pH-Responsive Polymeric Micelle for Theranostics of Ischemic Cerebral Area

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Statement of Purpose: Nanometer-scale particles are becoming extensive in biomedical applications, especially polymeric micelles as carriers of hydrophobic agents (drugs or contrast agents) for cancer diagnosis and therapy. Because pathologic cerebral ischemic area have more acidic environment, we designed a pH-responsive micelles as carriers for pathologic therapy and diagnosis.

Methods: Synthesis of the block copolymer: The PEG-PAE block copolymer was synthesized via a Michael-type step polymerization using monoacrylated PEG, 1,6-hexanediol diacrylate (HDD) and 4,4′-trimethylene dipiperidine (TDP) in chloroform at 10 % (w/v) concentration, and the reaction was carried on for 3 days at the temperature of 50 °C. The final product, PEG-PAE, was precipitated in the solution of ether and vacuum dried.

Preparation of Fe_3O_4 nanoparticles: Monodispersed Fe_3O_4 was prepared by the thermal decomposition from Fe(II)-oleate and Fe(III)-oleate mixture. Firstly the Fe(II)-oleate complex was prepared by Fe(II) chloride tetrahydrate and sodium oleate being heated to 70 °C for four hours in a mixture of ethanol, distilled water and n-hexane. Then the solution was washed three times with excessive distilled water and the product was obtained by evaporating organic solvents. The Fe(III)-oleate complex was also obtained by the same method. Subsequently, Fe_3O_4 nanoparticles were prepared by thermally decomposed of Fe(II)-oleate and Fe(III)-oleate in 1-octadecene at 300 °C with vigorous stirring for 30 min. The Fe_3O_4 nanoparticles was precipitated by adding excessive acetone, and dispersed in chloroform.

Fe_3O_4 loaded polymeric micelles: 10 mL of chloroform containing Fe_3O_4 (10mg) and PEG-PAE block copolymer (50mg) was evaporated to remove organic solvent, and incubated at 40 °C in vacuum for 30 min. After adding the PBS solution (pH 7.4) and filtered by 200 nm pore size, a clear and dark-brown suspension could be obtained and stored at 4 °C.

Results: In our system, the pH-responsive polymeric micelles were composed of hydrophilic methyl ether poly(ethylene glycol) (PEG) and pH-responsive degradable poly(β-amino ester) segments. After loading magnetite nanopartilces into the polymeric micelles, it can be used for rapid acidic pH-triggered magnetic nanoparticles release and aggregation at disease site for MRI diagnostic applications (Figure 1). From the in vivo result, this kind of pH-responsive can be used for the cerebral ischemic area diagnosis. It would improve the possibility to provide a new way to pathological diagnosis and therapy in the future.

Besides, a nano-sized protein-encapsulated polymeric micelle is formed by self-assembling human serum albumin (HSA) and degradable block copolymer methoxy poly(ethylene glycol)-poly(β-amino ester) (PEG-PAE) including imidazole components in PAE. To assess the ability of this polymeric micelle as an intelligent vehicle for highly efficient protein delivery to acidic tissues, we utilize a disease rat model of cerebral ischemia that produces acidic tissue due to its pathologic condition. The rat was intravenously injected with the Cy5.5-labled albumin-encapsulated polymeric micelle solution. We found gradual accumulation of fluorescence signals in the brain ischemic area, indicating that the protein-encapsulated polymeric micelle may be effective for targeting the acidic environment and diagnostic imaging of pathologic tissue.

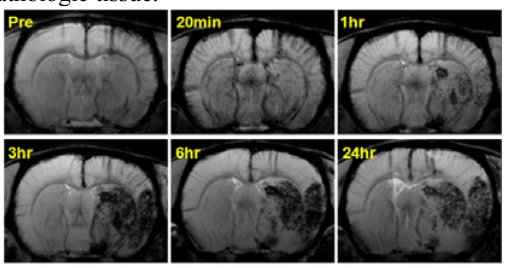

Figure 1. T^*_2-weighted MR imaging of the rat barin with MCAO treated and enhanced by Fe_3O_4-PEG-PAE.

Conclusions: In conclusion, we employed a facile and powerful pH-responsive polymeric micelle which was encapsulated with Fe_3O_4 nanoparticles as a pH-triggered targeting agent without any targeting ligands for MRI. The Fe_3O_4-PEG-PAE remained a micellar state at the physiological condition of pH about 7.4, whereas, it is dissolved in an acidic environment (pH < 6.8) of the pathological tissue. In this work, we demonstrated its initial application to a disease animal model, however, it is believed that more applications will be possible owing to its unique ability to target and simultaneously image the pH-stimuli pathologic environment.

Besides, a block copolymer PEG-PAE-API containing piperidine and imidazole rings was successfully synthesized and encapsulated a model protein albumin. The albumin-encapsulated polymeric micelle maintained a stable micellar state at physiological pH 7.4 with a particle size of around 56.0 nm in water. In the 0.15M NaCl solution, moreover, the particle size would increase from 56.0 nm to 65.7 nm. From the zeta potential results, when the pH is lower than pH 7.0, the albumin-encapsulated polymeric micelle bears a gradually increasing net positive charge due to the ionized amino groups in the chain of PAE blocks. As a result, the positive charge could enhance the uptake to the acidic pathologic area of middle cerebral artery occlusion. Thus, this strategy may be utilized in the design of general platforms for delivering and releasing numerous proteins in biomedical therapeutics and diagnostics.