Gelatin microcapsules for enhanced microwave tumor hyperthermia

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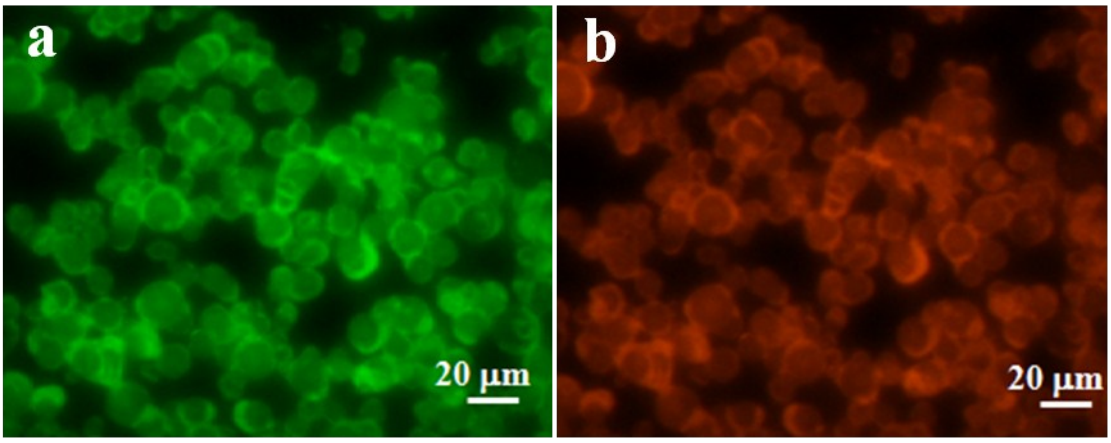

Figure S1. The optical microconfocal imaging of gelatin microcapsules with NaCl solution (0.7 M) under blue (a) and green (b) light excitation, respectively.

In order to define the microcapsules structure, we had used optical micro confocal fluorescence microscope (Olympus X71, Japan) to observe the morphology nature of the microcapsules. Gelatin contained a lot of protein, which resulting in bright green and red fluorescence. As shown in Figure S1, the as-prepared microcapsules exhibits strong green and red fluorescence under blue and green light excitation, respectively. The shell is labeled with strong green and red fluorescence, while weaker fluorescence is observed in the core of microcapsules, suggest that the microcapsules had shell-core structure, which is consistent the result of Figure 2b.

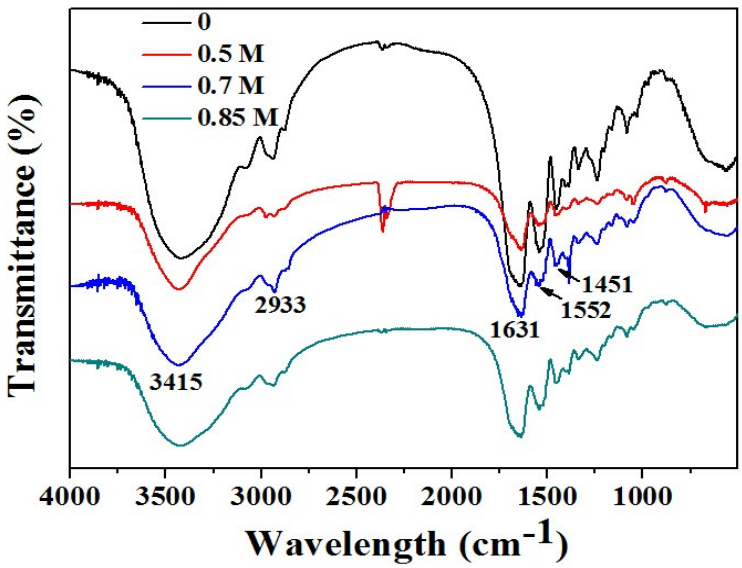

Figure S2. FT-IR spectra of microcapsules with different NaCl solution.

FT-IR analysis was carried out to confirm the possible groups of gelatin microcapsules. Figure S2 shows the FT-IR spectrum of gelatin microcapsules at different concentrations of NaCl, and all gelatin microcapsules were revealed different characteristic peaks ranging from 3415 cm^{-1} to 660 cm^{-1}. FT-IR spectrum of gelatin microcapsules reveals the presence of characteristic functional groups at 3415 cm^{-1} for amino group. The peaks at 2933 cm^{-1} indicate the presence of the carboxylic group. The absorption peaks at 1631 cm^{-1} are due to the stretching of carbonyl (C=O). The peaks at 1552 cm^{-1} are corresponds to the N-H (amide II) stretching frequency. A characteristic peak for C-N and N-H stretching vibrations in the region of 1451-1239 cm^{-1} is appeared in the microcapsules. These peaks are also present in the FT-IR spectrum of microcapsules show relatively similar patterns except intensity of the peak height.

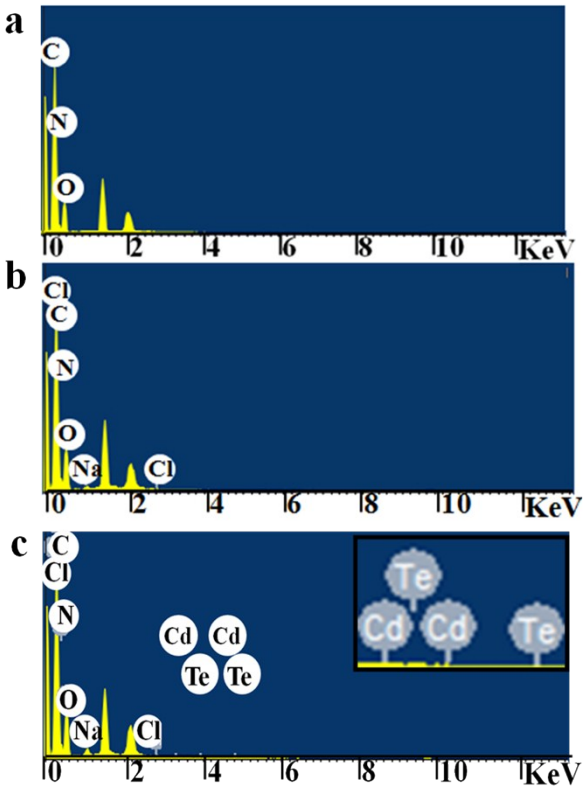

Figure S3. EDS spectrum of the gelatin microcapsules at different NaCl solution concentrations (a) 0, (b) 0.7 M and (c) CdTe-gelatin microcapsules (The inserts are amplification of their correspond image).

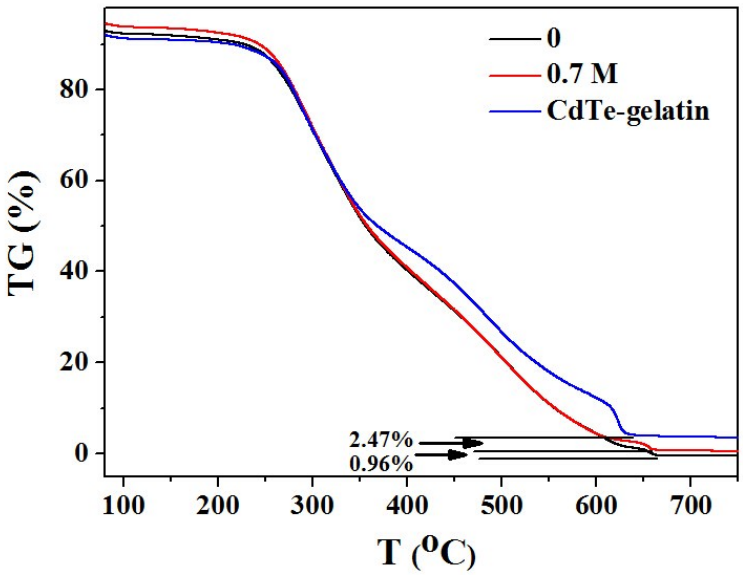

Figure S4. TG curve of gelatin microcapsules with different NaCl solution and CdTe-gelatin microcapsules.

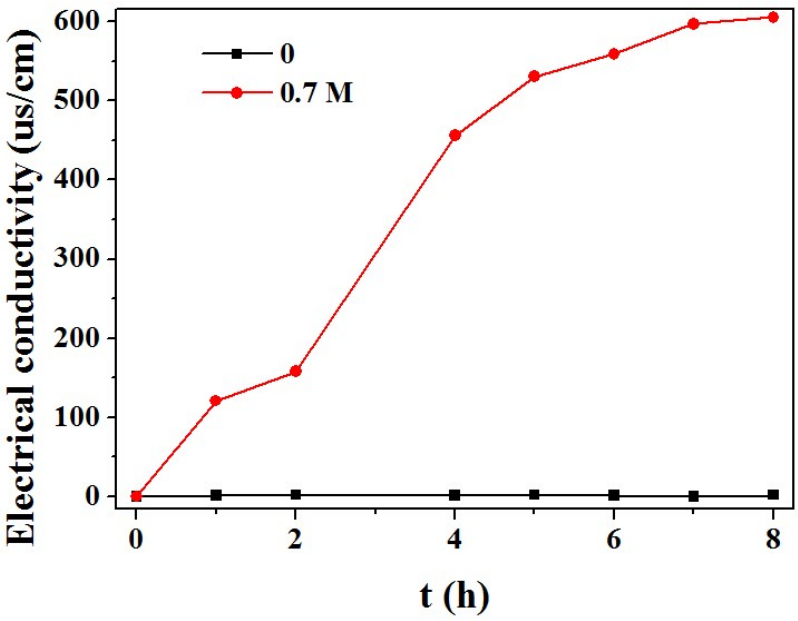

Figure S5. The electrical conductivity of microcapsules at different times.

The release of NaCl from the as-prepared microcapsules can be investigated by electrical conductivity measurement. For NaCl release assay, the samples (150 mg) were placed in 10 mL of pure water, stirring at room temperature. At given time intervals, 0.5 mL of the release medium was taken and added into 4.5 mL pure water. As presented in Figure S5, the electrical conductivity of microcapsules showed very low at 0 h. With increasing of the soaked time, the electrical conductivity of gelatin microcapsules with NaCl (0.7 M) reach to a high level, while the gelatin microcapsules showed no changes. According the result of electrical conductivity, the release of NaCl in pure water is slow till 2-4 hours. Thus all of the heating experiments in vitro and *in vivo* are finished in 1.5 hours.

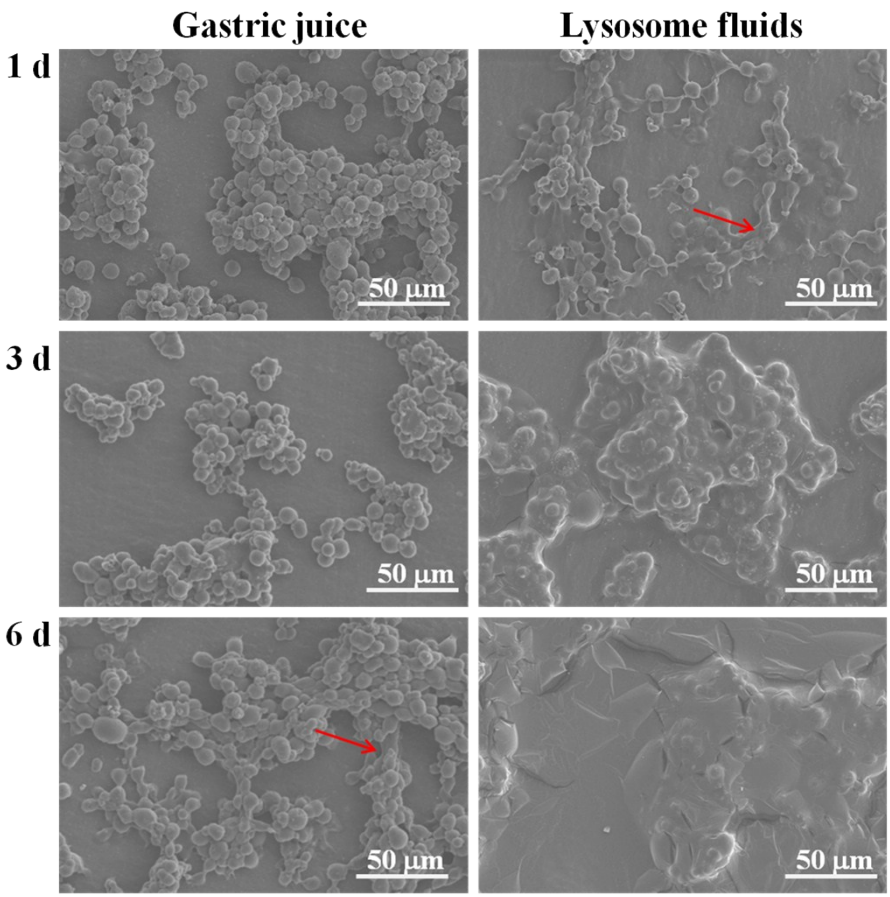

Figure S6. SEM images of microcapsules were immersed in gastric juice and lysosome fluid for different time.

In order to evaluate the stability and degradability of the microcapsules as function of time and pH, gelatin microcapsules are immersed in gastric juice (pH=1.6) and lysosome fluid (pH=4.5) for different time. 50 mg microcapsules were placed in 10 mL microcentrifuge tube, respectively. Then, 5 mL of gastric juice and 5 mL of lysosome fluid were added into each tube, respectively, and the samples were placed in 37 ^{o}C constant temperature water bath. The morphology of immersed microcapsules was also observed with scanning electron microscopy (S-4300, Hitachi). As shown in Figure S6, microcapsules began to degrade at the first 1 d in lysosome fluid, and as the time progressed to 6 d, the morphology of immersed microcapsules became more irregular. The degradation of microcapsules were obviously until 6 d in gastric juice. Thanks to their organic nature, the microcapsules were biodegradable and induced minimal acute toxicity during the treatment.

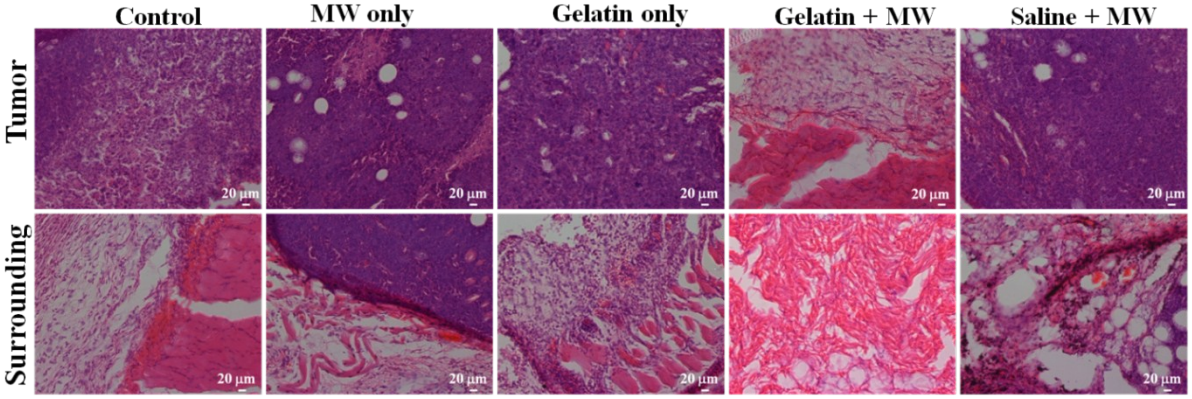

Figure S7 Histological examination of tumor tissue and the surrounding tissue from different treatment groups.

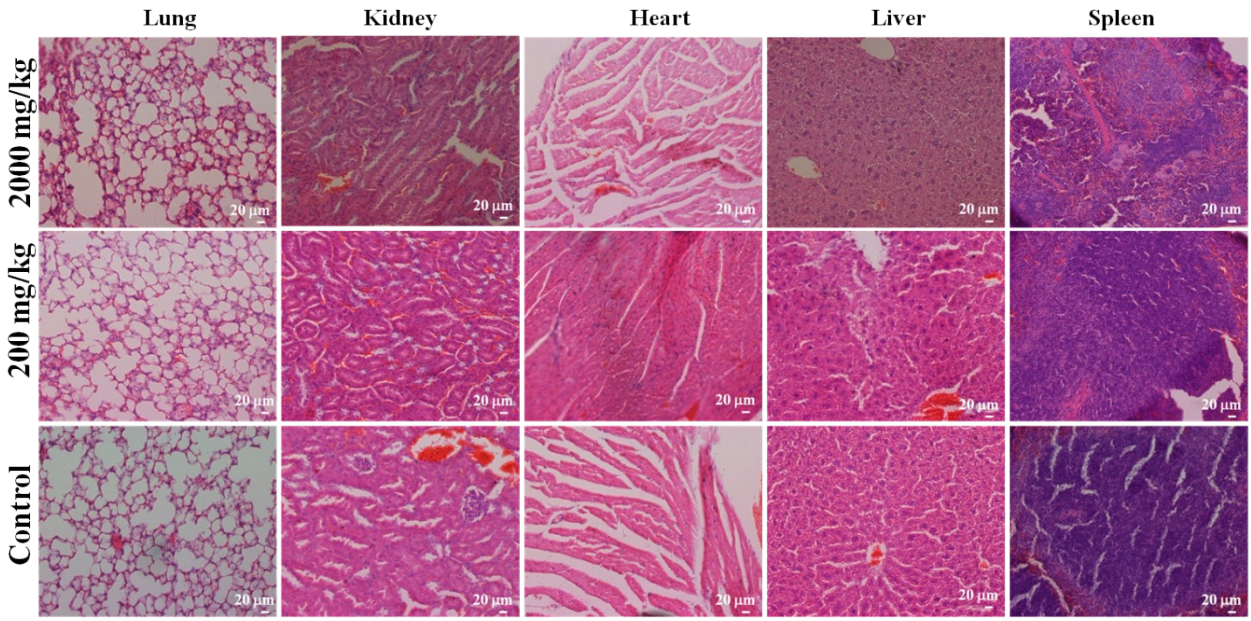

Figure S8. Representative histology images of organs from the healthy ICR mice via in vivo skin toxicity test with different dose of microcapsules.

A total 20 healthy ICR mice were randomly divided into 4 groups and injected subcutaneously in the axillary region with 2000 mg/kg, 200 mg/kg and without treatment. The measurement of body weight was made every three days. At day 16, all mice were sacrificed and collected their heart, liver, spleen, lung and kidney organs for histological examination with standard techniques. As depicted in Figure S8, microcapsules did not induce any changes in the appearance and micro-morphology of the lung, kidney, spleen, heart and liver at 16 days by different dose of microcapsules. Moreover, body weight of mice did not significant changes during the experiment (Figure S9). These results indicate that our experiment dose (200 mg/kg) is relatively safe.

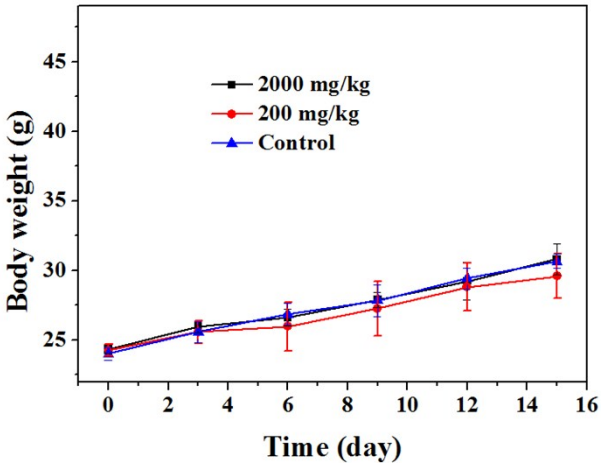

Figure S9. Mean body weight from the healthy ICR mice via in vivo skin toxicity test with different dose of microcapsules.

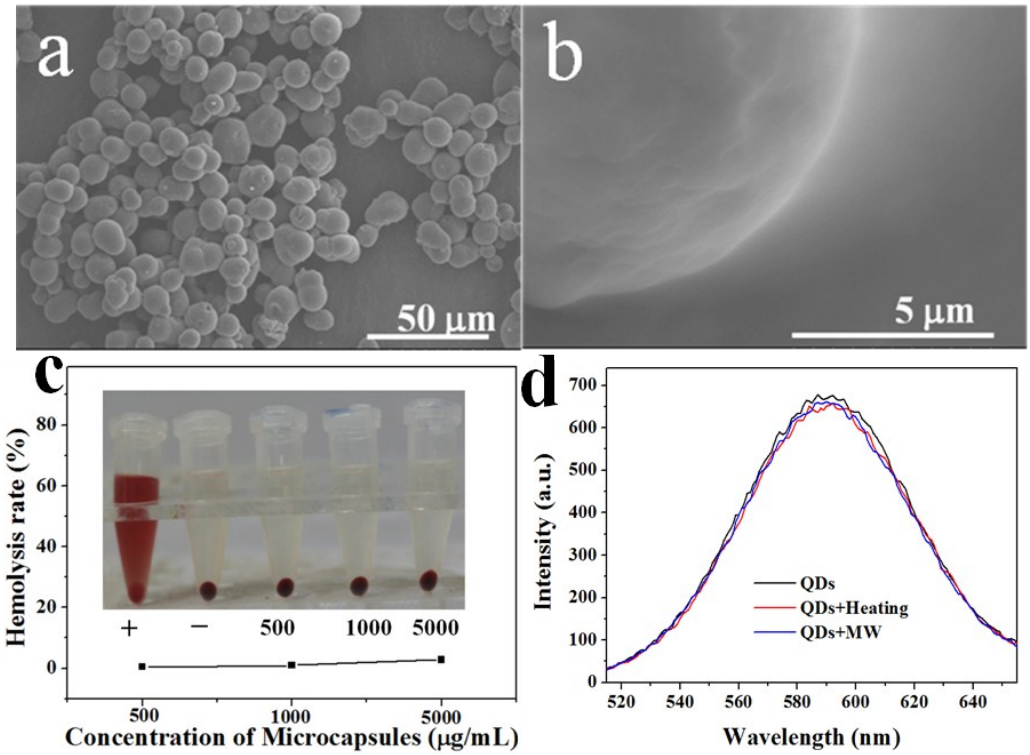

Figure S10. Characterization of CdTe-gelatin microcapsules. SEM images of (a) CdTe-gelatin microcapsules and (b) the amplification of CdTe-gelatin microcapsules. (c) Hemolytic percent of red blood cells incubated with CdTe-gelatin microcapsules at various concentrations for 3 h, using deionized water (+) and PBS (-) as positive and negative controls, respectively. Inset: Photographs of hemolysis test with different concentrations of microcapsules. (d) Photoluminescence of CdTe QDs under different conditions.

To evaluate the stability of the QDs under heating and microwave irradiation, 0.06 mL of QDs was taken and added into 6 ml pure water. Then, 2 mL of the solutions were heated at 60 ^{o}C for 10 min. Meanwhile, 2 mL of the solutions were exposed to MW irradiation(1.8 W, 10min). The fluorescence measurements were recorded with a Cary Eclipse fluorescence spectrophotometer (Varian, Inc.). As shown in Figure S10d, the fluorescence intensity did not change significantly under different conditions, which was suggested that the QD under heating and microwave irradiation is stable.