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Gold nanostar–polymer hybrids for siRNA delivery: Polymer design towards colloidal stability and in vitro studies on breast cancer cells

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ABSTRACT

To overcome the low bioavailability of siRNA (small interfering RNA) and to improve their transfection efficiency, the use of non-viral delivery carriers is today a feasible approach to transform the discovery of these incredibly potent and versatile drugs into clinical practice. Polymer-modified gold nanoconstructs (AuNCs) are currently viewed as efficient and safe intracellular delivery carriers for siRNA, as they have the possibility to conjugate the ability to stably entrap and deliver siRNAs inside cells with the advantages of gold nanoparticles, which can act as theranostic agents and radiotherapy enhancers through laser-induced hyperthermia.

In this study, AuNCs were prepared by coating Gold Nano Stars (GNS) with suitable functionalised polymers, to give new insight on the choice of the coating in order to obtain colloidal stability, satisfying in vitro transfection behaviour and reliability in terms of homogeneous results upon GNS type changing. For this goal, GNS synthesized with three different sizes and shapes were coated with two different polymers: i) α-mercapto-ω-amino polyethylene glycol 3000 Da (SH-PEG_{3000}-NH_2), a hydrophilic linear polymer; ii) PHEA-PEG_{2000}-EDA-LA (PPE-LA), an amphiphilic hydroxyethylaspartamide copolymer containing a PEG moiety. Both polymers contain —SH or —SS— groups for anchoring on gold surface and NH_2 groups, which can be protonated in order to obtain a positive surface for successive siRNA layering. The effect of the features of the coating polymers on siRNA layering, and the extent of intracellular uptake and luciferase gene silencing effect were evaluated for each of the obtained coated GNS. The results highlight that amphiphilic biocompatible polymers with multi-grafting function are more suitable for ensuring the colloidal stability and the effectiveness of these colloidal systems, compared to the coating with linear PEG.

1. Introduction

The growing families of gold nanoparticles (AuNPs), that comprise gold nanospheres, nanorods, nano-pyramids, nanocages, nanostars and other shapes, possess interesting properties such as the presence of a bio-inert surface, a versatile surface chemistry, a high degree of control on size and shape during the synthetic steps, and remarkable features such as intense extinction bands due to localized surface plasmon resonance (LSPR) and photothermal effects when irradiated at a proper wavelength (i.e. resonant with the LSPR bands), either in the visible or in the bio-transparent Near IR (NIR) window, i.e. 750–1100 nm (Huang et al., 2008). Other well-known features of AuNPs include chemical inertness, negligible toxicity, and high conjugation efficiency with various biomolecules and biocompatible polymers, leading to the formation of AuNanoconstructs (AuNCs). These are a class of promising platforms which can be used for various biomedical applications and in particular for cancer treatment (Lee et al., 2014; Muddineti et al., 2015). AuNCs can accumulate in tumour tissues either passively or actively. Passive accumulation takes place via the Enhanced Permeability and Retention (EPR) effect (Greish, 2010), which produces a selectively high local concentration of nano-sized

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anticancer drugs in tumour tissues, due to abnormalities of tumour vasculature (namely hyper vascularization, aberrant vascular architecture, extensive production of vascular permeability factors stimulating extravasation within tumour tissues, and lack of lymphatic drainage). Active accumulation takes place via AuNCs conjugation with a targeting motif, which can be specifically recognised by an hyper-expressed receptor on cells surface.

Through irradiation at the LSPR wavelength, AuNCs based on non-spherical AuNPs can induce through-tissues hyperthermia with NIR laser (Huang et al., 2008). Gold's high atomic number enables it to enhance the effect of radiotherapy, which in turn can be amplified by mild, laser-induced hyperthermia. When conjugated with drugs, AuNCs can also behave as drug carriers capable to enhance the uptake of the drug in the tumour tissue. Controlled release of the drug may be switched or enhanced by hyperthermal stimulation of AuNCs. An additional attractive property of AuNPs is their affinity for thiols, due to the high stability of the Au-thiolate bond, a feature that provides an effective and selective approach to controlled glutathione-mediated payload release via place-exchange reaction inside cells (Ghosh et al., 2008).

Gold nanostars (GNS) are branched gold nanoparticles that usually show LSPR absorptions which can fall in the NIR range and tuned during synthesis to fit in the bio-transparent window. Interest in GNS has increased thanks to the ease of synthesis for large scale production, the high surface-to-volume ratio useful for improving drug loading efficiency, the huge SERS effects due to the presence of sharp branch edges (Guerrero-Martínez et al., 2011) and the use of their TPL (two-photon luminescence) when irradiated in the NIR for deep tissue imaging (Liu et al., 2015). Moreover, their photothermal response on NIR irradiation is particularly efficient (Freddi et al., 2013). It was also reported that gold nanostars functionalized with TAT-peptides efficiently internalize in cells and it was demonstrated that these particles are promising agents for cancer therapy via photothermolysis (Yuan et al., 2012).

The potential of therapeutics for cancer based on RNA interference (RNAi) has received much attention. However, delivery of RNAi effectors, such as small interfering RNA (siRNA), remains an obstacle to clinical translation. Among the non-viral delivery vectors that have been investigated, AuNCs with different sizes, shapes, structures, chemistry and syntheses have shown the potential to enhance siRNA delivery in vitro and in vivo (Lee et al., 2008; Perche et al., 2016; Rahme et al., 2015; Wang et al., 2016; Zhao et al., 2015). Recently, it was also found that interferon-β levels in macrophage cells after treatment with densely functionalized oligonucleotide-modified gold nanoparticles were reduced by a factor of 25 when compared to the treatment with a lipoplex carrying the same oligonucleotide sequence, stating their significant lower innate immune response when compared with conventional DNA transfection materials (Massich et al., 2009). In the present study, three GNS types belonging to three different dimensional ranges (GNS TRITON: 80–90 nm; GNS LSB: 50–70 nm; shrunk GNS LSB: 20–30 nm, see Fig. 1) were synthesised, characterized and coated with two different polymers in order

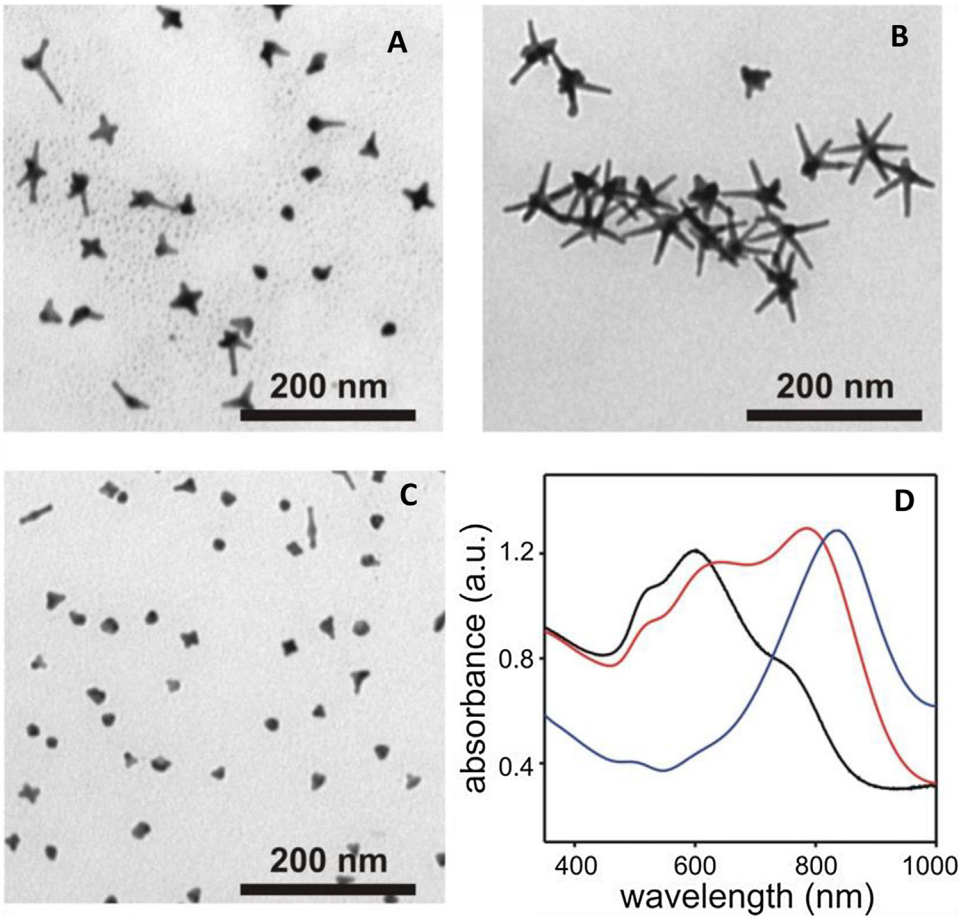

Fig. 1. Transmission Electron Microscopy (TEM). A: TEM image of GNS LSB. B: TEM image of GNS TRITON. C: TEM image of shrunk GNS LSB. D: extinction spectra of the colloidal solutions of uncoated GNS LSB (red), GNS TRITON (blue), and shrunk GNS LSB (black). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)