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An efficient nano-based theranostic system for multi-modal imaging-guided photothermal sterilization in gastrointestinal tract

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ABSTRACT

Since understanding the healthy status of gastrointestinal tract (GI tract) is of vital importance, clinical implementation for GI tract-related disease have attracted much more attention along with the rapid development of modern medicine. Here, a multifunctional theranostic system combining X-rays/CT/photothermal/photoacoustic mapping of GI tract and imaging-guided photothermal anti-bacterial treatment is designed and constructed. PEGylated $W_{18}O_{49}$ nanosheets (PEG-$W_{18}O_{49}$) are created via a facile solvothermal method and an in situ probe-sonication approach. In terms of excellent colloidal stability, low cytotoxicity, and neglectable hemolysis of PEG-$W_{18}O_{49}$, we demonstrate the first example of high-performance four-modal imaging of GI tract by using these nanosheets as contrast agents. More importantly, due to their intrinsic absorption of NIR light, glutaraldehyde-modified PEG-$W_{18}O_{49}$ are successfully applied as fault-free targeted photothermal agents for imaging-guided killing of bacteria on a mouse infection model. Critical to pre-clinical and clinical prospects, long-term toxicity is further investigated after oral administration of these theranostic agents. These kinds of tungsten-based nanomaterials exhibit great potential as multi-modal contrast agents for directed visualization of GI tract and anti-bacterial agents for phothothermal sterilization.

1. Introduction

Gastrointestinal tract (GI tract), also called digestive tract, acts as an organ system responsible for consuming and digesting foodstuffs, absorbing nutrients, as well as expelling waste. Characterization and understanding detailed status of GI tract is of the most significant issues in assessment of patient with GI tract-related diseases [1,2]. Thereby, accurate examination with visualization of the anatomy and pathology of GI tract and efficient treatment towards various personalized discomfort are generally mandatory in diagnosis, prognosis, and therapy of these diseases.

Instead of routinely used endoscope technique, exploration of GI tract has dramatically improved by the introduction of diversified non-invasive imaging modalities such as X-ray imaging, computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound (US) [3–5]. These imaging modalities, as expected, have revolutionized the way in definition and treatment planning of GI tract-related diseases in terms of their facile imaging process, no tissue damage, and painlessness to patients. To gain adequate contrast purpose and acquire conclusive description of disease status, orally administered contrast agents for GI tract imaging are extremely essential in daily imaging manipulation. Nowadays, barium meal has been used for X-ray contrast enhancement of GI tract. However, unknown median lethal dose and incidental false positive results partially confine the practical use of barium sulphate suspension. Meanwhile, small iodinated molecules have been employed as CT contrast agents to better differentiate imaging tissue against its surrounding. Due to their lack in absorbing X-ray, large doses of iodine-based contrast agents are usually required, which may cause serious iodine hypersensitivity reaction in patients. Accordingly, synthesis of novel iodine-free GI tract contract agents with low systemic toxicity and super imaging efficacy is extremely in demand. Moreover, information achieved from single-

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modality imaging can not satisfy the higher requirement for directly visualizing GI tract due to the inherent default rooted in each imaging modality [6–10]. In attempting to solve these problems, development of multi-modal contract agents for GI tract is of great interest and highly important.

Holding a great deal of microbial populations, GI tract has been figured as a site with the most complex environment in mammalian host. GI tract infection caused by bacteria is one of the most common diseases in clinic. To cure these GI tract infections, various antibiotics have been widely used [11]. Unfortunately, high-frequency use of antibiotics has promoted the multi-drug resistance and made antibiotic therapies less efficient. To circumvent antibiotic resistance, novel antibiotics have been designed and synthesized by pharmaceutical companies. Nevertheless, costly and time-consuming process usually restricts the above manipulations. As another clinical alternative, sterilization methods including dry/moist heat, microwave, and radiation have been routinely used [12–17]. Although promising, these methods tend to bring serious damage to the biological system of host. As a result, new and significant approaches for the treatment of GI tract infections caused by bacteria would be desirable and highly useful. Meanwhile, to locate the specific position of bacteria and reduce the additional side effect, it is preferred to design a targeted theranostic platform that combining multi-modal imaging ability of GI tract and capacity of imaging-guided killing of bacteria.

Of currently available therapeutic strategies, photothermal therapy (PTT) appears to be one of the most powerful techniques by using heat generated from absorbed near-infrared (NIR) light energy [18,19]. Different from traditional chemotherapy, PTT agent can effectively light up disease-related regions via photothermal imaging, and NIR beam can be successfully focused on these regions to gain ideal treatment, simultaneously. In the past decade, numerous types of NIR-absorbing agents have been demonstrated to effectively ablate solid tumors in vivo and kill bacteria in vitro by several groups including ours [20–24]. However, the use of these theranostic agents in multi-modal imaging-guided killing of bacteria in vivo has not been achieved thus far, which is extremely significant for the treatment of GI tract infections.

Herein, we demonstrate the first time that multi-modal imaging-guided PTT affords effective sterilization on a bacterial infection model. Scheme 1 illustrates our design of this targeted theranostic platform, which contains several essential sections as follows. Via a solvothermal method and an in situ probe-sonication approach, $W_{18}O_{49}$ nanosheets with biocompatible functionalization were rationally prepared. Owing to the excellent colloidal stability, low cytotoxicity, and neglectable hemolysis, these nanosheets could be used as an X-rays/CT/photothermal/photoacoustic multi-modal contrast agent to visualize the whole GI tract. Utilizing non-toxic GTA-modified PEG-$W_{18}O_{49}$ as an impactful NIR light-absorbing agent at a low dose, multi-modal imaging-guided photothermal killing of bacteria in vivo was well performed. In addition, long-term toxicity of these $W_{18}O_{49}$ nanosheets was investigated in detail, demonstrating their potential usage in future. It is worth noting that previous studies based on gold nanorods, graphene, and polypyrroles only focused on the application of PTT agents against bacteria in vitro, whereas, multifunctional tungsten-based nanomaterials were fabricated as an efficient targeted theranostic agent, more importantly, the multi-modal mapping of GI tract and targeted treatment against bacterial infections of GI tract both in vitro and in vivo have been established in the present study.

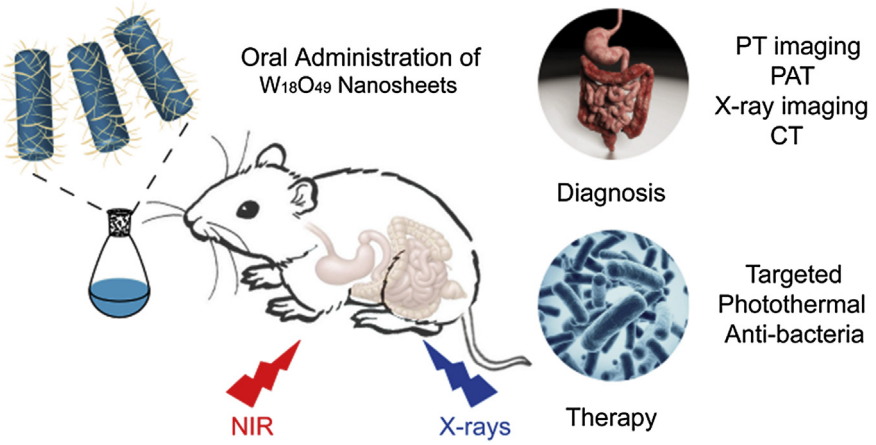
2. Materials and methods

2.1. Chemical and materials

Amino-terminated poly (ethylene glycol) (MW = 1500 Da) was purchased from Sigma–Aldrich. WCl_6, poly (ethylene glycol) (MW = 400 Da), and chloral hydrate were purchased from Aladdin Reagent. Trypsin, Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were obtained from Sangon. Other reagents and solvents were achieved from Beijing Chemicals. All chemical agents were of analytical grade and used directly without further purification. Water throughout all experiments was obtained by using a Milli-Q water system.

2.2. Preparation of PEGylated $W_{18}O_{49}$ nanosheet

PEGylated $W_{18}O_{49}$ nanosheets (denoted as PEG-$W_{18}O_{49}$) were fabricated by combining a one-pot solvothermal route and a probe-sonication method. PEGylated $W_{18}O_{49}$ nanowires were constructed at first via a solvothermal method. Typically, WCl_6 (0.25 g) was dissolved in a mixture containing 35 mL of poly (ethylene glycol) (MW = 400 Da) and 15 mL of ethanol under vigorous magnetic stirring to form a transparent yellow solution. Subsequently, above solution was transferred into a stainless Teflon-lined autoclave and kept at 180 °C for 24 h. After reaction, the resultant system was cooled down to room temperature. To achieve PEG-$W_{18}O_{49}$, an in situ probe-sonication method was applied with a period of 4 h. The



Scheme 1. Schematic illustration of the application of tungsten-based nanosheets as high-resolution targeted theranostic agents for four-modal imaging-guided photothermal sterilization.