Chitosan Coated Gold Nanoparticles for Hepatocellular Carcinoma Treatment via Photothermal Therapy

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Statement of Purpose: Gold nanoparticles (AuNPs) are attracting enormous attention in biomedical field for many applications including immunoassay, drug delivery, contrast enhancement and hyperthermia for tumor therapy. Photothermal therapy is a promising approach to cancer treatment, and the chemical design and synthesis of gold NPs is critical for this application. The purpose of this work is to develop a more potent photothermal therapeutic agent which will be specific for the treatment of Hepatocellular Carcinoma (HCC). To achieve this goal the toxicity of bare particles as well as those coated with different forms of chitosan were evaluated using an HCC cell line (Hep-G2) and controls cell line of human dermal fibroblast (HDF). Following toxicity evaluation, the degree of photothermal ablation at varying laser power was assessed for both cell lines.

Methods: Gold (AuNPs) with strong absorption in the near-infrared (NIR) region were prepared by the reaction of 2 mM $HAuCl_4$ with 3 mM $Na_2S_2O_3$. To obtain the biocompatible coating on the AuNP surface, chitosan (CS) and carboxymethylated chitosan (CMCS) were added into the $HAuCl_4/Na_2S_2O_3$ reaction solution when reaction is complete. Carboxymethylated chitosan (CMCS) was synthesized using the method described in reference [1]. Thiol terminated poly (ethylene glycol) (PEG) coated AuNP and bare AuNP were used as control samples. Also, AuNP with blended coating (CS+PEG) was investigated. The optical absorbances of AuNPs were measured by a UV-Visible spectrophotometer, (Cary 50 Bio, Varian). The hydrodynamic size and zeta potential were measured by Zetasizer (Nano-ZS90, Malvern). A FEI Tecnai F30 transmission electron microscope (TEM) operated at 200 KV was used to examine the particle's size and coating morphology. Chitosan coating was characterized using an IVATIR 360 FT-IR apparatus. Toxicity and laser ablation studies of AuNPs with CS, PEG, (CS+PEG), and CMCS coatings were evaluated on HDF and HepG2 cell lines. In toxicity studies AuNPs with different coatings were incubated with cells for 24 or 48 h without laser activation. Results were evaluated by live/dead stain images, silver stain images. Cellular activity was investigated by using a mitochondrial activity assay (MTT). Laser ablation was performed based on methods described in reference [2]. HDF and HepG2 Cells were incubated with Au NPs for 3h, media replaced and exposed to 817nm NIR laser for 2 min at power densities of 1, 3, & 5 W/cm^2. After 8h incubation live/dead staining was performed to examine degree of photothermal damage to cell line.

Results: From the spectral, TEM and DLS measurement, the NIR peak wavelength of as synthesized AuNPs as well as the size of the particles were correlated and showed an approximately linear increase with the molar ratio of $HAuCl_4$ /$Na_2S_2O_3$. This allowed precise modulation of the absorbance of Au NPs in the NIR region for subsequent studies. Figure1 shows the mitochondrial activity of the HepG2 and HDF cells after 24 h with different AuNPs. Hep-G2 cells show slightly lower activity and greater death when incubated with gold particles which have the CS+PEG blended coatings as compared to control cells and other particle formulations. Figure 2 presents the live/dead image of cells after 2 min laser ablation treatment with 5 W/cm^2 NIR laser activation. We can see that CMCS coated AuNPs allowed ablation of all HePG2 cells, with little damage to HDF cells.

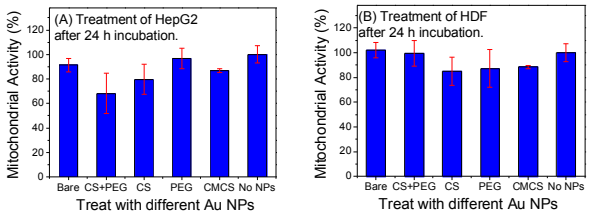

Figure 1 Normalized average mitochondrial activity of HepG2 and HDF after 24 h incubation with AuNPs.

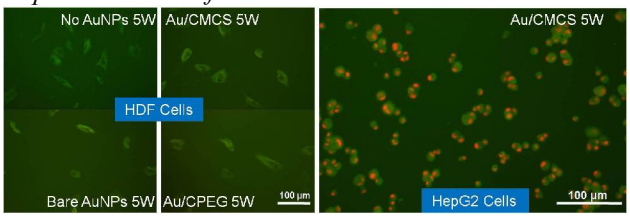

Figure 2. Live/dead stain image of HDF and HepG2 cells after the laser ablation treatment (5W/cm^2, 2min).

The percentage of dead cells after incubation with different AuNPs and laser ablation is shown in Figure 3. The AuNPs with the CMCS and CS+PEG coatings show the high efficiency for ablation of HepG2 cells, implying the surface coating may play an important role for the gold NPs employed as the photothermal therapeutic agent.

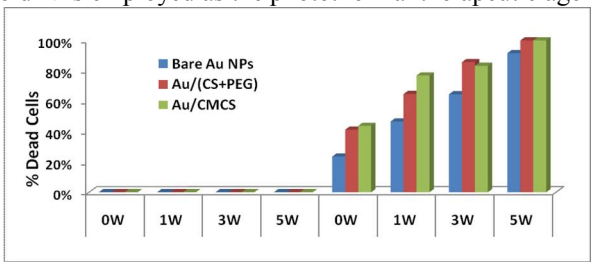

Figure 3: Death percentage of HDF and HepG2 cell treated with different Au NPs after the laser ablation.

Conclusions: Chitosan coated gold NPs with tunable NIR absorption were successfully synthesized. Toxicity and laser ablation studies show that AuNPs are more toxic to cancer cells than to normal cells. With suitable NIR laser activation, AuNPs with appropriate coatings may preferentially kill HCC cell lines with low or no damage to normal cells.

References:

1. Chen XG. Carbohydrate Polymers, 2003, 53: 355-359.
2. Gobin AM. Nano Lett. 2007, 7:1931-1933.