Functionalized Magnetic Force Enhances Magnetic Nanoparticle Guidance: From Simulation to Crossing of the Blood-Brain Barrier *in vivo*

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In recent studies, we introduced the concept of functionalized magnetic force as a method to prevent nanoparticles from sticking to vessel walls caused by extensive simulation and in vitro experiments involving a Y-shaped channel. In this study, we further investigated the effectiveness of the functionalized magnetic force with a realistic 3D vessel through simulations. For the simulations, we considered a more realistic continuous injection of particles with different magnetic forces and frequencies. Based on the results from our simulation studies, we performed in vivo mice experiments to evaluate the effectiveness of using a functionalized magnetic force to aid magnetic nanoparticles (MNPs) in crossing the blood-brain barrier (BBB). To implement the functionalized magnetic force, we developed an electromagnetic actuator regulated by a programmable direct current (DC) power supply. Our results indicate that a functionalized magnetic field can effectively prevent MNPs from sticking, and also guide them across the BBB. We used 770-nm fluorescent carboxyl MNPs in this study. Following intravenous administration of MNPs into mice, we applied an external magnetic field (EMF) to mediate transport of the MNPs across the BBB and into the brain. Furthermore, we evaluated the differential effects of functionalized magnetic fields (0.25, 0.5, and 1 Hz) and constant magnetic fields on the transport of MNPs across the BBB. Our results showed that a functionalized magnetic field is more effective than a constant magnetic field in the transport and uptake of MNPs across the BBB in mice. Specifically, applying a functionalized magnetic field with a 3 A current and 0.5 Hz frequency mediated the greatest transport and uptake of MNPs across the BBB in mice.

Index Terms— Blood-brain barrier (BBB), *In vivo* experiment, Mice, Targeted drug delivery, Electromagnetic actuation system, Simulation.

I. Introduction

MAGNETIC NANOPARTICLES (MNPs) are a class of nanoparticles that can be manipulated under the influence of an external magnetic field (EMF). For drug delivery to brain cells, MNPs have been used as a vehicle to cross the blood-brain barrier (BBB) [1-2]. They can be guided by non-invasive magnetic forces through the vasculature and concentrated at a localized site within the brain. However, using a constant magnetic force may cause the particles to aggregate or stick to vessel walls, which can lead to blockages. To address this concern, we previously published the use of a functionalized magnetic field, or a field function (FF), to replace the constant magnetic force [3-4]. By intentionally changing the direction of the magnetic field, sticking and aggregation no longer occur.

In this study, the effectiveness of the FF scheme was further evaluated using a realistic vessel. We chose a vessel from the human brain and created the vessel's exact geometry using magnetic resonance imaging (MRI) images and a special procedure [5]. Also, instead of considering by injecting MNPs only one time, this study used a more realistic initial condition by injecting MNPs continuously for 30 s. In addition, simulations were performed with different magnitudes and frequencies of FF. Based on the simulation results, we performed *in vivo* mice experiments to evaluate the effectiveness of the FF in mediating MNP transport across the BBB. Collectively, our results indicated that the FF is an effective method for guiding MNPs across the BBB in mice, with the most optimal FF at a frequency of 0.5 Hz.

II. Functionalized Magnetic Field to Avoid Sticking and Aggregation

Since a magnetic force is proportional to the cube of the particle's diameter, the force needed to navigate MNPs is not strong enough to overcome the drag force. Consequently, guidance of nanoparticles is not very effective. To effectively steer MNPs within blood vessels using existing magnetic/electromagnetic actuators, the direction of the magnetic force has to be perpendicular to blood flow [6]. The drag force exerted on a particle perpendicular to blood flow is less than that in the direction of the flow. Thus, a smaller magnetic force is required to steer the MNPs [6].

However, with a constant perpendicular magnetic force, particles easily aggregate and stick to vessel walls. To address this concern, the concept of an FF was introduced [3]. In brief, the concept involves release of MNPs from the vessel wall by reversing the magnetic direction periodically. The FF is a unitless multiplier function that varies with time, which determines how to change the magnetic field gradient over time under the following constraints:

$$\forall t \quad -1 \leq FF(t) \leq 1 \qquad (1)$$

Figure 1 illustrates the concept of FF. In this figure, T_{plus} and T_{minus} are the times during which the FF (as well as the magnetic force) is positive and negative, respectively. T is the time period of the FF, and the duty ratio (D) and frequency (f) of FF are defined as follows:
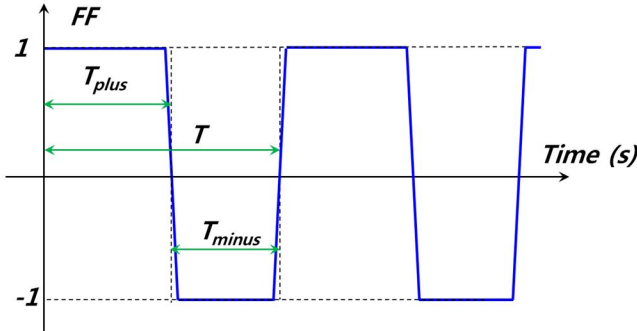
$$D = \frac{T_{plus}}{T}; f = \frac{1}{T} \qquad (2)$$

Fig. 1. Field function characteristic.

III. NUMERICAL SIMULATION STUDIES

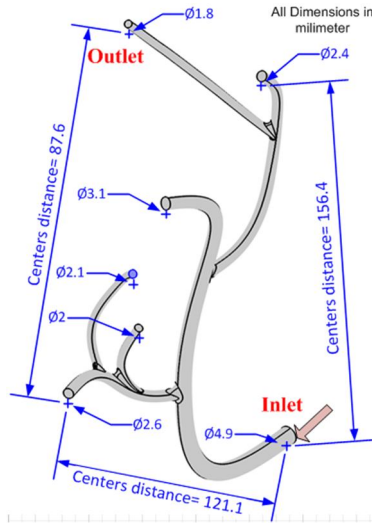### A. Simulation Model, Parameters, and Conditions

Fig. 2. 3D vessel model for simulation studies.

In this study, we first selected a vessel located in the human brain. Next, we extracted its geometry using MRI images via a special procedure [5]. Based on the extracted information, we created a three-dimensional (3-D) model of the vessel using computer-aided design (CAD) software. Finally, we imported this model into COMSOL software (COMSOL Inc., Palo Alto, CA, USA) prior to the simulation studies. The final 3-D model of the channel, with some of the geometric characteristics that were used for modeling, is shown in Fig. 2. We chose the outlet with the longest route and the most bifurcations (3).

Simulation conditions are summarized in Table I. Blood flow was at a steady, creeping flow, which flowed into the channel from the inlet and exited the channel from the outlets.

We selected fluid modeling parameters based on their similarity to blood behavior [6]. Blood flow inside the channels was simulated as a steady-state laminar flow and its velocity profile was calculated using the computational fluid dynamics (CFD) module of COMSOL. Unlike studies [3], [6] that had considered by injecting MNPs only one time, in this study, we considered a more realistic initial condition by injecting 100 particles into the inlet in each step of 0.1sec during 30 sec. By implementing this continuous injecting condition, our simulations become more realistic and consequently, the difference between simulations and experiments has been shortened.

Based on extensive simulation studies [3,6-7], the duty ratios that yielded optimal performances were in the range of 0.6–0.8. In this study, for simplicity, the duty ratio was fixed at 0.7.

TABLE I. MODELLING PARAMETERS

Parameter	Value	Unit
Blood density	1050	kg/m3
Blood viscosity	0.004	Pa.s
Air relative permeability	1	-
Blood relative permeability	1	-
Blood temperature	293.15	K
Particle diameter	770	nm
particle density	6450	kg/m3
Blood velocity	1	mm/s
Vessel length	10	mm
Vessel diameter	1	mm
Input current to the actuator	1 to 6	A
Saturation magnetization	106	kA/m

B. Particles Guidance Performance

TABLE II. PERCENTAGE OF PARTICLES AT THE CORRECT OUTLET IN SIMULATIONS

Current	1A	2A	3A	4A	5A	6A
0 Hz	0%	0%	0%	0%	0%	0%
0.25 Hz	2.66%	2.13%	1.96%	1.93%	1.86%	1.86%
0.5 Hz	6.51%	6.15%	10.0%	6.01%	5.98%	5.71%
1 Hz	0%	0%	2.15%	2.82%	1.13%	2.43%

Table II details the percentages of particles at the correct outlet during simulations using FF schemes with varying frequencies. The data in Table II clearly indicates that in cases in which the magnetic field is kept constant (0 Hz), particles are unable to reach the correct outlet regardless of the input current. This result occurs because all of the particles are stuck to vessel walls before they can reach the correct outlet. For the various FF schemes, we observed that the MNPs reach the correct outlet depending on the frequency and input current values. We observed optimal results at 0.5 Hz. This phenomenon can be explained as follows: as frequency increases, less particles are stuck at the vessel walls since the positive time T_{plus} is decreased. In this context, the negative time T_{minus} is long enough to release the particles from the walls. However, when the frequency is too high (> 0.5 Hz), the negative time T_{minus} is too small and the particles do not have enough time to release from the walls.

The results from these simulations suggest that the FF can

effectively enhance MNPs guidance by preventing sticking and aggregation at vessel walls. Furthermore, we observed optimal results when the frequency of the FF was 0.5 Hz.

IV. IN VIVO MICE EXPERIMENTS

A. Electromagnetic actuator for implementing FF

To implement the FF scheme, we used an electromagnetic magnetic actuator (EMA) that we designed in a previously study [4]. Figure 3 illustrates the experimental setup of the electromagnetic actuator system and its geometry. Geometric parameters were optimized via extensive simulation studies [6].
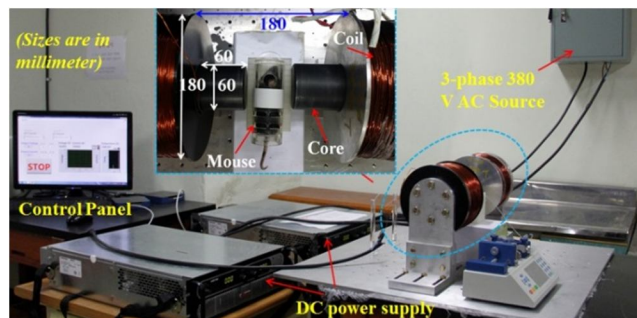

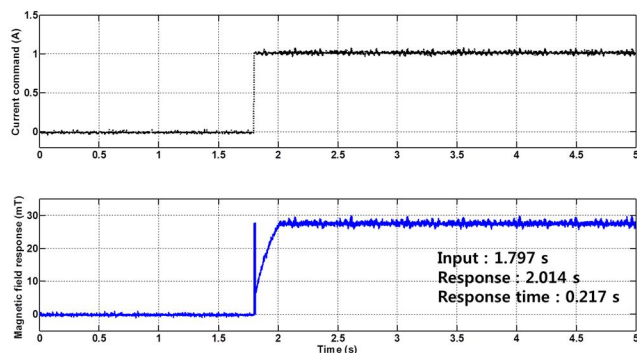Fig. 3. Experimental setup of the proposed electromagnetic actuator.

Fig. 4. Response time of the coil-core system.

The magnetic field at the region of interest (ROI) was 60 mm in diameter at the center of the actuation system, and was designed specifically for experiments involving mice. The magnetic force was controlled by a direct current (DC) power supply and the current in the coil. A current of up to 17 A could be applied to the coil-core system, which had a 20 Ω resistance and 0.42 H inductance. The rated power of the DC power supply was 6 kW. Figure 4 shows the response time of the coil-core system with a DC power supply. The 1 A current input command to the DC power supply was set as a step function at 1.797 s. At 2.014 s, the magnetic field response at the center of ROI reached 28.2 mT, meaning that the response time of our system was approximately 0.217s. This response time of EMA is mainly affected by the inductance of the coil and the response time of the DC power supply. Although this response time was quite slow, it was quick enough in the application to guide the particles (see B. Methods and conditions). In Fig. 5, we show the magnetic field surface in

the ROI of a coil with and without a core. As shown in the figure, the core strengthens the magnetic field and its gradient at the ROI by approximately 10-fold.
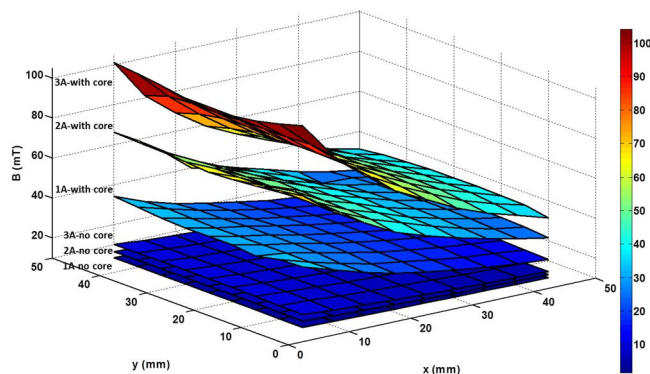

Fig. 5. Magnetic field surface of the left coil in the ROI with and without a core.

B. Conditions and Method

Yellow, fluorescent carboxyl MNPs (1% w/v) were purchased from Spherotech. The nanoparticles were 700–900 mm in diameter (mean diameter = 770 nm).

Male wild-type C57BL/6N mice (25–30 g, 8 weeks old) were purchased from Samtako Bio (Osan, South Korea). The mice were acclimatized for 1 week in the university animal house under a 12 h/12 h light/dark cycle (23ºC, 60% humidity), and were provided food and water ad libitum.

Mice were divided randomly into seven groups (n = 5 mice per group; see below). All groups were treated intravenously (I.V.) with 0.4 mL of fluorophore-labeled MNPs and then exposed to various EMF conditions (i–e) consisting of either a constant magnetic field (CMF) or a functionalized magnetic field (FMF) for 1 min. MNP uptake in the brain was verified by confocal microscopy (Fig. 6).

(1). I.V. injection of saline, no exposure to EMF (control group)
(2). I.V. injection of MNPs, no exposure to EMF
(3). I.V. injection of MNPs, exposure to CMF (1 A)
(4). I.V. injection of MNPs, exposure to CMF (3 A)
(5). I.V. injection of MNPs, exposure to FMF (3 A, 0.25 Hz)
(6). I.V. injection of MNPs, exposure to FMF (3 A, 0.5 Hz)
(7). I.V. injection of MNPs, exposure to FMF (3 A, 1.0 Hz)

Mice from all groups were sacrificed following treatment. All efforts were made to minimize suffering and the number of mice used. Experimental procedures were approved by the animal Ethics Committee of the Division of Applied Life Sciences, Department of Biology at Gyeongsang National University in South Korea.

Brain tissues from all of the above-mentioned groups were collected after treatment. Transcardial perfusion was performed using 1× phosphate-buffered saline (PBS) followed by 4% ice-cold paraformaldehyde. Brain tissues were post-fixed overnight in 4% paraformaldehyde and subsequently transferred to 20% sucrose until they settled at the bottom of the tube. Brains were frozen in optimal tissue cutting

temperature compound (Tissue-Tek O.C.T. compound; Sakura Finetek USA, Inc., Torrance, CA, USA) and then sectioned into 14 μm sections in the coronal plane with a CM 3050S cryostat (Leica, Wetzlar, Germany). Sections were thaw-mounted on Probe-On positively charged slides (Thermo Fisher Scientific Inc., Waltham, MA, USA) and stored at − 70°C. Brain tissue slides were dried overnight and washed twice with 0.01 M PBS for 15 min each. Tissue sections were stained with 4',6-diamidino-2-phenylindole (DAPI) for 10 min, rinsed with PBS, and glass coverslips were mounted on the tissue slides using fluorescent mounting medium. Images were captured with a confocal laser scanning microscope (FluoView FV 1000; Olympus, Tokyo, Japan), with argon ion laser. The power of laser was 20%. The fluorescent carboxyl MNPs excitation spectra were ranging from 400-500 nm and it showed highly efficient fluorescence in the FITC channel at 488 nm. The magnification was 10x with dried type lens.
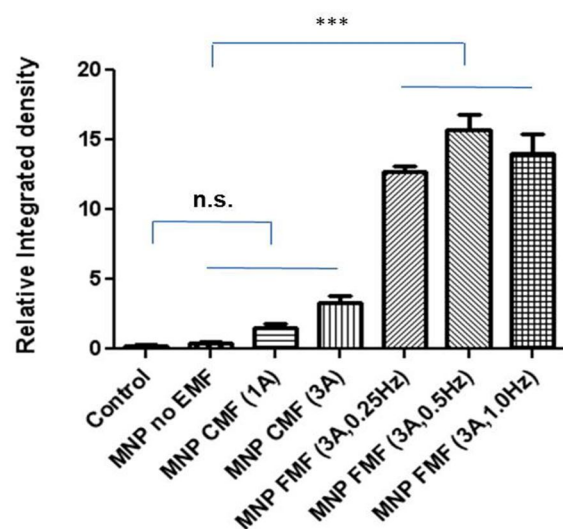

Fig. 6 Histogram showing the results of confocal microscopy analyzed by using the computer based Image J program. Data were expressed as the mean ± SEM of experiments performed in triplicate (n=3). Significant differences were evaluated using a one-way analysis of variance (ANOVA) followed by the student t-test. Differences were considered to be statistically significant. Symbol *** represents a significant difference compared to the control group and constant magnetic field (***p<0.0001).

Confocal analysis demonstrated that application of an EMF can facilitate the movement of MNPs across the BBB, resulting in MNP accumulation in the brain (Fig. 6). In the absence of an EMF, we observed no significant accumulation of MNPs in the brain. Under constant magnetic field conditions, when the value of the current increased from 1 A to 3 A, the magnetic field and magnetic field gradient increased, and as a result a greater magnetic force was produced. However, a higher magnetic force with a constant magnetic field increased particle aggregation, which negatively affected the particles' movement across the BBB [8]. This phenomenon also occurred in the normal control mice, as shown in Fig. 6. To address the issue of aggregation,

and to increase the uptake and transport of particles into the brain, we proposed the concept of a functionalized magnetic field [3]. By applying a functionalized magnetic field, the rate of particle transport across the BBB increased significantly compared to transport under a constant magnetic field with the same current (p < 0.0001). The results from this *in vivo* experiment suggest that using a functionalized electromagnetic field will reduce particle aggregation [3] and subsequently increase particle movement across the BBB.

V. DISCUSSION AND CONCLUSION

In this study, we showed that an FF can be utilized to prevent particle sticking and aggregation through extensive simulations by considering more realistic injection conditions of MNPs in a complex 3-D real-like vessel. The *in vivo* mice experiments were performed using an electromagnetic actuator that implemented the FF scheme. The various simulations and mice experiments showed that FF can be used to effectively prevent particle sticking and aggregation, subsequently resulting in particles crossing the BBB and accumulating in the brain.

ACKNOWLEDGMENT

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