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The application of hierarchy MoS$_2$ particles for NIR induced drug delivery towards liver cancer treatment

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Abstract

This paper applied hierarchy MoS$_2$ particles for near-infrared induced drug delivery towards liver cancer treatment. MoS$_2$ particles were used as the photothermal responsive agent, 1-tetradecanol was used as the phase change material, doxorubicin was encapsulated into the particles, and MoS$_2$/1-tetradecanol/doxorubicin composite was synthesized. The drug release was tested under near-infrared irradiation. Within the MoS$_2$/1-tetradecanol/doxorubicin composite, the photothermal response of MoS$_2$ to near-infrared facilitated the phase change of 1-tetradecanol and the release of doxorubicin. The release of doxorubicin could be regulated by both the MoS$_2$ concentration and the irradiation power. The cytotoxicity study indicated that MoS$_2$/1-tetradecanol had negligible toxicity to the HepG2 cells, while MoS$_2$/1-tetradecanol/doxorubicin had more tumor-killing effects than free doxorubicin. This research showed that hierarchy MoS$_2$ particles had the potential of delivering anti-tumor drugs through photothermal stimuli.

1. Introduction

Drug delivery systems based on stimuli-response have attracted enormous attention because precise drug release at tumor sites could be achieved in response to particular stimuli. In this case, drug leakage in healthy tissues could be prevented, and drug release could be focused on the tumor regions [1–5]. Both external stimuli (such as light and ultrasound) and internal stimuli (such as pH, temperature, and redox) could be applied for drug delivery [6–10]. Among these various stimuli, a light-responsive trigger has been widely used for medical applications [11–14]. Especially, near-infrared (NIR) light has been a particular interest because of its capability for deep tissue penetration. Several NIR sensitive materials, such as Au, MoS$_2$, and graphene-based materials, could potentially be or have already been used in drug delivery systems [15, 16]. In this system, the NIR irradiation is absorbed by the NIR sensitive material to generate heat, which stimulates the drug release at the targeted sites. Besides, the increased regional temperature could also be used for photothermal treatment so that synergetic treatment could be achieved.

In the NIR responsive drug delivery systems, phase change materials (PCMs) play a critical role. PCMs usually have a solid–liquid phase transition within a narrow temperature window. The drugs could be loaded to the solid PCMs, while the release could be achieved when the phase change is triggered by a temperature increase. Different PCMs, such as fatty acid, poly(N-isopropyl acrylamide), and poly(ethylene glycol), have been proposed and applied for drug delivery [17–21]. When combing the photo-absorbing agent with anti-tumor drug-loaded PCMs, the agent produces heat under irradiation, leading to the local temperature increase and phase transition of PCMs from a solid phase to a liquid phase. The phase change could release the loaded anti-tumor drug into the targeted region. 1-tetradecanol (TA) is a widely used PCM, because of its appropriate phase change temperature window, high biocompatibility, and low price [22–25]. Moreover, the medical application
of TA has been approved by the Food and Drug Administration (FDA). The phase change temperature of 39 °C is a little higher than human body temperature [26–28], which makes the material maintain a solid phase in the human body, and the phase change could also be easily induced by a mild temperature change.

Even though MoS2 has been widely used for NIR responsive drug release, most of the applications focus on the two-dimensional single-layer MoS2 nanosheets [29–32], which bring limitations for its wide applications because of complicated fabrication and exfoliating process, and the size is also difficult to be controlled. Generally, drug delivery requires the carriers to be less than 250 nm to ensure easy transportation through cell membranes and stabilization in the bloodstream [33–35]. Therefore, the application of MoS2 nanosheets for drug delivery purposes is still limited. In this paper, the hierarchy MoS2 particles with urchin-like shape are synthesized as a photothermal agent under NIR laser irradiation, 1-tetradecanol is used as the phase change material for doxorubicin (DOX) delivery and release. To the best of our knowledge, the reports regarding the application of hierarchy MoS2 particles in drug delivery are limited, even though they have been used in energy storage fields [36–38]. Finally, MoS2/TA@DOX drug delivery system is synthesized, and the DOX release is studied under NIR irradiation and various environmental temperatures.

2. Experimental

2.1. Hierarchy MoS2 particle synthesis

The hierarchy MoS2 particles were synthesized based on a previous report with modifications [39]. Compared with the report, it was found that a decreased precursor solution concentration resulted in decreased particle size. Specifically, hexaammonium heptamolybdate tetrahydrate ((NH4)6Mo7O24·4H2O, 1.24 g, Sigma Aldrich, 99.3%) and thiourea (NH2CSNH2, 2.28 g, Sigma Aldrich, ACS reagent, > 99%) were mixed in DI water (100 ml) with mechanical stirring for 60 min. The solution was then transferred to a Teflon coated 150 ml stainless steel autoclave, which was then heated to 220 °C for 6 h. Afterward, the reactor was cooled down, washed with DI water, and dried for future use.

2.2. MoS2/TA and MoS2/TA@DOX composite synthesis

1-tetradecanol (TA) and DOX were loaded to the surface of the hierarchy MoS2 particles as follows. Typically, TA (100 mg) and DOX methanol solution (5 ml) were mixed with MoS2 particles (1 mg) at room temperature with ultrasonication. The suspension was magnetically stirred for 30 min, and particles were separated with a high-speed centrifuge. The concentration of the DOX in the supernatant was determined by a UV–vis spectrophotometer. Finally, MoS2/TA@DOX composite was obtained. To investigate the relationship between the DOX solution concentration and the loaded DOX on MoS2, the initial DOX concentration was controlled as 10 to 100 μg ml⁻¹, with 10 μg ml⁻¹ as an interval. The DOX loading could be expressed as:

$$\text{DOX loading} = \frac{\text{the mass of the loaded DOX (μg)}}{\text{the mass of MoS2 (mg)}}$$  \hspace{1cm} (1)

The mass of the loaded DOX was calculated based on the concentration difference in the initial DOX solution and the supernatant DOX solution. In the controlled experiment, DOX was not added in the above procedures, and MoS2/TA composite was obtained. In the experiments of DOX releasing, the MoS2/TA@DOX was synthesized with the DOX concentration of 80 μg ml⁻¹.

2.3. Materials characterization

The surface morphology of MoS2 was determined by TEM (JEOL, JEM-2000), and the surface chemical states were studied by XPS (ESCALAB VG MK II). The crystal structure was studied by XRD (Shimadzu, LabX-6000) with Cu Kα radiation, with the 2θ between 10° and 70°. The specific surface area was determined by a BET tool (Micromeritics, ASAP 2460). Raman spectra were recorded by Laser Raman Spectrometer (ThermoFisher, DXR), and thermogravimetric analysis (TGA) was performed by a thermogravimetric analyzer (LABSYS EVO TG 1600). The UV–vis–NIR spectra were studied by a spectrophotometer (ThermoScientific, TU-1810). The Fourier transform infrared (FT-IR) spectroscopy was recorded by Nicolet (model: Nexus) spectrometer.

2.4. MoS2/TA Temperature change study under NIR irradiation

To determine the photothermal effect, MoS2/TA suspension (3.0 ml) was exposed to NIR irradiation (808 nm) for 10 min. Both the suspension concentration and laser power were controlled in the experiments. The instant suspension temperature was monitored by a digital thermometer at pre-defined time intervals. The same volume of DI water was also exposed to the irradiation as a control experiment. The photostability of MoS2/TA was studied by monitoring its temperature change in five ON/OFF irradiation cycles.
2.5. Drug release testing

DOX drug release from MoS$_2$/TA@DOX was studied in vitro, and it was tested in two different conditions, including different environment temperatures and constant environment temperature with/without NIR laser irradiation. For different environment temperatures, the temperature was controlled as 30 °C, 37 °C, 40 °C, 42 °C, and 45 °C, and the cumulative drug release was measured. For constant environment temperature, the temperature was controlled as 37 °C, the MoS$_2$/TA@DOX in PBS (1.0 mg ml$^{-1}$, 2 ml$^{-1}$) was exposed to a laser (808 nm, 2.0 W cm$^{-2}$), and the concentration of released DOX was investigated by fluorescence spectroscopy. As a control experiment, the MoS$_2$/TA@DOX in PBS was also incubated in the darkroom while the drug release was monitored.

2.6. Cytotoxicity testing

The cytotoxicity of MoS$_2$, MoS$_2$/TA, and MoS$_2$/TA@DOX was studied with human hepatoma cells (HepG2, obtained from the Institute of Biochemistry and Cell Biology at the Chinese Academy of Sciences, Shanghai, China) by using MTT assay. Specifically, HepG2 cells were cultured in 96-well plates for 48 h, and the density was around $1 \times 10^4$ cells/well. Different concentrations of MoS$_2$, MoS$_2$/TA, MoS$_2$/TA@DOX, and free DOX solution were introduced into the cells. The cells were cultured for 48 h under 37 °C, and with periodic NIR laser irradiation (300 s per hour) were applied under designed conditions. Finally, the cell viability was characterized.

3. Results and discussion

The SEM, TEM, and HRTEM images of as-synthesized MoS$_2$ particles are presented in figures 1(a)–(c). It can be seen that the particles have a hierarchy structure, with a size of ~200 nm. The surface consists of nanorods, which could increase the specific surface area of the particles. The lattice distance of 0.27 nm refers to the (100) plane of MoS$_2$ crystal structure. The XPS spectrum for the Mo element is presented in figure 1(d), and the two peaks at 229.4 eV and 232.8 eV refer to Mo 3d$_{3/2}$ and 3d$_{5/2}$, respectively. The XPS spectrum for the S element is exhibited in figure 1(e), and the two peaks at 162.4 eV and 163.6 eV are attributed to S 2p$_{3/2}$ and 2p$_{1/2}$, respectively. The powder XRD patterns of MoS$_2$ are presented in figure 1(f). The diffraction patterns match well with the standard diffraction peaks listed in the JCPDS card (No. 37–1492), and no impurity phases are observed.

The MoS$_2$ particles are modified with phase change material of TA for DOX drug loading. N$_2$ adsorption/desorption curves are measured to investigate the specific surface area changes of MoS$_2$ before and after TA modification, and the corresponding curves are presented in figures 2(a), and (b). The specific surface area is 254.3 and 238.9 m$^2$ g$^{-1}$, respectively, for MoS$_2$ and MoS$_2$/TA, equal to 5.9% decrease in specific surface area after the surface modification. Meanwhile, the pore size distributions are presented in the insets. The peak pore
size is decreased from 30.2 nm to 19.8 nm after the TA modification. The decrease of specific surface area and pore size could be attributed to that the TA partially blocks the holes of MoS₂ particles.

The Raman spectra of MoS₂ and MoS₂/TA are presented in figure 3(a). Both samples show two peaks of $E_{1}^{2g}$ and $A_{1g}$. However, due to the surface modification, peak broadening and shifting are observed in MoS₂/TA. The surface modification of TA could be further inspected by FT-IR spectra, which are presented in figure 3(b) for MoS₂ and MoS₂/TA. Compared with pure MoS₂, a new peak at 2938 cm⁻¹ is observed, which is contributed to the vibration and stretching of the C-H bond in TA.

Figure 2. N₂ adsorption/desorption curves of (a) MoS₂; (b) MoS₂/TA. (the insets show the pore size distributions).

Figure 3. (a). Raman spectra, and (b). FT-IR of MoS₂ and MoS₂/TA; (c). UV−vis spectra of DOX, MoS₂, MoS₂/TA, and MoS₂/TA@DOX; (d). DOX loading under different concentrations of DOX solution.

The UV−vis spectra of DOX, MoS2, MoS2/TA, and MoS2/TA@DOX are exhibited in figure 3(c). For pure DOX, a characteristic peak is observed at 489.9 nm. A similar peak is observed on MoS2/TA@DOX, but absent from MoS2/TA and MoS2, confirming that DOX is loaded on the surface of the MoS2/TA surface. The slight peak shift in MoS2/TA@DOX could be attributed to the interactions among MoS2, TA, and DOX.

The relationship between the loading DOX and the DOX concentration is presented in figure 3(d). In the beginning, an increased DOX concentration results in an increased DOX loading. However, when the DOX concentration exceeds 60 μg ml\(^{-1}\), a further increased DOX concentration does not increase the DOX loading, which could be attributed to the saturated surface area of MoS2.

To be a good candidate for a photothermal agent, it requires a sensitive response to the irradiation so that the light could be effectively converted into thermal energy [26, 40]. The UV−vis-NIR spectra of the aqueous MoS2/TA dispersions are presented in figure 4(a). It can be seen that MoS2/TA displays a strong absorption at 808 nm, which could be attributed to the surface plasma resonances of valence-band free carriers of MoS2 core [39, 41]. It is also observed that the absorption peak intensity is positively related to the concentration of MoS2/TA dispersion, and a larger concentration results in higher peak intensity.

To understand the response of MoS2/TA to NIR irradiation, the photothermal study is performed. In this research, various concentrations of MoS2/TA aqueous dispersion (3 ml) are exposed to NIR laser irradiation (808 nm, 2W cm\(^{-2}\)), and the corresponding temperature change is monitored and presented in figure 4(b). When the MoS2/TA concentration is 160 μg ml\(^{-1}\), the dispersion temperature is increased from 26.8 °C to 55.1 °C after irradiating for 10 min, equal to an increase of 28.3 °C. However, for pure water, the temperature is only increased by 1.1 °C. This result indicates that the photothermal effect plays a significant role in heating the dispersion. Besides, the temperature change is also related to the dispersion concentration, and a higher concentration results in a larger temperature increase.

Moreover, the temperature change could also be impacted by the laser irradiation power. The corresponding temperature changes of MoS2/TA dispersion (3 ml; 160 μg ml\(^{-1}\); 808 nm; 2.0 W cm\(^{-2}\)) in 5 NIR laser ON/OFF irradiation cycles are presented in figure 4(c). It can be seen that a higher power leads to a larger heating effect. The melting point of...
TA is 39 °C, and the local heating effect is high enough to induce the phase change of TA for drug release purposes.

Besides the one-time photothermal response, the photostability is another important evaluation for photothermal responsive drug carriers, and five laser ON/OFF irradiation cycles are performed on MoS\textsubscript{2}/TA, and the result is presented in figure 4(d). It is observed that the temperature change in five cycles is 29.5 °C, 28.7 °C, 28.9 °C, 29.1 °C, and 29.7 °C, respectively. Negligible temperature change is observed among the five cycles, indicating that MoS\textsubscript{2}/TA has relatively high photothermal stability over laser irradiation.

TA phase change (melting) is the driving force for the drug release. The thermosensitive release profiles are studied under different temperatures, and the result is presented in figure 5(a). The MoS\textsubscript{2}/TA@DOX composite suspension in PBS solution is stirred at a designed temperature. It can be seen in figure 5(a) that, the DOX release in 50 h is 10.2%, 10.4%, 34.5%, 63.4%, and 78.9%, respectively, at the temperature of 30 °C, 37 °C, 40 °C, 42 °C, and 45 °C. When the temperature exceeds the TA phase change point (39 °C), a dramatic increase in drug release is observed. These results indicate that TA is a great candidate as a gate-keeper for photothermal induced drug release.

Afterward, the NIR laser irradiation included drug release is presented in figure 5(b), and ON/OFF pulsed DOX release could be achieved by merely switching ON and OFF of the laser irradiation. When the laser irradiation is ON, the hierarchy MoS\textsubscript{2} particles are heated up, and TA is melted due to reaching its phase change temperature. TA melting leads to the release of DOX. In this process, the hierarchy MoS\textsubscript{2} particles still maintain their shapes, and the phase/shape change only happens to TA. When the laser irradiation is off, TA is resolidified, and the release of DOX was terminated. The phase change of TA is working as a switch for the DOX release.

The cytotoxicity of the drug delivery system is tested with human hepatoma cells (HepG2). The cell viability under different concentrations of MoS\textsubscript{2} and MoS\textsubscript{2}/TA for 48 h without laser irradiation is presented in figure 6(a). As a reference, the cells without any drug carriers (0 mg ml\textsuperscript{-1}) are also presented. After 48 h, the cell viability is over 80%, indicating that both MoS\textsubscript{2} and MoS\textsubscript{2}/TA have negligible toxicity to human hepatoma cells (HepG2). The cell viability under different concentrations of MoS\textsubscript{2} and MoS\textsubscript{2}/TA for 48 h with periodic laser irradiation (300 s/hour) is presented in figure 6(b). Compared to the results in figure 6(a), the periodic laser irradiation leads to a decreased cell viability, which is due to the photothermal effect, and a higher temperature results in the ablation of the cells [42].

The cytotoxicity of DOX from free DOX and MoS\textsubscript{2}/TA@DOX against HepG2 is shown in figure 6(c). Periodic layer irradiation (300 s per hour) is applied to both experiments. When the DOX concentration is 0 μg ml\textsuperscript{-1}, it means that neither DOX nor MoS\textsubscript{2}/TA@DOX is added to the cells, but the only periodic laser is applied. The cell viability is over 90%, indicating that the laser irradiation itself has little impact on cell life activity. It is observed that the cytotoxicity of MoS\textsubscript{2}/TA@DOX is higher than the free DOX in all controlled concentrations, which could be ascribed to the enhanced cellular update, and reduced active efflux of DOX molecules, together with the cell ablation confirmed in figure 6(b). It is concluded from figure 6 that MoS\textsubscript{2}/TA has low cytotoxicity, and MoS\textsubscript{2}/TA@DOX displays an efficient tumor cell killing effect for HepG2 cells.
4. Conclusions

MoS$_2$/TA@DOX based photothermal response drug delivery system was synthesized by using hierarchy MoS$_2$ particles as the photothermal responsive agent, TA as the phase change material, and DOX as the anti-tumor drug. The loading of TA and DOX on the surface MoS$_2$ was confirmed by the FT-IR spectrum and UV–vis spectrum. Under NIR irradiation, MoS$_2$ absorbed the light and produced heat, which induced the phase change of TA from the solid phase to the liquid phase, leading to the release of DOX at the targeted region. The impacts of laser irradiation power and MoS$_2$ concentration on the photothermal effect were investigated. The cytotoxicity study indicated that MoS$_2$/TA had negligible toxicity on the HepG2 cells, while MoS$_2$/TA@DOX had more tumor-killing effects than free DOX.

Disclosure

The authors report no conflicts of interest in this work.

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