The use of Werner complexes as remarkably versatile bioconjugation reagents in the synthesis of redox repsonsive albumin nanoparticles containing camptothecin derivatives

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ABSTRACT

We have begun applying classic coordination chemistry to the bioconjugation of amine-containing (bio)molecules in the context of targeted drug delivery. In addition to being an efficient conjugation strategy, the chemistry is completely reversible allowing for the synthesis of redox responsive materials. Co(3+) is inert to ligand exchange; however, upon reduction to Co(2+) labile ligand exchange occurs, which provides an opportunity to capitalize on the reducing nature of cytosol and hypoxic tumor microenvironments as stimuli for degradation of the delivery vector and concomitant release of therapeutic payload. We have applied this methodology to the synthesis of albumin nanoparticles (10-500 nm) and examined particle uptake in gastric carcinoma cells (SNU-5) by image-based flow cytometry. The particles were further loaded with camptothecin derivatives via either passive encapsulation (SN-38), or cobalt-mediated bioconjugation to albumin (topotecan). Details of drug loading and release will be discussed.

Keywords: nanoparticle therapeutic, cancer, cobalt, albumin

1 INTRODUCTION

SN-38 (7-ethyl-10-hydroxycampothecin) and irinotecan are campothecin derivatives that target topoisomerase I, an enzyme that plays a vital role in DNA replication and transcription.[1] Irinotecan has been approved for the treatment of metastatic colorectal cancer and is commercially available as Camptosar (Pfizer Inc, New York, New York).[2] SN-38 is the active metabolite of irinotecan, which exhibits a much stronger therapeutic effect (100-1000x compared to irinotecan).[3-4] However, despite its promising antitumor efficacy toward a wide range of experimental tumor models, SN-38 has limited application due to its poor aqueous solubility. The development of innovative nanoparticulate systems that allow for high drug loading and improved stability have remained a challenge.[5]

Nanoparticle therapeutics derived from albumin have emerged as promising tool in oncology because of its biocompatibility, lack of immunogenicity, and biodegradability.[6] Albumin is highly soluble in water, exhibits excellent stability, and is easily purified. Albumin posseses a variety of binding sites for small molecules, and has therefore been implicated in the binding and transport of a variety therapeutics.[7-8] Recent studies have also shown that some tumor cells consume large amounts of albumin as nutrient source to support for their rapid growth.[9] Albumin is especially attractive for targeting cancer cells because the cell surface receptor responsible for binding albumin, the 60kDa glycoprotein (gp60) (albondin), is overexpressed in a host of cancer cell types.[10]

2 RESULTS

2.1 Synthesis and Characterization of SN-38 loaded Co^{3+}-Alb NPs

Bovine serum albumin was prepared in ultrapure water as a 10mg/mL solution. Cobalt(II) chloride hexahydrate (100mM) and 25mM of NaOH solution was also prepared in ultrapure water. In a small glass vial, 200uL of bovine serum albumin (10 mg/mL aqueous) was mixed with 50uL of NaOH (25mM) and the solution was colorless. Cobalt(II) chloride hexahydrate (50 L, 100 mM) was added and the solution immediately turned blue and became turbid. The solution was sonicated for 5s and left undisturbed for 15 minutes at room temperature. Hydrogen peroxide (5 L, 30% aqueous) was added to the solution followed by 1 mL of ultrapure water. The solution immediately turned yellow without any changes in turbidity. The solution was centrifuged (Eppendorf, model #5810R) for 1 minute at 21,000 x g and the supernatant was discarded. The pellet was then resuspended into 1 mL of water, centrifuged at 21,000xg and the supernatant was discarded. The pellet was suspended in water and dynamic light scattering (DLS, Microtrac Nanotrac Ultra) was used to measure the size of the nanoparticle. The nanoparticles were 140-200 nm in diameter (Figure 1).

SN-38 (7-ethyl-10hydroxycampothecin, TCI Development Co., Shanghai, China) was dissolved in dichloromethane/ethanol (1:1) at 10mg/mL. An emulsification/solvent evaporation method was used to incorporate SN-38 into Co-Alb NPs, which was accomplished by mixing 5 mL of a Co-alb NP solution with 2.5 mL of the SN-38 solution. Sonication was applied for 2-3 minutes followed by stirring at RT for three hours to allow all the organic phase to evaporate. The solution was

then centrifuged at 21,000 x g for 5 minutes. The supernatant, which contained unbound SN-38 was removed and analyzed to determine drug loading. The pellet was then washed three times with ultrapure water. The particles were re-suspended in water and analyzed by DLS (Figure 1).

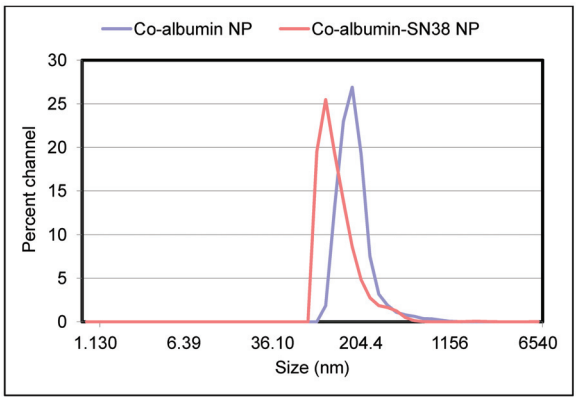

Figure 1. Dynamic Light Scattering results for Co-Alb NPs.

To determine SN-38 loading, the supernatants collected after centrifugation were analyzed by UV/Vis (Synergy 2, Biotek USA) based on a calibration curve of SN-38 (380 nm). Drug loading capacity and encapsulation efficiency was calculated according to Equations 1 and 2 below. Encapsulation efficiency was observed to be at 94% and loading capacity was approximately 31%. SN-38 loaded Co-Alb NPs exhibited minimal changes in size upon incubating in PBS; however when incubated in PBS plus reduced glutathione (GSH 10mM), the nanoparticles degraded rapidly (Figure 2). Representative SEM images of the particles are shown in Figure 3.

$$\text{Encapsulation Efficiency} = \frac{\text{Total drug - residual drug}}{\text{Total drug}} \times 100 \quad (1)$$

$$\text{Drug loading capacity} = \frac{\text{Loaded drug}}{\text{Total weight of NPs}} \times 100 \quad (2)$$

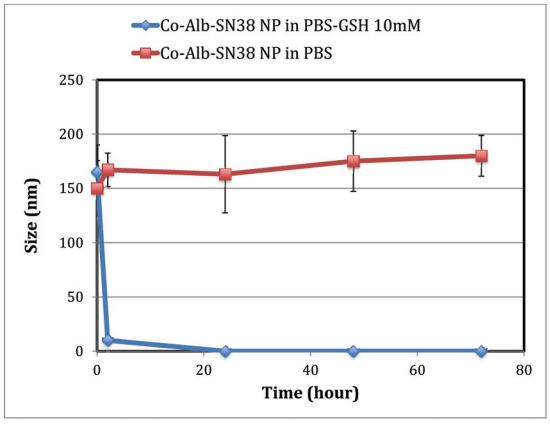

Figure 2. Stability of SN-38 loaded Co-Alb NPs

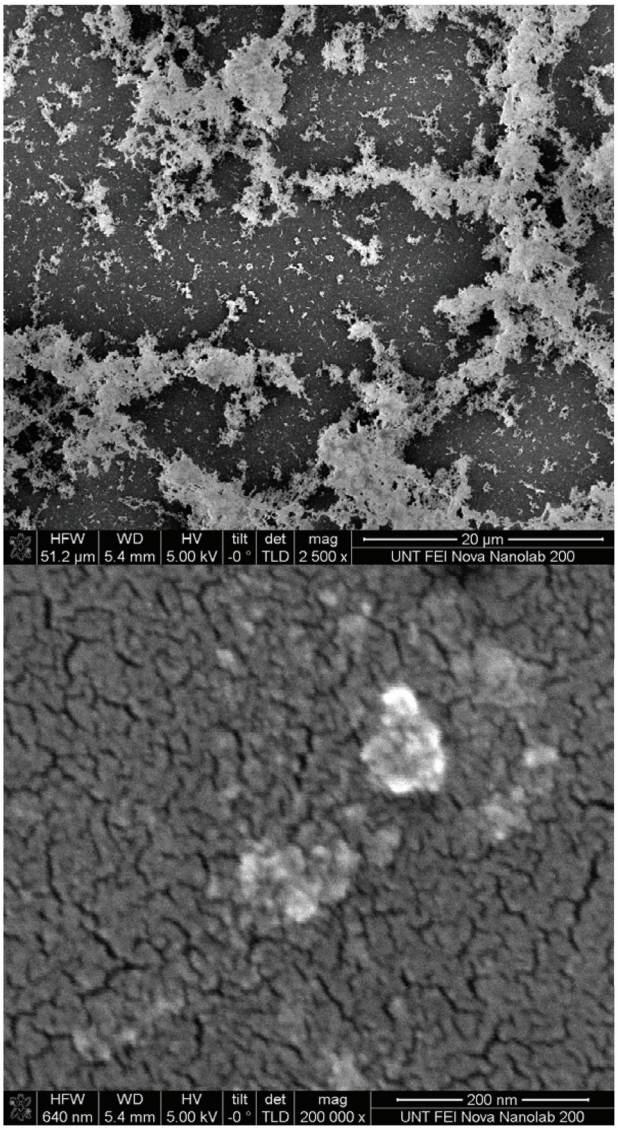

Figure 3. Representative SEM images of Co-Alb NPs.

3 CONCLUSIONS

SN-38 was successfully encapsulated in Co-Alb NPs. Drug loading could be achieved at very high levels (>30 wt%) in a highly efficient manner (>90%). The particles exhibited excellent stability under physiological conditions, but degraded rapidly under reducing conditions making this a promising formulation for further preclinical testing.

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