International Journal of Pharmaceutics 515 (2016) 729–739

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Depot system for controlled release of gold nanoparticles with precise intratumoral placement by permanent brachytherapy seed implantation (PSI) techniques

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ARTICLE INFO

Article history:
Received 29 August 2016
Received in revised form 28 October 2016
Accepted 1 November 2016
Available online 3 November 2016

Keywords:
Nanoparticle depot
Gold nanoparticles
Calcium alginate
Tissue-equivalent phantom
Tumor xenograft
Diffusion

ABSTRACT

We report the design of a nanoparticle depot (NPD) system for local delivery of gold nanoparticles (AuNP) that facilitates their controlled release and is implantable into tumors by permanent seed implantation (PSI) brachytherapy techniques. Various sizes (5, 15, 30, and 50 nm) of polyethylene glycol (PEG) coated AuNP and concentrations (6%, 8%, and 10% w/v) of calcium alginate used to form the NPD were studied. AuNP release rate, diffusion characteristics and spatial distribution were characterized in a tissue equivalent phantom model, and in a breast cancer tumor xenograft model and compared to a Fickian diffusion computational model, to identify the optimal NPD composition. In phantoms, 5 nm and 15 nm AuNP were released more rapidly than 30 nm or 50 nm AuNP but when implanted into tumor xenografts, AuNP exhibited slower release from NPD. Controlled prolonged release of AuNP was observed in tumor tissue over durations which were dependent on AuNP size. Maximum release and distribution in tumors were achieved using 5 nm AuNP incorporated into the NPD. These results demonstrate the potential for the NPD as an effective local delivery system for AuNP-based therapies.

1. Introduction

Gold nanoparticles (AuNP) are receiving considerable attention for delivery of chemotherapeutic drugs (Liang et al., 2014), radionuclides (Vilchis-Juarez et al., 2014; Yook et al., 2016) and photosensitizing drugs (Zhang et al., 2015) to tumors for cancer treatment. Furthermore, their ability to convert light to heat has led to interest in their application for photothermal therapy (Bao et al., 2016; Liu et al., 2016; Pekkanen et al., 2014) and their conversion of X-rays to more radiobiologically effective Auger and photoelectrons has generated interest in their use as sensitizers for radiation treatment of cancer (Chattopadhyay et al., 2013; Hainfeld et al., 2004; Huang et al., 2011). In almost all studies, AuNP were administered systemically by intravenous (i.v.) injection, based on extravasation of AuNP into tumors and their retenton by the enhanced permeability and retention (EPR) effect (England et al., 2015). In some studies, AuNP were surface-modified with targeting ligands such as monoclonal antibodies or peptides to promote active uptake into tumors (Llevot and Astruc, 2012). Nonetheless, there remain significant challenges to the effective delivery of AuNP to tumors after i.v. injection, since they are avidly recognized by the mononuclear phagocyte system (MPS) which causes sequestration by the liver and spleen (Zhang et al., 2016). Surface coating of AuNP with polyethylene glycol (PEG) chains minimizes MPS recognition and reduces liver and spleen uptake (Arnida et al., 2011). However, conjugation to targeting ligands may enhance MPS recognition resulting in diminished tumor uptake. For example, we previously reported that 30 nm diameter ^{111}In-labeled AuNP modified with trastuzumab to target subcutaneous

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http://dx.doi.org/10.1016/j.ijpharm.2016.11.001
0378-5173/

(s.c.) HER2-positive MDA-MB-361 human breast cancer (BC) xenografts in athymic mice exhibited 2-fold greater spleen uptake after i.v. injection than unmodified ^{111}In-AuNP [19.2 *vs.* 10.0 percent injected dose/g (% ID/g)] and tumor uptake was reduced compared to unmodified ^{111}In-AuNP by 2-fold (1.2 *vs.* 2.2% ID/g) (Chattopadhyay et al., 2012). A recent article reviewed the delivery to tumors of a variety of nanoparticles including AuNP, by analysing data from 117 publications and concluded that a median of <1% of systemically administered nanoparticles were taken up by tumors in mouse xenograft models (Wilhelm et al., 2016).

One strategy to overcome these limitations of i.v. injected AuNP may be intratumoral (i.t.) injection, particularly for tumors that are readily accessible, such as early stage BC which is confined mainly to the breast. We found that i.t. injected and trastuzumab-modified ^{111}In-AuNP yielded 25-fold higher tumor radioactivity (29.6% ID/g) than i.v. injected ^{111}In-AuNP and 10-fold lower spleen uptake (1.8% ID/g) and 1.7-fold lower liver accumulation (1.6% ID/g) (Chattopadhyay et al., 2012). More recently, we reported that very high tumor concentrations (>200% ID/g) were achieved for i.t. injected panitumumab-modified ^{177}Lu-labeled AuNP. These ^{177}Lu-AuNP deposited high radiation doses in s.c epidermal growth factor receptor (EGFR)-positive MDA-MB-468 human BC xenografts (>30 Gy) in athymic mice which arrested tumor growth (Yook et al., 2016). Moreover, the retention of ^{177}Lu-AuNP in tumors minimized redistribution to normal organs resulting in low radiation doses (<1 Gy) that caused no normal tissue toxicity. These results are very promising for maximizing the radiotherapeutic effects of ^{177}Lu-AuNP on tumors while minimizing their effects on normal tissues. However, a practical obstacle to advancing this approach to human studies is the feasibility in precisely positioning AuNP in tumors by i.t. injection to obtain predictable radioactivity distribution and dose deposition, especially since patient tumors are much larger than the mouse tumor xenografts previously studied. Treatment of tumors in patients would require multiple i.t. injections that need to be spatially distributed with high accuracy in order to minimize regional heterogeneities in the radiation doses deposited in the tumor.

To address this challenge, we report here the design of a novel nanoparticle depot (NPD) system composed of a porous calcium alginate reservoir into which AuNP may be loaded, and which enables their controlled local release and diffusion in tumor tissues. The NPD were designed to have the same dimensions as brachytherapy seeds that are routinely used in patients for local radiation treatment of tumors, so that they can be precisely positioned into a tumor using the pre-loaded needle technique used for permanent seed implantation (PSI) (Hepel and Wazer, 2012). PSI brachytherapy techniques involve careful insertion of multiple radioactive seeds that are preloaded into seeding needles and guided into the tissue using a template system to assure precise placement. The template allows selection of the insertion depth in tissue and the distance between adjacent seeds such that seed positioning is accurate to within a few millimeters (Morton et al., 2016; Pignol and Keller, 2009). Adapting the PSI technique for intra-tumoral placement of ^{177}Lu-AuNP would render the approach practical for tumor treatment in BC patients. Moreover, the approach could potentially be extended to local treatment of other cancers for which brachytherapy is currently used (*e.g.* prostate cancer) (Nicolae et al., 2016). We studied the release of AuNP from the NPD as a function of calcium alginate concentration and AuNP size using an *in vitro* tissue-equivalent phantom model, by modeling the release by Fick's diffusion law, and experimentally *in vivo* in a BC tumor xenograft mouse model with the NPD inserted by PSI techniques (Fig. 1). To our knowledge, this report is the first to describe local delivery of AuNP in a tumor using a NPD system inserted by PSI techniques.

2. Material and methods

2.1. Gold nanoparticle PEGylation

AuNP (5, 15, 30 and 50 nm; Ted Pella Inc., Redding, CA, USA) were PEGylated for 24 h at 4 °C by incubation with 0.235 g of mercaptopolyethylene glycol monomethyl ether (MeO-PEG-SH) (2 kDa; IRIS BioTech, Marktredwitz, Germany) in double distilled water (d.d. H_2O) per 500 mL of AuNP stock solution. The mean

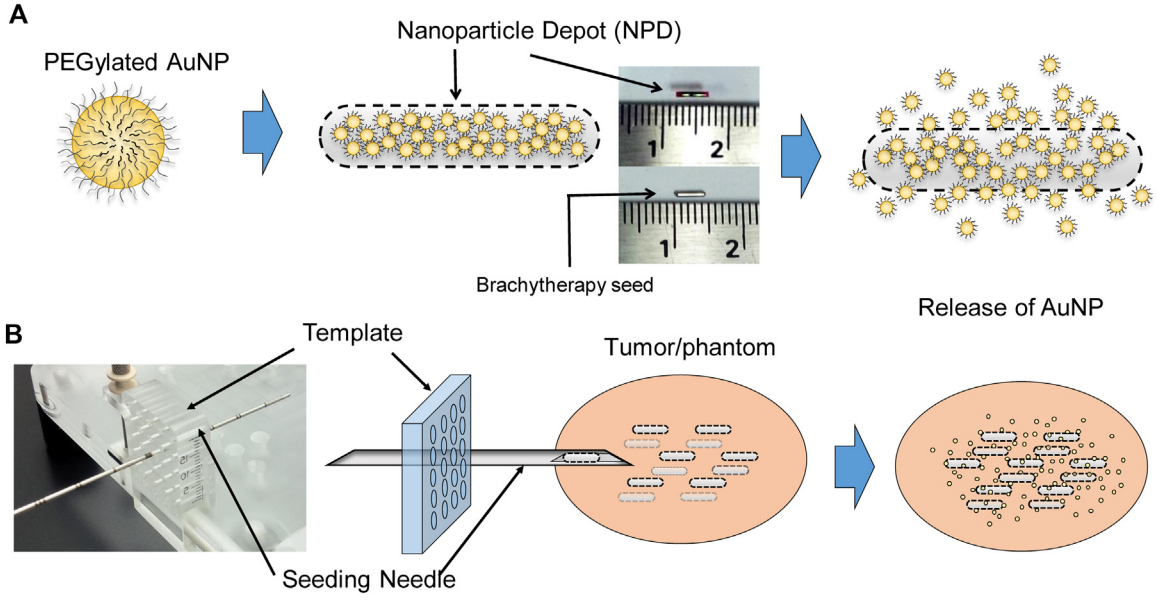

Fig. 1. (a) Conceptual illustration of a NPD loaded with PEGylated AuNP and release of AuNP from the NPD. A comparison of the dimensions of a NPD with those of a conventional titanium shell permanent brachytherapy seed is also shown. (b) The NPD was loaded in a 18 G seeding needle and deposited into a tissue-equivalent phantom or tumor xenograft in an athymic mouse using the template as a positioning guide. Once implanted, the NPD released AuNP into the surrounding phantom matrix or tumor tissue.