Supporting Information

MoS_2 nanosheets encapsulated in sodium alginate microcapsules as microwaveembolization agents for large orthotopic transplantation tumor therapy

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1. Experimental Section

1.1 Materials:

Sodium alginate (SA), Sodium molybdate ($Na_2MoO_4•2H_2O$) and thiourea (CH_4N_2S) were purchased from Aladdin Industrial Corporation (Shanghai, China). Hydrochloric acid (HCl), dimethyl sulphoxide (DMSO), Calcium chloride ($CaCl_2$) and Span 80 were obtained from Beijing Chemical Works (Beijing, China). Hematoxylin and eosin (H&E stain) were bought from Beijing Solarbio Science &Technology (China). Calcein-AM and propidium Iodide (PI) were obtained from Sigma. All reagents used in this work were analytical reagents (A.R.) without any further purification.

1.2 Preparation of MoS_2

MoS_2 were prepared according to our previous method. Typically, $Na_2MoO_4•H_2O$ (3.7 mmol) and CH_4N_2S (15.8 mmol) were dissolved in 25 mL DI water to form a homogenous solution with the assistance of ultrasonic. Afterwards, 12 mL HCl (1 M) was added into above solution. Then, the resultant mixed solution was transformed into a Teflon-lined stainless-steel autoclave and kept at 220 ºC for 22 h. Finally, the obtained black solution were centrifuged and washed repeatedly with distilled water to obtain the MoS_2.

1.3 Preparation of MSMC

Firstly, MSMC was prepared using micro emulsion method on the base of our previous study. Firstly, 10 mg MoS_2 were dispersed in 1 mL water solution and mixed with 5 mL sodium alginate solution at the mass ratio of 2 % with ultrasonic treatment. Then the resultant homogenous solution was added into the 15 mL atolin to form a two phase emulsion. Afterwards, 0.1 mL Span 80 was added under the magnetic stirring for 30 min to produce a milky homogenous W/O emulsion. Subsequently, 10 mL $CaCl_2$ solution (5 wt %) was gradually added to above W/O emulsion for 30 min under continual magnetic stirring. Finally,

microcapsules were obtained by destructing the W/O emulsion using isopropanol. The resultant microcapsules were washed twice with deionized water at 5000 rpm for 5 min (25 ºC). SA microcapsules were prepared by a same process without adding MoS_2 when emulsifying with atolin. All the samples were sterilized by the treatment of ultraviolet (UV) light for 1 h with the following immersion in 75% ethanol for 2 h.

1.4 Characterization and measurements

The morphology and size distribution of the obtained microcapsules were analyzed by Optical microscope (Nikon Eclipse Ti-S, CCD: Ri1), scanning electron microscope (SEM, Version, 4300 and 4800, Hitachi), and transmission electron microscopy (TEM, JEM-2100F, Japan Electronics Co., Ltd) operated at an acceleration voltage of 200 keV. For TEM observation, a drop of microcapsule dispersion in water was dropped on a copper mesh followed by drying at room temperature. The dispersion of MSMC was observed by SEM after a treatment of graded ethanol washing. Firstly, MSMC were fixed with the 3% glutaraldehyde for over 2 h at 4 ℃. Then MSMC were in turn to disperse in PBS, 50%, 70%, 80% and 90% ethanol for 15 min. In order to completely dehydration of MSMC, 100% ethanol was used to wash twice for 15 minutes per soak. Subsequently, tertiary butanol was replaced by ethanol. Finaly, the samples were freezed drying under vacuum for observation. EDX of the SEM were tested for detecting the elemental distribution. Thermogravimetric analysis (TG/DTA6300) was carried out to measure the composition of the materials from room temperature to 600 ºC with a heating rate of 10 k min^{-1} in the air flow. The temperature change *in vivo* and *in vitro* was monitored with the infrared thermal mapping apparatus during the process of microwave treatment. Electrochemical impedance spectroscope (EIS) was adopted to confirm the microwave heating mechanism in theory,

using a CHI 604B electrochemical workstation (Shanghai Chen Hua Company) through a conventional three-electrode system.

1.5 Microwave susceptibility of MSMC *in vitro*

The temperature elevation in the aline solution and the ablation of the gelatin water film were evaluated to examine the microwave susceptibility of as-prepared MSMC under microwave irradiation *in vitro*. To evaluate the temperature elevation of the saline solution, MSMC at the different concentrations from 2.5 to 10 mg mL^{-1} were added into a small plate with special treatment bottom. Afterwards, a round microwave source with a diameter of 0.5 cm was placed under the small plate to heat the solution at 2 W for 5 min. The heating curves were recorded to compare the microwave susceptible properties. The temperature change of the solution was recorded by fiber thermometers (Beijing Dongfangruizhe Technology Co., LTD). Furthermore, the ablation of gelatin water film was carried out to analyze the microwave susceptibility based on the emulsion method. During our experiment, the sample was dispersed in the 5% gelatin containing 1% BSA. Then the gelatin solution was freeze in ice-bath. The microwave needle was fixed in the center of the gelatin water film and irradiated with the output power of 5 W for 5 min. The elevated temperature was monitored by infrared thermal mapping apparatus (FLIR SC620) and the ablation zone was carefully observed. The biocompatibility of MSMC was evaluated via MTT method using HepG2, L929 and RAW264.7 cells. The microwave susceptible efficiency of MSMC was also carried out in the HepG2 cell stained with calcein AM/PI by the MTT assay. HepG2 cells suspension was mixed with MSMC at 0.5 mg mL^{-1}, MoS_2 at the concentration of 0.06 mg mL^{-1}, and DMEM containing 10% FBS as the blank test. All groups were exposed to microwave irradiation for 3 min at the outpower of 3 W. The cells without treatment were used as the

control group. Finally, the HepG2 cells were further incubated for 48 h at 37 °C before measurement.

1.6 Microwave susceptibility of MSMC *in vivo*

Animal studies were performed according to the local ethics committee. Female ICR mice, aged 4-6 weeks, purchased from Vital River Laboratory Animal Technology Co. Ltd, were used in the amimal experiments. 0.1 mL H22 cells (1×10^6) were injected subcutaneously in the right axillary region of the mice. After tumor grew to about 250 mm^3, the mice were allocated into five groups, namely, the control group without any treatment, the groups treated only microwave irradiation, and the group respectively treated with MC, MoS_2 MSMC and microwave irradiation. For the experimental groups, after intratumorally injecting of MC, MoS_2 and MSMC for 1 h, the mice were irradiated with microwave at 2 W for 5 min. The infrared thermal mapping apparatus were used to monitor the variation of the temperature. The body weight and tumor size were recorded during the whole experiment process. New Zealand White rabbits purchased from Vital River Laboratory Animal Technology Co. Ltd were injected with VX2 tumors in the hind limbs to propagate. When the diameter of VX2 tumors reached to about 3 cm, they were removed to prepare tissue suspension. Afterwards, a biopsy needle was performed to inject the VX2 tumor suspension into the left liver under ultrasound guidance. A 4 T MRI scanner was applied to monitor the tumor progression in the inoculated rabbits. When the diameter of tumor in the liver increased to 3 cm after 3 weeks observed from the T_2-weighted images, the animals were divided into three groups: a control group, a group with MW irradiation, and a group administrated with MSMC plus MW irradiation. The MSMC was injected through hepatic IA route at the concentration of 10 mg kg^{-1} before MW irradiation. The output power of microwave is 15 W at the way of continuous microwave irradiation for 2 min. The control

group was injected with equal-volume PBS solution under the same way. During the process of microwave irradiation, the temperature of the tumor was monitored by infrared thermal mapping apparatus. T_2-weighted images were obtained to monitor the tumor size at 1 d and 3 d after catheterization. DWI was also carried out to confirm the necrotic degree of the tumor after different treatment. The width (a) and length (b) of the tumor could be obtained from the T_2-weighted images. The tumor volume was calculated through the formula of $V = ab^2/2$.

1.7 Statistics

Results were expressed as a mean ± standard deviation (S.D). Multigroup comparisons of the means were performed by one-way analysis of variance (ANOVA) test using SPSS 14.0 (SPSS Inc., Chicago, IL). The statistical significance for all tests was set at $p < 0.05$

Results

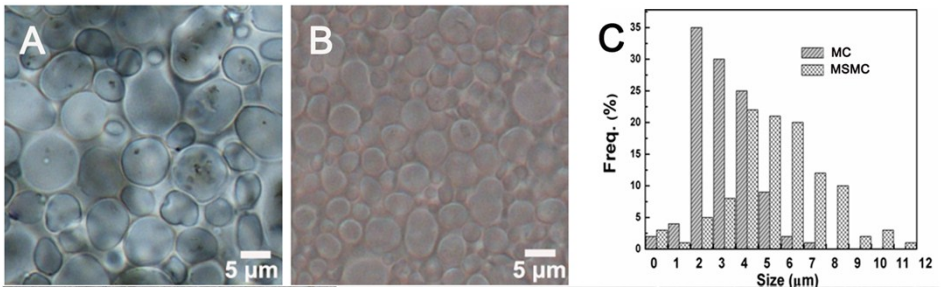

Fig. S1 (A-B) The optical photograph of MSMC and MC. (C) The size distribution of MSMC and MC.

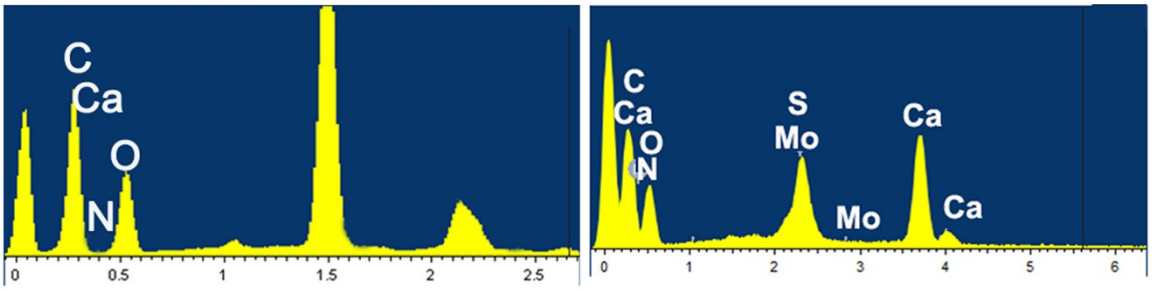

Fig. S2 EDS spectra of MC (A) and MSMC (B).

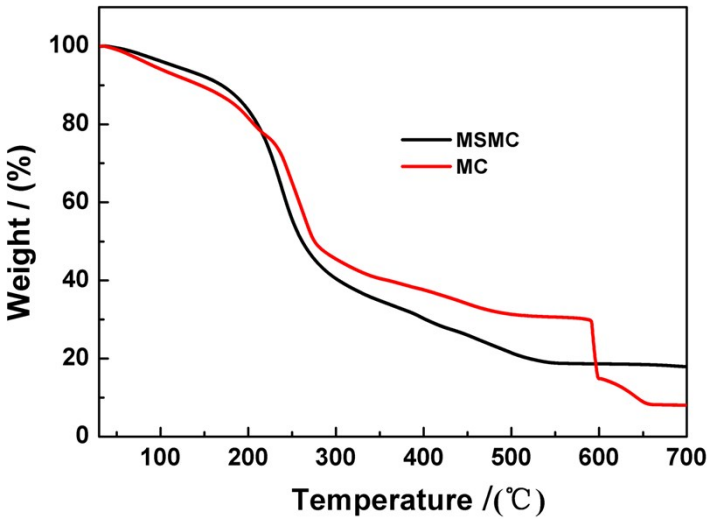

Fig. S3 TGA curves of MSMC and MC in the temperature range from room temperature to 600 ℃ with a heating rate of 10 ℃ per minute in the oxygen.

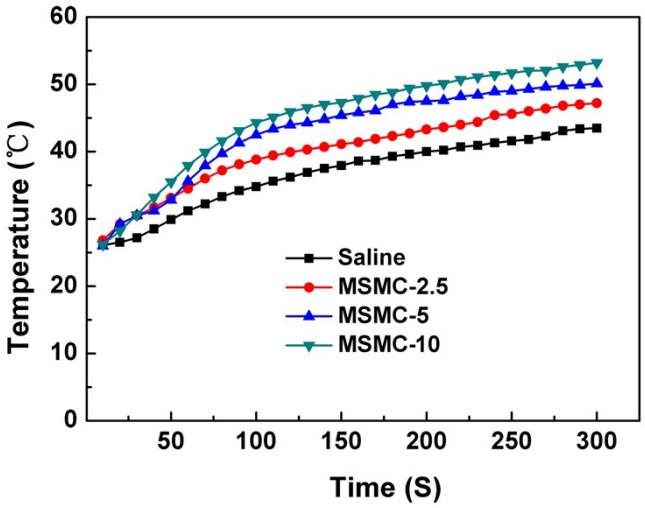

Fig. S4 Heating curves of saline with MSMC at 2.5, 5 and 10 mg mL^{-1}.

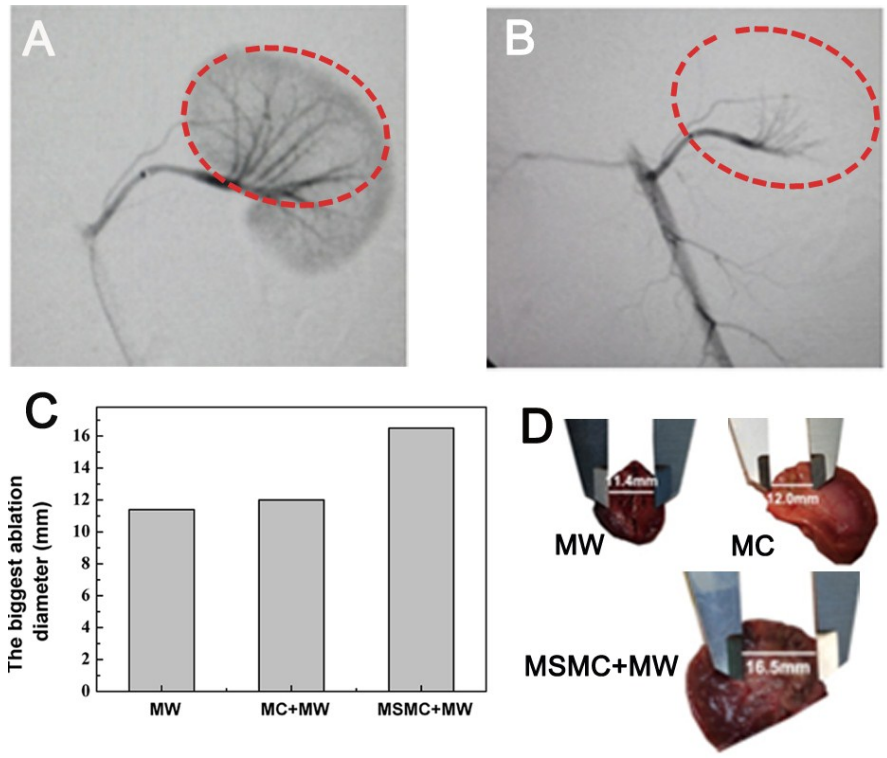

Fig. S5 (A-B) The DSA images of the normal kidney before embolization with MSMC. Red broken circle indicated the location of embolization. (C) The maximum ablation of kidney after embolization with MC and MSMC. (D) The optical photograph of kidney.

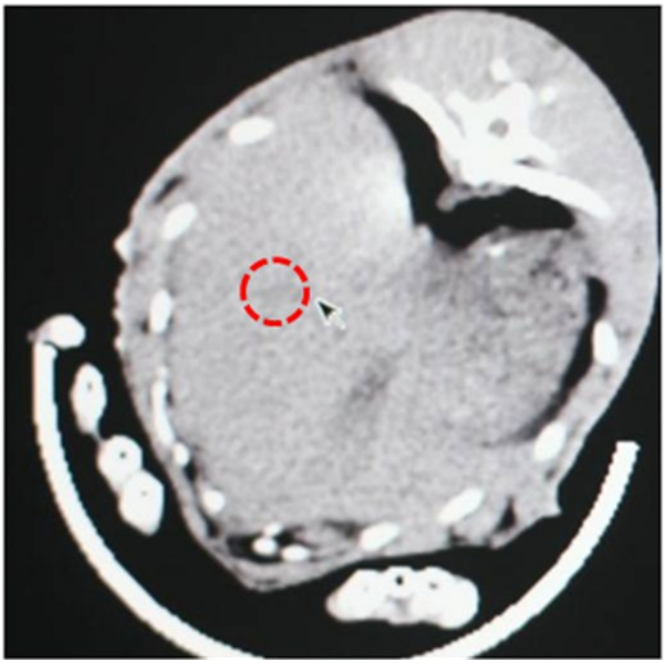

Fig. S6 The CT images of the tumor before embolization with MSMC. Red broken circle indicated the location of the tumor.

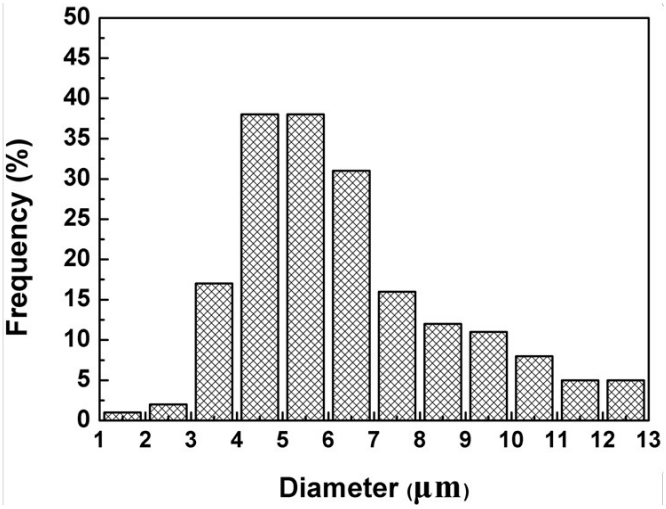

Fig. S7 The size distribution of MSMC observed in the liver;

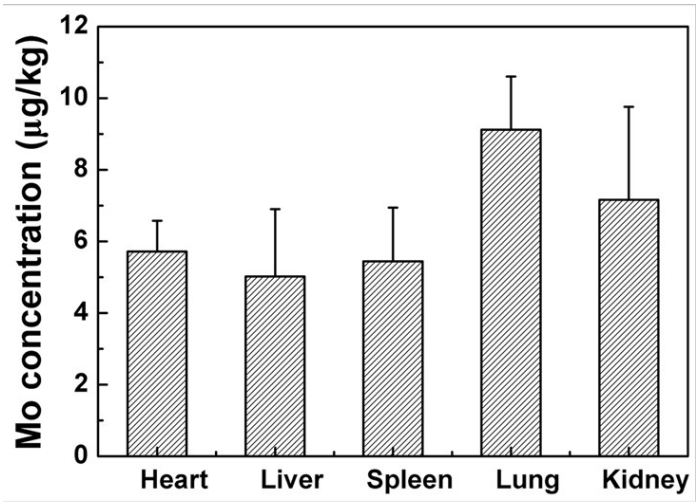

Fig. S8 Mo content in the heart, liver, spleen, lung and kidney.