Functionalizable and ultra stable nanoparticles coated with zwitterionic poly(carboxybetaine) in undiluted blood serum

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Statement of Purpose: Nanoparticle-based biotechnology is quickly heading to the forefront of drug delivery, diagnosis, and other areas. 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. Recently, zwitterionic materials such as carboxybetaine methacrylate (CBMA) have been shown to have ultra-low fouling properties. Furthermore, carboxybetaine groups are particularly attractive for convenient and effective protein immobilization via simple NHS/EDC chemistry under mild conditions, which is necessary to immobilize a bio-recognition element for targeting specific disease areas or selectively interacting with cells or biomolecules. Herein, we introduce polyCBMA surface chemistry presenting an abundance of functional groups for ligand immobilization in a nonfouling background all in one material for nanoparticles.

Methods: Initiator (11-mercaptoundecyl 2-bromoisobutyrate)-coated gold nanopartilces (SH-GNPs) were prepared following a previous method. [1] Then atom transfer radical polymerization (ATRP) was employed to coat GNPs. Different kinds of polymer-coated GNPs including oligo(ethylene glycol) methyl methacrylate (OEGMA)-GNPs via ATRP (OA-GNPs) and polyCBMA-GNPs via ATRP (CA-GNPs), along with bare GNPs, were prepared. Next, hydrogel was introduced to coat GNPs. The crosslinkers are ethyleneglycol dimethacrylate (EGDMA) and CBMA-based crosslinker (CBMAX) for OEGMA-hydrogel-GNPs (OC-GNPs) and polyCBMA-hydrogel-GNPs (CC-GNPs), respectively. The stability of all kinds of polymer-coated GNPs and bare GNPs was investigated in different media such as saline solution, single protein solutions, and diluted/undiluted blood serum at 25 °C and 37 °C by both UV–vis spectroscopy and dynamic light scattering (DLS). Then, a candidate cancer biomarker (ALCAM) was applied as a model antibody for ligand immobilization via NHS/EDC chemistry.

Results: After mixing with lysozyme or NaCl solution, the plasmon resonance peak of bare GNPs had a dramatically red shift. Only a slightly shift was observed for OA-GNPs and OC-GNPs, whereas the peak was the same for CA-GNPs and CC-GNPs. When polymer-coated GNPs were exposed to 10% blood serum, there was no agglomeration and all four samples showed good stability without obvious size increase after 72 h. Stability further studied in undiluted (100%) human blood serum at 37 °C (as indicated in Figure 1) showed OA-GNPs had a size increase of~140 nm at the end of 72 h, indicating significant protein adsorption and particulate aggregation. Although OC-GNPs were not stable in such extreme situations, the addition of EGDMA helped to enhance the stability. The diameter increase was ~30 nm after an incubation period of 72 h. However, for the two kinds of GNPs protected with polyCBMA coating (CA-GNPs and CC-GNPs), the interactions between proteins and nanoparticles did not cause any agglomeration and the particle sizes after their separation from blood serum proteins were almost the same as those without serum, indicating their excellent stability at harsh conditions. The stability in 10% and 100% blood serum was confirmed by UV–vis spectroscopy. Furthermore, anti-ALCAM can be easily conjugated to polyCBMA surfaces, which possessed effective detection to ALCAM in serum. The antibody/antigen ligand density on GNPs can be easily controlled by the antibody/antigen concentration.

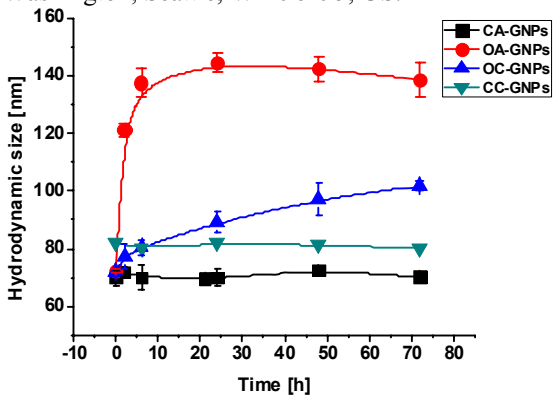

Figure 1. Hydrodynamic size of GNPs coated with different polymers in 100% blood serum.

Conclusions: A functionalizable and stable surface platform for nanoparticles has been demonstrated. Results show that polyCBMA-coated GNPs have superior performance in undiluted blood serum over GNPs with other conventional coatings including PEG, although their performance in 10% blood serum is comparable. This indicates that 10% serum commonly used to evaluate the stability of nanoparticles is not sufficient. Undiluted blood serum is recommended to screen nanoparticles before *in vivo* experiments. This new criterion will allow one to screen NPs effectively before *in vivo* experiments and save unnecessary *in vivo* work. In addition, the introduction of hydrogel coating could also be applied to increase the stability of nanoparticles in undiluted blood serum. Furthermore, bio-recognition elements such as anti-ALCAM can be easily conjugated to polyCBMA via NHS/EDC method. There are many more functional groups available for ligand immobilization onto polyCBMA. Ligand immobilization density can be varied by adjusting antibody/antigen concentrations. The uniqueness of polyCBMA (i.e., ultra-low fouling and multiple functionalities) makes this zwitterionic biopolymer very attractive as next-generation nanoparticle coatings for *in vivo* targeting drug delivery and diagnostics.

References: 1 Dong HC. J. Am. Chem. Soc. 2008; 130: 12852-12853.