Chapter 9

Preparation of Ionic Liquid-Modified Inorganic Nanoparticles and Their Biomedical Application

Asako Narita,*[,1,3] Kensuke Naka,[2] and Yoshiki Chujo[1]

[1]Department of Polymer Chemistry, Graduate School of Engineering, Kyoto University, Katsura, Nishikyo-ku, Kyoto 615-8510, Japan
[2]Department of Chemistry and Materials Technology, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan
[3]Advanced Software Technology & Mechatronics Research Institute of Kyoto (ASTEM), Shimogyo-ku, Kyoto 600-8813, Japan
***nariasa@chujo.synchem.kyoto-u.ac.jp**

Ionic liquid-modified inorganic nanoparticles were prepared, and their biomedical applications were discussed. The N-methylimidazolium cation-modified hydrophilic gold nanoparticles became hydrophobic one when the counter anion was exchanged with hydrophobic anions. The N-methylimidazolium cation-modified iron oxide nanoparticles also showed the same behavior. The physico-chemical change of the surface on the iron oxide nanoparticles is useful for biomedical applications such like magnetic resonance imaging (MRI). The ionic liquid-modified iron oxide nanoparticles showed high dispersibility in water and low toxicity. MR image of the aqueous solution of the N-methylimidazolium cation-modified nanoparticles appeared stronger signal comparing to those for commercial products. Furthermore, the interaction with bio-molecules such as DNA was found.

Background

Nano-sized particles are promising materials for various applications (*1*, *2*). One of the interesting characters of the nanoparticles is wide surface area

Malhotra; Ionic Liquid Applications: Pharmaceuticals, Therapeutics, and Biotechnology
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

compared to bulk-size materials one. The surface area of 1 cm cube is 6 cm^2. The total surface area of nanoparticles (10 nm) as total volume of 1 cm^3 is approximately 600 m^2. The highly concentrated interface and the small diameter are attractive characters for nano-sized physical chemistry and their applications. For example, dispersion of gold nanoparticles shows red-purple color because of the surface Plasmon absorption on the wide surface area. The spectra depend on the diameter or intermolecular distance. Nano-sized iron oxide nanoparticles (γ-Fe_2O_3, meghemite or Fe_3O_4, magnetite) are super paramagnetic depending on the diameter. Thus, the change of status of the nanoparticles can be detected as optical or magnetic signals. The nanoparticles are expected as environmental probes. Additionally, various molecules can be attached to the surface. The nano-sized particles are unstable comparing to bulk solids because of their higher surface energy. Therefore, most of nanoparticles need coating materials as stabilizer. The coating materials can be used as functional moieties or scaffolds to attach functional molecules. The surface modification of nanoparticles is one of important technologies to develop their applications.

In these days, imaging proves based on nanoparticles are desired in biomedical field simultaneously with the development of hardware. In a case of magnetic resonance imaging (MRI), there are some kinds of contrast reagents commercially available such as iron oxide nanoparticles and gadolinium ion complex reagents. However, both types of the commercial contrast reagents have not site recognition or stimuli response ability. The contrast reagents based on the iron oxide nanoparticles are usually coated by sugar chains such as dextran to keep high dispersibility in a living body. Therefore, addition of site recognition or stimuli responsive functions is strongly needed for the development of imaging probes. Such attempts to give recognition ability have been reported using also gold nanoparticles (*3*). One of major strategies to obtain site recognition ability is attachment of recognition molecules to marker molecules of the disease. However, there should be much more variations to recognition systems because the chemical environment exchange accompanying the change of pH or oxygen content around the diseases.

On the other hand, ionic liquids have quite interesting physico-chemical properties such as the melting points, the decomposition temperature, the vapor pressure, the viscosity, and the hydrophobicity. Their properties are attracted in various research fields and applications. However, although numerous reports discussed about these properties in bulk liquids so far, nano-sized physico-chemical properties and usage of ionic liquids still have great possibility to develop the researches. Especially, the hydrophobicity would be present even on the surface of nanoparticles as the same as bulk status. And the modified ionic liquids would act as stimuli response moieties. Thus, we have prepared inorganic nanoparticles modified with ionic liquids. Our nanoparticles modified with the ionic liquid have possibility of a new type of site recognition system in a living body.

Objective

The objective of this study is a preparation of the ionic liquids-modified nanoparticles and their analysis of stimuli response behavior. Especially, ionic liquid-modified iron oxide nanoparticles were evaluated their stimuli responses, toxicities, and the interaction with bio-molecules concerning bio-medical applications.

Experimental

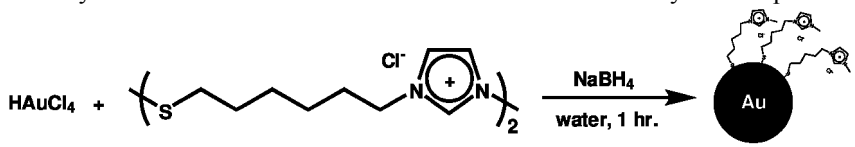
Preparation of Ionic Liquid-Modified Gold Nanoparticles

Preparation of N-methylimidazolium-modified gold nanoparticles ([MIm][Cl]-Au-NPs) was described in our previous report (*4*). Disulfide bearing N-methylimidazolium chloride (3.3'-[disulfanylbis(hexane-1,6-diyl)]bis(1-methyl-1H-imidazol-3-ium)dichloride) as shown in *Scheme* 1 was prepared as described in our paper. Into an aqueous solution of tetrachloroaurate and the disulfide, an aqueous sodium tetrahydroborate solution was added. The mixture was filtered through an ultrafiltration membrane. Modification of N-methylimidazolium chloride moiety on the gold nanoparticles surface was confirmed by FT-IR spectra.

Preparation of Ionic Liquid-Modified Iron Oxide Nanoparticles

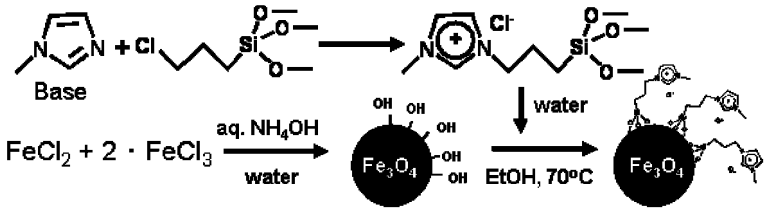
Preparation of N-methylimidazolium chloride modified iron oxide nanoparticles ([MIm][Cl]-IO-NPs) was described in our previous report (*6*) as shown in *Scheme* 2. An aqueous ammonium hydrate solution was added at once to an aqueous solution of iron chloride (II) tetrahydrate and iron chloride (III) hexahydrate under vigorously mechanical stirring. After the produced black precipitation was washed by water, the residue was re-dispersed in ethanol.

Ionic liquids were covalently modified via silane coupling on the ion oxide nanoparticles according to procedures reported previously (*6*) as shown in *Scheme* 2. The silane coupling agent (1-methyl-3-[3-trimethoxysilyl]propaneimidazolium chloride) was synthesized according to the procedures reported previously (*6*). The synthesized silane coupling agent and distilled water were added to the ethanol dispersion. After the mixture was stirred at 70 °C for 12 hours, generated brown particles were washed by ethanol using centrifuge. The covalent bonding between N-methylimidazolium chloride and iron oxide was confirmed by FT-IR spectra.



Scheme 1. Preparation of ionic liquid-modified gold nanoparticles.

Malhotra; Ionic Liquid Applications: Pharmaceuticals, Therapeutics, and Biotechnology
ACS Symposium Series; American Chemical Society: Washington, DC, 2010.



Scheme 2. Preparation of ionic liquid-modified iron oxide nanoparticles.

Analysis of the Stimuli Response

The details of the stimuli response analysis were described in each report(*4–6*). Basically, various kinds of acids or salts were added to aqueous dispersion of imidazolium-modified gold or iron oxide nanoparticles. The degree of aggregation of gold nanoparticles was observed by the appearance of the dispersion or UV-visible absorption spectra(*4*, *5*). In the case of iron oxide nanoparticles, the aggregated diameters were measured by dynamic light scattering (DLS) (*6*).

The Toxicity Assay

In vitro assay of [MIm][Cl]-IO-NPs was carried out as follows. The dispersion of the nanoparticles (0.5 mg/ml saline or PBS) was added to culture wells filled with medium containing HeLa cells (1×10^4 ml^{-1} / well). The volumes of nanoparticles dispersion were as follows (μl); 1, 2.5, 5, 10, 20, 40, 80, 100, 120, 140, 160. The microscope images of the culture wells were recorded at 3 days later. The cell death and aggregations of nanoparticles were observed in these microscope images.

Details of *in vivo* assay were described in the previous paper (*6*). [MIm][Cl]-IO-NPs were dispersed in 5 wt% aqueous glucose solution, saline or PBS. The dispersion was injected from tail vessel of mice. The mice injected the glucose containing dispersion of the nanoparticles were evaluated in various parameters (*6*). The general behaviors of mice were observed after [MIm][Cl]-IO-NPs dispersed in phosphate buffered saline (PBS).

Magnetic Resonance Imaging (MRI)

MR images of water dispersion of N-methylimidazolium chloride-modified nanoparticles were acquired with a 7 T Unity Inova MR scanner (Varian, Palo Alto, CA). A surface coil 20mm in diameter was used for signal acquisition. MR imaging parameters were with 3000 ms repetition time (TR), 100ms echo time (TE), 5mm slice thickness, 60 × 60mm field-of-view (FOV) and 256 × 256 matrices.

Analysis of Interaction between Nanoparticles and Biomolecules

The details about analysis of interaction between DNA and ionic liquid-modified iron oxide nanoparticles were described in our report (*6*). The aqueous dispersions of the nanoparticles were added to the DNA solutions (Tris-HCl-EDTA buffer, pH 8). After nanoparticles were removed by magnet or centrifuge, the supernatant solutions were analyzed by electrophoresis (0.7 % agarose gel, dyeing by ethydium bromide).

Results and Discussion

Characterization of Organic Salts Modified Nanoparticles

The diameter of [MIm][Cl]-Au-NPs measured in transmission electron microscopy (TEM) image was 5 nm. From thermogravimetric analysis (TGA) of [MIm][Cl]-Au-NPs, 380 of modification agent molecules were attached on the surface (*4*).

Characterizations of [MIm][Cl]-IO-NPs were described in our report (*6*). The average diameter of core iron oxide nanoparticles measured in TEM images were around 8 - 10 nm (standard deviation values were always around 1.5 nm) in every batch. The magnetite (Fe_3O_4) nanoparticles made from co-precipitation method (*7*) are tend to have higher deviation value on the diameters comparing to these for meghemite (γ-Fe_2O_3) nanoparticles made from thermal decomposition method (*8*). However, co-precipitation method does not require severe conditions such as high temperature and highly toxic reagents to produce via thermal decomposition method. From the TGA, the volume of organic compounds was around 5 wt% (*6*) even if an excess of the silane coupling agent was added to the nanoparticles. The volume means the nanoparticles have quite thin organic layer on the surface. It was confirmed that the crystal structure of Fe_3O_4 was stable before and after modification of N-methylimidazolium chloride from XRD patterns (*6*). The magnetization curve measured by SQUID also showed that the modification on the iron oxide nanoparticles with N-methylimidazolium chloride did not affect the magnetization values (*6*). As these results, these organic molecules on the surface did not change the properties of the core iron oxide nanoparticles.

Stimuli Response of [MIm][Cl]-Au-NPs

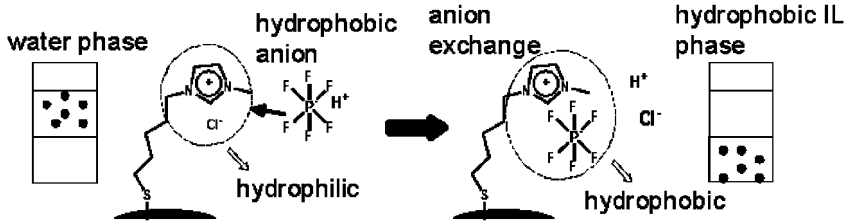
The most interesting behavior of the organic salts modified nanoparticles is the change of the hydrophobicity of the surface by anion exchange as shown in *Scheme* 3. The anion exchange between the surface and surrounding ions should be dominated by the degree of hardness of these ions. Therefore, the equilibrium tends to form ion pairs having large hydrophobic anion such like hexafluorophosphate and soft organic cations like imidazolium. Actually, after enough volume of hydrophobic anion was added to the aqueous dispersion

of [MIm][Cl]-Au-NPs, the nanoparticles transferred from aqueous phase to hydrophobic ionic liquid (1-methyl-3-butylimidazolium hexafluorophosphate) phase (*4*).

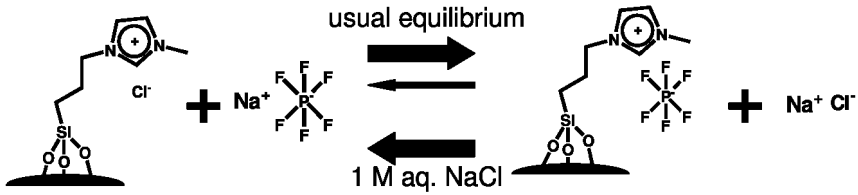
Other response mechanism was also found in [MIm][Cl]-Au-NPs. When an aqueous hydrochloric acid solution was added to the dispersion of [MIm][Cl]-Au-NPs and poly(acrylic acid), precipitation was observed depending on the pH. The change was likely that the re-formation of hydrogen bonding by addition of Brønsted acid (*5*). As a result, various mechanisms except for the anion exchange are expected by modification of organic salts on the surface.

Stimuli Response of [MIm][Cl]-IO-NPs

The same ionic stimuli response as [MIm][Cl]-Au-NPs was observed in an aqueous dispersion of [MIm][Cl]-IO-NPs (*6*). The degree of aggregation after salts were added to the dispersion depended on anion species (NaCl < NaI < $NaPF_6$). Basically, the equilibrium of the anion exchange on the surface depends on the degree of hardness of the combination of ion pairs. Ion exchange between N-methylimidazolium chloride and hydrophobic anion such as sodium hexafluorophosphate proceeds to form N-methylimidazolium hexafluoro-phosphate as soft-soft ion pair. However, reversed anion exchange depending on the concentration of surrounded ions was observed in aqueous dispersion of [MIm][Cl]-IO-NPs. When N-methylimidazolium TFSI-modified nanoparticles were washed by 1M aqueous sodium chloride solution, TFSI anions were replaced to chloride anions (*6*). Such anion exchange against to HSAB principles as shown in *Scheme* 4 should be difficult in balk ionic liquids. On the other hand, the organic salts on the surface behave as interface because the salts tethered to the surface of the nanoparticles face to surrounded molecules or ions. The fact would be the reason why such unusual anion exchange was observed. The reversed anion exchange is one of the impressive behaviors based on the wide surface area of nano-sized particles. Although the change of the hydrophobicity of the iron oxide nanoparticles could be observed by addition of both salts and acids, strong acid cause decomposition of iron oxide nanoparticles.



Scheme 3. Change of hydrophobicity of nanoparticles via anion exchange.



Scheme 4. The equilibrium of anion exchange on the surface.

In a living body, there are numerous kinds of ionic molecules or salts. And the acidity or an ionic environment could be changed in a specific area such like some kinds of disease. Ionic liquids are expected for sensing moiety tethered on the surface of nanoparticles. To optimize the chemical structure, specific environment in a living body would be detected.

Dispersibility of [MIm][Cl]-IO-NPs

[MIm][Cl]-IO-NPs have high dispersibility in water and alcohol. The dispersibility of nanoparticles is very important concerning to use in a living body. To keep high dispersibility, sugar chains such like dextran are often used (*9*) as a coating material for commercial products as MRI contrast agents. Ethylene oxide chains such as poly(ethylene glycol) are also used to give high dispersibility in a living body (*10*). Although these hydrophilic polymers are convenient to disperse in aqueous media, the coating layer is usually thick comparing to the diameter of the core iron oxide(*11*, *12*). On the other hand, organic salt layer on the surface should be thin according to the organic molecule contents as mentioned in the previous section. Although amino group-modified iron oxide nanoparticles were also reported as aqueous dispersed nanoparticles (*13*), the dispersibility was lower than that of [MIm][Cl]-IO-NPs as shown in *Fig.* 1. In an aqueous dispersion, it takes more than 2 weeks to collect [MIm][Cl]-IO-NPs using a neodymium magnet.

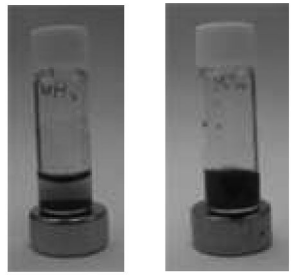


Figure 1. Photographs of the aqueous dispersion (5 mg/ml) of amino group-modified iron oxide nanoparticles (left) and [MIm][Cl]-IO-NPs(right) on neodymium magnets at 60 sec. later after dispersion by ultrasonic wave.

Toxicity of [MIm][Cl]-IO-NPs

Recently, the toxicity of ionic liquids is feared (*14*). Therefore, [MIm][Cl]-IO-NPs also make a suspicion about the toxicity in a living body. However, [MIm][Cl]-IO-NPs did not show serious toxicity in both *in vitro* and *in vivo* (*6*) assay. *Fig.* 2 and *Table* 1 show the results of *in vitro* assay of [MIm][Cl]-IO-NPs. When more than 80 Wl of the PBS dispersion of [MIm][Cl]-IO-NPs was added to the culture medium 1 ml, cell deaths were observed. Concerning of practical concentration of nanoparticles in a living body used as contrast agent for MRI, the concentrations should be lower than the wells added 40 el of dispersion. The result means that [MIm][Cl]-IO-NPs do not have serious toxicity for living cells in a practical concentration to use as a contrast agent.

In vivo assay was also carried out to evaluate sub-acute toxicity (*6*). After aqueous [MIm][Cl]-IO-NPs dispersions containing 0.5 % glucose (13.5 mg nanoparticles/kg body weight) were injected from tail vessel of mice, observation of general behavior and organs, and evaluation in various parameters were carried out. These results are almost same as commercial product (Resovist®) as control, even the concentration in a living body was 30 times higher than practical one actually using in a human body. Furthermore, in cases that the [MIm][Cl]-IO-NPs PBS dispersion (0.2 mg/ml) was injected with higher volume (19.8 and 31.4 mg/kg), also the general behavior of the mice was kept normal.

There would be some reasons that the [MIm][Cl]-IO-NPs showed low toxicity against the expectation. Generally, it is said that cationic molecules tend to act as toxic. Many of reports showed that the toxicity of imidazolium salts is increased with elongating the N-substituted alkyl group(*14–22*) because an increase of lipophilicity caused destroying lipid bilayer of cells(*14*, *22*), or interaction with the lipophilic active center of the enzyme (*16*). From this view point, short methyl substitution on the imidazolium ring on [MIm][Cl]-IO-NPs would not affect these lipophilic domains. The low volume of imidazolium salts also should be one of the reasons of the low toxicity. In the tested concentration (13.5 mg/kg), the organic component is 0.675 mg/kg according to 5 wt% as organic component measured by TGA. If 60 wt% of a mouse with 30 g as body weight was water, the total volume in the body is 2.0×10^{-2} mg, and the concentration of organic parts is 1.1×10^{-3} mg/ml. The value is lower than these for reported value that 1-butyl-3-methylimidazolium chloride showed toxicities(*15–18*). Additionally, the diffusion in living bodies was also low because the imidazolium cations were tethered via covalent bonding. These results imply that modification of cationic molecules via covalent bonding would lead to suppression of the toxicities even if the molecules were evaluated as toxic one.

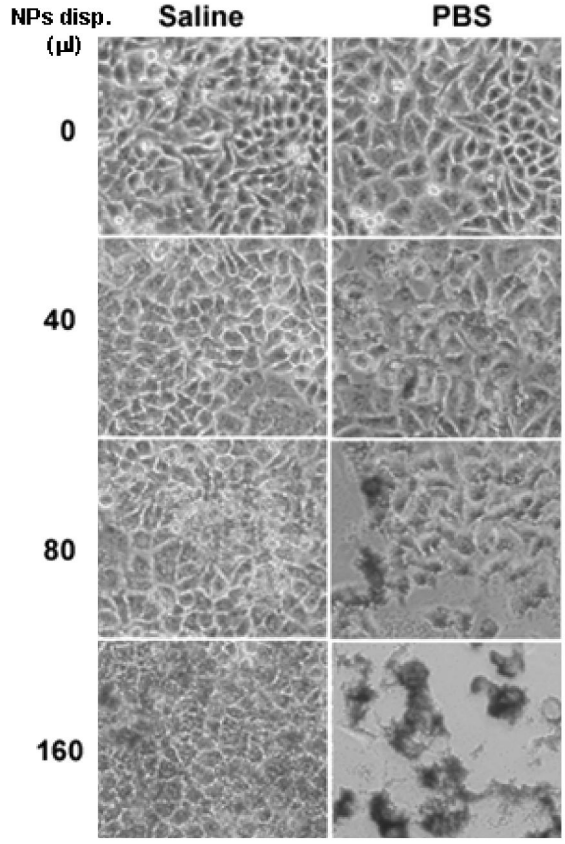


Figure 2. Microscope images of culture wells of HeLa cells at 3 days later after addition of [MIm][Cl]-IO-NPs dispersion.

MR Images of [MIm][Cl]-IO-NPs

MR images of aqueous [MIm][Cl]-IO-NPs dispersion are shown in *Fig.* 3. The results were the same as our previous report (*6*) comparing to a commercial product (Resovist®) in the same concentration. The MR images of aqueous [MIm][Cl]-IO-NPs dispersion were darker (stronger) than these of commercial product (Feridex®). This is likely that the aqueous [MIm][Cl]-IO-NPs dispersion includes higher iron content, or the thin organic layer of the surface of [MIm][Cl]-IO-NPs did not much inhibit the access of water molecules.

Table 1. Observation of cell death and aggregation of [MIm][Cl]-IO-NPs

NPs disp.	*disp. in saline*[a]		*disp. in PBS*[a]	
(μl/ml medium)	*cell death*	*aggregates*	*cell death*	*aggregates*
0	-	-	-	-
1	-	-	-	-
2.5	-	-	-	-
5	-	-	-	-
10	-	-	-	partly +
20	-	-	-	+
40	-	-	-	+
80	-	partly +	partly +	+
100	-	+	partly +	+
120	-	+	partly +	+
140	-	+	partly +	+
160	-	+	+	+

[a] Cell death and aggregates were judged in photographs.

Interaction with Biomolecules

Interaction between [MIm][Cl]-IO-NPs and DNA was analyzed. DNA sticking to [MIm][Cl]-IO-NPs was also found (*6*). It was reported that iron oxide nanoparticles coated with cationic polymer stuck to DNA (*23*). The volume of DNA sticking was depending on the chemical structure of the molecules on the surface. In comparison of [MIm][Cl]-IO-NPs and [MIm][TFSI]-IO-NPs, [MIm][Cl]-IO-NPs showed higher sticking ability. Amino group-modified iron oxide nanoparticles stuck less than [MIm][TFSI]-IO-NPs. The results mean that the degree of the interaction with bio-molecules such as DNA could be controlled by change of modified molecules on the surface. In the case of organic salts, change of the anion would affect the interaction. Actually, it was reported that the ion pair of imidazolium cation and phosphate anion in DNA was formed via anion exchange (*24*). If the anion exchange is included in the sticking forces, optimization of cation structure might be also effective. One of advantages of organic salts from the view points of control of the interaction and recognition is their numerous combinations of cations and anions. The control of the interaction would be useful not only as materials for imaging probes but for bioseparation systems.

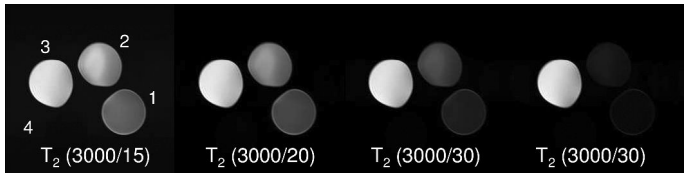


Figure 3. T2 enhanced MR images of aqueous iron oxide nanoparticles dispersions.1: [MIm][Cl]-IO-NPs (10 mg/ml aq. disp.5 μl was added to water 3 ml), 2: Feridex® (5 μl was added to water 3 ml), 3: water (H_2O), 4: water (D_2O). The numbers mean (TE/TR) values.

Conclusions

In summary, we have reported the preparation of ionic liquid-modified inorganic nanoparticles and discussed about their potential of biological applications such as contrast agents for magnetic resonance imaging (MRI) or supporting materials for bioseparation. The N-methylimidazolium chloride-modified gold nanoparticles were well-dispersed in water. The gold nanoparticles had ionic response ability due to the anion exchange between the anions on the surface and surrounded anions. The N-methylimidazolium chloride-modified iron oxide nanoparticles also showed the same ionic stimuli response. The iron oxide nanoparticles showed quite low toxicity as the same as commercial products both *in vitro* and *in vivo* assay. The aqueous solutions of the ionic liquid-modified iron oxide nanoparticles appeared stronger MR signals compared to the same concentration of commercial products due to their thin organic layer. The cationic surface was also useful to collect bio-molecules such as DNA.

Acknowledgments

The authors gratefully appreciate to Prof. Masahiro Hiraoka, Prof. Shinae Kizaka-Kondoh and Mr. Hiroyuki Tanaka (Kyoto University) for cooperation of toxicity assay, to Prof. Toshiro Inubushi and Dr. Masahito Morita (Shiga University of Medical Science) for cooperation of MR imaging, and to Prof. Susumu Kitagawa and Dr. Wakako Kaneko (Kyoto University) for cooperation of SQUID measurements.

This study is a part of joint researches, which are focusing on development of basis of technology for establishing COE of nano-medicine, carried out through Kyoto City Collaboration of Regional Entities for Advancing Technology Excellence (CREATE) assigned by Japan Science and Technology Agency (JST).

References

1. Laurent, S.; Forge, D.; Port, M.; Roch, A.; Robic, C.; Elst, L. V.; Muller, R. N. *Chem. Rev.* **2008**, *108*, 2064.
2. Daniel, M.-C.; Astruc, D. *Chem. Review.* **2004**, *104*, 293.
3. Dixit, V.; Van den Bossche, J.; Sherman, D. M.; Thompson, D. H.; Ronald, P.; Andres, R. P. *Bioconj. Chem.* **2006**, *17*, 603.
4. Itoh, H.; Naka, K.; Chujo, Y. *J. Am. Chem. Soc.* **2004**, *126*, 3026.
5. Naka, K.; Tanaka, H.; Chujo, Y. *Polym. J.* **2007**, *11*, 1127.
6. Naka, K.; Narita, A.; Tanaka, H.; Chujo, Y.; Morita, M.; Inubushi, T.; Nishimura, I.; Hiruta, J.; Shibayama, H.; Koga, H.; Ishibashi, S.; Seki, J.; Kizaka-Kondoh, S.; Hiraoka, M.; *Polym. Adv. Mater.*, 2008, in press.
7. Elmore, W. C. *Phys. Rev.* **1938**, *54*, 308.
8. Rockenberger, J.; Scher, E. C.; Alivisatos, A. P. *J. Am. Chem. Soc.* **1999**, *121*, 11595.
9. Shen, T.; Weissleder, R.; Papisov, M.; Bogdanov, Jr., A.; Brady, T. *Magn. Reson. Med.* **1993**, *29*, 599.
10. Bazile, D.; Prud'homme, C.; Bassoullet, M.-T.; Marlard, M.; Spenlehauer, G.; Veillard, M. *J. Pharm. Sci.* **1995**, *84*, 493.
11. For example, in a commercial product written in a description; Resovist® (Schering) in 1 ample includes 864 mg of fercarbotran as a main component containing 44.6 mg of iron.
12. Weissleder, R.; Kelly, K.; Sun, E. Y; Shtatland, T.; Josephson, L. *Nat. Biotechnol.* **2005**, *23*, 1418.
13. Yamaura, M.; Camiloa, R. L.; Sampaiob, L. C.; Macêdoc, M. A.; Nakamura, M.; Tomad, H. E. *Magn. Reson. Med.* **2004**, *279*, 210.
14. Jastorff, B.; Störmann, R.; Ranke, J.; Mölter, K.; Stock, F.; Oberheitmann, B.; Hoffmann, W.; Hoffmann, J.; Nüchter, M.; Ondruschka, B.; Filser, J. *Green Chem.* **2003**, *5*, 136.
15. Swatloski, R.P.; Holbrey, J. D.; Memon, S. B.; Caldwell, G. A.; Caldwell, K. A.; Rogers, R. D. *Chem. Commun.* **2004**, 668.
16. Stock, F.; Hoffmann, J.; Ranke, J.; Störmann, R.; Ondruschka, B.; Jastorff, B. *Green Chem.* **2004**, *6*, 286.
17. Docherty, K. M.; Kulpa, C. F., Jr. *Green Chem.* **2005**, *7*, 185.
18. Landry, T. D.; Brooks, K.; Poche, D.; Woolhiser, M. *Bull. Environ. Contam. Toxicol.* **2005**, *74*, 559.
19. Stolte, S.; Arning, J.; Bottin-Weber, U.; Matzke, M.; Stock, F.; Thiele, K.; Uerdingen, M.; Welz-Biermann, U.; Jastorffa, B.; Ranke, J. *Green Chem.* **2006**, *8*, 621.
20. Wang, X.; Ohlin, C. A.; Lu, Q.; Fei, Z.; Hub, J.; Dyson, P. J. *Green Chem.* **2007**, *9*, 1191.
21. Matzke, M.; Stolte, S.; Thiele, K.; Juffernholz, T.; Arning, J.; Ranke, J.; Welz-Biermannd, U.; Jastorff, B. *Green Chem.* **2007**, *9*, 1198.
22. Kulacki, K. J.; Lamberti, G. A. *Green Chem.* **2008**, *10*, 104.
23. McBain, S. C.; Yiu, H. H. P.; El Haj, A.; Dobson, J. *J. Mater. Chem.* **2007**, *17*, 2561.
24. Nishimura, N.; Nomura, N.; Nakamura, N.; Ohno, H. *Biomaterials* **2005**, *26*, 5558.