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Fe_3O_4/*o*-Carboxymethyl Chitosan/Curcumin-based Nanodrug System for Chemotherapy and Fluorescence Imaging in HT29 Cancer Cell Line

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A multifunctional nanodrug system containing Fe_3O_4, *o*-carboxymethyl chitosan (OCMCs), and curcumin (Cur) has been prepared and characterized by infrared and fluorescence spectroscopy, X-ray diffraction (XRD), and field-emission scanning electron microscopy (FE-SEM). The fluorescent staining experiments showed that this system not only had no effect on the cell internalization ability of curcumin but also successfully led curcumin into the HT29 cells as expected. From real-time cell analysis (RTCA), the effect of Fe_3O_4/OCMCs/Cur on this cancer cell line was found to be much stronger than that of pure curcumin. This system contained magnetic particles and, therefore, could be also considered for hyperthermia therapy in cancer treatment.

A great number of natural dietary compounds were investigated to look for therapeutic modalities with no or minimal side effects to normal organs in cancer treatment. Among these, curcumin, a yellow compound isolated from rhizomes of the herb *curcuma longa*, has received considerable attention because of its putative cancer prevention and anticancer activities which are mediated through influencing multiple signaling pathways.[1–4]

Although curcumin possesses these remarkable features, the extremely low solubility in aqueous solutions limits its bioavailability and chemical efficacy.[5,6] To deal with this obstacle, a variety of methods including the incorporation of curcumin into liposomes and into phospholipid vesicles are being studied.[7–9] More recently, the approach of biodegradable polymer nanoparticles has been developed.[10–12] This offers promising enhanced therapeutic performance of anticancer drugs by increasing their bioavailability, solubility, and retention time. These drug formulations are superior to traditional medicines with respect to control release, targeted delivery, and therapeutic impact.

OCMCs has a structure similar to chitosan, but the *o*-hydroxy group of each monomer is substituted by a carboxymethyl group through ether bond formation. It is an amphiprotic ether, exhibiting nontoxicity, biodegradability, biocompatibility, and strong bioactivity and has, therefore, garnered increasing interest in biomedical applications. More strikingly, it can load hydrophobic anticancer drugs effectively.[13,14]

Furthermore, magnetic nanoparticles with proper surface coatings have been widely developed because of their great applications. They can be used not only as magnetic resonance imaging contrast agents in medicinal diagnosis but also for therapeutic purposes such as drug delivery and hyperthermia treatment.[15–22]

In this work, we present the preparation of a multifunctional nanodrug system containing Fe_3O_4, OCMCs, and curcumin and the effect of this system on the viability of HT29 cancer cell line.

First, 150 mg of Fe_3O_4 was synthesized by chemical coprecipitation of Fe^{2+} and Fe^{3+} ions according to a procedure in the literature.[23] The Fe_3O_4 obtained was then ultrasonically vibrated in 50 mL of distilled H_2O to get 3 mg mL^{-1} Fe_3O_4 fluid. Next, OCMCs-coated Fe_3O_4 fluid was prepared using ex situ-grafting. 10 mL of Fe_3O_4 fluid was mixed with 5 mL of 2 mg mL^{-1} aqueous OCMCs solution, then ultrasonically vibrated for 1 h and stirred for 24 h to obtain an OCMCs-coated Fe_3O_4 fluid. To this fluid, 7.5 mL of 4 mg mL^{-1} ethanolic curcumin solution was added. The resulting solution was stirred for 48 h in a closed flask and then stirred in open air for further 24 h to evaporate ethanol completely. Subsequently, the solution was magnetically deposited to obtain 5 mL of Fe_3O_4/OCMCs/Cur fluid. It was dried at 60 °C to get a dark brown powder.

Figure 1 displays the FE-SEM images and XRD patterns of Fe_3O_4, Fe_3O_4/OCMCs, and Fe_3O_4/OCMCs/Cur.

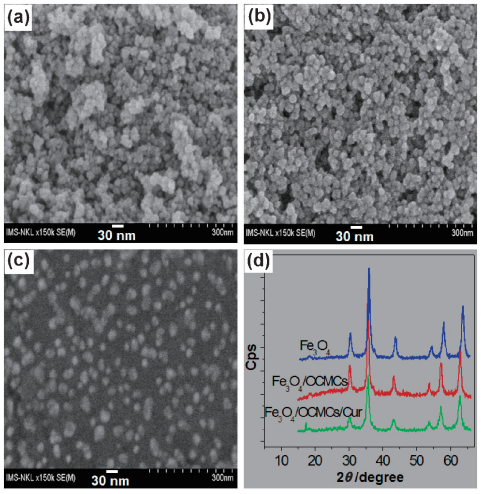

Figure 1. FE-SEM images of (a) Fe_3O_4, (b) Fe_3O_4/OCMCs, (c) Fe_3O_4/OCMCs/Cur, and (d) their XRD patterns.

Fe_3O_4 fluid contained aggregates, composed of spherical particles with a size of 10–20 nm. Fe_3O_4/OCMCs fluid was less aggregated from fairly uniform-sized particles ranging from 20 to 25 nm. Upon the encapsulation of curcumin, Fe_3O_4/OCMCs/Cur obtained had nearly the same size. However, different with the others, Fe_3O_4/OCMCs/Cur was of isolated particles; in this case the aggregation could not be observed.

From the XRD patterns of Fe_3O_4, Fe_3O_4/OCMCs, and Fe_3O_4/OCMCs/Cur nanoparticles, it was clear that all six diffraction peaks corresponded to faces of (200), (311), (400), (422), (511), and (440), characteristic for crystalline Fe_3O_4, which was the standard pattern for crystalline magnetite with a spinel structure.[24] The particle size of Fe_3O_4 calculated on the basis of the Scherrer formula was in the range of 10–20 nm, consistent with that from FE-SEM image. As could be seen from the diffraction patterns, after being encapsulated by OCMCs, the crystallinity of Fe_3O_4 was almost unchanged. Thus, Fe_3O_4 was apparently present in all samples under investigation.

The formation of Fe_3O_4/OCMCs/Cur nanoparticles was also evidenced by IR (see Supporting Information[25]) and fluorescence spectra. The peak at 584 cm^{-1} in the IR spectrum of Fe_3O_4, characteristic of Fe–O–Fe in the oxide,[23] was shifted to 564 cm^{-1} for OCMCs/Fe_3O_4 and to 570 cm^{-1} for Fe_3O_4/OCMCs/Cur. Because of the complexation of curcumin with the OCMCs, the wavenumbers corresponding to the characteristic peaks of OCMCs was shifted. Comparing Fe_3O_4/OCMCs and Fe_3O_4/OCMCs/Cur peak shifts were observed from 3440 to 3391 cm^{-1} and 1637 to 1626 cm^{-1}. This data confirmed the presence of curcumin in the OCMCs matrices. Curcumin is a strongly fluorescent compound. Therefore, this component in the Fe_3O_4/OCMCs/Cur system can be monitored by fluorospectrometry. Figure 2 showed fluorescence spectra of curcumin and Fe_3O_4/OCMCs/Cur. The fluorescence maximum of the latter was shifted by 27 nm compared with that of curcumin only. This result further confirmed that the microenvironment of OCMCs/Cur was changed after conjugation of OCMCs with curcumin.[6,26] Moreover, the fluorescence intensity of Fe_3O_4/OCMCs/Cur was also decreased probably due to the quenching effect of the electron transfer from the excited curcumin to ferric ion.[27]

The influence of Fe_3O_4/OCMCs/Cur on the cell internalization ability of curcumin was investigated. In these experiments, 2×10^5 cells were seeded on a coverslip placed in each well of the 24-well plate. After 24 h of culture, the cells were incubated with Fe_3O_4/OCMCs/Cur at the final concentration of 10 μg mL^{-1} for 15 h and then fixed with 4% PFA (Sigma). Fluorescent staining was carried out to label actins with Rhodamine–phalloidin and nuclei with Hoechst (Invitrogen). Coverslips were observed with an LSM 510 microscope (Carl Zeiss).

Fluorescent images taken by LSM 510 indicated the presence of curcumin as the green signal inside HT29 cells incubated with Fe_3O_4/OCMCs/Cur (Figure 3). The green signal was due to the autofluorescence of curcumin when excited by an argon laser. It, therefore, could not be seen in control cells. This result demonstrated that the conjugation did not affect the cell internalization ability of curcumin but also successfully led curcumin into the cells as expected.

An in vitro cytotoxicity evaluation of materials was carried out using an X-CELLigence system (Roche Inc.). The system measures electrical impedance across interdigitated microelectrodes integrated on the bottom of tissue culture E-plates. The impedance measurement provides quantitative information of cell number and viability. The real-time cell assay started with the background reading by adding 50 μL of DMEM media (Invitrogen) to each well of E-plate 96 and then monitored at 15 s intervals within 1 min. Next, 130 μL of DMEM media containing 10^4 HT29 cells was seeded into each well of the E-plate, and the cells were monitored every 15 min for 20 h to obtain the growth baseline reading. At the time point of treatment, 20 μL of conjugated curcumin or pure curcumin was added into each well to get 5 concentrations of the range from 0.01 to 100 μg mL^{-1}. Dynamic cell proliferation of cells was monitored in 30-min intervals from the time of treatment until the end of the experiment (72 h). Cell Index values were analyzed by RTCA software (Roche Inc.) to get IC_{50} and further evaluation.

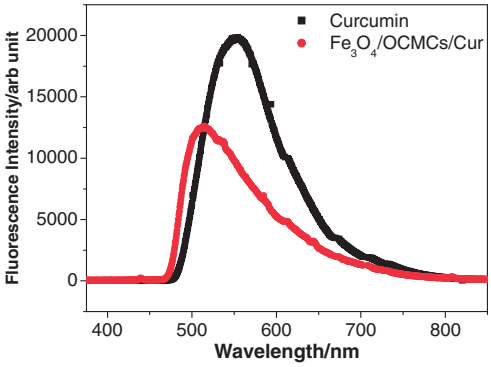

Figure 2. Fluorescence spectra of curcumin and Fe_3O_4/OCMCs/Cur.

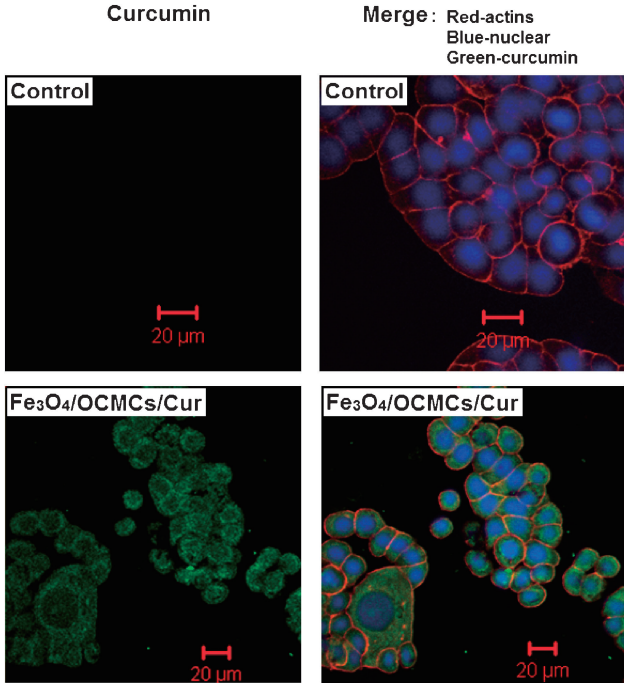

Figure 3. Fluorescent images of HT29 cells in normal culture conditions (control) and incubated with conjugated curcumin for 15 h.

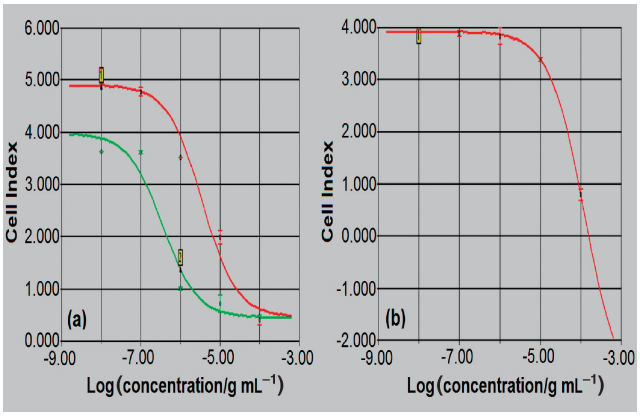

Figure 4. Dose-response curve of HT29 cell treated with pure curcumin (**a**, red curve), conjugated curcumin (**a**, green curve) and (b) Fe_3O_4/OCMCs.

The RTCA showed the cytotoxicity of Fe_3O_4/OCMCs/Cur on HT29 cells with the IC_{50} of $0.36\,\mu g\,mL^{-1}$ ($P < 0.05$), meanwhile the IC_{50} value of pure curcumin was $3.6\,\mu g\,mL^{-1}$. Dose-response curve of HT29 cells treated with pure curcumin was significantly higher than that with conjugated curcumin (Figure 4). This apparently suggested that our conjugate efficiently conducted curcumin and, therefore, enhanced its biological activity in cancer cells. Indeed, it was curcumin but not Fe_3O_4/OCMCs that determined the cytotoxicity of the conjugate. Fe_3O_4/OCMCs had a negligible impact on cancer cells ($IC_{50} = 125.610\,\mu g\,mL^{-1}$, $P < 0.05$). Its cytotoxicity effect was 350 times less than that of the whole conjugate. The improvement of cytotoxicity was probably due to the water solubility (curcumin in 1 mL of Fe_3O_4/OCMCs/Cur fluid was found to be 6 mg) and cell internalization ability of the conjugate.

Magnetic fluid hyperthermia is a promising tool in the therapy of various cancers. This is because tumor cells are more sensitive to temperatures in the range of 42–46 °C than normal tissue cells.[19] In this work, we just proved that our conjugate could satisfy criteria of temperature for hyperthermia therapy. All the samples enabled the temperature to increase up to 42 °C and even higher for 10 min (see Supporting Information[25]). The temperature retention lasted 10 min and could prolong when the heating conditions were held. Some experiments on magnetic hyperthermia therapy are in progress.

In conclusion, a Fe_3O_4/OCMCs/Cur-based nanodrug system could be successfully prepared by ex situ grafting. This system not only could be used as a tool for monitoring the drug circulation by the fluorescence technique but also in cancer treatment. The system was proven to successfully lead curcumin into HT29 cells, and its effect on this cancer cell line was much stronger than that of pure curcumin. It is promising to develop this conjugate as a new smart nanomaterial for drug delivery.

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References and Notes

1 L. M. Huong, H. P. Thu, N. T. B. Thuy, T. T. H. Ha, H. T. M. Thi, M. T. Trang, T. T. N. Hang, D. H. Nghi, N. X. Phuc, D. T. Quang, *Chem. Lett.* **2011**, *40*, 846.
2 G. Sa, T. Das, *Cell Div.* **2008**, *3*, 14.
3 P. Anand, C. Sundaram, S. Jhurani, A. B. Kunnumakkara, B. B. Aggarwal, *Cancer Lett.* **2008**, *267*, 133.
4 S. Karmakar, N. L. Banik, S. J. Patel, S. K. Ray, *Neurosci. Lett.* **2006**, *407*, 53.
5 G. Liang, L. Shao, Y. Wang, C. Zhao, Y. Chu, J. Xiao, Y. Zhao, X. Li, S. Yang, *Bioorg. Med. Chem.* **2009**, *17*, 2623.
6 L. D. Tran, N. M. T. Hoang, T. T. Mai, H. V. Tran, N. T. Nguyen, T. D. Tran, M. H. Do, Q. T. Nguyen, D. G. Pham, T. P. Ha, H. V. Le, P. X. Nguyen, *Colloids Surf., A* **2010**, *371*, 104.
7 M. Takahashi, D. Kitamoto, T. Imura, H. Oku, K. Takara, K. Wada, *Biosci., Biotechnol., Biochem.* **2008**, *72*, 1199.
8 K. Sou, S. Inenaga, S. Takeoka, E. Tsuchida, *Int. J. Pharm.* **2008**, *352*, 287.
9 L. Li, F. S. Braiteh, R. Kurzrock, *Cancer* **2005**, *104*, 1322.
10 P. Anand, H. B. Nair, B. Sung, A. B. Kunnumakkara, V. R. Yadav, R. R. Tekmal, B. B. Aggarwal, *Biochem. Pharmacol.* **2010**, *79*, 330.
11 M. M. Yallapu, B. K. Gupta, M. Jaggi, S. C. Chauhan, *J. Colloid Interface Sci.* **2010**, *351*, 19.
12 M. M. Yallapu, M. Jaggi, S. C. Chauhan, *Colloids Surf., B* **2010**, *79*, 113.
13 Z. Aiping, L. Jianhong, Y. Wenhui, *Carbohydr. Polym.* **2006**, *63*, 89.
14 A. Anitha, S. Maya, N. Deepa, K. P. Chennazhi, S. V. Nair, H. Tamura, R. Jayakumar, *Carbohydr. Polym.* **2011**, *83*, 452.
15 A. Kumar, P. K. Jena, S. Behera, R. F. Lockey, S. Mohapatra, S. Mohapatra, *Nanomedicine* **2010**, *6*, 64.
16 Q. A. Pankhurst, N. K. T. Thanh, S. K. Jones, J. Dobson, *J. Phys. D: Appl. Phys.* **2009**, *42*, 224001.
17 B. Koppolu, M. Rahimi, S. Nattama, A. Wadajkar, K. T. Nguyen, *Nanomedicine* **2010**, *6*, 355.
18 T. K. Jain, S. P. Foy, B. Erokwu, S. Dimitrijevic, C. A. Flask, V. Labhasetwar, *Biomaterials* **2009**, *30*, 6748.
19 J.-H. Park, K.-H. Im, S.-H. Lee, D.-H. Kim, D.-Y. Lee, Y.-K. Lee, K.-M. Kim, K.-N. Kim, *J. Magn. Magn. Mater.* **2005**, *293*, 328.
20 J.-H. Lee, J.-t. Jang, J.-s. Choi, S. H. Moon, S.-h. Noh, J.-w. Kim, J.-G. Kim, I.-S. Kim, K. I. Park, J. Cheon, *Nat. Nanotechnol.* **2011**, *6*, 418.
21 A. Jordan, R. Scholz, P. Wust, H. Fähling, R. Felix, *J. Magn. Magn. Mater.* **1999**, *201*, 413.
22 L.-Y. Zhang, H.-C. Gu, X.-M. Wang, *J. Magn. Magn. Mater.* **2007**, *311*, 228.
23 A. Zhu, L. Yuan, T. Liao, *Int. J. Pharm.* **2008**, *350*, 361.
24 Z. Ma, Y. Guan, H. Liu, *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *43*, 3433.
25 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.
26 H. Yu, Q. Huang, *Food Chem.* **2010**, *119*, 669.
27 J. S. Kim, D. T. Quang, *Chem. Rev.* **2007**, *107*, 3780.

