# Magnetic and MR Relaxation properties of Avidin-Biotin Conjugated Superparamagnetic Nanoparticles

**J-A. Park[1], Y. Chang[1,2], J-J. Lee[1], I-S. kim[1], G-H. Lee[3], J-H. Choi[4], and H-C. Ri[5]**

[1]Department of Biomedical Engineering, Kyungpook National University, Daegu, Korea, Republic of, [2]Department of Diagnostic Radiology, [3]Department of Chemistry, [4]Department of Natural Fiber Science, [5]Department of Physics, Kyungpook National University

## Introduction

The magnetic nanoparticles in colloid form are used as magnetic resonance imaging (MRI) contrast agents[1]. For application as a molecular imaging agent, the magnetic nanoparticles need to be conjugated with biological markers such as antibody, enzyme, and small peptides. The avidin -biotin linker system is a good candidate with strong protein-ligand interaction. In this study, we investigate the alteration of magnetic properties of magnetic nanoparticle by avidin-biotin system and the change of the MR relaxation property by synthesizing and characterizing a polycaprolactone coated iron oxide nanoparticles (PCLNP), avidin conjugated PCLNP (A-PCLNP) and avidin-biotin conjugated PCLNP (A-B-PCLNP).

## Material and Methods

***PCLNP*** : A mixed solution of 0.03M $FeCl_2 \cdot 4H_2O$ and 0.06M $FeCl_3 \cdot 6H_2O$ were precipitated with $NH_4OH$. The black precipitate that immediately formed was washed several times with distilled water. After washing, 15ml of 5.57%(w/w) PCL acetone solution and 215ml of 0.46%(w/w) PVP aqueous solution were added and the suspension was stirred for 1hr. After solvent evaporation, the resulting PCLNP was centrifuged to separate into different size and remove excessive polymer. ***A-PCLNP*** : <Solution 1> 6-aminocaproic acid 3.75mg was dissolved in 5ml 10mmol NaOH. PCLNP 2.27mg were added this solution and stirred for 2hr. $NaBH_4$ 15mmol dissolved in 50μl $H_2O$ was added in this solution and stirred for 1hr. <Solution 2> 5.5mg N-hydroxysuccinimide(NHS) and 9mg N-(3-dimethylaminopropyl)-N'-Ethylcarbodiimidehydrochloride(EDC) in 0.5ml N.N-dimethylformamide(DMF) were stirring until the solids were completely dissolved. The solution 1 and 2 were mixed and stirred for 30min. After 30min, avidin 2.5mg in phosphate buffer(pH7.4) 0.25ml was added and stirred for over night. ***A-B-PCLNP*** : The A-PCLNP solution includes 2.5mg avidin (10.8unit/1mg). Because biotin 1μg combines with the avidin 1unit, the equivalent amount of biotin was added to the A-PCLNP solution and stirred for 30min at room temperature.

## Results and Discussion

Fig. 1 shows TEM images of (a) PCLNP, (b) Avidin and (c) A-PCLNP. The average particle size of PCLNP, Avidin and A-PCLNP were about 20-40 nm, 100nm and 150nm, respectively. The morphology of A-PCLNP from TEM image suggests that avidin seems to surround iron oxide(black)core. Fig. 2 shows the magnetization curves of avidin, biotin and PCL. All three samples show diamagnetic property. Among three samples, avidin, which has the largest molecular weight, revealed the strongest diamagnetic property. The diamagnetic properties of these materials possibly work against to the superparamagnetism of iron oxide nanoparticle as shown in Fig. 3. This SQUID measurement show the magnetization curves for PCLNP, A-PCLNP, A-B-PCLNP and iron oxide($Fe_3O_4$) samples at 297K. At the same iron concentration of $Fe_3O_4$ the induced magnetization of iron oxide nanoparticle becomes to decrease by coating with PCL and conjugating with avidin and biotin. Fig. 4(a) shows the T2 relaxation curves for the three samples. The PCLNP shows faster T2 decay curve compared to A-PCLNP and A-B-PCLNP. Thus PCLNP shows highest R2 value. The measured T2 relaxation times and R2 relaxation rate of PCLNP, A-PCLNP and A-B-PCLNP were shown Table. 1. The effects of diamagnetic shielding on MR image intensity are well demonstrated in T2 maps (Fig. 4(b)). In T2 maps, A-B-PCLNP, which has lowest R2 value, shows most bright MR signal and suggests that the degree of diamagnetic shielding of the sample is proportional to the MR signal intensity. As a result, the MR image becomes more bright and the efficiency of the superparamagnetic iron oxide as a T2 contrast agent is reduced by conjugating diamagnetic biomolecules to superparamagnetic iron oxide. This finding is practically important in MR molecular imaging because it is difficult to distinguish any contrast change resulting from the accumulation of these bioconjugated superparamagnetic iron oxides. In conclusion, in the present work we have focused on the diamagnetic shielding effect of avidin-biotin on the magnetic properties of superparamagnetic iron oxide nanoparticles. The results show that the bioconjugation of diamagnetic avidin and biotin to the superparamagnetic iron oxide reduced the total magnetism of iron oxide nanoparticle. The magnetic shielding of diamagnetic materials hampers T2 shortening effect of the superparamagnetic iron oxide and the efficiency of the superparamagnetic iron oxide as a T2 contrast agent is reduced by conjugating diamagnetic biomolecules to superparamagnetic iron oxide. Therefore, although avidin-biotin system is most widely used for bioconjugation in many applications, their diamagnetic properties need to be considered in MR imaging and it is necessary to develop a new linker material with no or less diamagnetism.

[1] R. Weissleder, American journal of roentgenology,155 (1990) 1161

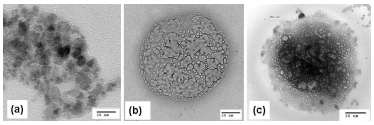



Fig.1. TEM images of (a) PCLNP, (b) Avidin and (c) A-PCLNP

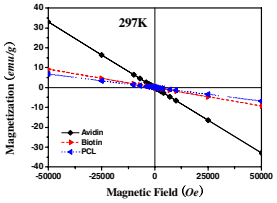



Fig. 2. Magnetization curves of avidin, biotin and PCL

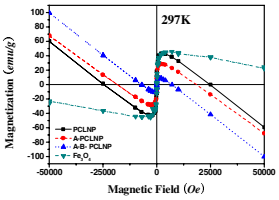
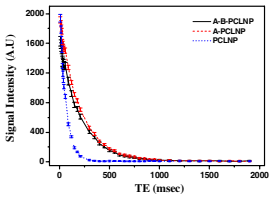



Fig. 3. Magnetization curves of PCLNP, A-PCLNP, A-B-PCLNP and $Fe_3O_4$




for

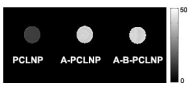



Fig. 4(a). The T2 relaxation curves PCLNP, A-PCLNP and A-B-PCLNP

Fig. 4(b). T2 map of PCLNP, A-PCLNP and A-B-PCLNP

Table 1. The T2 relaxation time and R2 relaxation rate of PCLNP, A-PCLNP and A-B-PCLNP