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Single-layer tungsten oxide as intelligent photo-responsive nanoagents for permanent male sterilization

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Permanent male sterilization has been recognized as useful tools for the development of neuter experimental animals and fattening livestock, as well as efficient control of pet overpopulation. Traditional routes such as surgical ways, chemical injections, and anti-fertility vaccines have addressed these crucial problems with idea outcomes. However, these routes usually bring out serious pain and infection towards animals, as well as induce long-term adverse reaction and immune suppression. Thus, a convenient, but non-surgical strategy for male sterilization under a mild manner is highly desirable. Here, for the first time, we demonstrate a novel platform for male sterilization by using single-layer $WO_{2.72}$ nanosheets as smart photo-responsive sterilants. Upon a 980 nm irradiation, these nanoagents can possess intrinsic NIR-induced hyperthermia and sensitize the formation of singlet oxygen due to the cooperation of photothermal and photodynamic effects. Mechanism of cellular injury can be attributed to the denaturation of protein and apoptosis-related death. Moreover, long-term toxicity and possible metabolism route after testicular injection are discussed, indicating the neglectable systemic toxicity and high bio-compatibility of our nanoagents. Overall, our strategy can extremely overcome the shortcomings in various routine routes and suggest the new biological application of nanomaterials.

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1. Introduction

During the rapid evolution of modern society and human civilization, male sterilization has emerged as a vital cornerstone of efforts to neuter experimental animals, fattening livestock, and pet overpopulation [1–3]. Several methods such as surgical techniques, injectable chemical routes, and anti-fertility vaccines have exhibited more potential in addressing the crucial aspect of sterilization with ideal outcomes [4–6]. However, gonadectomy in male is quite dependent on the skill proficiency of vets and usually comprises with complicated manipulations. Moreover, injectable chemical routes and anti-fertility vaccines can induce long-term adverse reactions and immune suppression [7–9]. In this regard, a convenient strategy for male sterilization under a mild manner is highly desirable to overcome above limitations.

Because of their unique physical properties and versatile functionality, novel nanomaterials have offered unprecedented opportunities for the fast development of disease diagnosis and therapy [10–16]. Among all these investigations, a variety of nano-based photothermal (PTT) agents such as gold nanorods, carbon nanotubes, graphene, and Pd nanosheets with localized surface plasmon resonance (LSPR) have exhibited huge promise for combating cancer [17–22]. Although promising, new usage of these materials in other biological fields may be developed. As well known, hyperthermia over normal body temperature can cause short-lived or irreversible injury in spermatogenesis [23–25]. In that case, male sterilization can be well mediated via the excellent PTT effect of these nano-based PTT agents. However, several present obstacles seriously limit the practical use of these nanomaterials. Gold nanorods often suffer significant loss of near-infrared (NIR) absorbance after laser irradiation due to their "melting effect" [26–28]. Carbon nanotubes and graphene are not biodegradable and may remain inside the body for a long period after systemic administration [29,30]. In addition, the high cost and low abundance of noble-metal-based nanoparticles may restrict their commercial

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translation from lab to clinic [31]. More importantly, a higher hyperthermia around testis can result in serious scrotal tenderness and testicular irritation, bringing unwanted pain to animals and excess risk towards operators. Thus, it is still a challenge to discover an intelligent nanoagent as an efficient sterilant for male sterilization under a mild manner.

Possessing a strong LSPR from the red edge to the NIR region of light, tungsten oxide-based nanomaterials have been applied as photothermal agents against tumors both in vitro and in vivo [32–34]. In addition, $WO_{2.72}$-based nanostructures hold a much higher LSPR band gap (1.26 eV) than the energy band gap of singlet oxygen (0.97 eV) at 980 nm, thereby facilitating their energy transfer from tungsten oxide to molecular oxygen to activate singlet oxygen [35–37]. Inspired by these crucial characters, we report the first platform for male sterilization by using single-layer $WO_{2.72}$ nanosheets as intelligent photo-responsive sterilants. In our design, photothermal and photodynamic effects of these novel nanomaterials exploit their advantages to the full. Upon a 980 nm irradiation, these nanosheets can sensitize the formation of reactive oxygen species (ROS) and possess an NIR-induced hyperthermia, which cause persistent male sterilization under a mild manner. Mechanism can be ascribed to the apoptosis-induced death of Sertoli cells with excess expression of caspase proteins. Our findings identify a new usage of $WO_{2.72}$ nanosheets and provide more significant insights for versatile functions of nanomaterials.

2. Materials and methods

2.1. Chemical and materials

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 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 single-layer $WO_{2.72}$ nanosheet

PEG-$WO_{2.72}$ NSs were fabricated by combining a one-pot solvothermal route and a probe-sonication method. PEGylated $WO_{2.72}$ nanowires were constructed via a solvothermal method at first. WCl_6 (0.25 g) was dissolved in a mixture containing PEG-400 (35 mL) and ethanol (15 mL) under vigorous magnetic stirring to form a transparent yellow solution. Subsequently, above solution was transferred into stainless Teflon-lined autoclaves and kept at 180 °C for 24 h. After reaction, the resultant system was cooled down to room temperature. To achieve PEG-$WO_{2.72}$ NSs, an in situ probe-sonication route was carried out with a period of 4 h. The final materials were separated via centrifugation, collected after washing with water and ethanol in sequence, and obtained via freeze-drying overnight.

2.3. Leaching study of metal ions from PEG-$WO_{2.72}$ NSs

Solution containing PEG-$WO_{2.72}$ NSs (1 mg mL^{-1}) was taken into a dialysis bag (50 kD cut-off) and dialyzed against 0.9% NaCl solution under stirring. Taking 0.5 mL of above solution out half a month later and inductively coupled plasma mass spectrometry (ICP-MS) was used to determine the content of tungsten.

2.4. Animal administration

Kunming mice were purchased from Laboratory Animal Center of Jilin University (Changchun, China). All animal procedures were in accord with the guidelines of the Institutional Animal Care and Use Committee.

2.5. Cell cultures

Sertoli cells were harvested from two/three-week-old Kunming mice. Mice were put to death by cervical vertebra dislocation and immersed in 75% ethanol solution for several minutes. Testes were separated from the mice and cut into pieces after the removal of subsidiary tissues including blood vessels and tunica albuginea. Trypsin containing EDTA and collagenase IV were used for the preparation of cell suspension. Obtained cells were cultured in DMEM containing penicillin (100 U mL^{-1}), streptomycin (100 U mL^{-1}), and 10% FBS in a humidified incubator at 37 °C and 5% CO_2. Due to the intrinsic adherent property of Sertoli cells, hypotonic solution was applied to purify Sertoli cells by discarding other cell suspension. Purified cells were harvested by trypsin and were re-suspended in medium before plating.

2.6. Cytotoxicity studies

Sertoli cells were cultured in a 96-well plate as a density of 5×10^3 per well for 12 h to allow the cells to attach. Solutions containing PEG-$WO_{2.72}$ NSs with different concentrations were added to the culture medium. After incubation for 24 h, the residual PEG-$WO_{2.72}$ NSs in the medium were removed. Cells were treated with MTT for another 4 h, which was followed by the addition of dimethyl sulfoxide (DMSO) to dissolve the formazan crystals. Bio-Rad model-680 microplate reader was used to measure the absorbance at a wavelength of 570 nm. The absorbance of PEG-$WO_{2.72}$ with different concentrations was measured as the deductible background. Six replicates were done for each treatment group and percent viability was normalized to the cell viability in the absence of PEG-$WO_{2.72}$ NSs.

2.7. Cellular modality and viability observation

Sertoli cells with a density of 1×10^4 were plated in a 6-well plate for 12 h to allow the cells to attach. After the cells were washed twice by 0.9% NaCl solution, PEG-$WO_{2.72}$ NSs with different concentrations were added to the cell culture medium. After incubation for 24 h, cells were washed with 0.9% NaCl solution to remove the remaining nanosheets. Then, cells were stained with calcein AM and propidium iodide (PI) to confirm the visualized viability. Fluorescence images were collected on an Olympus BX-51 optical system.

2.8. Hemolysis assay

Human blood stabilized by EDTA was obtained from the local hospital. Blood (1 mL) was added to 0.9% NaCl solution (2 mL), and then red blood cells (RBCs) were isolated from serum by centrifugation at 8×10^3 rpm for 10 min. After being washed several times with 0.9% NaCl solution, the purified blood was diluted to 1/10 of its volume with 0.9% NaCl solution. Diluted RBC suspension (0.2 mL) was then mixed with (a) 0.9% NaCl solution (0.8 mL) as a negative control, (b) water (0.8 mL) as a positive control, and (c) solutions containing PEG-$WO_{2.72}$ NSs (0.8 mL). All the mixtures were vortexed and kept at room temperature for 3 h. The mixtures were centrifuged at 8×10^3 rpm for 5 min. The absorbance of supernatants at 541 nm was determined by a UV–vis spectroscopy. The percent hemolysis of RBCs was calculated as following: percent hemolysis = [(sample absorbance − negative control absorbance)/(positive control absorbance − negative control absorbance)] × 100.

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