Surface Modifications of Poly(*N*-isopropylacrylamide) Microgels for Targeted Drug Delivery

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Statement of Purpose: Sophisticate drug delivery systems are being developed to optimize safety and efficacy of drugs. Thermo-responsive poly(*N*-isopropylarylamide) (PNIPAM) hydrogel is proposed to be an ideal drug carrier because its reversible volume transition (around 32˚C[1]) is close to the body temperature, therefore enabling controlled drug release. However, under physiological conditions the colloidal stability and biocompatibility of PNIPAM microgels need to be improved for realistic applications. In this study, PNIPAM particles are engineered by grafting poly(ethylene glycol) (PEG), resulting in a brush-like structure on the surface. This surface modification prevents particle aggregation and adsorption of biomolecules. Moreover, the end of the PEG chain was modified for specific binding with histidine-tagged proteins or peptides, which may be used as a signal for targeted delivery. Therefore, improved colloidal stability and biocompatibility as well as the ability of presenting signal biomolecules can be achieved by the simple surface modifications of PNIPAM microgels.

Methods: Carboxylic acid-terminated poly(ethylene glycol) monomethacrylate (PEGSA) was synthesized by reacting succinic anhydride with the hydroxyl group of poly(ethylene glycol) monomethacrylate (PEGMA, PEG MW=400, Polysciences, Inc.) as reported[2]. The modified macromer was mixed with *N*-isopropylacrylamide (NIPAM), *N,N'*-methylene bisacrylamide (BIS), and sodium dodecyl sulfate (SDS) in deionized water. The solution was purged with argon and heated to 60˚C. The polymerization was started by injection of potassium persulfate (KPS) and continued for 20 hours with stirring. Lysine-NTA ligand was synthesized as described previously[3] and conjugated to the end of PEGSA grafted on the particles by carbodiimide chemistry. The resulting particles were charged with $NiSO_4$ and dispersed in binding buffer containing His_6-Cys peptides. After overnight incubation at 4˚C, the particles were separated by centrifugation, and the supernatant was carefully collected for peptide concentration measurement. The pellet was washed and redispersed in elution buffer containing imidazole. After incubation the particles were removed by centrifugation and the supernatant was carefully collected for peptide concentration measurement. The peptide concentrations were measured by Pierce 660 assays according to the manufacturer's manual.

Results: PNIPAM particles containing 0-30 wt% PEGSA were synthesized and the transition behavior and colloidal stability were examined. For particles with lower than 1 wt% PEGSA, the transition temperature was around 32°C, but with increasing PEGSA amount it was shifted to higher temperatures gradually. In the binding buffer PNIPAM particles aggregated at 27°C, while with 0.1-5 wt% PEGSA the particles remained stable. When the PEGSA was increased to 10 wt%, the particles were stable at 29°C; while increasing the PEGSA level to 20 wt% causes particles to be stabilized up to 35°C. Finally, with 30 wt% PEGSA, the particles were stable up to 40°C. His_6-Cys peptide was used as a preliminary model to show the binding ability. As shown in Figure 1, both NTA ligand and nickel ion were required for peptide binding, indicating the binding is specifically through the formation of metal coordination complex. The reversibility was demonstrated by the eluted peptides, with elution efficiencies of 62%, 95% and 98% for particles with 10 wt%, 20 wt% and 30 wt% PEGSA respectively.

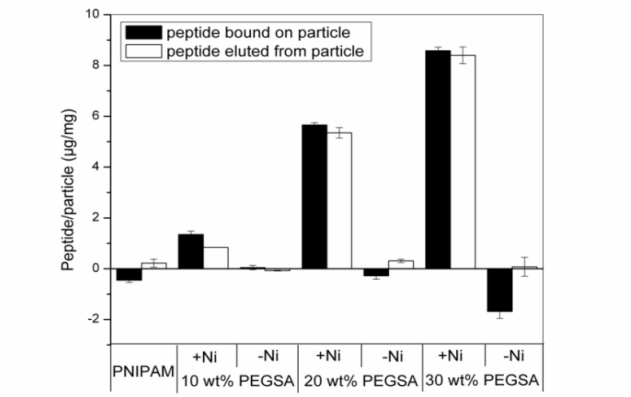

Figure 1. His_6-Cys peptides bound on the particles and eluted by imidazole.

Conclusions: With 30 wt% PEGSA, PEGSA-grafted PNIPAM microgel particles can be stabilized in the buffer over a wide range of temperature. The enhanced colloidal stability is attributed to the grafted hydrophilic PEG chains, since the temperature where particles aggregate was increased when higher PEGSA amount was applied. The transition temperature of the particles was also increased with PEGSA incorporation due to the hydrophilic units introduced by PEGSA incorporation. After conjugated with NTA ligand and charged with nickel, the particles were able to bind His_6-Cys peptides, and the binding is depend on the presence of PEGSA and nickel ion, indicating the interaction is through the NTA-Ni-His_6 complex. Moreover, the binding is reversible, as shown by the competition of imidazole. The results demonstrated that with the well-designed surface modifications, PNIPAM microgel particles can be colloidally stabilized under physiological conditions. The modifications also enable PNIPAM microgel particles to present signal biomolecules on the surface, which is advantageous for targeted drug delivery.

References:

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