Dextran-Coated Ultrasmall Superparamagnetic Iron Oxide (USPIO) Nanoparticles as Renal Clearable MRI Contrast Agents

Taekyung Yoon[1], Yoonhye Jeong[1], Jungyoon Kim[1], Sunghwi Kang[1,2], Jungsu Lah[1,3] and Minsuk Kwak[1,3]

[1]Center for Nanomedicine, Institute for Basic Science (IBS), Seoul, Republic of Korea
[2]Department of Chemistry, Yonsei University, Seoul, Republic of Korea
[3]Graduate Program of Nano Biomedical Engineering (NanoBME), Yonsei Advanced Science Institute, Yonsei University, Seoul, Republic of Korea

Abstract

Magnetic resonance imaging (MRI) is a powerful, non-invasive technique that produces detailed anatomical images for diagnosis among soft tissues. The use of magnetic contrast agents has allowed greater MRI sensitivity by producing a stronger magnetic signal that is projected as brighter spots on the image. One of the most widely used contrast agents is Gadolinium-based contrast agents (GBCAs). However, GBCAs have raised toxicity concerns and have short blood circulation time. As a substitute for GBCAs, ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles have been developed as contrast agents because of their biocompatibility and small size.

We coated commercial 5nm USPIO nanoparticles with Dextran, neutral-charged hydrophilic polysaccharide, using the ligand exchange method. After optimizing the coating method, we compared four different types of Dextran as coating material – Carboxy Methyl (CM) Dextran, Amino Dextran, Dextran nanoparticles (DNPs), and Dextran T10 - and measured their properties including the size, zeta potential, relaxivity, cell cytotoxicity, and in-vivo trials. DNP was selected as the coating material for 2nm USPIO and with this agent, we successfully observed renal excretion from in-vivo trials.

Introduction

T1 Contrast Agent - GBCAs

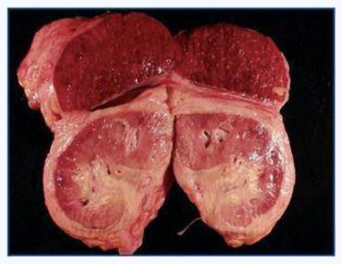


Figure 1. Gadolinium-based contrast agents (GBCAs) exhibit toxic side effects, including nephrogenic systemic fibrosis caused by gadolinium exposure in patients with impaired kidney function.

T1 Contrast Agent – Iron Oxides (IO)

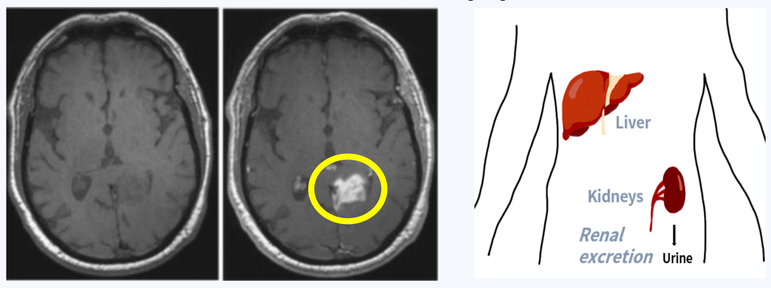


Figure 2. (a) IO T1 contrast agents shorten the relaxation time of nearby protons within tissues and produce a bright signal. (b) Upon intravenous administration, nanoparticles enter the vascular compartment and are distributed within the body. Depending on the size and surface chemistry, the IOs can be detected by the body's immune system and become accumulated in the liver or ideally become excreted via kidney. The smaller the size of the particle, the higher percentage to be excreted by the renal system.

Coating via Ligand Exchange method

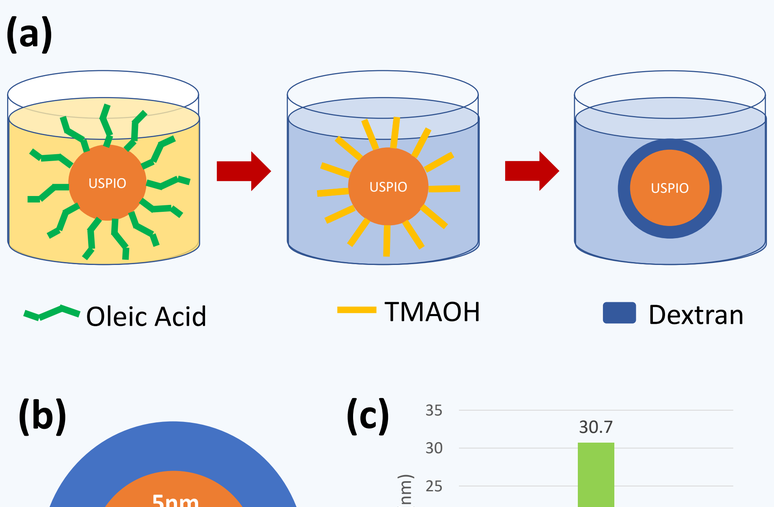


Figure 3. Three types of ligand exchange coating methods and their coating thickness. (a) Ligand exchange process for dextran coating. (b) All three experiments used 5nm IOs as the core and CM-dextran as the coating material. (c) The coating thickness of IOs from each experiment shows that Exp 1 enables the thinnest coating.

Dextran as Coating Material

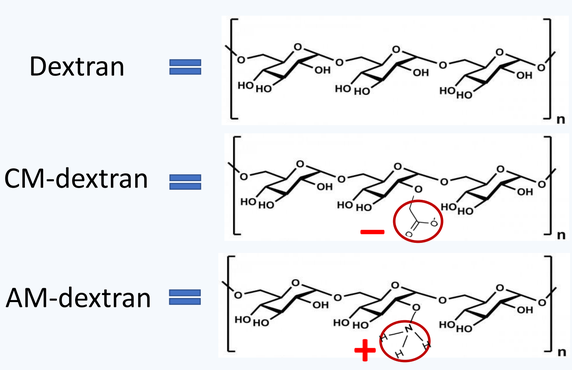


Figure 4. The more neutral the agent is, the less likely it is to be engulfed by macrophages.

Scheme

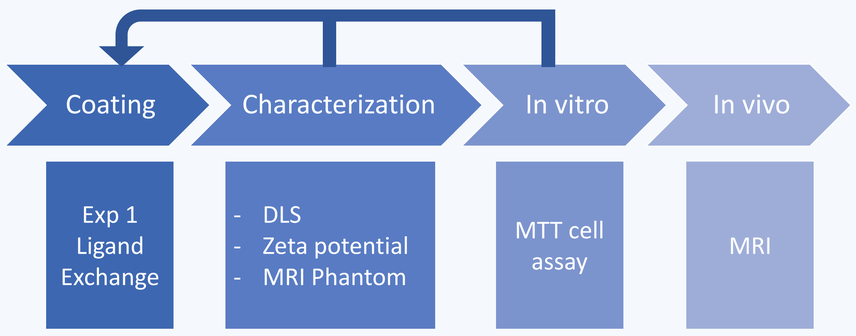


Figure 3. A brief overview of the experiment. We characterized the contrast agents by DLS, Zeta potential, MRI. Verified samples proceeded to in vitro cell cytotoxicity tests and in vivo MRI performance tests.

Results

Characterization Iron Oxide (IO) cores

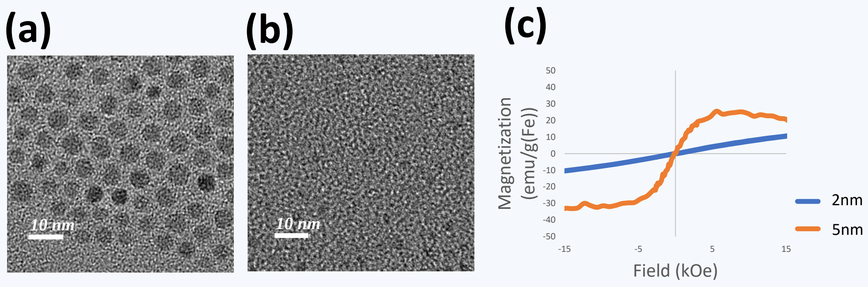
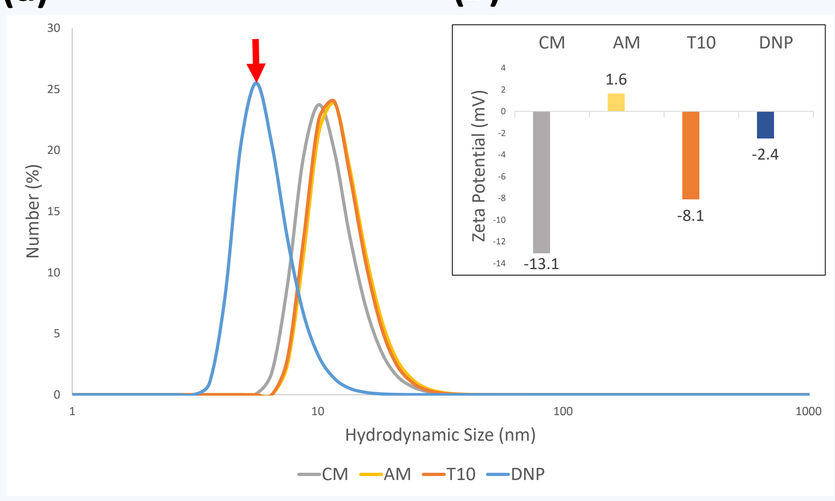


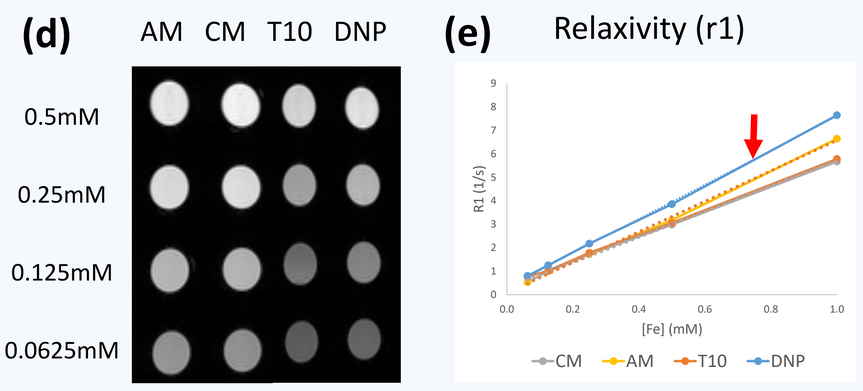
Figure 5. Characterization of 5nm and 2nm USPIO nanoparticles. (a) TEM image of 5nm IOs (b) TEM image of 2nm USPIOs. (c) VSM result shows the magnetization of 5nm and 2nm IOs.

Characterization of Dextran-coated 5nm IOs



(c)

	CM	AM	T10	DNP
Size Avg (nm)	11.3	12.7	12.4	6.3



(f)

	T10	CM	AM	DNP
r1 ($mM^{-1}sec^{-1}$)	5.4	5.4	6.5	7.3
r2/r1	2.7	3.3	3.2	3.1

Figure 6. Characterization of 5nm IOs coated with four types of Dextran.
(a) Hydrodynamic size distribution is shown with number peaks. (b) Zeta potential shows charge of each coated nanoparticles. (c) Table showing the values of the hydrodynamic size average in graph (a). (d) T1 weighted MR phantom images of AM, CM, T10, DNP coated IOs with respect to the concentration of Fe. The higher the concentration, the brighter the MR image. (d) The slope of the graph indicates the relaxivity (r1) value.

In Vitro - Cell Cytotoxicity

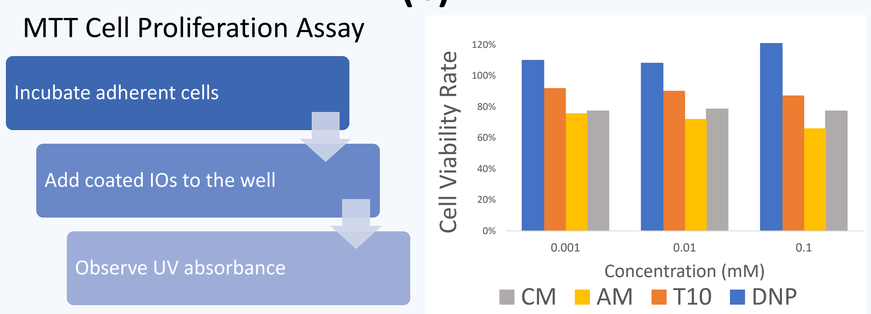
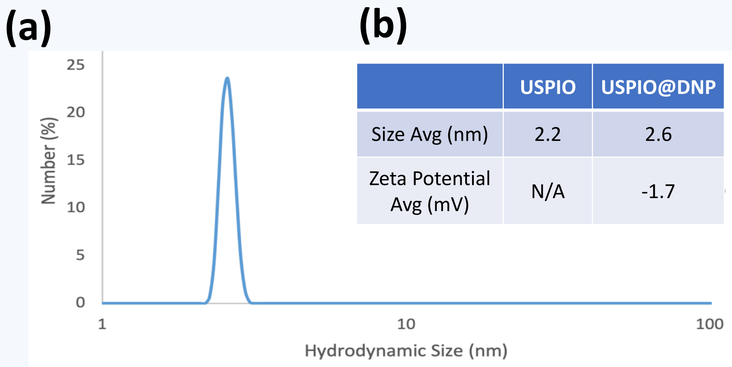


Figure 7. Cell viability rate acquired from in vitro cell cytotoxicity test. (a) A brief scheme of the MTT cell assay. We incubated tumor cells and added different concentrations of the coated IO agents to see their cytotoxic effects. (b) The cell viability rates are shown for each agent.

Characterization of DNP-coated USPIOs



	USPIO	USPIO@DNP
Size Avg (nm)	2.2	2.6
Zeta Potential Avg (mV)	N/A	-1.7

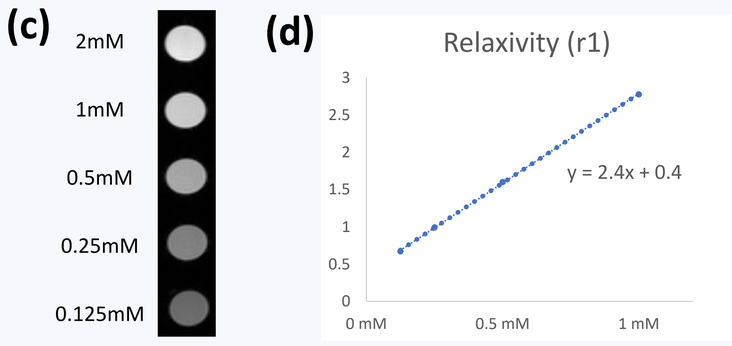


Figure 8. Characterization of DNP coated USPIO nanoparticles. (a) Hydrodynamic size average (nm) and (b) Zeta potential average (mV) is shown. (c) T1-weighted MR phantom image in the order of decreasing concentration starting from 2mM. (d) The relaxivity graph's slope shows r1 value.

In Vivo - MRI Performance

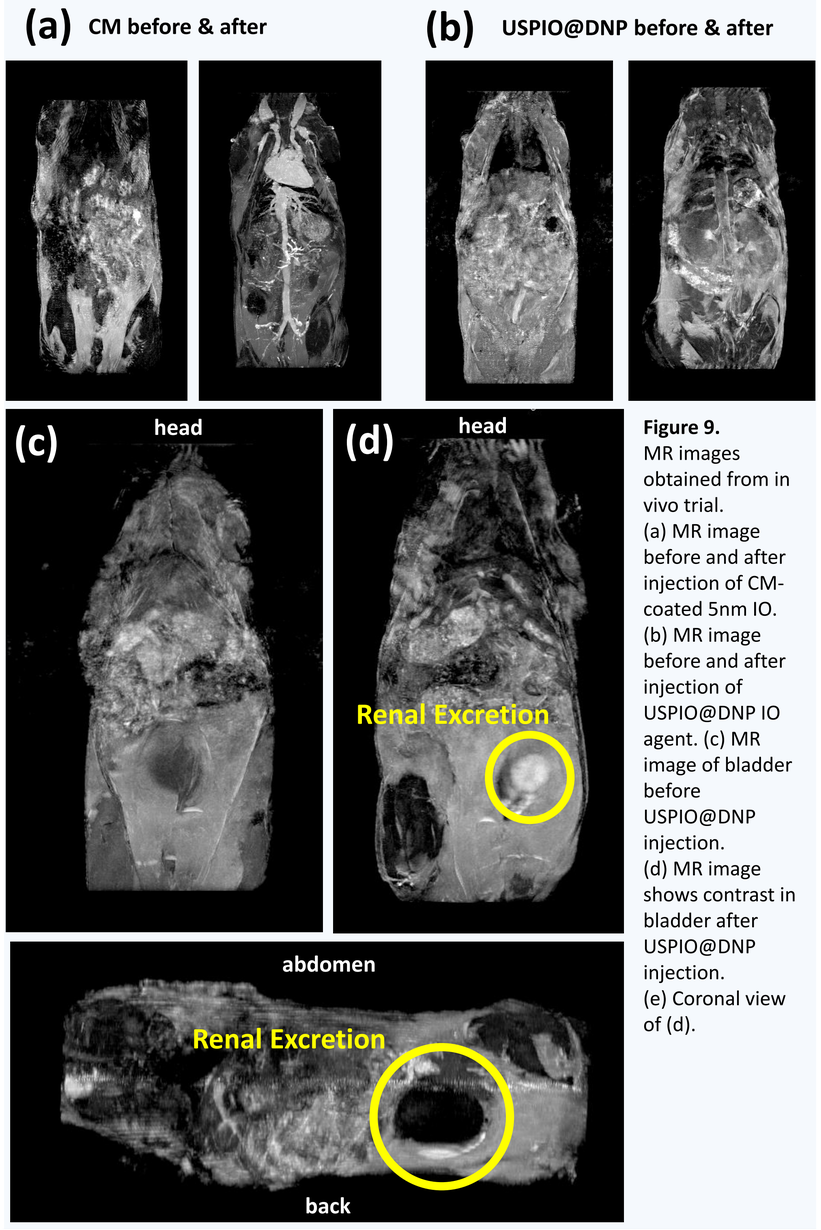


Figure 9. MR images obtained from in vivo trial.
(a) MR image before and after injection of CM-coated 5nm IO.
(b) MR image before and after injection of USPIO@DNP IO agent. (c) MR image of bladder before USPIO@DNP injection.
(d) MR image shows contrast in bladder after USPIO@DNP injection.
(e) Coronal view of (d).

Conclusion

In this study, we coated 5nm and 2nm USPIO nanoparticles with four types of Dextran. The size, surface charge, and relaxivity of the coated nanoparticles were studied, and biocompatibility was examined by cell cytotoxicity and MRI. Overall, the results demonstrated a successful coating of Dextran on IO nanoparticles. Especially, DNP as coating material showed the smallest hydrodynamic size, charge neutrality, small r2/r1 ratio, and good cell viability. In vivo MR images of DNP-coated USPIO contrast agent displayed enhanced contrast in the bladder, suggesting nanoparticle elimination through renal excretion instead of accumulation in the liver.

Our results demonstrate that Dextran-coated USPIO contrast agents are qualitatively different from previously reported IO contrast agents. This work may create new opportunities to develop effective T1 contrast agents from USPIOs as potential substitutes for GBCAs. For further improvement, the agents' pH and concentration may need to be optimized to ensure complete safety and to extend the blood circulation time for higher resolution.

References

Washer, J., et al. Chemistry of MRI Contrast Agents: Current Challenges and New Frontiers, Chem. Rev. **119,** 957-1057 (2019).
Jessica Wahsner, Eric M. Gale, Aurora Rodríguez-Rodríguez, and Peter Caravan Chemical Reviews 2019 119 (2), 957-1057
Caspani, S., Magalhães, R., Araújo, J. P., & Sousa, C. T. Magnetic nanomaterials as contrast agents for MRI, *Materials*, (2020)
Jornet, A. et al. Gadolinium-induced systemic fibrosis in severe renal failure. Nefrologia. 29, 285-378 (2009).
Anonymous, (n.d.) Megazyme. Retrieved from https://www.megazyme.com/dextran