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Insights into a microwave susceptible agent for minimally invasive microwave tumor thermal therapy

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ABSTRACT

This work develops a kind of sodium alginate (SA) microcapsules as microwave susceptible agents for *in vivo* tumor microwave thermal therapy for the first time. Due to the excellent microwave susceptible properties and low bio-toxicity, excellent therapy efficiency can be achieved with the tumor inhibiting ratio of 97.85% after one-time microwave thermal therapy with ultralow power (1.8 W, 450 MHz). Meanwhile, the mechanism of high microwave heating efficiency was confirmed via computer-simulated model in theory, demonstrating that the spatial confinement efficiency of microcapsule walls endows the inside ions with high microwave susceptible properties. This strategy offers tremendous potential applications in clinical tumor treatment with the benefits of safety, reliability, effectiveness and minimally invasiveness.

1. Introduction

Nowadays, advances in nano-biotechnology have allowed local artificial hyperthermia to be an emerging technique [1–10], which can induce apoptotic cancer cell death without harm to healthy tissues. Several techniques have been used to generate local heating for tumor repression, including near infrared photothermal therapy [11–16], magnetic thermal therapy [17–19], radiofreqency (RF) thermal therapy [20–22], ultrasonic hyperthermia [23–25] and microwave thermal therapy [26–28]. The microwave thermal therapy of tumors has attracted much interest recently, due to the maneuverability, faster heat generation from microwave radiation, depth of penetration in tissues, less susceptibility to local heat tissues and perfect ability of killing tumor cells. Progress toward microwave-induced thermal therapy of cancer has been made in the animal models and clinical studies. Some kinds of nanomaterials were employed as microwave susceptible agents to enhance the heating efficiency [29–32], such as ferrite [18,30], carbon black [29,33], rare earth compounds [34,35] and ferromagnetic metals [31,32,36]. While these materials were only studied *in vitro* and cannot be used directly *in vivo*, because of the poor thermal transformation efficiency of these materials and the excessive microwave power used in previous works as high as 50–100 W, which may induce serious damage to the surrounding normal tissue of tumor [22,29,37,38]. Moreover, these materials can induce some resultant adverse effects to organisms. Above of all, it remains a challenge to use nanomaterials for tumor treatment *in vivo*. To prevent normal tissues around the tumor from being damaged, local heating in the tumor tissues has been a serious hyperthermia challenge. Hence, there is a great potential of enhancing the thermal transducing efficiency of microwave radiation to heating in the tumor tissues while sparing normal tissues by biocompatible materials.

Herein, we present the biocompatible microcapsules for optimal heat production response to microwave irradiation, which is efficiently used in tumor microwave thermal therapy *in vivo* for the first time. The ultralow microwave irradiation power and noninvasive process strongly suggest the clinical feasibility of the newly

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developed microwave thermal therapy technique. The microcapsules are composed of liquid core of NaCl solutions and biocompatible sodium alginate as wall materials. In the presence of microwave, the absorbed microwave energy is transferred to the kinetic and interionic energies of ions and stored as Joule heating energy of salt ions via the interactions between salt ions, which leads to rapid temperature rise. Meanwhile, more frequently interactions between salt ions occur due to the microencapsulation process, resulting in the faster temperature rise in compare with the dissociative ions. It benefits from contributions of the confinement efficiency of the inside ions by microcapsules, the as-prepared microcapsules show ideal temperature rise and therapeutic efficiency when applied to microwave thermal therapy *in vivo*.

2. Materials and methods

2.1. Materials

Sodium alginate (SA) was obtained from Shanghai Chemical Reagents Company (China). Calcium chloride ($CaCl_2$) and Span 80 were purchased from Tianjin Jinke Fine Chemical Research Institute (China). Sodium chloride (NaCl), dimethyl sulphoxide (DMSO), isopropanol and dimethylbenzene were obtained from the Beijing Chemical Reagents Company (China). Hematoxylin and eosin (H&E stain) were purchased from Beijing Solarbio Science &Technology (China). Hochest33342 and Propidium Iodide (PI) were purchased from Sigma. All reagents used in this work were analytical reagents (A.R.) without any further purification.

2.2. Preparation of SA microcapsules encapsulated NaCl solution with different concentrations

The schematic illustration of preparing the microcapsule is as shown in Scheme S1. Plenty of literatures have reported that sodium alginate microcapsules can be prepared through physical method with drop-wise adding sodium alginate solution containing core materials into $CaCl_2$ solution. However, the size of the obtained microcapsules through physical method is too large, even to the level of millimeter. To solve this problem, Micro emulsion method was adopted to prepare SA microcapsules with small size. Through exploring the influence of various parameters, the optimization preparation condition was as below. Sodium alginate solution (5 mL, 2 wt%) was added into the atolin (15 mL) to obtain two independent phases. To achieve the formation of W/O emulsion, surfactant (Span 80, 0.1 mL) was added and magnetic stirring started simultaneously for 30 min. During the process, the two independent phases gradually became milky homogenous W/O emulsion. Subsequently, $CaCl_2$ solution (10 mL, 5 wt%) was added to form wall structure of microcapsules for 30 min under continual magnetic stirring. To make the obtained microcapsules separated from the original W/O emulsion, isopropanol (5 mL) was added as demulsified agent. During the process, the homogenous W/O emulsion became into two obvious independent phases immediately and the obtained microcapsules could be observed in the substratum water phase. The obtained microcapsules were centrifuged (5000 rpm, 5 min, 25 °C) and washed three times with deionized water. To encapsulate liquid core of NaCl solutions, the NaCl solutions (1 mL) with different concentrations were mixed with sodium alginate solution before the emulsion process. Prior to all animal experiments and cell experiments, the as-prepared microcapsules must be sterilized with medicinal alcohol (75% (V/V)) and ultraviolet lamp.

2.3. Characterization and measurements

Optical microscope (UB-100i, Chongqing optical instrument factory) and scanning electron microscope (SEM, Models 4300 and 4800, Hitachi) were used to characterize the morphology and size distribution of the microcapsules. Optical micro confocal fluorescence microscope (Olympus X71, Japan) played the role of observing the paraffin sections of embedded organs and stained cells. Fourier transform infrared spectrometry (FT-IR, Varian, Model 3100 Excalibur) was employed to examine the composition and surface functional groups of the microcapsules. The composition of microcapsules was further measured via thermogravimetric analysis (TG/DTA6300) in the temperature range from room temperature to 600 °C with a heating rate of 10 °C per minute in the nitrogen flow. Ultraviolet and visible spectrophotometer (UV–Vis) and enzyme-linked immunosorbent assay reader were adopted to measure the cell viability. Infrared thermal mapping apparatuss was used to monitor the change of mice temperature during the microwave thermal therapy process. Electrochemical impedance spectroscope (EIS) were performed to confirm the microwave heating mechanism in theory, using a CHI 604B electrochemical workstation (Shanghai Chen Hua company) containing a conventional three-electrode system.

2.4. In vitro microwave heating experiment

To evaluate the microwave susceptible properties of the as-prepared microcapsules, *in vitro* microwave heating preliminary experiment was designed as below. The as-prepared microcapsules were dispersed in saline solution to obtain solution of 50 mg mL^{-1}. 1 mL microcapsules solution was added into small plate and irradiated by microwave (1.8 W, 450 MHz) for 5 min. Two control groups were designed under the same conditions, including sodium alginate gel group (SAG) and simulated body fluid group (SBF). During the process, record the temperature values every 10 s with optical fiber probe to monitor the temperature change of the solution. The heating curves with time were plotted to compare the microwave susceptible properties.

2.5. In vitro hemolysis test

Hemolysis test was carried out using rabbit's heart blood to evaluate the cytotoxicity of the as-prepared microcapsules *in vitro*. 5 mL blood was taken from the heart of rabbit and 0.2 mL anticoagulant agent was added. The blood samples were washed with PBS (pH = 7.2) to remove external and lysed red blood cells. After the supernatant liquid was removed, 1 mL blood was diluted to 50 mL with PBS (pH = 7.2) to obtain 2% red blood cells. Then 0.5 mL cells were mixed with the as-prepared microcapsules (0.5 mL) diluted in PBS (pH = 7.2) at a wide concentration range of 5–1000 μg mL^{-1} (5, 50, 100, 500, 1000 μg mL^{-1}). The positive control group was the mixture of 0.5 mL cells and 0.5 mL deionized water, and the negative control group was the mixture of 0.5 mL cells and 0.5 mL PBS (pH = 7.2). Three parallel experiments were performed in duplicate for each group. The mixtures were centrifuged after placed at room temperature for 3 h incubation period. The absorbance of the supernatant was measured at 570 nm via UV–Vis. The hemolysis ratio was calculated through Formula 1 [39–41].

$$P = \frac{A_S - A_N}{A_P - A_N} \times 100\% \quad (1)$$

In this formula, A_S is the absorbance resulting from the mixture of microcapsules and red blood cells suspension. A_N and A_P represent the absorbance of negative and positive control, respectively.

2.6. MTT assay–cell viability test

HepG2 cell was used to evaluate the potential cytotoxicity of the as-prepared microcapsules and MTT assay was conducted. HepG2 cells were cultured in DMEM medium. They were maintained in a thermostat incubator at 37 °C in a humid atmosphere with 5% CO_2.

MTT assay was carried out in the procedure as below. 100 μL HepG2 cells were seeded onto 96-well plates (1×10^4 cells per well) and incubated for 48 h in culture conditions subsequently. Then the cells were further treated with the as-prepared microcapsules (100 μL) diluted in DMEM medium at a wide concentration range of 5–1000 μg mL^{-1} (5, 50, 100, 500, 1000 μg mL^{-1}) and maintained in the thermostat incubator at 37 °C in a humid atmosphere with 5% CO_2 for another 48 h. The control group was treated with equivalent DMEN medium and other conditions were consistent with the experimental groups. 20 μL MTT solution was added into each well. After incubation in the thermostat incubator at 37 °C for another 4 h, supernatant was removed and each well was washed for three times with PBS (pH = 7.2) to remove the redundant microcapsules and DMEM medium containing MTT solution. Then 150 μL DMSO was added into each well and the adsorption value at 492 nm was measured via an enzyme-linked immunosorbent assay reader after gently shaken for 10 s to make the purple solution be homogenous. All samples were designed for five repeating groups and the cell viability was calculated through Formula 2 [42,43].

$$V_{cell} = \frac{A_S}{A_C} \times 100\% \quad (2)$$

In this formula, A_S and A_C represent the absorbance of experimental and control groups, respectively.

2.7. Cell viability test when treated with microwave

To evaluate the influence of microwave to cells, the experiment was designed as below. 500 μL HepG2 cells (4×10^5 cells mL^{-1}) were mixed with 500 μL microcapsules (diluted in DMEM medium, 100 μg mL^{-1}) in a small plate. After gently shaken to make the cells and microcapsules be well-distributed, microwave (1.8 W, 450 MHz) was employed to irradiate the mixture for 5 min. Record the temperature values with optical fiber probe every 10 s to monitor the temperature change of the HepG2 cells. Two control groups were designed to contain the mixture of 500 μL HepG2 cells and 500 μL pure DMEM medium, one of which was treated with microwave and another was treated without microwave. The cells were stained with Hochest33342 and Propidium Iodide (PI) to identify the living and died cells. MTT assay was performed to evaluate the cell viability after treated with microwave and the procedure was as shown in MTT assay section.