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Cisplatin drug delivery using gold-coated iron oxide nanoparticles for enhanced tumour targeting with external magnetic fields

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ABSTRACT

The platinum-based chemotherapeutic drug cisplatin is highly effective in the treatment of solid tumours, but its use is restricted by poor bioavailability, severe dose-limiting side effects and rapid development of drug resistance. In light of this we have tethered the active component of cisplatin to gold-coated iron oxide nanoparticles to improve its delivery to tumours and increase its efficacy. Iron oxide nanoparticles (FeNPs) were synthesised via a co-precipitation method before gold was reduced onto the surface (Au@FeNPs). Aquated cisplatin was used to attach $\{Pt(NH_3)_2\}$ to the nanoparticles by a thiolated polyethylene glycol linker forming the desired product (Pt@Au@FeNP). The nanoparticles were characterised by dynamic light scattering, scanning transmission electron microscopy, UV–Vis spectrophotometry, inductively coupled plasma mass spectrometry and electron probe microanalysis. The nanoparticles increase in size as they are constructed, with the synthesised FeNPs having a diameter of 5–50 nm, which increases to 20–80 nm for the Au@FeNPs, and to 60–120 nm for the Pt@Au@FeNPs. Nanoparticle drug loading was found to be 7.9×10^{-4} moles of platinum per gram of gold. The FeNPs appear to have little inherent cytotoxicity, whereas the Au@FeNPs are as active as cisplatin in the A2780 and A2780/cp70 cancer cell lines. More importantly the Pt@Au@FeNPs are up to 110-fold more cytotoxic than cisplatin. Finally, external magnets were used to demonstrate that the nanoparticles could be accumulated in specific regions and that cell growth inhibition was localised to those areas.

1. Introduction

Cisplatin, *cis*-diamminodichloridoplatinum(II), is the most effective platinum based drug for the treatment of solid tumours [1–3]. It is indicated first line in malignancies of the lung, ovary, head and neck, bladder and cures over 90% of testicular cancers [1]. Cisplatin is activated when it enters the cell and subsequently binds directly to DNA, disrupting replication and transcription, which triggers an apoptotic response [1]. Following injection of cisplatin, most of the drug is excreted renally with only a fraction of the remaining dose converted to the active diaquo-platinum form [1], limiting the amount of drug that actually binds to DNA. The use of cisplatin is also restricted due to intrinsic and acquired resistance caused by reduced drug uptake and efflux, increased detoxification via thiol-containing biomolecules and increased DNA repair [1,4,5]. Additionally, it displays significant dose-related side effects such as nephrotoxicity, neurotoxicity, nausea and vomiting, which can be attributed to its indiscriminate attack on both healthy and cancerous cells [6].

Nanoparticle-based drug delivery vehicles have the ability to overcome some of these limitations by passively or actively targeting tumours. The disorganised vasculature and absence of effective lymphatic drainage in solid tumours allows nanoparticles to leak from the blood stream and accumulate in the cancer, a phenomenon known as the Enhanced Permeability and Retention (EPR) effect [7]. This allows nanoparticles to target tumours passively, reducing uptake into healthy cells.

Recently, tumour targeting using magnetic fields to direct the movement and localisation of drugs to solid tumours has generated much interest [8]. Magnetic nanoparticles offer the benefit of utilising both the EPR effect (passive targeting) whilst also ensuring a direct, guided delivery to the tumour (active targeting). Nanoparticles of iron oxide possess superparamagnetic properties, whereby magnetism is only present when under direct energy from an external magnetic field [9]. Other advantages of iron oxide include its ability to be used in magnetic resonance imaging and

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induce cytotoxicity through near infrared derived hyperthermia [10]. Unfortunately, iron oxide alone in physiological media is unstable, resulting in oxidation, aggregation and precipitation [10–13]. Moreover, it is a challenge to attach molecules to the surface of iron oxide. On attachment of cisplatin to iron oxide nanoparticles, it was observed that the drug rapidly dissociated due to inefficient binding [14]. In vivo, this may result in early release of cisplatin whilst still in the blood stream and failure of the drug to reach the tumour [14]. Similar fast release of cisplatin from iron oxide nanoparticles has been observed in a number of other studies as well [15,16]. There is therefore a need to develop safer, more stable iron oxide nanoparticles that can retain platinum drugs more strongly on their surfaces.

The use of gold nanoparticles as chemotherapeutic drug delivery vehicles is attractive as it is non-toxic, non-immunogenic, and provides a highly tunable surface to which drugs can be attached [17–19]. Previously we demonstrated that the active components of cisplatin and oxaliplatin can be tethered to gold nanoparticles with a drug loading of up to 70 000 cisplatin-like molecules per nanoparticle [20,21]. In addition, the oxaliplatin-nanoparticle conjugate showed a 6-fold increase in cytotoxicity compared with the drug alone [20]. We have also demonstrated that the gold nanoparticles can be reproducibly made and are relatively stable in solution; important features for their pharmaceutical approval as drug delivery vehicles [21]. Other platinum drugs have also been successfully attached to other gold-based nanoparticles, where cellular uptake and cytotoxicity was increased compared with the free drug [22,23].

By using both iron oxide and gold within the one drug delivery vehicle, a multifaceted system can be developed which exploits the surface chemistry of the gold whilst retaining the magnetic character of the iron oxide, allowing for biologically sound drug delivery and imaging. Lin et al. has demonstrated that a gold shell did not degrade the magnetic properties of their iron oxide nanoparticles [24]. A study whereby doxorubicin was successfully loaded onto gold-coated iron nanoparticles (Au@FeNPs) saw the same retention of magnetism and a sustained release of the drug [25]. Additionally, iron oxide and gold have been used in drug delivery and imaging to form dumbbell-like particles; these studies demonstrated attachment of a range of molecules to the nanoparticles and steady drug release profiles [26,27].

Taking the theme of this special issue (Metals in Medicine) to its limits, in this paper we give the first example of platinum anticancer drug delivery using gold-coated iron oxide nanoparticles (Fig. 1). The nanoparticles have been fully characterised using dynamic light scattering (DLS), scanning transmission electron microscopy (STEM), UV–Vis spectrophotometry, inductively coupled plasma mass spectrometry (ICP-MS), and electron probe microanalysis (EPMA). Their cytotoxicity was evaluated using in vitro growth inhibition assays with the human ovarian cancer cell lines A2780 and A2780/cp70 and the localisation of the nanoparticles to effect site specific growth inhibition has been demonstrated using an external bar magnet.

2. Results and discussion

2.1. Nanoparticle synthesis

The synthesis of magnetite-based (Fe_3O_4) iron oxide nanoparticles is well established in the literature [13,28]. The two most common methods for production are non-aqueous thermal decomposition and aqueous co-precipitation [29,30]. In synthesising our nanoparticles, we first made Fe_3O_4 cores by adding NaOH to a solution of iron(II) and iron(III) chloride salts, hence utilising the co-precipitation method. The concentration and type of salts, and the solution's pH and ionic strength, all contribute to the size and character of the nanoparticles created [31]. Recent literature has shown the oxidised maghemite form (γ-Fe_2O_3) preferentially binds gold compared with the magnetite form [11], and is a more stable and biocompatible form of iron oxide [31,32]. Subsequently we used nitric acid as an oxidising agent to convert the Fe_3O_4 nanoparticles to the γ-Fe_2O_3 form (from here onwards referred to as FeNPs) [33]. Iron oxide nanoparticles are known to be unstable in solution as their agglomeration and aggregation promote particle growth, inhibiting the formation of the gold shells on their surface. Addition of tetramethylammonium hydroxide (TMAOH) facilitated dispersion of the FeNPs, thus inhibiting aggregation and enforcing solution stabilisation through interaction between the $N(CH_3)^{4+}$ cations and the hydroxide anions that are absorbed onto the FeNP's surface [11,34].

Initial attempts to produce a gold coating onto the FeNPs with glucose as the reducing agent, which has been used by others, saw no development of the purple/pink colour associated with metallic gold and no change in the UV spectrum, indicating that this method was unsuccessful. Instead, mixing the FeNPs with citrate anions allowed for an exchange of the adsorbed hydroxide ions [35]. Drop wise addition of $HAuCl_4$ with strong heating was then used to ensure the gold coated the iron, rather than expand its own seeds and create pure gold nanoparticles. At the end of the reaction, the presence of a purple/pink solution was indicative of a gold coating on the nanoparticles [11,36,37]. Pure gold nanoparticles, which may form during the coating of the FeNPs, were separated from the Au@FeNPs by use of an external magnet (Fig. 2).

Next, a polyethylene glycol (PEG) linker was tethered to the Au@FeNPs. Polyethylene glycol is a highly flexible and hydrophilic molecule, and has widespread pharmaceutical use due to its stability and lack of toxicity [38,39]. Coatings of PEG on nanoparticles, polymers and liposomes can increase circulation time, improve

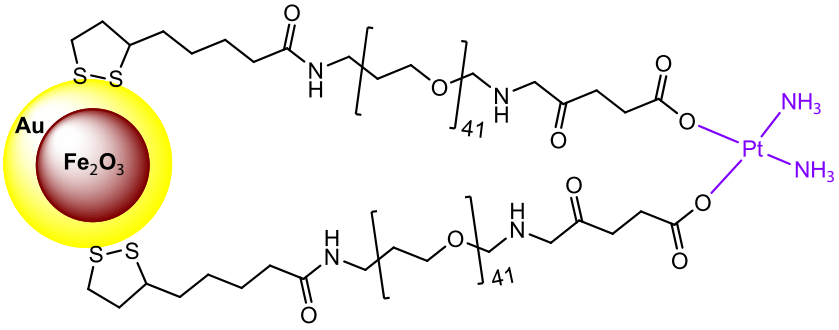

Fig. 1. The nanoparticle-based drug delivery system of gold-coated iron oxide nanoparticles functionalised with a thiolated polyethylene glycol (PEG) linkers to which the active component of the anticancer drug cisplatin, $\{Pt(NH_3)_2\}^{2+}$, is attached via the terminal carboxylate groups.