ELSEVIER

Materials Science and Engineering C 23 (2003) 113–116

MATERIALS SCIENCE & ENGINEERING C

www.elsevier.com/locate/msec

Synthesis of nir-sensitive Au–Au_2S nanocolloids for drug delivery

L. Ren[a,*], G.M. Chow[a,b]

[a] *Molecular Engineering of Biological and Chemical Systems, Singapore–Massachusetts Institute of Technology Alliance (SMA), National University of Singapore, 10 Kent Ridge Crescent 119260, Singapore*

[b] *Department of Materials Science, National University of Singapore, 10 Kent Ridge Crescent 119260, Singapore*

Abstract

Near IR (NIR) sensitive Au–Au_2S nanocolloids were prepared by mixing $HAuCl_4$ and Na_2S in aqueous solutions. An anti-tumor drug, *cis*-platin, was adsorbed onto Au–Au_2S nanoparticle surface via the 11-mercaptoundecanoic acid (MUA) layers. The results show that the degree of adsorption of *cis*-platin onto Au–Au_2S nanoparticles was controlled by the solution pH value, and the drug release was sensitive to near-infrared irradiation. The *cis*-platin-loaded Au–Au_2S nanocolloids can be potentially applied as NIR activated drug delivery carrier.

Keywords: Drug delivery; Near-infrared; Au–Au_2S; Nanoparticles; *cis*-platin

1. Introduction

Chemotherapy for the cancer treatment cannot be used to its full potential because it involves saturating the body with toxic drugs that produce harmful side effects such as reduced immune response to infection [1]. Drug delivery systems (DDS), in which the carriers incorporate the drug either through chemical bonding or passive adsorption, can deliver the drug to specific cells or release drugs optimally at predetermined rates [2,3]. The near-infrared (NIR) radiation, which is nondestructive to human tissues, has been developed as a DDS approach for cancer therapy [4,5]. In this approach, the photosensitizing drug is activated when irradiated by the NIR light during treatment. Moreover, tissue hyperthermia induced by NIR light, can be synergized for the release of photosensitizing drug. However, the traditional NIR photosensors are organic dyes that are harmful to human tissue, limiting the use of NIR radiation for cancer therapy.

In this study, NIR sensitive Au–Au_2S nanocolloids were investigated as drug delivery carriers. Zhou et al. [6] and Averitt et al. [7] reported that NIR sensitivity of Au–Au_2S nanoparticles, where Au_2S dielectric core was encapsulated by a thin gold shell, depended on the particle geometry. Gold is essentially a bio-inert material with applications ranging from dental surgery to arthritis treatments [8,9], Au (shell)–Au_2S (core) nanocolloids may also take advantage of the inherent biocompatibility of gold. Moreover, when using nanoparticles as drug carriers, the reduction of particle size to nanoscale not only enables an intravenous injection and minimizes possible irritant reactions at the injection site [10], but also allows the carriers to penetrate the membranes of the diseased cells, and delivers drugs to cancerous tumors [11].

In this paper, we report a preliminary study of the adsorption of *cis*-platin, a common drug applied in treatment of a broad range of solid cancers and lymphomas [12], to NIR sensitive Au–Au_2S nanocolloids. In order to functionalize the surface of NIR sensitive Au–Au_2S nanoparticles, 11-mercaptoundecanoic acid (MUA) was immobilized on the colloidal carriers before drug adsorption. The chemical structures of MUA and *cis*-platin are shown in Fig. 1. MUA molecules maybe adsorbed onto Au–Au_2S nanoparticles because the thiol (–SH) group is active to gold surface [13]. Another terminal –COOH group of MUA may provide further reactivity with *cis*-platin. The release of *cis*-platin from the Au–Au_2S nanocolloids under NIR light is also addressed.

2. Experimental

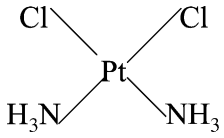
2.1. Synthesis

Chloroauric acid ($HAuCl_4 \cdot 4H_2O$) was obtained from Acros Organics. Sodium sulfide ($Na_2S \cdot 9H_2O$), 11-mercap-

* Corresponding author. Singapore–MIT Alliance, E4-04-10, 4 Engineering Drive 3, National University of Singapore, Singapore 117576, Singapore. Tel.: +65-68761266; fax: +65-67763604/67752920.

E-mail address: smarenl@nus.edu.sg (L. Ren).

0928-4931/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0928-4931(02)00247-3



Cis-Platin

$HSCH_2(CH_2)_8CH_2COOH$

11-mercaptoundecanoic acid (MUA)

Fig. 1. Chemical structures of *cis*-platin and MUA.

toundecanoic acid ($HS(CH_2)_{10}COOH$), and *cis*-platin (*cis*-$[Pt(NH_3)_2Cl_2]$) were obtained from Sigma. All reagents were used as purchased without further purification. The glassware were thoroughly cleaned and rinsed with deionized water. Following the literatures [6,7], the growth of Au–Au_2S nanocolloids was initiated when aqueous solutions of $HAuCl_4$ and Na_2S were mixed. Briefly, 20 ml of 1 mM Na_2S was mixed with 20 ml of 2 mM $HAuCl_4$, and stored at 25 °C for 1 day. The reaction was monitored using a UV–visible spectrophotometer at a range of 400–1100 nm. After centrifuging at 15,000 r.p.m., the Au–Au_2S nanoparticles were redispersed in a 100 mM 11-mercaptoundecanoic acid (MUA) solution in ethanol for 3 days at 40 °C. Excess MUA were removed from solution by at least three repeated cycles of centrifuging at 15,000 r.p.m., and subsequent redispersing in water. Finally, 10 mg of *cis*-platin was mixed with 10 ml MUA-modified Au–Au_2S nanocolloids by sonication. Afterwards, the flask containing the drug carriers was capped and left to stand for 2 days. Determination of the degree of loading *cis*-platin onto nanoparticles was carried out by separating the free *cis*-platin molecules from the supernatant fraction after centrifuging at 15,000 r.p.m. for 30 min. The concentration of *cis*-platin in supernatant was measured by high performance liquid chromatography (HPLC) method.

2.2. Drug release under NIR light

After centrifuging and rinsing, the *cis*-platin-loaded Au–Au_2S nanoparticles were transferred to glass vials containing 2 ml of water. Each vial was then irradiated along its long axis with a pulsed Nd:YAG laser (1064 nm, 100 mJ/pulse, 7 ns per pulse length, 10 Hz repetition rate, Surelite II, Continuum). The entire colloidal solution was exposed to the cross-sectional area of the beam. During the irradiation of 1 h, samples were moved from vials at different time intervals, and the amount of *cis*-platin released was subsequently determined using HPLC. Control experiments without laser irradiation were also performed.

2.3. Characterization

Samples for UV–Vis study were placed in quartz crystal cuvettes (path length 1 cm) and the absorption spectra were acquired at room temperature using a UV-1601 spectrophotometer (Shimadzu). Samples for TEM measurements were prepared by placing a drop of solution onto carbon-coated copper grids (200 mesh; 3 mm, Pelco) and left to dry prior to investigation using a microscope by JEOL 100CX (100 kV accelerating voltage). For Fourier transform infrared spectroscopy, nanoparticles were recovered from solution by centrifugation at 15,000 r.p.m. and completely dried by freeze-drying. The FTIR spectra were obtained by forming thin (~ 100 μm) transparent KBr pellets containing the materials of interest. The transmission spectra were recorded by using a Bio-red spectrometer (FTS 135) at a resolution of 4 cm^{-1}, and 256 scans were taken in the range of 400–4000 cm^{-1}. HPLC measurements were carried out in a liquid chromatograph instrument with a constant-flow-rate pump and diode array detector (model HP 1050, Hewlett Packard, USA). Samples were chromatographed using an analytical APS-Hypersyl column (Hewlett Packard of 20-cm length, 4.6-mm internal diameter). The mobile phase had a flow rate of 1.0 ml/min under isocratic conditions of acetonitrile–water (90:10). The UV detector was set at 210 nm. Each sample was injected three times and the results were averaged to obtain the value of the concentration.

3. Results and discussion

Fig. 2 shows a typical TEM bright field image of the *cis*-platin-loaded, MUA-modified Au–Au_2S nanoparticles. The drug-loaded spherical particles were about 40–50 nm in diameter, and a compact core of Au–Au_2S nanoparticles (dark contrast) was surrounded by a coating (light contrast). The coating presumably consisted of MUA and *cis*-platin. Fig. 3 shows the FTIR spectra of Au–Au_2S, MUA-modified Au–Au_2S, and *cis*-platin-loaded Au–Au_2S nanoparticles, respectively. Pure Au–Au_2S nanoparticles did not have the characteristic stretching vibration band of organic groups, whereas most of the IR bands of MUA were observed for MUA-modified Au–Au_2S nanoparticles. The strongest bands at 2920 and 1700 cm^{-1} were assigned to asymmetric

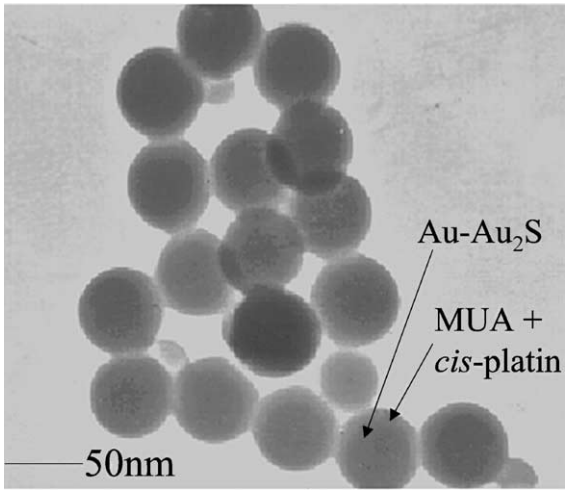


Fig. 2. TEM image of *cis*-platin-loaded MUA-modified Au–Au_2S nanoparticles. Core: Au–Au_2S; shell: MUA-cisplatin.

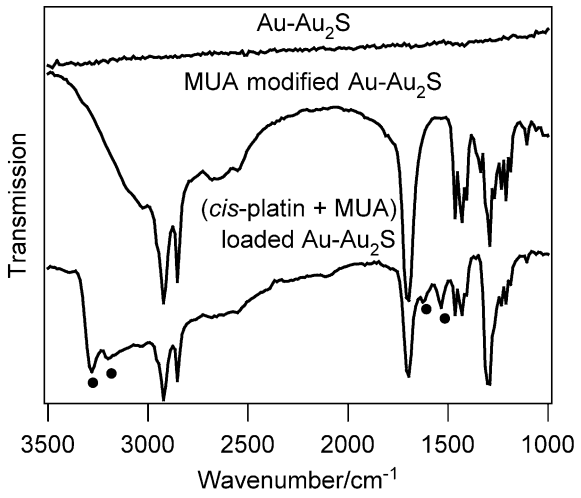


Fig. 3. FTIR spectra of Au–Au_2S nanoparticles, MUA-modified Au–Au_2S nanoparticles, and *cis*-platin-loaded, MUA-modified Au–Au_2S nanoparticles. ●: amine group.

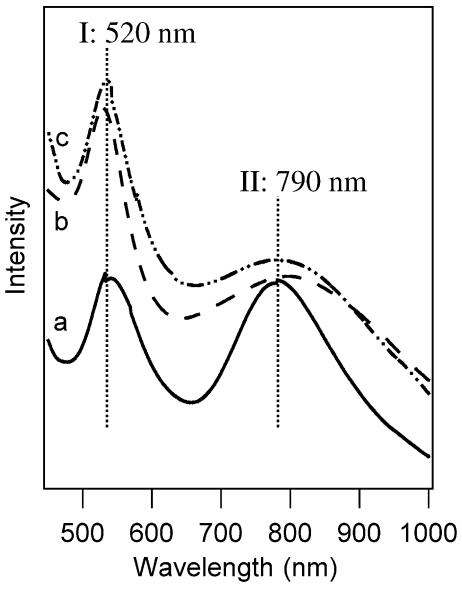


Fig. 4. UV–vis spectra of (a) Au–Au_2S nanocolloids, (b) MUA-modified Au–Au_2S nanocolloids, and (c) *cis*-platin-loaded Au–Au_2S nanocolloids.

and symmetric stretching vibrations ν_{CH} of the methylene groups and stretching vibration $\nu_{C=O}$ of the carboxylic acid groups of MUA, respectively, indicating that MUA was adsorbed on the Au–Au_2S nanoparticles. The FTIR spectra for the *cis*-platin-loaded, MUA-functionalized Au–Au_2S nanoparticles showed the appearance of new bands (indicated by closed circles) at 3280, 3200, 1614, and 1530 cm^{-1}, which were readily identified as the amine group. This indicates that a substantial amount of *cis*-platin was bound to Au–Au_2S nanoparticles through the MUA layer. It is suggested that MUA was adsorbed to Au–Au_2S nanoparticles via its –SH end group. The –COOH end group of MUA may be ionized to $-COO^-$ group, which may subsequently provide attachment sites for the protonated NH_3 (NH_4^+) group of *cis*-platin. This resulted in the adsorption of *cis*-platin to Au–Au_2S nanoparticles via the MUA layer.

Fig. 4 shows the UV–Vis spectra of Au–Au_2S, MUA-modified Au–Au_2S, and *cis*-platin-loaded, MUA-modified Au–Au_2S nanocolloids. All UV–Vis spectra consisted of two absorption bands. The band I at 520 nm is assigned to the surface-plasmon resonance of the Au nanoparticles, whereas the band II at 790 nm is due to Au-coated Au_2S nanoparticles [6,7]. Fig. 4 also shows that the band II for both of MUA-modified Au–Au_2S and *cis*-platin-loaded Au–Au_2S nanocolloids shifted to longer wavelength, which may due to the coating of MUA and *cis*-platin on Au–Au_2S nanoparticles. Note that Au–Au_2S nanocolloids were stable against aggregation even after 3 months when coated with MUA at pH 6, whereas the uncoated Au–Au_2S nanocolloids or MUA-coated Au–Au_2S nanocolloids in acidic conditions ($pH \leq 5$) aggregated in 1 week (data not shown). The long-term stability of Au–Au_2S nanoparticles against aggregation is important for future applications.

Fig. 5 shows the effects of solution pH on adsorption percentage of *cis*-platin to the MUA-modified Au–Au_2S nanoparticles. The maximum *cis*-platin adsorption value (~ 80%) was obtained at pH 6.0–7.0. However, below or above this pH, a smaller amount of *cis*-platin was absorbed. At $pH \leq 2.5$ or ≥ 8.5, the *cis*-platin was almost not adsorbed onto the MUA-modified Au–Au_2S nanoparticles. This pH-dependent behavior can be interpreted as follows: when the pH was decreased, the COO^- groups of the MUA layer became protonated; when the pH was increased, the NH_3 group of *cis*-platin was not protonated to NH_4^+. As a result, ionic bond between COO^- groups of MUA layer and NH_4^+ groups of *cis*-platin could not readily form in either acidic or basic solutions.

The release of *cis*-platin from MUA-modified Au–Au_2S nanoparticles over the observed period (≤ 1 h) under NIR

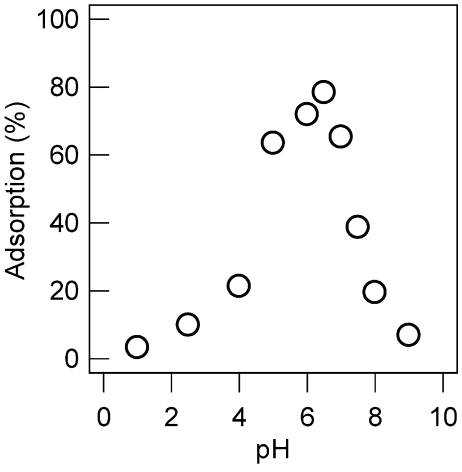


Fig. 5. Adsorption percentage of *cis*-platin onto the MUA-modified Au–Au_2S nanoparticles as a function of solution pH.

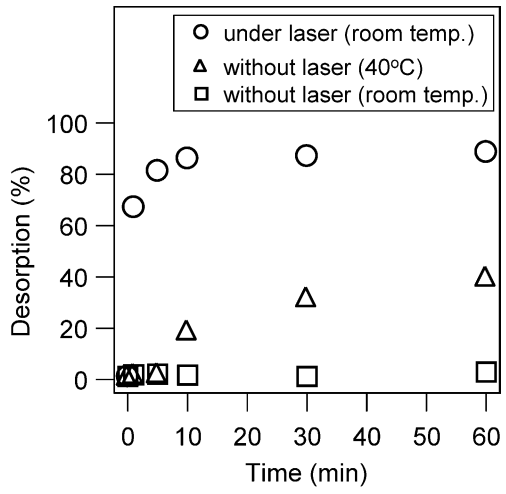


Fig. 6. Release of *cis*-platin from Au–Au_2S nanoparticles under Nd:YAG laser (room temperature) and heating (40 °C, without laser irradiation).

light irradiation and by heating (40 °C) without NIR irradiation is shown in Fig. 6. The NIR irradiation was performed at room temperature. The temperature of the solution during irradiation was not monitored. About 90% of *cis*-platin was released at the initial period (≤ 1 min) under NIR irradiation, and then the rate of release decreased with increasing time. For heating without NIR irradiation, the release of *cis*-platin started after heating at 40 °C for 10 min, and only 40% of *cis*-platin was released from Au–Au_2S nanoparticles during the period of 1 h. This shows that more *cis*-platin was released under NIR irradiation compared to heating without laser irradiation. The control experiments without laser irradiation or heating showed that only a little amount (1.2%) of *cis*-platin was released. It is thus suggested that the release of *cis*-platin from Au–Au_2S nanocolloids is sensitive to both of heating and NIR irradiation. However, such dissociative reaction is more readily initiated by the absorption of NIR light compared to heating without laser irradiation. The *cis*-platin-loaded Au–Au_2S nanocolloids may absorb the NIR photons, and this absorption with high energy may lead to photochemical interaction, thermal interaction, photoablation, plasma induced ablation, and photodisruption among nanocolloids [14,15]. These interactions may also occur to cause the cleavage of the ionic bond between COO^- and NH_4^+, and may finally release *cis*-platin from Au–Au_2S nanoparticles. However, the mechanism of the interactions between NIR light and NIR sensitive Au–Au_2S nanocolloids is presently unclear, and detailed work is in progress. The thermal effects in NIR irradiation also need to be understood. Based on the results shown in Fig. 5, it is suggested that the adsorption of *cis*-platin on Au–Au_2S nanoparticles is stable in physiological conditions where pH is about 7.2–7.4, however, when NIR light is applied in therapy, *cis*-platin will be released from the nanoparticles so that the cancerous cells will be killed. Hence, our preliminary study of drug release under NIR irradiation shows that *cis*-platin-loaded Au–Au_2S nanocolloids can be a potential drug delivery system for cancer treatment.

4. Summary

We have reported a preliminary study of preparing a drug carrier system in which MUA was used as a linker between drug and NIR sensitive Au–Au_2S nanoparticles. The degree of adsorption of *cis*-platin onto Au–Au_2S nanoparticles was controlled by the solution pH value, and *cis*-platin was released from nanoparticles under NIR irradiation at a greater rate than thermal activation without laser irradiation. The potential of this drug delivery system for cancer therapy warrants further investigation.

Acknowledgements

This work was supported by the Singapore-MIT alliance (SMA). L. Ren was supported by the SMA postdoctoral fellowship. We thank Dr. G. Chen in NTU for his help on using Nd:YAG laser. We also thank Dr. C.-L. Ho and Dr. S.-C. Li of the department of pharmacy of NUS for their advice and discussion.

References

[1] H. Calvert, I. Judson, W.J.F. Van der Vijgh, Cancer Surveys: Pharmacokinetics and Cancer Chemotherapy, Cold Spring Harbor Laboratory Press, New York, 1993.
[2] K. Park, Controlled Drug Delivery: Challenges and Strategies, American Chemical Society, Washington, DC, 1997.
[3] L. Domellof, Drug Delivery in Cancer Treatment: II. Symptom Control, Cytokines, Chemotherapy, Springer-Verlag, New York, 1989.
[4] R. Raghavachari, Near-Infrared Applications in Biotechnology, Marcel Dekker, New York, 2001.
[5] S. Sershen, S.L. Westcott, N.J. Halas, J.L. West, J. Biomed. Mater. Res. 51 (2000) 293.
[6] H.S. Zhou, I. Honma, J.W. Haus, H. Sasabe, H. Komiyama, J. Lumin. 70 (1996) 21.
[7] R.D. Averitt, D. Sarkar, N.J. Halas, Phys. Rev. Lett. 78 (1997) 4217.
[8] B. Merchant, Biologicals 26 (1998) 49.
[9] J.L. West, N.J. Halas, Curr. Opin. Biotechnol. 11 (2000) 215.
[10] F. Kratz, M.T. Schutte, Cancer J. 11 (1998) 176.
[11] S.M. Moghimi, A.C. Hunter, Crit. Rev. Ther. Drug Carr. Syst. 18 (2001) 527.
[12] B. Lippert, Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug, Wiley, New York, 1999.
[13] H. Schmidbaur, Gold: Progress in Chemistry, Biochemistry, and Technology, Wiley, Chichester, 1999.
[14] M.H. Niemz, Laser-Tissue Interactions: Fundamentals and Applications, Springer-Verlag, Germany, 1996.
[15] I.W. Boyd, R.B. Jackman, Photochemical Processing of Electronic Materials, Academic Press, London, 1992.