



# Glycol chitosan assisted *in situ* reduction of gold on polymeric template for anti-cancer theranostics

Asifkhan Shanavas<sup>a,\*</sup>, Aravind Kumar Rengan<sup>b,1</sup>, Deepak Chauhan<sup>c</sup>, Liya George<sup>c</sup>, Mukti Vats<sup>c</sup>, Navneet Kaur<sup>a</sup>, Pranjali Yadav<sup>a</sup>, Purvi Mathur<sup>a</sup>, Swaroop Chakraborty<sup>a</sup>, Appidi Tejaswini<sup>b</sup>, Abhijit De<sup>d</sup>, Rohit Srivastava<sup>c,\*</sup>

<sup>a</sup> Institute of Nano Science and Technology, Mohali, Punjab, India

<sup>b</sup> Department of Biomedical Engineering, Indian Institute of Technology Hyderabad, Telangana, India

<sup>c</sup> Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay, Powai, Mumbai, India

<sup>d</sup> Molecular Functional Imaging Lab, Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Tata Memorial Centre, Navi Mumbai, India

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## ABSTRACT

Multifunctional biodegradable nanomaterials that could be used for both imaging and therapy are being researched extensively. A simple technique to synthesize multifunctional nanoparticles without compromising on any of their functionality is a challenge. We have attempted to optimize a two-step procedure of gold coated polymeric template involving 1) Single pot synthesis of PLGA nanoparticles with cationic surface charge using glycol chitosan and 2) *in situ* gold coating for formation of gold coated PLGA nanoshell (AuPLGA-NS). These gold-coated PLGA nanoparticles were explored for photothermal therapy (PTT) and as X-ray/CT contrast agents. Biocompatibility and photothermal cytotoxicity of AuPLGA-NS were evaluated *in-vitro* and results confirmed the therapeutic efficacy of these particles resulting in 80% cancer cell death. Besides, it also showed potential X-ray/CT imaging ability with contrast equivalent to that of Iodine. The results demonstrated that these gold-coated PLGA nanoparticles synthesized by a simple approach could be used as a multifunctional nanosystem for cancer theranostics.

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## 1. Introduction

Gold nanoparticles has attracted much attention due to their unique physical properties combined with chemical inertness, biocompatibility and surface properties which permits conjugation with different chemical moieties [1]. Gold nanoparticles are also well known for their tunable surface plasmon resonance (SPR) ranging from 500 nm to 900 nm. SPR finds application in various techniques like SERS imaging, photothermal therapy, etc. [2,3]. Gold nanoshells are unique structures with very large optical absorption and scattering cross section that helps in absorption or scattering of light specifically at visible and near infrared (NIR) regions of electromagnetic spectrum. NIR absorption of gold nanostructures qualifies them to be used as diagnostic agents and for deep tissue therapy, as there is very low absorption or scattering from tissue

components between 650 nm to 1350 nm. This allows the penetrating light energy to be specifically absorbed by the nanoshells only which will be transduced into thermal radiation increasing the local tissue temperature above 42 °C. This photothermal phenomenon has been extensively utilized for specific ablation of solid tumor tissues [4,5].

Gold nanoshells were previously prepared by seed mediated growth method, where a dielectric core such as silica nanoparticle is first decorated with gold nanoseeds followed by further growth of gold to produce a continuous shell [6]. The thickness and roughness of the shell allows them to absorb at NIR region of the spectrum. For biomedical applications, apart from silica templates polymeric templates such as Poly (lactic-co-glycolic acid) (PLGA) nanoparticles were used for gold nanoshell fabrication to allow encapsulation of a drug molecule in the biodegradable core. Gold coated PLGA nanoparticles exhibiting NIR absorbance were first reported by Yang et al., in 2009 where they functionalized Doxorubicin loaded PLGA nanoparticles with cysteamine providing a thiol group for gold nanoseeds to cling onto for further growth to provide a continuous nanoshell on the polymeric nanoparti-

\* Corresponding authors.

E-mail addresses: [asifkhan@inst.ac.in](mailto:asifkhan@inst.ac.in) (A. Shanavas), [rsrivasta@iitb.ac.in](mailto:rsrivasta@iitb.ac.in) (R. Srivastava).

<sup>1</sup> Equally contributing authors.

cle that absorbs light at around 800 nm. Further with conjugation of anti-EGFR antibody to the nanoshell surface, the hybrid nanostructure proved to be an excellent material for receptor targeted photothermal chemotherapy in mammalian cancer cell lines [7]. An alternative to gold coating on polymeric nanoparticles has been reported recently where instead of thiol functionalization, cationic polyelectrolyte chitosan was used to recruit anionic gold nanoseeds for further growth of gold. Also, gold nanoshell along with NIR emitting dye, Indo cyanine green (ICG) was used for combined imaging and therapy [8]. An extension of this work with magnetic nanoparticles loaded in PLGA core prior to chitosan functionalization and gold coating helped in magnetic resonance imaging & guided the nanostructure with folic acid for targeted photothermal chemotherapy [9]. Our group has previously demonstrated growth of gold nanoshell on biodegradable liposomes for photothermal mediated cancer theranostics [10,11].

The seed mediated growth on PLGA nanoparticles involves several preparatory and synthetic steps such as (i) fabrication of template, (ii) functionalization of template surface with thiol or cationic groups, (iii) synthesis of gold nanoseeds, (iv) preparation of gold growth solution and (v) attachment of nanoseeds followed by growth into a continuous nanoshell. The present work is an attempt to optimize a two step procedure of gold coated polymeric template. This involves single pot synthesis of PLGA nanoparticles with cationic surface charge and *in situ* coating of gold nanoshell. Herein, we have chosen glycol chitosan as the aqueous soluble cationic agent for PLGA surface functionalization. The concentration of glycol chitosan plays crucial role in generation of the nanoshell, as at higher concentration they form polypod structures due to template aggregation (Fig. 1). The as prepared gold-coated PLGA nanoparticles were explored further for photothermal therapy and as X-ray/computed tomography contrast agents.

## 2. Materials and method

### 2.1. Materials

PLGA (50:50, 40KDa) was a kind gift from Corbion Purac Biomaterials, Netherlands. Polyvinyl alcohol (145KDa), Gold (III) chloride trihydrate, Glycol chitosan, d- $\alpha$ -tocopheryl polyethylene glycol 1000 succinate (TPGS), L-ascorbic acid were purchased from Sigma Aldrich, USA. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS) and antibiotics were procured from HiMedia, India. All chemicals used were of analytical quality. For all experiments, ultra-pure water from Evoqua Water Technologies, USA was used. Glass wares were cleaned with 100% aqua regia and rinsed with ultra-pure water before use. 808 nm NIR diode laser, 500 mW from Shanghai Dream Lasers Technology, China was used for photothermal experiments.

### 2.2. Synthesis of glycol chitosan functionalized PLGA nanoparticles

For synthesis of cationic PLGA nanoparticles, 25 mg of PLGA was dissolved in 5 ml tetrahydrofuran (THF) to prepare the organic phase. The aqueous phase was prepared by dissolving 2.5 or 0.25% (w/v) of glycol chitosan and 0.02% (w/v) TPGS in 2% (w/v) polyvinyl alcohol solution. Organic and water phase were mixed using a syringe pump at flow rate of 100  $\mu$ l/min and the resulting solution was allowed to stir for 2 h. The emulsion formed was sonicated for 10 min and was left overnight to stir for solvent evaporation. Nanoparticles formed were pelleted down at 4 °C, 15000 rpm for 60 min and washed thrice with water. Characterization of the glycol chitosan coated PLGA nanoparticles was performed using FEG-SEM (JEOL JSM-7600F), DLS (Brookhaven BI-200SM Goniometer)

and Zeta Potentiometer (Brookhaven ZetaPlus) to understand the nanoparticle's morphology, shape, size and cationic surface charge respectively.

### 2.3. In situ gold coating of cationic PLGA NPs

For *in situ* coating of gold on PLGA nanoparticles, 100  $\mu$ l of 5 mM gold hydrochloride was mixed with 200  $\mu$ l of 2 mg/ml cationic PLGA nanoparticle solution. 500  $\mu$ l of 20 mM ascorbic acid was added for *in-situ* reduction of gold ions over cationic PLGA nanoparticle surface. Characterization of the gold-coated PLGA nanoparticles (AuPLGA-NS) was performed for size and charge using DLS and Zeta potentiometer respectively whereas gold coating was confirmed using FEG-TEM (JEOL, 2100F), EDAX (Oxford instruments) and UV-vis spectroscopy (Lambda 25, Perkin Elmer).

### 2.4. Photothermal transduction

Light to heat converting efficacy of synthesized AuPLGA-NS were studied with the help of 808 nm NIR laser (500 mW, Shanghai Dream Laser). 200  $\mu$ l of AuPLGA-NS and controls (*i.e.* water and PLGA nanoparticles) were added in triplicates in a 96 well plate floating over a water bath at 37 °C. When the test and control solutions in the wells have attained temperature of 37 °C, NIR laser was irradiated over the wells. Samples were separated from one another to avoid any heat transfer. The rise in temperature was recorded with the help of a digital infra-red thermometer (Oakton Mini-InfraPro Infrared Thermometer) at different time intervals of 0, 2, 4, 6 and 8 min. Temperature increment was plotted against time of laser irradiation with baseline of 37 °C.

### 2.5. In-vitro biocompatibility test

Mouse fibroblast cells (L929) were seeded in a 96-well plate at concentration of  $\sim 10^4$  cells per well for *in-vitro* biocompatibility studies. After allowing 24 h for cell attachment, AuPLGA-NS were dispersed in fresh media at concentration ranging from (50  $\mu$ g ml<sup>-1</sup> to 200  $\mu$ g ml<sup>-1</sup>). 200  $\mu$ l of different concentrations of AuPLGA-NS in complete culture medium were added in triplicates. After 24 h, the existing media was carefully removed, 200  $\mu$ l media containing 10% (w/v) MTT dye was added and incubated for 5 h at 37 °C for formation of formazan crystals. Absorbance values at 570 nm and 690 nm were collected and percentage cell viability was calculated in reference to untreated cells.

### 2.6. In-vitro photothermal treatment

For the *in-vitro* photothermal therapy, breast cancer cells (MCF-7) were seeded in 96-well plates at cell density of  $\sim 10^5$  cells per well one day prior to irradiation experiment. Cells were washed thrice with PBS and following groups were treated accordingly: no treatment, NIR laser only (4 min of irradiation), AuPLGA-NS only and AuPLGA-NS with laser (4 min irradiation). Following the treatment, the cells were incubated at 37 °C for 24 h. Cells were washed with PBS and MTT dye was added for quantitative analysis of cell death. After 5 h, crystals formed were solubilized using DMSO and absorbance was taken using microplate reader. For qualitative examination, the protocol followed was similar to the one used for quantitative analysis except for the addition of propidium iodide (PI, dead stain) dye to visualize dead cells. Cells were observed using a Nikon Ti Eclipse fluorescence microscope of

### 2.7. X-ray/CT imaging

For phantom X-ray/CT imaging study, AuPLGA-NS (1 mg), Iodine (5 mg, positive control) and Water (100  $\mu$ l, negative control) were

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