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Phospholipase A2-responsive antibiotic delivery via nanoparticle-stabilized liposomes for the treatment of bacterial infection

Soracha Thamphiwatana[1,2], **Weiwei Gao**[1,2], **Dissaya Pornpattananangkul**[1,2], **Qiangzhe Zhang**[1], **Victoria Fu**[1,2], **Jiayang Li**[1], **Jieming Li**[1], **Marygorret Obonyo**[3], and **Liangfang Zhang**[1,2,*]

[1]Department of Nanoengineering, University of California, San Diego, La Jolla, California 92093, USA

[2]Moores Cancer Center, University of California, San Diego, La Jolla, California 92093, USA

[3]Department of Medicine, University of California, San Diego, La Jolla, California 92093, USA

Abstract

Adsorbing small charged nanoparticles onto liposome surfaces to stabilize them against fusion and payload leakage has resulted in a new class of liposomes capable of environment-responsive drug delivery. Herein, we engineered a liposome formulation with a lipid composition sensitive to bacterium-secreted phospholipase A2 (PLA_2) and adsorbed chitosan-modified gold nanoparticles (AuChi) onto the liposome surface. The resulting AuChi-stabilized liposomes (AuChi-liposomes) showed prohibited fusion activity and negligible drug leakage. However, upon exposure to either purified PLA_2 enzyme or PLA_2 secreted by *Helicobacter pylori* (*H. pylori*) bacteria in culture, AuChi-liposomes rapidly released the encapsulated payloads and such responsive release was retarded by adding quinacrine dihydrochloride, a PLA_2 inhibitor. When loaded with doxycycline, AuChi-liposomes effectively inhibited *H. pylori* growth. Overall, the AuChi-liposomes allowed for smart “on-demand” antibitoic delivery: the more enzymes or bacteria present at the infection site, the more drug will be released to treat the infection. Given the strong association of PLA_2 with a diverse range of diseases, the present liposomal delivery technique holds broad application potential for tissue microenvironment-responsive drug delivery.

Keywords

liposome; gold nanoparticle; phospholipase; enzyme responsive; antimicrobial delivery

INTRODUCTION

Liposomes are an established drug carrier with well-documented advantages including highly biocompatible lipid materials, readily tunable formulation properties, and high drug carrying capacity[1–3]. Owing particularly to their distinguishable bilayer structure, liposomes

*Corresponding author: Tel: 858-246-0999, zhang@ucsd.edu.

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are prone to fusion with bacterial membranes, making them a suitable delivery system for various antimicrobial treatments[4–6]. To further improve on the therapeutic efficacy of liposomal drugs, a myriad of environmentally responsive liposomal formulations have been developed that possess preferential liposome-bacterium fusion ability or triggered drug release at infection sites upon external stimulation[7, 8]. Common stimuli include temperature, pH, redox potential, and enzymatic activities, and these stimulus-responsive liposomal systems hold great promise to improve the current treatment regimes of bacterial infection[9–12].

Recently, nanoparticle-stabilized liposomes have emerged as a new and robust liposomal delivery system that involves the attachment of small charged nanoparticles onto the outer surfaces of phospholipid liposomes for liposome stabilization and triggered cargo release[13, 14]. The nonspecific adsorption of charged nanoparticles onto phospholipid bilayers provided steric repulsion that inhibited liposome fusion. It also reduced liposome surface tension and thus further enhanced liposome stability[15, 16]. Intriguingly, the charge and charge density of both the nanoparticle stabilizers and the liposomes could be precisely tailored to enable stimulus-responsive binding and detachment of the nanoparticles, thereby allowing for an on-demand control over liposome fusion activity for smart drug delivery. For instance, cationic liposomes bound with negatively charged gold nanoparticles only fused with bacteria at acidic pH, which made them suitable for treating various skin pathogens that thrive in acidic infection sites such as the case with *Propionibacterium acnes*[13]. Conversely, anionic liposomes stabilized by positively charged gold nanoparticles were highly stable in gastric acid, but capable of fusing with bacteria at physiological pH, making them suitable to treat gastric pathogens such as *Helicobacter pylori* (*H. pylori*)[14]. Even in the absence of such stimulus-induced detachment of the nanoparticle stabilizers, these liposomes still had a substantial fraction of their surface areas exposed and highly accessible to membrane-targeting biochemical molecules such as bacterial toxins and enzymes. In a previous study, it has been shown that pore-forming toxins could effectively punch holes in the exposed lipid membranes to trigger antibiotic release from the nanoparticle-stabilized liposomes[17]. Herein, we report their responsiveness to hydrolytic enzymes secreted by bacteria and demonstrate that the enzyme-triggered drug release subsequently kills or inhibits the growth of the enzyme-secreting bacteria. While in principle the enzyme-triggered antibiotic release from nanoparticle-stabilized liposomes can be applied to a broad range of pathogens that secrete membrane-damaging enzymes, particular interest is focused on *H. pylori* in this work.

H. pylori infects nearly half of the world population and is of a significant public health concern. Infection with *H. pylori* is the primary cause of chronic gastritis, peptic ulcers, and gastric malignancy[6, 18, 19]. However, eradication of *H. pylori* is challenging regardless of the treatment regimens, owing partly to the rapid emergence of *H. pylori* strains resistant to the antibiotics[20, 21]. *H. pylori* bacteria are known to secrete phospholipase A2 (PLA_2), a family of enzymes capable of hydrolyzing membrane phospholipids, causing mucosal damage and benefiting bacterial survival[22, 23]. Such enzymatic activity can be utilized as an environment cue to disrupt membrane integrity for triggered payload release from liposomes[24, 25]. In this study, we synthesized liposomes with lipid composition sensitive to PLA_2 and stabilized

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them with small chitosan-modified gold nanoparticles (AuChi). The adsorbed AuChi were effective in preventing liposome fusion and drug leakage, while leaving a considerable fraction of liposome surfaces accessible to PLA_2 enzyme. As shown in Figure 1, the cationic AuChi bind to the negatively charged liposome surfaces through electrostatic attraction and thus stabilize liposomes against fusion and avoid undesirable antibiotic leakage. When the stabilized liposomes are in the vicinity of *H. pylori* bacteria, the bacterium-secreted PLA_2 degrades phospholipids, compromises the membrane integrity, and subsequently releases the antibiotic payload. Such on-site release of antibiotics enables localized and rapid killing of *H. pylori* bacteria. We first demonstrated liposome stabilization upon AuChi adsorption and then examined the payload release kinetics of the AuChi-stabilized liposome (AuChi-liposome) in the presence of both purified PLA_2 and *H. pylori* culture, respectively. We further demonstrated that the released antibiotics from the liposomes in the presence of *H. pylori* were effective in inhibiting the growth of the bacteria.

RESULTS AND DISCUSSION

The preparation of AuChi-liposome can be divided into three steps. First, AuChi were synthesized by an ex-situ stabilization technique as previously described, where gold nanoparticles were made with a sodium borohydride reduction method and then stabilized by adding calculated amount of chitosan under ambient condition[14, 17]. Dynamic light scattering (DLS) measurements of AuChi showed a diameter of approximately 10 nm with a narrow size distribution and a strong positive surface charge of 35.5 ± 0.9 mV, indicating the presence of cationic amine groups of chitosan on gold surfaces. Second, 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) and 1,2-dioctadecanoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol) (sodium salt) (DSPG) in a molar ration of 9:1 were used to formulate anionic liposomes following a standard extrusion method in deionized water[6]. DSPG has been found susceptible to PLA_2-mediated degradation and liposomes made from DSPG are expected to be disrupted by PLA_2, leading to drug release from the liposomes[26, 27]. DLS measurements of the liposomes showed a diameter of 84.7 ± 0.8 nm with polydispersity index of 0.12 ± 0.02 and a surface zeta potential of −54.7 ± 2.6 mV. Lastly, the anionic liposomes were mixed with cationic AuChi nanoparticles at a liposome-to-AuChi molar ratio of 1:300 under bath sonication for 10 min[14, 17]. The resulting AuChi-liposomes showed a diameter of 95.0 ± 2.7 nm in water, which remained stable in PBS at pH = 6.5 with a diameter of 97.1 ± 2.4 nm. The size increase of AuChi-liposomes compared to bare liposomes corresponds to the adsorption of AuChi onto the liposome surface. Liposome surface charge also switched from the strong negative value of the bare liposomes to 36.9 ± 1.3 mV of AuChi-liposomes, further confirming the binding of positively charged AuChi to the negatively charged liposomes through electrostatic interactions (Figure 2). Further studies showed that the size and surface zeta potential AuChi-liposome remained stable upon loading small molecule cargoes such as rhodamine B (RhB) and doxycycline into the liposomes.

Adsorbed AuChi nanoparticles are expected to stabilize liposomes that are otherwise prone to fusion and drug leakage[14, 17, 28]. To test this, we first used a bacterium-liposome fusion assay[6, 14]. Specifically, 0.5 mM AuChi-liposomes containing 0.5 mol% DMPE-RhB were prepared and incubated with 5×10^8 CFU/mL *H. pylori* bacteria at 37°C for 30 min. Following the incubation, the bacteria pellets were collected and resuspended in PBS

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solution. The fusion ability of liposomes was then quantified by measuring the fluorescence intensity of the bacterial suspension. *H. pylori* bacteria incubated with bare liposomes without AuChi stabilizers showed strong fluorescence intensity In contrast, a much weaker florescence signal was detected from the bacteria incubated with AuChi-liposomes, indicating a significantly reduced liposome fusion activity upon the adsorption of small AuChi nanoparticles (Figure 3A). We next tested the inhibition of drug leakage by AuChi nanoparticles. For this study, rhodamine B (RhB) was used as a model drug. As shown in Figure 3B, bare liposomes released all the fluorescence dye in 48 hrs. In contrast, less than 2% of the encapsulated RhB was released in the same time span from AuChi-liposomes. Together, these results confirm strong stabilization effects conferred by adsorbing AuChi stabilizers onto the liposome surface.

We next proceeded to examine whether the drug release from AuChi-liposomes could be triggered by PLA_2. By adding purified PLA_2 into the RhB-loaded AuChi-liposome solutions, we found that the drug release rates increased with the increase of PLA_2 concentrations. When PLA_2 concentration was at 1, 10, and 100 μg/mL, approximately 5, 50, and 67% of encapsulated RhB molecules were released within 24 hrs, respectively (Figure 4A). In addition, accumulative RhB release profiles showed gradual increases with time without a burst release, implying that drug release kinetics from AuChi-liposomes in the presence of PLA_2 is dominated by diffusional liposome efflux [29, 30]. Therefore, we attempted to use a diffusion-dominant Higuchi model to analyze the drug release profiles: $M_t = Kt^{1/2}$, where M_t is drug release at time t in hours and K is the Higuchi constant [31, 32]. Plotting the drug release percentage against the square root of time yielded linear fittings with $R^2 = 0.97$ and 0.98 for 10 and 100 μg/mL PLA_2, respectively (Figure 4B). The goodness of the fit indicates a diffusion-controlled liposome release mechanism. On the basis of this analysis, the Higuchi constants of drug release with 10 and 100 μg/mL of PLA_2 were determined to be 10.57 ± 0.25, and 14.74 ± 0.26 $h^{-1/2}$, respectively.

To further verify that *H. pylori*-secreted PLA_2 can indeed trigger drug release from AuChi-liposomes, we incubated RhB-loaded AuChi-liposomes with *H. pylori* culture and monitored the release of RhB from the liposomes[33]. As shown in Figure 5, when AuChi-liposomes were incubated in fresh culture medium without *H. pylori* bacteria, less than 5% RhB was released in 24 hrs, confirming that AuChi-liposomes were stable. However, when the AuChi-liposomes were incubated in bacterial culture containing 1×10^7 CFU/mL *H. pylori* bacteria, 9.8%, 15.5%, and 17.0% of RhB was released in 1, 12, and 24 hrs, respectively. The drug release rate further increased when the bacterial concentration was increased. Specifically, when the bacterial concentration was increased to 1×10^8 CFU/mL, AuChi-liposomes released 13.4%, 30.3% and 28.7% of RhB in 1, 12, and 24 hrs, respectively. It seemed that the drug release has reached a plateau within 12 hrs and the observed release difference between 12 and 24 hrs was likely due to experimental variation. To further confirm that PLA_2 was indeed responsible for the accelerated drug release, 0.13 μM quinacrine dihydrochloride, a PLA_2 inhibitor, was added to the culture containing 1×10^8 CFU/mL *H. pylori*. Under this condition, a reduced drug release rate was observed; AuChi-liposomes released 6.8%, 9.7% and 15.1% of RhB in 1, 12, and 24 hrs, respectively. The incomplete inhibition of RhB release in the presence of quinacrine dihydrochloride was

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likely due to other *H. pylori*-secreted virulence factors such as CagA, VacA, and TlyA, which are all known to damage phospholipid membranes through various mechanisms[34–37].

After having verified the responsive drug release from AuChi-liposomes in the presence of both purified PLA_2 and PLA_2 secreted by *H. pylori* bacteria, we finally tested the antimicrobial activity of doxycycline-loaded AuChi-liposome against *H. pylori* bacteria. In the study, doxycycline-loaded AuChi-liposomes with a doxycycline concentration of 0.2 mM were incubated with *H. pylori* bacteria (5×10^7 CFU/mL) in 5% BHI for 12 hrs, followed by serial dilution of each sample for bacterial colony enumeration. For comparison, the same concentration of free doxycycline was tested in parallel as a positive control, and empty AuChi–liposomes (without drug) and PBS (1X) served as negative controls. As shown in Figure 6, empty AuChi–liposome did not show any inhibitory effect against *H. pylori*, as their incubation with the bacteria resulted in a comparable colony formation to the PBS (1X) control, whereas free doxycycline resulted a complete bacterial killing under the experimental condition. In contrast, doxycycline-loaded AuChi–liposomes showed excellent antimicrobial efficacy against *H. pylori* and such anti-*H. pylori* efficacy was significantly weakened when PLA_2 inhibitor (0.13 μM) was added to the bacterial culture. The incomplete killing of *H. pylori* by AuChi-liposomes was likely due to the partial release of doxycycline during the 12 hrs of incubation time. With bacterial enzyme-triggered drug release mechanism, the doxycycline-loaded AuChi-liposomes confer distinct advantages to treat bacterial infections. For example, with a high stability, the formulation improves on the shelf-time of the liposomal drug with minimum drug leakage prior to administration. In addition, AuChi-liposomes allow antibiotics to be delivered in a bacterium-targeted fashion: antibiotic payloads will only be released at the infection sites where the bacteria secrete hydrolytic enzymes. More importantly, by using cues from the target bacteria to trigger drug release, the dosage of the antibiotics is self-regulated by the severity of the infections: the more bacteria present at the infection site, the more drugs will be released to treat the bacteria.

CONCLUSION

In conclusion, we formulated a PLA_2-degradable liposome formulation and further adsorbed AuChi nanoparticles onto the liposome surfaces. The resulting AuChi-liposomes were stable under storage conditions but were susceptible to PLA_2 degradation at infection site. Such liposomal formulation effectively prevented undesirable liposome fusion and drug leakage. However, the presence of PLA_2, either in purified form or in *H. pylori* culture, caused rapid drug release due to the enzymatic degradation of phospholipids and the subsequent damage of liposome integrity. When incubated with *H. pylori* bacteria *in vitro*, AuChi-liposomes effectively inhibited the bacterial growth. Although aimed for anti-*H. pylori* treatment in this particular study, the critical role played by PLA_2 has been increasingly recognized in various disease pathogenesis including bacterial infections, viral infections, and cancer development[38–41]. Therefore, PLA_2-responsive AuChi-liposomes hold great potential for preferential drug delivery with minimized side effects and targeted therapeutic efficacy to treat a wide range of diseases.

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MATERIALS AND METHODS

Materials

1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1,2-dioctadecanoyl-sn-glycero-3-phospho-(1′-rac-glycerol) (sodium salt) (DSPG), and 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-N-lissamine rhodamine B sulfonyl (DMPE-RhB) were purchased from Avanti Polar Lipids, Inc (Alabaster, AL). 8-aminonaphthalene-1,3,6-trisulfonic acid disodium salt (ANTS) and *p*-xylene-bis-pyridinium bromide (DPX) were purchased from Life Technology (Carlsbad, CA). Rhodamine B, doxycycline, phospholipase A_2 (*Apis mellifera*), and quinacrine dihydrochloride were purchased from Sigma Aldrich (St Louis, MO). Brain-heart infusion (BHI) broth and Columbia agar were purchased from Becton Dickinson (Sparks, MD). Hydrogen tetrachloroaurate ($HAuCl_4$) and sodium borohydride ($NaBH_4$) were purchased from ACROS Organics (Geel, Belgium). Chitosan-50 was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Preparation of chitosan-modified gold nanoparticles (AuChi)

AuChi nanoparticles were prepared by sodium borohydride reduction technique as previously described [13, 14]. Briefly, an aqueous solution of $HAuCl_4$ (0.1 mM, 50 mL) was first reduced by 5 mg of $NaBH_4$ to form gold nanoparticles, followed by overnight incubation with 0.1% w/v chitosan that was pre-dissolved in 0.1 M acetic acid. The resulting AuChi nanoparticles were purified three times by using an Amicon Ultra-4 centrifugal filter with a molecular weight cut-off of 10 kDa and the final pH was adjusted to 6.5 by adding HCl. The nanoparticle size and surface zeta potential were determined by dynamic light scattering (DLS) measurements (Malvern Zetasizer ZS, Malvern Instruments, Worcestershire, UK).

Preparation of AuChi-stabilized liposomes (AuChi-liposomes)

Anionic liposomes were prepared by using a vesicle extrusion method. Briefly, DSPC, a zwitterionic phospholipid, and DSPG, an anionic phospholipid, were dissolved in chloroform and mixed at 9:1 molar ratio. The organic solvent was evaporated under a stream of nitrogen gas until the thin lipid film was formed. Then the dry lipid film was hydrated with deionized water, or 2 mM rhodamine B (RhB), or 20mM doxycycline, followed by 2 min of vortexing and 30 min of bath sonication (Fisher Scientific FS30D, Pittsburgh, PA) to produce multilamellar vesicles (MLVs). The solution was then sonicated for 1 min at 20 W by a titanium probe (Branson 450 sonifier, Danbury, CT) to produce unilamellar vesicles. Following the sonication, the solution was extruded through a 100 nm pore-sized polycarbonate membrane for 11 times at 60°C to form narrowly distributed small unilamellar vesicles (SUVs). The liposomes were purified by gel filtration through a Sephadex G-75 column. To prepare fluorescently labeled liposomes, DMPE-RhB (0.5% mol) was added to the lipid mixture prior to liposome preparation. To prepare AuChi-liposomes, the liposomes and AuChi were mixed at 1:300 molar ratio, followed by 12 hrs of vortexing. Hydrodynamic size, size distribution, and surface charge of the liposomes and AuChi-liposomes were characterized by DLS (Malvern Zetasizer ZS, Malvern Instruments, Worcestershire, UK). All measurements were repeated three times at 25°C.

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Helicobacter pylori (H. pylori) bacterial culture

H. pylori Sydney strain 1 (SS1) were routinely maintained on Columbia agar supplemented with 5% laked horse blood at 37°C under microaerobic conditions (10% CO_2, 85% N_2, and 5% O_2) [6]. For experiments, broth cultures of *H. pylori* were prepared by sub-culturing fresh colonies from agar plates into BHI containing 5% fetal bovine serum (FBS) overnight at 37°C under microaerobic conditions with moderate reciprocal shaking.

Liposome stability assay

A fluorescence method was used to study the fusion of AuChi-liposome with *H. pylori* bacteria. Specifically, 5×10^8 CFU *H. pylori* bacteria (determined by OD_{600} value, OD_{600} = 1.0 corresponding to approximately 1×10^8 CFU/mL) was washed three times with PBS by repeated centrifugation at 4,000 ×g. The bacteria pellet was collected and then mixed with 0.5 mM fluorescently labeled AuChi-liposome (containing 0.5 mol% of DMPE-RhB) at pH 6.5. After 10 min incubation, the bacteria pellet was collected by centrifugation at 4,000 ×g for 5 min and then resuspended in 1 mL PBS. The bacteria were then measured for fluorescence intensity at the range of 550–700 nm (DMPE-RhB's fluorescence emission range). DMPE-RhB-labeled bare liposomes (without AuChi stabilizer) were used as a control. The experiment was carried out in triplicate and average value was reported.

Phospholipase A_2 (PLA_2)-triggered drug release from AuChi-liposomes

RhB was used as a model drug for release study. RhB-loaded AuChi liposomes were formulated as described above and the unencapsulated RhB molecules were removed by gel filtration through a Sephadex G-75 column. The samples were added with PLA_2 (at a final enzyme concentration of 0–100 μg/mL) and the mixtures were incubated at 37°C. At predetermined time points, released RhB was separated by filtration using an Amicon Ultra-4 centrifugal filter with a molecular weight cut-off of 10 kDa at 14,000 × g for 20 min. RhB emission intensity at 585 nm was measured. To obtain 100% drug release, freshly prepared RhB-loaded AuChi-liposome suspension was disrupted by Triton-X-100 (1% v/v) to completely release the encapsulated drug, followed by drug quantification. Percentage of released drug was defined as following: Percentage of released drug (%) = $(I_{PLA_2} - I_{PBS})/(I_{Triton\text{-}X\text{-}100} - I_{PBS})\times100$, in which I_{PLA_2}, I_{PBS}, and $I_{Triton\text{-}X\text{-}100}$ represent fluorescence emission intensity at 585 nm of the samples incubated with PLA_2, PBS, and Triton-X-100, respectively. The experiment was performed in triplicate.

AuChi-liposome drug release in *H. pylori* bacterial culture

In the study, 190 μL of RhB-loaded AuChi-liposome was added with 10 μL of overnight broth from *H. pylori* cultures originally containing 1×10^7 CFU/mL and 1×10^8 CFU/mL bacteria, respectively. The mixture was incubated for 1, 12, and 24 hrs under gentle shaking. After incubation, released RhB was separated by the same filtration process as described above. Quinacrine dihydrochloride (final concentration 0.13 μM), a PLA_2 inhibitor, was used to inhibit PLA_2 activity. RhB-loaded AuChi-liposome incubated in 5% (v/v) fresh BHI broth without being used for *H. pylori* culture was taken as a negative control. To obtain 100% drug release, Triton-X-100 (1% v/v) was added to disrupt liposomes. The experiments were repeated there times.

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Anti-*H. pylori* activity study

Doxycycline-loaded AuChi-liposomes were prepared as described. Free doxycycline molecules were removed by using a Sephadex G-75 column. Doxycycline concentration was determined by measuring the absorbance at 273 nm and a standard curve of doxycycline was made. To quatnify encapsulated doxycycline, liposomes were disrupted by Triton-X-100 (1% v/v), followed by absorbance measurement. Then 10 μL bacterial suspension containing 1×10^7 CFU *H. pylori* bacteria was added to 190 μL of doxycycline-loaded AuChi-liposome. The mixture was incubated with gentle shaking at 37°C under microaerobic conditions. After 12 hrs incubation, a series of 10-fold dilutions of the bacterial suspension (1:10 to $1:10^5$) was prepared, and 5 μL from each diluted sample was inoculated onto a Columbia agar plate supplemented with 5% laked horse blood. The plates were cultured in the incubator for 4 days before colony counting. Free doxycycline served as a positive control, while empty AuChi-liposome (without doxycycline) and PBS (1X, pH=6.5) served as negative controls. All experiments were repeated three times.

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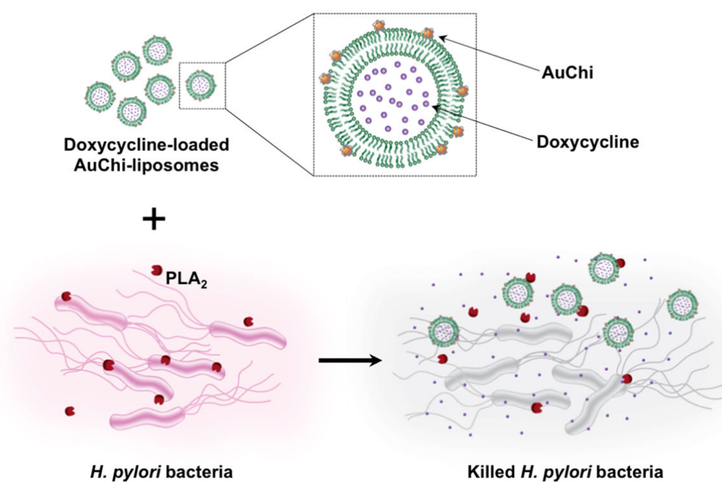

Fig. 1.

Schematic illustration of phospholipase A2 (PLA_2)-triggered antibiotic release from liposomes stabilized by chitosan-modified gold nanoparticles (AuChi-liposome) to treat bacteria (e.g., *H. pylori*) that secrete the enzyme. Antibiotic (*e.g.* doxycycline)-loaded liposomes are prohibited from fusion by absorbing AuChi nanoparticles onto their surface. Once the AuChi-liposomes encounter bacteria-secreted PLA_2, the enzyme cleaves the phospholipids that form the liposome membranes and thus release the encapsulated antibiotics, which subsequently kill or inhibit the growth of the bacteria.

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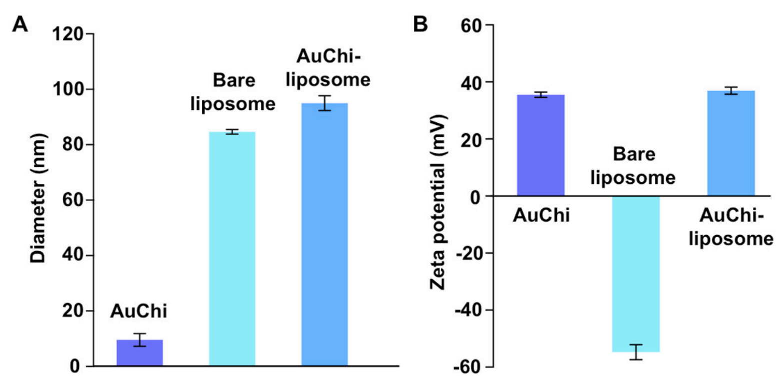

Fig. 2.

(A) Hydrodynamic size (diameter, nm) and (B) surface zeta potential of AuChi, bare liposome (without AuChi), and AuChi-liposome with an AuChi-to-liposome molar ratio of 300:1.

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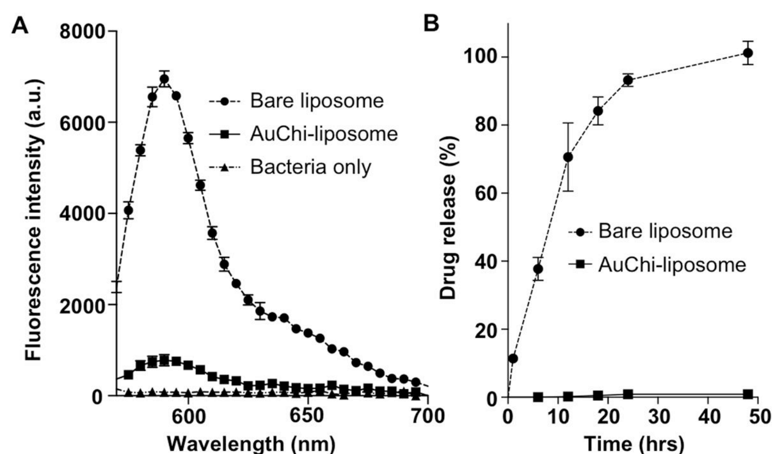

Fig. 3.

(A) Fusion ability of bare liposome and AuChi-liposome with *H. pylori* bacteria. Fluorescently labeled liposome (bare liposome or AuChi-liposome) was incubated with $5{\times}10^8$ CFU *H. pylori* bacteria at pH 6.5 for 10 min. After incubation, the bacteria pellet was collected and quantified for fluorescence intensity at the range of 550–700 nm. The same amount of bacteria without incubating with any liposome formulations was tested in parallel serving as background signal. (B) Accumulative drug release profile from bare liposome and AuChi-liposome. RhB was used as a model drug loaded inside the liposome. The released RhB was quantified by measuring the fluorescence intensity at 585 nm.

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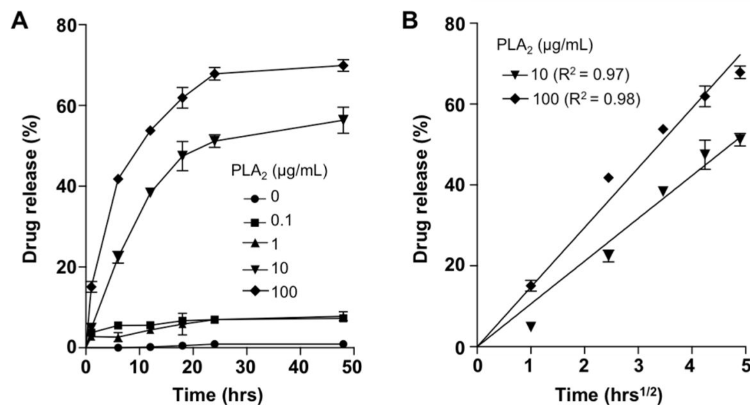

Fig. 4.

(A) Accumulative drug release kinetics from AuChi-liposome in the presence of various PLA_2 enzyme concentrations. (B) The drug release percentage was plotted against the square root of time, which yielded linear fittings using a diffusion-dominant Higuchi model. The fitted line was determined from the first order polynomial fitting assuming the y-interception at zero. The slope was then calculated by minimizing the sum of the squares of the vertical distances between the point and the line.

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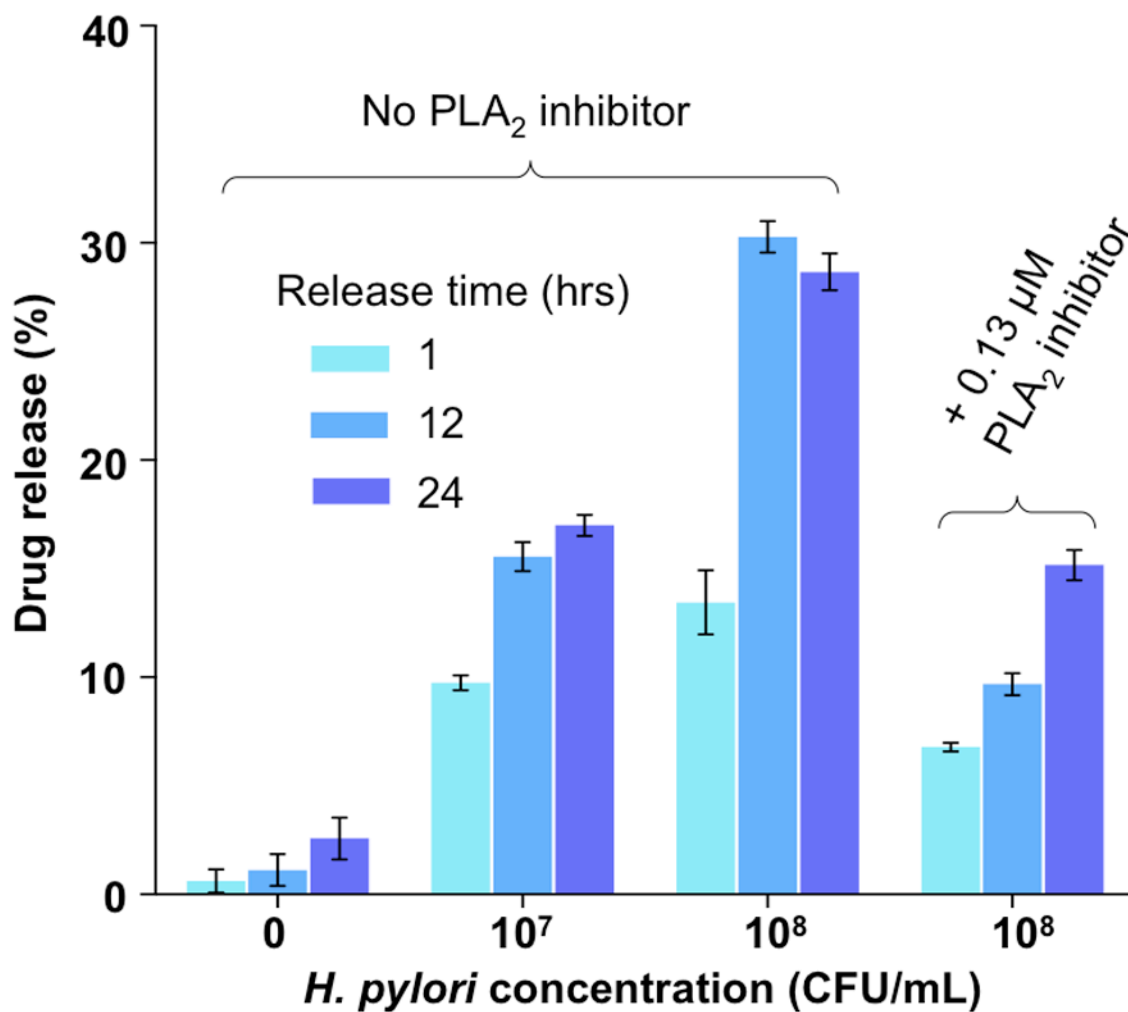

Fig. 5.

Drug release from AuChi-liposome at 1 hr, 12 hrs, and 24 hrs post incubation with 0, 1×10^7 and 1×10^8 CFU/mL *H. pylori* bacteria culture, respectively. As a control group, quinacrine dihydrochloride (0.13 μM), a PLA_2 inhibitor, was added to the bacterial culture to inhibit PLA_2 activity. Data represent mean ±SD (n = 3).

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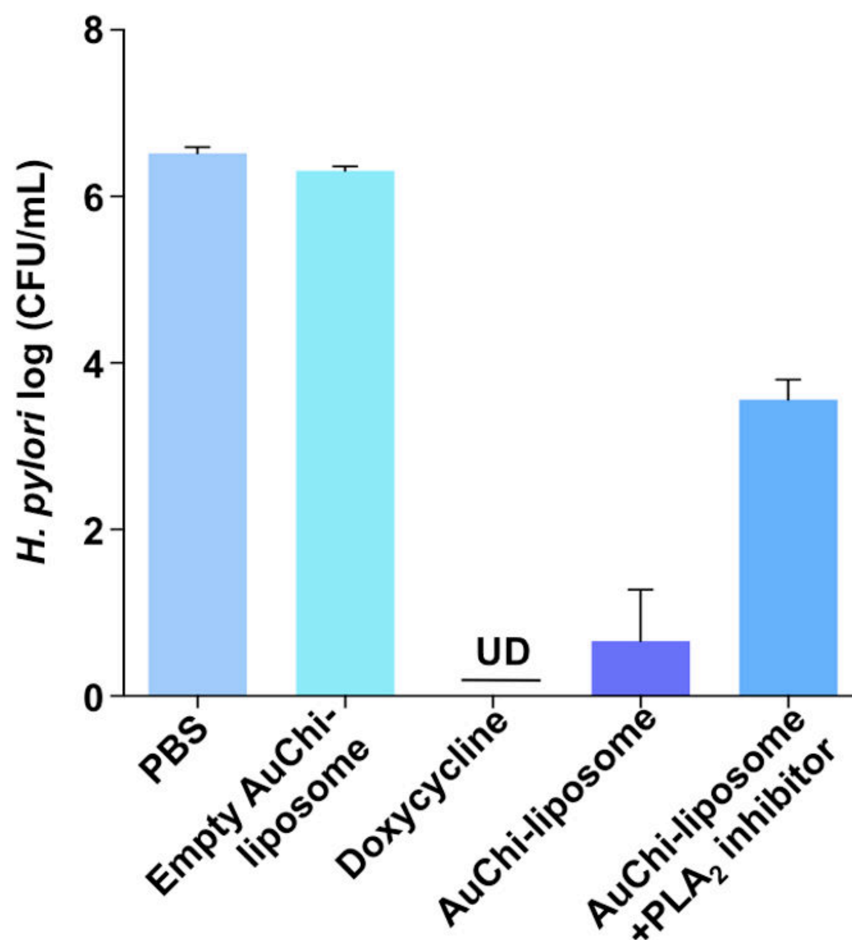

Fig. 6.

Antimicrobial activity of doxycycline-loaded AuChi-liposome against *H. pylori* bacteria. Doxycycline-loaded AuChi-liposome was incubated with *H. pylori* bacteria (5×10^7 CFU/mL) in 5% TSB for 24 hrs before the bacterium enumeration. To test the effect of PLA_2 on the observed antimicrobial activity, PLA_2 inhibitor (0.13 μM) was added the doxycycline-loaded AuChi-liposome and *H. pylori* mixture solution. Free doxycycline (0.2 mM) served as a positive control. Empty AuChi-liposome without doxycycline and PBS (pH = 6.5) served as two negative control groups. Data represent mean ±SD (n = 3).