Plasma Protein Interactions with Bovine Serum Albumin Nanoparticles

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Statement of Purpose: Site-specific delivery vehicles for therapeutics are actively being sought. These vehicles would greatly increase therapeutic efficacy while also reducing the quantity, and thus cost, of the therapeutics themselves. However, use of many delivery vehicles is often limited due to the general host response mounted against these foreign bodies by the host. Protein-based nanoparticles may prove to be viable materials because they can be biocompatible, facilitate cell uptake, are naturally eliminated, and have negligible side-effects. As protein adsorption initiates many deleterious host responses, it is crucial that the adsorbed protein profile be investigated as a function of the physiochemical properties of the material. This fundamental knowledge is critical for the development of all peptide based biomaterials.

Methods: Bovine serum albumin (BSA) nanoparticles were created using coacervation as described in the literature (1, 2). These nanoparticles were coated with 4.2 or 24kDa poly-L lysine (PLL) using the procedure previously described in literature (3). Coated nanoparticles were incubated at in human plasma, according to established protocols (4). After incubation, the nanoparticles were centrifuged to remove excess plasma and washed using a series of centrifugation and resuspension (in PBS) steps. The resuspended solution was then incubated in 2% SDS in PBS in order to solubilize adsorbed proteins. The system was centrifuged again to remove excess SDS. Western blotting was carried out in order to identify the proteins removed from the nanoparticle surface as well as to determine which, if any, plasma proteins remained on the nanoparticle surface after the SDS treatment.

Table 1. Adsorbed amount of plasma proteins determined using Western Blot analysis for BSA nanoparticles coated with 4 and 24 kDa PLL.

Protein Adsorbed	4.2 kDa	24 kDa
IgG (55 kDa)	*	*
IgG (27 kDa)	**	***
Human Albumin (66 kDa)	**	***
Plasminogen	**	***
Complement 3 (70 kDa)	***	***
Complement 3 (42 kDa)	*	*
Transferrin	***	***
Fibrinogen (Alpha 68 kDa)	***	***
Fibrinogen (Beta 56 kDa)	***	***
Fibrinogen (Gamma 48kDa)	***	***
Fibronectin	***	*****
Alpha 1 antitrypsin	****	****
Prothrombin	**	**
Antithrombin	****	****

Results: Preliminary results indicate that certain plasma proteins do adsorb to the nanoparticle surface and are easily removed by SDS. These proteins are listed in Table 1. Proteins which showed no response to the BSA surface include light and heavy kininogen, Factor 1, IgG heavy chain, Factor XII, Factor XI, beta lipoprotein, alpha$_2$-macroglobulin, hemoglobin, thrombin, protein C, vitronectin, protein S and prekallikrein. The molecular weight of the PLL has no effect on the adsorption of plasma proteins. Further analysis has indicated that after the 2% SDS incubation not all adsorbed proteins are removed from the nanoparticle surface. Unexposed nanoparticles show banding at about 66kDa on an SDS-PAGE gel yet there are many bands whose molecular weights are nowhere near 66kDa. Ongoing analysis is being carried out to remove these tightly bound proteins from the surface of the nanoparticles so that they may be identified.

Conclusions: Based upon our current results it has been shown that our current BSA and PLL nanoparticles do accumulate a layer of adsorbed protein on their surface. Some of these proteins are loosely bound while others are more tightly bound; so much so that a more vigorous washing procedure could be used. Because a layer of protein adsorbs onto the surface of the nanoparticles it is possible that the positively charged BSA and negatively charged PLL do not cancel each other out. These PLL molecular weights do stabilize the nanoparticles but do not carry enough charge to overcome the inherent charge of the BSA. In order to better understand the physiological process at play due to plasma protein interactions with these nanoparticles it is necessary to examine the adsorbed proteins on a case-by-case basis. Fibronectin, for example, is involved in the formation of clots along with fibrin. The notion that clotting could be activated by these nanoparticles is supported by the presence of fibrinogen and prothrombin loosely bound to the surface. In addition to a prospective clotting response, because human IgGs have been found bound to the nanoparticle surface, this suggests that a host would be successful in mounting an immune response against the BSA and PLL particles. With complement factor 3 present on the surface of the nanoparticles, it is possible that the alternative pathway of complement has been activated. Further analysis will clarify this by identifying whether or not factor 3 has been cleaved or not. Future work regarding these plasma protein interactions could include using a stronger detergent to remove the more tightly bound entities. The interplay between BSA and larger PLL should also be examined to determine if the inherent charge of BSA can be neutralized.

References: 1(Weber, C., et al., Int. J. Pharm. 2000, 194:91–102) 2 (Langer, K., et al. Int. J. Pharm. 2004, 257:169–180) 3 (Wang, G., et al., Pharm Res 2008, 25:2896–909) 4 (M. E. Price, et al., Biochim. Biophys. Acta 2001, 191-205)