International Journal of Pharmaceutics 519 (2017) 240–249

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Aerosolizable gold nano-in-micro dry powder formulations for theragnosis and lung delivery

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ARTICLE INFO

Article history:
Received 18 November 2016
Received in revised form 15 January 2017
Accepted 16 January 2017
Available online 19 January 2017

Keywords:
Pulmonary delivery
Gold nanoparticles
Nano-in-micro
Supercritical CO_2
Spray drying
Dry powder formulation
Lung cancer

ABSTRACT

Functionalized gold nanoparticles (AuNPs) have been widely investigated as promising multifunctional nanosystems for the theragnosis of lung cancer, the most common and prominent cause of cancer death worldwide. Nevertheless, nanoparticles are not in appropriate sizes for an accurate deep lung delivery and the lack of locally and effective delivery of therapeutic biomolecules to the deep lungs is, in fact, the major cause of low therapeutic outcome. Herein we incorporate, for the first time, AuNPs into respirable microparticles. AuNPs were functionalized with biocompatible oligo(2-oxazoline)-based optically stable fluorescent coatings, and conjugated with a laminin peptide (YIGSR) for targeted lung cancer delivery. These POxylated AuNPs were then incorporated into a chitosan matrix by a clean process, supercritical CO_2-assisted spray drying (SASD), yielding nano-in-micro clean ultrafine dry powder formulations. The engineered formulations present the adequate morphology and flowability to reach the deep lung, with aerodynamic sizes ranging 3.2–3.8 μm, and excellent fine particle fraction (FPF) (FPF of 47% for CHT-bearing targeted AuNPs). The optimal biodegradation and release profiles enabled a sustained and controlled release of the embedded nanoparticles, with enhanced cellular uptake, opening new prospects for future lung theragnosis.

1. Introduction

With the significant increase of lung cancer incidence in the past 50 years, among both women and men, there has been a huge demand for the development of new strategies to address this health problem. To overcome the limitations associated with the conventional treatment of several diseases, novel pulmonary therapeutics have been vastly investigated as they facilitate targeting the drug delivery directly to the lungs for both local and systemic treatments. Such type of treatment allows for drug sustained release, reduced therapeutic dose and improved patient compliance. However, the design of inhaled carriers with suitable properties for an accurate deep lung deposition and effective delivery is the major challenge that needs to be overcome. Respirable formulations should comprise aerodynamic sizes ranging from 1 to 5 μm (dry) in order to successfully reach the lungs, swell upon deposition in the moist lung, and provide a sustained controlled drug release through the polymeric matrix. Such features enable not only a proper delivery of the carriers, as also the avoidance of macrophage uptake (Chow et al., 2007; El-Sherbiny and Smyth, 2010; Li et al., 2010; Patil and Sarasija, 2012; Silva et al., 2014; Yang et al., 2008, 2009). Nanoparticles have been foreseen as effective platforms to provide a controlled release of drugs or other biomolecules in the lungs (Schütz et al., 2013; Van Rijt et al., 2014). Due to their nanometric scale, nanoparticles can

Abbreviations: AuNPs, GNPs: gold nanoparticles; YIGSR, laminin peptide (Cys-Asp-Pro-Gly-Tyr-Ile-Gly-Ser-Arg); SASD, supercritical assisted spray drying; CHT, chitosan; CHT-GNP, CHT bearing different formulations of gold nanoprobes; FPF, fine particle fraction; $scCO_2$, supercritical CO_2; OOxs, oligo(2-oxazolines); OEtOx, oligo(2-ethyl-2-oxazoline); OEtOx-OH, oligo(2-ethyl-2-oxazoline) end terminated with water; OEI-CS, oligo(2-ethyl-2-oxazoline) end terminated with polycationic oligo(ethyleneimine-N-chromylium salt; ACI, anderson cascade impactor; DPI, dry powder inhalers; EE, encapsulation efficiency; ED, emitted dose; MMAD, mass median aerodynamic diameter; $D_{v,50}$, volume mean diameter; GSD, geometric standard deviation.

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http://dx.doi.org/10.1016/j.ijpharm.2017.01.032
0378-5173/

penetrate into the deep tissue and then suffer cellular uptake. Moreover, innovative designs of nanoparticles can allow tailoring of multifunctional airway targeting nanocarriers enabling the integration of multiple functions, such as cell targeting, ultra-sensitivity imaging monitoring and therapy, all into one system, creating smart multifunctional nanosystems (Azarmi et al., 2008; Connor et al., 2005; El-Sherbiny and Smyth, 2010; Schütz et al., 2013; Silva et al., 2014; Van Rijt et al., 2014). Gold nanoparticles (GNPs, AuNPs) for instance, have been foreseen as candidate nanosystems for both local and systemic therapies for lung diseases (Bachler et al., 2015; Conde et al., 2013; Dreaden et al., 2012; Geiser et al., 2013). In fact, surface functionalized AuNPs have been extensively exploited to face the urgent need to further develop novel multifunctional nanosystem theragnosis of lung complications (Akiyama et al., 2009; Kurmi et al., 2010). However, nanoparticles are in a size range which is not suitable for deep lung delivery and can be easily exhaled or mucociliary cleared out before reaching the underlying epithelia and undergo macrophage phagocytosis (Adami et al., 2011; Geiser et al., 2013; Hu et al., 2013; Lai et al., 2010; Restani et al., 2016). Therefore, the major challenge for pulmonary delivery of nanoparticles is to find a proper carrier system. Increased effectiveness of an inhalation treatment may be achieved by the quick dissolution of powder particles in the surface lining fluid and by rapid diffusion to the destination tissue (Ruge et al., 2013). Hence, novel systems incorporating nanoparticles into swellable and respirable micro-scale structures have been engineered to overcome the issues of storing and delivering the drugs and other biomolecules to the lungs. From all the formulations available for inhalation, dry powders are usually preferred as they exhibit the most suitable behavior for pulmonary delivery such as stability and bioavailability of active ingredients, when compared to their aqueous counterparts (Al-Qadi et al., 2012; Hardy and Chadwick, 2000). In this contribution, new nano-in-micro platforms were developed for controlling pulmonary delivery and combine both the benefits of multifunctional nanoparticles and the respirable microspheres. Thus, targeted POxylated AuNPs (Silva et al., 2016) were micronized into a polymeric matrix of the natural biopolymer chitosan (CHT) in order to increase nanoparticles ability to reach the deep lungs, adhere to lung epithelium and avoid macrophage clearance. Moreover, due to CHT biodegradability properties and the innovative design of our POxylated AuNPs, that allow integration of both cell targeting (Mukhopadhyay et al., 2013; Wewer et al., 1987) and imaging (Boisselier and Astruc, 2009; Bonifácio et al., 2012; Correia et al., 2011; de Macedo et al., 2007), a controlled and sustained release of the targeted nanosystems can be achieved, improving their retention at the desired site, minimizing potential side effects and reducing health care costs with increased efficacy (Loira-Pastoriza et al., 2014; Lai et al., 2010). In fact, CHT has already been recognized as a promising excipient for the development of spray dry powders for pulmonary delivery due to its desirable advantages like non-toxicity, biocompatibility, mucoadhesive features and ability to improve drug absorption in lung tissues (Alvarenga, 2011; Domínguez-delgado et al., 2014; Jae et al., 2006; Temtem et al., 2012; Van Rijt et al., 2014). In this study, CHT bearing different formulations of gold nanoprobes (CHT-GNP) were synthesized in a laboratory scale using SASD apparatus. The resulting micronized powders were fully characterized and evaluated as novel biodegradable systems that confer a controlled and sustained release of the GNP entrapped. The optimal aerodynamic features and performances, nearly match the (dry powder inhalers) DPIs currently available on the market. Furthermore, the conjugation of targeted and always-on fluorescent gold nanoformulations with a biodegradable and biocompatible excipient enabled the production of inhaled systems able to interact with cancer cells (smart multifunctional nanosystems), bringing new insights for lung cancer screening and treatment.

2. Materials and methods

2.1. Materials

Gold in the form of tetra-chloroauric[III] acid trihydrate ($HAuCl_4 \cdot 3H_2O$) and trisodium citrate hydrate were purchase from Alfa Aesar. Dissodium hydrogen phosphate sodium and sodium chloride were purchased from AppliChem Panreac. Acetic acid glacial (99.7% purity) was purchased from Carlo Erba Reagents. 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) was purchased from Promega. Ethanol absolute anhydrous (99.9% purity) was purchased from Scharlau. Ethyl oxazoline monomer, boron trifluoride etherate ($BF_3 \cdot Et_2O$), cysteamine, anhydrous dimethylformamide (DMF) dihydrogen phosphate trisodium citrate hydrate, laminin fragment (Cys-Asp-Pro-Gly-Tyr-Ile-Gly-Ser-Arg) (YIGSR), and lysozyme chloride from Grade VI: from chicken egg white were purchased from Sigma-Aldrich. CHT (viscosity 5–20 mPa s, 0.5% in 0.5% acetic acid at 20 °C) was purchased from Tokyo Chemical Industry. Hydranalcoulomat AD from Sigma-Aldrich was used in Karl Fischer. All components were used as received without any further purification. Industrial carbon dioxide (purity ≥99.93%) from Air Liquid (Portugal) was used.

2.2. Nanoparticles formulation

The nanoparticles production was already described in our previous publication (Silva et al., 2016). Briefly, AuNPs were synthetized according to Frens's method (Frens, 1973): 100 mL of 0.01% (w/v) $HAuCl_4 \cdot 3H_2O$ were heated to boiling. Then, 1 mL of trisodium citrate hydrate 1% (w/v) was added to the boiling solution under constant stirring. After 25 s, a slightly yellow solution turned faintly blue. Subsequently, the solution colour changed to dark red, which is indicative of the formation of monodisperse spherical particles. The solution was boiled over 5 min, to allow for the complete reduction of the gold chloride (Frens, 1973). Afterwards, the nanoformulations were grafted with "green" oligomers synthetized using $scCO_2$, oligo(2-oxazolines) (OOxs). The oligomers oligo(2-ethyl-2-oxazoline) (OEtOx) were polymerized in a stainless-steel reactor using $BF_3 \cdot OEt_2$ as the initiator (de Macedo et al., 2007). The monomer/initiator ratio used for the polymerization was $[M]/[I]^1/_4 = 12$. The reactor cell was loaded with the monomer, the initiator, a magnetic stirring bar, and then immersed in a thermostatized water bath at 60 °C. Carbon dioxide was introduced in the reactor in order to achieve the desired reaction pressure (from 16 to 20 MPa). After 20 h of reaction, the pressure was slowly released until the reactor reach room temperature. Inside the reactor, a viscous foam was formed (OEtOx). At the end of the polymerization, the living oligomer was end-capped either with cysteamine or water, generating OEtOx-SH (**1**) and OEtOx-OH (**2**), respectively. Post-functionalization of **2** with 2,4-dihydroxybenzaldehyde produced a polycationic oligo (ethyleneimine-*N*-chromylium salt, OEI-CS (**3**). AuNPs were capped with OEtOx-SH (AuNP:OOx molar ratio 1:5000) and OEI-CS (AuNP:OOx molar ratios 1:2500) producing Au-OEtOx-SH and Au-OEI-CS, respectively. Afterwards, the mixtures were collected to 1.5 mL Eppendorfs and centrifuged at 13,500 × *g*, for 20 min, at room temperature. The supernatant was removed and the pellet was redispersed in milliQ water. Further conjugation of the produced particles with a YIGSR peptide, known to be recognized by the overexpress β1 integrins of A549 non-small cell lung cancer (Kang et al., 2011; Solis David et al., 2010), was also performed, producing Au-OEtOx-SH-YIGSR and Au-OEI-CS-YIGSR. The