Torre, L. Investigation of thermo-mechanical, chemical and degradative properties of PLA-limonene films reinforced with cellulose nanocrystals extracted from Phormium tenax leaves. *European Polymer Journal* **2014,** 56, 77-91 DOI: 10.1016/j.eurpolymj.2014.03.030.
127. Sanchez-Garcia, M. D.; Gimenez, E.; Lagaron, J. M. Morphology and barrier properties of solvent cast composites of thermoplastic biopolymers and purified cellulose fibers. *Carbohydrate Polymers* **2008,** 71 (2), 235-244 DOI: 10.1016/j.carbpol.2007.05.041.
128. Ten, E.; Turtle, J.; Bahr, D.; Jiang, L.; Wolcott, M. Thermal and mechanical properties of poly(3-hydroxybutyrate-co-3-hydroxyvalerate)/cellulose nanowhiskers composites. *Polymer* **2010,** 51 (12), 2652-2660 DOI: 10.1016/j.polymer.2010.04.007.

129. Jiang, L.; Wolcott, M. P.; Zhang, J. Study of Biodegradable Polylactide/Poly(butylene adipate-co-terephthalate) Blends. *Biomacromolecules* **2006,** 7 (1), 199-207 DOI: 10.1021/bm050581q.
130. Fortunati, E.; Luzi, F.; Puglia, D.; Petrucci, R.; Kenny, J. M.; Torre, L. Processing of PLA nanocomposites with cellulose nanocrystals extracted from Posidonia oceanica waste: Innovative reuse of coastal plant. *Industrial Crops and Products* **2015,** 67, 439-447 DOI: 10.1016/j.indcrop.2015.01.075.
131. Zhang, X.; Qin, L.; Su, J.; Sun, Y.; Zhang, L.; Li, J.; Beck-Broichsitter, M.; Muenster, U.; Chen, L.; Mao, S. Engineering large porous microparticles with tailored porosity and sustained drug release behavior for inhalation. *European Journal of Pharmaceutics and Biopharmaceutics* **2020,** 155, 139-146 DOI: 10.1016/j.ejpb.2020.08.021.
132. Klose, D.; Siepmann, F.; Elkharraz, K.; Krenzlin, S.; Siepmann, J. How porosity and size affect the drug release mechanisms from PLGA-based microparticles. *International Journal of Pharmaceutics* **2006,** 314 (2), 198-206 DOI: 10.1016/j.ijpharm.2005.07.031.
133. Wang, Y.; Xu, Z. Interaction mechanism of doxorubicin and SWCNT: protonation and diameter effects on drug loading and releasing. *RSC Advances* **2016,** 6 (1), 314-322 DOI: 10.1039/c5ra20866a.
134. Russell, L. M.; Hultz, M.; Searson, P. C. Leakage kinetics of the liposomal chemotherapeutic agent Doxil: The role of dissolution, protonation, and passive transport, and implications for mechanism of action. *Journal of Controlled Release* **2018,** 269, 171-176 DOI: 10.1016/j.jconrel.2017.11.007.
135. Ooi, S. Y.; Ahmad, I.; Amin, M. C. I. M. Cellulose nanocrystals extracted from rice husks as a reinforcing material in gelatin hydrogels for use in controlled drug delivery systems. *Industrial Crops and Products* **2016,** 93, 227-234 DOI: 10.1016/j.indcrop.2015.11.082.
136. Hossain, M. A.; Alam, S.; Paul, P. Development and evaluation of sustained release matrix tablets of Indapamide using methocel K15M CR. *Journal of Applied Pharmaceutical Science* **2013,** 3, 85-90 DOI: 10.7324/JAPS.2013.3516.
137. Quadir, M. A.; Morton, S. W.; Mensah, L. B.; Shopsowitz, K.; Dobbelaar, J.; Effenberger, N.; Hammond, P. T. Ligand-decorated click polypeptide derived nanoparticles for targeted drug delivery applications. *Nanomedicine: Nanotechnology, Biology and Medicine* **2017,** 13 (5), 1797-1808 DOI: 10.1016/j.nano.2017.02.010.