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Indium-111 labeled gold nanoparticles for *in-vivo* molecular targeting

Quinn K.T. Ng [a,b,1], Cristina I. Olariu [a,b,1], Marcus Yaffee [a], Vincent F. Taelman [a,b], Nicolas Marincek [a], Thomas Krause [a,b], Lorenz Meier [a,b], Martin A. Walter [a,b,c,*]

[a] Institute of Nuclear Medicine, University Hospital Bern, Bern 3010-CH, Switzerland
[b] Department of Clinical Research, University Bern, Bern 3010-CH, Switzerland
[c] Molecular and Medical Pharmacology, David Geffen School of Medicine, UCLA, Los Angeles, CA 90095-1722, USA

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ABSTRACT

The present report describes the synthesis and biological evaluation of a molecular imaging platform based on gold nanoparticles directly labeled with indium-111. The direct labeling approach facilitated radiolabeling with high activities while maintaining excellent stability within the biological environment. The resulting imaging platform exhibited low interference of the radiolabel with targeting molecules, which is highly desirable for *in-vivo* probe tracking and molecular targeted tumor imaging. The indium-111 labeled gold nanoparticles were synthesized using a simple procedure that allowed stable labeling of the nanoparticle core with various indium-111 activities. Subsequent surface modification of the particle cores with RGD-based ligands at various densities allowed for molecular targeting of the $\alpha_v\beta_3$ integrin *in-vitro* and for molecular targeted imaging in human melanoma and glioblastoma models *in-vivo*. The results demonstrate the vast potential of direct labeling with radioisotopes for tracking gold nanoparticles within biological systems.

1. Introduction

Radioisotopes are increasingly used in biomedical applications to help understanding of biological and pathological processes unattainable by other means [1,2]. For example, microdosing studies with radiolabeled drugs allow quantitative determination of the pharmacokinetics, drug metabolism and drug–drug interactions in humans, using doses below pharmacological concentration and prior to *phase I* clinical studies [3]. Additionally, tracking studies with radiolabeled cells facilitate monitoring of their migration and functionality, and provide paramount data for optimizing cell-based diagnosis and therapy [4,5].

Today, numerous radiotracers are clinically used [6], and the growing demand for imaging specific biological processes will further increase the development of new tracers. The efficiency of long-term imaging radiotracers depends on the optimization of several factors which include achieving high labeling yield, high labeling stability and low interference with the biological properties of the labeled molecule. However, few of the currently used clinical radiotracers fulfill these criteria.

Common radiolabeling techniques such as conjugation of tyrosine residues with iodine-123 [7], fluorination with fluorine-18 [8] and complexation with metal chelators [9] allow labeling with a single radioactive atom. Yet, actual conjugation and complexation ratios are commonly lower than one atom per molecule. Furthermore, complexation with radiometals can require harsh reaction conditions and has been shown to interfere with the biological properties of the labeled molecules [10]. Moreover, iodination [11] and complexation [12] are reversible, which limits quantitative imaging over long time periods. Consequently, clinical imaging with radioisotopes is mostly restricted to a few hours. To extend this window, imaging platforms that allow labeling of high activities at high labeling stability and low interference with its biological properties are highly desired. The present report describes the design, synthesis and biological evaluation of such an imaging tool.

An indium-111 labeled gold nanoparticle platform, modified with the tumor targeting sequence arginine-glycine-aspartate (RGD, Fig. 1A) was developed and utilized for tumor cell targeting *in-vitro* and *in-vivo*. Particles were designed to be injected intravenously, travel to the tumor cell (Fig. 1B), internalize via $\alpha_v\beta_3$ integrin mediated endocytosis (Fig. 1C) and allow imaging via subsequent gamma emission (Fig. 1D).

Gold nanoparticles were selected for use as platform cores due to their simple and efficient surface modification and reported use

* Corresponding author. University Hospital, 3010 Bern, Switzerland. Tel.: +41 31 63 23542; fax: +41 31 63 23137.
E-mail address: m.a.walter@gmx.net (M.A. Walter).
[1] QKTN and CIO contributed equally to the manuscript.

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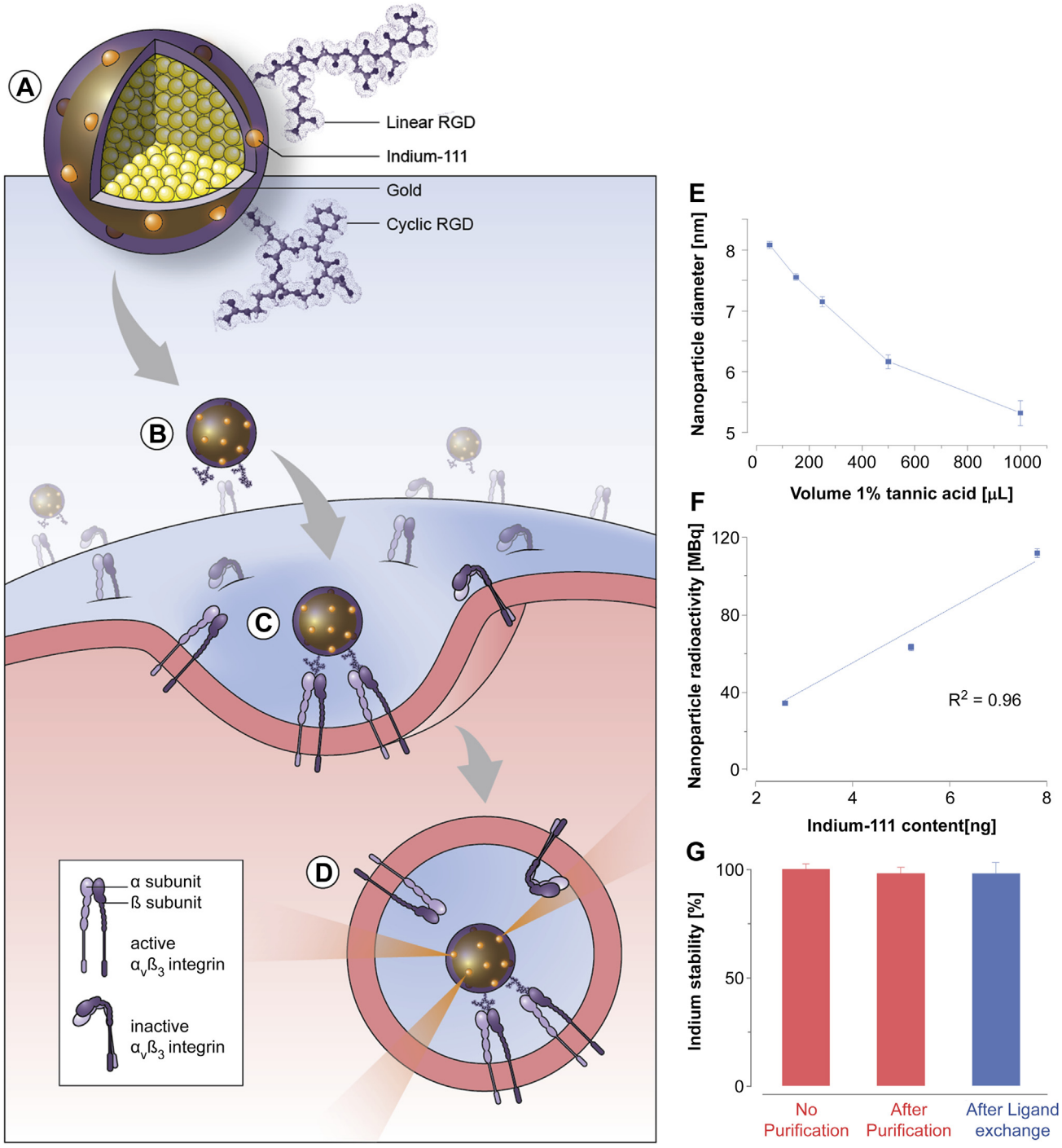

Fig. 1. Design of the indium-111 labeled gold nanoparticles. Gold nanoparticles were synthesized with the particle core stably labeled with the γ-emitter indium-111 and the surface modified with linear and cyclic RGD ligands (A). The particles were designed to be injected intravenously, travel to the tumor cell (B), internalize via $\alpha_v ß_3$ integrin mediated endocytosis (C) and allow imaging via γ-emission (D). Variation of the core size was achieved by adjusting the tannic acid concentration during synthesis (E). Variation of the particles' specific activity was achieved by varying the indium-111 activity during synthesis (F). Indium remained stably adsorbed before and after purification as well as after the ligand exchange reactions as derived from ICP-MS measurements (G).

in clinical applications [13,14]. Indium-111 was selected due to its FDA approval and its established clinical use for molecular imaging of somatostatin receptor [15] and CD20 expression [16]. Furthermore, the 2.8 day half-life of indium-111 allows for 3D whole-body imaging in humans over several days. Finally, RGD was selected, due to its multivalency effects for tumor targeting [17] and its successfully application for imaging of $\alpha_v ß_3$ integrin expressing tumors in humans [18]. As a result, the design of the indium-111 labeled gold nanoparticles exclusively comprised components already in clinical use.

2. Materials & methods

2.1. Materials

Hydrogen tetrachloroaurate trihydrate (99.9%), sodium citrate tribasic dehydrate (99%), indium chloride (99.9%) and diethylene triamine pentaacetic acid (DTPA, 99%) were purchased from Sigma–Aldrich and used without further