Complete surface control of peptide nanospheres with detachable and attachable polymer brush

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Statement of Purpose: Nanospheres have been widely studied for biomedical and biochemical applications such as drug delivery, diagnosis and imaging. To control their biodistribution, their surface characteristics including stealth property and biomolecules sorbability play an important role. The fabrication of controlled polymer brushes on the nanosphere surface represents one of the most effective approaches. Recently, we reported a novel method for the preparation of the peptide nanospheres with a high density poly(ethylene glycol) (PEG) brush layer.[1-4] The peptide nanospheres were synthesized by the one-step polymerization of L-phenylalanine *N*-carboxy anhydride with the dual initiators of *n*-butylamine and NH_2-monoterminated PEG (NH_2-PEG). The obtained peptide nanospheres showed amazing stealth properties due to the high density of PEG brush (0.21-1.8 chains/nm). Moreover, the target protein could be immobilized onto their surface functional groups. Developing this idea further, the surface properties of the peptide nanospheres are expected to be tuned through the control of surface PEG brush density. Here, we report the synthesis and unique properties of PEG-SS-peptide nanospheres possessing disulfide-conjugated high-density PEG brush layers (Figure 1).[5] The surface PEG chains were completely controlled from 100% to 0% based on thiol-disulfide interconversion. Furthermore, the PEG-SS-peptide nanospheres expressed bioinert and hemolytic activities depending on their PEG densities.

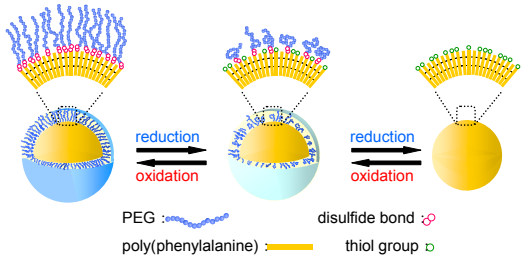

Figure 1. Schematic illustration of the surface control of PEG- SS-peptide nanospheres based on thiol-disulfide interconversion.

Methods: The PEG-SS-peptide nanospheres were synthesized based on our previous report.[5] A 5 mg/mL PEG-SS-peptide nanosphere dispersions were stirred with 0-42 mM dithiothreitol (DTT) as a reductant,. We also addressed the surface remodification of the 'hairless' nanospheres with SH-PEG (Mw = 2000). The hairless nanospheres (4 mg/mL) were stirred with 0.375 mM SH-PEG (Mw = 2000) in 0–10 mM iodine solution. To investigate the effect of PEG graft density on the membrane-disruptive activity, sheep red blood cells (RBCs) were incubated with 1 mg/mL PEG-SS-peptide nanospheres for 1 h at 37 °C at pH=5.5.

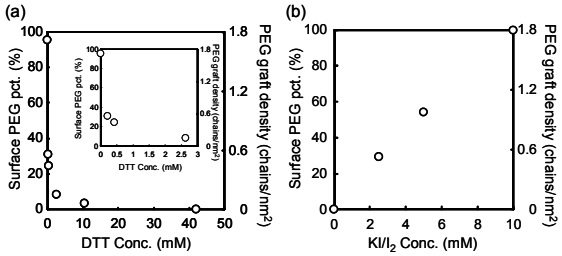

Figure 2. (a) Relationship between the concentration of DTT and the surface PEG percentage. (b) Relationship between the concentration of KI/I_2 and the surface PEG percentage.

Results: The addition of DTT rapidly induced the aggregation of the nanospheres. Interestingly, SEM and DLS measurements revealed that the nanospheres maintained their morphology and size after reduction. The ^{1}H NMR measurements indicated that the surface PEG density decreased with increasing DTT concentration (Figure 2a). Surprisingly, the surface PEG brushes on the hairless peptide nanospheres were recovered almost completely after oxidation with SH-PEG (Figure 2b). The PEG-SS-peptide nanospheres with a surface PEG percentages of 100, 94 and 69% showed low hemolytic activity (Figure 3). On the other hand, the PEG-SS-peptide nanospheres with surface PEG percentages of 39, 15 and 0% showed high hemolytic activity, indicating their biomolecular sorbability.

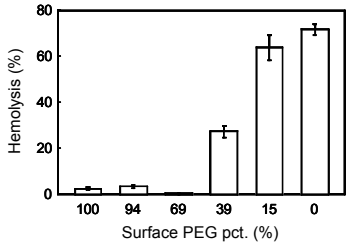

Figure 3. Effect of the surface PEG percentange of peptide nanospheres on hemolytic activity using RBCs.

Conclusions: The surface properties of PEG-SS-peptide nanospheres were precisely controlled, from being bioinert to biomolecular adsorptive, by detaching and attaching surface PEG brushes. These surface-controllable peptide nanospheres are expected to be a new class of nanocarriers.

References: 1) Matsusaki M. Langmuir. 2006;22: 1396-1399. 2) Waku T. Macromolecules. 2007; 40:6385-6392. 3) Waku T. Chem Lett. 2008;37:1262-1263. 4) Matsusaki M. J. Biomater. Sci. Polym. Edn. in press. 5) Waku T. Chem. Commun. 2010; 46:7025-7027.