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Magnetic Nanoclusters for T2 MR Imaging in Cancer using Xenograft Mice Model

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Abstract In this study, we tried to develop nanoprobe for molecular magnetic resonance (MR) imaging using magnetic nanoclusters (MNC). MNCs for magnetic resonance imaging were synthesized by thermal decomposition.

The size of the synthesized MNC was confirmed to be 73 ± 32.4 nm. Cytotoxicity test of the synthesized MNCs showed that the cell state of about 80% or more did not change in all the treatment ranges and cell survival rate was high even though the MNCs were injected. MNC was injected intravenously into the tail vein of nude mice. As a result, it was found that enhancement of the contrast was confirmed in xenograft mice model using MNC. These results will contribute to clinical application and related research through magnetic nanocluster in the future.

Key-word : MR Molecular Imaging, magnetic, Chemical exchange saturation transfer (CEST), Nanoclusters (MNC), Magnetic nanoparticles (MNPs)

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1. INTRODUCTION

Molecular imaging is a technique for diagnosing various changes at the cellular level. It is a field where advanced imaging technology and molecular cell biology are combined and has recently developed rapidly through a fusion of medicine, genetics, molecular biology, cytology, chemistry, pharmacology, physics, biomedical engineering, radiology, and nuclear medicine.

The imaging technologies and devices used in molecular imaging are Single Photon Emission Computed Tomography (SPECT), Positron Emission Tomography (PET), Magnetic Resonance Imaging (MRI), Ultrasonography, Fluorescence, and Bioluminescence. Molecular imaging has used for early diagnosis of cancer, new drug development,

gene therapy, stem cell research and treatment, and disease prognosis prediction[1]. MR molecular imaging can be performed noninvasively without radiation exposure. In addition, MR molecular imaging has excellent safety and image quality. Recently, chemical exchange saturation transfer (CEST), magnetic nanoparticles (MNPs) and magnetic nano clusters (MNCs) Research are being actively carried out.

MNCs are structures in which nanoparticles of 1 to 100 nanometers (10 to - 9 meters) in diameters, such as MNPs, gold nanoparticles, and quantum dots, are assembled. This structure has unique collective properties that are different from single nanoparticles. The MNCs showed a T_2 relaxation rate three times higher than that of the conventional MR imaging contrast agent Feridex and was well transferred to specific cells.

These results demonstrate that MNPs can be used in biomedical and medical applications such as MR molecular imaging, fluorescence imaging, and drug delivery [2].

This study is to develop a novel nanoprobe for MR molecular imaging of gastric cancer using MNCs based on MNPs which can be used for MR molecular imaging.

2. EXPERIMENTAL METHODS

2.1. Materials

Polysorbate, ethylenediamine, 1,4-dioxane, 4-dimethylamino-pyridine, triethylamine, and succinic anhydride(SA) were purchased from Sigma Aldrich Chemical Co. Phosphate buffered saline (PBS: 10 mM, pH 7.4) were purchased from Roswell Park Memorial Institute, and antibiotic-antimycotic solution Dialysis membrane was used. The gastric cancer cell line (American type culture collection) was incubated in an incubator containing fetal bovine serum and antibiotic antimycotic. Ultra pure deionized water was used for all synthesis.

2.2. Synthesis of Magnetic Nano Cluster

MNC was synthesized by the nanoemulsion method. The nanoemulsion process was carried out by following the two-step synthetic procedure. First, monocrystalline manganese iron nanoparticles are synthesized by pyrolysis from non-polar inorganic solvent to metal-inorganic precursors, and synthesized manganese iron is synthesized by seed-mediated growth method.

2.3. MNC Cytotoxicity Test

The gastric cancer cell line was cultured under the conditions of RPMI medium and antibiotics, and various concentrations of MNCs were treated for 4 hours. The cytotoxicity test of MNC on gastric cancer cell line was performed by measuring 3- (4,5-dimethyl-thiazole-2-yl) -2,5-diphenyltetrazolium bromide (MTT) assay.

2.4 Zenograft animal models and experimental procedures

All animal studies were approved and accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International. Six-week-old female nude mice were injected intravenously with an anesthetic Zolethyl / Rompun mixture. After anesthesia, 1.0×10^7 gastric cancer cell lines were dispersed in 200 mL saline solution and injected into the thighs of mice. MR images were obtained at 5th week after transplantation of gastric cancer cell line.

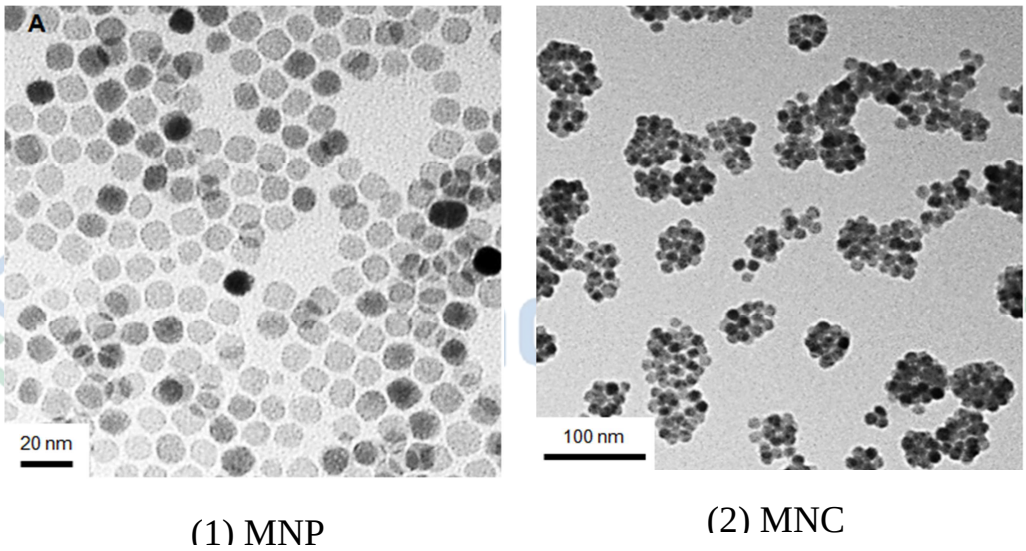
2.3 MR Imaging

Animal solution MRI was performed with 3 T Phillips clinical MRI equipment and T_2 weighted images were obtained using wrist coils. The conditions for obtaining T_2-weighted images were TR (repetition time): 700.85 ms, TE (echo time: 100.65 ms), Slice thickness: 1.0 mm, and FOV read: 100 mm.

3. RESULT & DISCUSSION

3.1. MNCs Characteristics

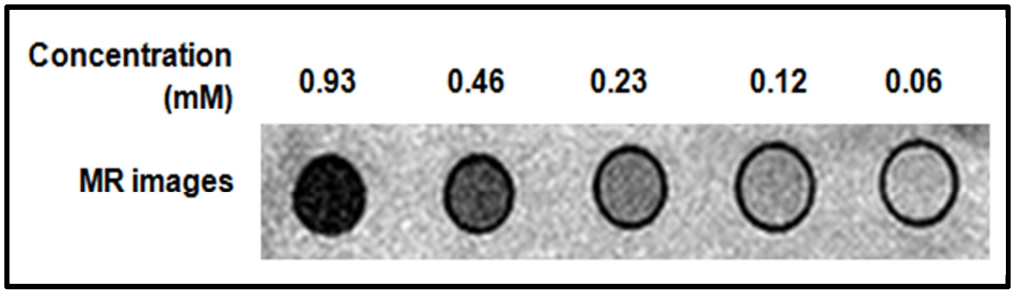
MNCs for MR contrast agents were synthesized by thermal decomposition methods. The manganese iron in the inorganic state was combined with PLI (Poly-L-Lysine) by emulsion method, and the remaining PLI was removed using Centrifuge and MNCs were synthesized with proper PLI ratio. As shown in [Figure 1], transmission electron microscope (TEM) was used to confirm the size distribution and morphology of MNCs. TEM confirmed that the size of the MNCs was 73 ± 32.4 nm.



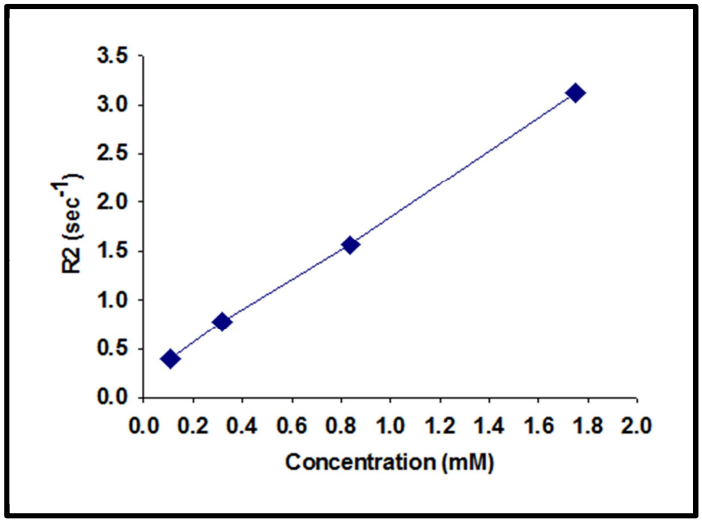
[Figure 1] TEM imaging of MNP and MNC

MRI was performed to confirm the characteristics of the synthesized cluster as a MRI contrast agent. Appropriate-sized clusters could adequately avoid the reticuloendothelial system (RES), allowing them to stay longer in the blood.

As shown in [Figure 2], T_2 images according to cluster concentration were confirmed through MRI, and it was confirmed that the T_2 value was increased with increasing concentration as shown in [Figure 3].



[Figure 2] MNCs T2 imaging



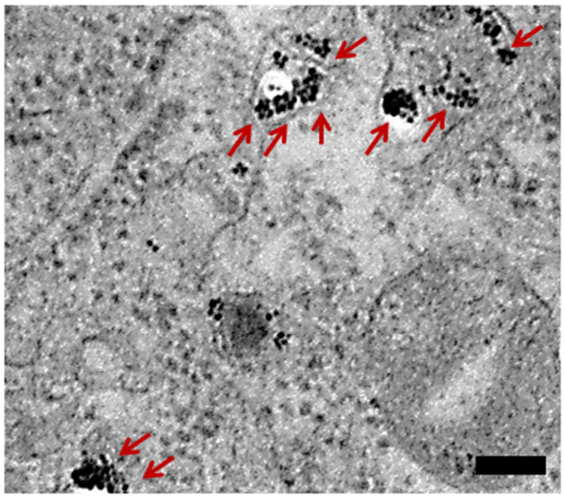
[Figure 3] R_2 relaxation rate with increasing MNCs concentration

3.2. MNC cytotoxicity test

Cytotoxicity tests should be carried out to determine the side effects of drugs intended to be injected into the living body including the contrast agent. Although there are objections to the sacrifice of animals, experiments through simulations have not been fully validated to date. Animal experiments are performed to reproduce the target concentration changes and effects of experimental cell lines during ADME (administration, distribution, metabolism, and emission) processes and processes in vivo with high accuracy.

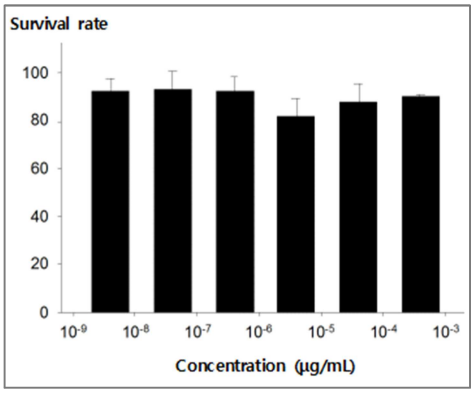
In this study, cell growth retardation was examined using animal experimental cell lines. We investigated normal cell damage and normal cell function after injection of MNCs contrast agent.

To evaluate cytotoxicity, 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT) test was performed on gastric cancer cell line. The gastric cancer cell line was treated with NMCs synthesized in the range of 10^{-7} to 10^{-1} and cultured for 24 hours.[Figure 4] shows the appearance of particle-type MNPs contrast agent and clustered MNCs contrast agent.



[**Figure** 4] MNCs MNC-74 cells (scale bar: 100 nm) – TEM image

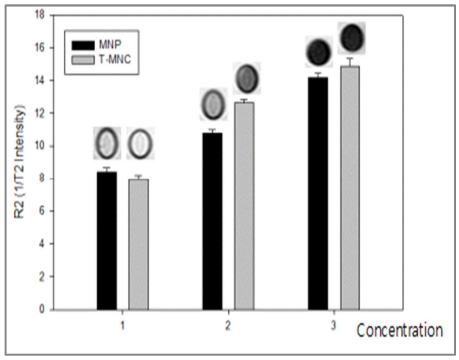
In all treatments of synthesized NMCs, no more than 80% of the cell states were changed. As shown in [Figure 5], cell survival rate was high even though MNCs was injected. As a result, there was no cytotoxicity of newly synthesized MNCs.



[**Figure** 5] Toxicity test results of MNC cells

[Figure 6] shows the evaluation of the degree of contrast enhancement by administering MNPs (black graph) contrast agent and clustered MNCs (gray graph) contrast agent to the cells, respectively.

Comparisons of contrast enhancement after administration of the two contrast agents at the same concentration showed that the enhanced contrast enhancement effect of the clustered MNCs contrast agent was greater.

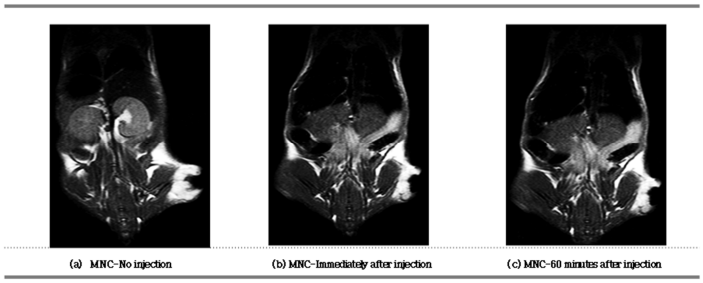


[Figure 6] Contrast enhancement of MNP and MNC

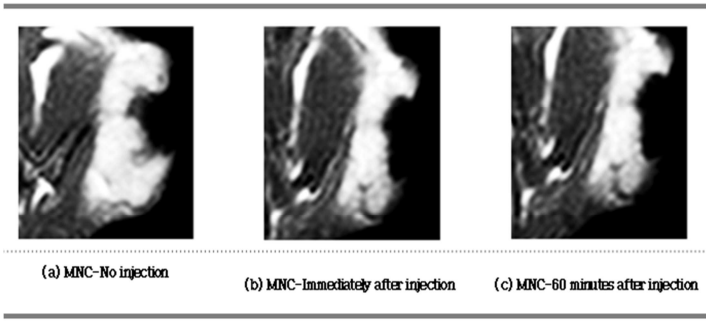
3.3. In vivo MRI

An animal model in which a gastric cancer cell line was transplanted into the thigh of a nude mouse was established. MRI was acquired using a T_2 pulse sequence after injecting the synthesized MNCs into the tail vein of a mouse. The intravenous injection into the tail vein of the nude mouse and observation of the passage of time showed that the grafted cancer cell site was specifically stressed. As shown in [Figure 7] and [Figure 8], non-MNCs images and MNCs images of cancer were acquired over time.

Over time, it was confirmed that the blood vessels in the periphery of the gastric cancer cell line were enhanced. As a result, it was found that the newly synthesized NMCs were suitable for the role of the MR molecular imaging probe for the early diagnosis of cancer.



[Figure 7] Comparison of non-MNCs image and MNCs images



[Figure 8] Comparison between non-MNCs and MNCs images (ROI)

4. CONCLUSION

In this study, we developed molecular MR imaging nano cluster for early diagnosis of cancer.

We designed Magnetic Nanocluster is more efficient than conventional Magnetic nanoparticles in contrast enhancement issue. In addition, since magnetic nanocluster which consisting of numerous magnetic nanoparticles was synthesized with a biocompatible polymer, the toxicity issue was also resolved. In our future studies, pathological confirmation will also be required. Also, biocompatible Magnetic nanoclusters will be actively developed in clinical applications and related research.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

[REFERENCE]

[1] Pack D. S, Choi G. R, Han B. S, & Ahn, B. J.,(2012). Feature values of DWT using MR general imaging and molecular imaging. Journal of the Korean Society of Radiology, 6(5), 409-414.

[2] Nirbhay, Y.(2014). Chemical Exchange Saturation Transfer(CEST) MRI : Theory and Applications, University or Western Sydney Previous Nanoscale Research Seminars.

[3] Mahwood U.(2003), Emerging Technologies That Will Change the World, Molecular Imaging. Tech. Rev., 106.

[4] Choi, G. R., & Lee, S. B.,(2014). Application and Prospects of Molecular Imaging. J. Korean. Soc. Radiol., 8(3), 123-136.

[5] Ha S.W.(2012). Preparation and evaluation of ultrasuperparamagnetic iron oxide nanoparticle for MRI contrast agent. Mater Thesis, University of Science and Technology.

[6] Esserman, L., Wolverton, D., Hylton, N.(2002). Magnetic resonance imaging for primary breast cancer management; current role and new applications, Endo. Rel. Cancer. 9. 141.

[7] A. Maiocchi.(2003). The Use of Molecular Descriptors in the Design of Gadolinium(Ⅲ) Chelates as MRI Contrast Agents, Mini Med. Chem. 3. 845.

[8] Wang, Y., Hussain, S., & Krestin, G.,(2001). Superparamagnetic iron oxide contrast agents: physicochemical characteristics and applications in MR imaging. Eur. Radiol., 11(11). 2319-2331.

[9] Nasongkla, N., Bey, E., Ren, J., Ai, H., Khemtong, C., Guthi, J. S., Chin, S. F. Sherry, A. D., Boothman, D. A. & Gao, J.,(2006). Multifunctional Polymeric Micelles as Cancer-Targeted, MRI-Ultrasensitive Drug Delivery Systems. Nano Lett., 6(11). 2427-2430.

[10] Tournier H, Hyacinthe R, Schneider M.,(2002). Gadolinium-containing mixed micelle formulations: A new class of blood pool MRI/MRA contrast agents. Acad. Radiol. 9. S20–S28.