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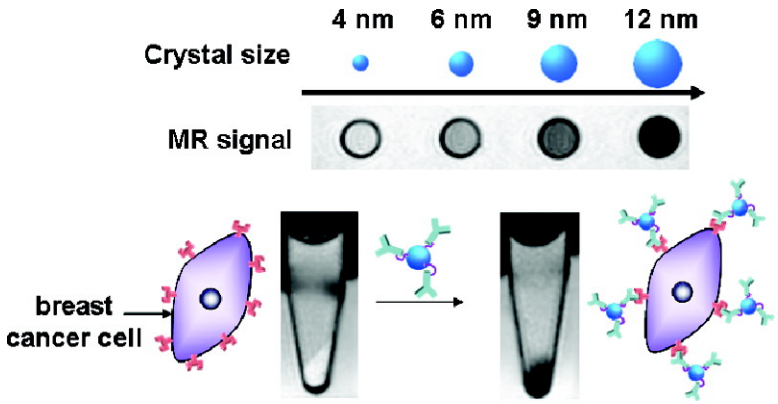

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Nanoscale Size Effect of Magnetic Nanocrystals and Their Utilization for Cancer Diagnosis via Magnetic Resonance Imaging

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The ability to synthetically tune the materials properties in terms of size, shape, and composition of inorganic nanocrystals makes them exhibit enhanced optical, magnetic, and electronic properties when compared to their bulk counterparts.[1] Such novel properties provide the potential for huge improvements in biomedical sciences including detection, diagnosis, and therapeutic systems.[2] Among theses nanocrystals, superparamagnetic nanocrystals are now emerging in biomedical applications with new possibilities.[3−5] In particular, magnetic nanocrystals have a capability as an excellent magnetic resonance (MR) signal enhancer that can resolve the weakness of current magnetic resonance imaging (MRI) techniques. Previously reported iron oxide-based magnetic materials (e.g., superparamagnetic iron oxide (SPIO) and its related systems) have been widely used in hospitals as MR contrast agents, and important studies including gene expression, cancer, angiogenesis imaging, and cellular trafficking have been well performed.[3] However, since most of such iron oxides are prepared through conventional water-phase synthetic protocols, there tends to be difficulty to achieve highly well-defined nanocrystalline size, stoichiometry, and magnetism, which in turn results in rather poor MR signal-enhancing effect.[6] Currently, the underdevelopment of synthetic methods for the high-quality biocompatible magnetic nanocrystals and, at the same time, the limited understanding of the nanoscale effects of magnetic nanocrystals for MRI applications make further progress difficult.

In this report, we present the development of a synthetically controlled magnetic nanocrystal model system that correlates the nanoscale tunabilities in terms of size, magnetism, and induced nuclear spin relaxation processes. Our magnetic model system further leads to the development of high-performance magnetic nanocrystal probe systems for diagnosis of breast cancer cell lines.

Magnetic Fe_3O_4 nanocrystals were synthesized through thermal decomposition of $Fe(acac)_3$ (acac = acetylacetonate) in a hot organic solvent by following previously developed methods.[7,8] The resulting nanocrystals are highly crystalline and stoichiometric Fe_3O_4 magnetite according to our spectroscopic analyses[8] including X-ray diffraction (XRD), X-ray absorption spectroscopy (XAS), and X-ray magnetic circular dichroism (XMCD), and the size of these nanocrystals is controlled from 4 to 6, 9, and 12 nm with high monodispersity ($\sigma = \sim 5\%$) (Figure 1a).

As synthesized, these high-quality magnetic nanocrystals are typically coated with hydrophobic capping ligands and are insoluble in water. Therefore, it was necessary to develop a multifunctional ligand system that allows one to transfer the nanocrystals from the organic to the aqueous phase and also provides high biostability and a conjugation moiety for a targeting ligand. For this purpose,

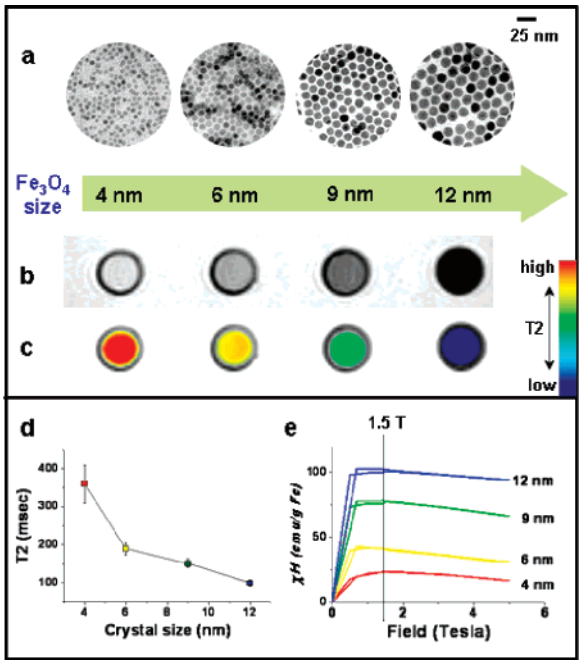

Figure 1. Nanoscale size effect of WSIO nanocrystals on magnetism and induced MR signals. (a) TEM images of Fe_3O_4 nanocrystals of 4 to 6, 9, and 12 nm. (b) Size-dependent *T*2-weighted MR images of WSIO nanocrystals in aqueous solution at 1.5 T. (c) Size-dependent changes from red to blue in color-coded MR images based on *T*2 values. (d) Graph of *T*2 value versus WSIO nanocrystal size. (e) Magnetization of WSIO nanocrystals measured by a SQUID magnetometer.

a simple but highly effective 2,3-dimercaptosuccinic acid (DMSA) ligand was exchanged onto the nanocrystal surface (Scheme 1). The DMSA first forms a stable coating through its carboxylic chelate bonding and further stabilization of the ligand shells is attained through intermolecular disulfide cross-linkages between the ligands under ambient conditions.[9] The remaining free thiol groups of DMSA ligand are used for the attachment of target-specific antibodies (vide infra).

Obtained Fe_3O_4 nanocrystals with the DMSA ligand are fairly stable in water (Scheme 1, inset) and phosphate-buffered saline (PBS) without any aggregation. This is further confirmed by size-dependent band migration results in the gel electrophoresis where larger nanocrystals show a higher retention time.

Before utilizing our water-soluble iron oxide (abbreviated as WSIO) nanocrystals for diagnostic applications, we examined their nanoscale size effects on the magnetism and induced MR signals. The spin−spin relaxation time (*T*2) weighted spin−echo MRI of WSIO nanocrystals (iron concentration 1 μM) at 1.5 T shows significant MR image changes from white to black via gray in the size regime from 4 to 12 nm (Figure 1b).[8] This effect is clearly

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Scheme 1. Schematic of 2,3-Dimercaptosuccinic Acid (DMSA)-Coated Water-Soluble Fe_3O_4 Iron Oxide (WSIO) Nanocrystals with Multifunctionalities; (Inset) Solubility Test of As-Synthesized (left) and WSIO (right) Nanocrystals in Water and Toluene

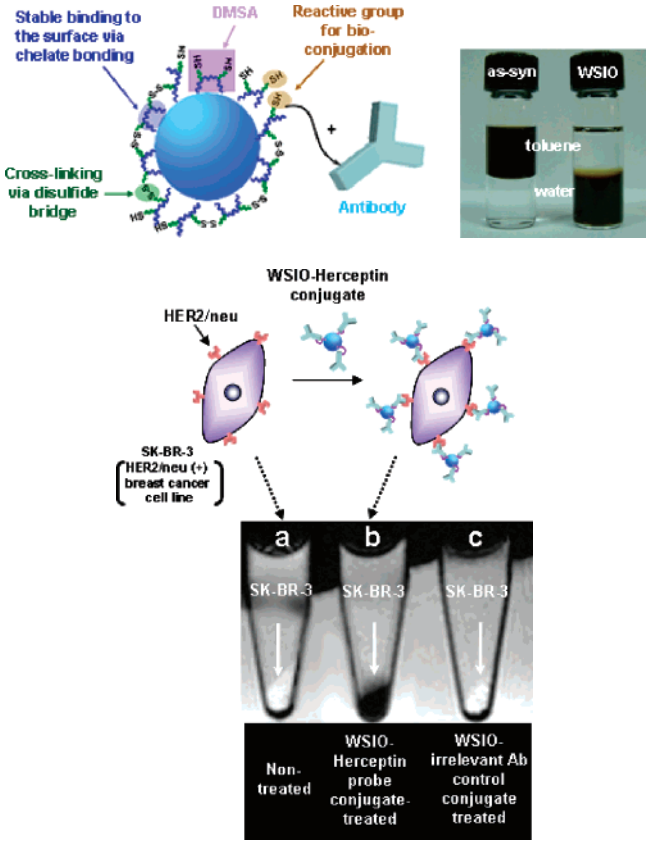

Figure 2. WSIO−Herceptin probe conjugate assisted diagnosis of breast cancer cells.

seen in color coded images based on *T*2 values (Figure 1c). The general trend of size dependent MR signal is that as WSIO size increases, the *T*2-weighted MR signal intensity continuously decreases (Figure 1d) which in turn appears as darker MR images. Since the spin−spin relaxation process of protons in the water molecules surrounding the nanocrystals is facilitated by the magnitude of magnetic spins in nanocrystals, it is reasonable to observe darker MR images (i.e. low *T*2 values) for bigger WSIO nanocrystals with larger mass magnetization values as measured by a superconducting quantum interference device (SQUID) magnetometer (Figure 1e).[10] Our mass magnetization value (χH) at 1.5 T changes from 25 to 43, 80, and 102 emu/(g Fe) as the size of WSIO nanocrystals increases from 4 to 6, 9, and 12 nm, respectively, and such a trend is clearly observed from our MR signals. Our nanocrystals exhibit promising magnetic properties for MRI enhancement while their sizes are extremely small compared to conventional iron oxide-based MR signal enhancers.

Since our WSIO magnetic nanocrystals were well dispersed without any aggregation in an aqueous media and retained their strong MR signals, we investigated their utility for cancer diagnosis. As a model study, the 9 nm sized WSIO was chosen, and its DMSA ligand shell was conjugated with the cancer-targeting antibody, Herceptin (Scheme 1). Herceptin was selected due to its specific binding properties against a HER2/neu receptor overexpressed from breast cancer cells.[11] Conjugation of the WSIO nanocrystals with Herceptin was performed through a known method.[8,12] To evaluate the cancer cell diagnosis activity of WSIO−Herceptin probe conjugates, we examined the binding specificity by treating them to a breast cancer cell line, SK-BR-3, which possesses overexpressed HER2/neu cancer markers. In the *T*2-weighted MR images, treatment of WSIO−Herceptin probe conjugates to the cell lines results in the significant darkening of the MR images (Figure 2b) compared to nontreated cell lines, which indicates selective binding of the probe conjugates to the target cancer cells. In contrast, no noticeable difference is observed between the nontreated (Figure 2a) and WSIO-irrelevant antibody control conjugate-treated cells (Figure 2c), which further confirms the minimal nonspecific adhesion of the conjugates with excellent selectivity.

In summary, we have established a biocompatible magnetic nanocrystal model system which correlates nanoscale size-dependent magnetism and MR properties. Subsequently, these nanocrystals are demonstrated as excellent MRI contrast agents for the breast cancer diagnosis. This study should be used as a general protocol for various types of cancer diagnosis and can be further extended for in vivo live animal systems.

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Supporting Information Available: Experimental procedures of Fe_3O_4 nanocrystals, phase transfer, and bioconjugation of WSIO nanocrystals with Herceptin, XRD, XAS, XMCD, and IR analyses of nanocrystals, MR imaging procedures, and electrophoretic analyses of various sizes of WSIO nanocrystals. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Murray, C. B.; Norris, D. J.; Bawendi, M. G. *J. Am. Chem. Soc.* **1993**, *115*, 8706. (b) Alivisatos, A. P. *Science* **1996**, *271*, 933. (c) Zeng, H.; Li, J.; Liu, J. P.; Wang, Z. L.; Sun, S. *Nature* **2002**, *420*, 395.
(2) (a) Alivisatos, A. P. *Nat. Biotechnol.* **2004**, *22*, 47. (b) Elgahanian, R.; Storfoff, J. J.; Mucic, R. C.; Lestinger, R. L.; Mirkin, C. A. *Science* **1997**, *277*, 1078.
(3) (a) Josephson, L.; Tung, C. H.; Moore, A.; Weissleder, R. *Bioconjugate Chem.* **1999**, *10*, 186. (b) Zhao, M.; Beauregard, D. A.; Loizou, L.; Davletov, B.; Brindle, K. M. *Nat. Med.* **2001**, *7*, 1241. (c) Kang, H. W.; Josephson, L.; Petrovsky, A.; Weissleder, R.; Bogdanov, A., Jr. *Bioconjugate Chem.* **2002**, *13*, 122. (d) Artemov, D.; Mori, N.; Okollie, B.; Bhujwalla, A. M. *Magn. Reson. Med.* **2003**, *49*, 403. (e) Weissleder, R.; Moore, A.; Mahmood, U.; Bhorade, R.; Benveniste, H.; Chiocca, E. A.; Basilion, J. P. *Nat. Med.* **2000**, *6*, 351. (f) Perez, J. M.; Josephson, L.; O'Loughlin, T.; Hogemann, D.; Weissleder, R. *Nat. Biotechnol.* **2002**, *20*, 816. (g) Bulte, J. W. M.; Zhang, S.-C.; van Gelderen, P.; Herynek, V.; Jordan, E. K.; Duncan, I. D.; Frank, J. A. *Proc. Natl. Acad. Sci.* **1999**, *96*, 15256.
(4) Kronick, P. L.; Campbell, G. L.; Joseph, K. *Science* **1978**, *200*, 1074.
(5) Goodwin, S.; Peterson, C.; Hoh, C.; Bittner, C. *J. Magn. Magn. Mater.* **1999**, *194*, 132.
(6) Sjögren, C. E.; Johansson C.; Naevestad A.; Sontum P. C.; Briley-Saebo K.; Fahlvik, A. K. *Magn. Reson. Imaging* **1997**, *15*, 55.
(7) Sun, S.; Zeng, H.; Robinson, D. B.; Raoux, S.; Rice, P. M.; Wang, S. X.; Li, G. *J. Am. Chem. Soc.* **2004**, *126*, 273.
(8) Experimental procedures including the nanocrystal synthesis, spectroscopic characterization, bioconjugation with Herceptin, and MRI procedures were described in the Supporting Information.
(9) Fauconnier, N.; Pons, J. N.; Roger, J.; Bee, A. *J. Colloid Interface Sci.* **1997**, *194*, 427.
(10) Koenig, S. H.; Kellar, K. E. *Magn. Reson. Med.* **1995**, *34*, 227.
(11) Hudziak, R. M.; Lewis, G. D.; Winget, M..; Fendly, B. M.; Shepard, H. M.; Ullrich, A. *Mol. Cell Biol.* **1989**, *9*, 1165.
(12) Hermanson, G. T. *Bioconjugate Techniques*; Academic Press, Inc.: New York, 1996; p 235.

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