Loading and Release of Doxorubicin with Magnetic Nanotubes#

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In this work, we study magnetic nanotubes (MNTs) as drug carriers to control the loading and release of doxorubicin (Dox). The inner surfaces of MNTs where Dox molecules are stored are modified with C18-silane and pyridine–silane. By tuning the interaction between the drug molecules and inner surfaces of MNTs via pH, Dox can be effectively encapsulated at pH 7.2 and released at pH 4.5. The successful loading of Dox is confirmed with confocal microscopy studies. The release profiles of Dox from modified MNTs are detected by spectrofluorophotometry, with bare MNTs as control. With proper modifications, MNTs can be used for pH-dependent, controlled release of drug molecules.

Keywords: Magnetic nanotubes, Superparamagnetic iron oxide nanoparticles, Doxorubicin, pH-dependent controlled release, Encapsulation, Degradation

Introduction

Magnetic nanotubes (MNTs), which are silica nanotubes loaded with superparamagnetic iron oxide nanoparticles, have been demonstrated as excellent magnetic resonance contrast agents.[1] With their unique features such as biocompatibility of the constituent materials and distinctive inner and outer surfaces that can be functionalized respectively for drug loading and targeting,[2,3] MNTs have shown great potential as candidate for an ideal therapeutic system. However, reports on drug loading and release studies with MNTs are rare.

Endocytosis, as the main pathway for the uptake of macromolecules and particles from the surrounding medium, has been well documented.[4] This process involves endosome and lysosome internalization, where the pH can be as low as 5.5–6.0 in the endosome and 4.5–5.0 in lysosomes.[5,6] Therefore, drug carriers responsive to pH variation can be designed to selectively release their payload in tumor tissue cells for lysosomotropic delivery.[7] Several approaches have been reported. In summary, they can be classified into two routes. One is achieved by covalently conjugating drug molecules to drug carriers through a proteolytically cleavable bond or a hydrolytically unstable bond.[8–11] The other is using noncovalent bonding, such as electrostatic interactions. An example is to incorporate amines or carboxylic acids into block copolymers to form pH-sensitive micelles whose formation is altered by the protonation of these groups.[12–14]

Doxorubicin (Dox) is a commonly used drug to treat breast, ovarian, and bronchial cancers by inhibiting the synthesis of nucleic acids in cancer cells. However, it has a very narrow therapeutic index, which has highly limited its use.[15] It is, therefore, critical to control the release profile of Dox. The use of nanovectors, such as nanotubes or vesicles, can provide protection of drugs from degradation and reduce toxicity through selective targeting and controlled release.[16–19] A study of the impact of pH on the partition of Dox in aqueous/chloroform phases by Kataoka *et al*. showed that the Dox partition coefficient varies with pH.[13] At pH >7, Dox partitioning into the chloroform phase is >90%. At pH <5.5, the partition into chloroform phase is ~10%. Based upon these results, the release of Dox can be controlled by pH. Ideally, nanotubes loaded with Dox will not release the drug during circulation in the blood, where the pH is >7. Once the nanotubes are taken up by the cancer cells, the drug will be released in an acidic environment. Lysosomotropic delivery can thus be achieved.

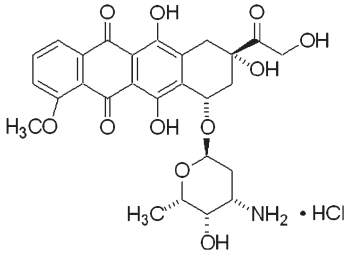
In this paper, Dox was used as the model anticancer drug to test the pH-dependent loading and release features of MNTs. The loading and release profile of Dox is studied by modifying the inner surface of MNTs to vary the bonding strength of the drug molecules to MNTs according to pH.

Experimental

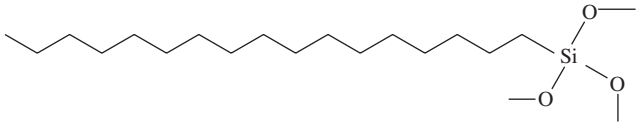
Materials. Dox in the form of hydrochloride salt was purchased from Sigma-Aldrich (St. Louis, MO, USA). Dox base was prepared by mixing Dox with 3 equiv of triethylamine (TEA, Fisher Scientific, Pittsburgh, PA, USA) in dimethylsulfoxide (DMSO, Fisher Scientific), and then diluted with chloroform to get a solution with a Dox concentration of 5 mg/mL. The solution was kept in the dark at 4 °C overnight to allow the neutralization of Dox. Chloroform was purchased

This paper is dedicated to Professor Kwan Kim on the occasion of his honorable retirement.

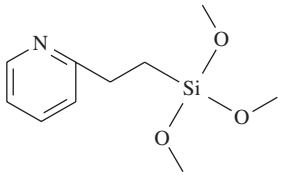
from Fisher Scientific. Silane agents were obtained from Gelest (Arlington, VA, USA), including *n*-octadecyltrimethoxysilane (95%, C18-silane), 2-(trimethoxysilylethyl)pyridine (95%, pyridine–silane), and tetraethoxysilane (99+%, TEOS).



Doxorubicin hydrochloride



n-octadecyltrimethoxysilane (C18-silane)



2-(trimethoxysilylethyl)pyridine (pyridine-silane)

Acetic acid (J.T. Baker, Philipsburg, NJ, USA) and sodium acetate (ACS reagent) were obtained from Fisher Scientific. HEPES buffer solution (1 M) was obtained from Invitrogen (Grand Island, NY, USA). Piranha solution was prepared by mixing concentrated sulfuric acid (H_2SO_4, Fisher Scientific) and hydrogen peroxide (H_2O_2, 30%, Fisher Scientific) with a volume ratio of 3:1. Deionized (DI) water was obtained by a Milli-Q A10 (Billerica, MA, USA) system and used for all the experiments. MNTs (silica nanotubes loaded with magnetic nanoparticles) of length 2 μm were used for the study on Dox release at different pH values.

Modification and Characterization of Nanotubes. MNTs were prepared using a template synthesis method, as reported previously.[1] The inside surfaces of the nanotubes were modified with pyridine–silane together with C18-silane to adjust the bonding strength between Dox molecules and the nanotubes by varying the pH. Three pyridine–silane/C18-silane ratios were used: (1) 100% C18-silane (100C18), (2) 75% C18-silane and 25% pyridine–silane (75C18), and (3) 50% C18-silane and 50% pyridine–silane (50C18). The final concentration of silane was 5% prepared in 95% ethanol/water solutions. The silica-coated anodic aluminum oxide (AAO) templates were immersed in the pyridine–silane and C18-silane mixture for 20 min, washed with ethanol and water, and then baked in an oven at 120 °C for 20 min. MNTs were released by selectively dissolving the AAO template with 0.1 M sodium hydroxide solution and collected with filtration.

MNTs were characterized by a ZEISS EM10CA (Thornwood, NY, USA) transmission electron microscope (TEM). High-resolution TEM images were taken with a JEM-2100F field-emission TEM operating at 200 kV with STEM (scanning tunneling) capability and an Oxford energy dispersive X-ray spectrometer (EDS). The iron content was measured with a flame atomic absorption spectrometer (SOLAAR S Series, Thermo Elemental Corp., thermoscientific. webhelp@thermofisher.com) using acetylene as the fuel and compressed air as oxidant.

Confocal Fluorescence Microscopy Studies. Cover slips were cleaned with Piranha solution initially, and then modified with a layer of TEOS to immobilize the nanotubes. Specifically, the cover slips were immersed in 1:1:4 mixture of 1 M HCl/TEOS/ethanol. After being sonicated for 5 min, cover slips were rinsed with ethanol. Twenty microliters of the nanotube solution was dropped on the TEOS-coated cover slips and dried overnight at 25 °C. An inverted scanning confocal microscope (Axiovert 200, Carl Zeiss, Gottingen, Germany) with a 100× oil-immersion objective lens was used to obtain the fluorescence images. The instrument setup has been described previously.[20]

Release Profiles. The concentration of Dox was measured by a spectrofluorophotometer (RF-1501, Shimadzu, Columbia, MD, USA) with an excitation wavelength of 490 nm and emission wavelength of 560 nm. Considering the potential effect of Fe^{3+} released from iron oxide nanoparticles in MNTs on the measurement of Dox fluorescence intensity, we prepared Dox buffer solutions containing $FeCl_3 \cdot 6H_2O$ with different concentrations.

The amount of the nanotubes used for loading and release studies was in the range $2–3 \times 10^{10}$ for each set of MNTs. It was calculated based on the multiplication of the pore density of the template (10^{10} per cm^2) by the template area. Nanotubes were immersed in the Dox base solution (5 mg/mL) and incubated overnight at 4 °C to attain equilibrium. The MNTs were then collected and dried at 60 °C. To study the Dox release profiles, MNTs loaded with Dox were dispersed in 20 mM HEPES (pH 7.2) buffer solutions at room temperature. The release profiles at acidic pH were obtained in 20 mM acetate buffer (pH 4.5). For the fluorescence intensity measurement, fractions were taken out from the buffer solutions containing MNTs loaded with Dox over time. The fluorescence intensity was measured for each fraction to determine the amount of released Dox. After each measurement, the fraction was put back to keep the total amount of Dox constant.

Two control samples were prepared by dissolving the Dox base with HEPES (20 mM) buffer solutions and acetate buffer solutions (20 mM), respectively.

Results and Discussion

The fluorescence images of the nanotubes loaded with Dox are shown in Figure 1. The images indicate that the loading was successful.

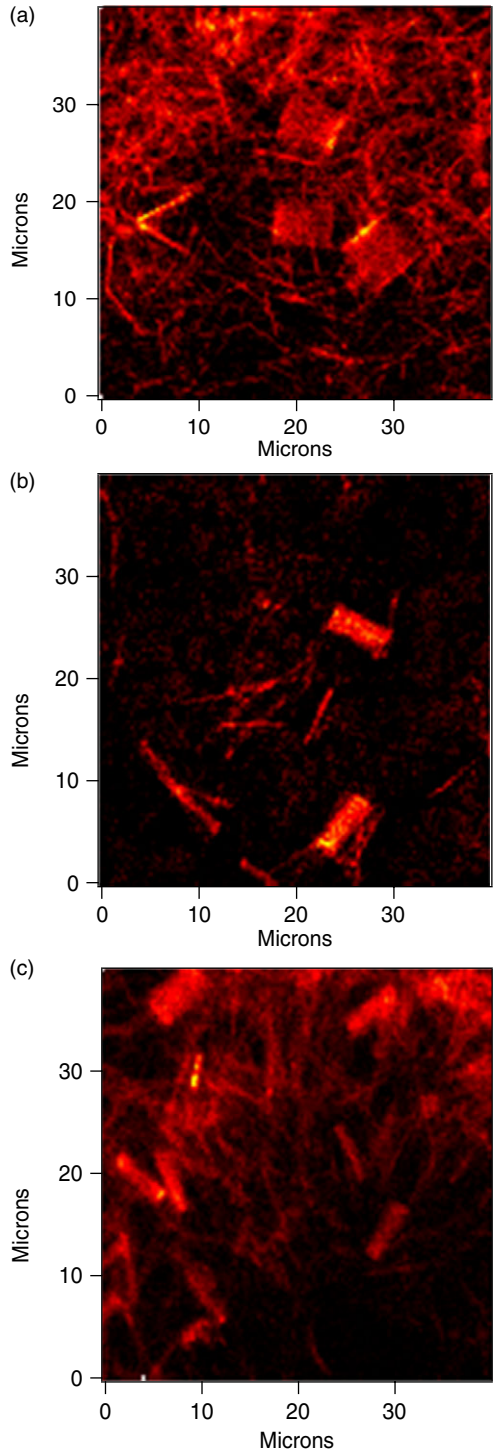


Figure 1. Fluorescence data collection images of NTs loaded with Dox. (a) NTs modified with 100% C18-silane. (b) NTs modified with 75% C18-silane and 25% pyridine–silane. (c) NTs modified with 50% C18-silane and 50% pyridine–silane.

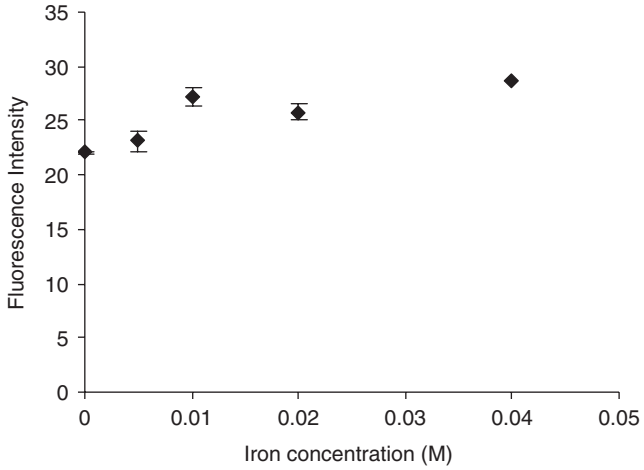


Figure 2. Effect of Fe^{3+} on the fluorescence intensity of Dox.

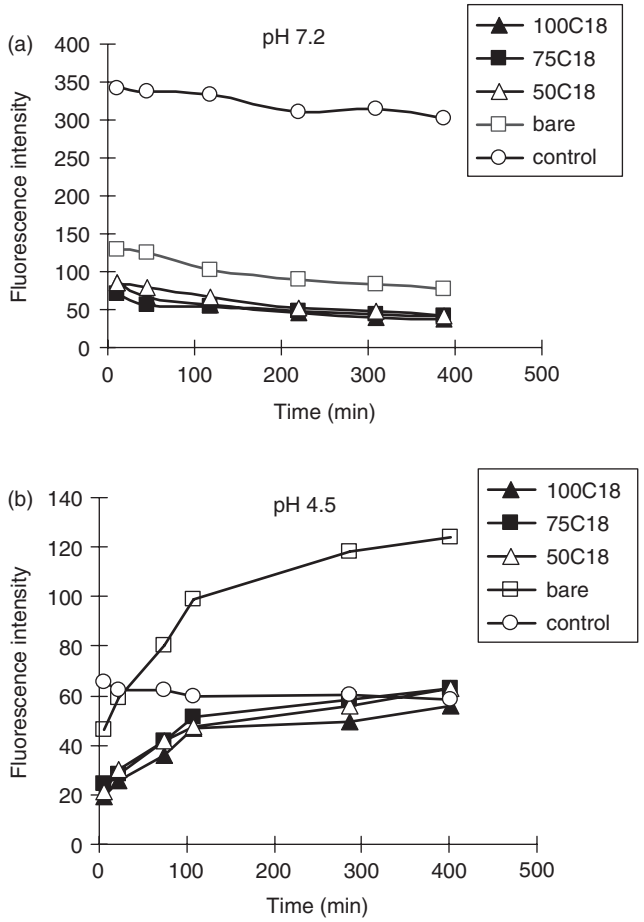


Figure 3. Effect of pH on the release profile of Dox from MNTs with different inner surface modifications. (a) pH 7.2. (b) pH 4.5.

The fluorescence spectroscopy measurement results did not show any significant effect of Fe^{3+} on the fluorescence intensity of Dox (Figure 2).

The release profiles of Dox-loaded nanotubes at different pH values are illustrated in Figure 3. The fluorescence intensity in Figure 3 is expressed as the intensity per 10^{10} MNTs, which was calculated by dividing the measured fluorescence intensity by the number of incubated MNTs.

In HEPES buffer (at pH = 7.2), the fluorescence intensity of Dox decreased slightly over time. When compared with the controlled sample, this decrease can be explained as due to

the decay of the Dox molecule itself in the buffer solutions. Therefore, there is no significant release of Dox at pH 7.2.

At pH 4.5, the fluorescence intensity of Dox increased with time. The difference between the release profiles at pH 7.2 and 4.5 can be explained by the change of bonding strength between Dox and the modification layer of MNTs at different pH values. The pK_a value of pyridine in water is 5.3.[21] For pyridine–silane-modified MNTs, the inner surfaces of the MNTs will be protonated when the pH is <5.3 and will be in neutral form when the pH is ~7. The pH also has an effect on the hydrophobicity of Dox. At pH >7, Dox is in basic form and tends to stay in hydrophobic phase, as mentioned before. Hence, there will be a hydrophobic interaction between the Dox molecules and the modification layers that contain the pyridine group and the C18 chain from silane. This leads to the retardation of Dox release. When the pH is <5.3, both Dox and pyridine functional group in the modification layers of MNTs will be protonated and hence charged positively. Therefore, there will be charge–charge repulsion between Dox and pyridine. Consequently, Dox tends to leave the nanotubes at pH <5.3.

The concentrations of Dox at pH 4.5 and 7.2 buffers were measured against calibration curves obtained at pH 4.5 and 7.2, respectively. The total amount of Dox loaded in the nanotubes, m_T, was calculated by multiplying the concentration of the stock Dox base solution M_s (5 mg/mL) with the total volume of the nanotubes, V_{NT} ($m_L = M_s \times V_{NT}$). This gave 0.38 μg per 10^{10} nanotubes for the payload of Dox in MNTs. The release percentage (%Release) is defined to be the released amount ($m_{pH4.5}$) over the amount of Dox remaining (m_{remain}) in the nanotubes after release in HEPES buffer solutions ($m_{pH7.2}$). It is expressed as Eq. (1):

$$\%\text{Release} = \frac{m_{pH4.5}}{m_{remain}} \cdot 100\% = \frac{m_{pH4.5}}{m_T - m_{pH7.2}} \cdot 100\% \quad (1)$$

where $m_{pH\,4.5}$ is the amount of Dox released in acetate buffer solutions (pH = 4.5). This value is determined by the fluorescence intensity compared to the standard curve obtained in pH 4.5 buffer solutions. $m_{pH\,7.2}$ is the amount of Dox released in HEPES buffer solutions, which was determined by the same method as $m_{pH4.5}$. The percentage (%Release) release profiles of Dox in acetate buffer solutions are shown in Figure 4.

The amount of Dox remaining in the nanotubes after release in HEPES buffer, m_{remain}, was determined by $m_{remain} = m_L - m_{pH\,7.2}$. As can be seen from Table 1, there was no significant difference between the modified MNTs and bare MNTs based on the statistical *F*-test. The results indicated that physical trapping with iron oxide nanoparticles in MNTs, instead of chemical interactions, played the main role in loading Dox molecules in the MNTs.

Table 1. Amount of Dox remaining in the MNTs after release in HEPES buffer, m_{remain} (g/10^{10} MNT).

100C18	75C18	50C18	Bare
0.274 ± 0.044	0.260 ± 0.047	0.272 ± 0.038	0.249 ± 0.046

The release profiles showed that the modified nanotubes had a lower release rate than the bare nanotubes. This indicates that the modifications can change the release rate of Dox.

Considering that iron oxide nanoparticles might degrade at pH 4.5, and hence have an effect on the release profiles, we carried out TEM studies. TEM images of MNTs were taken before and after incubation in the acetate buffer solution for 55 h. Figure 5(a) and (c) is the TEM images of MNTs before incubation in acetate buffer solutions with a low and high magnification, respectively. Figure 5(b) and (d) shows the TEM images of MNTs after the incubation in acetate buffer solutions with a low and high magnification, respectively. There was a slight difference between the MNTs before and after

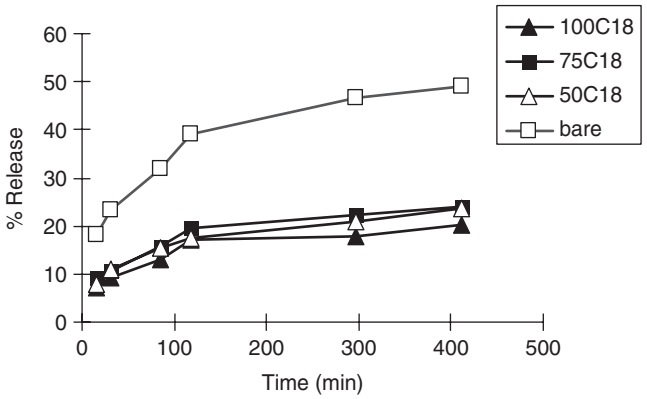


Figure 4. Dox release profiles from MNTs with different inner surface modifications at pH 4.5.

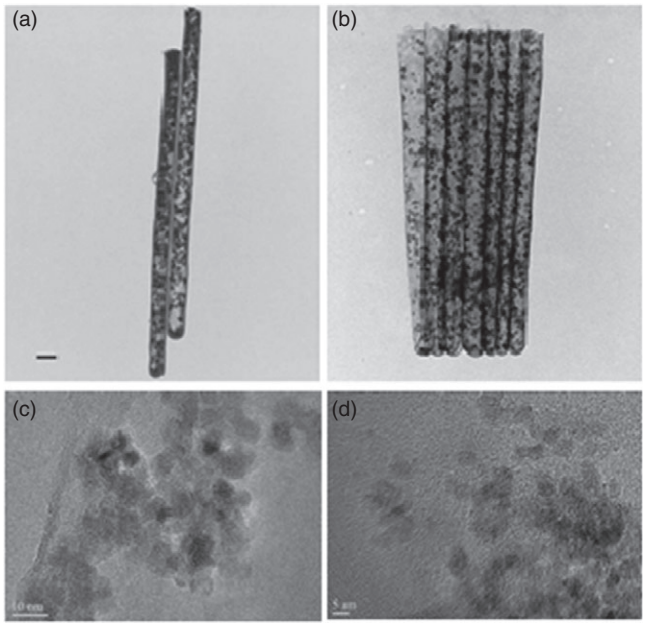


Figure 5. TEM (a and b) and HRTEM (c and d) images of MNTs loaded with Dox before release (a and c) and after incubation (b and d) in acetate buffer solutions (pH 4.5) for 55 h.

the incubation. The size of the iron oxide nanoparticles in MNTs was observed to be 5.4 ± 1.7 and 4.7 ± 0.6 nm before and after the incubation in acetate buffer, respectively. In addition, the iron content of the MNTs after the incubation was 0.593 fg/MNT, which is lower than that of MNTs before the incubation, 1.367 ± 0.473 fg/MNT. The results indicate that iron oxide nanoparticles degraded during the incubation in pH 4.5 buffer solutions. The degradation of the iron oxide nanoparticles in MNTs might be contributing to Dox release at pH 4.5.

Conclusion

In summary, MNTs can be used as drug reservoirs. Dox molecules are found to be stable in MNTs at pH 7.2. They can be released at pH 4.5. The physical trapping with the iron oxide nanoparticles in MNTs could play a main role in drug loading. The modification of the inner surfaces of the nanotubes is effective in changing Dox release rates. The iron oxide superparamagnetic nanoparticles might also contribute to Dox release at acidic pH as a result of their slow degradation. This work is highly complementary to the recent stimulated drug delivery method in silica nanotubes.[22]

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