Biomaterials xxx (2014) 1–15

Contents lists available at ScienceDirect

Biomaterials

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

Leading opinion

A safe-by-design approach to the development of gold nanoboxes as carriers for internalization into cancer cells

Dania Movia [a], Valerie Gerard [a,c], Ciaran Manus Maguire [b], Namrata Jain [b], Alan P. Bell [d], Valeria Nicolosi [a,c,e], Tiina O'Neill [f], Dimitri Scholz [f], Yurii Gun'ko [a,c], Yuri Volkov [a,b], Adriele Prina-Mello [a,b,*]

[a] Centre for Research on Adaptive Nanostructures and Nanodevices (CRANN), Trinity College Dublin, Dublin, Ireland
[b] School of Medicine, Trinity College Dublin, Dublin, Ireland
[c] School of Chemistry, Trinity College Dublin, Dublin, Ireland
[d] Advanced Microscopy Laboratory, Trinity College Dublin, Dublin, Ireland
[e] School of Physics, Trinity College Dublin, Dublin, Ireland
[f] UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, D4 Dublin, Ireland

ARTICLE INFO

Article history:
Received 26 November 2013
Accepted 19 December 2013
Available online xxx

Keywords:
Gold nanoboxes
Lung cancer barrier
High content screening and He-ion microscopy
Safe-by-design
Tiered approach
Three-dimensional culture

ABSTRACT

Gold nanomaterials are currently raising a significant interest for human welfare in the field of clinical diagnosis, therapeutics for chronic pathologies, as well as of many other biomedical applications. In particular, gold nanomaterials are becoming a promising technology for developing novel approaches and treatments against widespread societal diseases such as cancer. In this study, we investigated the potential of proprietary gold nanoboxes (AuNBs) as carriers for their perspective translation into multifunctional, pre-clinical nano-enabled systems for personalized medicine approaches against lung cancer. A safe-by-design, tiered approach, with systematic tests conducted in the early phases on uncoated AuNBs and more focused testing on the coated, drug-loaded nanomaterial toward the end, was adopted. Our results showed that uncoated AuNBs could effectively penetrate into human lung adenocarcinoma (A549) cells when in simple (mono-cultures) or complex (co- and three-dimensional-cultures) *in vitro* microenvironments mimicking the alveolar region of human lungs. Uncoated AuNBs were biologically inert in A549 cells and demonstrated signs of biodegradability. Concurrently, preliminary data revealed that coated, drug-loaded AuNBs could efficiently deliver a chemotherapeutic agent to A549 cells, corroborating the hypothesis that AuNBs could be used in the future for the development of personalized nano-enabled systems for lung cancer treatment.

1. Introduction

Gold nanomaterials have been used in biomedical applications since the discovery of gold colloids more than three centuries ago [1] and, as stated by Dreaden *at al.* in a recently published review [2], "a new 'Golden Age' of biomedical nanotechnology is upon us". The recent, growing interest in gold nanomaterials arises from their unique combination of photophysical properties (such as large and tuneable light extinction, localized surface plasmon resonance (LSPR), surface-enhanced Raman scattering (SERS) and efficient thermal ablation, to name a few), which can find exploitation in a wide range of diagnostic, biomedical and therapeutic applications.

To date, nanometer-sized gold materials are employed in the healthcare market as part of *in vitro* diagnostic devices [3] (e.g., First Response® pregnancy test – Carter-Wallace; Duopath® Verotoxins – Merck Millipore; ImmunoCAP® Rapid test – Phadia, Inc; and Verigene® platform – Nanospheres [4]) allowing the detection of biologically relevant molecules from complex fluids, such as blood plasma, with unprecedented limits of detection. In addition, the use of gold nanomaterials is currently under clinical evaluation for the treatment of solid tumours by laser-induced thermal ablation (e.g. the AuroLase® Therapy [5] by Auroshell™ particles [6] – Nanospectra Biosciences, at present in phase I of clinical trials [4,7]) or by delivering anti-cancer agents (e.g. Aurimmune – CytImmune Sciences, currently in phase II of clinical trials [4,7]). Finally, gold nanomaterials (including nanoparticles, nanoclusters, nanorods,

* Corresponding author. Centre for Research on Adaptive Nanostructures and Nanodevices (CRANN), Trinity College Dublin, College Green, D2 Dublin, Ireland. Tel.: +353 1 8963259, +353 1 8963087; fax: +353 1 8963037.
E-mail address: prinamea@tcd.ie (A. Prina-Mello).

0142-9612/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2013.12.057

nanoshells and nanoporous surfaces) are employed in biomedical research [1,8] as *in vitro*/*in vivo* imaging tools [2,9], optical sensors [10,11], photothermal therapeutics [12] and drug delivery systems [2,13,14]. By exploiting the high affinity of gold surfaces for thiols, gold nanomaterials provide in fact optimal chemical reactivity conditions for functionalization of their surface with biologically active moieties (e.g. targeting and therapeutic molecules) [1]. In the near future, safely applicable (e.g. non-toxic) gold nanotechnology-products are expected to improve patients' treatment through a more adaptive and personalized approach to medicine [15], where a single construct can accumulate in the site of interest (targeting component) while allowing simultaneous diagnosis of the disease, tracking of the drug delivery (imaging component) and selective drug release to the target cells/tissue (therapy component). The personalized treatment approach through gold nanomaterials will therefore offer the opportunity to decrease the adverse side-effects of drugs, focussing the medical efforts at the target tissue/organ level [16].

The aim of our present experimental study was to evaluate the biological interactions of uncoated gold nanoboxes [17] (AuNBs) with *in vitro* models representative of the human alveolar barrier, thus defining their potential as candidates for perspective functionalization and translation into pre-clinical nano-enabled chemotherapeutic agents for the targeted and personalized treatment of lung cancer. AuNBs, which are hollow nanostructures in the shape of triangular prisms, were selected as nanoprisms are known to show a much higher degree of enhancement of the plasmon resonance energy than nanospheres [18], finding application, for example, in Surface Enhanced Fluorescence (SEF) and Surface Enhanced Raman Spectroscopy (SERS) and opening up the opportunity of using AuNBs as imaging component of a perspective nano-enabled personalized treatment against lung cancer. Worldwide, lung cancer is one of the leading causes of cancer-related mortality, with more than 157,000 deaths only in 2010 [19,20]. The survival rate of patients with lung cancer is poor, with less than 15% of patients surviving 5 years after diagnosis [21]. Lung cancer is also the third most common cancer in Ireland, accounting for 13.9% of cancers diagnosed in men and 9.4% in women during the 1994–2008 period [21]. The statistical data on the survival rate of lung cancer patients clearly evidence the current lack of effective treatments to cure lung cancer [22] and the pressing need of developing new therapeutic approaches, such as personalised nano-enabled treatments, against this disease.

In order to achieve our aim, a cross-disciplinary, three-tiered approach was used (Fig. 1). In Tier 1 the internalization of two types of uncoated AuNBs (differing from each other in size, ranging from 30 to 70 nm ca.) was assessed in *ín vitro* mono-, co- and three-dimensional (3D)-culture models of human lung adenocarcinoma (A549) cells, thus allowing an evaluation of their capability as carriers to enter into the cancer cells. The mechanism and time-dependence of cell internalization, the cytotoxicity and the biodegradation of uncoated AuNBs were also determined for assessing the safety of these nanostructures once they have entered the cells surrounding the targeted tumour tissue. Despite the fact that the Au nanoparticles with diameter above 2 nm are generally considered inert and non-toxic [23–25], dispersions of gold nanomaterials can in fact induce cytotoxicity [2,24]. Such toxicity can rise from residual chemicals of the synthesis process [23,26–28], free small molecules and metal ions present in the solution [29] or from the degradation of the gold core [23]. In Tier 2 and 3, through a safe-by-design approach [16], the long-term biocompatibility provided by a gelatine coating [30] was combined with the targeting of cancerous cells over-expressing folic acid (FA) receptors with the aim of assessing *in vitro* the ability of AuNBs to deliver a chemotherapeutic agent, namely Paclitaxel, following purpose-specific functionalization.

Our results demonstrated that uncoated AuNBs were internalised by A549 cells in mono-, co- and 3D-cultures. Although significant AuNBs internalization was achieved after 5–7 h exposure, AuNBs showed to be biologically safe in A549 cells up to 24 h. Concurrently, preliminary *in vitro* data suggested that AuNBs could indeed be a suitable carrier for the delivery of a chemotherapeutic agent following purpose-specific functionalization, thus ultimately advocating for further pre-clinical research on AuNBs as candidate for nano-enabled targeted treatment of lung cancer.

2. Materials and methods

Chemicals and solvents were purchased from commercial sources (Sigma–Aldrich, Fisher Scientific, Invitrogen and Calbiochem) and used as provided, unless otherwise specified in the manuscript.

For clarity purposes, uncoated AuNBs tested in Tier 1 will be referred to as $AuNB_1$ and $AuNB_2$, gelatine-coated AuNBs used in Tier 2 as $AuNB_3$, and, in Tier 3, gelatine-coated Paclitaxel-loaded AuNBs and gelatine-coated Paclitaxel-loaded AuNBs to which FA was conjugated will be indicated as $AuNB_4$ and $AuNB_5$, respectively (Fig. 2).

2.1. Synthesis of uncoated AuNBs ($AuNB_1$ and $AuNB_2$)

AuNBs in two different size ranges were synthetized according to previously published protocols [17,31]. A complete description of the synthetic procedure is reported in the Supporting Data.

2.2. Physico-chemical characterization of uncoated AuNBs

Table 1 reports the main properties of uncoated AuNBs ($AuNB_1$ and $AuNB_2$). Uncoated AuNBs were characterised by transmission electron microscopy (TEM), He-ion microscopy (HIM), Nanoparticles Tracking Analysis (NTA) and pH measurements, as described below.

2.2.1. Transmission electron microscopy (TEM) of AuNBs

The TEM specimens were prepared on 200-mesh Cu lacey carbon grids by drop-casting and were visualized under a FEI Titan Transmission Electron Microscope (FEI,

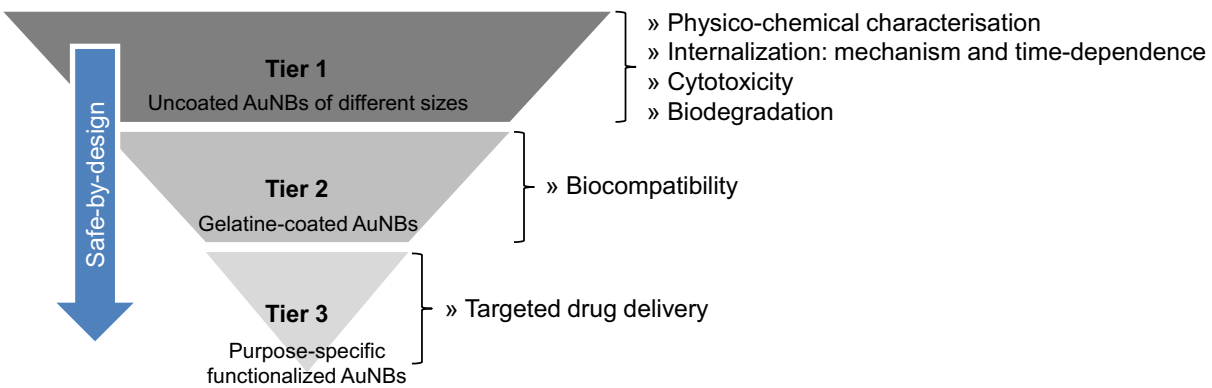

Fig. 1. Schematic of the safe-by-design, tiered approach used in this study to design a non-toxic nano-enabled carrier for therapeutic applications.

Please cite this article in press as: Movia D, et al., A safe-by-design approach to the development of gold nanoboxes as carriers for internalization into cancer cells, Biomaterials (2014), http://dx.doi.org/10.1016/j.biomaterials.2013.12.057