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Movement of magnetic nanoparticles in brain tissue: mechanisms and impact on normal neuronal function

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Abstract

Magnetic nanoparticles (MNPs) have been used as effective vehicles for targeted delivery of theranostic agents in the brain. The advantage of magnetic targeting lies in the ability to control the concentration and distribution of therapy to a desired target region using external driving magnets. In this study, we investigated the behavior and safety of MNP motion in brain tissue. We found that MNPs move and form nanoparticle chains in the presence of a uniform magnetic field, and that this chaining is influenced by the applied magnetic field intensity and the concentration of MNPs in the tissue. Using electrophysiology recordings, immunohistochemistry and fluorescent imaging we assessed the functional health of neurons and neural circuits and found no adverse effects associated with MNP motion through brain tissue.

From the Clinical Editor: Much research has been done to test the use of nanocarriers for gaining access across the blood brain barrier (BBB). In this respect, magnetic nanoparticles (MNPs) are one of the most studied candidates. Nonetheless, the behavior and safety of MNP once inside brain tissue remains unknown. In this article, the authors thus studied this very important subject.

Key words: Magnetic nanoparticles; Transport; Drug delivery; Brain; Safety

Nanotechnology based solutions for the treatment of brain tumors have been developed in recent years to address the challenges faced by conventional cancer therapeutics[1] such as surgery,[2,3] chemotherapy[4–6] and radiation therapy.[7,8] Drugs such as doxyrubicin[9] and oxantrazole[10] can be combined with appropriate nanocarriers to penetrate the blood brain barrier (BBB) to increase the intracellular concentration of drugs in tumor cells.[11–13] Magnetic nanoparticles (MNPs) have been investigated as effective nanocarriers for targeted drug delivery in the brain.[14–17] MNPs coated with pharmacological agents, proteins, and genes can potentially be imaged using MRI technology and guided toward brain tumor locations using external magnets.

MNPs with an aminosilane coating have been investigated in human trials for targeting glioblastoma multiforme cells and have been shown not to cause any adverse effects in patients. In the presence of an alternating magnetic field, the MNPs were found to extend tumor necrosis with minor or no side effects in the patients.[17] Hassan and Gallo showed that after a systemic injection of magnetic chitosan microspheres coated with oxantrazole, while in the presence of a 0.6 T magnetic field, microspheres accumulated in a targeted region of rat brain tissue.[10] Thus, MNPs have been shown to cross the BBB and reach targets in brain tissue without disrupting the barrier in rat models.[15,18]

Furthermore, endothelial progenitor cells (EPCs) from humans have been loaded with MNPs and guided to targets in mouse brains.[19] These EPCs loaded with MNPs have shown increase in secretion and migration of growth factors such a vascular endothelial growth factor (VEGF) and fibroblast growth factor

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(FGF), in vitro, thereby promoting angiogenesis for neural regeneration. Various in vitro studies have shown that cancer cells can be made to internalize a higher level of nanoparticles with drugs by appropriate targeting of receptors.[20–23] This illustrates that MNPs can be used as a potential option to circumvent the challenges faced by conventional drug delivery techniques.

Most of the work mentioned above has focused on the motion of MNPs through blood vessels and the observation of MNP presence in living tissue.[15,24,25] The motion of MNPs in brain tissue surrounding the blood vessels is expected to differ from its motion in the vessels. Hence there is a need for a better understanding of the motion of MNPs in brain tissue after extravasating from blood vessels. To be appropriate for therapeutic purposes, MNP movement cannot induce cytotoxic effects, nor should it adversely influence circuit function. Addressing these needs will result in better nanotherapeutic schemes to target tumors in brain tissue diminishing permanent side effects following drug delivery.

Here, we examine the movement MNPs in brain tissue under an applied magnetic field. The movement of MNPs throughout this work includes the interactive motion of MNPs toward each other caused by the influence of an external magnetic field. Using whole-cell patch recordings, immunohistochemical staining and confocal imaging, we found that the motion of MNPs did not cause any detrimental effects on the functional health of the neurons or the circuit function in the main olfactory bulb.

Methods

Characterization of magnetic nanoparticles

The physical properties (mean hydrodynamic diameter, polydispersity index) of MNPs (nano-screenMag, Chemicell, listed as 300 nm diameter) used in our experiments were determined using dynamic light scattering. The MNPs were required to be monodispersed to avoid non-uniformity in their motion in the tissue caused by particle size variations. For the dynamic light scattering measurements, the stock concentration of MNPs (25 mg/mL in double distilled water) was diluted with de-ionized water to a concentration of 0.25 mg/mL. Three samples of 3 mL of the diluted solution were used for the measurement assays. The particle size distribution curve was plotted for these samples and used to calculate the polydispersity index (Figure 1, *A* in Supplementary materials).

The magnetic properties of the nanoparticles including magnetic susceptibility and saturation magnetization were measured using a vibrating sample magnetometer (Lake Shore Cryotronics Inc.). Sample volumes of 60 μL of MNPs in DI water were pipetted into the sample holder (Kel-F) and the holder was placed in the vibrating sample magnetometer setup. The experiments were performed at room temperature (298 K). The samples were exposed to a cycle of different magnetic field values in the range of −1.5 to +1.5 tesla and the corresponding net magnetization produced in the samples was recorded. The magnetic properties (susceptibility and saturation magnetization) of the samples were then calculated from the magnetization versus magnetic field (M vs H) plot obtained from the vibrating sample magnetometer (Figure 1, *B* in Supplementary materials).

Uniform magnetic field using a two magnet setup

A system was created to apply a uniform magnetic field to magnetic nanoparticles inside brain tissue slices. A uniform magnetic field was desired so that all MNPs in the tissue would experience the same magnetic field irrespective of their location in the tissue. Two permanent magnets, appropriately sized and placed as shown in Figure 1, *A*, were sufficient to create a uniform magnetic field. The uniformity of the field was verified by a 3-channel Gaussmeter (Lake Shore Inc.) mounted on a piezo positioning stage (VXM Motor Inc.). The Gaussmeter measured the spatial distribution of the magnetic field intensity between the two magnets and it was found that the deviation from the mean magnetic field intensity in the tissue sample volume was less than 1%. These data are displayed in Figure 2 in the Supplementary Materials.

Motion of MNPs in the brain slices

The motion of MNPs toward each other under the influence of an applied uniform magnetic field was studied in rat brain slices using a total of 12 rats (Sprague Dawley). Each different motion experiment was repeated three times using tissue from different rats to ensure that the data were independent of animal to animal variability. The rat brains were dissected out and immediately stored at 4 °C in 1X Phosphate Buffer Saline (PBS) solution to increase their viability. After 15 minutes, the brains were injected in the prefrontal cortex with 4 μL of the MNPs, using a 10 μL micro-syringe (Hamilton). Following this injection we obtained cortical slices using a razor blade. The slicing was facilitated by the low temperature storage of the brain samples. The slices containing the injected MNPs were then stabilized at room temperature in 1X PBS solution in a Petri dish. The MNPs were visualized by fluorescence using a lipophilic dye coating (Texas Red, Chemicell) with excitation and emission wavelengths of 578 nm and 613 nm respectively. The Petri dish containing the brain slices, immersed in PBS, was placed in the uniform magnetic field region of the two magnet setup. The effect of the uniform magnetic field on the MNPs in the brain slices was observed using a fluorescence microscope (Zeiss) with ×40 magnification and recorded using a video camera (Hamamatsu). The videos were post-processed in MATLAB (Mathworks) to quantify the movement of the MNPs in the uniform magnetic field.

Electrophysiological recordings

All animal studies were conducted in accordance with the policies and recommendations of the National Institute of Health Guide for the Care and Use of Laboratory Animals, and under approval from the Institutional Animal Care and Use Committee of the University of Maryland. The electrophysiological recordings were performed in brain slices extracted from wild-type BL6/C57 mice (Jackson Labs), or 4-6-week-old transgenic mice expressing green fluorescent protein (GFP) and subjected to MNP motion. Specifically, we used the ChAT-Tau-GFP line, generously provided by Dr. Sukumar Vijayaraghavan.[26] We performed these electrophysiology experiments in mice because of the feasibility of transgenic modification in a mouse model compared to a rat model. All the functional experiments involved whole-cell