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Kinetics of Drug Release from Drug Carrier of Polymer/TiO_2 Nanotubes Composite—pH Dependent Study

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ABSTRACT: This study was to investigate the kinetics of drug release from polymer/TiO_2 nanotubes composite. Lidocaine and carprofen were selected as model drugs to represent weak base and weak acid drugs, respectively. Mathematical models used to fit the *in vitro* drug release experimental data indicate that at higher pH, the drug release was first order diffusion controlled. At lower pH, the release of the two drugs exhibits two staged controlled release mechanism. The first phase is due to drug diffusion and the second stage is a result of poly(lactic-co-glycolic acid) (PLGA) polymer degradation. The rate of drug release from polymer/TiO_2 nanotubes drug carrier was mainly controlled by three pH dependent factors: the solubility of the drug, the degree of polymer swelling/degradation, and the electrostatic force between polymer and drug. This study suggests that controlled release could be achieved for polymer/TiO_2 nanotubes drug carrier via the modulation of pK_a values of polymers and drug solubility. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41570.

KEYWORDS: biodegradable; drug delivery systems; kinetics

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INTRODUCTION

Titanium (Ti) is the most common used 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.[1] The drug release from pure TiO_2 nanotubes is very quick. In our previous study, we have demonstrated the improved drug release by adding poly(lactic-co-glycolic acid) (PLGA) into TiO_2 nanotubes. However, the drug release mechanism from the drug carrier of composite PLGA/TiO_2 nanotubes is not known. The drug release profile varies with types of drugs and is pH dependent.[2–4] This study selects carprofen and lidocaine as the model drugs representing the weak base and weak acid, respectively, to investigate the drug release mechanism from PLGA/TiO_2 nanotubes. Both drugs are frequently used in canine implant surgeries. Canine implant surgeries (e.g., orthopedic and dental) are performed to treat hip and 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 h in dogs.[5] This short half-life means that more frequent dosing 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 and are 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 6000 reports to FDA of the adverse reactions of carpofen in causing gastrointestinal, liver, and kidney problems in dogs.[5] 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 relief 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 requirement for drug dosage and the reduced organ toxicity. During implant surgeries, local anesthesia is usually conducted. 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.[6] 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,[6] adding vasoconstrictor,[7] 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.[7] The developed PLGA/TiO_2 nanotubes composite drug carrier in this study will not have any toxic effect because the TiO_2 nanotubes is inherent grown on Ti implant surface and adding polymer enhances its mechanical properties as demonstrated in our previous study.[1] In fact, any clinical Ti implant in the body has naturally formed TiO_2 surface coating which is left with the body implant in today's clinical practice. It has been also proven in this work that the TiO_2 nanotube has the excellent chemical stability in phosphate-buffered saline (PBS) solution. Even though TiO_2 nanotubes falls off the Ti surface, the human immunity system should be able to clean it up on its own because of its insignificant solubility. In addition, the toxicity of TiO_2 is very controversial in the nanotoxicology field.[8,9] Furthermore, TiO_2 nanotubes can serve as a barrier layer to prevent Ti ion dissolution from Ti implant surface. Once the sustained lidocaine release time is achieved by using the PLGA/TiO_2 nanotubes as drug carriers, lower dosage and longer duration of anesthesia effect can be achieved and allergic reaction and side effect to local anesthesia can be minimized.

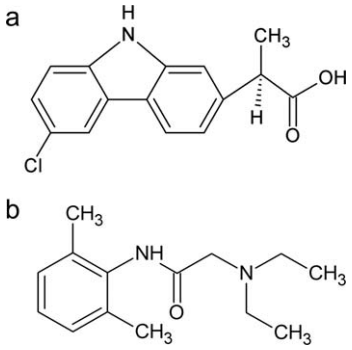
MATERIALS AND METHODS

Preparation of Drug Carrier

The drug carrier used in this study consists of structures of TiO_2 nanotubes infiltrated with PLGA/drug matrix. To make this drug carrier, it involves the experimental steps of (1) synthesis of TiO_2 nanotubes film, (2) loading of PLGA/drug into TiO_2 nanotubes.

Synthesis of TiO_2 Nanotubes Film. TiO_2 nanotubes on Ti foil were synthesized by a two-step anodization method as described in our previous study.[1] About 0.25 mm thick Ti foil (99.97% purity, Sigma-Aldrich) was cleaned ultrasonically in DI water, ethanol, and acetone for 5 min each and dried by 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 % NH_4F, and 3 vol % H_2O under stirring. The first anodization was carried out at 50 V for 24 h. Then, the TiO_2 nanotubes layer was removed in DI water ultrasonically. The second anodization was performed with the pretreated Ti foil at 50 V for 3 h. Highly ordered TiO_2 nanotubes arrays could be obtained by this two-step anodization. Finally, the Ti foil with TiO_2 nanotubes was cleaned in ethanol to remove the electrolyte and dried in air.

Loading of PLGA/Drug into TiO_2 Nanotubes. Lidocaine and PLGA of molecular weight 66,000–107,000 Da 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 TiO_2 nanotubes/Ti foil by a dip coating process for 3 days, and then dried in air for 24 h similar to the procedure described in our previous study.[1] Good infiltration depth of polymer in TiO_2 nanotubes was demonstrated by a technique



Scheme 1. Chemical structure of carprofen (a) and lidocaine (b).

developed in our previous study.[1] For drug loaded pure TiO_2 nanotubes, TiO_2 nanotubes/Ti foil was soaked in 6 mg/mL lidocaine or carprofen acetone solution for 3 days and then dried in air. The weight difference between initial TiO_2 nanotube/Ti foil weight and that after drug loading was measured to roughly estimate the drug loading capacity. The drug loading capacity is calculated based on per gram of TiO_2 nanotube/Ti foil. For lidocaine, the loading capacities in TiO_2 nanotubes and PLGA/TiO_2 nanotubes are 4.4 mg/g of carrier and 5.1 mg/g, respectively. For carprofen, the loading capacities are 3.6 mg/g and 3.9 mg/g, respectively. The slight increase of loading capacity when PLGA is used is due to the adsorption of more drugs at the surface of PLGA.

Infrared (IR) spectroscopy was performed using Perkin Elmer Spectrum One spectrometers with an attenuated total reflectance (ATR) accessory to study the possible chemical bonding interaction between drugs and PLGA/TiO_2 nanobubes. Scanning electron microscopy (SEM, Zeiss Supra 35) was used to characterize the morphology of the drug carrier.

In Vitro Drug Release Studies

Lidocaine and carprofen (their chemical structures are shown in Scheme 1) releases were studied in (a) sodium acetate buffer with pH 3.5, (b) PBS with pH 7.4, and (c) phosphate buffer with pH 10.5, respectively. All the buffer solution concentrations were 0.01*M*. The release studies were performed in bottles with buffer solutions. The bottles were incubated in a shaking incubator at 37°C. The release medium was the same volume for drug/TiO_2 nanotubes and drug/PLGA/TiO_2 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 and carprofen at 237 nm 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.

Chemical Stability Study

To be used clinically, drug carrier needs to have good mechanical and chemical stability. The excellent mechanical strength

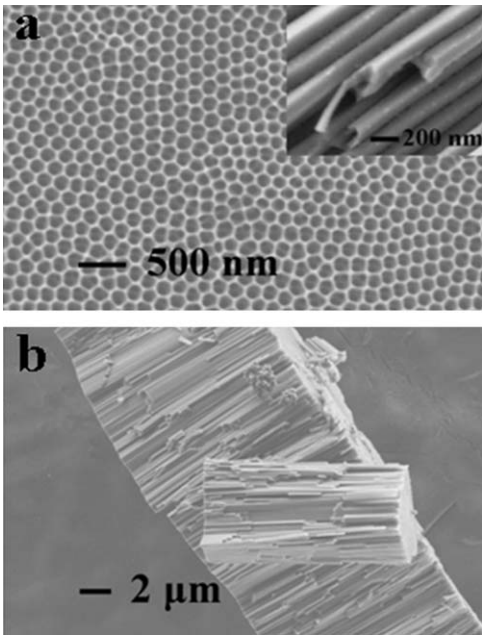


Figure 1. SEM images of TiO_2 nanotubes array (a) top view and (b) side view.

and stability of the PLGA/TiO_2 nanotube drug carrier in this study have been demonstrated in our previous study.[1] To test the chemical stability, the drug carrier was tested in aqueous environment. The TiO_2 nanotubes with PLGA and drug were soaked in PBS solution of pH 7.4 at 37°C for 40 days. 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 examine any morphological change of TiO_2 nanotubes before and after soaking in PBS.

RESULTS AND DISCUSSION

Chemical Stability

Figure 1 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 to be 120 nm [Figure 1(a)]. Figure 1(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 μm as seen in Figure 1(b). The open top and hollow nature of the TiO_2 nanotubes as confirmed by the SEM suggests the possibility of serving as carrier for drug and polymer loading. Figure 2(a–c) shows the SEM images of TiO_2 nanotubes after incubation in PBS solution at 37°C for 40 days. It can be seen that TiO_2 nanotubes retain the nanotubular structure after soaking in PBS for 40 days, and the surface appears to be the same as the one before incubation shown in Figure 1(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 ~120 nm with an average tube length of 10 μm. 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 biomedical implant materials for the long-term use inside human body.

***In Vitro* Drug Release Profiles of Lidocaine and Carprofen Release from Pure TiO_2 Nanotubes.** 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 sets of 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 are shown in Figures 3 and 4. It was apparent that the drug release profiles and kinetics were different for these two drugs. Lidocaine (Figure 3) exhibits a burst release at all pH levels while carprofen (Figure 4) has a burst release only at pH = 10.5 and pH = 7.4. Carprofen exhibits almost linear release at pH = 3.5 from pure TiO_2 nanotubes. Figures 3 and 4 also indicate that the rate of releases of both drugs has the pH-dependent behavior. In the case of lidocaine (Figure 3), lowering the pH of buffer solution to 3.5 greatly accelerated the lidocaine release rate from TiO_2 nanotubes. As we can see, nearly 96% of lidocaine was released after 5 min. When pH increased to 10.5, the lidocaine release became much

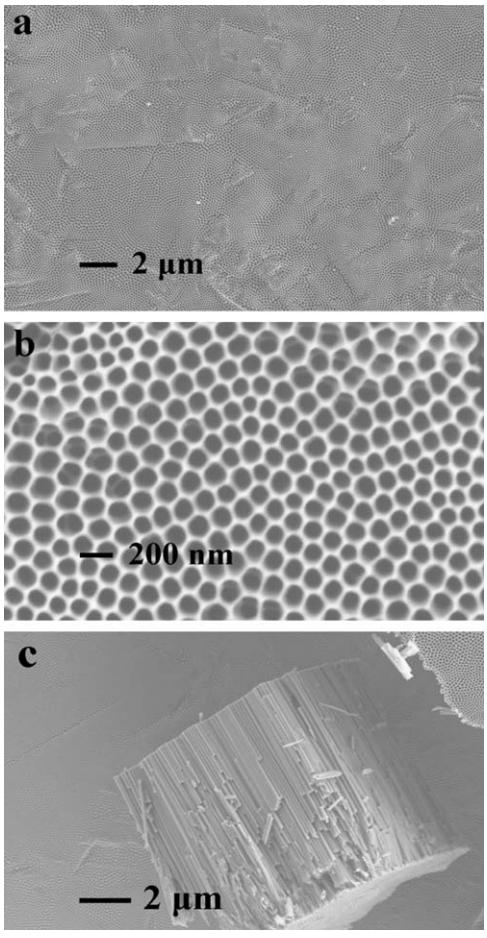


Figure 2. SEM images of TiO_2 nanotubes after soaked in PBS solution at 37°C for 40 day, (a) top view, (b) top view at higher magnification, and (c) side view.

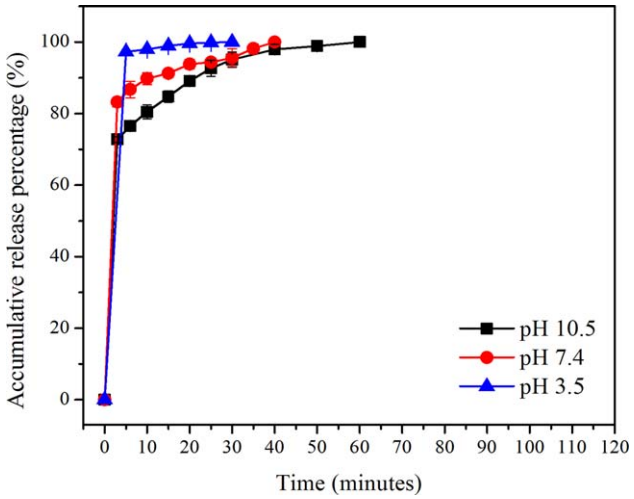


Figure 3. Accumulative percentage of lidocaine releases from pure TiO_2 nanotubes in different pH solutions. Each point presents mean ± S.D. of three experiments. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

slower and only 75% was released in 5 min. In contrast, carprofen burst release rate (Figure 4) was significantly lowered in acidic medium (pH 3.5) compared in neutral and basic solutions and the release process lasted for more than 2 h at pH = 3.5. In basic medium (pH 10.5), carprofen showed enhanced release rate and 80% of the drug was released in 5 min.

Drug Release from PLGA/TiO_2 Nanotubes Composite. Now adding polymer into the carrier matrix, the typical release profiles of lidocaine and carprofen from drug/PLGA/TiO_2 nanotubes composite at 37°C in different pH buffer solutions are presented in Figures 5 and 6. Due to the considerable restriction effect from polymer chains on drug molecular movement, the total release duration was extended to 12 days for lidocaine and

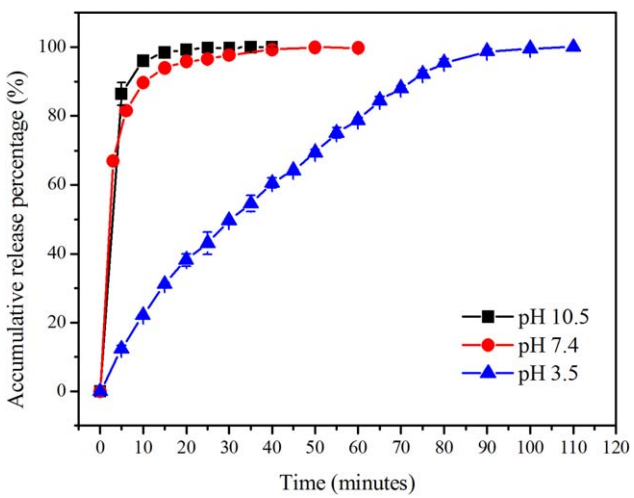


Figure 4. Accumulative percentage of carprofen releases from pure TiO_2 nanotubes in different pH solutions. The experimental conditions are the same as illustrated in Figure 3. Each point presents mean ± S.D. of three experiments. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

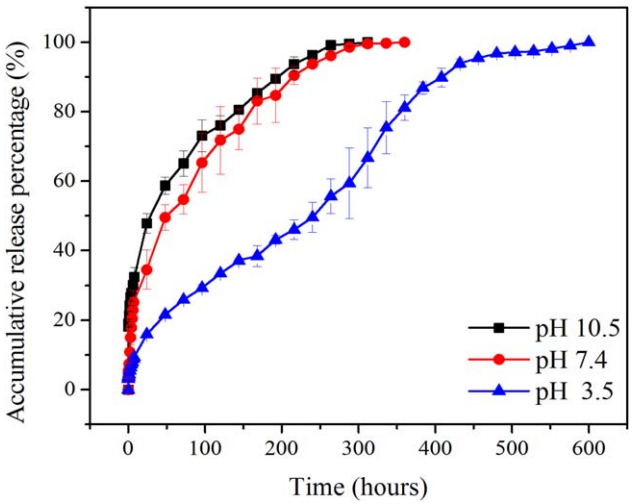


Figure 5. Accumulative percentage of lidocaine releases from PLGA (M_w 66,000–107,000 Da)/TiO_2 nanotubes in different pH solutions. Each point presents mean ± S.D. of three experiments. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

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 (Figure 5) loaded PLGA/TiO_2 release profile shows a slightly better controlled initial burst release than carprofen (Figure 6). The initial burst release during the first 10 h is only 35% of the total loaded lidocaine while it is 50% for carprofen in 10 h. During the following stage after burst release, it took 240 h to release 95% of lidocaine (Figure 5) in a trend close to linear release with much slower burst release rate at pH of 7.4. The time for 95% carprofen (Figure 6) release was 100 h, suggesting a much faster diffusion rate of carprofen at the same pH value of 7.4. Both carprofen and lidocaine drug release rates were faster at pH of 10.5 than that at

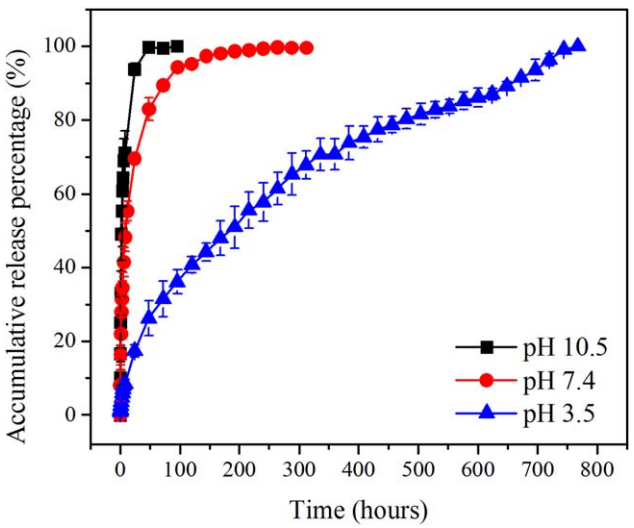


Figure 6. Accumulative percentage of carprofen releases from PLGA (M_w 66,000–107,000 Da)/ TiO_2 nanotubes in different pH solutions. The experimental conditions are the same as illustrated in Figure 5. Each point presents mean ± S.D. of three experiments. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table I. Comparison of Lidocaine and Carprofen Release from TiO_2 Nanotubes and PLGA/TiO_2 Nanotubes

	Drug carrier	
	Pure TiO_2 nanotubes	PLGA/TiO_2 nanotubes
Lidocaine	(1) First order release at all pH levels.	(1) Multiple phase release at all pH levels;
	(2) Release rate pH 10.5 < pH 7.4 < 3.5	(2) Release rate pH 3.5<<pH 7.4≤ pH 10.5
Carprofen	(1) First order release at pH=10.5 and 7.4	(1) First order release at pH=10.5 and 7.4
	(2) Zero order release at pH=3.5	(2) Two phase release at pH=3.5;
	(3) Release rate pH 3.5<< pH 7.4≤ pH 10.5	(3) Release rate pH3.5<<pH7.4< pH10.5

pH 7.4. For carprofen, in the first 24 h, 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 released at pH 10.5, compared to 40% at pH 7.4 within 24 h. When the buffer solution pH was lowered to 3.5, it was found that both lidocaine and carprofen initial burst release and overall release rates became significantly lower than those at pH 7.4 and 10.5. These observations are summarized in Table I.

Mechanisms for Controlling Lidocaine and Carprofen Drug Releases

Drug Release from Pure TiO_2 Nanotubes. We first investigated the chemical bonding interaction between the two drugs and PLGA/nanobubes or pure TiO_2 nanotubes using IR characterization. Such interactions could cause shift in peak position or change the peak shape or induce new peaks. Figure 7(a) shows the IR spectra of pure individual components in drug carrier matrix, namely, pure TiO_2 nanotubes, pure PLGA, and pure drugs. Figure 7(b) is the IR spectra of their hybrid structures. As seen from Figure 7(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 shows a strong peak at 1760 cm^{-1}. The peaks at 1080–1300 cm^{-1} of pure PLGA are associated with C—O—C stretching. The IR spectrum of composite PLGA/TiO_2 nanotubes does not show any new peaks indicating that the chemical bonding between PLGA and TiO_2 is not significant. The IR spectrum of pure lidocaine in Figure 7(a) shows characteristic amide group (H—N—C═O) peaks in 3000–3500 cm^{-1}. The R3—N stretching of pure lidocaine produces 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 is indicative of C═O stretching mode. For carprofen presented in Figure 7(a), the peak at 3410 cm^{-1} belongs to N—H stretching, while the peak at 1698 cm^{-1} is 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} correspond to —OH deformation. The IR spectra in Figure 7(b) of drug/PLGA loaded TiO_2 show no shift of peak positions and are simply the combination of individual polymer, drug, and TiO_2 nanotubes. These spectral analyses confirm that the specific functional groups of the polymer (drug) molecules in the composite material have the same chemical characteristics as the pure samples. Therefore, it is concluded that there are no strong intermolecular interactions among drug, TiO_2 nanotubes, and polymer molecules though week van der Waals attraction still exists. In our previous study,[1] we have found that the role of TiO_2 nanotubes in drug delivery profile was its capability to

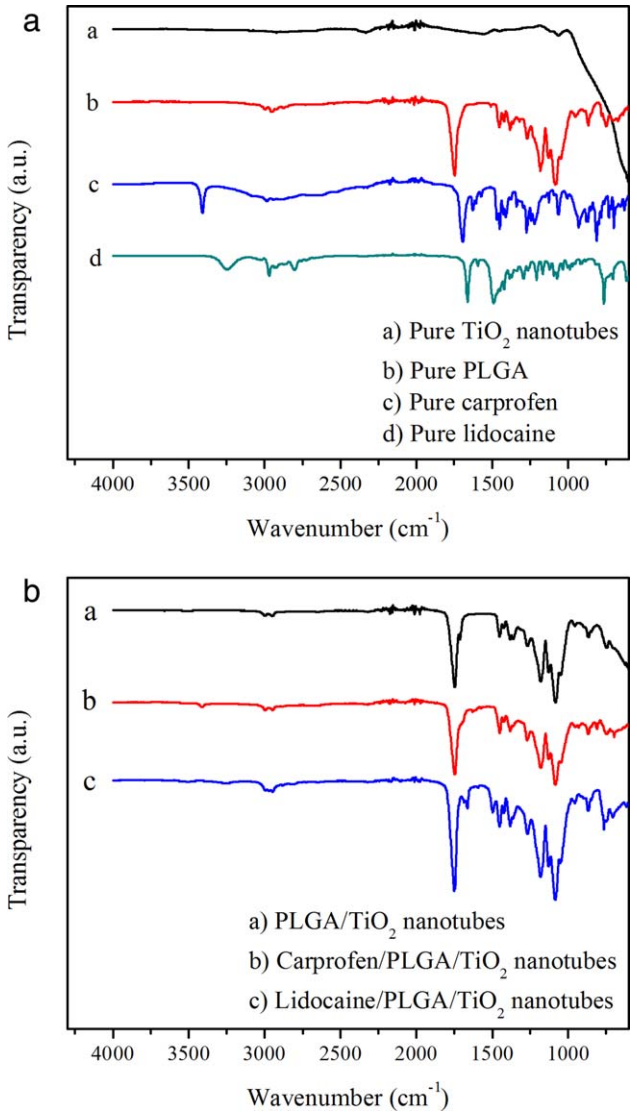


Figure 7. IR spectra of (a) pure TiO_2 nanotubes, PLGA, carprofen and lidocaine, (b) hybrid structures. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table II. Ionization Degree and Solubility of Lidocaine and Carprofen at Different pH

	Lidocaine ($pK_a = 7.9$)[a]		Carprofen ($pK_a = 4.3$)[a]	
	Degree of ionization	Final solubility (S)	Degree of ionization	Final solubility (S)
pH = 3.5	≈100	73.33 mg/mL	13.68	~5.3 μg/mL
pH = 7.4	75.97	14.56 mg/mL	99.92	6.68 mg/mL
pH = 10.5	0.25	~3500 μg/mL	≈100	66.73 mg/mL

[a] Lidocaine intrinsic solubility(S_0) is 3500 μg/mL,[11] and the intrinsic solubility of carprofen is 5.3 μg/mL.[12]

increase drug loading. TiO_2 nanotubes also have the potential to improve the bone tissue integration.[10]

Drug ionization degree greatly affects the drug solubility which plays significant role in the drug release. Ionized drugs will have increased drug solubility in release medium. When using pure TiO_2 nanotubes as drug carrier, the drug release is diffusion controlled. Higher drug solubility induces higher concentration gradient and results in a faster drug release. Lidocaine is a weak base with an amine group and carprofen is a weak acid containing one carboxylic group as shown in Scheme 1. At different pH, lidocaine and carprofen present different ionization degree and solubility which are listed in Table II. The degree of ionization of a drug is calculated by eq. (1a) and (1b).[13]

$$\text{For weak acids, Ionized/Nonionized} = 10^{(\text{pH}-\text{p}K_a)} \quad (1a)$$

$$\text{For weak bases, Nonionized/Ionized} = 10^{(\text{pH}-\text{p}K_a)} \quad (1b)$$

The pH dependence of solubility of a drug is calculated by eq. (2a) and (2b).[14]

$$\begin{gathered}\text{For weak acids,}\\ \log S = \log S_0 + \log\left(1+10^{\text{pH}-\text{p}K_a}\right) \text{ for pH} < \text{p}K_b\\ \log S = \log S_0 + \log\left(1+10^{\text{p}K_b-\text{p}K_a}\right) \text{ for pH} > \text{p}K_b\end{gathered} \quad (2a)$$

$$\begin{gathered}\text{For weak bases,}\\ \log S = \log S_0 + \log\left(1+10^{pK_a-\text{pH}}\right) \text{ for pH} > \text{p}K_b\\ \log S = \log S_0 + \log\left(1+10^{\text{p}K_a-\text{p}K_b}\right) \text{ for pH} < \text{p}K_b,\end{gathered} \quad (2b)$$

Equation (2) is developed by modifying the Henderson–Hasselbalch equations.[14] We have found that for the base drug, as pH > pK_b and for the acidic drug, as pH < pK_b, the Henderson–Hasselbalch equations do not fit the experimental data well. The final solubility listed in Table II is calculated using these modified Henderson–Hasselbalch equations.

For the diffusion controlled release from pure TiO_2 nanotubes, the diffusion is determined by the drug's pK_a and its intrinsic solubility. For carprofen which is a weak acid with pK_a of 4.3, as the pH of the medium was lower than this value (e.g., at pH of 3.5), only small amount of carprofen was ionized, resulting in a very limited solubility and a very slow and almost linear release rate at pH = 3.5 as shown in Figure 4. As the pH increases above the pK_a of carprofen, 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 as seen in Figure 4. At pH of 7.4, 99.92% of carprofen undergoes deprotonation and becomes ionized, so the total release time is largely shortened (40 min release duration compared with 2 h at pH of 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 with pK_a of 7.9, 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 as seen in Figure 3. The much lower intrinsic solubility (S_0) of carprofen compared to lidocaine resulted in lower final solubility of carprofen shown in Table II. This explains the overall slower release rate of carprofen compared to that of lidocaine. From the above discussion, we conclude that the releases of lidocanine and carprofen from pure TiO_2 nanotubes depend mainly on the drug solubility which is determined by its intrinsic solubility and degree of ionization.

Drug Releases from PLGA/TiO_2 Nanotubes. Adding polymer into drug delivery matrix, as seen from Figures 5 and 6 which are summarized in Table I, we observed:

1. Overall sustained drug release to days at all pH levels due to the polymer matrix hindering the drug diffusion.
2. For both lidocaine and carprofen, release rate increased when increasing the medium pH. At pH 7.4 and 10.5. Carprofen showed faster release rate and higher initial burst release than lidocaine. While the opposite is true for pH = 3.5, the lidocaine release rate and initial burst were higher.
3. 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.

Explanation of pH dependent shape of drug release profile: Single stage vs. multiple stage. Drug releases from PLGA/TiO_2 nanotubes at different pH are complicated process and could not simply be explained by the drug solubility. Therefore, we fitted the experimental release data by two mathematical models to understand the release mechanism. The two models are the first order[15,16] and Gallagher–Corrigan[17] as shown below. These two models were selected and discussed because they generated the best fit to our experimental data.

1. First order model: $M_t/M_\infty = 100 - e^{b-kt}$
   where M_t/M_∞ is the cumulative drug release amount at time t and infinite time, k is the first order release constant, b is constant.

Table III. Kinetic Models Equations and Best-Fit Parameters of Lidocaine and Carprofen Release from PLGA/TiO_2 Nanotubes

	Lidocaine								
	pH= 3.5			pH= 7.4			pH= 10.5		
	K	b	R^2	k	b	R^2	k	b	R^2
$M_t/M_\infty = 100 - e^{b-kt}$	0.003933	4.577	0.9579	0.0105	4.498	**0.9877**	0.0112	4.344	**0.9902**
	pH	f_B	k_1	f_{tmax}	k_2	t_{2max}		R^2	
$f_t = f_B \cdot (1 - e^{-k_1 t}) + (f_{tmax} - f_B) \cdot \left(\frac{e^{k_2 t - k_2 t_{2max}}}{1 + e^{k_2 t - k_2 t_{2max}}}\right)$	3.5	42	0.0102	100	0.01845	312		**0.9923**	

	Carprofen								
	pH= 3.5			pH= 7.4			pH= 10.5		
	K	b	R^2	k	b	R^2	k	b	R^2
$M_t/M_\infty = 100 - e^{b-kt}$	0.003519	4.558	0.9927	0.04452	4.40	**0.9839**	0.1957	4.486	**0.9821**
	pH	f_B	k_1	f_{tmax}	k_2	t_{2max}		R^2	
$f_t = f_{tmax} \cdot (1 - e^{-k_1 t}) + (f_{tmax} - f_B) \cdot \left(\frac{e^{k_2 t - k_2 t_{2max}}}{1 + e^{k_2 t - k_2 t_{2max}}}\right)$	3.5	50	0.009832	100	0.006351	336		**0.9968**	

This model represents the drug dissolution in pharmaceutical dosage, for example, water-soluble drug releases from porous matrices.[18] 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:

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

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_{tmax} is the maximum drug release percentage during the whole process, t_{2max} is the time at which drug release rate reaches the maximum.

This model proposed by Gallagher and Corrigan[17] 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 demonstrate the drug release from biodegradable carriers.[17,19–21]

These two models were examined with experimental data to determine the best model and mechanism of drug release from PLGA/ TiO_2 nanotubes. The correlation coefficient (R^2) was used as an indicator of the best fitting for each model considered. The model could be used to describe the release kinetics when R^2 is higher than 0.97.[22] Matlab was employed to fit the two models to the experimental drug release data. Based on the models, lidocaine and carprofen release curve fitting results are displayed in Table III and Figures 8 and 9.

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 as seen in Figure 8(a) (lidocaine) and Figure 9(a) (carprofen), 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. It is worthy to note that though R^2 obtained are satisfactory in fitting to the first order, the actual release seems to be deviated from the first order release as seen in Figures 8(a) and 9(a). This is especially true for lidocaine release which might be due to the amino group interaction of lidocaine with PLGA in aqueous environment. Thus, we can only say that at pH 10.5 and 7.4, the lidocaine and carprofen releases are approximated as the first order release. At pH = 3.5, however, the fitting of first order kinetics to the experimental data of lidocaine release was not satisfactory, demonstrating that the drug release in the medium with the low pH value have a different release mechanism compared with those of releases at higher pH values. Therefore, the Gallagher–Corrigan model was employed to fit lidocaine release experiments at pH 3.5. The results are shown in Figure 8(b) and Table III. It seems that this Gallagher–Corrigan model approximated the experimental points of lidocaine much better than the first order. The release constants for the first and second stage are 0.009681 h^{-1} and 0.01843 h^{-1}, respectively. This lidocaine release kinetics suggests that the process was driven by the diffusion during the initial release and then polymer degradation became the controlling factor.

For carprofen release at pH 3.5 although the first order kinetics presented a good fitting result ($R^2 = 0.9927$), the release profile was slightly better fitted to Gallagher–Corrigan model ($R^2 = 0.9968$) and results are included in Figure 9(b). Release

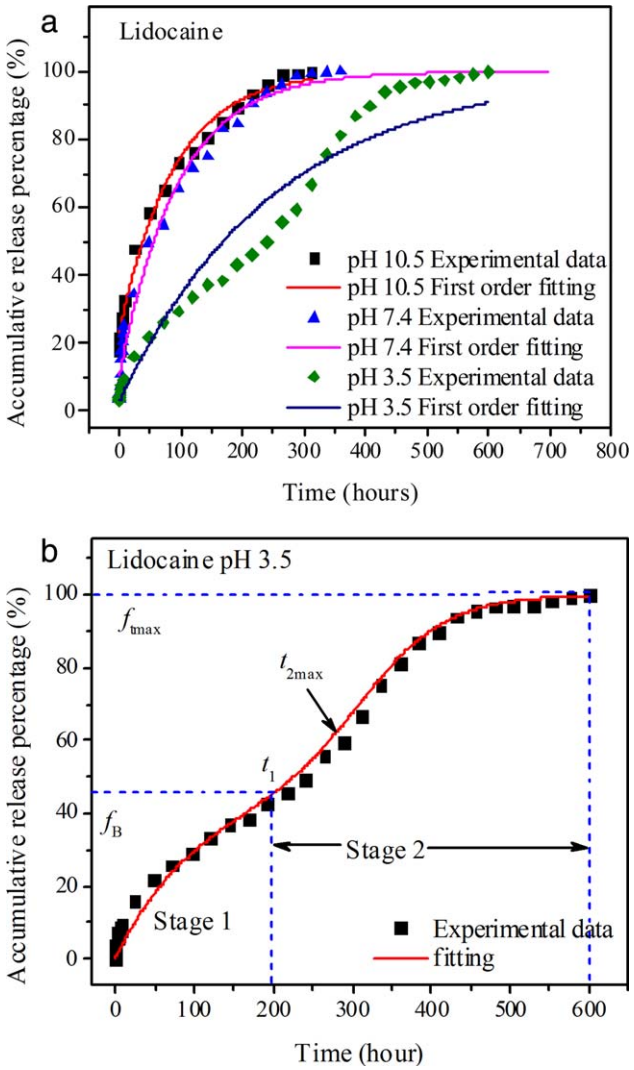


Figure 8. Comparison between experimental data and model fitting (a) using first order kinetics referring to lidocaine releases from PLGA/TiO_2 nanotubes at pH of 10.5, 7.4, and 3.5, and (b) using Gallagher–Corrigan kinetics referring to lidocaine releases from PLGA/TiO_2 nanotubes at pH of 3.5. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

constants of $k_1 = 0.006632\ h^{-1}$ and $k_2 = 0.005751\ h^{-1}$ were obtained for carprofen at pH 3.5. One can observe that lidocaine presented a higher diffusion drug release rate (k_1) and polymer degradation drug release rate (k_2) compared to carprofen. When polymer is put in a solution, it goes through two stages of change, swelling which is due to water absorption followed by polymer degradation through a hydrolysis process. As discussed above from the mathematical model fitting, drug release from PLGA/TiO_2 nanotubes could follow two mechanisms: (a) diffuse through the pores of swollen polymer matrix and (b) drug escapes from the eroded polymer (polymer degradation) matrix. The shape of release profile is very similar between polymer/drug and polymer/drug/TiO_2 nanotubes matrix according to our previous study.[1] In addition, the IR results in this study demonstrated no chemical interaction between TiO_2 nanotubes and polymer/drug. Based on these, we hypothesize that the drug release mechanism of polymer/drug/TiO_2 nanotubes is the same as that of polymer/drug. Thus, the drug release mechanism from PLGA/TiO_2 nanotubes is described in Scheme 2 without TiO_2 nanotubes. 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 diffuse out to the solution. This explains why we observed sustained drug release for both drugs of days at all pH levels when polymer is used in drug delivery. This effect of the polymer matrix hindering the drug diffusion is more profound than the solubility contribution as seen from the changing of carprofen release from near linear release in pure TiO_2 nanotubes to first order diffusion at the 1st stage [Figure 9(b)] at pH = 3.5 in PLGA/TiO_2 nanotubes.

PLGA contains carboxylic terminal groups and has a pK_a of 3.8 (lactic acid pK_a is 3.8). At higher pH values of 7.4 and 10.5,

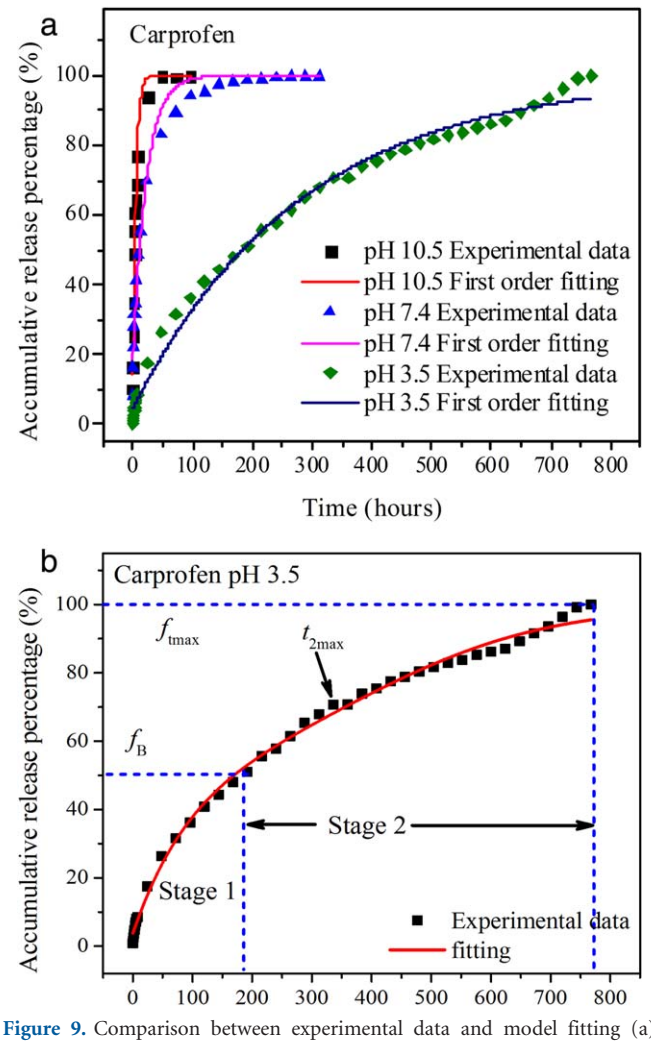
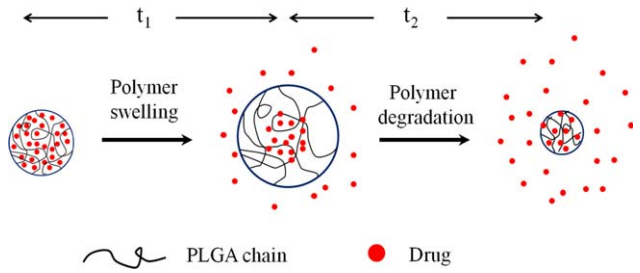


Figure 9. Comparison between experimental data and model fitting (a) using first order kinetics referring to carprofen releases from PLGA/TiO_2 nanotubes at pH of 10.5, 7.4 and 3.5, and (b) using Gallagher–Corrigan kinetics referring to carprofen releases from PLGA/TiO_2 nanotubes at pH of 3.5. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]



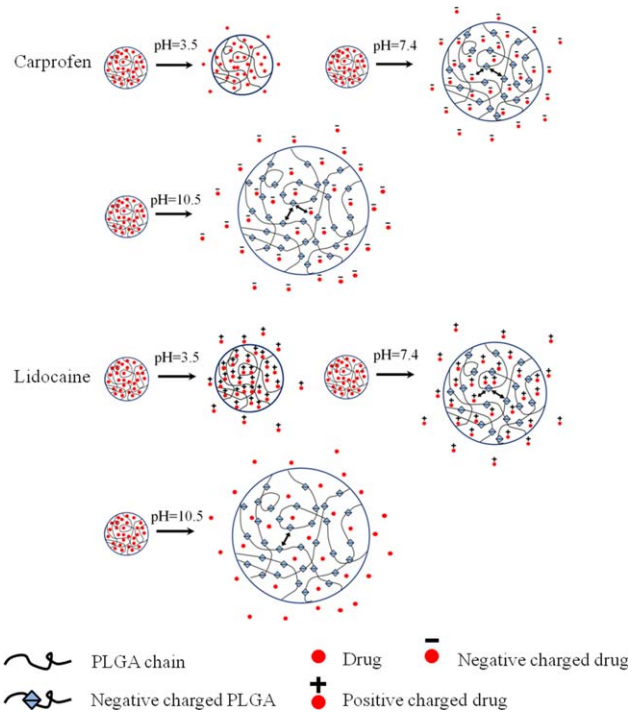
Scheme 2. Schematic diagram explaining pH dependent drug release shape. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

most of the carboxylic groups of PLGA are ionized which lead to higher degree of swelling due to the more hydrophilic properties of the ionized carboxylic groups as well as the electrostatic repulsion between the ionized carboxylic groups. In addition, the repulsive electrostatic repulsive force between charged PLGA will contribute to even more opening space and further increase in the polymer network mesh size. This high degree of swelling of PLGA at pH = 10.5 and 7.4 is reflected by the higher release constant, k compared to k_1 values of pH = 3.5 as seen in Table III. Therefore, at higher pH, the drug can easily diffuse through the swelling polymer and nearly all the drugs will be released to the medium within the critical time t_1 shown in Scheme 2 before the polymer degradation occurs. In this case, we observe a single phase first order release process like the case for both drug releases at pH = 7.4 and 10.5. At pH of 3.5, only small portion of PLGA underwent ionization. The polymer swelling degree was much lower compared to the ones at higher pH values, resulting in a significantly decreased release rate. Thus, the time needed to complete drug release extends to the t_2 time region in Scheme 2 where the contribution of polymer degradation comes into play to allow further release of the remaining drugs that did not finish during previous diffusion phase. In this case, we observe a two phase Gallagher–Corrigan release profile. Thus, we conclude that the PLGA swelling and degradation are the predominant factors in determining the shape of drug release profile from PLGA/TiO_2 nanotubes carrier. As seen from Figures 8(b) and 9(b), the t_1 is 198 h for lidocaine release which is similar to that of carprofen (~185 h). This finding suggests an innovative and easy way to determine the time of polymer degradation in drugs by the generation of two-staged drug release through the pH selection of solution medium in references to the pK_a values of polymer and drugs.

Explanation of pH-dependent drug release rate. At pH of 7.4 and 10.5, both drugs are diffusion controlled. At pH = 3.5, the first stage is diffusion controlled. This indicates that the drug diffusion rate is important in determining the drug release rate. Since polymer is incorporated to the drug carrier matrix, we need to take into account of drug/polymer interaction along with the drug solubility as discussed earlier for the case of releases from pure TiO_2 nanotubes. Here, we use Scheme 3 which illustrates the drug release mechanisms of polymer/TiO_2 nanotubes to explain the release rate for the diffusion controlled release process.

At pH = 3.5 which is lower than the pK_a of PLGA, the ionization of carboxylic terminal groups of PLGA was restricted dramatically as shown in Scheme 3, and this would in turn reduce the repulsion among the ionized PLGA and reduce the PLGA water uptake (swelling) significantly. Therefore, the drug diffusion through the polymer matrix became difficult. For carprofen, its solubility was also lowered in the acid medium. Together, the release rate of carprofen from PLGA/TiO_2 nanotubes at pH 3.5 was reduced by both factors. On the other hand, lidocaine became fully ionized in this buffer which would benefit its diffusion. However, the limited opening space of neutral PLGA seemed to be the predominate factor to determine the drug diffusion rate. Thus, the lidocaine release rate similar to that of carprofen was slow at pH 3.5 as seen in Figures 5 and 6.

At pH of 7.4 and 10.5 which are higher than the PLGA pK_a of 3.8, a large percentage of PLGA will undergo deprotonation and carry the negative charges and thus become swelled as shown in Scheme 3. 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 illustrated by Scheme 3 for the case of carprofen at pH = 7.4 and 10.5. This resulted in a fast carprofen burst release (Figure 6) similar to that of pure TiO_2 nanotubes (Figure 4) at these two pH values. 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.[3,23,24] Consequently, the drug release was accelerated at pH = 10.5 compared to pH = 7.4. In contrast, at pH = 7.4 and 10.5, lidocaine has much improved and slower burst release (Figure 5) compared to that of without polymer



Scheme 3. Schematic diagram to explaining pH dependent drug release rate. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

(pure TiO_2 nanotubes, Figure 3). For the release at pH 7.4 lower than lidocaine's pK_a value of 7.9, there are more positively charged lidocaine than the uncharged one. This will lead to a stronger electrostatic attraction to PLGA as seen in Scheme 3, which hinders the lidocaine diffusion from polymer matrix into the buffer solution, resulting in a slower release rate (Figure 5). 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.[25] For the release at pH 10.5, lidocaine is less charged and the release is mainly controlled by the degree of polymer network swelling.

It is known that the PLGA degrades by a hydrolysis process[26] and the degradation rate is controlled by the rate of water diffusion into the matrix and the rate of hydrolysis reaction. For the second release stage at pH = 3.5 which is PLGA degradation controlled, drug with a higher solubility will be released faster to the medium, thereby the rate of water diffusion into polymer will increase which will expedite the polymer degradation (hydrolysis) rate accordingly,[27] which could explain that lidocaine releases faster than caprofen in this stage.

In summary, the pH-dependent drug release rate is determined by

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

CONCLUSIONS

In vitro release of lidocaine and carprofen loaded pure TiO_2 nanotubes and PLGA/TiO_2 nanotubes were described in this study. Adding PLGA to TiO_2 nanotube can greatly sustain drug release. In pure TiO_2 nanotube, the drug release is determined by the drug solubility. The drug release from PLGA/TiO_2 nanotube is much more complicated. The *in vitro* release experiments from PLGA/TiO_2 nanotube showed that the shape of the drug release is determined by the pH-dependent PLGA swelling and degradation. The drug release rate from PLGA/TiO_2 is dominated by pH-dependent parameters of the drug solubility, opening space in the polymer, and electrostatic force between polymer and drug. In addition, the TiO_2 nanotubes showed excellent chemical stability in aqueous environments which promises a great potential to be used as Ti implant surface coating for drug carrier. These pH-dependent findings suggest that modulation of pK_a values of polymers and drug solubility can lead to the controlled release from polymer/TiO_2 drug carriers. It worth to note here that though we employed pH = 3.5 and achieved a sustained release at this pH level in the study which does not represent most of the real body pH environments, it only provides a scenario for an environment to be below polymer pK_a value. The pH = 3.5 value is only used here to see how a polymer will behave kinetically at an environment below its pK_a value. Therefore, if one picks a polymer with a pK_a value higher than pH = 7.4 with proper drug solubility, then this polymer might have a similar behavior in human body as PLGA in pH = 3.5 in this work. Thus, a controlled release could be achieved at pH = 7.4 by carefully selecting, designing, and synthesis of polymers for the drugs of interest. Our immediate future work is to carefully design and synthesize polymers with desirable pK_a at the physiological pH for both carprofen and lidocaine releases based on the findings from this paper.

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