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Imaging-guided synergetic therapy of orthotopic transplantation tumor by superselectively arterial administration of microwave-induced microcapsules

Shunsong Tang [a, b, 1], Changhui Fu [a, 1], Longfei Tan [a], Tianlong Liu [a], Jingsong Mao [d], Xiangling Ren [a], Hongying Su [d], Dan Long [a], Qianqian Chai [a], Zhongbing Huang [b, **], Xudong Chen [c, ***], Jingzhuo Wang [e], Jun Ren [a], Xianwei Meng [a, *]

[a] Laboratory of Controllable Preparation and Application of Nanomaterials, CAS Key Laboratory of Cryogenics, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing, 100190, PR China
[b] College of Materials Science and Engineering, Sichuan University, Chengdu, 610065, PR China
[c] 2nd Clinical Medical College of Jinan University, Shenzhen People's Hospital, 518020, PR China
[d] Department of Radiology, First Hospital of China Medical University, Shenyang, 110001, PR China
[e] Department of Electronic Engineering, Huaihai Institute of Technology, Lianyungang, 222005, PR China

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ABSTRACT

It is an ambitious target to improve overall Hepatocellular Carcinoma therapeutic effects. Recently, MW ablation has emerged as a powerful thermal ablation technique, affording favorable survival with excellent local tumor control. To achieve better therapeutic effects of MW ablation, MW sensitizers are prepared for enhanced MW ablation to preferentially heat tumor territory. However, it is still not practicable for treatment of the orthotopic transplantation tumor. Herein, biocompatible and degradable methoxy poly(ethylene glycol)-poly(lactic-*co*-glycolic acid) (mPEG-PLGA) microcapsules with hierarchical structure have been designed for microwave-induced tumor therapy. Chemical drug doxorubicin hydrochloride (DOX·HCl), microwave (MW) sensitizers and CT imaging contrast MoS_2 nanosheets and MR imaging contrast Fe_3O_4 nanoparticles are co-incorporated into the microcapsules. *In vitro/vivo* MR/CT dual-modal imaging results prove the potential application for guiding synergetic therapy and predicting post-therapy tumor progression in the orthotopic transplantation tumor model. After blocking the tumor-feeding arteries, these microcapsules not only exclude the cooling effect by cutting off the blood flow but also enhance MW heating conversion at tumor site. The focused MW heating makes microcapsules mollescent or ruptured and releases DOX·HCl from the microcapsules, achieving the controlled release of drugs for chemical therapy. Compared with MW ablation, 29.4% increase of necrosis diameter of normal liver in rabbit is obtained under MW ablation combined with transcatheter arterial blocking, and the average size of necrosis and inhibition rate of VX-2 liver orthotopic transplantation tumor in rabbit has increased by 129.33% and 73.46%. Moreover, it is proved that the superselectively arterial administration of the as-prepared microcapsules has no recognizable toxicity on the animals. Therefore, this research provides a novel strategy for the construction of MW-induced microcapsules for orthotopic transplantation tumor ablation with the properties of MW sensitizing, superselective arterial blocking, control release and enhanced accumulation of DOX·HCl, and MR/CT dual-modal imaging, which exhibits great potential applications in the field of HCC therapy.

* Corresponding author. Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, No. 29 East Road Zhongguancun, Beijing, 100190, PR China.
** Corresponding author. No. 24, South 1st Section, 1st Ring Road, Chengdu, 610065, PR China.
*** Corresponding author. No. 1017, East Gate North Road, Luohu District, Shenzhen, Guangdong, 518020, PR China.
E-mail addresses: zbhuang@scu.edu.cn (Z. Huang), chenxudong9999@126.com (X. Chen), mengxw@mail.ipc.ac.cn (X. Meng).
[1] These authors contributed equally to this work.

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1. Introduction

Hepatocellular Carcinoma (HCC) is the second most prevalent cause of cancer-related deaths in China. Mortality from HCC in USA had increased by about 60% in 2015. Despite of continued efforts and some advances in pathogenesis and therapeutics of HCC, it is still a highly aggressive malignant tumor [1,2]. Recently, MW ablation has emerged as a powerful thermal ablation technique, affording favorable survival with excellent local tumor control in select patients [3–5]. MW ablation is prevalently used for HCC therapy in Far East countries, and increasingly utilized worldwide [6].

To achieve better therapeutic effects of MW ablation, MW sensitizers based on ion confinement mechanism were prepared for enhanced MW ablation to preferentially heat tumor territory [7,8]. The high MW-heating conversion efficacy of MW sensitizers endowed the tumor with specificity for MW irradiation, which was of great importance to localize the MW energy in the tumor site. The MW ablation enhanced by sensitizers could increase the volume of tumor necrosis, allowing the complete ablation of the marginal tumor area including microscopic metastasis adjacent to the primary tumor [9]. For example, a MW sensitive theranostic agent was prepared by a simple nanostructure of ionic liquid@ZrO_2 (IL@ZrO_2) nanoparticles [10]. The IL@ZrO_2 nanoparticles exhibited powerful tumor inhibition rate (>90%) in mice model under a very low MW irradiation of 1.8 W. Hollow polydopamine nanoparticles were also loaded with IL (IL@PDA) for enhancing the selectivity and targeting of MW ablation [11]. Both *in vivo* and *in vitro* experiments implied that the IL@PDA nanoparticles had favorable sensitization effect to tumor MW ablation. However, the above enhanced MW ablation was only achieved in the tumor therapies of subcutaneous tumor models. These sensitizers were used to ablate subcutaneous tumor model, which was far less accessing clinical human cancer than the orthotopic transplantation tumor with regard to location, onset, and histological and biological characteristics. There are plenty of blood vessels in an orthotopic transplantation tumor [12,13]. The abundant blood flow from the host organs would carry away the heat from the MW ablation zone. The obvious cooling effect had a distinctive impact on the final volume of the necrosis zone. Thus the insufficient local heating led to decrease of necrosis diameters obviously and microscopic metastasis adjacent to the primary tumor being residual. Therefore, a comprehensive solution excluding the cooling effect caused by blood flow in a simple way would be really encouraging. Based on the physiological characteristics of orthotopic transplantation tumor, a micrometer-sized microwave sensitizers which could be delivered into the artery feeding the tumor and blocked up there, was highly desirable to completely ablate the orthotopic transplantation tumor.

Following the optimal MW responsive structure simulated by a computer model, a MW-induced microcapsule was designed for MR/CT imaging-guided synergetic therapy of the orthotopic transplantation tumor, which was mPEG-PLGA microcapsule incorporated with DOX·HCl, MoS_2 nanosheets, and Fe_3O_4 nanoparticles. Firstly, it was demonstrated that the hierarchical structure endowed the as-prepared microcapsule (named as mPEG-PLGA@DMF) with high MW-heating conversion efficiency. Next, in combination with transarterial administration, the as-prepared microcapsules were delivered into the artery feeding the tumor via the femoral. It could be observed that the micrometer-sized materials allowed to superselectively blocking up the blood vessels, resulting in the reduction of heat loss caused by blood flow. The mPEG-PLGA@DMF microcapsules enables a novel tumor treatment strategy by combining enhanced MW ablation based on sensitizers, superselective arterial blocking, enhanced accumulation of DOX·HCl in tumor tissue. Importantly, since the integration of MoS_2 nanosheets and Fe_3O_4 nanoparticles in the mPEG-PLGA@DMF microcapsules was sufficient for CT imaging and MR imaging of tumor, it was found that the mPEG-PLGA@DMF microcapsules could be used for imaging-guided synergetic therapy of the orthotopic transplantation tumor. Based on these findings, the mPEG-PLGA@DMF microcapsules were recommended for treatment of the orthotopic transplantation tumor.

2. Materials and methods

2.1. Materials

The mPEG-PLGA (mPEG, M_w 1000, PLGA, lactide:glycolide 60:40, M_w 100,000) are purchased from Daigang Biomaterial Co., Ltd (Jinan, China). Polyvinyl acetate (M_w 30,000–70,000, 87–89% hydrolyzed) is purchased from Sigma Aldrich (St. Louis, MO, USA), DOX·HCl is bought from Huafeng United Technology Co., Ltd. Sodium molybdate ($Na_2MoO_4·2H_2O$) and thiourea (CH_4N_2S) are obtained from Aladdin Industrial Corporation. Ferrous chloride ($FeCl_2·4H_2O$), ferric chloride ($FeCl_3·6H_2O$) and dichloromethane (DCM) are bought from Lanyi Reagent Co., Ltd (Beijing, China). Hydrochloric acid (HCl) and aqua ammonia ($NH_3·H_2O$) are commercially available products. In this work, all agents are analytical reagents and not further purified.

Experimentation with animals was governed by the Regulations of Experimental Animals of Beijing Authority and approved by the Animal Ethics Committee of the Peking University.

2.2. Computer-simulated modal

The COMSOL software was used for the computer-simulation, in which the RF Module's Port boundary condition was employed for the wave propagation problem [7]. In the computer-simulated modal, two microcapsule modals were designed. One was a saline solution ball, which can be simulated as the situation of the spare mPEG-PLGA microcapsules. Another was a saline solution ball with 4 compartments, which can be simulated as the situation of mPEG-PLGA@DMF with hierarchical structure.

2.3. Synthesis of Fe_3O_4 nanoparticles and MoS_2 nanosheets

In our work, the method of co-precipitation of $FeCl_2$ and $FeCl_3$ in basic solution is used to prepare Fe_3O_4 nanoparticles. Briefly, appropriate amount of Fe^{2+} and Fe^{3+} with molar ratio 1:2 are dissolved in deionized water under the protection of N_2 and rigorously stirred at 80 °C. The reaction (alkalescent pH adjusted by $NH_3·H_2O$) is conducted 2 h in water bath at 80 °C, and the products are collected by centrifugation and washed 5 times with deionized water and dried in freeze dryer for 24 h. The MoS_2 nanosheets are manufactured by a facile hydrothermal route followed the procedure published previously [14]. Final washed products are dispersed into deionized water at a concentration of 5.7 mg/mL.

2.4. Preparation of microcapsules

The preparation of mPEG-PLGA@DMF microcapsules are used a double emulsion method with a slight modification. In detail, 200 mg mPEG-PLGA is accurately weighted and dissolved into 4 mL DCM as oil phase. 1 mL aqueous solution (inner water phase) containing 15 mg DOX·HCl, 5.7 mg MoS_2 and 21 mg Fe_3O_4 is emulsified with oil phase for 90 s in an ice bath using a probe type sonicator to form homogeneous primary emulsion. The emulsion is then dropped into 50 mL water (outer water phase) containing 0.1% PVA agitated by a mechanical stirrer with a sharp blade for 4 h at speed of 1200 rpm. Pure mPEG-PLGA and mPEG-PLGA@MF