Stability of Zwitterionic-Modified Gold Nanoparticles in Complex Media: Effect of Surface Packing Densities

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Introduction:

Nanoparticles have shown potential to be tools for advancement in the medical world, such as in drug delivery, diagnosis and other areas. Despite their promise, one of the largest obstacles to these applications is nonspecific protein adsorption, which can result in cellular uptake, nanoparticle aggregation, immune system response, and other disastrous problems for *in vivo* applications. The current solution for combating this protein adsorption is to coat the nanoparticles in a non-fouling poly(ethylene glycol) (PEG) or oligo(ethylene glycol) (OEG) shell. Recent studies have shown zwitterionic materials such as poly(carboxybetaine) (pCB) have ultra-low fouling properties. To achieve high surface resistance to nonspecific protein adsorption, the surface packing density and film thickness are important.

In this study, an atom transfer radical polymerization (ATRP) reaction was employed to coat nanoparticles with pCB. Different sized particles and surface packing densities were obtained by adjusting the ratio of reactants and the reaction time in the ATRP process.

Experimental Procedure:

Synthesis of Bare and PEG-Coated Gold Nanoparticles (GNPs). Bare GNPs and PEG-5000-SH-coated GNPs were prepared by using a previous method [1].

Synthesis of Initiator-Modified GNPs. Initiator-modified GNPs were synthesized according to literature [2]. After reaction, the system was dried with a Rotary evaporator and washed with ethanol to precipitate the GNPs. The precipitate was collected and re-dispersed in acetone.

ATRP Reaction. In a typical reaction, 200 mg CBMA, 61.707 mg 2,2 bipyridiyl, 28.533 mg copper(I) bromide, and 4.4 mg copper(II) bromide were placed in a reaction tube. The tube was thoroughly purged by vacuum and flushed with nitrogen before N_2-purged methanol was added to the flask via syringe. The reaction mixture was homogenized by agitation using a vortex mixer and ultrasound for 5 min and 1 min, respectively. The deoxygenated initiator-coated GNPs (0.4 mL) in toluene were mixed with the above solution under N_2 protection. The final mixture was stirred (13, 200 rpm) at room temperature. In this reaction 1:3, 1:1, and 2:1 volume ratio of methanol to acetone was applied.

Analysis. After polymerization, the pCB-GNPs were washed through centrifuging and re-dissolved in water. Using diffraction light scattering (DLS) and a syringe filter the size of the nanoparticles in solution was measured. Confocal laser scanning microscopy (CLSM) was further applied to gain some insight into the interaction between cells and nanoparticles. In this experiment, COS-7 cells were exposed to different ATRP product ratios and their interactions observed under microscope.

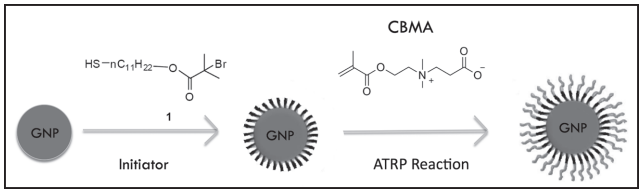

Figure 1: Scheme of the preparation of initiator-coated GNPs and pCB-coated GNPs.

Hydrodynamic sizes of GNPs in different media.

MeOH:Acetone	Bare GNPs	PEG-GNPs	pCB-GNPs 2:1	pCB-GNPs 1:1	pCB-GNPs 1:3
Reaction Time	-	-	2 Hours	2 Hours	2 hours
Size: H_2O	18.5 nm	53.4 nm	50 nm	40 nm	65 nm
Size: Lysozyme 1mg/mL	160 nm	85 nm	95 nm	40 nm	65 nm
Size: 10% Serum in PBS	50 nm	54 nm	55 nm	55 nm	65 nm

	Lysozyme 1mg/mL	10% Serum in PBS
Size	4 nm	55 nm

Table 1: Hydrodynamic sizes of GNPs in different media.

Results:

Stability of Polymer-Coated GNPs in Complex Media. As observed from Table 1, the hydrodynamic size of PEG-GNPs in water was 53.4 nm. However, after the addition of lysozyme solution, nanoparticles showed an increase of 20 nm in size compared to those in water. In the case of pCB-GNPs, their diameters did not change, indicating the high *in vitro* stability of pCB-GNPs in high ionic strength or in the presence of proteins under physiological conditions. Results also show that pCB-GNPs with different packing densities exhibit different stability when exposed to complex media and those with 1:3 ratio exhibits the best performance.

Cell Morphology. Cell density and morphology was observed by microscopy. In the bare-GNP case there were few to no living cells on the surface, whereas all other samples did not inhibit growth. PEG-GNPs and pCB-GNPs (1:1) exhibited similar morphology, widely dispersed and fewer cells. PCB-GNPs (1:3) and pCB-GNPs (2:1) allowed greater cell growth, and the highest density of cells was observed in the pCB-GNPs (2:1) ratio.

Conclusions and Future Work:

Conclusions. In this work, we investigated the stability and cell interactions of pCB-coated GNPs coated with different surface packing densities. It was found that the surface resistance to nonspecific protein adsorption highly depends on the surface packing density. We also observed GNPs made with different ATRP ratios caused dissimilar cell morphology in their respective cultures. In addition, when mixed with cells, the bare GNPs proved to be toxic.

Future Work. We will continue to focus on the ratios that have shown the most promising experimental results. This focus will include repeating experiments and working to understand why a particular ratio works, or does not work. Future research into the best performing ratios will yield further insights as to the effects of SPDs on coated GNPs. Ideally, results will help prove that pCB is a more effective and longer circulating nonfouling coating.

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References:

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