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Local Retention and Combination Effects of Biocompatible Doxorubicin-Loaded and Radioiodine-Labeled Microhydrogels in Cancer Therapy

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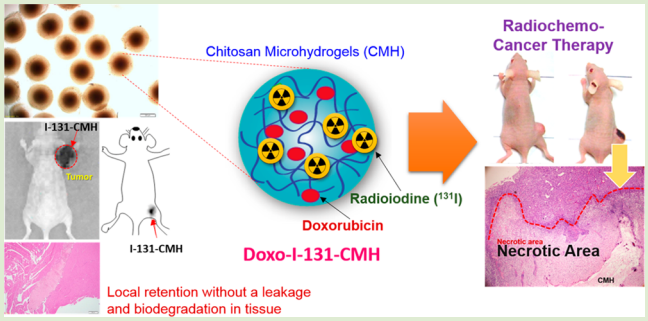
Supporting Information

ABSTRACT: I-131-labeled chitosan microhydrogels (I-131-CMH) that are retained at an injection site without leaking free I-131 into normal tissue can provide opportunities to improve cancer therapy. This study focuses on the development of doxorubicin-loaded I-131-CMH (Dox-I-131-CMH) for use in radiochemotherapy against cancer. The radiolabeling of I-131-CMH was found to be stable over a period of 2 weeks with no disassociation of free I-131, and Dox showed a sustained release from the CMH. When I-131-CMH were injected into the thigh muscle or tumor tissue, in vivo gamma imaging showed a retention at the injection site with no significant leakage of I-131 into other areas of normal tissue, and after an intrahepatic arterial injection, I-131-CMH were selectively retained in the liver. Dox-I-131-CMH had significant synergistic therapeutic effects of radiation and chemotherapy on mouse breast cancer models. In this regard, Dox-I-131-CMH may be a new alternative agent for cancer therapy.



Radioiodine (I-131) therapy has been widely used to treat thyroid cancer. I-131 normally accumulates in thyroid tissue as a result of the human sodium/iodide symporter (hNIS), and it then induces apoptosis and necrosis of the cancer cells.[1−4] I-131 is a radioisotope that is used for both therapeutic and imaging purposes. It emits both β- and γ-rays and has a physical half-life of 8.05 days. It has several advantages over various cancer treatments since it has a lower cost and longer half-life.[5] However, I-131 cannot be extensively used for cancer therapy (aside from that for thyroid cancer) because healthy tissue and organs can be exposed to the radiation, producing adverse effects.[6] Many studies have attempted to address these problems and to expend the clinical use of I-131.[7−9] One such effort is to develop a carrier that can accumulate I-131 in the target lesion without exposing healthy tissue to the radiation and separating the radioisotope (RI) from the carrier. For example, lipiodol radio-labeled with I-131 has been recently developed as a treatment for hepatocellular carcinoma.[10,11] Except for that, I-131 labeling was used for tracing chitosan microparticles to delivery an angiogenic peptide in a rodent model of acute myocardial infarction.[12] Chitosan is a polysaccharide that is deacetylated from chitin, and it consists of glucosamine and *N*-acetylglucosamine. It has received considerable attention in various medical fields as a result of its high biodegradability and biocompatibility, with low toxicity.[13−16] Chitosan has many groups of primary amines, and chemical modifications can be easily performed to conjugate functional groups, including drugs, target moieties for specific binding, hydrophobic moieties for self-assembled particles, and chelators for RI labels.[17] In addition, chitosan is a polysaccharide that is suitable for use in hydrogel-based drug delivery systems because it has a net cationic charge, thereby forming cross-linked networks against negatively charged molecules.[18,19] This study focuses on the preparation and characterization of doxorubicin-loaded and I-131 labeled chitosan microhydrogels (Dox-I-131-CMH) prepared for use in cancer therapy. Dox-I-131-CMH are designed for local retention at the tumor site without any leakage of I-131 into other areas of normal tissue. The advantages of using hydrogels include having an ability to deliver a high dose of radiation from I-131 to the target site without exposing healthy tissue or organs and its feasibility of producing a combination with other anticancer therapeutic drugs. In tumor therapy using RI, it is important to develop a carrier that can prevent the RI from

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draining into normal healthy tissue or organs and that can stay at the injection site.[20] A schematic diagram of the preparation of I-131-CMH and Dox-I-131-CMH is presented in Figure 1.

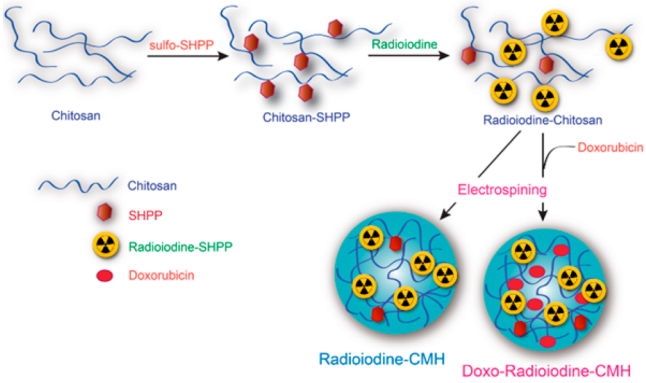

Figure 1. Schematic diagram for the preparation of I-131-CMH and Dox-I-131-CMH.

First of all, chitosan was chemically modified using sulfo-succinimidyl-3-[4-hydroxyphenyl]propionate (SHPP) in order to label a radioiodine (Figure 2a). ^{1}H NMR data based on our

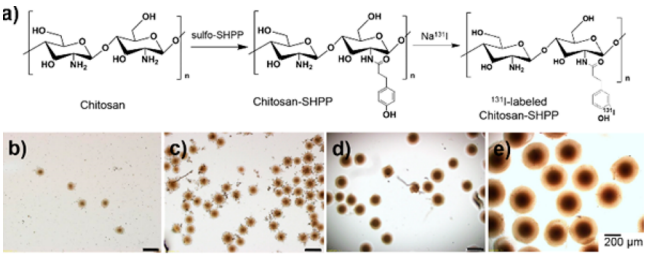

Figure 2. (a) Schematic diagram for the synthesis of the chitosan-SHPP conjugate and the radiolabeling of I-131. Images of CMH prepared using an electro-spinning system at an electric field intensity of (b) 13, (c) 10, (d) 9, and (e) 8 kV (all scale bars indicate 200 μm).

previous research indicate that the degree of substitution of SHPP per 100 sugars of chitosan was 6.04.[12] The chitosan-SHPP conjugate was then radiolabeled with Na·I-131. After radiolabeling, the I-131-labeled chitosan-SHPP conjugate solution was dropped into a tripolyphosphate solution by using an electro-spinning system, which formed spherical microhyrogels. As shown in Figure 2b–e, their diameters ranged from 50 to 300 μm and were controlled by adjusting the intensity of the electric field of the electro-spinning system. The size of microhydrogels showed a significant decrease with as the voltage increased from 8 to 13 kV. The radiolabeling efficiency of I-131-CMH was determined using ITLC and was found to be higher than 90%. The radiolabeling of I-131-CMH was observed to be stable over a one-week period in PBS buffer and showed stability in serum for 16 h without dissociation of free I-131.

The CMH were added into the cell media from 0.25 to 1 mg/mL. The CMH showed no cytotoxicity in cell viability studies for various cells (Figure 3a). The cell viability was more than 92.8% within 24 h for all cell lines, indicating that CMH had no significant adverse effects on cell viability. We investigated the cell viability for I-131-CMH at various radioactivities of 3, 5, 10, 15, 20, and 30 μCi (the results at 3, 5, and 10 μCi are not shown here). All cell lines showed a

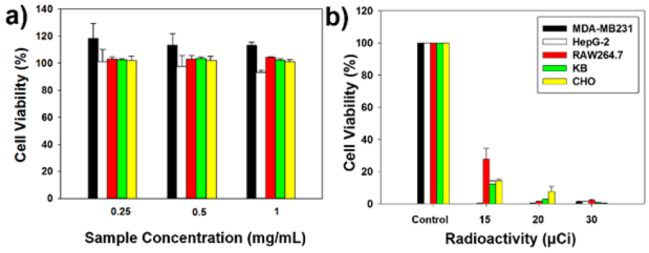

Figure 3. In vitro cell viability of various cells treated with (a) CMH and (b) I-131-CMH by an MTT assay.

high cell viability 24 h after the addition of 10 μCi I-131-CMH, whereas the viability of tumor cell lines was less than 20%. In particular, the zero percent of HepG-2 and MDA-MB231 cells were alive after the addition of 15 μCi I-131-CMH (Figure 3b). Beta particles from I-131 damage biological cells through direct and indirect effects. The radiation may directly hit a particularly sensitive molecule in the cell or can indirectly interact with water molecules in the body through reactive oxygen species (ROS).[21] Recently, Almeida et al. reported that high concentrations of I-131 above 10 μCi showed cytotoxic and mutagenic effects in *Rattus norvegicus* hepatoma cells.[22]

Next, Dox was contained physically into CMH to add a chemotherapeutic effect. Fluorescent signals from Dox-CMH were observed, and in particular, a fluorescent spherical shape was observed via fluorescent microscopy (Figure S1 in Supporting Information). The loading efficiency of Dox in CMH was 86.7% of the total drug weight used for loading, and Dox content in CMH was 34.7% of the total weight of the Dox-CMH. Figure S2 in Supporting Information shows the drug release pattern of Dox-CMH. The Dox released at pH 7.4 and 5.4 was 38.7 ± 5.6% and 67.3 ± 2.3% of the total Dox loaded at 24 h, respectively. The release of Dox from CMH at an acidic pH may be attributed to a stronger protonation of the amino groups of chitosan and the higher solubility of Dox at an acidic pH.[23] This indicates that Dox was released from the CMH in greater quantities at acidic tumor sites than in normal tissue, reducing the release of Dox into the bloodstream.

During radiochemotherapy against cancer, the question of whether I-131-CMH accumulate at the injection site without any leakage into normal tissue is an important issue. To follow the retention of CMH over a longer period of time, I-125 with a half-life of 60 days was used for CMH instead of I-131. After radiolabeling with I-125, I-125-CMH were injected into the normal thigh muscle, and gamma images of the rats were obtained afterward. As shown in Figure 4a, the injected I-125-CMH were retained at the injection site without any leakage to other areas of normal tissue over a 25-day period. Moreover, I-131-CMH were retained in liver tissue without any leakage of free I-131 to other areas of normal tissue 10 days after a hepatic arterial injection with a catheter (Figure 4b and video file of Supporting Information). At 10 days after injection into the hepatic artery, 96.3% of the total injected I-131-CMH were retained in the liver tissue (Figure S3 in Supporting Information).

To investigate the biocompatibility and biodegradation in vivo, the CMH were intramuscularly implanted into the thigh muscle of rats for a period of 1, 3, or 5 months. The host cells infiltrated the CMH and fibrotic capsules formed around these microhydrogels 1 month after intramuscular implantation (Figure S4a in Supporting Information). The CMH were agglomerated into a mass after their implantation into the

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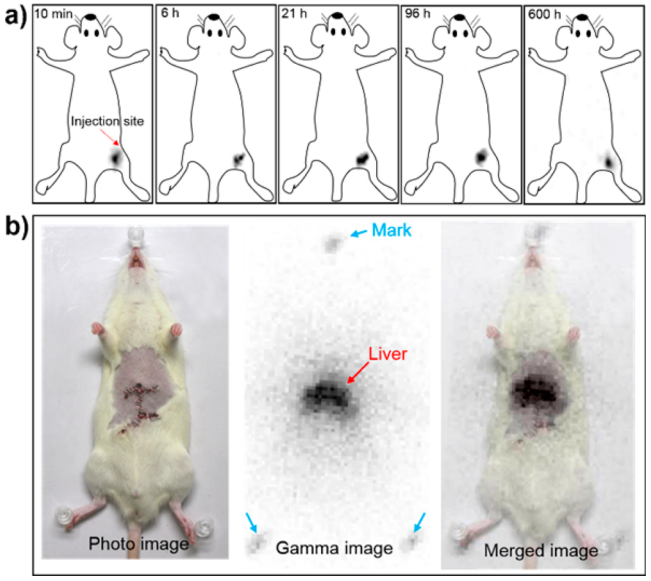

Figure 4. (a) Gamma images of rats with I-125-CMH injected intramuscularly into the thigh muscle. (b) A photograph of a rat after a microsurgical procedure, a gamma image 1 h after a hepatic arterial injection of I-131-CMH, and a merged image photograph and gamma image.

muscle. At 3 months after implantation, they were divided and wandered in the capsule. As shown in Figure S4c, the CMH disappeared, and degenerated muscular tissue was seen at the implantation site. It is well-known that chitosan is degraded by enzymatic hydrolysis.[24] However, the properties of chitosan can change through chemical modification or by changing conditions during fabrication. Although chitosan was conjugated with SHPP and formulated into a microspheres, CMH showed biodegradability and had high biocompatibility in vivo.

The retention of I-131-CMH at the tumor site was investigated before evaluating the therapeutic effects against tumors in an MDA-MB231 tumor model. As shown in Figure 5a, the I-131-CMH injected directly into the tumor site were retained in the lesion without any leakage to other areas of normal tissue over five-day period. Figure 5b shows a tumor growth in the mouse model over a 21-day period for different groups. At 21 days after direct injection of I-131-CMH with 60 μCi radioactivity and Dox-I-131-CMH with 70 μCi radioactivity, the tumor growth rate decreased by 39% and 58%, respectively, relative to the tumor growth rate of mice injected with PBS. In particular, the tumor growth rate of mice injected with Dox-I-131-CMH with 90 μCi radioactivity decreased by 64% relative to that of mice injected with PBS. It is well-known that I-131 and Dox induce apoptosis and necrosis of tumor cells. These results reveal that the simultaneous use of I-131 and Dox has a synergistic effect for tumor therapy through the injection of CMH. Tumor tissue was immunostained to investigate the therapeutic effect of I-131-CMH. As shown in Figure 5c, a necrotic area was observed around I-131-CMH injected into the tumor tissue.

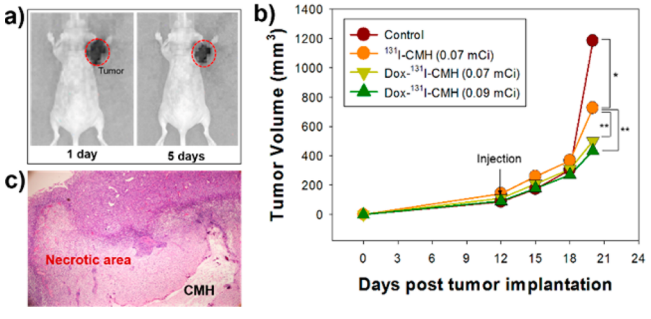

Figure 5. (a) Merged images of photographs and gamma images of the MDA-MB231 mouse model after a tumoral injection of I-131-CMH. Red dotted line indicate tumor region, and those regions in dark black indicate the presence of I-131-CMH. (b) The tumor growth of MDA-MB231 mouse models after a tumoral injection of PBS, I-131-CMH, or Dox-I-131-CMH for 21 days (n = 4; *p < 0.01, **p < 0.03). (c) Microscopic images of tumor tissue stained and selected 9 days after a tumoral injection of I-131-CMH (400× magnification).

In summary, this study presents I-131-CMH and Dox-I-131-CMH as therapeutic agents against cancer through a local injection. CMH showed very low cytotoxicity in vitro and high biocompatibility in vivo. The results based on in vivo gamma-imaging studies after local injection of I-125-CMH verify that I-131-CMH are retained at the injection site of the thigh muscle and tumor tissue without any leakage of free I-131 into other areas of normal tissue after a local injection. Dox-I-131-CMH, with a combination of radiation and chemotherapy, had significant synergistic therapeutic effects against tumors through a local injection into the tissue, suggesting that Dox-I-131-CMH may be a useful combination therapeutic agent against cancer through a local injection into tissue. In particular, they are well within the realm of a retention therapeutic agent for liver cancer.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedure and the results including the fluorescence microscopic image, Dox release, biodistribution data, and biodegradation of CMH in normal thigh muscle, as well as additional video file of the procedure for a hepatic arterial injection of the CMH. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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