Synthesis, Integrity, and Biocompatibility of scFvD2B Coated Gold Nanoparticles in Human Blood

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Abstract: In this study, ~ 26 nm gold nanoparticles (AuNPs) were synthesized in water and characterized using ultraviolet-visible spectroscopy (UV-Vis), dynamic light scattering (DLS) and Zeta potential analysis. AuNPs were coated with Thiol functionalized polyethylene glycol **(**PEG-SH**)** and scFvD2B (single chain antibody fragment of D2B MAb) recognizing an extracellular epitope of the human prostate specific membrane antigen (hPSMA). scFvD2B binds PSMA and induces its endocytosis, thereby marking PSMA as a docking site for the delivery of therapeutic agents. AuNPs successful coating was confirmed by UV-Visible spectroscopy via a surface plasmon resonance (SPR) band shift of about2 nm for scFvD2B-AuNPs and 4 nm for PEG-scFvD2B-AuNPs. Likewise, DLS revealed an increase in the citrate-AuNPs size from 26 to ~29 nm for scFvD2B-AuNPs and from 29 to ~50 nm for PEG-scFvD2B-AuNPs. Moreover, Zeta potential of citrate-AuNPs increased from -34 mV to -19 mV for scFvD2B-AuNPs and from -19 mV to -3 mV for mixed PEG-scFvD2B-AuNPs. Conjugated AuNPs stability *in-vivo,* was characterized post-incubation with human blood plasma using gel electrophoretic separation, zeta potential and DLS measurements. PEG-AuNPs and PEG-scFvD2B-AuNPs showed similar reduced variation in charge and binding affinity to plasma proteins. However, citrate-AuNPs and scFvD2B-AuNPs revealed a drastic change in size compared with their pre-plasma incubation state. Additionally, neutrophil function test and pyridine formazan extraction showed lower neutrophils activation by PEG- and PEG-scFvD2B-AuNPs (9 &10 %) compared with citrate and scFvD2B-AuNPs (~14%). All AuNPs were blood-compatible, with < 10% hemolysis. In conclusion, our data provide a novel scFvD2B-AuNPs (± PEG) with proof that PEG-scFvD2B-AuNPs serve as promising vehicles for drug delivery with minimal protein adsorption affinity, insignificant charge and size variation, low immunorecognition, and reduced hemolytic activity.

Keywords: gold nanoparticles; prostate cancer; PSMA; D2B antibody; scFvD2B antibody; polyethylene glycol **(**PEG**)**