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Tunable, biodegradable gold nanoparticles as contrast agents for computed tomography and photoacoustic imaging

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ABSTRACT

Gold nanoparticles (AuNP) have been proposed for many applications in medicine. Although large AuNP (>5.5 nm) are desirable for their longer blood circulation and accumulation in diseased tissues, small AuNP (<5.5 nm) are required for excretion *via* the kidneys. We present a novel platform where small, excretable AuNP are encapsulated into biodegradable poly di(carboxylatophenoxy)phosphazene (PCPP) nanospheres. These larger nanoparticles (Au-PCPP) can perform their function as contrast agents, then subsequently break down into harmless byproducts and release the AuNP for swift excretion. Homogeneous Au-PCPP were synthesized using a microfluidic device. The size of the Au-PCPP can be controlled by the amount of polyethylene glycol-polylysine (PEG-PLL) block co-polymer in the formulation. Synthesis of Au-PCPP nanoparticles and encapsulation of AuNP in PCPP were evaluated using transmission electron microscopy and their biocompatibility and biodegradability confirmed *in vitro*. The Au-PCPP nanoparticles were found to produce strong computed tomography contrast. The UV–Vis absorption peak of Au-PCPP can be tuned into the near infrared region *via* inclusion of varying amounts of AuNP and controlling the nanoparticle size. In vitro and *in vivo* experiments demonstrated the potential of Au-PCPP as contrast agents for photoacoustic imaging. Therefore, Au-PCPP nanoparticles have high potency as contrast agents for two imaging modalities, as well as being biocompatible and biodegradable, and thus represent a platform with potential for translation into the clinic.

1. Introduction

Nanoparticle contrast agents have gained increasing interest over the past decade [1–5]. Nanoparticles have several advantages over small molecule contrast agents. They can carry payloads that have contrast properties unavailable in small molecules, and their size, shape and surface characteristics can be specifically engineered. These features can be used to form nanoparticle contrast agents that have long circulation times, accumulation in disease sites, and provide contrast generation for novel imaging methods. Nanoparticle platforms also allow the formation of multimodal and multifunctional agents for imaging with different modalities, as well as simultaneous imaging and therapeutic applications using theranostic nanoparticles [6]. Gold nanoparticles (AuNP) have been extensively studied as contrast agents for a range of biomedical imaging techniques, and as therapeutic agents [7]. AuNP are attractive as materials for use in medicine, since they are inert, biocompatible and can be easily modified with surface capping ligands [7–9]. Furthermore, AuNP have a number of other characteristics that have encouraged their use in biomedical applications. For example, due to gold's high X-ray attenuation, many AuNP formulations have been developed as contrast agents for computed tomography (CT) [9–15]. CT is one of the most extensively used medical imaging methods, and has the capability of producing images of high spatial and temporal resolution at relatively low cost. Limitations of the current, iodinated small molecule blood

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pool CT contrast agents, which include short circulation half-lives [16] and potential damage to the kidneys [17], are motivations for developing newer contrast agents such as AuNP [13]. Photoacoustic (PA) imaging is an experimental technique that couples the high sensitivity of optical imaging with the depth penetration of ultrasound. Gold nanostructures that absorb near infrared light have found many applications as contrast agents in PA imaging [17–20]. Other imaging modalities such as fluorescence, surface enhanced Raman spectroscopy (SERS) and optical imaging take advantage of properties of gold nanostructures, such as inherent fluorescence, electromagnetic field enhancement and strong light scattering, respectively [7,8]. Therapeutic applications of gold nanostructures include drug delivery, nucleic acid delivery, photothermal therapy and radiation therapy [7,8]. Furthermore, clinical trials have shown no adverse effects of gold nanoparticles for therapeutic applications [21]. Despite these attractive features, no gold agent has yet been FDA-approved, for reasons including concerns over the excretion of AuNP.

The vast majority of the AuNP developed for applications in nanomedicine have been larger than 5.5 nm [7,8]. Larger AuNP are desirable because of their longer circulation times and accumulation in disease sites. Long circulating nanoparticles are especially advantageous in the case of blood pool contrast agents [13]. In addition, for applications that involve the use of near infrared light, the types of AuNP structures that absorb strongly in those wavelengths (rods, shells, etc.) are much larger than 5.5 nm [7,8,22–24]. However, AuNPs larger than 5.5 nm cannot be excreted by the kidneys [25] and data shows that about 20–30% of the injected dose is retained in the liver for months after injection [26–28], which would be a concern for FDA-approval and eventual clinical application [29]. Furthermore, long-term retention of contrast agents may interfere with subsequent imaging sessions. For nanoparticles to have better translational potential, their size should be less than 5.5 nm in diameter on average to allow them to be excreted rapidly [13]. Furthermore, in order to avoid renal damage and lower the burden on kidneys, it may be beneficial for the injected contrast agent dose to be filtered *via* the kidney gradually, *i.e.* over a period of days, as opposed to minutes as is the case with smaller than 5.5 nm nanoparticles. Therefore, a potentially translatable formulation of long-circulating gold nanoparticles would be larger than 5.5 nm, but would slowly break down into sub-5.5 nm components that could be excreted *via* the urinary system. Such an approach would result in low concentrations of gold nanoparticles reaching the kidneys over an extended time, minimizing potential nephrotoxicity. Agents of such design may prove useful for many of the applications mentioned above.

Others have previously reported biodegradable gold nanoparticle platforms [20,30,31]. However, these platforms have suffered from issues such as a lack of ability to tune the nanoparticle size, low gold to polymer ratios, the need to use large gold nanoparticles or the use of gold nanoparticles that are not stable in serum when released. In this study we sought to address these issues. We synthesized sub-5.5 nm gold nanoparticles and incorporated them into biodegradable poly-di(carboxylatophenoxy) phosphazene (PCPP) nanospheres to form Au-PCPP nanoparticles. The resulting Au-PCPP would maintain their size and structure in the short-term after injection, allowing for their diagnostic potential to be fulfilled. After this period, the polymer will degrade into harmless byproducts such as phosphate, ammonia, and 4-hydroxybenzoic acid [32–36] and release the small gold cores for excretion (Fig. 1). Our synthesis method allows for control over the size of the Au-PCPP, the amount of AuNP loaded and variation in the surface ligand coating of the AuNP incorporated into the PCPP nanospheres. Varying the payload and nanoparticle size allowed the absorbance to be shifted into the near infrared. We have shown that Au-PCPP are biocompatible and biodegradable *in vitro* and are strong dual imaging contrast agents for CT and PA imaging.

2. Materials and methods

2.1. Materials

Gold (III) chloride trihydrate, sodium borohydride, poly(bis(4-carboxyphenoxy)phosphazene) disodium salt (PCPP, 1 MDa), L-glutathione reduced, 11-mercaptoundecanoic acid (11-MUA), spermine tetrahydrochloride, and titanium oxide were purchased from Sigma-Aldrich (St. Louis, MO). Methoxy-poly(ethylene glycol)-block-poly(L-lysine hydrochloride) (PEG-PLL, PEG MW 5000, PLL MW 4900) was purchased from Alamanda Polymers (Huntsville, AL). HepG2, J774A.1, Renca, and SVEC4-10 cell lines were purchased from ATCC (Manassas, VA). LIVE/DEAD assay kits were purchased from Life Technologies Invitrogen (Grand Island, NY). MTS assay kits (CellTiter 96) were purchased from Promega (Madison, WI). Polyvinyl chloride plastisol (PVCP) was purchased from M-F Manufacturing Co. (Fort Worth, TX, USA).

2.2. Gold nanoparticle synthesis

The gold nanocrystals were synthesized *via* a modification of the method of Turkevich [37,38]. Gold (III) chloride was reduced by addition of sodium borohydride in small batches, followed by surface modification with a capping ligand. For each batch, 8 mg of $AuCl_3$ was dissolved in 100 ml of DI water while stirring. A 5 mg/ml solution of $NaBH_4$ was prepared and 2 ml was added in a dropwise manner. After 20 min of stirring, 1 ml of a 17 mM solution of 11-MUA or glutathione was added as capping ligand. The solution was left to incubate overnight at 4 °C. Using 10 kDa molecular weight cut off (MWCO) tubes, and centrifugation at 2500 rcf, the AuNP suspension was concentrated, washed three times with DI water and suspended in DI water to the desired concentration. The final concentration of AuNP was determined using inductively coupled plasma optical emission spectroscopy (ICP-OES) performed on a Spectro Genesis ICP.

2.3. Au-PCPP nanoparticle synthesis

For PCPP nanoparticle synthesis, 2 ml of 0.1% PCPP in PBS (PH 7.4) was loaded in a 10 ml syringe. A separate 2 ml solution of 2 mg spermine and 0–0.2 mg PEG-PLL in PBS was prepared and loaded in a second 10 ml syringe. The PEG-PLL amount determines the size of the PCPP nanoparticles formed. The two syringes were attached *via* polytetrafluoroethylene (PTFE) tubing (ID: 0.5 mm, OD: 1 mm, microfluidic ChipShop, Jena, Germany) to a microfluidic chip (Herringbone Mixer, microfluidic ChipShop, Jena, Germany) with two 200 μm × 300 μm inlets, a 200 μm × 600 μm staggered herringbone mixer (SHM) channel, and a 200 μm deep × 600 μm outlet directed to a conical tube (Fig. S1). The syringes were loaded on syringe pumps and the solutions mixed at a combined speed of 12 ml/min. The resulting solution was collected in the conical tube and immediately transferred to a 100 ml stirred solution of 8.8% (w/v) $CaCl_2$ in DI water. After 30 min of stirring, the solution was transferred to conical tubes and centrifuged at 600 rcf for 10 min. The supernatant was discarded and the pellet was re-suspended in DI water. After repeating this process three times, the PCPP solution was re-suspended in DI water and stored at 4 °C. For Au-PCPP nanoparticles, the 0.1% PCPP solution was mixed with the desired amount of AuNP suspension solution prior to being loaded into the syringe.