Journal of Chromatography A, 1402 (2015) 1–7

Contents lists available at ScienceDirect

Journal of Chromatography A

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

# Online open-tubular fractionation scheme coupled with push–pull perfusion sampling for profiling extravasation of gold nanoparticles in a mouse tumor model

Cheng-Kuan Su, Po-Jen Tseng, Meng-Han Lin, Hsien-Ting Chiu, Andrea del Vall, Yu-Fen Huang, Yuh-Chang Sun*

*Department of Biomedical Engineering and Environmental Sciences, National Tsing-Hua University, 30013 Hsinchu, Taiwan*

ARTICLE INFO

*Article history:*
Received 8 February 2015
Received in revised form 20 April 2015
Accepted 6 May 2015
Available online 12 May 2015

*Keywords:*
Extravasation
Fractionation
Gold nanoparticle
Push–pull perfusion
Tumor extracellular fluid

ABSTRACT

The extravasation of administered nano-drug carriers is a critical process for determining their distributions in target and non-target organs, as well as their pharmaceutical efficacies and side effects. To evaluate the extravasation behavior of gold nanoparticles (AuNPs), currently the most popular drug delivery system, in a mouse tumor model, in this study we employed push–pull perfusion (PPP) as a means of continuously sampling tumor extracellular AuNPs. To facilitate quantification of the extravasated AuNPs through inductively coupled plasma mass spectrometry, we also developed a novel online open-tubular fractionation scheme to allow interference-free determination of the sampled extracellular AuNPs from the coexisting biological matrix. After optimizing the flow-through volume and flow rate of this proposed fractionation scheme, we found that (i) the system's temporal resolution was 7.5 $h^{-1}$, (ii) the stability presented by the coefficient of variation was less than 10% (6-h continuous measurement), and (iii) the detection limits for the administered AuNPs were in the range 0.057–0.068 $\mu g\,L^{-1}$. Following an intravenous dosage of AuNPs (0.3 mg $kg^{-1}$ body weight), in vivo acquired profiles indicated that the pegylated AuNPs (PEG-AuNPs) had greater tendency toward extravasating into the tumor extracellular space. We also observed that the accumulation of nanoparticles in the whole tumor tissues was higher for PEG-AuNPs than for non-pegylated ones. Overall, pegylation appears to promote the extravasation and accumulation of AuNPs for nano-drug delivery applications.



## 1. Introduction

Gold nanoparticles (AuNPs), thanks to their diverse range of surface functionalization, unique optical properties, and high biocompatibility, are among the most popular metal-based drug delivery systems for cancer treatment applications [1–7]. After delivery through either active or passive targeting strategies and then evading clearance by the mononuclear phagocyte system (MPS), the administered AuNPs may (i) extravasate through its large vascular pores and into the tumor extracellular space, (ii) become lodged as a result of diminished lymphatic drainage and low diffusivity, or (iii) penetrate the dense extracellular matrix to induce pharmacological effects [8–11]. Extravasation, one of the most critical transportation steps, of AuNPs through the enlarged endothelial gaps into the tumor extracellular space would have a strong impact on their consequent biodistribution pattern and treatment efficacy [10–13]. Hence, a better understanding of the extravasation phenomena of administered AuNPs across these physiological barriers, as well as improvements in correlating kinetic extravasation profiles with biodistribution data, will contribute to provide more effective and selective drug delivery platforms.

Unfortunately, conventional strategies for examining the time-dependent accumulation of administered AuNPs in tumor tissue involve (i) determining total Au concentrations in digested tumor tissues using conventional elemental analysis equipment or (ii) measuring the photon emissions from labeled fluorescent dyes or radioactive tracers on particles [14]; both approaches focus on assessment or semi-quantification of the total Au content deposited in the entirety of the tumor tissues. Intrinsically limited by these

* Corresponding author. Tel.: +886 3 5727309; fax: +886 3 5723883.
*E-mail address:* ycsun@mx.nthu.edu.tw (Y.-C. Sun).

http://dx.doi.org/10.1016/j.chroma.2015.05.008
0021-9673/

methods' spatial resolution, only intravital imaging techniques (e.g., confocal microscopy and two-photon excitation microscopy) have enabled the direct and dynamic observation of the extravasation of fluorescently labeled nano-sized materials (NMs) and their detailed distributions within biological compartments (e.g., blood stream, extracellular fluid, and association with cells) [15–17]; in contrast, quantitative descriptions of extravasated AuNPs in tumor extracellular space have remained impossible, even after the resolution of these image-based techniques has been improved down to the subcellular scale.

Push–pull perfusion (PPP) sampling has the attractive feature of allowing continuous acquisition of extracellular substances in vivo without the restriction of molecular weight cut-off (MWCO) [18–20]; furthermore, coupling with elemental analysis using, for example, atomic absorption spectrometry or inductively coupled plasma mass spectrometry (ICP-MS) can allow not only quantification of extracellular NMs sampled from living subjects but also measurements of their transfer kinetics among the blood stream, the extracellular compartments, and the cellular interactions. Nevertheless, the contents of samples acquired from PPP sampling are extremely complicated because the many dissolved inorganic salts and biomolecules in the extracellular fluid are sampled simultaneously through the configuration of an open-tubular PPP sampling cannula [19,20]. Effectively eliminating the complicated matrices from the samples from PPP sampling while retaining the sampled NMs prior to its hyphenation with the desired detection system will be a prerequisite for quantitation of extravasated NMs.

Based on current scientific reports [21,22], the online sample pretreatment methods available to isolate AuNPs from aqueous solutions are primarily size-based discrimination schemes [e.g., diafiltration, field-flow fractionation (FFF), and liquid chromatography] [23–27], although selective chemical extraction methods also allow the direct extraction of AuNPs from complex aqueous media [28–30]. Nevertheless, few of these approaches can be used for practical online pretreatment of biological samples containing administered AuNPs and complicated biological matrices—such AuNPs would be covered by a thick layer of protein corona, causing them to exhibit physicochemical properties completely different from those of pristine AuNPs [31–33]. In addition, because the perfusion flow rates of PPP sampling are usually in the range of several microliters per minute, there is a need to develop more suitable online sample pretreatment schemes for handling PPP samples having sample volumes in the range of several tens of microliters and for separating the administered AuNPs from coexisting biological matrices to facilitate online profiling of their extravasation in living animals.

To satisfy the requirements of quantitative in vivo monitoring of AuNPs, in this study we developed a new sample pretreatment scheme—employing a piece of hollow PFTE tubing without any surface functionalization and packing materials as a simple fractionation column with the water carrier stream inside as a mobile phase—for separating AuNPs from high-salt-content matrices. This experimental device and its design are simpler than those of flow FFF devices, because neither a flow across liquid nor a liquid filtration membrane for the fractionation channel is necessary, dramatically decreasing the overall experimental complexity. Furthermore, because of its open-tubular configuration, the liquids within the manifold can also be driven by a conventional peristaltic pump, rather than using a high-pressure pump. After optimizing this proposed open-tubular chromatographic fractionation scheme and the PPP sampling process, we demonstrated the system's applicability through online monitoring of the concentrations of tumor extracellular AuNPs in living mice and associating the extravasation profiles to their total tumor accumulations.

## 2. Materials and methods

### 2.1. Chemicals

50-nm pegylated (notated as PEG-AuNPs, 765686; OD 50, methyl terminated, $PEG_{5000}$ coated, dispersion in water) and 50-nm non-pegylated AuNPs (notated as AuNPs, 753645; OD 1, stabilized suspension in 0.1 mM PBS), and phosphate-buffered saline (PBS; P3813) were purchased from Sigma–Aldrich (St. Louis, USA). Dulbecco's modified Eagle's medium (DMEM; 11965-092), fetal bovine serum (FBS; 16000-044), and penicillin-streptomycin (15140-122; 10,000 U $mL^{-1}$) were purchased from Life Technologies (Carlsbad, CA, USA). A gold ion stock solution (1000 mg $L^{-1}$), nitric acid ($HNO_3$; 9598-34), and hydrochloric acid (HCl; 9530-33) were purchased from J. T. Baker (NJ, USA).

### 2.2. AuNP characterization and preparation

10% FBS/DMEM solution was selected as the primary dispersion medium to avoid aggregation of AuNPs before and immediately after their administration into living mice. For imaging through transmission electron microscopy (TEM), the stock AuNP solutions were diluted using 10% FBS/DMEM solution, and then one drop of the diluted sample was deposited and dried on a formvar-coated copper grid. Imaging was performed using a Hitachi HT7700 transmission electron microscope operated at an acceleration voltage of 80 kV. Analysis of the size distribution of the AuNPs was performed using ImageJ software (v. 1.46) downloaded from the NIH website (http://rsbweb.nih.gov/ij/index.html). Their hydrodynamic diameters (HDs) before and after dispersion in 10% FBS/DMEM were also measured using dynamic light scattering (DLS; Zetasizer NanoZS, Malvern Instruments, Worcestershire, UK). The Au concentrations in the purchased suspensions were determined through ICP-MS after their complete dissolution in aqua regia.

### 2.3. Apparatus and methods

A concentric PPP probe was acquired after removing the membrane from the tip of a CMA 20 microdialysis probe (CMA Microdialysis, Solna, Sweden), which featured an inner withdraw polyimide tube (o.d./i.d., 380/280 μm) and an outer infusion polyurethane tube (o.d./i.d., 770/580 μm). Prior to performing daily experiments, the push and pull flow rates were adjusted using two identical peristaltic microdialysis pumps (MAB 20, Microbiotech/se AB, Stockholm, Sweden) to precisely control the withdrawal flow rate such that it was equal to the infusion flow rate. A PBS solution containing heparin sodium (20 IU $mL^{-1}$; B. Braun, Melsungen AG, Germany) was infused into the desired tumor region; simultaneously, a sample was collected through the pull cannula, online diluted with 10% FBS/DMEM (10-fold), and loaded into a piece of the hollow PFTE tubing (49 cm long × 0.02 inch i.d.; Alltech, Virginia, USA; inner volume: 100 μL) through manipulation of a six-port valve (C22Z-2186, Valco, Lucerne, Switzerland). The sampled AuNPs and the sample matrix were then online fractionated in the loaded PTFE tubing using a water carrier stream driven by a peristaltic pump (Miniplus 3, Gilson, USA). As the coexisting high-salt-content matrix was fractionated, the residual AuNPs were eluted, using a solution of 0.5% $HNO_3$/0.5% HCl, into an Agilent 7500a ICP mass spectrometer (Agilent Technologies, CA, USA) through a Micromist nebulizer (AR35-1-EM04EX, Glass Expansion, Victoria, Australia) for time-resolved scanning at $m/z$ 197. The overall configuration of this sample pretreatment system is presented in Fig. 1; it comprised two six-port valves and an ten-port valve (C22Z-3180, Valco), all of which were programmed and controlled by a personal computer through a serial valve interface (SIV-110, Valco). The operational sequence of the online pretreatment system is provided