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PREPARATION AND CHARACTERIZATION OF A NEAR-INFRARED LIGHT RESPONSIVE MICROCAPSULE SYSTEM FOR CANCER THERAPY

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A novel near-infrared light responsive microcapsule system, gold nanorod-covered DOX-loaded hollow $CaCO_3$ microcapsule (AuNR-HM-DOX) is developed for cancer therapy. The hollow $CaCO_3$ microcapsules were prepared based on the self-assembly between chitosan and sodium alginate on $CaCO_3$ particles via layer-by-layer technique, and then covered with gold nanorods to obtain the microcapsule system. Upon near-infrared (NIR) irradiation, microcapsule with gold nanorods can convert the absorbed NIR light into heat. Meanwhile, doxorubicin (DOX), a chemotherapy drug, is loaded into the microcapsule system *via* electrostatic adsorption for combined photothermal therapy and chemotherapy. Properties of AuNR-HM-DOX including grain diameter, optical spectra were characterized. Confocal fluorescence imaging was performed to observe the morphology of the capsules and existence of DOX in the core, confirming the successful loading of DOX. The release of DOX from the capsules under continuous NIR irradiation was investigated to evaluate the temperature responsiveness of AuNR-HM-DOX. Results indicate that AuNR-HM-DOX microcapsules possess uniform particle size and high light responsiveness. The combination of chemical and physical therapy of AuNR-HM-DOX features great potential as an adjuvant therapeutic alternative material for combined cancer therapy.

Keywords: Microcapsules; self-assembly; NIR-irradiation; cancer research.

1. Introduction

In the past decades, various treatment modalities have been developed for cancer therapy. Chemotherapy and physical therapy are the two main categories.[1] However, for the reason of unfavorable side effects and drug resistance, simple chemotherapy has been greatly limited for treatment of cancers. On the other hand, physical therapy like

photothermal therapy may overcome these shortcomings.[2] But these physical approaches tend to cause damage to the biological system due to the nontargeted treatments. In this context, a composite material combining the features of chemical drugs and physical therapy is highly called for cancer research, while at the same time avoiding the above limitations.

Compared with the traditional material, microcapsule has recently attracted extensive attention.[2] It has favorable biocompatibility and high capacity to load drugs. Polyelectrolytes can be deposited on the surface of particle core layer-by-layer (LbL) to form microcapsules, with the total polymer thickness being determined by the number of deposited layers. Generally, the LbL approach[3–5] applies two oppositely charged polymers on the substrate or particles mixed. Once deposited, the layer of polyelectrolyte inverts the surface charge of the microcapsules, ensuring a subsequent polyelectrolyte with opposite charges to be deposited from the second solution.[6] The process can be repeated for many times to form a uniform multilayered film of polymeric microcapsules. The LbL process has been applied to a multitude of substrate cores, including different solid supports and a range of submicron to micron-sized particles. In addition, polyelectrolyte solutions can be replaced with similarly charged species such as proteins, clays, nanoparticles, etc. The LbL technique also ensures a high surface area[7] being used to modify some functional groups. Meanwhile, to meet the needs of carrying capacity for drugs, microcapsules are usually etched to obtain a hollow structure by dissolving the cores. Then the hollow microcapsules become suitable to serve as a novel drug carrier. Anti-cancer drugs, such as doxorubicin, can be loaded in the microcapsules.

Gold nonmaterial has been widely used in cancer research by local heating in a wide variety of nanoshapes, e.g., nanoshell particles,[8,9] nanocages[10,11] and nanorods.[12,13] Among these nanoparticles, gold nanorods (AuNRs) emerged as an excellent photothermal therapy agent due to a more efficient photothermal energy conversion and tunable aspect ratios than most other nonmaterial.[13–15] Moreover, Au nanorod has two characteristic absorption peaks, a weak transverse plasmon band (500–550 nm) and a strong longitudinal surface plasmon resonance wavelength (700–850 nm) known as near-infrared light-responsive.[16] As the near infrared light possess the deep tissue penetration depth in living subjects, therefore, gold nanorods-covered microcapsules can strongly absorb near-infrared light and then realize NIR photothermal ablation in the deep pathological tissues.

In this study, we develop a cooperative AuNR-HM-DOX microcapsule system. The fabrication is based on the layer-by-layer (LbL) self-assembly of different charged polyelectrolyte thin films on $CaCO_3$ core and yielding hollow capsules with ethylene diamine tetraacetic acid (EDTA). Thereafter, an anti-cancer drug, doxorubicin (DOX), is loaded in the AuNR-covered hollow microcapsules to form the AuNR-HM-DOX microcapsule system. Under NIR irradiation, microcapsules convert the absorbed NIR light into heat to play the role of photothermal therapy.[17] Meanwhile, the heat induce the destruction of microcapsule system,[18] which allow the anticancer drug DOX being released from the microcapsules for chemotherapy. This AuNR-HM-DOX microcapsule system combines chemotherapy with photothermal therapy for the localized cancer therapy.

2. Experiment Section

2.1. *Materials*

$Ca(NO_3)_2{\cdot}4H_2O$, $NaCO_3$, $AgNO_3$, EDTA, Sodium Boride ($NaBH_4$), absolute ethyl alcohol, uranin, chloroauric acid ($HAuCl_4$), cetyl trimethyl ammonium bromide (CTAB) and ascorbic acid (AA), (analytically pure) were purchased from China National Medicine Corporation Ltd. Glutaraldehyde (GA) was purchased from Shanghai Wulian chemical. Sodium polystyrenesulfonate (PSS) and rhodamine 6G were purchased from Sigma-Aldrich and used as starting materials without further purification. Wahaha pure water and DI water were used throughout.

2.2. *Synthesis of gold nanorod-covered DOX-loaded $CaCO_3$ microcapsules*

2.2.1. *Preparation of hollow $CaCO_3$ microcapsules*

Microcapsules were made using calcium carbonate ($CaCO_3$) microparticles as a sacrificial template. $CaCO_3$ microparticles were synthesized according

to Volodkin *et al.* by mixing Ca $(NO_3)_2$ and Na_2CO_3 solutions (0.025 M) with vigorous stirring and ultrasound for 15 s, followed by extensive washing with pure water to remove unreacted reagents. Spherically shaped $CaCO_3$ particles with an average diameter of 2 μm were obtained. The $CaCO_3$ particles were coated between chitosan and sodium alginate via the layer-by-layer (LbL) technique.[19] $CaCO_3$ particles were dispersing in 1 ml water with 200 μl of chitosan (1-mg-ml^{-1}) added. After shaking for 15 min, microparticles were collected by centrifugation, and residual chitosan was removed by washing twice with pure water. Thereafter, the microparticles were suspended in water (1 ml) with sodium alginate (200 μl) added and shaken for 15 min, followed by centrifugation and two washing steps. This procedure was repeated until eight layers.

Hollow $CaCO_3$ capsules were obtained by removing the $CaCO_3$ core by incubating the coated microparticles for 30 min in 2 ml of 0.2 M EDTA solution to dissolve the $CaCO_3$ core. Then the dissolved ions were removed by two centrifugation and washing steps. Finally, the hollow microcapsules were resuspended in pure water.

2.2.2. *Preparation of the gold nanorods*

Gold nanorods were prepared by a seed-mediated method.[16] The seed solution was prepared by rapidly adding ice cold $NaBH_4$ solution (0.01 M, 0.6 mL) into a mixture containing 5 mL $HAuCl_4$ (0.0005 M) and 5 mL CTAB (0.2 M), followed by strong stirring for 1 min.

Then 12 μL seed solution was injected into the growth solution containing 0.004 M $AgNO_3$, 0.001 M $HAuCl_4$, 0.0788 M AA in 5 ml CTAB solution (0.2 M). This solution was left undisturbed for 30 min. The obtained gold nanorods solution was centrifuged before use at 12,000 rpm for 10 min (twice) to remove any excess CTAB surfactant.

2.2.3. *Gold nanorod-covered DOX-loaded hollow $CaCO_3$ microcapsule*

Gold nanorod-covered $CaCO_3$ microcapsules were prepared by mixing 2.0 mL of the above gold nanorod solution and 1.0 mL of microcapsule solution. Then the products were vortex and left overnight. The obtained microcapsule system was washed twice and redispersed in pure water before use.

To prepare the DOX-loaded microcapsules, microcapsules (1 ml) were mixed with 300 μL DOX solution (1.55 mg-ml^{-1}). The obtained mixture was vortexed for 24 h and then centrifuged at 4500 rpm for 5 min to remove the excess DOX (twice). The obtained DOX-loaded microcapsules were saved in pure water before use.

2.3. *Characterization*

The morphology of microcapsules and gold nanorods were viewed by using scanning electron microscopy (SEM) and transmission electron microscope (TEM). AuNRs-covered microcapsules were confirmed by the strong ultraviolet absorption in the NIR region. The UV-vis absorbance indicated that microcapsules had been covered with Au nanorods. Confocal fluorescence imaging was also performed to observe the morphology of the capsules and confirm the successful loading of DOX.

2.4. *Photothermal destruction of gold nanorod-covered DOX-loaded $CaCO_3$ microcapsule*

To investigate the photothermal destruction behavior of AuNR-HM-DOX microcapsules, a fixed amount of the DOX-loaded microcapsules were incubated in different temperatures and irradiated under continuous near-infrared light.[20] The release of DOX was assessed through the fluorescence intensity of liquid supernatant.

3. Results and Discussion

3.1. *Fabrication of the AuNR-HM-DOX microcapsules*

Figure 1 illustrated a six-step process for the preparation of gold nanorod-covered DOX-loaded $CaCO_3$ microcapsule. $CaCO_3$ particles were obtained by the reaction between Ca $(NO_3)_2$ and Na_2CO_3 as described in Sec. 2.2.1. The positively charged CS and negatively charged SA were deposited on the surface of the $CaCO_3$ layer by layer to form the microcapsule. EDTA degradated $CaCO_3$ particles to form the hollow structure. Gold nanorods were subsequently coved on the microcapsule system. An anticancer drug, doxorubicin, was choosed to be loaded in the microcapsules by interaction of static electricity. Then we got the DOX-loaded gold-covered microcapsule.

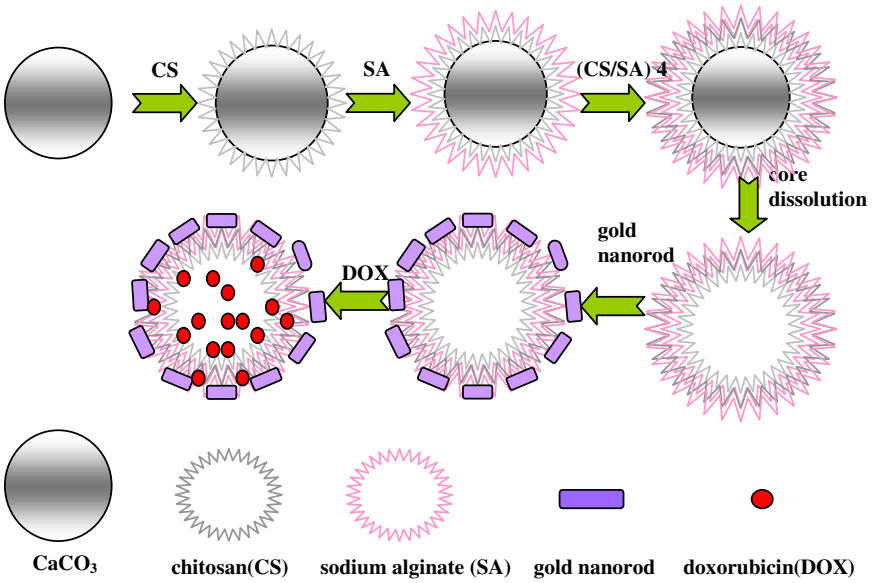


Fig. 1. Schematic of the fabrication of gold nanorod-covered DOX-loaded hollow $CaCO_3$ microcapsules.

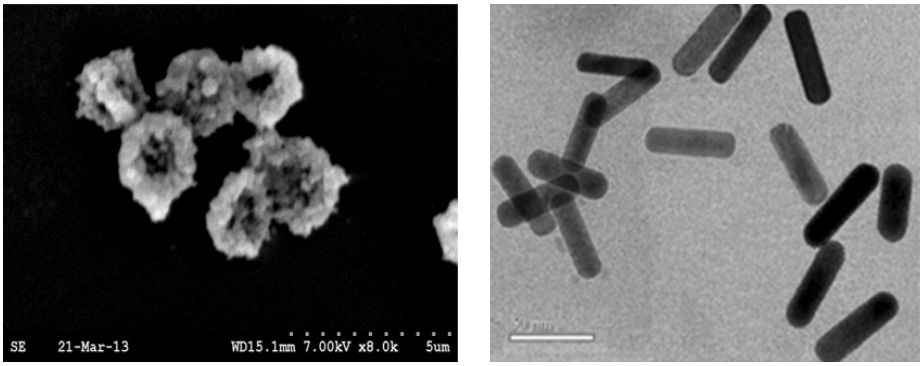
3.2. *Characterization of microcapsules and AuNRs*

Hollow $CaCO_3$ microcapsules were prepared based on the self-assembly between chitosan and sodium alginate on $CaCO_3$ particles via layer-by-layer technique. The SEM image of hollow microcapsules was shown in Fig. 2(a). Microcapsules had an average particle diameter of 2 μm. And in Fig. 2(a), SEM image of hollow microcapsules clearly indicated the shells and dissolved pores, which allowed DOX to pass through and load in. The result strongly demonstrated that $CaCO_3$ microcapsules remained intact after core removal and could disperse separately in water.

Au nanorods were prepared by the seed-mediated growth method. TEM image of the gold nanorods was shown in Fig. 2(b). The nanorods had an average aspect ratio (length/diameter) of 4.0, resulting in a strong longitudinal surface plasmon resonance wavelength of 765 nm and a weak transverse plasmon band at 530 nm.

3.3. *Optical characterization of AuNR-covered microcapsules*

AuNR-HM microcapsules were obtained by electrostatic adsorption between gold nanorods and hollow microcapsules. In Fig. 3, AuNR-covered microcapsules showed a strong absorbance in the NIR region, while microcapsule without gold nanorods did not show any characteristic absorption peaks. It indicated that gold nanorods had been linked to the



(a) (b)

Fig. 2. (a) SEM image of hollow $CaCO_3$ microcapsules, (b) TEM image of Au nanorods.

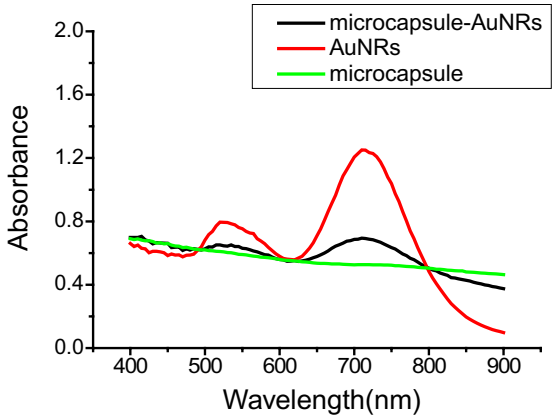


Fig. 3. UV-vis spectra of AuNR-HM microcapsule, AuNRs and microcapsule.

microcapsules successfully. Then AuNR-HM microcapsule could convert the absorbed NIR light into heat.

The drug-loading was performed by incubating hollow microcapsules in a doxorubicin sulfate solution (1.55 mg mL^{-1}) for 24 h. Doxorubicin could be excited by laser at 543 nm wavelength to emit the red fluorescence. As shown in Fig. 4, red fluorescence from DOX could be observed in microcapsules. After removing the core, microcapsules still maintained the integrity of the core-shell structure. So the doxorubicin could be loaded completely in the intact shells and cores of AuNR-HM microcapsules.[21]

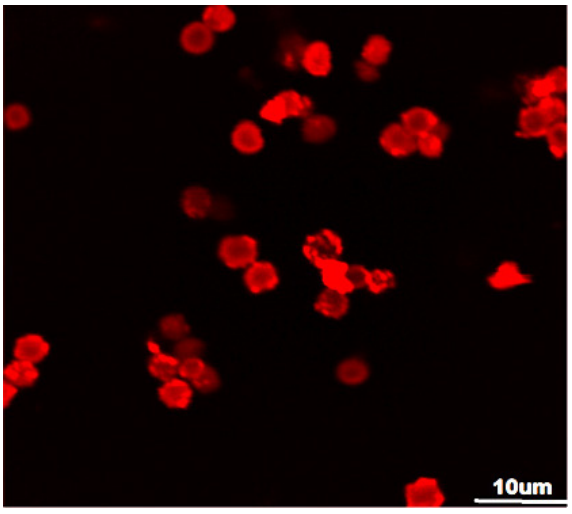


Fig. 4. CLSM images of AuNR-HM-DOX microcapsules with red fluorescence DOX loaded in the core.

3.4. *In vitro photothermal destruction of the AuNR-HM-DOX microcapsules*

In order to demonstrate the destruction of the AuNR-HM-DOX microcapsules for further study, the release of DOX from the capsules was evaluated. The photothermal effect of the AuNR-HM-DOX microcapsules was investigated by incubating them in the water bath with different temperatures, followed by measuring the fluorescence intensity of DOX. In Fig. 5, fluorescence intensity and doxorubicin release increased gradually with the increased temperature.[22] 80% of doxorubicin had been released at 45°C, and when the temperature reached to 70°C, most of microcapsules had been destructed.

Thereafter, AuNR-HM-DOX microcapsules were irradiated with continuous NIR light for 30 min. As shown in Fig. 6(a), the red fluorescent cores of microcapsules did not show obvious changes at first. In comparison with the images presented in Fig. 6(a), the red fluorescence [Fig. 6(b)] wore off gradually in the core. Finally, after 30 min of NIR irradiation, the red fluorescent cores had almost completely disappeared[23,24] [Fig. 6(c)].

As illustrated in Fig. 7, quantitative data of the released DOX was evaluated. The temperature increased rapidly from ambient temperature, 16°C, to 45°C within an irradiation time of 30 min, and afterwards the temperature remained virtually constant when further increasing the irradiation time up to 60 min. Figure 7(b) indicated the doxorubicin release behavior from the microcapsules along with

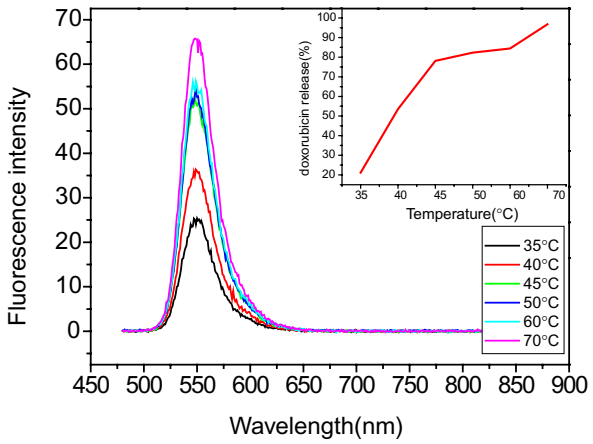
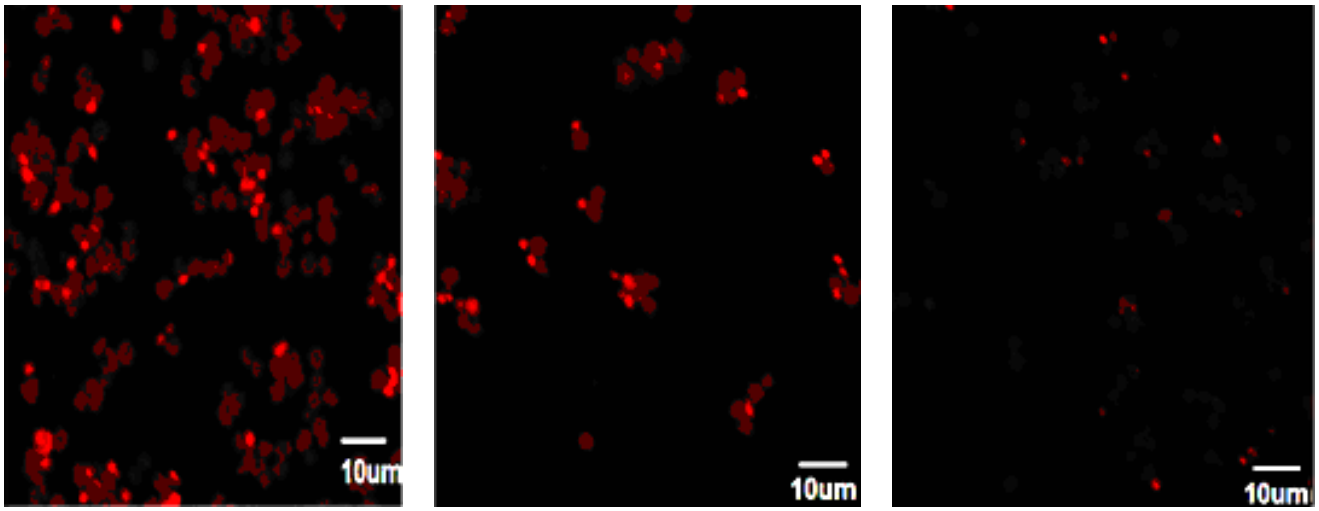


Fig. 5. Photothermal properties of the AuNR-HM-DOX microcapsules with different temperatures.



(a) Before (b) During (c) After

Fig. 6. CLSM images of destruction of AuNR-HM-DOX microcapsules (a) before, (b) during, (c) after continue NIR irradiation for 30 min.

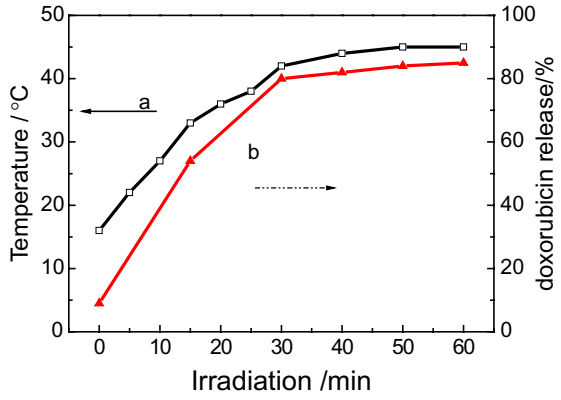


Fig. 7. Profile of temperature-irradiation time of the AuNR-HM-DOX microcapsules (a) and the drug-release behavior from microcapsules under NIR irradiation (b).

an increase of the irradiation time. It showed that the release of DOX was temperature-dependent, and its release could be regulated by manipulating the NIR irradiation. When exposing the microcapsules to NIR irradiation for 30 min and then vortex vibration for 5 min, more than 80% release of the drug was recorded. It indicated the expected drug release ability of AuNR-HM-DOX microcapsules. All the results indicated the strong photothermal effect of AuNR-HM-DOX microcapsules.

4. Conclusion

In this study, we have synthesized AuNR-HM-DOX microcapsules via the layer-by-layer technique for NIR responsive drug delivery. SEM and CLSM have indicated that microcapsule system remained intact and loaded doxorubicin successfully. The anti-cancer drug, DOX, was loaded in the microcapsules for the chemotherapy. Upon NIR irradiation, the microcapsule system exhibited excellent photothermal properties and therapeutic effect. Compared with drug and photothermal treatment alone, the anti-cancer capacity by combined treatment with AuNR-HM-DOX microcapsules will have great potential to be much higher than individual therapy.[25] Future work will involve the application of these microcapsules in cells and in vivo for the cancer research.

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