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Nanocomposite hydrogel incorporating gold nanorods and paclitaxel-loaded chitosan micelles for combination photothermal–chemotherapy

Nan Zhang, Xuefan Xu, Xue Zhang, Ding Qu, Lingjing Xue*, Ran Mo, Can Zhang*

State Key Laboratory of Natural Medicines and Jiangsu Key Laboratory of Drug Discovery for Metabolic Diseases, Center of Drug Discovery, China Pharmaceutical University, Nanjing 210009, China

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ABSTRACT

Development of combination photothermal–chemotherapy platform is of great interest for enhancing antitumor efficacy and inhibiting tumor recurrence, which supports selective and dose-controlled delivery of heat and anticancer drugs to tumor. Here, an injectable nanocomposite hydrogel incorporating PEGylated gold nanorods (GNRs) and paclitaxel-loaded chitosan polymeric micelles (PTX-M) is developed in pursuit of improved local tumor control. After intratumoral injection, both GNRs and PTX-M can be simultaneously delivered and immobilized in the tumor tissue by the thermo-sensitive hydrogel matrix. Exposure to the laser irradiation induces the GNR-mediated photothermal damage confined to the tumor with sparing the surrounding normal tissue. Synergistically, the co-delivered PTX-M shows prolonged tumor retention with the sustained release of anticancer drug to efficiently kill the residual tumor cells that evade the photothermal ablation due to the heterogeneous heating in the tumor region. This combination photothermal–chemotherapy presents superior effects on suppressing the tumor recurrence and prolonging the survival in the Heps-bearing mice, compared to the photothermal therapy alone.

1. Introduction

Nanotechnology-based photothermal ablation (PTA) has been intensively explored as a minimally invasive treatment for solid tumors in recent years. This technique using the photothermal nanostructures, such as gold nanoshells, gold nanorods (GNRs), gold nanocages and carbon nanotubes as strong optical absorbers to convert harmless near infrared (NIR) light into localized heat, holds great promise to achieve selective and confined thermal damage within tumor while minimizing damage to the surrounding healthy tissue and preserving critical structures (Huang et al., 2008; Qin and Bischof, 2012; Shanmugam et al., 2014; Thakor and Gambhir, 2013). The gold nanoshells mediated PTA to treat refractory head and neck cancer is currently undergoing in clinical trial (Singh and Torti, 2013). GNRs have also received considerable attention due to their efficient large-scale synthesis, facile bioconjugation, strong and tunable plasmonic absorption (Chen et al., 2013a; Dickerson et al., 2008; Huang et al., 2009; Vigderman et al., 2012; von Maltzahn et al., 2009). The efficacy of tumor-specific thermal damage is highly dependent on the tumor-specific accumulation and localization of photothermal nanostructures following intravenous or local administration (Kennedy et al., 2011; Shenoi et al., 2011; Soni et al., 2014; Xu et al., 2013). Intravenously injected photothermal nanoparticles are known to be preferentially delivered to tumor *via* passive or active targeting mechanism, but their dynamic concentration and distribution in tumor fluctuate in terms of various factors associated with particle characteristics and physiological barriers, posing problems in nanoparticle dosimetry to obtain a reproducible heating profile in PTA (Alkilany et al., 2012; Chauhan et al., 2011; Khlebtsov and Dykman, 2011; Wicki et al., 2015). Local administration affords preferable nanoparticle dose control for PTA, but may be compromised by the rapid diffusion and distant migration of photothermal nanostructures away from tumor, inability to distribute throughout the whole tumor due to pressure gradient and matrix constraint within tumor (Le Renard et al., 2010; Meenach et al., 2010; Mooney et al., 2014; Redolfi Riva et al., 2014). A single dose of PTA may be difficult to achieve complete ablation due to the heterogeneous heat distribution caused by uneven distribution of photothermal nanostructures, the gradual attenuation of NIR light energy as it travels deeper into tissue, highly

* Corresponding authors. Fax: +86 25 83271171.
E-mail addresses: xuelingjing65@163.com (L. Xue), zhangcan@cpu.edu.cn (C. Zhang).

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variable local tissue factors, including location, geometries of tumor, optical and thermal properties of tissue, and heat sink effect (Bhatia et al., 2010; Lu et al., 2011; Oliva et al., 2015; Ren et al., 2013).

The potential risk of incomplete photothermal ablation often results in local tumor recurrence, which requires an alternative therapeutic intervention to achieve the completeness of tumor ablation. The combination of thermal ablation with chemotherapy that is increasingly adopted in the experimental and clinical levels has been demonstrated able to increase intratumoral drug accumulation, tumor destruction volume within the peripheral zone of sub-lethal temperature, and improve antitumor efficacy (Ahmed et al., 2012; Issels, 2008; Li et al., 2014; Sun et al., 2014). In addition, a sustained and localized chemotherapy offers many advantages of allowing accurate dosage and one-time administration, facilitating efficient delivery of anticancer agents, reducing the systemic toxicity, prolonging drug exposure, and ensuring more cytotoxic to tumors cells over multiple cell cycles (Mo et al., 2014; Weinberg et al., 2008; Wolinsky et al., 2012).

Herein, we report an injectable hydrogel incorporating photothermal nanostructures and chemotherapeutics for combined PTA and localized chemotherapy to achieve controlled and selective thermal tumor damage with reduced the risk of local recurrence and systemic cytotoxicity (Fig. 1). This nanocomposite hydrogel (designated as PTX/GNR/gel) co-encapsulates PEGylated (PEG = polyethylene glycol) GNRs and paclitaxel (PTX)-loaded chitosan polymeric micelles (PTX-M) in a thermal-reversible Poly(F127) hydrogel matrix, in which both of the PEGylated GNRs and PTX-M could be homogeneously mixed on a demand basis. The PEGylated GNRs with the superior colloidal stability serve as photothermal transducer to convert the NIR light energy into heat to destroy tumor. The strength of the heat can be readily tailored by adjusting the amount of the PEGylated GNRs and the power intensity of the NIR laser. A synthetic amphiphilic chitosan derivative is used to load PTX, a model chemotherapeutic for treatment of many types of cancer, and then incorporated into the hydrogel matrix, which can prevent the precipitation of PTX that causes a poor dose control and uneven drug distribution in the matrix. The polymeric hydrogel matrix also renders a longer retention capacity of PTX-M at the tumor site, compared to the flowable solution without the hydrogel support. After intratumoral injection, PTX-M/GNR/gel is immobilized in the tumor region due to the thermal gelling properties of the Poly(F127) polymer. Under NIR laser irradiation, the heat generated by the GNR-mediated photothermal conversion was confined within tumor. Following the photothermal treatment, PTX-M/GNR/gel as a localized drug depot could liberate PTX-M into the tumor tissue, which plays an important role in eradicating the potential remaining viable tumor cells, thereby achieving an improved completeness of tumor destruction. Such a synergistic effect of photothermal–chemotherapy would finally contribute to a reduction and delay of tumor recurrence.

2. Material and methods

2.1. Materials

Cetyltrimethyl ammonium bromide (CTAB) was purchased from NanShi Chemical Reagent Co., Ltd. (Jiangsu, China). Tetrachloroaurate (III) acid hydrate ($HAuCl_4 \cdot 4H_2O$) were purchased from Sinopharma Group Co., Ltd. (Shanghai, China). mPEG-SH (MW 5 kDa) was purchased from Sinopeg Biotech Co., Ltd. (Xiamen, China). Chitosan with deacetylation degree of 92% and viscosity average molecular weight of 70 KDa was purchased from Shuangling Biochemical Co., Ltd. (Nantong, China). PTX was purchased from Yew Pharmaceutical Company Ltd. (Jiangsu, China). F127 was provided by Badische Anilin and Soda-Fabrik (BASF). Stannous 2-ethyl-hexanoate ($SnOct_2$) and hexamethylene diisocyanate (HDI) were purchased from Aladdin Reagent Company (Shanghai, China).

2.2. Synthesis and characterization of PEGylated GNRs

CTAB-stabilized GNRs (CTAB-GNRs) were synthesized using a seed-mediated silver ion-assisted growth method (Ni et al., 2008). PEGylation of GNRs was performed as previous described (Liao and Hafner, 2005). In brief, mPEG-SH was added into a concentrated CTAB-GNRs solution to a final concentration of 10 mM, and the pH of the mixture was adjusted to 8.5 by K_2CO_3 (0.1 M). The mixture was sonicated for 4 h and incubated overnight, followed by two cycles of centrifugation–redispersion in deionized (DI) water.

The absorption spectra of GNRs were determined using a spectrophotometer (UV-5300, Metash, China), and the dimensions

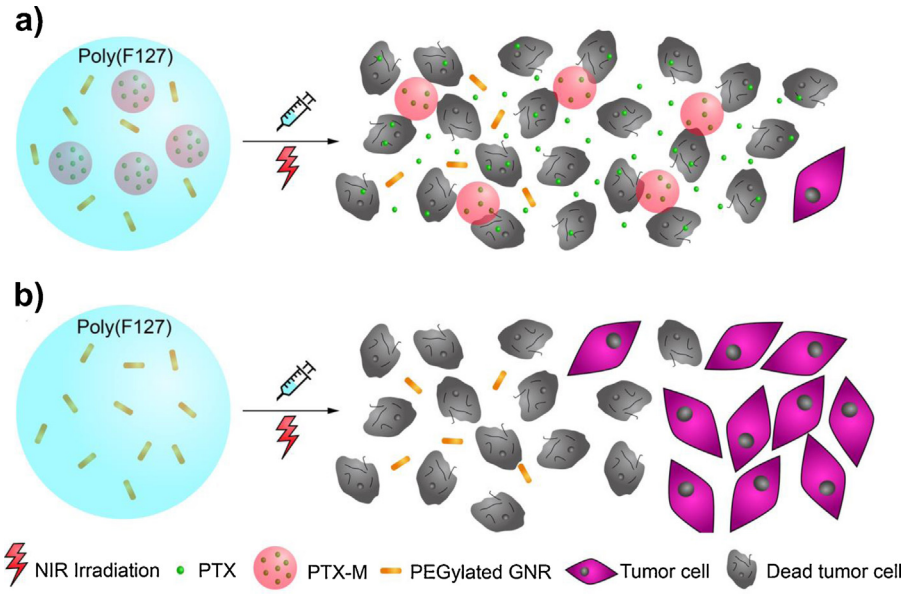

Fig. 1. Schematic design of the PTX-M/GNR/gel mediated photothermal–chemotherapy (a) for enhancing antitumor efficacy and inhibiting tumor recurrence compared to the GNR/gel mediated photothermal ablation alone (b).