TiO$_2$ nanotubes have great potential to improve the performance of Ti implants as a surface coating due to their high surface area, ability to promote bone growth and biocompatibility. However, there are two issues needed to be solved before further advancing TiO$_2$ nanotubes technology as drug carrier: uncontrolled drug release and poor mechanical properties. In this study, a drug carrier using composite of biodegradable polymer/TiO$_2$ nanotubes is engineered. Ibuprofen, carprofen and lidocaine were selected as test drugs. A simple characterization method is developed to investigate the infiltration of polymer into TiO$_2$ nanotubes. The synthesized drug carrier demonstrated much better sustained drug release profile, greatly improved mechanical strength and flexibility compared to pure TiO$_2$ nanotubes coating. It has also been found that the drug release kinetics using the synthesized drug carrier is controlled mainly by the drug solubility, polymer degradation and electrostatic force between drug and polymer which are pH dependent.
ANODIZED TiO$_2$ NANOTUBE FILM FOR CONTROLLABLE DRUG DELIVERY

A Thesis

Submitted to the
Faculty of Miami University
in partial fulfillment of
the requirements for the degree of
Master of Science
Department of Chemical and Paper Engineering
by
Huiying Jia
Miami University
Oxford, Ohio
2013

Advisor  Lei Kerr

Reader   Shashi Lalvani

Reader   Catherine Almquist
Table of Contents

1. Introduction ......................................................................................................................... 1
   1.1 Introduction of drug delivery and current state of art in drug delivery .................. 1
   1.2 Advantages of using TiO₂ nanotube in drug delivery and their current state of art research 3
   1.3 Problem statement ........................................................................................................ 4
   1.4 Hypothesis .................................................................................................................... 6

2. Sustained Ibuprofen Release Using Composite Poly(Lactic-co- Glycolic Acid)/Titanium Dioxide Nanotubes from Ti Implant Surface ................................. 7
   2.1 Introduction .................................................................................................................. 7
   2.2 Experimental section .................................................................................................... 9
      2.2.1 Anodized TiO₂ nanotube film synthesis ............................................................ 9
      2.2.2 Polymer/ibuprofen loading onto TiO₂ nanotubes ............................................ 10
      2.2.3 Scanning electron microscopy (SEM) imaging of PLGA/nanotubes............... 10
      2.2.4 Drug loading and release study ........................................................................ 11
      2.2.5 Tensile bonding strength test .......................................................................... 12
   2.3 Results and discussion ................................................................................................ 13
      2.3.1 TiO₂ nanotubes synthesized by anodization in NH₄F based organic electrolyte 13
      2.3.2 Scanning electron microscope (SEM) characterization of PLGA/drug infiltration in TiO₂ nanotubes ................................................................. 14
      2.3.3 Drug release study .............................................................................................. 15
      2.3.4 Mechanical properties ...................................................................................... 19
   2.4 Conclusions .................................................................................................................. 21

3. Study of the Mechanism to Control Carprofen and Lidocaine Release from TiO₂ Nanotubes ................................................................................................. 22
   3.1 Introduction .................................................................................................................. 22
   3.2 Experimental section .................................................................................................. 23
3.2.1 Preparation of Drug Carrier ................................................................. 23
3.2.2 In vitro drug release studies ................................................................. 24
3.2.3 Chemical stability study ...................................................................... 26

3.3 Results and discussion ........................................................................... 26
3.3.1 Chemical stability ............................................................................... 26
3.3.2 In-vitro drug release profiles of lidocaine and carprofen ..................... 28
3.3.3 Mechanisms for controlling lidocaine and carprofen drug releases ....... 32
3.3.4 Kinetics models .................................................................................. 39

3.4 Conclusion .............................................................................................. 47

4. Future work ............................................................................................. 48

5. References .................................................................................................. 49
List of Tables

Table 2.1 Tensile strength test results .......................................................................................................................... 19

Table 3.1 Comparison of lidocaine and carprofen release from TiO$_2$ Nanotubes and PLGA/TiO$_2$ Nanotubes ........................................................................................................................................ 32

Table 3.2 Ionization degree and solubility of lidocaine and carprofen at different pH .............................. 35

Table 3.3 Kinetic models equations and best-fit parameters of lidocaine and carprofen release from PLGA/TiO$_2$ nanotubes ........................................................................................................................................ 40
List of Figures

Figure 1.1 (a) The TiO$_2$ nanotube layer shows cracks and partial fracturing of the layer at the bending edge[26]; (b) The TiO$_2$ membrane will curl and break into shards after detached from Ti and dried in air[27]. ................................................................. 5

Figure 1.2 Cisplatin released amount (right axil e) and the fraction of total drug released (left axile) from nanotubes filled with 75, 150, and 225 μg of cisplatin (a, b and c, respectively)[26]. 5

Figure 1.3 Schematic description of the fabrication of drug/polymer loaded TiO$_2$ nanotube array 6

Figure 2.1 (a) Uv-vis spectrum of ibuprofen (b) calibration curve of ibuprofen......................... 11

Figure 2.2 Set-up of the bonding strength test of TiO$_2$ nanotube arrays................................. 12

Figure 2.3 SEM images of TiO$_2$ nanotube array generated in an ethylene glycol electrolyte containing 0.3 wt. % NH$_4$F and 3 vol.% H$_2$O at 50 V for 2 hours (a) top view, (b) top view at higher magnification, (c) side view.............................. 13

Figure 2.4 SEM images of the remaining PLGA nanotubes after removing the TiO$_2$ nanotube using 5 vol. % HF solution. (a) High molecule weight PLGA nanotubes (Molecular weight. 24,000-38000) at lower magnification. (b) High molecule weight PLGA nanotubes (Molecular weight. 24,000-38000) at higher magnification and inset shows the bottom part of PLGA nanotubes at higher magnification. (c) Low molecule weight PLGA nanotubes (Mw. 4000-15000)................................................................. 15

Figure 2.5 (a) Ibuprofen releases from pure TiO$_2$ nanotubes, from low molecular weight PLGA/TiO$_2$ nanotubes and from high molecular weight PLGA/TiO$_2$ nanotubes. (b) Ibuprofen releases from pure TiO$_2$ nanotubes, from low molecular weight PLGA/TiO$_2$ nanotubes and from high molecular weight PLGA/TiO$_2$ nanotubes during the initial 2 hours. (c) Accumulative percentage of ibuprofen releases from low molecular weight PLGA/TiO$_2$ nanotubes and from low molecular weight PLGA coated Ti foil during the first 50 hours. (d) The accumulative amount of ibuprofen release from the two samples in (c). (e) Accumulative percentage of ibuprofen releases from high molecular weight PLGA/TiO$_2$ nanotubes, and from high molecular weight PLGA coated Ti foil. (f)The accumulative amount of ibuprofen release from the two samples in (e). For each time interval, three samples were tested and the mean and standard deviation of these three samples were used in data analysis. The standard deviation was represented by the error bars in drug release profile graphs. ........................................................ 18

Figure 2.6 (a) Picture of PLGA/TiO$_2$ nanotubes and pure TiO$_2$ nanotubes on Ti foil after bending. (b) SEM image of PLGA/TiO$_2$ nanotubes at bending site showing no cracks; and (c) SEM image of pure TiO$_2$ nanotubes at bending site showing cracks. ......................................................... 20

Figure 3.1 UV-Vis spectra of lidocaine (a) and carprofen (b) in PBS pH 7.4. ......................... 26

Figure 3.2 SEM images of TiO$_2$ nanotube array (a) top view and (b) side view.................... 27
Figure 3.3 SEM images of TiO$_2$ nanotubes after soaked in PBS solution at 37 °C for 40 day.... 28

Figure 3.4 Accumulative percentage of lidocaine releases from pure TiO$_2$ nanotubes with different pH. Each point presents mean ± S. D. of three experiments.......................................................... 30

Figure 3.5 Accumulative percentage of carprofen releases from pure TiO$_2$ nanotubes with different pH. The experimental conditions are the same as illustrated in Figure 3.4. Each point presents mean ± S. D. of three experiments.......................................................... 30

Figure 3.6 Accumulative percentage of lidocaine releases from PLGA (Mw.66,000-107,000 Da)/TiO$_2$ nanotubes with different pH. Each point presents mean ± S. D. of three experiments........ 31

Figure 3.7 Accumulative percentage of carprofen releases from PLGA (Mw.66,000-107,000 Da)/TiO$_2$ nanotubes with different pH. The experimental conditions are the same as illustrated in Figure 3.6. Each point presents mean ± S. D. of three experiments........................................ 31

Figure 3.8 IR spectra of (a) pure TiO$_2$ nanotubes, PLGA, carprofen and lidocaine, (b) hybrid structures...................................................................................................................................... 34

Figure 3.9 Comparison between experimental data and model fitting using first order kinetics referring to lidocaine releases from PLGA / TiO$_2$ nanotubes at pH of 10.4. ......................... 41

Figure 3.10 Comparison between experimental data and model fitting using first order kinetics referring to lidocaine releases from PLGA / TiO$_2$ nanotubes at pH of 7.4. ......................... 42

Figure 3.11 Comparison between experimental data and model fitting using first order and Gallagher-Corrigan kinetics referring to lidocaine releases from PLGA / TiO$_2$ nanotubes at pH of 3.5.......................................................................................................................... 43

Figure 3.12 Comparison between experimental data and model fitting using first order kinetics referring to carprofen releases from PLGA / TiO$_2$ nanotubes at pH of 10.5. ......................... 43

Figure 3.13 Comparison between experimental data and model fitting using first order kinetics referring to carprofen releases from PLGA / TiO$_2$ nanotubes at pH of 7.4. ......................... 44

Figure 3.14 Comparison between experimental data and model fitting using first order and Gallagher-Corrigan kinetics referring to carprofen releases from PLGA / TiO$_2$ nanotubes at pH of 3.5.......................................................................................................................... 45
Acknowledgements

First and foremost, I would like to express my deepest and sincere gratitude to Dr. Lei Kerr, for her invaluable guidance, continuous support and constant encouragement during my graduate studies. Without her incredible support and encouragement, this thesis would not have been possible. I hardly can find words to thank her enough.

I would also like to extend my appreciation to committee members, Dr. Lalvani and Dr. Almquist, for their valuable advice, insightful feedback and thoughts on my research.

My sincere appreciation is extended to Mrs. Laurie Guest and Mr. Douglas Hart for all the help and assistance in the past few years.

I would specially like to thank Dr. Richard Edelmann and Mr. Matthew Duley for their support and valuable time in conducting SEM experiments.

I would like to thank all the faculty members and graduate students for their generous help and assistance throughout my graduate studies.

Last but not least, I would like to thank my parents, my husband, Jianbo and my little girl, Sophie, for all their love, support, and encouragement in my life.
1. Introduction

1.1 Introduction of drug delivery and current state of art in drug delivery

Applications of controllable drug delivery have drawn tremendous attention of scientist, and the rapid expansion in the fields of biomedical as well as material science has resulted in a remarkable progress. To control drug delivery is to administer the necessary amount of drug safely and effectively to specific sites in the human body and to regulate the temporal drug profile for maximum therapeutic benefits[1]. Contemporary, almost all of the medical implant procedures require some type of drug therapy regiments. In general, oral antibiotic administration is not effective to prevent bacterial infection. This has to do with the adsorption of the drug as it travel through the entire body of the patient. As a result the requisite amount of drug cannot reach the infection site in implant surface. In addition, increasing drug doses cannot solve this problem because it will lead to organ toxicity. Thus, delivery of drugs locally from an implant surface has become a commonly used option to prevent the infection. This drug delivery method has several merits over the traditional ways: (a) taking effect at a lower dose; (b) avoiding the possibility of organ toxicity and reducing the possibility of antibiotic resistance; and (c) realizing the controllable drug release.

The most common used carrier substance for antibiotics and other antibacterial substances to allow drug release from an implant surface in a controllable rate is the biodegradable polymer drug carriers such as poly(lactic-co-glycolic acid) (PLGA), poly(lactic acid) (PLA), and polyglycolides (PGA) which have several advantages over the traditional used polymer carriers. For example, most biodegradable polymers will undergo hydrolysis degradation and be decomposed into biologically acceptable, much smaller compounds within the body, without the need to remove a drug delivery system after the release process[2, 3]. However, like most of the polymeric materials, biodegradable polymers such as PLGA, PLA and PGA are not bioactive, and they cannot promote cell growth when they are used in implant
coating. Besides, the biodegradable polymers usually have lower tensile strengths and moduli (stiffness), and may be subjected to wear which make them less suitable for load-bearing applications[4, 5].

The newest developments in drug release systems are based on inorganic nanomaterials drug delivery platforms. Inorganic nanomaterials such as nanotube alumina[6], porous silicon[7], nanostructured ceramics and nanostructured TiO$_2$[8] have already shown great potential in biomedical coatings and implants. The requirement for the nanomaterial for drug delivery is that they must be biocompatible, mechanical and chemical stable. In addition, the most important aspect is to be able to incorporate a drug, preserve it and delivers it gradually over the time. One of the advantages of application of nanomaterials in drug delivery is the ability to control the material properties precisely. Drug loading and release rate can be adjusted by varying the pore size, pore distribution and the film thickness. The accommodation of various drug molecules can also be realized by changing the surface into hydrophobic or hydrophilic by modifying surface charges of the pores. Thus, nanostructured materials may provide constant delivery of a pharmacologic agent to the site in the body where it is needed, providing appropriate treatment over an extended time. For example, Stigter studied the release of different antibiotics (gentamicin, cefamandol, tobramycin and cephalothin) loaded in carbonated HA coatings on titanium alloy implants. They found that antibiotics can band with calcium through the carboxylic and release at a slower rate as a result[9]. However, the longest drug release achieved on calcium phosphates or synthetic HAs coating is no longer than 3 days[10]. Besides, many inorganic nanomaterial have biocompatibility issues and are not suitable for using as drug delivery material. Several studies have shown that association between aluminum concentration in the brain and Alzheimer’s disease[11, 12] although aluminum and its oxides have been proven to be suitable for the orthopedic implants. Nanostructured TiO$_2$ and porous silicon are the most promising material to be used in drug delivery implant coating[1]. This thesis focused mainly on the nanostructured TiO$_2$. 
TiO₂ is one of the most important compounds of titanium and oxygen, and has many important applications such as photocatalysis and solar cell. Besides, Ti based alloys and TiO₂ are the most common used implant materials in the human body (such as cardiovascular stents, joint replacements and dental implants), and about 40% of today’s biomedical implant materials are made by Ti or Ti alloys. TiO₂ is chemically stable, relatively nontoxic, and environment friendly. Especially, TiO₂ particles show weak or no toxicity in vivo[13]. Thus, TiO₂ nanostructure is expected to be a more favorable material for realizing drug delivery to prevent bacterial infection around the implant.

1.2 Advantages of using TiO₂ nanotube in drug delivery and their current state of art research

Several methods such as sol-gel, hydrothermal, vapor deposition and anodization have been used to the synthesis of nanostructured TiO₂, from nanopores to nanowires and nanotubes. In 1999, Zwilling and coworkers successfully synthesized self-organized titanium nanotube by electrochemical anodization[14, 15]. This finding stimulated intense research on growth, modification, properties, and applications of these unique one dimension nanostructure materials. Today, scientists have realized the precisely control the nanotubes diameters, spacing between the nanotubes, crystallinity, and length of the tubes by varying anodization parameters including electrolyte concentration, pH, voltage, and bath temperature[16]. For example, when using aqueous electrolytes, TiO₂ nanotubes with diameter of ~ 40 nm can be obtained at applied voltage of 10 V, and the diameter increased to ~ 110 nm when the voltage changed to 25 V.

The nanotubular structures of TiO₂ with open volume indicate themselves as one of the ideal candidates serving as carrier for drug load and release and it is also confirmed that TiO₂ nanotube could be used as drug release carrier[17]. Much effort has been devoted to investigation the controllable drug release using TiO₂ nanotube. Shrestha filled TiO₂ nanotubes with Fe₃O₄ and such magnetic nanoparticles can be guided to desired locations by the magnet field[18]. TiO₂ tubes can also be filled with drugs which are attached to the nanotube wall by linker molecules. Drug release could be triggered by UV light which could break the bonds
between the linker molecules and the TiO2 nanobube[19]. Song et al. produced amphiphilic layers on the top of the TiO2 nanotube which prevented water to enter into the tubes[20]. After UV light irritation, the hydrophobic layer was removed from the nanotube and drugs filled in the tube could be released.

It is also found that TiO2 nanotube coatings of Ti are not only biocompatible but even promote bone growth compared to bare titanium[21]. Ti implants with smooth surface has been shown to initiate detrimental fibrous tissue encapsulation which is one of the common results of implant failures. The cell culture experiments demonstrated that osteoblast adhesion increased significantly on TiO2 nanotube samples comparing to bare TiO2 surface[22]. This phenomenon might be attributed to several reasons: (1) the similarity between the structure of TiO2 tube and bones which is composed of collagen fibers with nanostructure inorganic minerals; (2) the optimal initial protein interactions happening on the anodized titanium. In addition, TiO2 nanotube can be grown directly from the native underlying Ti implant. Compared with other commonly used bioactive implant coating methods such as calcium phosphates, synthetic HAs coating and TiO2 nanoparticle coating which introduce foreign ceramic and spray coatings on Ti implant surfaces, much greater adhesion strength is obtained from the nanotube coating on the Ti substrate[23]. Thus, improved long term performance of the implants is expected based on such nanotubular TiO2 modified surface. In addition, growth of uniform nanotube layers can be easily realized on complex shaped surfaces (such as dental-implant screws or hip implants) which is the most striking feature of this technique.

1.3 Problem statement

TiO2 nanotube has some unsolved problems when applied in drug delivery. For example, TiO2 nanotube film has poor mechanical flexibility as we can see from Figure 1.1. Besides, the drug elusion time is only several hours in most of reports when using the TiO2 nanotubes as drug carriers[15-17, 24]. For example, Xiao[25] found that 90% of the total loaded cisplatin released from the TiO2 nanotube at the first 100 min, no matter how much drug was
loaded (see Figure 1.2). In these reports, drugs were filled inside the nanotubes by casting or dip coating the drug solution. There is a big difference between the pore size of the TiO₂ nanotube which is between 20-100 nm and the size of loaded small drug molecules which is usually several nanometers. The diffusion of drug cannot be hindered or restricted by the TiO₂ nanotube alone. Realizing both sustained and controllable drug release is of great importance for the extensive application of TiO₂ nanotubes in drug delivery.

**Layer cracking off**

![Layer cracking off](image)

(a) (b)

Figure 1.1 (a) The TiO₂ nanotube layer shows cracks and partial fracturing of the layer at the bending edge[26]; (b) The TiO₂ membrane will curls and break into shards after detached from Ti and dried in air[27].

![Graph](image)

Figure 1.2 Cisplatin released amount (right axile) and the fraction of total drug released (left axile) over time.
axile) from nanotubes filled with 75, 150, and 225 μg of cisplatin (a, b and c, respectively)[26].

Therefore, achieving controlled release kinetics of drugs from TiO₂ nanotube and improving the mechanical properties of the nanotube are extremely important to explore the application of titanium nanotubes as novel drug delivery carriers.

1.4 Hypothesis

In this thesis we developed a method for controllable drug release based on the anodized TiO₂ nanotube film. A drug carrier using a hybrid structure which TiO₂ nanotubes were filled with biodegradable polymers is designed. The rate and duration of drug release could be controlled by choosing proper polymers. The fabrication process is described in Figure 1.3.

![Figure 1.3 Schematic description of the fabrication of drug/polymer loaded TiO₂ nanotube array](image)

This hybrid structure will combine the advantages of both the biodegradable polymer and the TiO₂ nanotube arrays. Biodegradable polymers will be degraded into biologically acceptable small molecule as the process of hydrolysis, avoiding the danger of macromolecular polymer accumulation in the body. Besides, when the TiO₂ nanotubes incorporate with the polymer, the mechanical properties of the material such as flexibility and the toughness could be greatly improved by forming the interpenetrating structures. In addition, the TiO₂ nanotube in this hybrid material will promote bone growth compared to bare titanium.
2. Sustained Ibuprofen Release Using Composite Poly(Lactic-co- Glycolic Acid)/Titanium Dioxide Nanotubes from Ti Implant Surface

Huiying Jia and Lei Kerr
Department of Chemical & Paper Engineering, Miami University
Oxford, Ohio 45056, United States

2.1 Introduction

Titanium (Ti) implant is the most widely used implant material. The dissolution of Ti into body is very insignificant. The Ti metal surface can spontaneously form a stable and inert layer of titanium oxide (TiO$_2$, titania) which will prevent Ti metal from reacting with body fluid. The excellent biocompatibility (high corrosion resistance, low ion-formation tendency, low level of electronic conductivity, etc.) owing to this oxide layer made titanium one of the most widely-used metallic implant materials[28]. Delivery of drugs locally from a Ti implant surface has become a commonly used option to prevent infection, reduce organ toxicity and minimize drug resistance. The benefit of sustained drug release will not only significantly reduce the undesired side effects and the frequency of administering drugs. It may also eliminate the need for self-administration and improve patient compliance. Technology development like this work to modify Ti implant surface with TiO$_2$ nanotubes as drug carrier is important to achieve the sustained local drug delivery right at the Ti implant surface. The newest developments in drug release systems are based on inorganic nanomaterial drug delivery platforms. The advantage of the application of nanomaterials in drug delivery is the ability to control the nanomaterial properties precisely. The TiO$_2$ nanotubes are one of the most attractive nanomaterials for this application because of their biocompatibility, high surface area, ability to promote direct tissue and bone growth [21, 29]. Researchers have also used other bioactive implant coatings such as calcium phosphates [9] and synthetic carbonated hydroxyapatite[10]. However, these coatings
introduce foreign materials on Ti implant surfaces and have much lower adhesion strength compared to the TiO₂ nanotubes coating directly grown on the Ti surface[23]. Thus, improved long-term performance of the implants is expected based on such nanotubular TiO₂ modified Ti implant surface compared to other implant coatings. Growth of uniform nanotube layers can be easily realized on complex shaped surfaces (such as dental-implant screws or hip implants), which is the most striking feature of this technique. In this study, ibuprofen is selected as the concept drug. It is a non-steroidal anti-inflammatory drug (NSAID) and is an extensively used analgesic, antipyretic, and anti-inflammatory drug. Pain management and reduction of inflammation after implant surgeries are important. However, ibuprofen has a fairly short duration of action due to its plasma half-life of only 1-3 hours and requires frequent oral or parenteral administration [30]. Therefore, prolonging ibuprofen release time is needed.

Several methods such as sol-gel, hydrothermal, vapor deposition and anodization have been used in the synthesis of nanostructured TiO₂, from nanopores to nanowires and nanotubes. In 1999, Zwilling and coworkers successfully synthesized self-organized TiO₂ nanotubes by electrochemical anodization[14, 15]. This finding stimulated intensive research on growth, modification, properties, and applications of these unique one-dimension nanostructure materials. The nanotubular structures of TiO₂ with open volume indicate themselves as one of the ideal candidates as carrier for drug loading and release[17]. However, the issue with TiO₂ nanotubes as the drug carrier is the short drug elusion time of only several hours in most of the reports [25, 31]. For example, Xiao found that 90% of the total loaded cisplatin released from the TiO₂ nanotubes in the first 100 min, no matter how much drug was loaded. Song et al.[31] found that 90% of horseradish peroxidase was released in the first minute from untreated TiO₂ nanotubes and adding hydrophobic monolayer of octadecylphosphonic acid only improved the release rate by less than two orders. In these reports, drugs were filled inside the nanotubes by cast or dip coating. There was a big difference between the diameter of the TiO₂ nanotubes (20-100 nm) and the size of loaded small drug molecules (~several nanometers). Thus, the diffusion of drug cannot be hindered or restricted by the TiO₂ nanotubes alone. Polymer coating has been proven
to be an effective method to improve the drug elution from TiO₂ nanotubes [32, 33]. In these studies, polymers were just cast coated directly on the top of TiO₂ nanotubes which were already loaded with drugs [32, 33]. The role that polymer plays was a cap to slow down the drug release from the TiO₂ nanotubes [32, 33]. However, this way, the potential benefit of TiO₂ nanotubes to promote bone growth is diminished since TiO₂ nanotubes are buried underneath the polymer cap and are not exposed to the blood and bone cells. Another issue with TiO₂ nanotubes coated Ti implant is their poor mechanical properties [34, 35]. In our study, a composite of drug loaded Poly(lactic-co-glycolic acid) (PLGA) infiltrated TiO₂ nanotubes was synthesized and could improve the ibuprofen drug release time from 30 minutes to 6 days (low molecular weight PLGA) and 9 days (high molecular weight PLGA). PLGA approved by FDA for therapeutic devices [36] is chosen as model polymer because of its bioresorbable and biocompatible properties, which enable it as a promising drug carrier for clinical applications. The interactions between polymer and TiO₂ nanotubes and their influence on the drug release performance of such composite were investigated. The advantages of using the matrix network of drug/polymer/TiO₂ nanotubes in our study are that (1) the use of TiO₂ nanotubes to promote bone growth can be realized; (2) The use of TiO₂ nanotubes/polymer provides a way to control the drug loading and release via tuning the lengths and diameters of the nanotubes and employing different types of polymers. In this work, improved mechanical strength and flexibility were demonstrated for the first time in literature by using PLGA/TiO₂ nanotubes composite.

2.2 Experimental section

2.2.1 Anodized TiO₂ nanotube film synthesis

Anodized TiO₂ nanotube films were synthesized by a two-electrode DC anodization system using 0.25mm thick pure Ti foil (99.97% purity, Sigma-Aldrich). Prior to anodization, Ti foils were ultrasonically cleaned in deionized water, ethanol, and acetone. A Ti foil acted as the working anode and a graphite rod was used as the cathode. The two electrodes were separated by a distance of approximately 2 cm. Anode and cathode were put into a beaker containing the
desired electrolyte, and the whole system was magnetically stirred. The organic electrolyte contains ethylene glycol, 0.3 wt. % NH₄F, and 3 vol. % H₂O. The fabrication of the TiO₂ nanotubes was processed by a two-step anodization. In the first step, the Ti foil was anodized in 100 ml electrolyte at 50 V for 12 hours. Then the TiO₂ nanotube layer was ultrasonically removed in deionized water. In the second step, anodization was performed using the pretreated Ti foil from first step at 50 V for 2 hours to get defect free nanotubes. Finally, the sample was rinsed with ethanol and then dried at room temperature.

2.2.2 Polymer/ibuprofen loading onto TiO₂ nanotubes

Ibuprofen and PLGA were obtained from Sigma-Aldrich. Three types of samples were prepared. The first type was made by directly loading ibuprofen into pure TiO₂ nanotubes on Ti foil (ibuprofen/pure TiO₂ nanotubes/Ti foil). The ibuprofen was loaded into TiO₂ nanotubes by dipping TiO₂ nanotube film/Ti foil in a solution of 30 mg/ml ibuprofen in ethanol for 3 days and then dried in air. The second type was made by loading mixture of ibuprofen/PLGA into TiO₂ nanotubes on Ti foil (ibuprofen/PLGA/TiO₂ nanotubes/Ti foil). Two types of PLGA with different molecular weights (Molecular weight 4,000-15,000 and 24,000-38,000) were selected. PLGA and ibuprofen were dissolved in dichloromethane. The concentration of PLGA and ibuprofen in the solution were 12 mg/ml and 6 mg/ml, respectively. The polymer/drug mixture was loaded into TiO₂ nanotubes/Ti foil by a dip coating process in the PLGA/ibuprofen solution for 3 days at 40 °C, and then dried in air. The third type was made by loading PLGA/ibuprofen mixture directly on bare Ti foil, which served as a bench mark sample to investigate the effect of TiO₂ nanotubes.

2.2.3 Scanning electron microscopy (SEM) imaging of PLGA/nanotubes

In this study, SEM was used to demonstrate the good infiltration of PLGA polymer into TiO₂ nanotubes. First, the top side of PLGA filled TiO₂ nanotubes were attached to a scotch tape. Then the tape was peeled off to separate the TiO₂ nanotube film from the Ti foil. The tape with
TiO$_2$ nanotubes was soaked in 5 vol. % HF solution for 10 minutes until all the TiO$_2$ nanotubes templates were etched off. The remaining PLGA was examined by SEM.

2.2.4 Drug loading and release study

1. Calibration curve

To generate calibration curve, ibuprofen with known concentration in PBS was characterized by UV-Vis spectrometer (WGS-9 Chromatic). Ibuprofen has typical absorption at wavelength of 222 nm when dissolved in phosphate buffered solution (PBS) as shown in Figure 2.1 (a). The intensity of absorption as a function of ibuprofen concentration was then recorded to generate the calibration curve. The calibration curve is shown in Figure 2.1 (b).

![Uv-vis spectrum of ibuprofen](image1.png) ![Calibration curve of ibuprofen](image2.png)

Figure 2.1 (a) Uv-vis spectrum of ibuprofen (b) calibration curve of ibuprofen.

2. Drug release study

The drug release experiments were performed by immersing the above three types of samples into PBS of pH 7.4 at 37°C in water bath. At pre-determined time intervals, samples were withdrawn and analyzed with UV-Vis spectroscopy. Absorbance was measured at 222 nm and the corresponding ibuprofen concentration was found from the calibration curve of ibuprofen in PBS. The percentage of drug release was calculated by dividing the accumulated amount of released drug by the total drug loading amount. The total drug loading amount was the amount of
drugs released at the end of experiment when the UV-Vis absorption did not change any more. For each time interval, three samples were tested and the mean and the standard deviation of these three samples were used in data analysis. The standard deviation was represented by the error bars in drug release profile graphs.

2.2.5 Tensile bonding strength test

The tensile bond strength test between TiO$_2$ nanotube layer and Ti substrate was conducted. TiO$_2$ nanotube films with/without polymer on Ti foil were glued onto the surface of clean Ti bar using epoxy adhesives as shown in Figure 2.2. Please note that the as-deposited anodization sample has TiO$_2$ nanotubes grown on both sides of Ti foil. After 24 hours curing and hardening of the glue, the samples were loaded in the INSTRON universal tester. A ball joint was used to keep a good axial alignment during the test. A crosshead speed of 1 mm/min was set during the tensile test. The bonding strength was obtained by dividing the maximum load with TiO$_2$ nanotubes area attached to the Ti bar. The tensile bond test was also done on the samples after immersion in PBS solution at 37°C. After the immersion, samples were removed and washed with deionized water and then dried. The tension strength test followed the same
procedure mentioned above.

2.3 Results and discussion

2.3.1 TiO$_2$ nanotubes synthesized by anodization in NH$_4$F based organic electrolyte

Figure 2.3 shows the TiO$_2$ nanotube arrays by a two step anodization of Ti foil in a 24 hours aged ethylene glycol electrolyte containing 0.3 wt % NH$_4$F and 3 vol% H$_2$O. The top view of nanotube layer at different magnification is shown in Figure 2.3(a) and (b). It is apparent that highly ordered nanotube arrays with a diameter of 100 ~120 nm were formed throughout the whole Ti foil. Figure 2.3(c) shows the side view of the nanotube layers. The surface of nanotube layer presented a high degree of smoothness and was free of surface debris. The thickness of the nanotubes is approximately 10 μm.

Figure 2.3 SEM images of TiO$_2$ nanotube array generated in an ethylene glycol electrolyte containing 0.3 wt. % NH$_4$F and 3 vol.% H$_2$O at 50 V for 2 hours (a) top view, (b) top view at
higher magnification, (c) side view.

2.3.2 Scanning electron microscope (SEM) characterization of PLGA/drug infiltration in TiO$_2$ nanotubes

Optimizing the infiltration of the mixture of PLGA and drug into TiO$_2$ nanotubes is of particular importance for improving the performance of the composite structure, such as mechanical properties. It has always been a challenge to characterize the extent of polymer infiltration into TiO$_2$ nanotubes. Transmittance electron microscope (TEM) is by far the most popular technique used. However, by using TEM only single TiO$_2$ nanotube can be investigated and does not give the idea of whole sample. In addition, the preparation of TEM sample requires tedious procedures including separating nanotubes. The sample preparation procedures question the reliability of TEM results. In this work, we use a simple method to give a direct illustration of the degree of polymer infiltration into TiO$_2$ nanotubes. In this method, the PLGA infiltrated nanotube film on scotch tape (see the experimental section) was immersed in 5 vol. % HF solution to etch away the TiO$_2$ nanotubes and the remaining polymer membrane was kept for SEM measurement. Figure 2.4 shows the SEM images of the remaining PLGA of different molecular weights after the removal of the TiO$_2$ nanotubes. We can see from Figure 2.4(a) that the remaining PLGA of high molecular weight forms nanotubes (or nanowires) homogenously in a large area. The top part of the PLGA nanotubes (or nanowires) is closely packed. The length of the tube (wire) is about 10 μm which is of the same length of TiO$_2$ tube. It could be conclude that the PLGA has excellent infiltration depth into the TiO$_2$ nanotubes. Figure 2.4(b) and the inset show the top and bottom PLGA nanotubes (or nanowires) at a higher magnification. More tubes of PLGA are formed than wires. The tube structure of PLGA indicates that PLGA does not completely fill the TiO$_2$ nanotubes and rather form a thin layer onto the wall of the TiO$_2$ nanotubes. In addition, the thickness of the PLGA tube wall decreases gradually from the top (surface) to the bottom (towards the Ti substrate) indicating that more PLGA fills into the top part of TiO$_2$ nanotubes than into the bottom. We also examined the PLGA nanotubes formed by
lower molecular weight and we can see in Figure 2.4(c) that the nanotubes have the same structure as those of tubes formed by higher molecular weight PLGA.

Figure 2.4 SEM images of the remaining PLGA nanotubes after removing the TiO$_2$ nanotube using 5 vol. % HF solution. (a) High molecule weight PLGA nanotubes (Molecular weight. 24,000-38000) at lower magnification. (b) High molecule weight PLGA nanotubes (Molecular weight. 24,000-38000) at higher magnification and inset shows the bottom part of PLGA nanotubes at higher magnification. (c) Low molecule weight PLGA nanotubes (Mw. 4000-15000).

2.3.3 Drug release study

In order to reveal the potential of using ibuprofen loaded PLGA/TiO$_2$ nanotubes as carriers for the controlled release of ibuprofen, we compared the drug release profiles of three different samples: ibuprofen loaded pure TiO$_2$ nanotubes, ibuprofen loaded low molecular weight
PLGA/TiO₂ nanotubes, and ibuprofen loaded high molecular weight PLGA/TiO₂ nanotubes. As we can see in Figure 2.5(a) and (b), the three drugs release profiles are quite different. For the drug release directly from nanotubes, as expected, the release rate is very fast and almost all the drug embedded in the TiO₂ nanotubes is released in the first 30 minutes according to Figure 2.5(b). This result is in consistence with the literature results that TiO₂ nanotubes alone have little influence on controlling the eluding of small drug molecules [25]. Compared with the burst release of ibuprofen loaded pure TiO₂ nanotubes, the drug release rate of ibuprofen from PLGA/TiO₂ nanotubes composite is significantly lowered. Ibuprofen release time lasted 5 days (Figure 2.5(c)) by using low molecular weight PLGA (Molecular weight 4,000-15,000)/TiO₂ as carrier, and extended to 9 days (Figure 2.5(e)) when using higher molecular weight PLGA (Molecular weight 24,000-38,000). This could be ascribed to the considerable restriction from polymer chains to drug molecule movement, as well as the deceleration of diffusion due to polymer swelling which will reduce the volume of pores and channels available for drug diffusion inside the polymer matrix[37]. It is also obvious that the increase of PLGA molecular weight will decrease the drug eluding rate due to the decreased ibuprofen diffusivity. Biodegradable polymers are degraded into oligomers and monomers in the human body which leave vacancies in the polymer matrix. For low molecular weight polymer, the oligomers and monomers are generated and dissolved more quickly than the high molecular weight polymer. The more vacancies in low molecular weight polymer allow the drug molecules to travel in the polymer matrix much more easily. Figure 5(b) shows that there are two stages in the drug loaded polymer/TiO₂ release profile: initial burst release and an extended region with a much lower release rate. For drug loaded low molecular weight PLGA/TiO₂ nanotubes, the initial burst release during the first 6 hour is 85% of the total loaded drug. While the extent of the drug burst is significantly decreased to 65% when increasing PLGA molecular weight from 4,000 to 28,000. The burst release could be explained in two ways. First, ibuprofen molecules which are loosely associated with the PLGA surface or embedded in the surface layer are responsible for the burst release[38]. The high concentration gradient between the drug carrier and release medium is
another possible cause of the high initial burst. From the release profiles, it could be concluded that the PLGA molecular weight has a significant influence on the burst drug release and total release time. High molecular weight polymer will reduce the burst release rate and provide longer overall release time compared with the low molecular weight polymer. Figure 2.5(c) and (e) show the release kinetics of ibuprofen from low molecular weight and high molecular weight PLGA/TiO$_2$ nanotubes and PLGA/Ti foil. It is apparently that release profiles from nanotubes and foil are quite similar, implying that the release behaviors of drug molecules from these two structures are similar. Combining the results from Figure 2.5(b), it could be inferred that the drug release from PLGA/TiO$_2$ nanotubes or PLGA/Ti is different in comparison with drug delivered directly from TiO$_2$ nanotubes. When polymer exists in this system, the drug release is mainly controlled by the transport of drug through the polymer matrix and by the rate of polymer degradation [33, 39, 40]. We also studied the accumulative amount of drug release from polymer/TiO$_2$ nanotubes and polymer/Ti foil which is shown in Figure 2.5(d) and Figure 2.5(f). Obviously, a much higher amount of drug (0.14 mg for low molecular weight and 0.27 mg for high molecular weight) is released from PLGA/TiO$_2$ nanotubes compared to that (0.10 mg for low molecular weight and 0.14 mg for high molecular weight) from PLGA/Ti foil. This indicates that employing ordered TiO$_2$ nanotubes surface could capture more drug molecules due to the large surface area of TiO$_2$ nanotubes available for PLGA/ibuprofen infiltration.
Figure 2.5 (a) Ibuprofen releases from pure TiO$_2$ nanotubes, from low molecular weight PLGA/TiO$_2$ nanotubes and from high molecular weight PLGA/TiO$_2$ nanotubes. (b) Ibuprofen releases from pure TiO$_2$ nanotubes, from low molecular weight PLGA/TiO$_2$ nanotubes and from high molecular weight PLGA/TiO$_2$ nanotubes during the initial 2 hours. (c) Accumulative percentage of ibuprofen releases from low molecular weight PLGA/TiO$_2$ nanotubes, and from low molecular weight PLGA coated Ti foil during the first 50 hours. (d) The accumulative amount of ibuprofen release from the two samples in (c). (e) Accumulative percentage of ibuprofen releases from high molecular weight PLGA/TiO$_2$ nanotubes, and from high molecular weight PLGA coated Ti foil. (f) The accumulative amount of ibuprofen release from the two samples in (e). For each time interval, three samples were tested and the mean and standard
deviation of these three samples were used in data analysis. The standard deviation was represented by the error bars in drug release profile graphs.

In this study, the drug release was performed via in vitro method at 37 °C intended to simulate the drug release environment in the human body. However, practical issues such as the influences of the fluid dynamics around the implant area, cellular and enzymatic activities on the drug release profile need to be considered before this technology could be adapted. This will require intensive in vivo studies in the future. Nevertheless, in vitro results provide a useful approach to predict the drug release profile and provide a guideline for planning in vivo studies.

2.3.4 Mechanical properties

TiO$_2$ nanotubes adhesion strength and flexibility are important factors in evaluating the potential application of the TiO$_2$ nanotube film as implant coating. Here, we used the tensile strength test and bending test to investigate the mechanical properties’ changes before and after the polymer infiltration. Table 2.1 gives the tensile bonding strength test results. The bonding strength of pure TiO$_2$ nanotubes is 1.38 MPa, while the strength of the polymer infiltrated nanotubes is four times more than that of pure TiO$_2$ nanotube film which is 4.4 MPa. This indicates that the polymer infiltration could greatly improve the adhesion strength by forming interpenetrating structures as seen from Figure 2.4. After immersion in PBS solution at 37°C for 28 days, the decrease of the bonding strength of both pure and polymer infiltrated TiO$_2$ nanotube film are found to be negligible. This indicates the superior mechanical and chemical stability of using TiO$_2$ nanotubes as Ti implant surface coating for drug carrier applications.

<table>
<thead>
<tr>
<th>Soaking Time (days)</th>
<th>Without polymer</th>
<th>With polymer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tensile Strength (Pa)</td>
<td>1.38×10$^6$</td>
<td>1.37×10$^6$</td>
</tr>
</tbody>
</table>
A bending test is also used to study the mechanical properties of the TiO$_2$ nanotubes. When bend to the same angle which is about 60°, pure TiO$_2$ nanotubes array layer cracks and part of nanotube film detaches from the Ti substrate as seen from Figure 2.6(a); while the polymer infiltrated TiO$_2$ nanotubes sample shows no cracks. In addition, SEM is used to study the microscopic morphology at the bending site. As illustrated in Figure 2.6(b) and (c), pure nanotube film shows many continuous cracks while PLGA infiltrated nanotube film has few cracks at the same magnification. These tensile strength and bending tests indicate that the incorporation of PLGA into TiO$_2$ nanotubes enhances the mechanical flexibility of TiO$_2$ nanotubes. To the best of our knowledge, this is the first study that reports the mechanical properties of polymer infiltrated TiO$_2$ nanotubes.

Figure 2.6 (a) Picture of PLGA/TiO$_2$ nanotubes and pure TiO$_2$ nanotubes on Ti foil after bending. (b) SEM image of PLGA/TiO$_2$ nanotubes at bending site showing no cracks; and (c) SEM image
of pure TiO$_2$ nanotubes at bending site showing cracks.

2.4 Conclusions

Ibuprofen drug release time was prolonged to 5 days (low molecular weight PLGA) and 9 days (high molecular weight PLGA) using the composite of PLGA/TiO$_2$ nanotubes as drug carriers versus 30 minutes of pure TiO$_2$ nanotubes. The simple characterization method developed in this study showed good PLGA polymer infiltration into TiO$_2$ nanotubes. The drug release is mainly controlled by the transport of drug through the polymer. TiO$_2$ nanotubes was able to capture more drugs during drug loading on to Ti foil compared to pure PLGA coating on Ti foil. The tensile strength and bending test also demonstrate much improved mechanical properties of PLGA/TiO$_2$ nanotubes compared to pure TiO$_2$. 
3. Study of the Mechanism to Control Carprofen and Lidocaine Release from TiO$_2$ Nanotubes

3.1 Introduction

Titanium (Ti) implant is a widely used implant materials. Titanium is the most common implant material due to its strong resistance to body fluid, high mechanical strength, light weight and most importantly biocompatible. There are numerous Ti implant surgeries performed in both human and animals each year. Nanotechnologies such as coating Ti implants with TiO$_2$ nanotubes have attracted great research interests in the past few years and have demonstrated to be a promising technology for controlled local drug delivery[41]. However, the drug release mechanism from these novel drug carriers is not known. This study selects carprofen and lidocaine as the model drugs to investigate the drug release mechanism from TiO$_2$ nanotubes due to their frequent use in canine implant surgeries. Canine implant surgeries (e.g. orthopedic and dental) are performed to treat hip, elbow dysplasia, osteosarcoma, and tooth replacement, etc. Carprofen is a commonly used nonsteroidal anti-inflammatory drug (NSAIDS) on dogs after orthopedic surgeries to manage the pain and reduce the inflammation. However, the plasma half-life of carprofen is only 8–9.8 hours in dogs [6]. This short half-live means that more frequent dosage and drug administration is required. Organ toxicity e.g. kidney poison has prevented many drugs that approved for humans from being used on animals. Oral anti-inflammatory or pain management drugs are not effective for preventing inflammation and reducing the pain around the implants because most drugs travel through the whole body as well as be absorbed by the liver, intestine, kidneys, or lungs. As a result sufficient dosage does not reach the infection site in the implant surface. Increasing drug doses cannot solve this problem because it will lead to organ toxicity. Especially, there have been 6,000 reports to FDA of the adverse reactions of carprofen in causing gastrointestinal, liver and kidney problems in dogs [6]. Thus, technology like the drug carrier developed in this study is needed to deliver carprofen
locally from the Ti implant surface. Delivering drugs locally will have less demand in the drug dosage to achieve the effective relieve of inflammation and management of the post-surgery pain and thus allow the veterinary doctors to have wider drug selections for dogs due to the reduced required drug dosage and organ toxicity. During implant surgeries, local anesthesia is usually conducted on dogs. There is an urgent clinical need for providing prolonged duration local anesthesia from single injection. The local anesthetic effect generally lasts only a few hours [7]. Lidocaine is a commonly used local anesthetic drug on both human and dogs. Various techniques have been used to prolong the duration of effect of lidocaine, such as nanoparticle drug carrier [7], adding vasoconstrictor [8], repeated injection, etc. These approaches have disadvantages to prevent them from being used clinically. For example, nanoparticle drug carrier travels through the whole body and induces further cytotoxicity. Vasoconstrictor can cause serious side effects such as tachycardia, arrhythmias, allergic reaction to sulfite and seizures [8]. The developed drug carrier in this study will not have any toxic effect because the TiO$_2$ is inherent grown on Ti implant surface with excellent adhesion and will not fall off the Ti surface and travel through the whole body. In addition, TiO$_2$ can actually serve as a barrier layer to prevent Ti ion dissolution from Ti implant surface. We anticipate that sustained lidocaine release time can be achieved by using the TiO$_2$ nanotube drug carrier. Thus, lower dosage and longer duration of effect can be achieved and allergic reaction and side effect to local anesthesia can be minimized.

3.2 Experimental section

3.2.1 Preparation of Drug Carrier

The drug carrier used in this study consists of structures of TiO$_2$ nanotube infiltrated with PLGA/drug matrix. To make this drug carrier, it involves the experiments steps of (1) synthesis of TiO$_2$ nanotube film (2) loading of PLGA/drug into TiO$_2$ nanotube

(1) Synthesis of TiO$_2$ nanotube film

TiO$_2$ nanotubes on Ti foil were synthesized by a two step anodization method as described in our previous study[41]. 0.25 mm Ti foil (99.97% purity, Sigma-Aldrich) was cleaned
ultrasonically in DI water, ethanol and acetone for 5 minutes each and dried in air stream. The anodization was performed in a two electrode (a graphite rod as cathode, a Ti foil as anode) electrochemical cell containing ethylene glycol, 0.3 wt. % NH₄F, and 3 vol. % H₂O under stirring. The first anodization was carried out at 50 V for 24 hours. Then, the TiO₂ nanotubes layer was removed in DI water ultrasonically. The second anodization was performed with the pretreated Ti foil at 50 V for 3 hours. Highly ordered TiO₂ nanotubes arrays could be obtained by this two step anodization. Finally, the Ti foil with TiO₂ nanotubes was cleaned in ethanol to remove the electrolyte and dried in air.

(2) Loading of PLGA/drug into TiO₂ nanotube

Lidocaine, and poly(lactic-co-glycolic acid) (PLGA, Molecular weight: 66,000-107,000) were obtained from Sigma-Aldrich. Carprofen was purchased from Fluka. Solutions of PLGA/drug of 12 mg/ml and 6 mg/ml were loaded into TiO₂ nanotubes/Ti foil by a dip coating process for 3 days, and then dried in air for 24 hours. Good infiltration depth of polymer in TiO₂ nanotube was demonstrated by a technique developed in our previous study[41]. For drug loaded pure TiO₂ nanotubes, TiO₂ nanotubes/Ti foil was soaked in 6 mg/ml lidocaine/carprofen acetone solution for 3 days and then dried in air. Infrared (IR) spectroscopy was performed using Perkin Elmer Spectrum One spectrometers with an ATR accessory to study the possible chemical bonding interaction between drugs and PLGA/nanobubes. Scanning electron microscopy (SEM, Zeiss Supra 35) was used to characterize the morphology of the drug carrier.

3.2.2 In vitro drug release studies

The lidocaine and carprofen drug releases were studied in (a) sodium acetate buffer, pH 3.5, (b) phosphate-buffered saline (PBS), pH 7.4, and (c) phosphate buffer, pH 10.5, respectively. All the buffer solution concentrations were 0.01 M. The release studies were performed in plastic bottles with buffer solutions. The bottles were incubated in a shaking incubator at 37 °C. The release media was 20 ml for drug/TiO₂ nanotubes and 100 ml for drug/PLGA/TiO₂ nanotubes. At given intervals, 1.5 ml drug solutions were withdrawn and analyzed with UV-Vis spectrometer
(WGS-9 Chromatic) and then put back in the bottle. To establish the relationship between the drug concentration and absorbance, the standard calibration curves of lidocaine at the wavelength of 262 nm (Figure 3.1 (a)) and carprofen at 237 nm (Figure 3.1(b)) in buffer solutions were developed. The amount of drug released can be calculated from the measured UV-Vis spectrum and corresponding calibration curves. The percentage of drug release was defined by dividing the accumulated amount of released drug by the total drug loading amount, which was the amount of drug released at the end of experiment when the UV-Vis absorption does not change any more.

Scheme 3.1 Chemical structure of carprofen (a) and lidocaine (b).
3.2.3 Chemical stability study

To be used clinically, drug carrier needs to have good mechanical and chemical stability. The excellent mechanical strength and stability of the drug carrier in this study have been demonstrated in our previous study[41]. To test the chemical stability, the drug carrier was tested in aqueous environments. The TiO$_2$ nanotubes with PLGA and drug were soaked in PBS solution of pH 7.4 for 40 day. Then the sample was removed from PBS, rinsed with water and dried in oven at 100 °C. The excess PLGA on TiO$_2$ nanotubes was removed by rinsing with acetone. SEM was subsequently used to exam any morphological change of TiO$_2$ nanotubes before and after soaking in PBS.

3.3 Results and discussion

3.3.1 Chemical stability
Figure 3.2 shows the SEM images of the as grown TiO$_2$ nanotubes. As illustrated, uniform, well aligned nanotubes arrays with smooth surface were formed over the whole Ti substrate. The average inner pore size of the nanotubes was estimated as 120 nm (Figure 3.2(a)). Figure 3.2 (b) shows the cross-sectional views of the nanotubes after they are detached from the Ti substrate. The thickness of the nanotubes is approximately 10 $\mu$m as seen in Figure 3.2(b). The open top and hollow nature of the TiO$_2$ as confirmed by the SEM suggests the possibility of serving as carrier for drug and polymer loading. Figure 3.3(a)-(c) shows the SEM images of TiO$_2$ nanotubes after incubation in PBS solution for 40 days. It can be seen that TiO$_2$ nanotubes remains the nanotubular structure after soaking in PBS for 40 days, and the surface looks the same as the one before incubation shown in Figure 3.2(a). There are no cracks, collapse or defects present after PBS incubation. The nanotubes have preserved smooth walls, flat surface and uniform pores. TiO$_2$ nanotubes before and after soaking both have the similar diameter of approximately 120 nm with an average tube length of 10 $\mu$m. No obvious morphology change associated with the soaking process was observed. The SEM analysis suggested that the TiO$_2$ nanotubes in our study feature an excellent structural stability in aqueous environment and thus promises to be implant biomedical materials for the long-term use inside human body.

Figure 3.2 SEM images of TiO$_2$ nanotube array (a) top view and (b) side view.
3.3.2 In-vitro drug release profiles of lidocaine and carprofen

In order to reveal the mechanism and potential of using TiO$_2$ nanotubes as carriers for the sustained release of lidocaine and carprofen, the drug release profiles were obtained for two different samples: drug loaded pure TiO$_2$ nanotubes and drug loaded PLGA/TiO$_2$ nanotubes. Lidocaine and carprofen released from pure TiO$_2$ nanotubes in different buffer solution were shown in Figure 3.4 and 3.5. It was apparent that the drug release profiles and kinetics were different for these two drugs. Lidocaine exhibits burst release at all pH levels while carprofen has burst release only at pH=10.5 and pH=7.5. Carprofen exhibits almost zero order release at pH=3.5 from pure TiO$_2$ nanotubes. Figure 3.4 and 3.5 also indicate that the rate of releases of both drugs demonstrated pH dependent behavior. In the case of lidocaine (Figure 3.4), lowering the pH of buffer solution to 3.5 greatly accelerated the lidocaine release rate from TiO$_2$ nanotube.
As we can see, nearly 96 % of lidocaine was released after 5 minutes. When pH increased to 10.5, the lidocaine release becomes much slower and only 75 % was released in 5 minutes. In contrast, carprofen burst release rate (Figure 3.5) was significantly slowed in acidic media (pH 3.5) compared in neutral solution and the release process lasted for more than 2 hours at pH=3.5. In basic media (pH=10.5), carprofen showed enhanced release rate and 80 % of the drug was released in 5 minutes.

Now adding polymer into the carrier matrix, the typical release profiles of lidocaine and carprofen from drug/PLGA/TiO₂ nanotubes hybrid at 37 °C with different pH buffer solutions are presented in Figure 3.6 and 3.7. Due to the considerable restriction effect from polymer chains to drug molecule movement, the total release duration could be extended to 12 days for lidocaine and 10 days for carprofen in PBS of pH 7.4. In addition, lidocaine and carprofen exhibited obviously pH dependent release profiles. At pH 7.4, the lidocaine loaded PLGA/TiO₂ release profile shows a better controlled initial burst than carprofen. The initial burst release during the first 10 hours is only 35% of the total loaded lidocaine while it is 50 % for carprofen in 10 hours. During the following stage, it took 240 hour to release 95 % of lidocaine in a manner of linear release. The time for 95 % carprofen release was 100 hour, suggesting a much faster diffusion rate of carprofen at the same pH value of 7.4. At pH 10.5, both carprofen and lidocaine drug release rates were faster than that at pH 7.4. For carprofen, in the first 24 hours, the percentage of released drug was about 95 % at pH 10.5 and 75 % at pH 7.4, respectively. In the case of lidocaine, 50 % was release at pH 10.5, compared to 40 % at pH 7.4 within 24 hours. When the buffer solution pH is lowered to 3.5, it was found that both lidocaine and carprofen initial burst release and overall release rates become significantly lower than those at pH 7.4 and 10.5. The observations are summarized in the Table 3.1.
Figure 3.4 Accumulative percentage of lidocaine releases from pure TiO$_2$ nanotubes with different pH. Each point presents mean ± S. D. of three experiments.

Figure 3.5 Accumulative percentage of carprofen releases from pure TiO$_2$ nanotubes with different pH. The experimental conditions are the same as illustrated in Figure 3.4. Each point presents mean ± S. D. of three experiments.
Figure 3.6 Accumulative percentage of lidocaine releases from PLGA (Mw.66,000-107,000 Da)/TiO$_2$ nanotubes with different pH. Each point presents mean ± S. D. of three experiments.

Figure 3.7 Accumulative percentage of carprofen releases from PLGA (Mw.66,000-107,000 Da)/TiO$_2$ nanotubes with different pH. The experimental conditions are the same as illustrated in Figure 3.6. Each point presents mean ± S. D. of three experiments.
Table 3.1 Comparison of lidocaine and carprofen release from TiO$_2$ nanotubes and PLGA/TiO$_2$ nanotubes

<table>
<thead>
<tr>
<th>Drug Carrier</th>
<th>Pure TiO$_2$ Nanotubes</th>
<th>PLGA/TiO$_2$ Nanotubes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lidocaine</td>
<td>(1) First order release at all pH levels.</td>
<td>(1) Multiple phase release at all pH levels;</td>
</tr>
<tr>
<td></td>
<td>(2) release rate pH10.5 &lt; pH7.4 &lt; 3.5</td>
<td>(2) release rate pH3.5&lt;&lt;pH7.4≤ pH10.5</td>
</tr>
<tr>
<td>Carprofen</td>
<td>(1) First order release at pH=10.5 and 7.4</td>
<td>(1) First order release at pH=10.5 and 7.4</td>
</tr>
<tr>
<td></td>
<td>(2) Zero order release at pH=3.5</td>
<td>(2) Two phase release at pH=3.5;</td>
</tr>
<tr>
<td></td>
<td>(3) release rate pH3.5&lt;&lt; pH7.4≤ pH10.5</td>
<td>(3) Release rate pH3.5&lt;&lt;pH7.4&lt; pH10.5</td>
</tr>
</tbody>
</table>

3.3.3 Mechanisms for controlling lidocaine and carprofen drug releases

It has been reported that the drugs release patterns could be significantly affected by the drugs’ physical and chemical characterizations[42, 43]. Thus, we first investigated the chemical bonding interaction between carprofen/lidocaine and PLGA/nanobubes using IR characterization. Such interactions could cause shift in peak position, or change in peak shape or have new peak. Figure 3.8(a) shows the IR spectra of pure individual components in drug carrier matrix namely, pure TiO$_2$ nanotubes, pure PLGA and pure drugs. Figure 3.8(b) is the IR spectra of their hybrid structures. As seen from Figure 3.8(a), pure TiO$_2$ nanotubes do not show any strong peaks in the range of 4000-600 cm$^{-1}$. The characteristic peaks of pure PLGA are around 3000 cm$^{-1}$ which was attributed to the carboxylic acid end groups. The C=O stretching mode of pure PLGA showed a strong peak at 1760 cm$^{-1}$. The peaks at 1080-1300 cm$^{-1}$ of pure PLGA were associated with C-O-C stretching. The IR peaks of composite PLGA/TiO$_2$ nanotube do not show any new peaks which indicates the chemical bonding between PLGA and TiO$_2$ is not significant. IR spectrum of
pure lidocaine in Figure 3.8(a) showed characteristic amide group (H-N-C=O) peaks in 3000-3500 cm\(^{-1}\). The \(\text{R3-N}\) stretching of pure lidocaine produced peaks in the range of 1020-1360 cm\(^{-1}\). The strong signals at 1488 cm\(^{-1}\) of pure lidocaine is associated with C=C stretching. The peak at 1660 cm\(^{-1}\) of pure lidocaine was indicative of C=O stretching mode. For carprofen presented in Figure 3.8(a), the peak at 3410 cm\(^{-1}\) belonged to N-H stretching, while the peak at 1698 cm\(^{-1}\) related to C=O bond. The peaks at 1627, 1572, 1126, 878, 810 and 697 cm\(^{-1}\) were associated with aromatic ring. The peaks at 1450 and 930 cm\(^{-1}\) corresponded to –OH deformation. The IR spectra in Figure 3.8(b) of drug/polymer loaded TiO\(_2\) shows no shift of peaks positions and is simply the combination of individual polymer and drug and TiO\(_2\) nanotubes. Spectral analysis confirms that the specific functional groups of the polymer (drug) molecules in the hybrid material have the same chemical characteristics as the pure samples. Therefore, it is concluded that there are no intermolecular interactions among drug, TiO\(_2\) nanotubes and polymer molecules.
Figure 3.8 IR spectra of (a) pure TiO$_2$ nanotubes, PLGA, carprofen and lidocaine, (b) hybrid structures.

As concluded from IR experiments, the chemical bonding does not change as the drug is loaded into the drug carrier. Thus, we investigated the physical interaction of drug carrier and drug or drug polymer matrix. Lidocaine is a weak base with an amine group and carprofen is a weak acid containing one carboxylic group as shown in Scheme 1. Drug ionization degree greatly affects the drug solubility. Ionized drugs will have increase drug solubility in PBS. When using pure TiO$_2$ nanotube as drug carrier, the drug release is diffusion controlled. Higher drug solubility induces higher concentration gradient and result in faster drug release. Table 3.2 lists the degree of ionization and the final solubility of the drugs in different pH solutions. The degree of ionization of a drug is calculated by equation 1(a) and (b)[44].

For weak acids, \[ \frac{\text{Ionized}}{\text{Nonionized}} = 10^{(pH - pK_a)} \]  

(a)
For weak bases, $\frac{\text{Nonionized}}{\text{Ionized}} = 10^{(pH-pKa)}$  

The pH dependence of solubility of a drug is calculated by equation 2(a) and (b)[45].

For weak acids, 
\[
\log S = \log S_0 + \log(1+10^{pH-pK_a}) \quad \text{for } pH<pK_b
\]

For weak bases, 
\[
\log S = \log S_0 + \log(1+10^{pK_b-pK_a}) \quad \text{for } pH>pK_b
\]

The equation 2 is developed by modifying the Henderson-Hasselbalch equations[45]. We have found that for base drug, as pH<pKb and for acidic drug, as pH< pKb, the Henderson-Hasselbalch equations do not fit the experimental data well. The final solubility listed in Table 1 is calculated using these modified Henderson-Hasselbalch equations.

Table 3.2 Ionization degree and solubility of lidocaine and carprofen at different pH

<table>
<thead>
<tr>
<th>pH</th>
<th>Degree of Ionization</th>
<th>Final Solubility (S)</th>
<th>Degree of Ionization</th>
<th>Final Solubility (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>0.25</td>
<td>3500 μg/ml</td>
<td>0.25</td>
<td>66.73 mg/ml</td>
</tr>
<tr>
<td>7.4</td>
<td>0.25</td>
<td>3500 μg/ml</td>
<td>0.25</td>
<td>66.73 mg/ml</td>
</tr>
<tr>
<td>10.5</td>
<td>0.25</td>
<td>3500 μg/ml</td>
<td>0.25</td>
<td>66.73 mg/ml</td>
</tr>
</tbody>
</table>

*Lidocaine intrinsic solubility($S_0$) is 3500 μg/ml[46], and the intrinsic solubility of carprofen is 5.3 μg/ml[47].

The pKa of carprofen is 4.3, as the pH of the media was lower than this value (at pH 3.5), only small amount of carprofen was ionized, resulting in a very limited solubility and a very slow
and almost zero order release rate at pH=3.5 as shown in Figure 3.5. As the pH increases above the pKa, a greater percentage of the carprofen would be ionized and consequently the drug solubility would be increased and release rates were dramatically enhanced at pH=7.4 and 10.5 seen in Figure 3.5. For example, at pH of 7.4, 99.92% of carprofen undergoes deprotonation and become ionized, so the total release time is largely shortened (40 min release duration compared with 2 hours at pH 3.5). The almost overlapping release profiles of carprofen at pH=7.4 and 10.5 is a result of the close degree of ionization of carprofen at these two pH values (99.92% at pH=7.4 and 100% at pH=10.5). For lidocaine which is a weak base and has a pKa of 7.9, at 7.4, about one third of lidocaine molecules still exist in their free base form. As the pH dropped to 3.5, nearly all the amine group in lidocaine become protonated, hence the solubility and release rate were increased compared to that at pH=7.4 and 10.5. The much lower intrinsic solubility ($S_0$) of carprofen compared to lidocaine resulted in lower final solubility of carprofen as seen in Table 3.2. This explains the overall slower release rate of carprofen to lidocaine. For the above discussion, we conclude that the releases of lidocaine and carprofen from pure TiO$_2$ nanotubes depend mainly on the drug solubility which is determined by its intrinsic solubility and degree of ionization.

Adding polymer into drug delivery matrix, as seen from Figure 3.6 and 3.7, we observed,

1. Overall sustained drug release to days at all pH levels due to the polymer matrix hindering the drug diffusion.
2. At pH=3.5, both carprofen and lidocaine showed slow release with multiple-phase release mechanism while single phase releases were observed at higher pH for both drugs.
3. Significantly improved initial burst release of lidocaine compared to that of release from pure TiO$_2$ while carprofen still has the same very fast initial burst release profile as that without polymer (pure TiO$_2$).

Due to the addition of polymer, we have to take drug/polymer interaction into mechanism explanation along with consideration of the drug solubility as discussed earlier for the case of releases from pure TiO$_2$ nanotubes. In our previous study[41], we have found that the role of
TiO₂ in drug delivery profile is the ability to increase drug loading. TiO₂ nanotubes also has the potential to improve the bone tissue integration[21]. The shape of release profile is very similar of polymer/drug and polymer/drug/TiO₂ matrix according to our previous study [41]. Thus, we hypothesis that the drug release mechanism of polymer/drug/TiO₂ is the same as that of polymer/drug. Here, we use Scheme 2 of the drug release mechanisms illustration inside a polymer/TiO₂ nanotubes to explain the drug delivery mechanism for the drug carrier of polymer/drug/TiO₂ used in this study.

Neutral PLGA restricts the drugs release because drugs need to go through the pores and open space inside the polymer network to be able to be diffused out to the solution. This explains why we observed sustained drug release for both drugs to days at all pH levels when polymer is used in drug delivery. Charged PLGA will swell and have larger opening space due to the repulsive electrostatic force. PLGA contains carboxylic terminal group and has a pKa of 3.8 (lactic acid pKa is 3.8). At pH=3.5 as shown in Scheme 2, which is lower than pKa of PLGA, the ionization of carboxylic terminal groups of PLGA was restricted dramatically, and this would in turn reduce the repulsion between the ionized PLGA and the water uptake (swelling) significantly. Therefore, the drug diffusion through the polymer matrix became difficult. For carprofen, its solubility also lowered in the acid medium. Together, the release rate of carprofen from PLGA/TiO₂ at pH 3.5 was reduced by both factors. On the other hand, lidocaine became full ionized in this buffer which would benefit its diffusion. However, in this case, the limited opening space of neutral PLGA was still the predominate factor to determine the drug diffusion rate. Thus, the lidocaine release rate was decreased at pH 3.5 instead of increased. When comparing lidocaine and carprofen released at pH 7.4 and 10.5, we need to consider the drug solubility and polymer drug interaction as we described in Scheme 2. When the pH of the buffer solution was higher than the PLGA pKa, a large percentage of PLGA would undergo deprotonation and carries negative charges and thus becomes swelled. If the drug is also ionized with negative charges, there will be an electrostatic repulsion between the negatively charged drug and the PLGA carboxylic groups, leading to the fast diffusion of drugs. This is the case of
fast carprofen release at pH=10.5 and 7.4 (Figure 3.7). The slightly increased drug release rate at pH=10.5 compared to that of pH=7.4 for both drugs is due to the full ionization of PLGA at pH of 10.5, leading to a stronger electrostatic repulsion between the carboxylic groups as well as a
higher degree of PLGA network swelling and water uptake which is usually seen in pH sensitive polymers [48-50]. Consequently, the drug release was accelerated at pH=10.5 compared to pH=7.4 for carprofen. In contrast, at pH=3.5 and 7.4, lidocaine has much improved and slower burst release (Figure 3.6) compared to that of without polymer (pure TiO$_2$, Figure 3.4). This is because lidocaine is positively charged and is highly likely have a strong electrostatic attraction to PLGA, which simultaneously hinder the lidocaine diffusion from polymer matrix into the buffer solution, resulting slower release rate (Figure 3.6). It has been also reported that the electrostatic interaction between its amine groups and polymer carboxylic terminal groups will significantly affect the drug release process [51]. This strong attractive force between lidocaine and PLGA changed to lidocaine release from first order release (Figure 3.4) to two-stage release (Figure 3.6) at all pH=7.4 and 10.5. This indicates that the electrostatic forces also play a role in the drug release of lidocaine from polymer matrix.

In summary, the drug release profile is determined by

1. Solubility of the drug
2. Opening space inside a polymer which is pH dependent
3. Electrostatic force between polymer and drug which is also pH dependent.

3.3.4 Kinetics models

In this study, two mathematical models were used for describing drug release. They are first order [52, 53], and Gallagher-Corrigan [54] as following.

1. First order model: $M_t / M_\infty = 100 - e^{b-kt}$

where $k$ is the first order release constant.

This model represents the drug dissolution in pharmaceutical dosage. For example, water-soluble drug releases from porous matrices [55]. In the first order kinetics, the drug dissolution rate is proportional to the difference between the amount of drug remaining for delivery and the drug concentration in the liquid phase.

2. Gallagher-Corrigan model:
\[ f_t = f_B \cdot (1 - e^{-k_1 t}) + (f_{t_{\text{max}}} - f_B) \cdot \left( \frac{e^{k_2 t - k_2 t_{2_{\text{max}}}}}{1 + e^{k_2 t - k_2 t_{2_{\text{max}}}}} \right) \]

where \( f_t \) is the accumulative drug release percentage at time \( t \), \( k_1 \) is the first order release constant (stage 1), \( k_2 \) is the second stage release constant due to the polymer degradation, \( f_B \) is the accumulative drug release percentage during the stage 1, \( f_{t_{\text{max}}} \) is the maximum drug release percentage during the whole process, \( t_{2_{\text{max}}} \) is the time at which drug release rate reaches the maximum.

This model proposed by Gallagher-Corrigan[54] describes a two-stage drug release kinetics. The first part of the equation reflects the diffusion controlled dissolution of drug to the medium, which is characterized by the first order kinetics. The second part describes that the drug release rate depends on the polymer degradation. This model has been used to demonstrated the drug release from biodegradable carriers [54, 56-58].

These two models were examined with experimental data to determine the best model and mechanism of drugs release from PLGA/ TiO$_2$ nanotubes. The correlation coefficient (R$^2$) was used as an indicator of the best fitting for each of the model considered. The model could be used to describes the release kinetics when R$^2$ is higher than 0.975. Matlab was employed to fit the three models to the data. Based on the models, lidocaine and carprofen release curve fitting results were displayed in Table 3.3 and Figure 3.9 to 3.14.

Table 3.3 Kinetic models equations and best-fit parameters of lidocaine and carprofen release from PLGA/TiO$_2$ nanotubes

<table>
<thead>
<tr>
<th>pH</th>
<th>( M_t / M_{\infty} = 100 - e^{b \cdot k t} )</th>
<th>( f_B )</th>
<th>( k_1 )</th>
<th>( f_{t_{\text{max}}} )</th>
<th>( k_2 )</th>
<th>( t_{2_{\text{max}}} )</th>
<th>( R^2 )</th>
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<tr>
<td>3.5</td>
<td>0.003933 4.577 0.9579</td>
<td>0.0105 4.498</td>
<td>0.9877</td>
<td>0.0112 4.344</td>
<td>0.9902</td>
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<td></td>
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<tr>
<td>7.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
\[
f_t = f_{B} \cdot \left(1 - e^{-k_1 t} + ft_{max} - f_{B} \cdot \frac{e^{k_2 t - k_2 t_{2max}}}{1 + e^{k_2 t - k_2 t_{2max}}} \right)
\]

<table>
<thead>
<tr>
<th>( f_t )</th>
<th>( k )</th>
<th>( b )</th>
<th>( R^2 )</th>
</tr>
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<tbody>
<tr>
<td>3.5</td>
<td>38</td>
<td>0.0174</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01815</td>
<td>312</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.9956</td>
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Carprofen

<table>
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<th>( b )</th>
<th>( R^2 )</th>
</tr>
</thead>
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<tr>
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<td>0.003519</td>
<td>4.558</td>
<td>0.9927</td>
</tr>
<tr>
<td>pH= 7.4</td>
<td>0.04452</td>
<td>4.40</td>
<td>0.9839</td>
</tr>
<tr>
<td>pH= 10.5</td>
<td>0.1957</td>
<td>4.486</td>
<td>0.9821</td>
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</tbody>
</table>

\[
M_t / M_{\infty} = 100 - e^{b-kt}
\]

<table>
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<tr>
<th>pH</th>
<th>( k )</th>
<th>( b )</th>
<th>( R^2 )</th>
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<td>4.558</td>
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<tr>
<td>pH= 7.4</td>
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</tr>
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<td>pH= 10.5</td>
<td>0.1957</td>
<td>4.486</td>
<td>0.9821</td>
</tr>
</tbody>
</table>

\[
f_t = ft_{max} \cdot \left(1 - e^{-k_1 t} \right) + (f_{tmax} - f_{B}) \cdot \frac{e^{k_2 t - k_2 t_{2max}}}{1 + e^{k_2 t - k_2 t_{2max}}}
\]

<table>
<thead>
<tr>
<th>( f_t )</th>
<th>( k )</th>
<th>( f_B )</th>
<th>( k_1 )</th>
<th>( f_{tmax} )</th>
<th>( k_2 )</th>
<th>( t_{2max} )</th>
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<tr>
<td>3.5</td>
<td>50</td>
<td>0.009832</td>
<td>100</td>
<td>0.006351</td>
<td>336</td>
<td>0.9968</td>
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Figure 3.9 Comparison between experimental data and model fitting using first order kinetics referring to lidocaine releases from PLGA / TiO₂ nanotubes at pH of 10.4.
Figure 3.10 Comparison between experimental data and model fitting using first order kinetics referring to lidocaine releases from PLGA / TiO$_2$ nanotubes at pH of 7.4.
Figure 3.11 Comparison between experimental data and model fitting using first order and Gallagher-Corrigan kinetics referring to lidocaine releases from PLGA / TiO₂ nanotubes at pH of 3.5.

Figure 3.12 Comparison between experimental data and model fitting using first order kinetics referring to carprofen releases from PLGA / TiO₂ nanotubes at pH of 10.5.
Figure 3.13 Comparison between experimental data and model fitting using first order kinetics referring to carprofen releases from PLGA / TiO$_2$ nanotubes at pH of 7.4.
Based on the highest regression coefficient value, data from lidocaine and carprofen release experiments performed at pH 10.5 and 7.4 were better fitted to the first order release, indicating that the release rate depends on the difference between the amount of drug remaining for delivery and the drug concentration in the release medium. These results suggested that the dissolution of drugs is diffusion controlled and the swelling of polymer is very fast and opens the pores for drug to easily diffuse through. However, the fitting of first order kinetics to the experimental data of lidocaine release at pH 3.5 was not satisfactory; demonstrating that the drug release in medium with low pH value might has a different mechanism compared with those of release at a higher pH. Therefore, the Gallagher-Corrigan model was employed to fit lidocaine release experiments at pH 3.5. The results were showed in Figure 3.11 and Table 3.3. It seemed that this model approximated the experiment points of lidocaine much better than the first order does, as the $R^2$ is 0.9579 for first order fitting and 0.9956 when using Gallagher-Corrigan model. The release constant for the first and second stage was 0.0174 and 0.01815, respectively. This obtained
lidocaine release kinetics suggests that the process was driven by diffusion during the initial release and then polymer degradation becomes the controlling factor. These two mechanisms seem play similar role in the release process as $k_1 \approx k_2$.

For carprofen release at pH 3.7, although the first order kinetics presented a good fitting result ($R^2 = 0.9927$), the release profile was also fitted to Gallagher-Corrigan model and results are included in Figure 3.14. It can be seen that this model approximated the data better than the first order did ($R^2 = 0.9968$), suggesting drug release is actually controlled by two different mechanisms. Besides, $k_1 = 0.006632$ and $k_2 = 0.005751$ was obtained for carprofen, indicating that both mechanisms had comparable contribution to the overall release which is similar to the results of lidocaine. One can also observe that lidocaine presented much higher diffusion drug release rate ($k_1$) and polymer degradation drug release rate ($k_2$) compared to carprofen. The above results could be explained by the higher solubility of lidocaine and lower solubility of carprofen in pH 3.5 medium as mentioned in the previous part.

In brief, drug release from PLGA could follow two mechanisms: (1) diffuse through the pores of swollen polymer matrix and (2) drug escapes from the eroded polymer matrix as we described in Scheme 3. As we discussed before, at higher pH, most of the carboxylic groups of PLGA are ionized which lead to higher degree of swelling and further increase the polymer network mesh size. Therefore, the drug can easily diffuse through the polymer and nearly all the drugs will be released to the medium within time $t_1$ shown in Scheme 3 before the polymer degradation occurred. In this case, we observed a single phase first order release process. At pH of 3.5, only small portion of PLGA underwent ionization, so the polymer swelling degree was much lower compared to the one at higher pH, resulting a significantly decreased release rate and the time for drug release extends to the $t_2$ time region in Scheme 3 where the polymer degradation contribution comes into play. In this case, we observed a two phase Gallagher-Corrigan release profile. Thus, we conclude that the PLGA swelling and degradation are the predominant factors in determine the drug release mechanism.
3.4 Conclusion

In this study, lidocaine and carprofen loaded TiO₂ nanotubes and PLGA/TiO₂ nanotubes hybrid structures were fabricated and the drugs release kinetics was investigated. It was observed that in vitro release of these two drugs depends on the drug property, carriers’ type and the pH value of the medium. Lidocaine is a weak base and carprofen is a weak acid. When lidocaine and carprofen released from pure TiO₂ nanotubes, the drug solubility in the medium determined their release rate. Results obtained from lidocaine and carprofen release from PLGA/TiO₂ nanotubes at different pH values showed that both of the drug release rate increased when increasing the medium pH. In addition, lidocaine displayed a much slower release rate at pH of 7.4 and 10.5 compared with those of carprofen. While in the medium with pH of 3.5, carprofen showed a longer release time. The release mechanism was studied by fitting the experimental data to two mathematical models. The analysis indicated that at pH 7.4 and 10.5, the two drug transport followed the first order release. At a lower pH of 3.5, both diffusion and polymer degradation controlled the release. Overall, the release of these two drugs from PLGA/TiO₂ was controlled by solubility of the drug, polymer swelling degree and the electrostatic force between polymer and drug. All the three factors were pH dependent.
4. Future work

In the present thesis we developed a method for controllable drug release of ibuprofen, carprofen and lidocaine based on the anodized TiO$_2$ nanotube film on Ti implant surface (common orthopedic and dental implant material). A novel drug carrier using a hybrid structure of TiO$_2$ nanotubes filled with biodegradable polymers was engineered.

This novel technology will have broad applications on preventing and treating various canine diseases associated with orthopedic and dental implant surgeries such as hip, elbow dysplasia, osteosarcoma, etc. The novel drug carrier developed in this study will provide the implant surgery community a new tool to more effectively deal with the pain, inflammation, drug administration, osteosarcoma disease. Our future plans are to collaborate with biologists and veterinary doctors to perform in-vitro study of how gemcitabine delivered by this novel drug carrier interacts with canine osteosarcoma cell lines and to perform in-vivo study of drug release of gemcitabine, carprofen and lidocaine using this novel drug carrier and evaluate its effectiveness on osteosarcoma disease control, inflammation reduction, pain management and bone tissue growth.
5. References


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