nanoPARCEL probe : soft nanoparticles containing MRI and fluorescence imaging probes

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Introduction Dual or multi-probe molecules, which are composed of combinations of two or more functional molecules, are attractive for use as tools for noninvasive diagnosis and therapy. Such dual and multi-probes improve the reliability and safety of the diagnoses as they mutually complement one another.

We have developed a method for preparing polyethylene glycol (PEG)-based nanoparticles that contain various kinds of probe molecules, as reported previously.[1,2] The nanoparticles were prepared from monomer molecules that consisted of four long PEG chains with acryloyl groups at each chain end. Simply adding the probe molecules to a solution of the monomers resulted in the formation of uniformly sized nanoparticles that encapsulated the probe molecules. Because the encapsulated molecules are physically trapped by the mesh structure of the nanoparticles and because no chemical bonding between the molecules and PEG is required for encapsulation, we concluded that there are no limitations to the types of molecules that can be encapsulated using this technique. Only two acryloyl groups were required to form a mesh structure for physical encapsulation of molecules within the nanoparticles, and thus we expected that the one of the two remaining acryloyl groups on each monomer could be used for chemical reactions with the probe molecules. To test this hypothesis, we encapsulated probe molecules in the PEG-based nanoparticles, by chemical means, in addition to the physical encapsulation. We speculated that if we encapsulated two different probes using two different (i.e., chemical and physical) methods simultaneously, a dual probe could be prepared without limitation of the encapsulated molecules. In this study, we prepared three different dual probe nanoparticles containing physically and chemically encapsulated probe molecules. The physical properties of these nanoparticles were examined, and the nanoparticles were used for animal experiments to examine their pharmacokinetics and toxicity.

Materials and Methods The PEG-based 4armed linker and fluorescent linkers were prepared as described in our previous report.[3,4] The controlled radical polymerization of these linker with physically encapsulated physically encapsulated molecule (MRI probe or proteins) provide nanoparticles containing dual probes. The nanoparticles were administrated to Female BALB/c nude mice and the pharmacokinetics of the nanoparticles was analyzed by means of MRI (7.0 Tesla horizontal magnet Kobelco and Jastec, Tokyo, Japan), fluorescence spectroscopy (Maestro EX, PerkinElmer, MA, USA), Atomic force microscopy (NanoWizard II JPK Instrument, Berlin, Germany), transmission electron microscopy (H-7000 electron microscope Hitachi, Tokyo, Japan) and Inductively Coupled Plasma-Atomic Emission Spectrometry (iCAP6300 Duo, Thermo Scientific, MA, USA). Detailed experimental conditions are showed in our paper.[4]

Results and Discussion The soft nanoparticles were excreted by the mice rapidly through the urine without collapse of the nanoparticles and without leaking of the probe molecules, and no accumulation of the nanoparticles in the body was observed. The pharmacokinetics of the nanoparticles was not changed by the encapsulated molecules and acute toxicity to mice was negligible. (Fig. 1)

The size of these nanoparticles in liquid was about 200 nm by DLS. (Fig. 2a) Interestingly the diameter and shape of same nanoparticles was examined by means of TEM and AFM with sizes in the range of 600–800 nm, which was 3 to 4 times the diameter measured by DLS. Three dimensional detailed AFM measurements showed the height of the nanoparticle was only 15–20 nm. (Fig. 2b-d) In general, nanoparticles less than 10 nm were excreted from urine, however, nanoparticles with diameters greater than 10 nm are difficult to excrete in urine because the pore size of the kidney glomerulus is too small for penetration of these larger nanoparticles. However, our results indicated that the 200 nm nanoparticles were excreted in urine; a similar phenomenon has been reported by He[5] and Lu[6], who observed the excretion of 100 nm nanoparticles through mice urine. We hypothesized that the nanoparticles could change their shape to penetrate the kidney glomerulus, because the nanoparticles were very soft and flexible, as indicated by AFM images. (Fig.2 e)

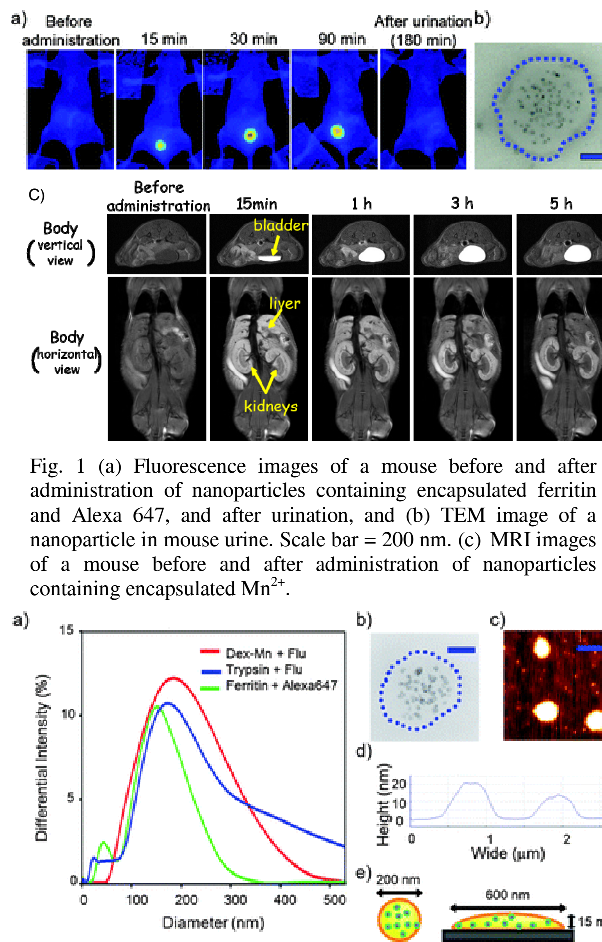

Fig. 1 (a) Fluorescence images of a mouse before and after administration of nanoparticles containing encapsulated ferritin and Alexa 647, and after urination, and (b) TEM image of a nanoparticle in mouse urine. Scale bar = 200 nm. (c) MRI images of a mouse before and after administration of nanoparticles containing encapsulated Mn^{2+}.

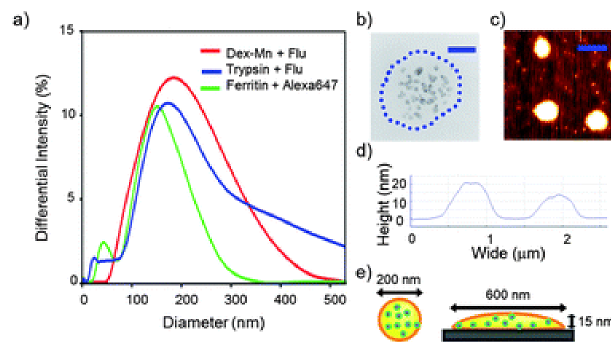

Fig. 2 (a) DLS measurements of the three types of nanoparticles prepared in this study, (b) TEM image of a nanoparticle containing ferritin, scale bar = 200 nm (c) AFM phase contrast image of the nanoparticles, scale bar = 1000 nm (d) cross-sectional topological profile obtained from image (c), and (e) schematic images of a nanoparticle dispersed in solution and of a nanoparticle affixed to a plate.

Conclusion In this study we developed a method to prepare soft PEG-based nanoparticles containing different probe molecules. The advantages of the nanoparticles were that (1) various probe molecules could be physically or chemically encapsulated within the nanoparticles, (2) different probe molecules were encapsulated within the nanoparticles simultaneously, (3) the nanoparticles could be detected in mice using various analytical techniques, depending on the type of probe molecules encapsulated, and (4) the soft nanoparticles were rapidly excreted through the urine, with low accumulation in the body. For these reasons, we concluded that these nanoparticles containing dual probes are promising for the reliable analysis of living animals.

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