Design of tailored response biomaterials for controlled release of bio-reagents in diagnostic devices

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**DESIGN OF TAILORED RESPONSE BIOMATERIALS FOR
CONTROLLED RELEASE OF BIO-REAGENTS IN
DIAGNOSTIC DEVICES**

CHENG TING

SCHOOL OF MATERIALS SCIENCE AND ENGINEERING

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SCHOOL OF MATERIALS SCIENCE AND ENGINEERING

A thesis submitted to the Nanyang Technological University
in partial fulfilment of the requirement for the degree of
Doctor of Philosophy

2017

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research and has not been submitted for a higher degree to any other University or Institution.

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27 Jul 2016

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Cheng Ting

Abstract

Chemical release kinetics from polymeric matrix can be modified by changing physical or chemical properties of polymers. However, it is pre-determined with the fabrication of the drug delivery system and cannot be modified further once launched. Herein polymer based release systems with tunable release kinetics adjusted via pulse width modified optical stimulation have been developed and satisfied following conditions :1) tunable release kinetics; 2) on demand release and 3) sustained controllable period.

Polyesters (poly (DL-lactide-co-glycolide)/PLGA, poly lactic acid/PLA) were fabricated into thin films ($<50\text{ }\mu\text{m}$) with additives of photocatalytic nanoparticles, LiYF₄ upconversion nanoparticles (UCNP), or combination thereof and irradiated with either ultraviolet (UV) light (365 nm) light emitting diodes or near infrared (NIR) light (980 nm) laser diodes at varied duty cycles. Photocatalytic nanoparticles can generate free radicals under UV light irradiation. UCNP is feasible of converting NIR radiation to UV radiation. When compared to non-irradiated polyester (PLGA) thin film without photocatalytic nanoparticles, UV ($2.6\pm0.3\text{ mW/cm}^2$) irradiated polyester thin film with photocatalytic nanoparticles showed 10 times higher release kinetics and NIR ($42\pm3\text{ mW/cm}^2$) irradiated polyester thin film with both photoadditives had enhanced release kinetics up to 30 times for an extended release period of 28 days. Tunable release kinetics had been achieved via adjusting duty cycle applied. UV/NIR induced photocatalysis reaction had modified release kinetics.

Polyester matrix had been changed to PLA and irradiated with $110\pm3\text{ mW/cm}^2$ NIR irradiation. Polyester films displayed a 500 times increase in drug release from the NIR irradiated polyester/photoadditive film compared to non-irradiated polyester films for 17 days with photocatalysis as primary degradation mechanism. Release kinetics had

reduced to 1/20 upon removal of NIR irradiation afterwards for the subsequent 14 days. On-demand release over extend period of 31 days had been achieved.

In-situ UV irradiation had been adjusted by control loading of UCNP. However, release kinetics in synergistic system with UCNP and photocatalytic nanoparticles didn't correlate with In-situ UV generated. Transmission electron microscopy images showed coaggregation of neat photocatalytic nanoparticle and UCNP in polyester thin film. UV light were speculated to transit from UCNP to photocatalytic nanoparticle via two energy transfer mechanisms, Förster resonance energy transfer mechanism (FRET) and reabsorption, simultaneously. Colloidal photocatalytic nanoparticle was mixed with UCNP to isolate reabsorption from FRET as nanoparticles disperse homogeneously in polyester matrix. Result suggested FRET benefits photocatalysis efficiency. Meanwhile, colloidal photocatalytic nanoparticle can be used to optimize formulation.

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Abbreviations

DC	Duty-cycles
EDX	Energy Dispersive X-Ray Analysis
FDAC	Fluorescein Diacetate
FRET	Förster Resonance Energy Transfer
LED	Light Emitting Diodes
NIR	Near Infrared
ONB	<i>O</i> -Nitrobenzyl
PCB	Printed Circuit Board
PLA	Poly Lactic Acid
PLGA	Poly (DL-Lactide-co-Glycolide)
PTX	Paclitaxel
PWM	Pulse Width Modulation
ROS	Reactive Oxygen Species
TEM	Transmission Electron Microscopy
TPA	Two Photon Absorption
SEC	Size Exclusion Chromatography
SEM	Scanning Electron Microscopy
UCNP	Upconversion Nanoparticles
UV	Ultraviolet

Chapter 1

Introduction

Release profile of polyester matrix is fixed upon fabrication and cannot be further modified. Motivated by lack of dynamic release kinetics over extended periods, herein we designed matrices depot encapsulations upon optical stimulation. Polyester matrices have been produced with photocatalytic nanoparticles and upconversion nanoparticles, irradiated by ultraviolet light (365 nm) and near infrared light (980 nm). It is hypothesized photocatalysis reaction under light irradiation will degrade polyester carrier, leading to chemical depot. We target to build up a platform using polyester thin films as carrier whose release kinetics is tunable by optical stimulation. This chapter covers the motivation, hypothesis and project objectives. It briefly overviews each chapter and scientific contributions.

1.1 Hypothesis/Problem Statement

Polymers, either natural or synthetic, due to the versatility in chemical or physical properties, have been exploited in the field of biomedical, cosmetic, pharmaceutical or food industries [1,2]. Emerging demand for controlled release highlights the importance of polymers as they have been widely utilized as vehicles for delayed chemical release. Pharmaceutical drugs, antibiotics, vaccines, DNA, gas molecules etc. have been encapsulated into polymers and released out in a controlled manner for clinical, agriculture, or environmental usage [3-5].

Chemical release kinetics from polymeric matrix can be modified by changing physical (i.e. films or particles) or chemical properties (hydrophobicity). However, release profile is fixed upon fabrication and cannot be further modified. The lack of dynamic release kinetics over prolonged periods is the motivation for this project. We have designed matrices that allow dynamic chemical release upon light stimulation with tunable release kinetics. Once light stimulation is removed, the release kinetics should be reduced as well. The chemical release system proposed herein is made possible by encapsulating hydrophobic chemicals in polyester matrix that is fabricated with photocatalyst nanoparticles (photoadditives) which will produce free radicals under photon stimulation. To broaden the application, upconversion nanoparticles (UCNP) that can convert photons with lower energy (longer wavelength) to those with higher energy (shorter wavelength) are employed.

The free radicals generated from light stimulated photocatalyst nanoparticles will lead to degradation of polyester carrier, hence releasing encapsulated chemicals into the surrounding medium. Figure 1.1 and 1.2 schematically illustrates the hypothesis with ZnO as the photocatalytic nanoparticle. The release kinetics is controlled based on the hypothesis that stimulation strength (irradiation dose) and photocatalysis of the polyester matrix will force the release of encapsulated chemical [6].

Figure 1.1 Hypothesis: ZnO-mediated photocatalysis generates free radicals under UV irradiation, leading to polymer degradation

Figure 1.2 Hypothesis: ZnO-mediated photocatalysis generates free radicals under in situ UV from UCNP under NIR light, leading to polymer degradation

The following hypotheses are proposed under this project.

Covered in Chapter 4,

Hypothesis #1: Chemical release from polyester thin films can be accelerated under UV light with additives of photocatalytic nanoparticles.

Covered in Chapter 5,

Hypothesis #1: The release kinetics within polyester thin films with additives of ZnO nanoparticles is tunable through static light intensity through pulse width modification.

Hypothesis #2: Chemical release from polyester thin films can be accelerated under NIR light with additives of ZnO and UCNP.

Covered in Chapter 6,

Hypothesis #1 Dynamic chemical release is achieved through *in-situ* UV irradiation via NIR irradiated UCNPs.

Hypothesis #2 Photocatalytic ZnO scissors nearby polyester into soluble oligomers rather than degrade whole matrices evenly under irradiation.

Hypothesis #3 Chemical release can be reduced with removal of NIR light after irradiation if photocatalysis is the primary mechanism of degradation.

Hypothesis #4 *In-situ* UV irradiation transfers from UCNPs to neat ZnO in polyester thin film via two mechanisms simultaneously: Förster resonance energy transfer (FRET) and reabsorption. Former one leads to high efficiency in synergistic photocatalysis though majority of nanoparticles transfer UV via latter one.

Hypothesis #5 More photocatalysis surface of colloidal ZnO should benefit photocatalysis though reabsorption is the only energy transfer mechanism.

Covered in Chapter 7,

Hypothesis #1: Chemical release from polysaccharide/polyanhydride thin films can be tuned under UV/NIR light with additives of photoactive nanoparticles.

The design and hypothesis are proposed based on the following suppositions and observations:

1. The degradation (rate) of polyester macromolecules determine the chemical release from carrier.

Taking poly (lactic-co-glycolic acid) (PLGA) as an example, the dynamic delivery process caused by molecular weight change during the delivery process showed how the degradation/molecular weight can influence the chemical delivery process. Additives that can catalyze the degradation was also proved to be able to change the release profile [7,8]. It can be deduced that the polymer degradation could generally accelerate the chemical release from carrier. Once oligomer fragments become soluble, they then allow release of chemicals.

2. The free radicals can cleave the polyester matrix.

Free radical degradation mechanisms has been observed to cleave C-C covalent bonds with subsequent polymer degradation [9]. The high reactivity of alkyl and superoxide free radicals induce beta-scission and C-C cleavage on polymer matrices.

3. Photocatalyst nanoparticles are able to generate free radicals upon UV light.

Highly oxidative superoxide free radicals are generated (among other radical species) from photocatalyst surfaces of TiO₂ and ZnO under UV light. It has also been reported that the light intensity directly affects the reaction rate [10], hence can be used to modulate release kinetics.

4. NIR light is has less tissue absorbance than UV light.

UV light has poor penetration into tissues (to millimeter) compared with NIR light (to centimeter) hence the biomedical application will be limited to surfaces under UV light. Ionizing UV light can damage DNA and protein macromolecules, thus stimulation sources should employ non-ionizing NIR light as the triggering source.

5. Upconversion nanoparticles allow collimated NIR light for *in situ* UV light generators.

Upconversion nanoparticles have been reported to be able to emit photons with shorter wavelength (including UV emission overlapping photocatalyst excitation wavelengths) under the excitation of photons with longer wavelength (980 nm or 800 nm). The capability for those nanoparticles to convert NIR light to UV light introduces the possibility of tuning the photocatalyst nanoparticles to generate free radicals under NIR light with the presence of upconversion nanoparticles, limiting UV exposure to the polyester matrix, with little to no intensity transmitted to the tissue biomacromolecules.

1.2 Objectives and Scope

We target to build up a platform using polyester thin films as carriers whose release kinetics are tunable by optical stimulation. Photocatalytic nanoparticles will degrade covalent

bonds by generating free radicals upon exposure to ionizing UV irradiation. To broaden the application, lanthanide based upconversion nanoparticle that can emit UV light is incorporated. As shown in Figure 1.3, K1, K2 and K3 represents diverse chemical release kinetics dependent upon optical stimulation. More specifically,

- Hydrophobic small molecule (fluorescein diacetate, FDAC) that encapsulated in polyester films would be released out at controllable kinetics.
- The release kinetics is controlled by the degradation of the polymeric carrier via photocatalysis reaction under UV light
- The kinetics could be tunable by light stimulation, including the duty cycle of the light source, out-put power of the light source, wavelength of the light source. The ideal extendable release kinetics should be varied from fractional percentage per day (sustained release) to total release on-demand.

Figure 1.3 Controllable release kinetics by out-source stimulation

The scopes for this project include

1. The release behavior of polyester thin films with photocatalytic nanoparticles (ZnO , TiO_2 , Fe_2O_3 , ZnFe_2O_4) and films without any nanoparticles either exposed to UV light (365 nm) or protected from it will be compared to support the hypothesis that photocatalytic nanoparticles will lead to chemical release under photon stimulation.

The most effective nanoparticle under the given conditions (ZnO) would be chosen for as the prime photoadditive.

2. The release behavior of polyester thin films with/without ZnO under UV light of varied duty-cycles (related to optical dose) will be compared to check the dependence of pulse width modulation vs. release kinetics.
3. The release behavior of polyester thin films of neat, with ZnO nanoparticles, with upconversion nanoparticles and combination thereof, will be assessed to identify the primary release mechanisms.
4. The release behavior of polyester thin films of neat, with ZnO nanoparticles, with upconversion nanoparticles and combination thereof, will be compared after exposure to NIR light to assess presence of autocatalysis within polyester matrix.
5. To compare our photoadditive strategies against current state of the art polymeric release strategies.

1.3 Dissertation Overview

This thesis compromises of eight chapters.

Chapter 1 provides rationale and hypothesis for the research. It also outlines the goals and scope for the project.

Chapter 2 describes and compares the project relevant developments of controlled release and justifies near infrared (NIR) light based stimulated release. Background information of photocatalytic reaction and upconversion relates to release mechanism is given.

Chapter 3 discusses the strategy and rationale for materials and methods selection in the dissertation. Design and set up of high throughput release study relevant for chapter 4, 5, 6 and 7 with light stimulation varied by pulse width modulation (PWM) is illustrated. The strategy used to analyze the release kinetics pathway to support/nullify hypotheses is described.

Chapter 4 elaborates a screening of different photocatalytic nanoparticles' effect on chemical release behavior from polyester under UV irradiation. The most efficient biocompatible nanoparticle was selected for subsequent analyses.

Chapter 5 demonstrates for the first time chemical release from a polyester thin film can be modulated under UV light either with or without inclusion of photocatalytic nanoparticles via pulse width modulation. It also illustrates how the chemical release from polyester film can be tuned under NIR light with inclusion of both photocatalytic and upconversion nanoparticle.

Chapter 6 illustrates release kinetics independent of autocatalysis employing a more labile-resistant polyester matrix. It tests dependence of release kinetics nanoparticle loading and light intensity. It compares efficiency of FRET and reabsorption (between photocatalytic and upconversion nanoparticle) in accelerating release via synergistic photocatalysis.

Chapter 7 extends the research to polysaccharide and polyanhydride matrices.

Chapter 8 concludes and summaries the major findings within this project, suggests future work that extends and further supports the findings herein.

1.4 Findings and Outcomes/Originality

This research led to several novel outcomes by:

1. Demonstrated for the first time photocatalytic reaction can be incorporated into polyester based chemical release.
2. Demonstrated for the first time upconversion and photocatalytic reaction can be linked for thin film polyester matrix based chemical release.
3. Employed duty cycle as a method to tune release kinetics allows control of both chemical release kinetics, and the dominant degradation mechanism

4. Developed polyester based sustained release systems whose release kinetics can be tuned under either UV light or NIR light, dose dependently, with same formulation.
5. Developed polyester based sustained release systems whose release kinetics can be reduced with removal of light stimulation, after more than two weeks of irradiation.

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Chapter 2

Literature Review

This chapter reviews the concept and requirement for controlled release. Briefly introduces polyesters release systems that can tune the release kinetics over sustained period and describes current development of release triggered by stimulation. Development of NIR light stimulated release system is justified. Background information about mechanisms of photocatalytic reaction and upconversion process relating to proposed release system is provided for the reader's convenience. Current literature shows gap in controlled/tunable/on demand release over sustained periods (from week to months). Our strategy incorporates sustained release (polyester matrices) with tunable/on demand release (light stimulated release).

2.1 Background — Controlled release

Controlled release refers to the relationship between amount of chemical compounds delivered from carrier upon stimuli or time; this technique could be applied in various fields, including agriculture, cosmetics, water treatment, sensors, and medical implants. Conventional dosing methods in medicine (injection, oral administration etc.) tend to spike drug concentrations followed by complex clearance rates. Maintaining specific concentration requires multiple administrations to maintain the plasma drug levels. However, the spikes and systemic exposure raises the possibility of side effects. Localized, controlled release systems attempt to improve efficacy, reduce systemic toxicity and maintain the plasma drug levels within the therapeutic index for extended periods [1-4]. Previously developed biocompatible polymers can be selected as carriers, including natural polymers (e.g. polysaccharides alginate, collagen, silk) to synthetic polymers such as polyester (e.g. PLGA, PLA) and polyanhydride [5].

2.2 Polyester based chemical release systems

Aforementioned polyesters, especially poly (D,L-lactic-co-glycolic acid) (PLGA) and poly (lactic-acid) (PLA) , can be considered as in the gold standard for (sustained) controlled release[6,7]. Polyester has attractive attributes like being tissue resorbable, biocompatible, commercially available and considered GRAS (generally recognized as safe) by the US Food and Drug Administration. They have been applied in implants [8,9], bone defect healing [10], and tissue engineering [11,12]. With respect to drug release, hydrophilic macromolecules [13,14], hydrophobic small molecules [15,16], and even hydrophilic molecules [17] have demonstrated successful release from polyesters.

Degradation of polyesters is dependent upon molecular weight, hydrophilicity, autocatalysis, terminal group, degree of crystallinity, size and geometry of the polymer matrix, composition in a copolymer system and environment as temperature or pH [18-26]. Correspondingly, chemical release kinetics from polyesters have been modulated by modifying the degradation of the matrix via changing the molecular weight of the matrix

[27], including hydrophilic additive into the system [28] or alternating the terminal end-groups [29] etc. Release profile from polyester carriers is determined by the formulation of the carrier and can be varied and predicted systematically. Table 2.1 lists chemical release kinetics from a range of polyester thin films (<200µm) with different formulations in literature. Hydrophilic compounds (e.g. ibuprofen) tend to release out from polyester matrix at higher release kinetics due to low solubility in hydrophobic polyester matrix. As a result, release kinetics can only be modulated in a small range. In comparison, hydrophobic molecules (e.g. paclitaxel) are well dissolved in polyesters. Release kinetics are correlated with polyester degradation and can be modified by changing the physical or chemical properties of matrix. As degradation takes place, polyesters will become soluble and release hydrophobic drugs when degraded to oligomeric fragments under 10 kDa. Therefore, a method that would induce scission to under 10 kDa would allow drug release.

Table 2.1 A comparison of mass release kinetics from static polyester thin films (<200µm).

	Polyester Matrix:	Molecular Weight (Mw):	Release Additives:	Chemical Released:	Release kinetics (µg.cm⁻².d⁻¹):	Ref.
(1)	PLGA M	110kDa	NA	PTX	0.33	[28]
(2)	PLGA M	110kDa	PEG 400Da 20%	PTX	1.36	[28]
(3)	PLGA M	110kDa	PEG 400Da 30%	PTX	1.98	[28]
(4)	PLGA M	110kDa	PEG 10KDa 20%	PTX	3.57	[28]
(5)	PLGA M	110kDa	PEG 10KDa 30%	PTX	9.33	[28]
(6)	PLGA M	110kDa	NA	FDAC	0.83	[29]
(7)	PLGA M	20kDa	NA	FDAC	1	[29]
(8)	PLGA A	40kDa	NA	FDAC	6.43	[29]
(9)	PLGA A	15kDa	NA	FDAC	6.25	[29]
(10)	PLGA M/A	110kDa/15kDa	NA	FDAC	2.68	[29]
(11)	PLGA M/A	110kDa/40kDa	NA	FDAC	1.6	[29]
(12)	PLA/EVA	121.5kDa	NA	PTX	14.86	[30]
(13)	PLGA	56kDa	NA	Ibuprofen	160	[31]
(14)	PLGA porous	56kDa	NA	Ibuprofen	160	[31]
(15)	PLGA	56kDa	PEG 3.3KDa 10%	Ibuprofen	178	[31]
(16)	PLGA porous	56kDa	PEG 3.3KDa 10%	Ibuprofen	178	[31]
EVA, polyethylene vinyl acetate; PLGA, poly (lactic-co-glycolic acid) with lactide/glycolide ratio 50/50; PLA, poly-L-lactic acid; PLGA M, PLGA with methyl ester end group; PLGA A, PLGA with carboxylic acid end group; PTX, paclitaxel; FDAC, fluorescein diacetate; NA, no additives						

2.3 Stimuli-responsive release systems

Stimuli-responsive release systems, have been developed to provide dynamic release kinetics under specific circumstances or stimuli. Drug encapsulated hydrogels, mesoporous silica, liposomes, polymersomes, polymer matrices [32-38] have been fabricated into various geometries e.g. films, particles, capsules. Upon an external stimulus, the physical and/or chemical properties of the carrier will undergo a shift that allows encapsulated compounds to be released into the local environment. Stimuli responsive systems can be classified into closed-loop and open-loop systems [39]. Closed-loop release systems are self-regulated in which the kinetics are adjusted by the system as response to stimuli without external intervention, such as pH, ionic strength, glucose etc. [37,40,41]. For example, healthy tissues can maintain the pH value around 7.4 while tumor tissues exhibit lower pH. Polymeric micelles, having a release rate 30 times as fast [40], takes advantage of the lower pH and enhanced permeability of tumor cells [42]. This increases the drug efficacy and reduces systemic side effects. In contrast, open-loop release systems are independent of the local environment and rely on external stimuli, such as magnetic fields, ultrasound, temperature and light irradiation [36,43-49]. Closed-loop systems are limited in providing accurate dosage while open-loop systems can overcome the problem.

2.4 Light-responsive chemical release

Light stimulated release has been emerging as an attractive topic as it can remotely trigger the release system with high spatial and temporal precision. Another advantage of light stimuli release system is the user friendly, portable, economical affordable stimulation source: it can simply be a laser diode. In light stimulated chemical release, a photoactive component is incorporated which forces a physical or chemical shift is linked to chemical release when exposed to light of specific wavelengths [50].

2.4.1 UV light stimulated chemical release

Photon-triggered release can be categorized into several groups according to their photoactive mechanisms, including photoisomerization, hydrophobicity transition, photocleavage, and photothermal conversion. Majority of the photoreactions rely on UV light activation, due to its potential to overcome the activation energies required for various photoreactions, such as photoisomerization, beta-scissions, and photocatalysis.

Photoisomerization is a process in which the molecules experience conformational transition under light irradiation. For example, azobenzenes' configuration can be transitioned from *trans* to *cis* under UV light (300-380 nm) and reverted from *cis* to *trans* configuration under visible light (>400 nm) [51]. *Trans* form of azobenzenes moieties are more hydrophobic compared with *cis* form. Azobenzenes molecules have been grafted on mesoporous silicon nanoparticle (MSN) in *trans* form, acting as "caps". On-demand release of encapsulations from MSN has been achieved under UV/Vis light irradiation when "caps" open/closed as azobenzenes configuration was converted between *trans/cis* [47,52].

O-nitrobenzyl (ONB) and coumarin derivatives [35,53,54] are representative photocleavable linkers that have been utilized towards light triggered release. *O*-nitrobenzyl photolabile groups are ester moieties known to cleave into acetal and acid groups under UV light (365nm) exposure [55-57]. The ONB derivatives can be utilized as a linker that connects the carrier and prodrug. Light irradiation subsequently cleaves the linkage, forming a depot of the chemical [58]. Or it can be synthesized as part of hydrophobic polymer carrier that under UV irradiation the exposure of acid group will convert the hydrophilicity of the polymer matrix hence the chemical delivery process [59,60]. Taking advantage of the fact that coumarin derivatives can be dimerized into cyclobutane under UV irradiation > 310nm and cyclobutane undergoes photocleavage when exposed to UV light of shorter wavelength (<280 nm) [45], prodrugs have been directly attached to coumarin functionalized polymer backbone under UV light and released under light of shorter wavelength [61]. Researchers have developed on-demand release system based on reversible reaction of coumarin families as well [54]. Light triggered release systems taking advantage of photon responsive functional usually involve

synthesis of chemical carrier or prodrug with certain functional groups, limiting the range of application. Dynamic release kinetics or on demand release is achievable by changing the irradiation dose. However, most developed release system will depot all encapsulations within 24 hours' irradiation.

Photocatalytic materials such as TiO_2 have also been applied in chemical release. Low molecular weight organic materials are easily broken up by photocatalysis products leading to release of encapsulated chemicals [62,63]. Photocatalysis can be applied as a more general mechanism in chemical release compared with photoisomerization and photocleavage, which rely on specific chemical functional groups that cannot be applied across most polymeric matrices. The detailed mechanism for photocatalysis will be reviewed in section 2.7.

Table 2.2 shows some of UV/Vis stimulated release system of above mentioned mechanisms. We compare release from following aspects: tunable kinetics, on demand release, and controlled time scale. For the purpose of comparison, we define on-demand release as less than 1/20 (5%) release kinetics with removal of stimulation. None of those systems can combine properties dynamic release kinetics, on demand release and sustained release (in period of weeks to months).

Table 2.2 Comparison of UV stimulated drug release systems.

	Released chemicals	Photosensitive formulation	Matrix	Tunable kinetics	On demand release	Controlled time scale	Ref.
(1)	Rh6G	Azobenzene	MSN		√ ‡	10 hrs	[52]
(2)	siRNA	<i>O</i> -nitrobenzyl	PEG-DPA	√		15 days	[58]
(3)	5-FU	coumarin	PEO-b-P(BMA-co-CMA)			6 hrs	[61]
(4)	PTX	TiO_2	MTNP			3 hrs	[64]
(5)	PTX	TiO_2	FA-PEI-PTX-MTNP	√		5 days	[64]

Rh6G: rhodamine 6G; 5-FU: 5-Fluorouracil; PTX: paclitaxel; MSN: mesoporous; PEG-DPA: poly (ethylene glycol)-di (photolabile acrylate); PEO-b-P (BMA- co-CMA): poly (ethylene oxide)-b-poly (n-butyl methacrylate-co-4-methyl-[7-(methacryloyl) oxyethyloxy] coumarin); MTNP: modified porous TiO_2 nanoparticles; FA-PEI-PTX-MTNP: folate coated hybrid polyethylenimine modified porous TiO_2 nanoparticles; polyethylenimine ‡: with both UV and visible light; mins: minutes; hrs: hours; ref.: reference;

2.4.2 NIR light stimulated chemical release

Towards triggering chemical release within bulk phases (i.e. treatment tanks, subcutaneous implants) UV light inherent properties limit its application to anything but surfaces. UV light has limited penetration depth (up to microns, $<650\text{nm}$, penetration depth $<1\text{cm}$) over most organic compounds, because it is strongly absorbed by any compounds with pi-conjugated chemistries.[51,65] In contrast, NIR light would be better choice for penetration into dense bulk phases such as mixed aqueous solutions and tissues. Tissues are known to have an ‘optical window’ in the 800-1200nm region as biomolecules are relatively “transparent” in this range, but they still allow light scattering. Despite this scattering, NIR light can penetrate up to few centimeters depending on composition and light intensity [66,67]. NIR light is also known to be more bioinert towards tissues and cells compared with UV light.[68] For these reasons, it has been applied to chemical release via various mechanisms: photothermal conversion of NIR light, multi-photon absorption of NIR light in photoactive moieties, and upconversion of NIR light to UV light.

Gold nanostructures, including nanoshell nanoparticle, hollow structure, nanorods etc., show plasmon resonant effect and convert light into heat efficiently, aka photothermal conversion.[69-71] Associated with different size and nanostructures, Au shows resonant peaks at different wavelengths range from Vis light (e.g. 514nm) to NIR light (e.g. 730nm, 912nm). Au nanostructures can be incorporated as thermal sensitive parts in liposomes, polyelectrolyte lipids and polymers like PLGA and poly(N-isopropylacrylamide) as part of their release system. On-demand release of these encapsulations can be controlled under light irradiation with local raised temperatures from the photothermal effect [72-78].

NIR light also can induce photothermal effect without inclusion of Au nanostructures in the release system. Absorption coefficient of water molecule shows lowest in the NIR light of 650nm-900nm wavelengths while increases after 900nm and shows peaks at 980nm, 1200nm, 1450nm and 1950nm [68,79], owing to the vibrational movement of water molecules. Taking advantage of heat induced by water molecules’ resonance effect, water droplets have been confined into PLGA nanoparticles and on-demand release encapsulations from it under NIR light of 980nm [80].

UV light stimulated processes can be initiated NIR light as well. Two photon absorption (TPA) and upconversion are observed to reach the required activation energy [35,81,82]. ONB derivatives and coumarin derivatives can simultaneously absorb two low NIR photons via virtual energy states and undergo UV photochemical reactions [83-86]. However, the TPA process requires femtosecond pulsed laser with photon density from several to several hundred W/cm^2 to take place and is less efficient than UV. For example, 3.5 hours NIR irradiation displayed similar chemical release as 75 seconds UV irradiation of same intensity ($500 \text{ W}/\text{cm}^2$) [87]. In comparison, upconversion process with lanthanide-doped nanomaterials reduces the power requirement for the reaction to take place via multiple metastable energy levels. As a result, upconversion process can be accomplished under continuous wave laser diode with relatively low photon intensity up to several hundred mW/cm^2 [88,89], showing efficiency with several orders of magnitude compared with TPA. Table 2.3 shows some of NIR stimulated release system (without UCNP). None of those systems can combine properties dynamic release kinetics, on demand release and sustained release (in period of weeks to months).

Table 2.3 Comparison of NIR stimulated drug release systems.

	Released chemicals	Photosensitive formulation	Matrix	Tunable kinetics	On demand release	Controlled time scale	Ref.
(1)	Nile red	H_2O	PLGA	✓	✓	90 mins	[80]
(2)	DOX	Au	Au- pNIPAAm			4 mins	[72]
(3)	FTIC	Au	Au@MSN–rotaxane		✓‡	3 hrs	[73]
(4)	PTX	coumarin	PEO ₁₁₄ -b-P(LGA _{0.62} -co-COU _{0.38}) ₃₄			5 days	[83]
(5)	Nile red	<i>O</i> -nitrobenzyl	(ONB-alt-PEB)-g-PEG			1 hr.	[86]
DOX: doxorubicin; FTIC: Fluorescein isothiocyanate; PTX: paclitaxel; Au-pNIPAAm: Au cage with poly(N-isopropylacrylamide); Au@MSN–rotaxane: azobenzene-based rotaxane coated Au nanorod–mesoporous silica core–shell hybrid; PEO ₁₁₄ -b-P(LGA _{0.62} -co-COU _{0.38}) ₃₄ : poly(ethylene oxide) and poly(L-glutamic acid) with 6-bromo-7-hydroxycoumarin-4-ylmethyl groups; (ONB-alt-PEB)-g-PEG:3-(((4-((2,5-Bis(3-hydroxypropoxy)-4-methylphenyl)ethynyl) benzoyl) oxy) methyl)-2-nitrobenzyl-4-methylbenzoate ; ‡: with both NIR and UV light; mins: minutes; hrs: hours; ref.: reference;							

Nevertheless, NIR irradiation will lead to local tissue hyperthermia [115]. Moderate hyperthermia ($41^\circ\text{C} - 46^\circ\text{C}$) is beneficial in photothermal therapy (PTT) for cancer

treatment while overheating is undesired [116]. To avoid side-effect, several approaches can be used. Standards provided by authorities (e.g. American National Standards Institute) serve as safety guideline for irradiating human with NIR (e.g. 0.72 W/cm^2 for 980 nm or 0.42 W/cm^2 for 1064 nm for skin exposure). Pulsed laser has often been employed to keep the average power low [117]. Reducing output density of irradiation can decrease elevated temperature in tissue as well [118]

2.5 Upconversion nanoparticle

Upconversion nanoparticle (UCNP) absorb photons at high wavelengths (e.g. 980 nm) and emit photons at lower wavelengths (i.e. 365 nm) through a nonlinear excitation-emission process [90,91]. More than one photon is absorbed in the excitation leading to the emission of shorter wavelength light with higher energy. Figure 2.1 illustrates this process [92].

Figure 2.1 Nonlinear optical excitation-emission of upconversion process. E_{ex} : excitation energy, E_{em} : emission energy, λ_{ex} : excitation wavelength, λ_{em} : emission wavelength.

Lanthanide doped UCNPs show multiple energy levels in the form of a ladder with equal energy gaps, as a result, single wavelength radiation can be used to promote electrons through multiple excited states, as climbing up a ladder with multiple steps, leading to the emission of shorter wavelength. UCNPs are composed of a host matrix and two trivalent lanthanide ion dopants, one as a sensitizer and the other as an activator. Sensitizers can be excited effectively by the incident light, transferring the energy to activator where radiation emission takes place. Yb^{3+} with simple energy level is usually doped as sensitizers while Er^{3+} , Tm^{3+} , and Ho^{3+} with ladder spacing energy states are most frequently doped as activators. [90] Several emission peaks can be achieved with one excitation wavelength.

For example, the UCNP applied in this dissertation, with LiYF_4 as the host matrix and doped with Yb^{3+} (sensitizer) and Tm^{3+} (activator), shows dominant emission in the region of UV light under NIR light excitation with multiple emission peaks from UV light to NIR light, as displayed in Figure 2.2 [93].

Figure 2.2 Upconversion emission spectrum of (0.5 mol %) Tm^{3+} (25 mol %) Yb^{3+} -doped LiYF_4 nanocrystals spanning the UV to NIR regions. Emission spectrum from UCNP overlaps the absorbance spectrum of ZnO nanoparticles.

2.6 Current UCNP based NIR stimulated release

With the development of UCNPs, NIR stimulated chemical release systems have been designed by linking UV/Vis reactions and UCNPs [94]. Responsive molecules include previously mentioned azobenzene derivatives, coumarin derivatives, and ONB derivatives etc.

Azobenzene molecules have been grafted on UCNP-MSN core-shell structure where UCNPs served as core and coated with MSN (UCNP-MSN@Azo). Anticancer drug doxorubicin, which can adsorb to silica via strong hydrogen bond and charge interactions with the silanol, were loaded into mesoporous structure. As mentioned above, the structure of azobenzene will change from *trans* to *cis* under UV light irradiation, and convert from *cis* to *trans* under irradiation of visible light. Upon NIR light stimulation, the UCNP will emit light in both UV and visible region simultaneously, which can trigger the reversible

photoisomerization of azobenzene molecules continuously, creating a rotation–inversion movement, propelling the release of Dox [95].

Abovementioned photocleavable linkers coumarin [96], ONB derivatives [97-99] have been applied to conjugate drug onto silica coated UCNPs or UCNPs directly, the *in-situ* generated UV light from UCNPs can cleave the linker leading the release of conjugated drugs. Table 2.4 selects some current developed drug release system with UCNP. Complex synthesis route and non-sustainable released limits the application.

Table 2.4 Comparison of NIR stimulated drug release systems with UCNPs.

	Released chemicals	Photosensitive formulation	Matrix	Tunable kinetics	On demand release	Controlled time scale	Ref.
(1)	FTIC-BSA	O-nitrobenzyl	Polyacrylamide–PEG	✓	✓	50mins	[100]
(2)	FTIC-BSA	Succinimidyl and acetylene group photolabile linker	UCNPs@SiO ₂ @GPS@CH/PhL/FTC-BSA/PEGBA	✓	✓	12 mins	[66]
(3)	β-gal	Spiropyran dye	Hollow structured UCNP	✓	✓	20mins	[101]
(4)	DOX	Stilbene	Core-shell UCNP-MSN	✓	✓	17 hrs	[95]
(5)	5-FU	O-nitrobenzyl	UCNP	✓	✓	20 mins	[102]
FTIC-BSA: Fluorescein isothiocyanate labelled bovine serum albumin; β-gal β-galactosidase; DOX: doxorubicin; 5-FU: 5-Fluorouracil; PEG: poly (ethylene glycol); GPS: (3-glycidyloxypropyl) trimethoxysilane; CH: chitosan; PhL: photosensitive formulation PEGBA: polyethylene glycol-bisazide; mins: minutes; hrs: hours; ref.: reference							

2.7 Photocatalysis

Metal oxides have been extensively applied as photocatalysts since several decades [103]. TiO₂ and ZnO photocatalysts are the most widely used among the metal oxides due to their high photocatalytic activity and are economically attractive [104]. Photocatalysis chemical reaction equations are listed Table 2.5. Photocatalytic metal oxides (e.g. ZnO, TiO₂) are semiconductors whose valence band and conduction band are separated by an energy gap. Illuminating the oxides promotes valance electrons into the conduction band. This generates a free negatively charged electron and a positively charged hole. This is referred as “electron-hole” pair generation (equation (1)). For example, ZnO and TiO₂ have an energy gap of 3.2eV, hence UV light with wavelength shorter than 378 nm can initiate

“electron-hole” pair generation. We use 365 nm UV light to initiate photocatalysis reaction. The electrons in conduction band and holes in valence band generated may either be recombined in the bulk and release heat (equation (2)) or migrate to the surface of photocatalysis for redox reactions to take place. In the redox reaction, reactive oxygen species (ROS), including superoxide oxygen free radical ($\bullet O_2^-$), hydroperoxide free radical ($\bullet HO_2$), and hydroxyl free radical ($\bullet OH$), can be generated at the surface with presence of water and oxygen. (equations (3) – (7)). Those ROS (especially hydroxyl free radical) have been reported effectively degrade organics from small dye molecules to long polymers chains [105-107].

Table 2.5 Chemical reactions in photocatalysis.

Electron-hole pair generation	$MO + h\nu \rightarrow e_{cb}^- + h_{vb}^+$	(1)
Electron-hole pair recombination	$e_{cb}^- + h_{vb}^+ \rightarrow heat$	(2)
Free-radical generation	$h_{vb}^+ + H_2O \rightarrow H^+ + \bullet OH$	(3)
	$2h_{vb}^+ + 2H_2O \rightarrow 2H^+ + H_2O_2$	(4)
	$H_2O_2 \rightarrow 2 \bullet OH$	(5)
	$e_{cb}^- + O_2 \rightarrow \bullet O_2^-$	(6)
	$\bullet O_2^- + H^+ \rightarrow \bullet HO_2$	(7)
Organic degradation	$RH + ROS \rightarrow \bullet R$	(8)

Where: MO: Metal oxide; e_{cb}^- : Free electrons in conduction band, h_{vb}^+ : Free holes in valence band, ROS: reactive oxygen species.

Herein we hypothesized the photocatalysis reaction can accelerate the drug release by decomposing the polymer carrier through oxidation. ROS generated in photocatalysis can attack the backbone of polyester and cleave them to shorter chains or oligomeric fragments [106,107], leading to the release of encapsulation. Compared with light triggered release using photon responsive functional groups, this is foreseen as a strategy with more general application without specific synthesis route. Controlled release over sustained period (several weeks) is also achievable with polyester matrices.

2.8 Hypothesized strategy vs. literature

A comparison of hypothesized strategy in this dissertation versus other's publication is displayed in Table 2.6. Compared with other's work with similar polymer carrier, stimulation source, hypothesis as illustrated throughout Table 2.1-2.3, simple fabrication method and flexible release profile for polyester thin films is unprecedented [108-114]. Tunable release refers to release kinetics from a same polymer can be adjusted via external stimulation (optical stimulation). On demand release is defined as reduction in kinetics till 5% with removal or stimulation. Sustained release means biomedical device release encapsulations over extend period from several weeks to months. Our strategy incorporates sustained release from polyester matrices with tunable and on demand release from light triggered release systems.

Table 2.6 Controlled release in this project vs. others' publication.

	Materials	Fabrication	Tunable release	On demand release	Sustained release
This project	PLGA/ PLA/ other commercialized polymers ZnO, UCNP	Physical mixture raw materials	✓	✓	✓
Table 2.1	PLGA/PLA or different properties	Physical mixture raw materials			✓
Table 2.2	Different synthetic polymers/nanostructures, light responsive group	Chemical synthesis contained certain functional group	✓	✓	
Table 2.3	Different synthetic polymers/nanostructures, light responsive group, UCNPs	Chemical synthesis contained certain functional group	✓	✓	

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Chapter 3

Experimental Methodology

This chapter covers the strategy and rationale for materials and methods selection in the dissertation. It also illustrates design and set up of high throughput release study with light stimulation varied by pulse width modulation (PWM). The scope to support or nullify the hypotheses is described in detail as well. It describes the methods to characterize polymer matrix and phase separation of nanoparticles in the film respectively.

3.1 Overview of the strategy

The chemical release system reported in the dissertation is inclusive of two nanoparticles, UCNP and ZnO. Five mechanisms may contribute to chemical release under NIR irradiation as illustrated in Figure 3.1. To resolve the distribution of each mechanism, it is necessary to prepare film neat (no photoadditives), film with either nanoparticle, and experimental formulations as displayed in Figure 3.2. Formulations can be conducted via high throughput techniques as described in section 3.2. Detailed algorithm for isolation of release kinetics is section 3.3.

Figure 3.1 Distribution of release mechanisms for a polymer film containing both UCNP and ZnO under NIR irradiation

	No light	Light stimulation
Polymer		NIR
Polymer/photocatalysis		NIR-photocatalysis
Polymer/photogenerator		NIR-photogenerator
Polymer/photogenerator /photocatalysis	Diffusion	NIR-photogenerator- photocatalysis

Figure 3.2 Formulations and groups setting up in release study

Our hypothesis is based on observation that polymeric matrix degradation correlates to the chemical release [1,2]. Polyesters were chosen for the proof of concept study. Poly (DL-lactide-co-glycolide) (PLGA 50/50), degrades from weeks to months [3,4], was purchased from Purac (Netherlands). Poly (lactide-acid) (PLA), degrades from months to years [4,5], was purchased from Dongguan Pioneer (China). Alginate and poly(maleic anhydride-alt-1-octadecene were received from FMC biopolymer (Thailand) and Chevron Phillips Chemical (Singapore) respectively. Photocatalytic ZnO powder was purchased from Sigma (Singapore). Tm^{3+} , Yb^{3+} doped LiYF_4 upconversion nanoparticle (UCNP) with high UV emission under low energy NIR was synthesized by collaborator Prof. Vetrone in Université du Québec, Canada [6]. Other nanoparticles as Fe_2O_3 and ZnFe_2O_4 were purchased from Sigma (Singapore) and TiO_2 was purchased from Fluka. Figure 3.3 summaries objective in each chapter and the corresponding experimental set-up for it.

Figure 3.3 Flow chart for each chapter

3.2 High throughput in vitro chemical release

A 96 well microplate based high throughput release study, using 6 mm diameter sample, enabling multiple formulations and stimulated conditions to be tested and compared at the

same time, was designed. Fluorescein diacetate (FDAC), purchased from Sigma-Aldrich (Singapore), was selected as the model drug [7]. Light emitting diodes (LED), with peak wavelength at 365 nm, 6 mW power output, were purchased from Roithner Lasertechnik (Austria), as the source for UV light. Laser diodes, with peak wavelength at 980 nm, 10 mW or 50 mW power output, were purchased from Thorlabs (Singapore, China) and Bob Laser (China), as the source for NIR light. Other than the economical preference, LED and laser diode show advantages in their small features (<6 mm diameter) and monochromatic emission. Multiple LEDs/laser diodes can be integrated in an electronic assembly fitting into the dimension of microplate with each row/column controlled separately, allowing high throughput release study design to be realized. Trade off of using LED /laser diode is light intensity variation from individual light source, which may affect the utilization of light energy and photocatalytic reaction rate in ZnO [8] and the quantum yield of light emission from UCNPs [6]. Intensity was normalized in the project and optical input was adjusted by pulse width modulation (PWM).

3.2.1 Preparation of neat polyester thin films

Polyester thin films were prepared by knife casting technique [9]. Polymer solution of 10 % (w/v) polymer solution was prepared with 10% (w/w) FDAC/polymer added in. For example, 800 mg PLGA, 80 mg FADc was dissolved in 8 mL DCM. Solution was stirred overnight and casted on polyethylene terephthalate (PET) sheet on glass substrate using 80 mm film casting knife applicator at 50 mm/sec. Film was dried in vacuum hood at ambient temperature for 1 day then transferred to vacuum oven at 37 °C for a week.

3.2.2 Preparation of thin films with photoadditives

Photoadditives were added into solvent, sonicated for 30 minutes before the addition of polymer and FDAC. For example, 8 mg of ZnO (1% w/w ZnO/PLGA) was added in 8 mL DCM prior adding 800 mg PLGA and 80 mg FDAC. The solution was stirred overnight, vortexed, and immediately knife casted.

Sodium alginate films were fabricated in modified method. Briefly, 1 g sodium alginate was dissolved in 50 mL of DI water (2% w/v), 50 mg FDAC and 50 mg of nanoparticles (TiO_2 , ZnO or Fe_2O_3) were added into the solution. The solution was stirred until homogenous solution. The solution was poured into glass petri-dish (12 cm in diameter) and put in oven of 40°C for 2 days. Subsequently, the dried film was immersed into 5% w/w of CaCl_2 (15 mL) for 5 minutes and put in oven for another 6 hours.

3.2.3 LED/laser diode array for high throughput chemical release study

An 8x12 LEDs/laser diodes array with 9 mm pitch was made on a custom printed circuit board (PCB) to match the dimension of 96 well plates. LEDs/laser diodes in each column are connected in series to a constant current sink. The reference current is manually adjustable through a trimmer, determine the optical output. The current was set to 20 mA for both UV LED and 10 mW NIR laser diode, and 40 mA/70 mA for 50 mW NIR laser diode. The average optical dose of each column is individually controlled by a PWM signal. PWM commands are generated with a Raspberry Pi using the pi-blaster software. The duty cycle had a resolution of 1000 steps and the modulation frequency was set to 100 Hz in chapter 4 and 586 Hz in chapter 5. A schematic for the PCB is displayed in Figure 3.4.

Figure 3.4 Schematic for the printed circuit board

The picture illustrating electrical diagram for high throughput release is displayed Figure 3.5. A power supply (1) is connected to PCB board via three cords (2), (3) and (4) to supply constant voltage; 5 volts to PCB via cord (2), 30/12 volts to LEDs/laser diodes via cords (3) and (4) when eight of them are connected in series in one column. The optical output of LED/laser diode is determined by adjusting current via a trimmer (5). LEDs/laser diodes connected to PCB (6) are fitted in to customized supportive 96 well microplate (7) with bottom drilled away to align the light to microplate (8) where the release study take place. PCB is connected to Raspberry Pi (10) via cord (9). A 220 volts voltage is supplied via (11) for Raspberry Pi to work. Representative LED/laser diode connected in PCB are displayed in panel B and C respectively.

Figure 3.5 A) Electrical diagram for high throughput release; B) LED connected to PCB; C) laser diode connected to PCB

3.2.4 High-Throughput *In-Vitro* FDAC release study under light stimulation

Films were punched out into 6 mm diameter disk (n=4), placed in Costar 96 well black flat-bottom polystyrene plate, immersed into 200 μ L phosphate buffered saline (PBS)

spiked with 2% v/v Tween 80 (pH=7.4), then incubated at 37°C for the release study. Light stimulation for each formulation varied from 0.0 (no light) to 1.0 duty cycle. At predetermined time interval, the concentration of FDAC released was analyzed by basic hydrolysis of FDAC to fluorescein and fluorescence was immediately assessed with an Infinite M2 Tecan Microplate spectrofluorometer (Tecan Asia PTE LTD, Singapore) within a Costar 96 well black polystyrene flat-bottom plate, 20 uL buffer media were taken and quantified for FDAC release. Fresh release medium (0.2 mL) was subsequently added in to maintain sink conditions. Quantitation of FDAC was described in previous publications. [10] The amount of the dye was quantitatively analyzed under different gain setting using three calibration curves for 0.01-0.1 µg/mL, 0.1-1 µg/mL and 1-10 µg/mL respectively. At the end of release study, films were dissolved in acetone and diluted with PBS buffer to measure the FDAC residue. The cumulative release profiles represented in this dissertation were expressed in percentage of total encapsulated FDAC, as it allows for a more accurate comparison across sample formulations. Formulated films and light stimulation condition presented in each chapter are listed in Table 3.1.

Table 3.1 Formulations and light stimulation for each chapter.

<i>Chapter</i>	<i>Polymer matrix</i>	<i>Photocatalytic additives</i>	<i>UCNP</i>	<i>Light stimulation</i>	<i>Purpose</i>
Chapter 4	PLGA	<i>neat</i>	-	365 nm UV	Proof of concept: chemical release and high throughput design
		TiO ₂ , ZnO, Fe ₂ O ₃ , ZnFe ₂ O ₄	-		
Chapter 5	PLGA	<i>-neat</i>	-	365 nm UV	Tune release kinetics under UV light
		ZnO (1% w/w)	-		
Chapter 5	PLGA	<i>-neat</i>	-	980 nm NIR	Stimulated under NIR light
		ZnO (1% w/w)	-		
		-	UCNP (100% w/w)		
		ZnO (1% w/w)	UCNP (100% w/w)		
Chapter 6	PLA	<i>-neat</i>	-	980 nm NIR	Tune release kinetics under NIR light; on demand release
		ZnO (1% w/w)	-		
		-	UCNP (1% w/w)		
		ZnO (1% w/w)	UCNP (1% w/w)		
		<i>-neat</i>	-	365 nm UV	

Chapter 7	Sodium Alginate	TiO ₂ , ZnO,Fe ₂ O ₃ , ZnFe ₂ O ₄	-		Stimulated immediate release
	Polyanhydride	-neat	-	980 nm NIR	Stimulated zero order release
		ZnO (1% w/w)	-		
		-	UCNP (1% w/w)		
		ZnO (1% w/w)	UCNP (1% w/w)		

3.3 Release mechanisms analysis

We hypothesized the chemical release from polymeric matrix can be accelerated under light stimulation with inclusion of photoactive photoadditives into the matrix, resulting from the degradation of the polymeric matrix. In a release system involving photoadditives and light stimulation, the release mechanism can be a complicated mixture of various release mechanisms: 1) intrinsic diffusion release from matrix, 2) accelerated release under light, 3) accelerated release from photoadditive + light. To support or nullify the hypothesis, chemical release via photoadditive + light need to be resolved. Thus we fabricated the sample with or without photoadditives, stimulating them with light, comparing the release from sample that not exposed to light. Release is an additive sum of each mechanism under light stimulation.

3.3.1 Kinetics assessment

Release kinetics K of each formulation under each illumination condition was determined as the slope of cumulative release profiles verses time from day 1 to day 15. It is assumed to be degradation dependent and the increment in release kinetics can be assigned to individual degradation mechanism. After assessment to individual mechanism contribution in release kinetics, we can support or nullify our hypothesis.

3.3.2 UV light stimulated release

Only two film formulations are involved in a UV light stimulated release study, the polymeric matrix without photoadditives and the matrix with photoadditives, for example,

PLGA and PLGA with ZnO additives (PLGA/ZnO). Additive release mechanisms for each formulation under UV light stimulation are illustrated in Figure 3.6. In the most complicated situation when PLGA is inclusive of ZnO additive and irradiated under UV light, the FDAC is assumed to be released as a result of three release mechanisms additively, 1) diffusion from matrix; 2) UV-light mediated photooxidation; and 3) UV-ZnO photocatalysis reaction; as seen in equation 3.1.

$$K = K_{diff} + K_{UV} + K_{UV/ZnO} \quad (\text{Equation 3.1})$$

Where

K is the release kinetics calculated as the slope from release profile;

K_{diff} is the release kinetics contributed via diffusion from polymeric matrix;

K_{UV} is the release kinetics contributed via UV-mediated photooxidation;

$K_{UV/ZnO}$ is the release kinetics contributed via photocatalysis reaction.

Equation 3.1 Release kinetics as additive result of different mechanisms in UV stimulated release.

The diffusion release kinetics K_{diff} is measured from films in control group with no UV light exposure. It is considered to be equivalent in same formulation across different illumination conditions because they are punchouts from a same film and in same batch of release study.

For PLGA film, when stimulated with UV light, $K_{UV/ZnO}$ is zero, the release kinetics K is the additive result of diffusion release K_{diff} and UV-mediated release K_{UV} only. Hence the UV-mediated release K_{UV} is calculated by subtracting diffusion release from total release. $K_{UV} = K - K_{diff}$.

It is difficult to define the energy utilization in this project. In addition, not all the light can be absorbed by photoadditives as they didn't form a continuous film. To simplify the analysis, the energy the UV light mediated photooxidation is assumed to be consistent across formulation. With that assumption, K_{UV} keeps constant under identical irradiation condition regardless the presence of photoadditives.

Figure 3.6 Isolation of release mechanisms in UV stimulated release

3.3.3 NIR light stimulated release

In NIR stimulated release system, two photoadditives were added in, UCNP [11-13] and ZnO [6,14-16]. Local UV light would be generated *in situ* from UCNP under NIR irradiation. Additive release mechanisms for each formulation under NIR light stimulation is illustrated in Figure 3.7. In the most complicated system inclusive of both UCNP and ZnO, release kinetics is assumed to be additive result of five mechanisms, under NIR irradiation: 1) diffusion, K_{diff} ; 2) NIR-mediated photooxidation or thermal effect, noted as K_{NIR} ; 3) NIR-ZnO interaction mediated release, noted as $K_{NIR/ZnO}$; 4) photooxidation mediated by *in situ* UV light generated from UCNP, noted as $K_{NIR/UCNP}$; 5) synergistic photocatalysis effect from ZnO under UV light generated from UCNP under NIR irradiation, noted as $K_{NIR/UCNP/ZnO}$, as stated in equation 3.2. K is the measured release kinetics from release profile. To analyze individual effect in a film contains both UCNP and ZnO (PLGA/UCNP/ZnO, take PLGA as example for matrix), three control films are required: 1) PLGA film without photoadditives, 2) PLGA film with ZnO (PLGA/ZnO) and 3) PLGA film with UCNP (PLGA/UCNP). All the samples are divided into groups that not exposed to NIR light as control and exposed to certain NIR illumination condition.

Figure 3.7 Isolation of release mechanisms in NIR stimulated release

$$K = K_{diff} + K_{NIR} + K_{NIR/ZnO} + K_{NIR/UCNP} + K_{NIR/UCNP/ZnO} \quad (\text{Equation 3.2})$$

Where

K is the release kinetics calculated as the slope from release profile;

K_{diff} is the release kinetics contributed via diffusion from polymeric matrix;

K_{NIR} is the release kinetics contributed via NIR-mediated photooxidation or thermal effect;

$K_{NIR/ZnO}$ is the release kinetics contributed via NIR-ZnO interaction;

$K_{NIR/UCNP}$ is the release kinetics contributed via *in situ* UV light generated from UCNP;

$K_{NIR/UCNP/ZnO}$ is the release kinetics contributed via synergistic photocatalysis effect from ZnO under UV light generated from UCNP under NIR irradiation.

Equation 3.2 Release kinetics as additive result of different mechanisms in NIR stimulated release.

The assumption that the diffusion release keeps equivalent regardless of illumination is still valid. Diffusion release kinetics K_{diff} is measured from group not exposed to NIR light.

Similar with UV-mediated photooxidation release, NIR mediated photooxidation release K_{NIR} is determined from PLGA films without additives by subtracting diffusion release.

With adding ZnO to the PLGA thin film, three release mechanisms are proposed under NIR irradiation: 1) diffusion release K_{diff} , 2) NIR mediated release K_{NIR} and 3) NIR-ZnO mediated release $K_{NIR/ZnO}$. Again, diffusion release is measured from PLGA/ZnO in control group and NIR mediated release is assumed to be same as the portion from PLGA with identical NIR stimulation. Thus, the NIR-ZnO mediated release is calculated by subtracting release kinetics via the other two mechanisms $K_{NIR/ZnO} = K - K_{diff} - K_{NIR}$.

With adding UCNPs to the film, three release mechanisms are assumed under NIR illumination; 1) diffusion release K_{diff} , 2) NIR mediated release K_{NIR} and 3) *in situ* UV light mediated release, $K_{NIR/UCNP}$. Similarly, $K_{NIR/UCNP} = K - K_{diff} - K_{NIR}$. The $K_{NIR/UCNP}$ is indicative of UV light generated from UCNPs. With $K_{NIR/UCNP} > 0$, there is UV light generated from UCNP and when $K_{NIR/UCNP} \leq 0$, there is no UV light emission.

In a PLGA/UCNP/ZnO film, it is hypothesized that the encapsulated chemicals will release upon matrix degradation via the photocatalysis reaction from ZnO taking place with UV light generated from UCNPs under NIR irradiation. Five release mechanisms are proposed:

thus $K = K_{diff} + K_{NIR} + K_{NIR/ZnO} + K_{NIR/UCNP} + K_{NIR/UCNP/ZnO}$. It is assumed the interaction of NIR-PLGA, NIR-ZnO, and NIR-UCNP would not change with formulation, regardless of UV transfer from UCNP to ZnO. Thus, K_{NIR} , $K_{NIR/ZnO}$, and $K_{NIR/UCNP}$ are calculated from three control films: PLGA, PLGA/ZnO and PLGA/UCNP under identical NIR irradiation respectively. The rest of released FDAC is a result of synergistic degradation via photocatalysis. $K_{NIR/UCNP/ZnO} = K - K_{diff} - K_{NIR} - K_{NIR/ZnO} - K_{NIR/UCNP}$. With $K_{NIR/UCNP/ZnO} > 0$, there is FDAC released as polymer matrix degradation caused by synergistic photocatalysis of UCNP and ZnO under NIR, the hypothesis is true. In contrast, if $K_{NIR/UCNP/ZnO} \leq 0$, no FDAC is released out due to synergistic photocatalysis effect, the hypothesis is null.

3.4 Size Exclusion Chromatography (SEC) to determine molecular weight of polymer matrix

The molecular weight of polymeric matrix was determined by size exclusion chromatography (SEC), as a direct evidence of polymer degradation of the film.

At predetermined time points, punchouts were rinsed with water and dried in 25°C vacuum oven for 1 week to remove salt residue. Dried punchouts (around 0.7 mg) were dissolved in 300 μ L chloroform overnight. Polymer solutions were centrifuged at 17,000 xG (Sorvall Legend Micro 17, Thermo Scientific, Singapore) for 15 minutes to remove nanoparticles, supernatant was filtered through a 0.2 μ m PTFE filter (Agilent Technologies, IT Tech, Singapore) and injected into vial with 300 μ L microvolume insert and immediately capped. An Agilent 1100 series high-performance liquid chromatography pump complete with degasser and PLGel mixed C (Polymer Standards Service, Mainz, Germany) in a 35 °C temperature-controlled oven was connected in-line with a Wyatt MiniDawn 3-angle light scattering detector and an Agilent 1100 refractive index detector (Agilent Technologies, Santa Clara, CA, USA). The elution buffer was chloroform at a flow rate of 1.0 mL min⁻¹. The injection volumes were typically 50 μ L. Polystyrene standards (Polymer Standards Service, Mainz, Germany) were used as calibration for molar mass analyses. Elution time for each sample was used to compare the hydration radius of the polymer chain, thus

indicative of molar mass.

3.5 Transmission electron microscopy (TEM) and energy dispersive X-ray Analysis(EDX) to determine phase separation of UCNP and ZnO in polyester film

The distribution of nanoparticles, speculated to determine the energy transfer mechanism in the system, was characterized by transmission electron microscopy (TEM). TEM shows advantage in providing morphology characterization via transmitted electron beam through thin specimens. Scanning electron microscope (SEM), requires coating, was not selected as that interferes with nanoparticles features. A comparison of morphology obtained in FESEM and TEM is displayed in Figure 3.8. Other techniques, such as laser ablation inductively coupled plasma mass spectrometry or Raman spectroscopy, were out of consideration due to the nanometer-size features.

Polyester films were embedded in Araldite epoxy microtomed into 100 nm cross-sections (Ultracut UCT Microtome, Leica, Singapore), placed on a 300 mesh copper grid (Ted Pella, USA) and imaged with a transmission electron microscope (Carl Zeiss Libra 120 Plus, Carl Zeiss). The operating voltage was 120 kV and the emission current was adjusted to 4 μ A. Lower energy is adapted to avoid burning in polymer specimen. Energy dispersive X-ray analysis (EDX), taking advantage of unique X-ray emission spectrum from atomic structure of individual elements, attached in TEM, is used to identify element in specimen to ZnO and UCNP. TEM (JEOL JEM2100F) attached with a Schottky Field Emission Gun at 200KV with Annular Dark Field Mode attached with an EDAX/EDX was used for EDX analysis.

Figure 3.8 Image of PLA/ZnO taken from A) FESEM and B) TEM.

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Chapter 4

Screening of photocatalytic nanoparticles

This chapter shows proof-of-concept that the chemical release from a polyester (PLGA) matrix can be accelerated under UV (365 nm) light irradiation with addition of photocatalytic nanoparticles. Assessment of release kinetics show portion of the chemicals released via photocatalysis mechanism as hypothesized. Different photocatalytic nanoparticles, TiO_2 , ZnO , Fe_2O_3 and ZnFe_2O_4 , were added into the matrices and compared. ZnO is the most efficient, reflecting the highest release kinetics under similar irradiation conditions. Hence it was selected for further studies.

4.1 Rationale and Introduction

Hypothesis #1: Chemical release from polyester thin films can be accelerated under UV light with additives of photocatalytic nanoparticles.

Release behavior of hydrophobic small molecules from polyester matrices, such as FDAC from PLGA, has been observed to be dependent on matrix erosion [1]. Thus, if the erosion of the polyester matrix can be accelerated, the release kinetics are predicted to be accelerated as well. It is hypothesized that chemical release can be accelerated under UV light with photocatalytic nanoparticles [2-4]. Reactive hydroxyl radicals will initiate oxidative degradation of the polymeric matrix through hydrogen abstraction [4-6], as shown in Figure 4.1.

Figure 4.1 Degradation of PLGA via hydroxyl free radicals

4.2 Result and Discussions

To support or nullify the hypothesis, neat PLGA thin films or with photocatalytic nanoparticles were exposed to UV light (365 nm) or dark conditions. Temporal FDAC release into the surround medium was subsequently measured. Various metal oxide photocatalytic nanoparticles, including TiO_2 , ZnO , Fe_2O_3 and ZnFe_2O_4 , were analyzed to determine the most efficient chemical release under the given matrix conditions.

4.2.1 TiO₂ vs. ZnO

TiO₂ and ZnO, known for high photocatalysis efficiency under UV light [7,8], were added into PLGA matrix and compared. PLGA thin films, PLGA with 10% w/w TiO₂ (PLGA/TiO₂) and PLGA with 10% w/w ZnO (PLGA/ZnO), were fabricated and divided into two groups, one group kept in dark as the control and the other group exposed to UV light of $0.5 \pm 0.1 \text{ mW/cm}^2$. Irradiation of the samples lasted for 6 days.

Cumulative FDAC release profiles from PLGA and PLGA/TiO₂ under UV irradiation are displayed in Figure 4.2.A. For comparison, films in control group are also included, accounting for diffusion based release. Cumulative release showed no significant difference ($p < 0.05$) between neat film ($1.2 \pm 0.1 \%$) and PLGA/ZnO thin film ($1.2 \pm 0.2 \%$) under dark conditions (control). UV light increased release from neat film ($3.1 \pm 0.1 \%$). Addition of TiO₂ further increased FDAC release under UV light ($3.9 \pm 0.1 \%$).

Figure 4.2 Cumulative release profiles (up to day 6) of FDAC vs time from PLGA thin film and A) PLGA/TiO₂ thin film and B) PLGA/ZnO thin film in control group and under UV light of $0.5 \pm 0.1 \text{ mW/cm}^2$.

Release kinetics calculated from day 1 to day 6 is plotted and compared in Figure 4.3. Release kinetics showed no significant difference from PLGA and PLGA/TiO₂ thin films under dark conditions. FDAC released from both films were accelerated under exposure to UV. UV light increased release kinetics of PLGA thin film from $0.19 \pm 0.02 \text{ \%} \cdot \text{d}^{-1}$ to $0.52 \pm 0.09 \text{ \%} \cdot \text{d}^{-1}$, via photooxidation. Adding TiO₂ nanoparticles further accelerated the

release kinetics to $0.8 \pm 0.1 \text{ \%} \cdot \text{d}^{-1}$ under UV irradiation. Release kinetics assessment in Table 4.1 analyzed release contribution via different mechanisms as described in Chapter 3. It shows 23.7 % (>0) FDAC released via photocatalysis reaction of TiO_2 under UV exposure in UV-irradiated PLGA/ TiO_2 film. Hence, it can be concluded that photocatalysis does accelerate the chemical release as proposed.

Figure 4.3 Comparison of FDAC release kinetics from PLGA thin films with different nanoparticles in control group and under UV light of $0.5 \pm 0.1 \text{ mW/cm}^2$. * indicates significant differences as p value is less than 0.05.

Cumulative FDAC release profiles from PLGA and PLGA/ ZnO under UV irradiation are displayed in Figure 4.2.B. Significant higher cumulative release was observed from PLGA/ ZnO thin film ($4.5 \pm 0.3 \text{ \%}$) compared with PLGA thin film ($1.2 \pm 0.1 \text{ \%}$) in under dark conditions. Release kinetics is plotted in Figure 4.3 and analyzed in Table 4.1. Unlike TiO_2 , ZnO additive accelerated the chemical release intrinsically without any light stimulation, from $0.19 \pm 0.02 \text{ \%} \cdot \text{d}^{-1}$ to $0.77 \pm 0.07 \text{ \%} \cdot \text{d}^{-1}$, speculated owing to Lewis acidic nature of ZnO , as degradation of PLGA is acid catalyzed [9]. Other researcher groups have also reported faster chemical release from PLGA with evidence of faster degradation of polymer carrier. [10-13] Release kinetics from PLGA/ ZnO is $1.99 \pm 0.04 \text{ \%} \cdot \text{d}^{-1}$ under UV irradiation, 3-fold more than PLGA/ TiO_2 and 4-fold compared with neat PLGA. The

contribution of FDAC released via ZnO-mediated photocatalysis is 53.1 %, twice the efficiency of TiO₂.

Compared with TiO₂, ZnO is more effective in UV stimulated release with respect to higher release kinetics and higher contribution of chemicals released via photocatalysis.

Table 4.1 Assessment of FDAC release kinetics for PLGA, PLGA/TiO₂, PLGA/ZnO thin films under UV irradiation.

Formulation	K^a (%.d ⁻¹)	K_{diff}^b (%.d ⁻¹)	K_{UV}^c (%.d ⁻¹)	$K_{UV/NP}^d$ (%.d ⁻¹)	$K_{UV/NP}/K^e$ (%)
PLGA	0.52±0.09	0.19±0.02	0.33±0.02	N.A ^f	N.A
PLGA/TiO ₂	0.7±0.1	0.19±0.03	0.33±0.02	0.2±0.1	23.7
PLGA/ZnO	1.99±0.04	0.77±0.07	0.33±0.02	1.06±0.05	53.1

^a K Release kinetics (per cent per day) calculated as slope of day 1 to 6 when irradiated with UV

^b K_{diff} Diffusion release kinetics (per cent per day) calculated as slope of day 1 to 6 without UV irradiation

^c K_{UV} Contribution of release kinetics via UV-mediated mechanism (per cent per day), assigned as described in methodology

^d $K_{UV/NP}$ Contribution of release kinetics via UV-nanoparticle (photocatalysis, either UV-TiO₂ or UV-ZnO) mechanism (per cent per day), assigned as described in methodology

^e Percent contribution of FDAC release via photocatalysis

^f Not Applicable

4.2.2 Fe₂O₃, ZnFe₂O₄ vs. ZnO

ZnO is observed to more efficiently release FDAC in polyester films by photocatalysis compared with TiO₂. We speculated ZnO and Zn²⁺ role as a Lewis acid may increase ester hydrolysis, leading to faster polymer backbone cleavage. In the regard, other metal cation Lewis acid with photocatalytic properties were selected, such as trivalent iron (Fe³⁺). Nanoparticles of Fe₂O₃, and ZnFe₂O₄ which have also been reported as photocatalyst [14,15], were added into release system and compared with ZnO. They have extended magnetic and visible light photocatalysis properties [16,17]. PLGA thin films, PLGA with 1% w/w Fe₂O₃ (PLGA/Fe₂O₃), PLGA with 1% w/w ZnFe₂O₄ (PLGA/ZnFe₂O₄) and PLGA

with 1% w/w ZnO (PLGA/ZnO), were fabricated and divided into two groups, exposure to 2.6 ± 0.3 mW/cm² UV light and control under dark conditions. Irradiation of the samples lasted for 28 days.

Cumulative FDAC release profiles from Fe₂O and ZnFe₂O₄ are plotted and compared in Figure 4.4. The release kinetics from day 1-14 is plotted in Figure 4.5 and analyzed in Table 4.1. All films with nanoparticles showed enhanced release kinetics under UV light via photocatalysis mechanism. With inclusion of ZnO, highest release kinetics and most contribution (55%) of FDAC released stimulated mechanism (photocatalysis) under UV irradiation were obtained, thus ZnO is considered most effective in stimulated release among the three nanoparticles.

Figure 4.4 Cumulative release profiles (up to day 28) of FDAC vs time from PLGA thin films and A) PLGA/Fe₂O₃ thin films, B) PLGA/ZnFe₂O₄ thin films, C) PLGA/ZnO thin films in control group and under UV light.

Figure 4.5 Comparison of FDAC release kinetics from PLGA thin films with different nanoparticles under UV light of $2.6 \pm 0.3 \text{ mW/cm}^2$. * indicates significant differences as p value is less than 0.05.

Table 4.2 Assessment of FDAC release kinetics for PLGA, PLGA/TiO₂, PLGA/ZnO thin films under UV irradiation.

Formulation	K^a (%.d ⁻¹)	K_{diff}^b (%.d ⁻¹)	K_{UV}^c (%.d ⁻¹)	$K_{UV/NP}^d$ (%.d ⁻¹)	$K_{UV/NP}/K^e$ (%)
PLGA	0.53 ± 0.05	0.32 ± 0.02	0.21 ± 0.06	N.A ^f	N.A
PLGA/Fe ₂ O ₃	0.81 ± 0.09	0.32 ± 0.03	0.21 ± 0.06	0.30 ± 0.10	37.0
PLGA/ZnFe ₂ O ₄	0.84 ± 0.09	0.29 ± 0.02	0.21 ± 0.06	0.34 ± 0.11	40.5
PLGA/ZnO	1.49 ± 0.04	0.45 ± 0.02	0.21 ± 0.06	0.82 ± 0.07	55.0

^a K Release kinetics (per cent per day) calculated as slope of day 1 to 6 when irradiated with UV

^b K_{diff} Diffusion release kinetics (per cent per day) calculated as slope of day 1 to 6 without UV irradiation

^c K_{UV} Contribution of release kinetics via UV-mediated mechanism (per cent per day), assigned as described in methodology

^d $K_{UV/NP}$ Contribution of release kinetics via UV-nanoparticle (photocatalysis, UV-Fe₂O₃, UV-ZnFe₂O₄, or UV-ZnO) mechanism (per cent per day), assigned as described in methodology

^e Percent contribution of FDAC release via photocatalysis mechanism

^f Not Applicable

4.3 Conclusion

Four different photocatalytic nanoparticles, TiO_2 , ZnO , Fe_2O_3 and ZnFe_2O_4 , were added into PLGA thin films respectively. The FDAC from PLGA with nanoparticles were compared with those without nanoparticles. All films with nanoparticles showed enhanced release kinetics under UV light via photocatalysis mechanism. Among all of them, ZnO is the most effective one reflected as highest release kinetics and most contribution of FDAC released stimulated mechanism (photocatalysis) under same UV irradiation. ZnO is also known to be a biocompatible and bioresorbable additive, a key advantage for environmental applications or implantable medical devices, especially when compared to TiO_2 . ZnO was selected as candidature for next sessions.

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Chapter 5

Tunable chemical release from PLGA thin films

This chapter demonstrates the tunable release kinetics of polyester thin film under UV (365 nm) and NIR (980 nm) light irradiation via pulse width modification. PLGA and PLGA/ZnO was irradiated with 6 mW UV LED at varied duty cycles. As a result, release kinetics was tuned up to 6 times in PLGA thin film and 8 times in PLGA/ZnO thin film. In addition, for both formulations, release kinetics displayed linear R^2 dependence of 0.9 towards duty cycle. Photocatalytic ZnO nanoparticles, doped LiYF₄ upconversion nanoparticles, or combination thereof were added into PLGA to induce polymer degradation under NIR light via in situ UV light generation. PLGA without nanoparticles also responds to NIR light, increasing release kinetics under irradiation. Inclusion of photoadditives further enhanced release kinetics up to 30 times more than non-irradiated, neat films with extended release time of 28 days. NIR intensity further increased release kinetics in neat PLGA thin films up to 45 times.

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5.1 Rationale and Introduction

Hypothesis #1: The release kinetics within polyester thin films with additives of ZnO nanoparticles is tunable through static light intensity through pulse width modification.

Results in Chapter 4 supports the prediction that adding photocatalytic metal oxides can accelerate the chemical release from polyester thin films (PLGA). Photocatalysis reaction rate is irradiation dose dependent. Irradiation dose is proposed to tune the chemical release kinetics in polyester thin films via pulse width modulation of LED.

Hypothesis #2: Chemical release from polyester thin films can be accelerated under NIR light with additives of ZnO and UCNP.

Short penetration depth (up to millimeter) through tissue and ionizing nature of UV light limits its application to surfaces. NIR light allows larger penetration depth through tissue and biomolecules such as enzyme and proteins are more transparent.

Doped LiYF₄ based UCNPs have been reported to emit UV light when irradiated with NIR light [1, 2]. Herein we proposed that with inclusion of both UCNPs and ZnO into the polymer matrix, UV light will be *in situ* generated from UCNPs under NIR irradiation and initiate photocatalysis reaction of ZnO, hence regulating release kinetics under NIR light. Laser diode was applied as the light source and the optical dose was regulated via pulse width modification.

5.2 Result and Discussions

5.2.1 Tunable chemical release is observed with UV LED pulse width modification

Thin films of neat PLGA and PLGA with 1% w/w ZnO (PLGA/ZnO) were manufactured and irradiated with 6 mW UV LED from 0.1 to 1.0 duty cycle (DC) until day 28. Cumulative FDAC release profiles for each duty cycle are displayed in Figure 5.1. For comparison, neat PLGA and PLGA/ZnO films at 0.0 DC were also included, accounting

for diffusion release. At all DC applied, UV light accelerated release by photooxidation and ZnO photocatalysis enhanced the acceleration further. Autocatalysis was noted with application of UV light (15-25 days) and appeared earlier with addition of ZnO (7-15 days).

Figure 5.1 Cumulative release profiles (up to day 28) of FDAC vs time from PLGA thin films and PLGA/ZnO thin film in control group (0.0 DC) and under UV light of A) 0.1 DC; B) 0.3 DC; C) 0.5 DC; D) 0.7 DC; E) 0.9 DC; F) 1.0 DC.

FDAC release as a function of time and DC applied for neat PLGA and PLGA/ZnO thin films is plotted in 3D contour in Figure 5.2.A. A shift in release profile with addition of 1% w/w ZnO was noted. When irradiated under UV light of identical DC, the time required to release 40, 60, or 80% FDAC was shifted 4-6 days earlier with addition of ZnO. Cumulative release of FDAC can be modified by the additive and light dose: 40% FDAC release line from PLGA overlapped with 80% FDAC release line from PLGA/ZnO and 60% FDAC release line from PLGA overlapped with 90% FDAC release line from PLGA/ZnO. It also illustrates the versatility in PLGA/ZnO formulation induced by outsource UV stimulation—varying the applied DC, 40% release could be tuned from day 8 -28 and 80% release at day 14 -28. Alternatively, at day 15, 10%-90% release could be chosen by the end user.

Figure 5.2 A) Temporal and duty cycle as a function of FDAC release. Solid lines, neat PLGA. Dotted lines, PLGA/ZnO ; B) Release kinetics (through 15 days) of FDAC released from PLGA sample either with or without ZnO under various DC UV exposure. Linear fit from 0.3 to 1.0 duty cycle.

A plot of release kinetics of per cent per day (%.d⁻¹) versus duty cycle for both formulations is displayed and compared in Figure 5.2.B. At 0.1 DC, no statistical differences in release kinetics were noted with 0.0 DC (no UV). From 0.3 DC to 1.0 DC, linear R² correlation is 0.966 and 0.997 for UV-mediated (PLGA thin film) and ZnO-UV mediated (PLGA/ZnO thin film) film, respectively, suggesting dose/photon-dependent release kinetics, which is ideal for control of light stimulated chemical depots. Varying duty cycle of light source

applied, PLGA displays controllable release kinetics ranged from 0.55 %. d^{-1} to 3.7 %. d^{-1} while PLGA/ZnO shows controllable release kinetics ranged from 0.76 %. d^{-1} to 6.3 %. d^{-1} . Two parallel curves in Figure 5.2.B would have been expected if the photocatalytic nature of ZnO nanoparticles was ineffectual under UV light. Statistical increase in slope with inclusion of ZnO compared with neat PLGA (6.5 ± 0.2 vs. 4.0 ± 0.4) further suggests *in situ* photocatalysis was activated within the polyester matrix.

We assumed FDAC release was limited to the following quantifiable mechanisms: 1) surface diffusion, 2) UV-mediated photooxidation); 3) UV-mediated photooxidation and photocatalysis with inclusion of ZnO (PLGA/ZnO). Contribution of each release mechanisms in both formulations is assessed as described in Chapter 2, listed in Table 5.1.

Table 5.1 Assessment of FDAC release kinetics for PLGA and ZnO/PLGA films subjected to UV LED of various duty cycles.

Duty cycle (DC, UV)	PLGA		PLGA/ZnO				
	K^a (%. d^{-1})	K_{UV}^c (%. d^{-1})	K^a (%. d^{-1})	K_{UV}^c (%. d^{-1})	$K_{UV/ZnO}^d$ (%. d^{-1})	K_{UV}/K^e (%)	$K_{UV/ZnO}/K^f$ (%)
0.0 (off)	0.54 ± 0.03^b	-	0.76 ± 0.02^b	-	-	NA ^g	NA
0.1	0.92 ± 0.05	0.38 ± 0.06	1.01 ± 0.04	0.38 ± 0.06	-0.13 ± 0.08	NA	NA
0.3	0.85 ± 0.04	0.31 ± 0.05	1.59 ± 0.06	0.31 ± 0.05	0.52 ± 0.08	19.5	32.7
0.5	1.30 ± 0.06	0.76 ± 0.07	2.8 ± 0.1	0.76 ± 0.07	1.3 ± 0.1	27.1	46.4
0.7	2.4 ± 0.1	1.9 ± 0.1	4.1 ± 0.1	1.9 ± 0.1	1.5 ± 0.2	46.3	36.6
0.9	3.2 ± 0.1	2.7 ± 0.2	5.5 ± 0.2	2.7 ± 0.2	2.1 ± 0.3	49.1	38.2
1.0	3.7 ± 0.1	3.2 ± 0.2	6.3 ± 0.2	3.2 ± 0.2	2.4 ± 0.3	50.8	38.1

^a K Release kinetics (per cent per day) calculated as slope from day 1 to day 15

^b Diffusion release kinetics (K_{diff}) without UV irradiation

^c K_{UV} Contribution of release kinetics via UV-mediated mechanism, assigned as described in methodology

^d $K_{UV/ZnO}$ Contribution of release kinetics via photocatalysis mechanism, assigned as described in methodology

^e Percent contribution of FDAC release via UV-mediated (photooxidation) mechanism

^f Percent contribution of FDAC release via photocatalysis mechanism

^g Not Applicable

At duty cycle of 0.1, no photocatalysis mediated release was noted with inclusion of ZnO, enhanced release was observed from UV-mediated photooxidation only. With higher duty

cycles, FDAC released via UV mediated photocatalysis, noted as $K_{UV/ZnO}$, increases with duty cycle applied, and shows dose-dependent behavior. FDAC released via UV mediated photooxidation, noted as K_{UV} , also increases with duty cycle applied and is dose-dependent. Photocatalysis peaks at the 0.5 duty cycle while photooxidation increases with duty cycle. This correlates with the time shift at 20% cumulative release lines in Figure 5.2.A; time shifted up to 8 days at 0.3-0.5 duty cycles with inclusion of ZnO, as the result of photocatalysis, however, as the duty cycle increased past 0.5 and reached 1.0, time shifted 1 day only, because UV mediated photooxidation became dominant. Employing duty cycle as a method to tune release kinetics allows control of both chemical release kinetics, and the dominant degradation mechanism.

5.2.2 Tunable chemical release under 42 ± 3 mW/cm² NIR light with photocatalytic ZnO and/or UV emitting upconversion nanoparticles (UCNP)

Four possible quantifiable mechanisms contributing to chemical release under NIR light irradiation were assumed and four formulated PLGA films were manufactured correspondingly: 1) NIR-mediated release (PLGA film, no additives); 2) NIR-ZnO mediated release (PLGA/ZnO); 3) NIR-UCNP $\rightarrow$ UV photooxidation (PLGA/UCNP); and 4) NIR-UCNP-ZnO photocatalysis (PLGA/UCNP/ZnO). Those films were irradiated under NIR light (average intensity: 42 ± 3 mW/cm²) of 0.3 and 1.0 DC until day 28. 1% w/w ZnO was added in PLGA thin film (PLGA/ZnO), same as in section 5.2.1; 100% w/w UCNP was added in PLGA (PLGA/UCNP), deemed necessary to shorten the distance to ZnO to increase the UV transfer efficiency in reabsorption or even via Förster resonance energy transfer (FRET). TEM image of PLGA/UCNP (1/1) is displayed in Figure 5.3.A. TEM images display complete saturation of UCNP (analyzed by image J). TEM image of PLGA/UCNP/ZnO (100/100/1) is displayed in Figure 5.3.B. Again, more than 90% area of the film was covered with nanoparticles. It is hard to distinguish UCNP and ZnO in the mixture owing to saturated loading. As a result, the real distance between UCNP and ZnO is not measurable. However, as more than 90% area of the matrix was filled with UCNP as indicated from Figure 5.3.A, it is assumed the energy transfer efficiency between UCNP

and ZnO has a high probability.

Figure 5.3 TEM images show distribution of nanoparticles inside PLGA matrix for formulations of A) PLGA/UCNP (1/1) and B) PLGA/UCNP/ZnO (100/100/1) at 16kX magnification.

Figure 5.4 Cumulative release profiles (up to day 28) of FDAC vs time from PLGA thin films and with A) ZnO; B) UCNP; C) UCNP and ZnO in both control group (0.0 DC) and under NIR light of 1.0 DC

Cumulative release profiles of FDAC from neat PLGA and PLGA/ZnO under NIR irradiation is displayed in Figure 5.4.A. For simplicity, only profiles under 1.0 DC irradiation are displayed. Again, films with both formulations at 0.0 DC (no NIR light) were included as comparison for diffusion release. Figure 5.5 summarizes and compares release kinetics of each films under NIR irradiation of 0.0, 0.3 and 1.0 DC and linear correlation of release kinetics towards NIR. Assessment of release kinetics for each film under 0.0, 0.3 and 1.0 DC NIR irradiation is displayed in Table 5.2. Minimal difference in release kinetics was expected when PLGA was exposed to NIR light as it is transparent in the NIR range. Surprisingly, irradiation of NIR light on neat PLGA shows an increase in release kinetics up to 6-fold compared with no NIR stimulation. Previous investigators have attributed accelerated release to photo- or thermal-oxidation [3]. Also, the water uptake by PLGA has been reported to show increase in temperature by NIR light, further accelerating the degradation of PLGA [4]. As these films were incubated at 37 °C, which lays in the glass transition temperature (T_g) of the PLGA (36-41 °C), any temperature transitions in this range may attribute to fluctuations in release behavior. However, unlike UV light, NIR induced release showed non-linear dose dependent behavior. The R^2 correlation of release kinetics ($\% \cdot d^{-1}$) versus duty cycle was 0.69.

PLGA/ZnO shows release kinetics of little difference compared with PLGA when no NIR was applied (0.0 DC). It was assumed the release kinetics would not be significantly increased with NIR irradiation, as NIR photons contain only one third of the energy required (3.3 eV) to initiate photocatalysis effect in ZnO [5]. However, with irradiation of NIR light, the presence of ZnO additive raised the release kinetics more than two times higher compared to PLGA film at both 0.3 and 1.0 DC. This effect likely results from the increase in optical density in the PLGA/ZnO films, as the ZnO scattering coefficient is known to increase the light scattering efficiency of polymer/ZnO composites [6,7]. Materials with sufficient optical density have been seen to increase temperature up to 1°C under NIR stimulation [8]. The release kinetics displayed non-linear dependence on NIR light dose with a linear R^2 of 0.78, No significant increase in release kinetics was noted from 0.3 to 1.0 duty cycle ($p > 0.05$).

Figure 5.5 Comparison of release kinetics (up to day 15 days) of FDAC released from PLGA sample either with or without UCNP and/or ZnO at 0.0, 0.3, and 1.0 DC NIR exposures. Linear R^2 correlation of release kinetics versus DC is displayed. * indicates significant differences as p value is less than 0.05.

Figure 5.4.B displays cumulative FDAC release profile of neat PLGA and PLGA/UCNP under NIR irradiation at 0.0 and 1.0 DC. At 0.0 DC, release kinetics were 1 %·d⁻¹ from PLGA/UCNP film, 8 times faster compared to neat PLGA. The comparatively fast release kinetics is due to the saturated loading of UCNP, which was formulated to provide a high probability of ZnO activation. The Lewis acid nature of lanthanide elements [9] are likely responsive for the high diffusion release. Release kinetics was tuned to more than 4 per cent per day under NIR stimulation with UCNP additives, the highest among the four formulations. More than 70% of the increase is attributed to UV light generation under NIR light. Dose-dependent release under NIR was absent and release kinetics increased only 7% when DC was elevated from 0.3 to 1.0.

The cumulative release profiles for PLGA/UCNP/ZnO film irradiated under 0.0 DC and 1.0 DC NIR laser diode are displayed Figure 5.4.C. Release kinetics are correlated with NIR duty cycle with linear $R^2 = 0.98$. This suggests kinetics are highly dependent on irradiation dose. PLGA with both additives is the only formulation showed dose/photon-dependent release behavior, as ZnO under UV light. PLGA/UCNP/ZnO had

PLGA/UCNP/ZnO had release kinetics capped at $3.6\% \cdot d^{-1}$, lower than the additive effect of PLGA/ZnO and PLGA/UCNP, making it difficult to conclude FDAC released via synergistic photocatalysis mechanism directly. Release kinetics synergistically increased $1.6\% \cdot d^{-1}$ when NIR irradiation varied from 0.3 DC to 1.0 DC. This is $1.0\% \cdot d^{-1}$ more increment compared with PLGA films with either photoadditive ($0.3\% \cdot d^{-1}$ for each).

The result corroborates the hypothesis, that adding both UCNP and ZnO additives to the system can tune the release kinetics under NIR light. However, the additives ratios will need to be adjusted to show the clear contribution in release mechanisms. Saturated addition of UCNP nanoparticles may increase surface diffusion. NIR light applied was 42 mW/cm^2 , which is below the limit set for human skin exposure (726 mW/cm^2 at 980 nm) [10]. Higher NIR intensity would be used to increase UV emission from UCNP in next sections.

Table 5.2 Assessment of FDAC release kinetics for PLGA and PLGA/ZnO, PLGA/UCNP, and combination thereof PLGA/UCNP/ZnO subjected to 50 mW NIR laser diode of various duty cycles. Linear correlation (R^2) was > 0.97 for all samples through day 15.

PLGA					
Duty cycle (DC, NIR)	K^a (%.d ⁻¹)	K_{NIR}^c (%.d ⁻¹)	K_{NIR}/K^g (%)		
0.0 (off)	0.15±0.01 ^b	-	NA ^k		
0.3	0.98±0.06	0.83±0.06	84.7		
1.0	0.92±0.06	0.77±0.06	83.7		
PLGA/ZnO					
	K^a (%.d ⁻¹)	K_{NIR}^c (%.d ⁻¹)	K_{NIR}/ZnO^d (%.d ⁻¹)	K_{NIR}/K^g (%)	$K_{NIR}/ZnO/K^h$ (%)
0.0 (off)	0.14±0.01 ^b	-	-	NA	NA
0.3	2.0±0.1	0.83±0.06	1.0±0.1	41.5	50
1.0	2.18±0.09	0.77±0.06	1.3±0.1	35.5	59.6
PLGA/UCNP					
	K^a (%.d ⁻¹)	K_{NIR}^c (%.d ⁻¹)	$K_{NIR}/UCNP^e$ (%.d ⁻¹)	K_{NIR}/K^g (%)	$K_{NIR}/UCNP/K^i$ (%)
0.0 (off)	1.30±0.04 ^b	-	-	NA	NA
0.3	4.4±0.3	0.83±0.06	2.3±0.3	18.9	52.3
1.0	4.7±0.3	0.77±0.06	2.6±0.3	16.3	55.3
PLGA/UCNP/ZnO					
	K^a (%.d ⁻¹)	K_{NIR}^c (%.d ⁻¹)	$K_{NIR}/ZnO + K_{NIR}/UCNP + K_{NIR}/UCNP/ZnO^f$ (%.d ⁻¹)	Synergistic Increase ^j (%.d ⁻¹)	
0.0 (off)	0.76±0.05 ^b	-	-		
0.3	2.1±0.1	0.83±0.06	0.5±0.1		
1.0	3.6±0.2	0.77±0.06	2.1±0.2	1.0±0.4	

^a K Release kinetics (per cent per day) calculated as slope from day 1 to day 15

^b Diffusion release kinetics (K_{diff}) without NIR irradiation

^c K_{NIR} Contribution of release kinetics via NIR-mediated mechanism, assigned as described in methodology

^d $K_{NIR/ZnO}$ Contribution of release kinetics via NIR-ZnO mechanism, assigned as described in methodology

^e $K_{NIR/UCNP}$ Contribution of release kinetics via NIR-UCNP mechanism (*in situ* UV), assigned as described in methodology

^f Additive contribution of $K_{NIR/ZnO} + K_{NIR/UCNP} + K_{NIR/UCNP/ZnO}$, $K_{NIR/UCNP/ZnO}$ Contribution of release kinetics via NIR-UCNP-UCNP mechanism (synergistic photocatalysis)

^g Percent contribution of FDAC release via NIR-mediated mechanism

^h Percent contribution of FDAC release via NIR-ZnO mechanism

ⁱ Percent contribution of FDAC release via NIR-UCNP mechanism

^j Percent contribution of FDAC release via NIR-UCNP-ZnO mechanism

^k Not Applicable

5.2.3 Tunable chemical release under $110 \pm 3 \text{ mW/cm}^2$ NIR light from PLGA thin films

PLGA thin films were irradiated with NIR light of higher intensity ($110 \pm 3 \text{ mW/cm}^2$), but still lower than the toxic level, at duty cycle varied from 0 to 1.0 for 27 days. Cumulative release profiles are displayed in Figure 5.6. The release profiles shifted significantly under NIR irradiation even without any nanoparticles added into the film. From less than 10% release in 27 days at 0 DC to 100% release in 5 days at 0.7 DC. For sustained release last longer than 2 weeks, this system might not be a good candidate as it is too sensitive towards NIR light and will depot all the chemicals in short time with high burst release.

Figure 5.6 Cumulative release profiles (up to day 27) of FDAC vs time from PLGA thin films irradiated with NIR light ($110 \pm 3 \text{ mW/cm}^2$) of 0 to 1.0 duty cycles.

However, this system does illustrate tunable release for a short period, dose dependently. Cumulative release at day 2 and day 5 versus duty cycle is displayed in panel A and B of Figure 5.7, respectively. At day 2, the cumulative release of FDAC ranged from 2% up to 95% with linear R^2 correlation of 0.951 from 0 DC to 1.0 DC. At day 5, the cumulative release shows linear R^2 correlation of 0.990 until 0.7 DC. Other advantages of this system includes free of photoactive additive, making the system more biocompatible, and less irradiation energy (shorter time) required to achieve higher release.

Figure 5.7 Tuned cumulative release by DC at A) day 2 and B) day 5 from PLGA thin film with NIR irradiation.

As mentioned in section 5.2.2, water molecules have been reported being heated up under 980nm irradiation by absorbing incident energy via vibrational motion. To verify that, water temperature under NIR irradiation of different DC was measured. 200 μ L PBST was added into 96 well microplate and irradiated with 980nm laser diode of different DC. Microplate was incubated at 37 °C for 2 hours. After that, temperature of PBST solution was measured. Result is listed in Table 5.3. Heat generated couldn't be dissipated from microplate. As a result, temperature of PBST solution increased from 36 °C to 44 °C, overlapping with the glass transition of PLGA (36-41 °C). Elevated temperature accelerated PLGA degradation, and FDAC release as well [11].

Table 5.3 Temperature raise of water under NIR illumination of different duty cycle applied

Duty Cycle, DC	0	0.1	0.3	0.7	1.0
Temperature, °C	36	37-38	38-39	40-41	43-44

We target to extend dynamic chemical release with tunable kinetics from polyester over longer period. In addition, the release kinetics should be reduced with optical stimulation removed. This is achievable when photocatalysis is the dominate degradation mechanism. However, early onset of autocatalysis (<15 days) in PLGA leads to uncontrolled release at higher kinetics. High sensitivity of PLGA towards NIR irradiation limits photocatalysis' role as primary degradation mechanism, further restricts application in on-demand release over sustained period. We hypothesized that the sensitivity towards NIR can be reduced by using materials with Tg higher than elevated temperature under NIR irradiation. PLA, another typical polymer in the family of polyesters, with slow degradation, retarded autocatalysis [12], and higher glass transition temperature (60-65 °C), could be a good candidature for tunable sustained release. The monomer for PLA, lactic acid, a monomer in PLGA as well, was proposed susceptible to hydrogen abstraction via hydroxyl free radicals in Chapter 4. Comparison of glass transition and structure of PLGA and PLA is displayed in Figure 5.8 and Figure 5.9 respectively.

Figure 5.8 Differential scanning calorimetry analysis for PLGA (Mw 110 kDa) and PLA (Mw 128 kDa).

Figure 5.9 Structure of PLGA and PLA

5.3 Conclusion

Herein, UV, NIR, and *in situ* UV irradiation has been demonstrated being able to modulate release kinetics from PLGA either with or without photoadditives. Pulse width modulation was assessed as a function of release kinetics. A strong linear correlation (>0.9) with respect to duty cycle and release kinetics ($\% \cdot d^{-1}$) was present for UV LED stimulated release from PLGA thin films, either with or without ZnO nanoparticles. Release kinetics via both UV-photooxidation and photocatalysis mechanism increases with applied duty

cycle, collaborated with hypothesis #1. Dominate degradation mechanism is tuned by PWM as well. Under 42 ± 3 mW/cm² NIR light, neat PLGA release kinetic are significantly increased. Inclusion of photoadditives increased release kinetics by up to 30 times versus that of non-irradiated PLGA films. ZnO + UCNP displayed synergistic increase attributed to NIR→UV photocatalysis. Under high intensity (110 ± 3 mW/cm²) NIR light, PLGA displayed high burst release, due to photothermal effects. In order to build up tunable release system for a sustained period, PLA was selected as candidature for next sections.

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Chapter 6

On demand chemical release from PLA thin films

This chapter demonstrates that PLA based release system is feasible of increasing release kinetics under high intensity NIR light stimulation and while limiting autocatalysis and surface diffusion kinetics. FDAC displayed 500 times release kinetics from 110 ± 3 mW/cm² NIR irradiated PLA matrix with inclusion of both UCNP and ZnO compared with non-irradiated PLA matrix without adding any nanoparticle.

6.1 Rationale and Introduction

Hypothesis #1 Dynamic chemical release is achieved through in-situ UV irradiation via NIR irradiated UCNP.

Results in Chapter 5 support the hypothesis that release kinetics can be tuned via UV dose from polyester thin films (PLGA) via photocatalysis and NIR light can accelerate the release with inclusion of UCNP photoadditive. Thus, it is proposed that dynamic release kinetics (of PLA) can be imposed with *in-situ* UV dose through photoadditives of UCNP and ZnO.

In-situ UV dose is a function of three properties:

1. Control NIR light dose via duty cycle
2. Employ various NIR laser diode intensities
3. Control the concentration of UCNP within the PLA film

Hypothesis #2 Photocatalytic ZnO scissors nearby polyester into soluble oligomers rather than degrade whole matrices evenly under irradiation.

The free radicals are known to be short-lived, from nanosecond (hydroxyl free radical) to seconds (peroxyl free radical) [1,2], and short-travelled in polymer matrix, from a few Angstrom units to tens of Angstrom units [3]. It is hypothesized that rather than degrading the whole film, which would be reflected as decrease in molecular weight, free radicals would attack regions nearby only, locally degrading regional polyester to soluble oligomers. Freed encapsulations will be released with eluted oligomers.

Hypothesis #3 Chemical release can be reduced with removal of NIR light after irradiation if photocatalysis is the primary mechanism of degradation.

If hypothesis #2 is verified. Since the molecular weight of the whole release device would not be affected, the release kinetics should be reduced with removal of the outsource stimulation. This hypothesis can be true only if photocatalysis is the primary mechanism of degradation. To verify this, PLA with slow degrading and retarded autocatalysis is used.

Hypothesis #4 *In-situ* UV irradiation transfers from UCNP to neat ZnO in polyester thin films via two mechanisms simultaneously: Förster resonance energy transfer (FRET) and reabsorption. Former one leads to high efficiency in synergistic photocatalysis though majority of nanoparticles transfer UV via latter one.

In-situ UV transfer from UCNP to neat ZnO via either FRET or photon reabsorption, depending on the distance between two nanoparticles. Former one shows higher energy transfer efficiency compared with the latter one [4]. It is hypothesized small portion of nanoparticles transfer majority of *in-situ* UV via FRET. Colloidal ZnO is added into thin film to control particle to particle distance in the film, isolating reabsorption from mixed mechanisms.

Hypothesis #5 More photocatalysis surface of colloidal ZnO should benefit photocatalysis though reabsorption is the only energy transfer mechanism.

6.2 Result and Discussions

PLA thin films (<50 μm thick) loaded with FDAC were prepared with and without additives of photocatalytic zinc oxide nanoparticles (ZnO), upconversion nanoparticles (UCNP), and a combination thereof (ZnO+UCNP). Laser diodes with power ratings of 10 mW and 50 mW were used, both of which have been shown to activate UCNP [5]. Subsequent FDAC release was monitored during 17 days of NIR irradiation and a further 14 days under dark conditions to investigate the responsiveness to NIR irradiation. Release kinetics K_{light} and K_{dark} were evaluated during NIR irradiation (day 2-15) and under dark conditions (day 18-31), respectively, and are reported as per cent per day ($\% \cdot \text{d}^{-1}$); the 100% value was calculated by dissolving the remaining film after 31 days. Value of $K_{\text{light}}/K_{\text{dark}}$ indicates the responsiveness of film towards removal of NIR stimulation. $K_{\text{light}}/K_{\text{dark}}$ ratio was also calculated for those samples irradiated with NIR light of 0 DC (off), to account for the burst release, diffusion, autocatalysis, or combination thereof. For each formulation, an increase in $K_{\text{light}}/K_{\text{dark}}$ ratio relative to that for 0.0 duty cycle indicates a controlled, on demand reduction in release kinetics. Otherwise it may be contributed from diminished

release via diffusion. Higher value indicates a better responsiveness towards removal of triggering event as stopping of releasing. Higher portion of photocatalysis in degradation mechanisms (in PLA/UCNP/ZnO) is predicted leading to higher value of $K_{\text{light}}/K_{\text{dark}}$ and more favored “on demand off”.

FDAC release from polyester films containing ZnO additives has been shown to correlate with UV dose [6]. Under NIR irradiation, UCNPs act as *in situ* sources of UV light for ZnO photocatalysis. As such, we expected that FDAC release could be controlled by UCNP concentration as well as NIR dose. Two UCNP concentrations were investigated, equal mass ratios of UCNPs to ZnO (1% w/w PLA) and an excess of UCNPs (5% w/w PLA).

6.2.1 Accelerated chemical release under 15 ± 3 mW/cm² NIR light with photocatalytic ZnO and/or UV emitting upconversion nanoparticles (UCNP)

Films were exposed to 10mW NIR light at 0, 0.5 and 1.0 duty cycle. Release profiles for each formulation are displayed in Figure 6.1. Contribution of release mechanisms for first 17 days (K_{light}) is assessed as described in Chapter 2, listed in Table 6.1 to Table 6.4. Without NIR irradiation (0 DC), cumulative FDAC released via diffusion in 30 days was around 0.1% with exception for PLA with 1% ZnO and 5% UCNP showed total release around 1.5%. In general, the 10mW laser diode accelerated the release; most contribution comes from NIR photooxidation on film. The isolation of release kinetics showed the FDAC released via NIR photooxidation mechanism, K_{NIR} , is correlated with NIR dose, 0.0018%.d⁻¹ at 0.5 DC and 0.0058%.d⁻¹ at 1.0 DC. Without adding nanoparticles, K_{NIR} increases release kinetics 3 times. While FDAC released via other mechanisms, interaction of ZnO-NIR, UV emission from UCNP and photocatalysis, with adding ZnO or UCNP, noted as $K_{\text{NIR/ZnO}}$, $K_{\text{NIR/UCNP}}$, and $K_{\text{NIR/UCNP/ZnO}}$, showed absence under NIR irradiation. No NIR dose dependency was observed. In this case, no *in-situ* UV generation from UCNPs can be concluded under this irradiation nor photocatalysis. With removal of NIR light, only FDAC released from PLA without nanoparticles showed responsiveness as ratio of $K_{\text{light}}/K_{\text{dark}}$ showed higher value at non zero DC compared with 0 DC.

Under NIR irradiation of $15 \pm 3 \text{ mW/cm}^2$, the only controllable mechanism is NIR induced photooxidation. FDAC might not release via other NIR-related mechanisms. To increase the portion of FDAC released under stimulation, conditions that induce photocatalysis are required, notably higher intensity NIR.

Table 6.1 Assessment of FDAC release kinetics for PLA films subjected to 10 mW NIR laser diodes of various duty cycles

PLA			
Duty cycle (DC, NIR)	K^a (%.d ⁻¹)	K_{NIR}^c (%.d ⁻¹)	K_{NIR}/K^g (%)
0.0 (off)	0.0028 $\pm$ 0.0004 ^b	-	-
0.5	0.0046 $\pm$ 0.0003	0.0018 $\pm$ 0.0006	40 $\pm$ 10
1.0	0.0086 $\pm$ 0.0007	0.0058 $\pm$ 0.0008	68 $\pm$ 9

^a K Release kinetics (per cent per day) calculated as slope from day 1 to day 15

^b Diffusion release kinetics (K_{diff}) without NIR irradiation

^c K_{NIR} Contribution of release kinetics via NIR-mediated mechanism

^g Percent contribution of FDAC release via NIR-mediated mechanism

Table 6.2 Assessment of FDAC release kinetics for PLA/ZnO films subjected to 10 mW NIR laser diodes of various duty cycles

PLA/ZnO					
Duty cycle (DC, NIR)	K (%.d ⁻¹)	K_{NIR}^c (%.d ⁻¹)	$K_{NIR/ZnO}^d$ (%.d ⁻¹)	K_{NIR}/K^g	$K_{NIR/ZnO}/K^h$ (%)
0.0 (off)	0.0071 $\pm$ 0.0008 ^b	-	-	-	-
0.5	0.010 $\pm$ 0.003	0.0018 $\pm$ 0.0006	0.001 $\pm$ 0.001	18 $\pm$ 6	10.0 $\pm$ 10
1.0	0.008 $\pm$ 0.001	0.0009 $\pm$ 0.0008	NA	10 $\pm$ 10	NA ^k

^d $K_{NIR/ZnO}$ Contribution of release kinetics via NIR-ZnO mechanism, assigned as described in methodology

^h Percent contribution of FDAC release via NIR-ZnO mechanism

Table 6.3 Assessment of FDAC release kinetics for PLA/UCNP films subjected to 10 mW NIR laser diodes of various duty cycles

PLA/UCNP(100/1)					
Duty cycle (DC, NIR)	K (%.d ⁻¹)	K_{NIR} (%.d ⁻¹)	$K_{NIR/UCNP}^c$ (%.d ⁻¹)	K_{NIR}/K (%)	$K_{NIR/UCNP}/K^i$ (%)
0.0 (off)	0.0030 $\pm$ 0.0005 ^b	-	-	-	-
0.5	0.0068 $\pm$ 0.0005	0.0018 $\pm$ 0.0006	0.0020 $\pm$ 0.0009	27 $\pm$ 9	30 $\pm$ 10
1.0	0.0050 $\pm$ 0.0003	0.0020 $\pm$ 0.0008	NA	40 $\pm$ 20	NA

PLA/UCNP(100/5)					
Duty cycle (DC, NIR)	K (%.d ⁻¹)	K_{NIR} (%.d ⁻¹)	$K_{NIR/UCNP}^e$ (%.d ⁻¹)	K_{NIR}/K (%)	$K_{NIR/UCNP}/K^i$ (%)
0.0 (off)	0.0062±0.0008 ^b	-	-	-	-
0.5	0.0099±0.0008	0.0018±0.0006	0.002±0.001	18±6	2±1
1.0	0.014±0.001	0.0058±0.0008	0.002±0.001	41±6	41±7

^e $K_{NIR/UCNP}$ Contribution of release kinetics via NIR-UCNP mechanism (*in situ* UV),

assigned as described in methodology

ⁱ Percent contribution of FDAC release via NIR-UCNP mechanism

Table 6.4 Assessment of FDAC release kinetics for PLA/UCNP/ZnO films subjected to 10 mW NIR laser diodes of various duty cycles

Duty cycle	K (%.d ⁻¹)	K_{NIR} (%.d ⁻¹)	$K_{NIR/ZnO}^d$ (%.d ⁻¹)	$K_{NIR/UCNP}^e$ (%.d ⁻¹)	$K_{NIR/UCNP/ZnO}^f$ (%.d ⁻¹)	$K_{NIR/UCNP/ZnO}^g$ (%)
PLA/UCNP/ZnO(100/1/1)						
0.0	0.0046±0.0006	-	-	-	-	-
0.5	0.0084±0.0007	0.0018±0.0006	0.001±0.001	0.0020±0.0009	0.002±0.001	20±10
1.0	0.0097±0.0004	0.0058±0.0008	NA	NA	NA	NA
PLA/UCNP/ZnO(100/5/1)						
0.0	0.024±0.0008	-	-	-	-	-
0.5	0.038±0.002	0.0018±0.0006	0.001±0.001	0.002±0.001	0.011±0.002	29±5
1.0	0.029±0.001	0.0058±0.0008	NA	NA	NA	NA

^f $K_{NIR/UCNP/ZnO}$ Contribution of release kinetics via NIR-UCNP-UCNP mechanism

(synergistic photocatalysis), assigned as described in methodology

^j Percent contribution of FDAC release via NIR-UCNP-ZnO mechanism

^k Not Applicable

Table 6.5 K_{light}/K_{off} for films under 10 mW NIR irradiation from 0 to 1.0 duty cycle.

Film formulation	Duty Cycle		
	0*	0.5	1.0
PLA	2.7	6.6	6.9
PLA/ZnO (100/1)	2.4	2.6	3.9
PLA/UCNP (100/1)	2.7	1.0	1.0
PLA/UCNP (100/5)	3.9	1.9	2.5
PLA/UCNP/ZnO (100/1/1)	2.5	1.9	5.4
PLA/UCNP/ZnO (100/5/1)	0.9	1.3	1.0

* Ratio at 0 duty cycle, indicating stabilization of release kinetics.

Figure 6.1 FDAC cumulative release profiles up to 31 days under 10mW NIR light varying duty cycle from 0 to 1.0 duty cycle as a function of time from PLA thin films containing A) no nanoparticle, B) 1% w/w ZnO, C) 1% w/w UCNP, D) 5% w/w UCNP E) 1% w/w UCNP and 1% w/w ZnO, F) 5% w/w UCNP and 1% w/w ZnO. For those groups exposed to NIR light, NIR light was turned on from day 1 to day 17 and turned off from day 17 to day 31.

6.2.2 On demand chemical release under 110 ± 3 mW/cm² NIR light with photocatalytic ZnO and/or UV emitting upconversion nanoparticles (UCNP) with tunable kinetics

Cumulative release profiles of PLA films with or without nanoparticles under 110 ± 3 mW/cm² NIR light are displayed in Figure 6.2. The release kinetics K_{light} and K_{dark} for each formulation are plotted in Figure 6.3 and the ratios ($K_{\text{light}}/K_{\text{dark}}$) of them are listed in Table 6.6.

6.2.2.1 110 ± 3 mW/cm² NIR light provides duty cycle dependent release

For all the formulations, K_{light} is increasing with applied duty cycle when irradiated with 110 ± 3 mW/cm² NIR light, positively correlated, nonlinearly. Highest K_{light} is obtained from PLA/UCNP/ZnO (100/1/1) exposed to NIR light with 1.0 duty cycle, $1.4\% \cdot \text{d}^{-1}$, more than 300 times compared with non-irradiated film with same formulation (0 DC), $0.0046\% \cdot \text{d}^{-1}$; and 500 times compared with non-irradiated PLA film without any nanoparticles, $0.0028\% \cdot \text{d}^{-1}$.

6.2.2.2 Release kinetics reduces by 95% after removal of NIR irradiation

K_{dark} is calculated from day 18 - 31, representing the release kinetics after removal of light. Release kinetics for non-irradiated sample (0 DC) for the same period (day 18 - 31) is also calculated, as a reference of diffusion release. For convenience, it is recorded as K_{dark} as well. K_{dark} shows no obvious correlation towards duty cycle. NIR irradiated sample showed higher K_{dark} compared with non-irradiated sample (0 DC), as a consequence of degradation of polymer matrix. Most K_{dark} shows lower value compared with K_{light} . This is also reflected from the turning point at day 17, after which the slope of cumulative release reduced in all the samples with removal of NIR light. However, it cannot be concluded as responsiveness towards removal of NIR light as diffusion controlled release devices also show diminished kinetics over time [7]. To evaluate whether the reduction in release

kinetics can be regarded as system's responsiveness to removal of stimulation, ratio of K_{light} and K_{dark} , is analyzed. Release kinetics from non-irradiated samples (0 DC) is assumed to be surface diffusion only, higher value of $K_{\text{light}}/K_{\text{dark}}$ compared with 0 DC group indicates FDAC release slowed down as responsiveness towards removal of NIR light. Although $K_{\text{light}}/K_{\text{dark}}$ ratio shows no dependence on duty cycle. Highest $K_{\text{light}}/K_{\text{dark}}$ value of 21.4 was obtained from PLA/UCNP/ZnO (100/1/1) films exposed to NIR light with 1.0 duty cycle. Up to 26.1% of encapsulated FDAC was released during 17 days of NIR irradiation of 1.0 duty cycle, compared to 1.24% release during the following 14 days without NIR irradiation.

Table 6.6 $K_{\text{light}}/K_{\text{dark}}$ for films under 50 mW NIR irradiation from 0 to 1.0 duty cycle.

<i>Film formulation</i>	<i>Duty Cycle</i>				
	0*	0.1	0.3	0.7	1.0
PLA	2.7	3.5	9.1	7.1	10.8
PLA/ZnO (100/1)	2.4	3.7	9.5	14.9	4.5
PLA/UCNP (100/1)	2.7	3.1	1.0	3.1	4.5
PLA/UCNP (100/5)	3.9	6.1	0.8	2.2	18.0
PLA/UCNP/ZnO (100/1/1)	2.5	0.6	1.9	16.7	21.4
PLA/UCNP/ZnO (100/5/1)	0.9	2.6	3.7	5	3.5

Figure 6.2 FDAC cumulative release profiles up to 31 days under 50 mW NIR light varying duty cycle from 0 to 1.0 duty cycle as a function of time from PLA thin films containing A) no nanoparticle, B) 1% w/w ZnO, C) 1% w/w UCNP, D) 5% w/w UCNP E) 1% w/w UCNP and 1% w/w ZnO, F) 5% w/w UCNP and 1% w/w ZnO. For those groups exposed to NIR light, NIR light was turned on from day 1 to day 17 and turned off from day 17 to day 31.

Figure 6.3 FDAC release kinetics (K_{light} and K_{dark}) of PLA thin films containing A) no nanoparticle, B) 1% w/w ZnO, C) 1% w/w UCNP, D) 5% w/w UCNP E) 1% w/w UCNP and 1% w/w ZnO, F) 5% w/w UCNP and 1% w/w ZnO under 50mW NIR irradiation from 0 to 1.0 duty cycle, * K_{light} showed significant difference ($p < 0.05$) compared with PLA film not exposed to NIR; ** for the same sample, significant difference ($p < 0.05$) is found between K_{light} and K_{dark} .

6.2.2.3 Synergism of photoadditives ZnO and UCNP increases kinetics over two orders-of-magnitude

Different NIR associated mechanisms are involved under irradiation. Assessment of release kinetics K_{light} for each film under NIR irradiation is conducted in Table 6.7-6.10, and plotted in Figure 6.4. FDAC released via NIR stimulated mechanisms, which are K_{NIR} , $K_{\text{NIR/ZnO}}$, $K_{\text{NIR/UCNP}}$, and $K_{\text{NIR/UCNP/ZnO}}$, increase with duty cycle. When irradiated with NIR light of 1.0 DC, NIR-mediated photooxidation became the dominated mechanism in PLA films without nanoparticles, 100% of FDAC released via it, release kinetics increased up to 20 times. For PLA/ZnO and PLA/UCNP, FDAC released via $K_{\text{NIR/ZnO}}$ and $K_{\text{NIR/UCNP}}$ also increase with duty cycle applied, while NIR-mediated photooxidation (K_{NIR}) still acts as dominated mechanism at 1.0 DC. In a release system with both UCNP and ZnO, photocatalysis was dominant after 0.1 DC, noted as $K_{\text{NIR/UCNP/ZnO}}$. Portion of FDAC release via photocatalysis increases with duty cycle and reached 100% at 1.0 DC. Photoadditive combination of ZnO and UCNP synergistically increases kinetics over two orders-of-magnitude. Elaborated with our hypothesis, when photocatalysis dominates degradation mechanism, higher value of $K_{\text{light}}/K_{\text{dark}}$ and more favored “on demand off” is achieved as mentioned in section 6.2.2.

Table 6.7 Assessment of FDAC release kinetics for PLA films subjected to 50 mW NIR laser diodes of various duty cycles

PLA			
Duty cycle (DC, NIR)	K^a (%.d ⁻¹)	K_{NIR}^c (%.d ⁻¹)	K_{NIR}/K^g (%)
0.0 (off)	0.0028±0.0004 ^b	-	-
0.1	0.0043±0.0004	0.0015±0.0005	30±10
0.3	0.0070±0.0009	0.004±0.001	40±10
0.7	0.014±0.002	0.012±0.002	90±10
1.0	0.06±0.01	0.06±0.01	100±20

^a K Release kinetics (per cent per day) calculated as slope from day 1 to day 15

^b Diffusion release kinetics (K_{diff}) without NIR irradiation

^c K_{NIR} Contribution of release kinetics via NIR-mediated mechanism, assigned as described in methodology

^g Percent contribution of FDAC release via NIR-mediated mechanism

Table 6.8 Assessment of FDAC release kinetics for PLA/ZnO films subjected to 50 mW NIR laser diodes of various duty cycles

PLA/ZnO					
Duty cycle (DC, NIR)	K^a (%.d ⁻¹)	K_{NIR}^c (%.d ⁻¹)	$K_{NIR/ZnO}^d$ (%.d ⁻¹)	K_{NIR}/K^g (%)	$K_{NIR/ZnO}/K^h$ (%)
0.0 (off)	0.0071±0.0008 ^b	-	-	-	-
0.1	0.0090±0.0007	0.0015±0.0005	0.000±0.001	17±6	0±10
0.3	0.016±0.001	0.004±0.001	0.004±0.002	25±6	30±10
0.7	0.052±0.005	0.012±0.002	0.033±0.006	23±4	60±10
1.0	0.09±0.01	0.06±0.01	0.02±0.01	90±10	20±10

^d $K_{NIR/ZnO}$ Contribution of release kinetics via NIR-ZnO mechanism, assigned as described in methodology

^h Percent contribution of FDAC release via NIR-ZnO mechanism

6.2.2.4 Higher dosing of UCNP increases release kinetics via *in-situ* UV generation

PLA loaded with UCNP at two concentrations, 1% w/w and 5% w/w of UCNP are assessed for the dependence on release kinetics. UCNP loading is predicted to be a factor in release kinetics, due to the *in-situ* generated UV light [6]. When irradiated under NIR light of identical DC, $K_{NIR/UCNP}$, increased with UCNP loading. This suggests more *in-situ* UV light generation, supporting our hypothesis. The ratio of $K_{NIR/UCNP}$ in two films didn't correlate with UCNP loading and increase with DC.

Table 6.9 Assessment of FDAC release kinetics for PLA/UCNP films subjected to 50 mW NIR laser diodes of various duty cycles

PLA/UCNP(100/1)					
Duty cycle (DC, NIR)	K (%.d ⁻¹)	K_{NIR} (%.d ⁻¹)	$K_{NIR/UCNP}^c$ (%.d ⁻¹)	K_{NIR}/K (%)	$K_{NIR/UCNP}/K^i$ (%)
0.0 (off)	0.0030±0.0005 ^b	-	-	-	-
0.1	0.0029±0.0002	NA	NA	NA	NA
0.3	0.011±0.001	0.004±0.001	0.004±0.002	36±9	40±20
0.7	0.023±0.002	0.012±0.002	0.009±0.003	52±9	40±10
1.0	0.069±0.006	0.06±0.01	0.01±0.01	90 ±10	10±10

PLA/UCNP(100/5)					
Duty cycle (DC, NIR)	K (%.d ⁻¹)	K_{NIR} (%.d ⁻¹)	$K_{NIR/UCNP}^c$ (%.d ⁻¹)	K_{NIR}/K (%)	$K_{NIR/UCNP}/K^i$ (%)
0.0 (off)	0.0062±0.0008 ^b	-	-	-	-
0.1	0.0099±0.0009	0.0015±0.0005	0.002±0.001	15±5	20±1
0.3	0.013±0.001	0.004±0.001	0.003±0.002	31±8	20±20
0.7	0.059±0.004	0.012±0.002	0.042±0.004	20±3	71±7
1.0	0.14±0.02	0.06±0.01	0.08±0.02	43±7	50±10

^c $K_{NIR/UCNP}$ Contribution of release kinetics via NIR-UCNP mechanism (*in situ* UV), assigned as described in methodology

ⁱ Percent contribution of FDAC release via NIR-UCNP mechanism

6.2.2.5 Higher dosing of UCNP with ZnO does not increase release kinetics

Regardless of UCNP loading (1% w/w or 5% w/w), release kinetics was increased two-orders-of-magnitude under completely on NIR irradiation (1.0 DC) in PLA/UCNP/ZnO. NIR-UCNP-ZnO synergistic photocatalysis dominated the release mechanism. $K_{NIR/UCNP/ZnO}$ shows correlation with $K_{NIR/UCNP}$. This suggests kinetics were predominantly controlled by photocatalysis. The higher UV emission for PLA/UCNP/ZnO films with 5% w/w UCNP was not observed to improved release kinetics. At 1.0 DC, PLA/UCNP/ZnO (100/1/1) showed 40% higher release kinetics than PLA/UCNP/ZnO (100/5/1). Two factors affect photocatalysis efficiency: photocatalysis concentration and UV light intensity. Higher *in-situ* UV emission has been proved with higher UCNP loading. Lower photocatalysis efficiency in PLA/UCNP/ZnO (100/5/1) can be resulted from: 1) lower ZnO cluster concentration or 2) lower UV transfer efficiency from UCNP to ZnO. Both scenarios can take place and lead to lower release kinetics in PLA/UCNP/ZnO (100/5/1). Nanoparticle distribution in the film can tell us the which reason is responsible. TEM was used to observe UCNP and ZnO in PLA in section 6.2.4. Thus duty cycle remains the primary method of tuning release kinetics, as various concentrations in UCNP displayed no release kinetic dependence.

Table 6.10 Assessment of FDAC release kinetics for PLA/UCNP/ZnO films subjected to 50 mW NIR laser diodes of various duty cycles

Duty cycle (DC, NIR)	K (%.d ⁻¹)	K_{NIR} (%.d ⁻¹)	K_{NIR}/ZnO^d (%.d ⁻¹)	$K_{NIR}/UCNP^e$ (%.d ⁻¹)	$K_{NIR}/UCNP/ZnO^f$ (%.d ⁻¹)	$K_{NIR}/UCNP/ZnO/K^g$ (%)
PLA/UCNP/ZnO(100/1/1)						
0.0 (off)	0.0046±0.0006 ^b	-	-	-	-	-
0.1	0.0081±0.0007	0.0015±0.0005	0.000±0.001	0.0000±0.0005	0.0020±0.0009	20±10
0.3	0.016±0.002	0.004±0.001	0.004±0.002	0.004±0.002	0.007±0.002	40±10
0.7	0.48±0.04	0.012±0.002	0.033±0.006	0.009±0.003	0.46±0.04	96±8
1.0	1.4±0.2	0.06±0.01	0.02±0.01	0.01±0.01	1.4±0.2	100±10
PLA/UCNP/ZnO(100/5/1)						
0.0 (off)	0.024±0.0008 ^b	-	-	-	-	-
0.1	0.51±0.02	0.0015±0.0005	0.000±0.001	0.002±0.001	0.48±0.02	94±4
0.3	0.56±0.03	0.004±0.001	0.004±0.002	0.003±0.002	0.53±0.03	95±5
0.7	0.75±0.02	0.012±0.002	0.033±0.006	0.042±0.004	0.75±0.02	100±3
1.0	1.09±0.03	0.06±0.01	0.02±0.01	0.08±0.02	1.00±0.03	92±3

^f $K_{NIR}/UCNP/ZnO$, Contribution of release kinetics via NIR-UCNP-UCNP mechanism (synergistic photocatalysis), assigned as described in methodology

^j Percent contribution of FDAC release via NIR-UCNP-ZnO mechanism

Figure 6.4 Contribution of release kinetics (K_{light}) from PLA thin films containing A) no nanoparticle, B) 1% w/w ZnO, C) 1% w/w UCNP, D) 5% w/w UCNP E) 1% w/w UCNP and 1% w/w ZnO, F) 5% w/w UCNP and 1% w/w ZnO under 50mW NIR irradiation from 0 to 1.0 duty cycle.

6.2.3 Elution time in SEC shows no correlation with FDAC release

Elution time in SEC for all samples after release study is displayed and compared in Figure 6.5. For comparison, the elution time before release study was also included to exclude nanoparticle aberrations. No correlation between FDAC release and molar mass (as assessed by SEC elution time) was observed, nor statistic difference under different radiation condition. Thus no bulk degradation was observed with inclusion of nanoparticles or under irradiation. The results suggest matrix nearby ZnO was degraded into oligomers by free radicals. The accelerated release resulted from the eluted oligomers and freed FDAC. Removal of light supports the eluted oligomers hypothesis. If the bulk matrix was evenly degraded, the release kinetics would likely continue at the rate observed at the moment irradiation was removed. This property is particularly important for medical implant drug depots: release can be “turned on” to therapeutic range when required, and “turned off” when the drug exceeds a local setpoint.

Figure 6.5 SEC retention time versus total release after release study from PLA films containing A) no nanoparticle, B) 1% w/w ZnO, C) 1% w/w UCNP, D) 5% w/w UCNP E) 1% w/w UCNP and 1% w/w ZnO, F) 5% w/w UCNP and 1% w/w ZnO under 50mW NIR irradiation from 0 to 1.0 duty cycle

6.2.4 Nanoparticle distribution in PLA films

In section 6.2.2 we compared two concentrations of UCNP and observed an increased release kinetics attributed to *in-situ* UV generation; however, more UV light from UCNP didn't increase release kinetics via photocatalysis with ZnO. We speculated colloidal distribution of the photoadditives with the matrix will affect the particle to particle energy transfer. Energy transfer is affected by distance between UCNP and ZnO [4]. thus 100 nm thin section of were characterized by TEM to assess particle to particle distance and colloidal distribution.

6.2.4.1 ZnO form aggregations in PLA film

Morphology of ZnO and UCNP are displayed in panel A and B of Figure 6.6 respectively. Nanoparticles can be distinguished by the needle-like morphology of ZnO and regular morphology of UCNPs. When casted in PLA films, ZnO formed aggregates within PLA bulk phase with aggregate size in nanometers to micrometers, as displayed in panel C, Figure 6.6. The size distribution 54 ZnO clusters in a 100 nm thick film slice are displayed in Figure 6.7. Though only small portion (<2%, 1 cluster out of 54) of ZnO cluster is of 3 μm , but it contains of the majority of ZnO (77%), estimated from the size of cluster. That means, only a minor percentage of ZnO is distributed in nanometer clusters. ZnO clusters had a volume concentration of 0.9 ± 0.3 clusters/ μm^3 (~1 cluster per cubic micrometer PLA)

6.2.4.2 UCNPs distributed homogeneously in PLA film

UCNPs in PLA film were distributed uniformly at both concentrations, as displayed in panel D and E of Figure 6.6. PLA loaded with 1% w/w (primary) UCNP showed concentration at 14 ± 4 nanoparticles/ μm^3 while 5% w/w UCNP showed concentration at 60 ± 10 nanoparticles/ μm^3 , the volume concentration correlates with the % dosing.

Figure 6.6 TEM images show morphology nanoparticle (A) ZnO at 63kX magnification, B)UCNPs at 125kX magnification; and distribution of nanoparticles inside PLA matrix for formulations of C) PLA/ZnO (100/1), D) PLA/UCNP (100/1), E) PLA/UCNP (100/5) at 4kx magnification

Figure 6.7 Distribution of size of ZnO clusters in PLA film

6.2.4.3 Mixture of ZnO and UCNP formed coaggregation in PLA film

Figure 6.8 displayed ZnO and UCNP in PLA films casted with both nanoparticles. Panel A and B are TEM images for PLA with 1% w/w UCNP and 1% w/w ZnO while panel C and D are TEM images for PLA with 5% w/w UCNP and 1% w/w ZnO, aggregations of ZnO, UCNPs and coaggregations of both nanoparticles were found.

Figure 6.8 TEM images show phase separation of ZnO and UCNP inside PLA matrix with UCNP at concentration of A) 1% w/w, C) 5% w/w at 4kx magnification, panel B) and D) show image at 16kX magnification respectively.

The concentration of ZnO clusters, which are the sites for photocatalysis, has been calculated for both formulations. It is 0.5 ± 0.2 clusters/ μm^3 in PLA/ZnO/UCNP (100/1/1) and 0.4 ± 0.1 clusters/ μm^3 in PLA/ZnO/UCNP (100/5/1), displayed no statistical difference in two formulations. Thus both samples should have same amount of centers for free radical

generation. Release kinetics of PLA/ZnO/UCNP (100/5/1) was not confined by ZnO cluster concentration. It was limited by UV light transferred to ZnO as mentioned in section 6.2.2.

To support/nullify that UV light transfer to ZnO limited release kinetics in PLA/ZnO/UCNP (100/5/1), the distribution of UCNP around ZnO has been analyzed. From TEM image at 4000x magnification, total number of non ZnO coaggregated UCNP clusters and fraction of UCNP that are within certain distance of a ZnO cluster was counted. Five images have been used for sampling the particle to particle distance. The calculated results are displayed in Figure 6.9.

Figure 6.9 Distribution of UCNP towards ZnO in A) PLA/UCNP/ZnO (100/1/1) and B) PLA/UCNP/ZnO (100/5/1) and concentration of UCNP located within 1 μm of ZnO in C) PLA/UCNP/ZnO (100/1/1) and D) PLA/UCNP/ZnO (100/5/1)

No statistical conclusions are possible due to the limited sample counts. However, they can be considered as representative for both samples due to random sampling. In PLA/UCNP/ZnO (100/1/1), concentration of UCNPs clusters (rather than primary UCNPs) not coaggregated with ZnO is 3 clusters/ μm^3 , while in PLA/UCNP/ZnO (100/5/1) is 10 clusters/ μm^3 . Most UCNPs in PLA/UCNP/ZnO (100/5/1) have a particle to particle distance further than 1 μm away to ZnO. Within the PLA/UCNP/ZnO (100/1/1), the majority of UCNPs had a distance of less than 100nm from ZnO. The concentration of UCNPs clusters located within 20nm around ZnO is even higher with less dosing of UCNPs.

To elementally distinguish UCNPs and ZnO, EDX mapping in representative clusters is demonstrated in Figure 6.10 and Figure 6.11. ZnO is composed of zinc (Zn) and oxygen (O). UCNPs employed herein have the chemical composition of LiYF_4 doped with 0.5% mol Tm and 25% Yb. The following elements could be chosen for elemental mapping: lithium (Li), yttrium (Y), fluorine (F), thulium (Tm) and ytterbium (Yb). EDX for each element was conducted for all elements except Li, which is too light to be analyzed. Overlap of Zn and O shows the location of ZnO particles while overlap of Y, F and Yb confirms UCNPs particles. The concentration of Tm was too low to generate any signal above background. Elemental mapping overlays of UCNPs and ZnO elements was confirmed, thus UCNPs and ZnO particles appear to spontaneously coaggregate. The concentration of primary UCNPs in the coaggregation shows no correlation with its original percent dosing. Local concentration of UCNPs in cluster is 154 nanoparticles/ μm^3 in PLA/ZnO/UCNP (100/1/1) (Figure 6.10) and 77 nanoparticles/ μm^3 in PLA/ZnO/UCNP (100/5/1) (Figure 6.11), much higher than the PLA/UCNP dosing (14 ± 4 and 60 ± 10 nanoparticles/ μm^3 at 1 and 5%, respectively).

SEI and EDX of a cluster in PLA/UCNP/ZnO (100/1/1)

Figure 6.10 Secondary electron image (SEI) and energy dispersive X-ray analysis (EDX) of a cluster of nanoparticle in film PLA/UCNP/ZnO (100/1/1) .

Figure 6.11 Secondary electron image (SEI) and energy dispersive X-ray analysis (EDX) of a cluster of nanoparticle in film PLA/UCNP/ZnO (100/5/1) .

We speculated the distribution of UCNP and ZnO in PLA film affected the release kinetics via efficiency of UV light transfer from UCNP to ZnO. UV light transits from UCNPs to ZnO by two established mechanisms: the Förster resonance energy transfer (FRET) mechanism and photon/radiation reabsorption. FRET has been reported more efficient in energy transfer and hence more free radical generation from photocatalysis [4,8-10], but distance from UCNP to ZnO is required to be less than 10 nm. Reabsorption efficiency is dependent on the inverse square relationship with distance between nanoparticles. From the TEM image of PLA/UCNP/ZnO, we found regions of coaggregation of UCNPs and ZnO in the film where two kinds of nanoparticles are overlapping with each other or within distance of 10 nm, regions where the more efficient FRET will be dominant. However, the majority of UCNPs weren't within the FRET distance to ZnO, so reabsorption is likely to be the main mechanism of energy transfer. With more UCNPs dosed into the film, we didn't observe increase in the regions of UCNP-ZnO coaggregation nor decrease in the distance between UCNP and ZnO nanoparticles. The efficiency in UV transfer between UCNP and ZnO could be the main limiting factor for enhancing release kinetics further in PLA/UCNP/ZnO (100/5/1) film with more UV emission from it.

Two energy transfer mechanisms, FRET and reabsorption, exist in PLA/UCNP/ZnO. It is hypothesized that FRET leads to high efficiency in synergistic photocatalysis though majority of UCNPs transfer UV to ZnO via reabsorption. To verify this, colloidal ZnO was added into PLA film with UCNP in next section. Colloidal ZnO didn't aggregate in PLA film, particle to particle distance is thus controlled by homogeneous dispersion of UCNP and ZnO. FRET can only happen within coaggregation of UCNP and ZnO where distance between them is less than 10 nm. It is predicted that energy transfer mechanism will be limited to photon reabsorption by using colloidal ZnO. Meanwhile, more photocatalyst surface area is available in colloidal ZnO, which is hypothesized being able to compensate low energy transfer efficiency of reabsorption mechanism. As a result, comparable or even higher release kinetics might be obtained in PLA/UCNP/ZnO (colloid) compared with PLA/UCNP/ZnO (neat).

6.2.5 ZnO Colloid vs. ZnO

PLA thin films (<50 μm thick) loaded with FDAC were prepared with 1% w/w ZnO colloid, a combination of ZnO colloid and UCNP (1% w/w). Nanoparticle distribution and drug release from them have been compared with those presented in section 6.2.2.

6.2.5.1 Colloidal ZnO stay as primary nanoparticles in PLA film

Colloidal ZnO disperse uniformly and most of them remain in form of primary nanoparticles when casted in PLA film, as displayed in panel A of Figure 6.12. The concentration of colloidal ZnO is 120 ± 10 clusters/ μm^3 . Compared with PLA/UCNP/ZnO, more photocatalysis ZnO surface area was available in PLA/UCNP/ZnO (colloid). Panel B displays distribution of UCNP and ZnO when both added in PLA film. Unlike neat ZnO, no coaggregation of UCNP and colloidal ZnO was observed—both were homogeneously dispersed. EDX analysis of a random area from the film is displayed in Figure 6.13, distance between UCNP and ZnO is more than 50nm, energy transfer mechanism is limited to photon reabsorption.

Figure 6.12 Distribution of nanoparticles inside PLA matrix for formulations of A) PLA/ZnO (100/1), B) PLA/UCNP/ZnO (100/1/1), ZnO is stable in colloid.

Figure 6.13 Secondary electron image (SEI) and energy dispersive X-ray analysis (EDX) of a cluster of nanoparticle in film PLA/UCNP/ZnO (100/1/1), distance from UCNPs to ZnO is more than 50 nm.

6.2.5.1 Lower release kinetics without coaggregation of UCNPs and ZnO

FDAC has been released from PLA with ZnO colloid (PLA/ZnO (colloid)), PLA with UCNPs and ZnO colloid (PLA/UCNP/ZnO (colloid)) in two groups, one group kept in dark as the control and the other group exposed to NIR light of $110 \pm 3 \text{ mW/cm}^2$. FDAC release profile is displayed in Figure 6.14. NIR irradiated samples release more FDAC compared with non-irradiated sample.

Figure 6.14 FDAC cumulative release profiles up to 17 days as a function of time from PLA thin films containing A) 1% w/w ZnO colloid, B) 1% w/w UCNP and 1% w/w ZnO colloid in control group and under NIR light of $110 \pm 3 \text{ mW/cm}^2$

Release kinetics for both formulations (PLA/ZnO, and PLA/UCNP/ZnO) using neat and colloidal ZnO are compared in Figure 6.15. For PLA with neat and colloidal ZnO, release kinetics is higher with application of colloidal ZnO for NIR irradiated film as well as non NIR irradiated film. Result supports theory of Lewis acid ZnO catalyses polyester degradation, as acidic surface area would increase in colloidal ZnO compared with neat ZnO. It also implies that NIR-ZnO interaction catalyses degradation, probably photocatalysis via simultaneous three-photon absorption [11]. For PLA with both UCNP and ZnO, release kinetics is comparable when no NIR light was applied. With exposure to NIR light, release kinetics from PLA/UCNP/ZnO (colloid) using colloidal ZnO is as half

as release kinetics from PLA/UCNP/ZnO using neat ZnO. Assessment of release kinetics from PLA/UCNP/ZnO (colloid) in Table 6.11 shows when irradiated with NIR light, $K_{NIR/UCNP/ZnO}$, assigned as FDAC released via photocatalysis under synergistic effect of UCNP and ZnO, is about 0.5 per cent per day. While under same NIR irradiation, $K_{NIR/UCNP/ZnO}$ is about 1.4 per cent per day from PLA film with same dosing of UCNP (1%w/w) and ZnO (1% w/w) with neat ZnO.

Figure 6.15 Comparison of FDAC release kinetics from PLA thin films containing ZnO colloid and ZnO nanoparticles in control group and under NIR light of $110 \pm 3 \text{ mW/cm}^2$. * indicates significant differences as p value is less than 0.05.

Table 6.11 Assessment of FDAC release kinetics for PLA/ZnO (colloid) film and PLA/UCNP/ZnO (colloid) when subjected to 50 mW NIR laser diodes

PLA/ZnO(colloid)					
K^a (%.d ⁻¹)	K_{diff}^b (%.d ⁻¹)	K_{NIR}^c (%.d ⁻¹)	$K_{NIR/ZnO}^d$ (%.d ⁻¹)	K_{NIR}/K^g (%)	$K_{NIR/ZnO}/K^h$ (%)
0.198±0.005	0.039±0.001	0.06±0.01	0.10±0.01	30±5	50±5
PLA/UCNP/ZnO(colloid)					
K^a (%.d ⁻¹)	K_{diff}^b (%.d ⁻¹)	K_{NIR}^c (%.d ⁻¹)	$K_{NIR/UCNP}^e$ (%)	$K_{NIR/UCNP/ZnO}^f$ (%)	$K_{NIR/UCNP/ZnO}/K^j$ (%)
0.65±0.03	0.008±0.0004	0.06±0.01	0.01±0.01	0.47±0.03	72±5

^a K Release kinetics (per cent per day) calculated as slope from day 1 to day 15

^b K_{diff} Diffusion release kinetics (K_{diff}) without NIR irradiation

^c K_{NIR} Contribution of release kinetics via NIR-mediated mechanism, assigned as described in methodology

^d $K_{NIR/ZnO}$ Contribution of release kinetics via NIR-ZnO mechanism, assigned as described in methodology

^e $K_{NIR/UCNP}$ Contribution of release kinetics via NIR-UCNP mechanism (*in situ* UV), assigned as described in methodology

^f $K_{NIR/UCNP/ZnO}$ Contribution of release kinetics via NIR-UCNP-UCNP mechanism (synergistic photocatalysis), assigned as described in methodology

^g Percent contribution of FDAC release via NIR-mediated mechanism

^h Percent contribution of FDAC release via NIR-ZnO mechanism

ⁱ Percent contribution of FDAC release via NIR-UCNP-ZnO mechanism

Thus, photocatalysis is more efficient when adding UCNP and neat ZnO compared with adding UCNP and colloidal ZnO. However, without UCNP, colloidal ZnO is more efficient in increasing release kinetics under NIR irradiation compared with neat ZnO. Coaggregation of UCNP and ZnO is formed in knife casted PLA film with adding UCNP and ZnO nanoparticle, according to the distribution of UCNP and ZnO, it can be deduced two energy transfer mechanisms are involved in UV transfer from UCNP to ZnO, photon reabsorption and FRET. In PLA film added with UCNP and colloidal ZnO, UCNP and ZnO is homogeneously dispersed and UV transfer from UCNP to ZnO is dominated by photon reabsorption. Concentration of sites for photocatalysis is higher in PLA film added with colloidal ZnO compared with PLA film added with neat ZnO. Result supports the hypothesis that FRET leads to high efficiency in synergistic photocatalysis. Thus, the major limitation of our system comes from efficiency of *in-situ* generated UV light transfer from UCNP to ZnO. Energy transfer mechanism with higher efficiency benefits the synergistic photocatalysis. Coaggregation of neat ZnO and UCNP benefits efficiency of photocatalysis via FRET. However, limited by the fabrication process, the coaggregation of ZnO and UCNP is not necessarily correlated with nanoparticle dosing. As a result, optimized formulation would never be achieved if ZnO was added into the matrix. With current

colloidal ZnO loading, increased photocatalyst surface area wouldn't be able to compensate the less efficient energy transfer mechanism from UCNP to ZnO. Deduced from homogeneous dispersion of UCNP and colloidal ZnO, particle to particle (UCNP, colloidal ZnO) distance is supposed to be controllable. Reabsorption efficiency can be adjusted. Formulation can be optimized by adjusting loading of photoadditives. In this way, A system with lowest loading of UCNP yet high photocatalysis efficiency would be preferred with regarding to biocompatibility.

6.3 Conclusion

Herein we have demonstrated a tailorable chemical release system based on the synergistic effect of UCNP and ZnO. Release kinetics was increased up to two-orders-of-magnitude over two weeks under NIR irradiation. Moreover, the photoadditives within PLA displayed dependence on NIR light. After removing NIR irradiation, release kinetics dropped to 5% of the illuminated rate. Other than controlling release with NIR dosage, adding more UCNPs into the system to increase local UV emission didn't enhance release kinetics further. To evaluate two energy transfer mechanisms (FRET and reabsorption) involved in PLA/UCNP/ZnO, reabsorption was isolated by using colloidal ZnO. Result supports the hypothesis of FRET benefits photocatalysis efficiency. Meanwhile, colloidal ZnO makes it possible to optimize formulation by modulating particle to particle distance. PLA matrix show advantages in on demand release. Limited by its slow degradation, highest achievable release kinetics is 1.4 per cent per day. PLA based release can't fulfill the requirement for immediate on demand release. Hence, polymer matrix with more free radical liable bond will be used as matrix in next chapter.

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Chapter 7

Chemical release from polysaccharide and polyanhydride

This chapter illustrates the application of proposed mechanism on non-polyester based release systems. Polysaccharide (sodium alginate) and polyanhydride (poly (maleic anhydride-alt-1-octadecene)) have been selected as polymer matrix. Same as previous chapters, release kinetics of photon irradiated polymer matrix with photocatalytic nanoparticles was compared with release kinetics of non-irradiated neat polymer matrix. No statistical difference in release kinetics was revealed from abovementioned systems/conditions. Hence, it has been concluded that the proposed mechanism was not able to tune release kinetics in these two polymer based release systems.

7.1 Rationale and Introduction

Hypothesis #1: Chemical release from polysaccharide/polyanhydride thin films can be tuned under UV/NIR light with additives of photoactive nanoparticles.

Chapter 4-6 demonstrates that with the inclusion of photoactive nanoparticles, release kinetics can be tuned from polyester matrices up to three-orders-of-magnitude under either UV or NIR light irradiation as free radicals generated from photocatalytic nanoparticles can induce degradation of matrices via photocatalysis reaction. As free radicals can abstract hydrogen from backbone of polymer matrix non-specifically, herein proposed release from other polymer based systems can be tuned with inclusion of photoactive nanoparticles under UV or NIR light as well.

Two systems were designed:

1) Immediate release from free radical sensitive alginate

Polysaccharides are known to be sensitive to free-radicals [1] and are good candidates. Hydroxyl radical has been reported capable to generate carbon-center radicals by abstraction of hydrogen atom from ring C-H bond of monosaccharide such as aldoses, uronic acids etc. Those carbon-center radicals which form glycosidic bonds can undergo β -scission resulting in the breakdown of the polysaccharide chains; hopefully the chemical release can be controlled by the degradation rate of the polysaccharide carrier. [2,3]

Sodium alginate (NaAlg), a linear unbranched polysaccharide that composed of (1-4)-linked β -D-mannuronate (M) and C-5 epimer α -L-guluronate (G) residues, can be crosslinked by the chelate formed between metal ions and surrounding hydroxyl, oxygen, carboxylic acidic group from G residues due to the configuration of monomer. The M residues will not form chelates with metal ions, as illustrated in Figure 7.1

Figure 7.1 Chelate formed between multivalent metal ion and G residues on alginate

On the backbone of NaAlg, a hydrogen atom can be abstracted from a non-glycosidic bond forming carbon, leading to ring-opening of the uronic acid (Figure 7.2.A). It can also be abstracted from a glycosidic bond forming carbon (C1), leading to the breakdown of glycosidic bond and decrosslinking of NaAlg backbone (Figure 7.2.B) [4]. As a result, encapsulated chemicals will be released out.

Figure 7.2 Hydrogen atom on carbon ring of polysaccharide (alginate) backbone susceptible to be abstracted (a) draw from ring C (non glycosidic bond forming carbon) (b) draw from carbon forms glycosidic bond

2) Release kinetics from zero-order release system can be tuned under light stimulation with inclusion of photoactive nanoparticles

In last chapter, FDAC release from PLA can be “on demand” on with NIR stimulation and “on demand” off as responsiveness towards removal of NIR light. However, release kinetics was unpredictable after the NIR light was reduced, most probably due to the fact that the polyesters went through bulk degradation. It was hypothesized the release kinetics would be predictable with turning off the NIR light if the polymer matrix express surface degradation, such as polyorthoester and polyanhydride. Commercial available polyanhydride, poly (maleic anhydride-alt-1-octadecene) (PA18) was utilized as matrix. The structure of the polymer is displayed in Figure 7.3.

Figure 7.3 Chemical structure of poly (maleic anhydride-alt-1-octadecene)

7.2 Result and Discussion

7.2.1 Chemical release from sodium alginate films with addition of different photocatalytic nanoparticles (TiO₂, ZnO and Fe₂O₃) under UV light

Photocatalytic TiO₂, ZnO and Fe₂O₃ nanoparticles, which have been proved able to increase release kinetics of PLGA via photocatalysis degradation in Chapter 4, were added into NaAlg based release system and compared again. NaAlg thin films, NaAlg with 5% w/w TiO₂ (NaAlg/TiO₂), NaAlg with 5% w/w ZnO (NaAlg/ZnO), and NaAlg with 5% w/w Fe₂O₃ (NaAlg/Fe₂O₃) were fabricated and divided into two groups, one group kept in dark as the control and the other group exposed to $2.6 \pm 0.3 \text{ mW/cm}^2$ UV light. The experiment lasted for 14 hours.

Cumulative FDAC release profiles versus time from NaAlg film and NaAlg film with nanoparticles under UV irradiation are displayed in Figure 7.4. For comparison, films kept in the dark are also included. Alginate films released 90% of FDAC in 14 hours regardless of the formulation and light stimulation. Standard deviation sharply increased after 10 hours.

Figure 7.4 Cumulative release profiles (up to 14 hours) of FDAC vs time from sodium alginate (NaAlg) thin film contains A) no nanoparticles, B) TiO_2 , C) ZnO and D) Fe_2O_3 in control group and under UV light.

Release kinetics calculated as slope between 2 to 10 hours from each sample in both group is analyzed in Table 7.1. No statistic difference was noted in FDAC release kinetics across formulations when no UV light was applied. Under UV irradiation, UV-mediated degradation increased FDAC release kinetics from $5\% \text{ hr}^{-1}$ to $9\% \text{ hr}^{-1}$ in alginate film

without nanoparticles. However, adding nanoparticles into alginate film didn't increase release kinetics under UV irradiation. On the contrary, FDAC released at lower kinetics from NaAlg/nanoparticles compared with NaAlg film under UV light, which means, photocatalysis effect reduces the release. A possible explanation could be the dissolution of metal ions (Zn^{2+} etc.) from nanoparticles under UV irradiation crosslinked the alginate via chelation, thus reduced the release kinetic.

Due to the complexity of crosslinking, decrosslinking and the degradation involved in the alginate system with inclusion of nanoparticles and UV light, it is difficult the control release kinetics by the technology we applied in polyester system.

Table 7.1 Assessment of FDAC release kinetics for NaAlg, NaAlg/TiO₂, NaAlg/ZnO and NaAlg/Fe₂O₃ thin films under UV irradiation.

Formulation	K^a (%.hr ⁻¹)	K_{diff}^b (%.hr ⁻¹)	$K_{UV}^c + K_{UV/NP}^d$ (%.hr ⁻¹)	$K_{UV/NP}^d$ (%.hr ⁻¹)	$K_{UV/NP}/K^e$ (%)
NaAlg	9±1	5±1	4±1	N.A ^f	N.A
NaAlg/TiO ₂	4.2±0.5	4.8±0.4	N.A	N.A	0
NaAlg/ZnO	6±3	4.5±0.6	2±3	N.A	0
NaAlg/Fe ₂ O ₃	8±1	5±2	3±2	N.A	0

^a K Release kinetics (per cent per hour) calculated as slope of hour 1 to 10 when irradiated with UV

^b K_{diff} Diffusion release kinetics calculated as slope of hour 1 to 10 without UV irradiation

^c K_{UV} Contribution of release kinetics via UV-mediated mechanism, assigned as described in methodology

^d $K_{UV/NP}$ Contribution of release kinetics via UV-nanoparticle (photocatalysis), assigned as described in methodology

^e Percent contribution of FDAC release via photocatalysis mechanism

7.2.2 Chemical release from polyanhydride films with inclusion of UCNP and ZnO under NIR light

Four films were fabricated, polyanhydride (PA18), polyanhydride with ZnO (PA18/ZnO), polyanhydride with UCNP (PA18/UCNP) and polyanhydride with both UCNP and ZnO (PA18/ZnO/UCNP). Films were irradiated with NIR light ($110 \pm 3 \text{ mW/cm}^2$) for 10 days.

FDAC released profile from different formulated PA18 under varied NIR illumination are displayed in Figure 7.5. Burst release ranged from 30% to 60% for all samples in the first 2 hours. Standard deviation for burst release is about 20%. More than 90% FDAC released 3 days regardless of the formulation and light stimulation.

Figure 7.5 Cumulative release profiles (up to day 11) of FDAC vs time from polyanhydride (PA18) thin film contains A) no nanoparticles, B) ZnO, C) UCNP and D) UCNP and ZnO under 50mW NIR light with different duty cycle

Table 7.2 compared the FDAC release kinetics from day 1 to day 3 from PA18 samples under different NIR illumination. A distinctive behavior for polyanhydride based release system is the linear release profile of FDAC. Linear R^2 correlation with respect to FDAC release towards time is higher than 0.95 in all samples from day 1 to day 3. Release kinetics showed the trend of increasing with applied duty cycle. However, no significant different kinetics can be concluded due to the huge standard deviation, except for sample contained both UCNP and ZnO. To analyze if the proposed mechanism worked on polyanhydride,

the release kinetics at 0 and 1.0 duty cycle is accessed in Table 7.3. FDAC released via synergistic photocatalysis mechanism at kinetics of four per cent per day, suggesting proposed mechanism worked. The value is higher than $K_{NIR/UCNP/ZnO}$ in PLA/UCNP/ZnO with identical NIR irradiation. However, synergistic photocatalysis contributed almost 100% of the release in PLA/UCNP/ZnO and contributed 10% in release in PA18/UCNP/ZnO. That explains why the release kinetics in PA18 cannot be tuned under NIR light as in PLA. The contribution of controllable mechanism plays a minor part in release, most FDAC released via hydrolysis of the matrix at faster kinetics. This shows our technology can be applied in other matrices, but the matrices need to be less labile and more hydrophobic. However, when the system itself can release encapsulations expeditiously, inherent matrix release mechanisms will outrun any mechanisms additionally supplied by the photoadditives, limiting any control by light stimulation.

Table 7.2 FDAC release kinetics for PA18, PA18/ZnO, PA18/UCNP, and PA18/UCNP/ZnO subjected to NIR laser diode at 0 to 1.0 duty cycle.

Duty Cycle, (NIR, DC)	$K(\% \cdot d^{-1})$	Linear regression (R^2)
PA18		
0.0 (off)	21±1	0.999
0.1	17±1	1
0.3	22±2	0.995
0.7	25±2	0.997
1.0	28±5	0.987
PA18/ZnO		
0.0 (off)	23±2	0.998
0.1	23±2	0.995
0.3	26±2	0.990
0.7	26±4	0.996
1.0	33±10	0.953
PA18/UCNP		
0.0 (off)	21±1	0.999
0.1	22±2	0.995
0.3	21±2	0.995
0.7	23±2	0.995
1.0	27±5	0.979
PA18/UCNP/ZnO		
0.0 (off)	17±1	0.999
0.1	18±3	0.990
0.3	17±1	0.999
0.7	24±2	0.995
1.0	31±1	0.999

Table 7.3 Assessment of FDAC release kinetics for PA18 and PA18/ZnO, PA18/UCNP, and combination thereof PA18/UCNP/ZnO subjected to NIR laser diode.

PA18						
Duty cycle (DC, NIR)	K^a (% $\cdot$ d $^{-1}$)	K_{NIR}^c (% $\cdot$ d $^{-1}$)				
0.0 (off)	21 $\pm$ 1 ^b	-				
1.0	28 $\pm$ 5	7 $\pm$ 5				
PA18/ZnO			PA18/UCNP			
	K^a (% $\cdot$ d $^{-1}$)	K_{NIR}^c (% $\cdot$ d $^{-1}$)	$K_{NIR/ZnO}^d$ (% $\cdot$ d $^{-1}$)	K^a (% $\cdot$ d $^{-1}$)	K_{NIR}^c (% $\cdot$ d $^{-1}$)	$K_{NIR/UCNP}^e$ (% $\cdot$ d $^{-1}$)
0.0 (off)	23 $\pm$ 2 ^b	-	-	21 $\pm$ 1 ^b	-	-
1.0	33 $\pm$ 10	7 $\pm$ 5	3 $\pm$ 11	27 $\pm$ 5	6 $\pm$ 5	0 $\pm$ 5
PA18/UCNP/ZnO						
	K^a (% $\cdot$ d $^{-1}$)	K_{NIR}^c (% $\cdot$ d $^{-1}$)	$K_{NIR/ZnO}^d$ (% $\cdot$ d $^{-1}$)	$K_{NIR/UCNP}^e$ (% $\cdot$ d $^{-1}$)	$K_{NIR/UCNP/ZnO}^f$ (% $\cdot$ d $^{-1}$)	$K_{NIR/UCNP/ZnO}/K^g$ (%)
0.0 (off)	17 $\pm$ 1 ^b	-	-			
1.0	31 $\pm$ 1	7 $\pm$ 5	3 $\pm$ 11	0 $\pm$ 5	4 $\pm$ 11	10 $\pm$ 40

^a K Release kinetics (per cent per day) calculated as slope from day 1 to day 3^b Diffusion release kinetics (K_{diff}) without NIR irradiation^c K_{NIR} Contribution of release kinetics via NIR-mediated mechanism, assigned as described in methodology^d $K_{NIR/ZnO}$ Contribution of release kinetics via NIR-ZnO mechanism, assigned as described in methodology^e $K_{NIR/UCNP}$ Contribution of release kinetics via NIR-UCNP mechanism (*in situ* UV), assigned as described in methodology^f $K_{NIR/UCNP/ZnO}$ Contribution of release kinetics via NIR-UCNP-UCNP mechanism (synergistic photocatalysis), assigned as described in methodology^g Percent contribution of FDAC release via NIR-UCNP-ZnO mechanism

7.3 Conclusion

Herein, we extended the application of the proposed mechanism (photocatalysis) to non-polyester based release systems. NaAlg and PA18 have been used as chemical carrier with inclusion of photoactive nanoparticles and stimulated with UV/NIR light. Photocatalysis failed to accelerate release from NaAlg but was able to accelerate the release from PA18, suggesting our technology cannot be universal applied to any polymer system as proposed.

Though photocatalysis does accelerate release from PA 18, it plays as minor part compared to hydrolysis, thus the controllability over the system is limited. Our technology seems to be more suitable to control release from a slow release system, such as polyesters as demonstrated in previous chapters.

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Chapter 8

Summary and future suggestions

The objectives of chapter 8 are to (1) summarize overall performance for tunable chemical release and (2) suggest the future work for tunable chemical release. Photoactive nanoparticles can accelerate drug release from polyester thin films upon optical stimulation with dose dependent tunable release kinetics. Furthermore, on-demand release with reduced release kinetic upon removal of stimulation source is achieved for a sustained period (one month) with non-autocatalysis effect from polyester matrix. Meanwhile, future work addressing experimental limitations and new hypotheses is suggested. New core-shell structures are proposed to improve the photocatalysis efficiency.

8.1 Summary

A study of tailored response biomaterials for controlled release of bio-reagents in diagnostic devices was reported in this dissertation. Both UV and NIR have been applied as stimuli towards the release system, with intensity adjusted by pulse width modification. Polyester thin films with or without photoactive nanoparticles have been fabricated with different formulations. Drug release study of them has been carried out with exposure to different optical dosage. Tunable release behavior from those films has been obtained under external optical stimulation.

The major conclusions of this work are as follows:

1. UV light accelerates release kinetics from PLGA thin films via UV mediated oxidation. Photocatalytic nanoparticles can accelerate release kinetics from PLGA thin films under UV light exposure via photocatalysis reaction. Four photocatalysts, ZnO, TiO₂, Fe₂O₃ and ZnFe₂O₄ have been compared and result showed ZnO is most efficient in accelerating drug release via photocatalysis.
2. Pulse width modification has been applied to regulate the optical output of stimulation source. Release kinetics from a same formulated film is tuneable by photon dosage/duty cycle of optical stimulation. Assessment of release kinetics showed release via different optical stimulated mechanisms (UV mediated oxidization and photocatalysis) also positively correlated with photon dosage but ratio varies. Release kinetics, as well as degradation mechanisms can be tuned by duty cycles.
3. NIR light accelerates release kinetics from polyester thin films without any nanoparticle via photothermal effect through molecular vibration of water molecule. Addition of photoactive ZnO and UCNPs further increase the release kinetics under NIR light. Inclusion of both UCNP and ZnO in polyester thin film increases release kinetics via synergistic photocatalysis. Two kinds of polyesters, PLGA and PLA displayed unique profiles towards NIR irradiation. PLGA had the advantage of elevated NIR sensitivity, leading to higher release kinetics with lower optical dose. While PLA had the advantage in low diffusion release, as a result, most chemicals released through stimulated mechanism. Additive free PLGA system showed cumulative release

- adjusted from 2% to 95% by duty cycle in 2 days under NIR irradiation of 110 ± 3 mW/cm². Chemical release from PLA with higher glass transition temperature is less affected by NIR light, but it was more controlled by designed mechanism. Adding both nanoparticles in PLA increased release kinetics under 110 ± 3 mW/cm² NIR light up to 500 times compared with non-irradiated thin film without any nanoparticles for 17 days. Subsequently, as autocatalysis degradation of polymer matrix has not yet started, release kinetics was decreased with removal of optical stimulation. Compared with PLGA, PLA, a slow degrading polymer matrix benefits the release system from two respects: 1. to be more controllable by stimulated mechanism and 2. on-demand release for a sustained period.
4. UCNP and ZnO have been observed assembled into coaggregation in casted film. It has been speculated the coaggregation enhance the UV transfer efficiency from UCNP to ZnO, thus release kinetics. Adding more UCNPs alone in release system increased release kinetic via more local UV generation while adding more UCNPs with ZnO didn't increase release kinetic via photocatalysis. Coaggregation of nanoparticles showed no correlation with ratio of UCNP/ZnO added in. A control experiment with disperse distribution of UCNP and ZnO nanoparticle showed lower release kinetics, supporting the speculation.
 5. The proposed mechanism can regulate release kinetics from polyester thin films (PLGA and PLA) under optical stimulation. With other polymer based release systems, such as polysaccharide and polyanhydride, it might not be able to regulate release, as intrinsic release of the system overruns any stimulated release kinetics.

The comprehensive investigation of two photoadditives can tune drug release from polymer carrier by photon stimulation with tuneable release kinetics via photocatalysis reaction. A combination of upconversion material and photocatalyst material enabled the system to be stimulated by NIR irradiation, thus develop the potential *in vivo* application. We also found that stimulated mechanism can dominate release in polyester based release systems that show intrinsic sustained release with slow degrading profile but not in release systems that perform faster release with fast degrading. Thus the proposed technique shows potential application in sustained on demand release. As fabrication of release system

involved in this technique can be as simple as physical mixture of all ingredients (drug, carrier, functional nanomaterial), no specific synthesis is involved, thus theoretically speaking, drugs/chemicals can be delivered in a controlled way by this technique as long as they can be encapsulated in a matrix suitable for this technique (i.e. PLGA or PLA).

8.2 Verified/nullified hypothesis

Covered in Chapter 4,

Hypothesis #1: Chemical release from polyester thin films can be accelerated under UV light with additives of photocatalytic nanoparticles.

This hypothesis was supported by result. Release kinetics from PLGA thin films was accelerated under 365 nm UV light with photocatalytic nanoparticles. Assessment of release kinetics with/without photoadditive with/without UV irradiation showed portion of encapsulated FDAC released out due to photocatalysis. (Table 4.1 and Table 5.1)

Covered in Chapter 5,

Hypothesis #1: The release kinetics within polyester thin films with additives of ZnO nanoparticles is tunable through static light intensity through pulse width modification.

This hypothesis was supported by result. Release kinetics displayed linear R^2 dependence of 0.9 towards duty cycle. (Figure 5.2)

Hypothesis #2: Chemical release from polyester thin films can be accelerated under NIR light with additives of ZnO and UCNP.

This hypothesis was supported by result. PLGA/UCNP/ZnO enhanced release kinetics up to 30 times more than non-irradiated, neat PLGA with extended release time of 28 days. (Figure 5.5)

Covered in Chapter 6,

Hypothesis #1: Dynamic chemical release is achieved through *in-situ* UV irradiation via NIR irradiated UCNP.

This hypothesis was partially supported with results. *In-situ* UV dose was controlled by three methods: 1) control NIR light dose via duty cycle; 2) Employ various NIR laser diode intensities and 3) Control the concentration of UCNP within polyester film. Release kinetics showed non-linear positive relationship towards duty cycle applied. (Figure 6.4) Control concentration of UCNP in film did manipulate *in-situ* UV generated while synergistic photocatalysis didn't show correlation with UCNP loading. (Table 6.9 and Table 6.10) Other than *in-situ* UV generation via UCNP, UV absorption by ZnO could be another variable affects chemical release.

Hypothesis #2: Photocatalytic ZnO scissors nearby polyester into soluble oligomers rather than degrade whole matrices evenly under irradiation.

This hypothesis was supported by result. SEC confirmed no static difference in molecular weight (elution time) existed in polyester matrix with photoadditives after NIR irradiation. (Figure 6.5) Release kinetics was not correlated with molecular weight.

Hypothesis #3: Chemical release can be reduced with removal of NIR light after irradiation if photocatalysis is the primary mechanism of degradation.

This hypothesis was supported by result. Release kinetics reduced by 95% by removal of NIR light in the matrix showed highest release kinetics under irradiation (more than 250 times compared with non-irradiated matrix of same formulation). (Figure 6.3) Assessment of release kinetics showed 100 ± 10 % of release contributed from photocatalysis. (Table 6.10)

Hypothesis #4: *In-situ* UV irradiation transfers from UCNP to neat ZnO in polyester thin film via two mechanisms simultaneously: Förster resonance energy transfer (FRET) and reabsorption. Former one leads to high efficiency in synergistic photocatalysis though majority of nanoparticles transfer UV via latter one.

This hypothesis was supported by result. TEM image showed coaggregation of UCNP and neat ZnO in PLA film (Figure 6.8 and Figure 6.9). Distance between nanoparticles indicates UV transfers from UCNP to ZnO via both FRET (in coaggregation) and

reabsorption (majority of nanoparticles). Colloidal ZnO that won't form coaggregation with UCNP in film was used to control particle to particle distance, limiting energy transfer mechanism to reabsorption. (Figure 6.12 and Figure 6.13) Release kinetics of PLA/UCNP/ZnO (neat) was more than 2 times compared with PLA/UCNP/ZnO (colloid) when irradiated with identical NIR light. (Figure 6.15)

Hypothesis #5: More photocatalysis surface of colloidal ZnO should benefit photocatalysis though reabsorption is the only energy transfer mechanism.

This hypothesis was nullified by results (of current nanoparticle loading). Photocatalysis showed higher efficiency when mixing UCNP and neat ZnO compared with colloidal ZnO. However, without UCNP, colloidal ZnO is more efficient in increasing release kinetics under NIR irradiation compared with neat ZnO. (Table 6.11)

Covered in Chapter 7,

Hypothesis #1: Chemical release from polysaccharide/polyanhydride thin films can be tuned under UV/NIR light with additives of photoactive nanoparticles.

This hypothesis was nullified by results. Inherent degradation mechanism of polysaccharide/ polyanhydride thin film dominated release, limiting stimulated mechanism to control release.

8.2 Implication and future suggestions

Based on the current result and conclusion from drug delivery characteristics of polyester systems incorporated with photocatalytic and upconversion nanoparticles under optical stimulation, it will provide directions for further studies to be conducted. The following work are thus suggested be done in the near future to explore and substantiate the research work presented in the dissertation. These studies will provide a wider scope and better understanding of the photoactive nanoparticle assisted drug delivery system, they will also help to facilitate the technique towards application.

Future suggestions for this work are as follows:

1. Optimization of formulation via FRET

The coaggregation of ZnO and UCNP has been observed effectively improving the chemical release kinetics under NIR light via more efficient energy transfer mechanism, FRET. To achieve the best effect of the controlling from NIR light, integrated structure of UCNP and ZnO illustrated in Figure 8.1 can be synthesized. Chemical release from systems with UCNP-ZnO integration can be compared with those with heterogeneous mixture of UCNP and ZnO under NIR irradiation. Concerning about the potential nanotoxicity of nanoparticles, different concentration of nanoparticles added in the system should also be compared to optimize the whole system: with least nanoparticles added in to achieve relative higher release kinetics under irradiation.

Figure 8.1 Optimization of formulation by adding in A) heterogeneous of UCNP and ZnO to B) integrated structure of UCNP and ZnO

2. Optimization of formulation via reabsorption

Homogeneous dispersion of colloidal ZnO and UCNP has been observed. It is hypothesized particle to particle (UCNP, colloidal ZnO) distance can be controlled by changing loading of nanoparticles as coaggregation can be avoided. As a result, reabsorption efficiency, which is inversely related to distance between nanoparticles, can

be adjusted [1,2]. Formulation can be optimized by adjusting loading of photoadditives as shown in Figure 8.2. A system with lowest loading of UCNP yet high photocatalysis efficiency would be preferred with regarding to biocompatibility.

Figure 8.2 Optimization of formulation by changing particle to particle distance from A) lower concentration of colloidal ZnO to B) higher concentration of colloidal ZnO

3. Optimization of irradiation

In our experiment we have showed that 50mW NIR laser diode can effectively cause synergistic photocatalysis between UCNP and ZnO. The quantum yield of UV light emission from UCNP has been reported increased with irradiation intensity. In future, NIR laser diode with higher output power can be applied as light source. The FDA approved commercial NIR red therapy equipment utilizes light source of 100mW peak power (per diode), indicating the relative “safe” range of the light source can be applied. The conservative limits set for human skin exposure to 980 nm light (726 mW/cm^2). Comparison of the release kinetics control would be made under the same total energy (photons) from different light sources to check if the light source with lower output power can show same controllability as the one with higher output power. NIR Optical fiber may also be applied as the waveguide for light source in later stage.

4. Different delivery pattern

We have tested two delivery patterns: 1. continuous irradiation for sustained period (28 days) and 2. continuous irradiation for sustained period (17 days) followed by removal of irradiation for another sustained period (14 days). In future, chemical release behavior with more irradiation on/off cycles of different period can be conducted. Higher portion of photocatalysis in degradation mechanisms is to be achieved to benefit “on demand off” of the release.

5. Degradation study of polyester system for prolonged period

We have studied the release behavior from polyester system with inclusion of nanoparticle under optical irradiation systematically. Only molecular weight of polymer matrix at certain point has been measured and no degradation study of polyester has been conducted systematically. In the future, a systematical degradation study of polymer matrix under optical irradiation monitoring water absorption rate, mass change, molecular weight change over time can be conducted. It could help us to comprehend the mechanism in depth.

6. Specific drug release study

In this dissertation, we release a model drug FDAC from the system. Next step, a real drug can be released from the system to match the clinic requirement. For example, anticancer drug paclitaxel can be a good candidate to start with as it shares same logP value of 4.0 with FDAC.

Reference

- [1] Y. Tang; W. Di; X. Zhai; R. Yang; W. Qin. *ACS Catalysis* **2013**, 3 (3), 405-412.
- [2] X. Guo; W. Song; C. Chen; W. Di; W. Qin. *Physical Chemistry Chemical Physics* **2013**, 15 (35), 14681-14688.