Stability of Biodegradable Peptide Nanospheres with PEG Brush Layer for Hydrolysis and Sterilization

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Statement of Purpose: Polymeric nanoparticles have been widely studied in biomedical fields such as the drug delivery system (DDS), medical diagnosis, and bioimaging. For the long-term drug delivery system, stability for hydrolysis, stealth property for inhibition of unspecific protein adsorption, and surface functionality for immobilization of specific molecules to target tissues are required. Furthermore, stability for sterilization is also important property for drug delivery carriers. However, there is no report about nanoparticles which satisfy the above requirements.

Recently, we reported a novel method for the preparation of the peptide nanospheres with a high density poly(ethylene glycol) (PEG) brush layer.[1-3] The peptide nanospheres were synthesized by the one-step polymerization of L-phenylalanine *N*-carboxyanhydride (Phe-NCA) with the dual initiators of hydrophobic *n*-butylamine and hydrophilic NH_2-monoterminated PEG (NH_2-PEG). The peptide nanospheres can avoid nonspecific protein adsorption owing to the surface PEG corona layer, while they can immobilize covalently the target molecules on their surface functional groups (Figure 1). This PEG brush peptide nanosphere is expected to have high stability for hydrolysis due to stable hydrophobic phenylalanine (Phe) core and high density PEG brush. In the present study, we investigated stability of the peptide nanospheres for hydrolysis and autoclaving sterilization.

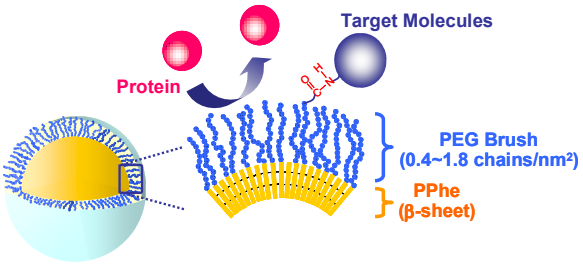

Figure 1. Schematic illustration of the PEG brush peptide nanospheres, which have stealth property and chemical functionality.

Methods: The peptide nanospheres were synthesized based on our previous report.[1] For hydrolysis experiment at neutral pH condition, the nanospheres dispersed in phosphate buffered saline (PBS, pH 7.4) and incubated at 37 °C for 14 days. For accelerated hydrolysis test, the peptide nanospheres were incubated in 5 M NaOH solution at 80 °C for 21 days. After the incubation, the morphology and size of the peptide nanospheres were investigated by scanning electron microscopy (SEM) and dynamic light scattering (DLS), respectively. The amounts of Phe degraded from poly(phenylalanine) (PPhe) in the supernatant were determined by the ninhydrin method. The composition of the remained particles was evaluated by ^{1}H NMR measurement.

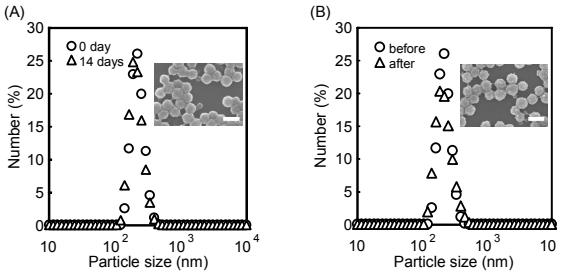

Figure 2. Size distributions and SEM images (insets) of the peptide nanospheres before and after the incubation in PBS for 14 days (A), and the autoclaving sterilization at 121 °C for 15 minutes under 1.21 atm (B). The scale bars in the insets are 300 nm.

Results: After the incubation in PBS and the sterilization, the peptide nanospheres were maintained their morphology, size, and dispersibility completely (Figure 2). To analyze hydrolysis property of the peptide nanospheres, accelerated hydrolysis experiment was performed at alkaline conditon in 5M NaOH at 80 °C. The remaining weight and surface PEG density decreased to 70% and 40 %, respectively, after 21 days of incubation (Figure 3). However, the PEG chain density on the surface of the hydrolyzed nanospheres was still remained at high brush density (> 0.1 chains/nm^2) even after such extreme hydrolysis condition. The reason of the extremely high stability of this peptide nanosphere is not clarified yet, but probably due to the hydrophobic PPhe core and high amount of bound water on high density PEG brush layer.[4]

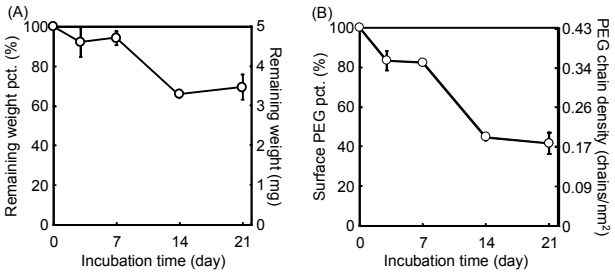

Figure 3. Remaining weight (A) and estimated PEG percentage (B) of the peptide nanospheres after the hydrolysis in 5 M NaOH solution at 80 °C.

Conclusions: The peptide nanospheres were extremely stable for hydrolysis and autoclaving sterilization, and thus it can be useful as a drug carrier for long-term DDS.

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) Yamauchi T. J Appl Polym Sci. 1993;49:1653-1658.