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# Rotating magnetic field induced oscillation of magnetic particles for *in vivo* mechanical destruction of malignant glioma

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ABSTRACT

Magnetic particles that can be precisely controlled under a magnetic field and transduce energy from the applied field open the way for innovative cancer treatment. Although these particles represent an area of active development for drug delivery and magnetic hyperthermia, the *in vivo* anti-tumor effect under a low-frequency magnetic field using magnetic particles has not yet been demonstrated. To-date, induced cancer cell death *via* the oscillation of nanoparticles under a low-frequency magnetic field has only been observed *in vitro*. In this report, we demonstrate the successful use of spin-vortex, disk-shaped permalloy magnetic particles in a low-frequency, rotating magnetic field for the *in vitro* and *in vivo* destruction of glioma cells. The internalized nanomagnets align themselves to the plane of the rotating magnetic field, creating a strong mechanical force which damages the cancer cell structure inducing programmed cell death. *In vivo*, the magnetic field treatment successfully reduces brain tumor size and increases the survival rate of mice bearing intracranial glioma xenografts, without adverse side effects. This study demonstrates a novel approach of controlling magnetic particles for treating malignant glioma that should be applicable to treat a wide range of cancers.



## 1. Introduction

The field of nanotechnology for cancer therapy has expanded drastically in the last decade with micro and nanoparticles successfully used as therapeutic or diagnostic materials [1,2]. As part of this progress, magnetic nanomaterials are now integrated in biomedical applications such as contrast agents in magnetic resonance imaging, therapeutic agents enabling magnetic hyperthermia, and drug delivery [3–6]. One advantage of these platforms is that they can induce the physical destruction of cancer cells (e.g. by hyperthermia, mechanical force), offering an alternative to molecule-based therapeutic approaches like chemotherapy agents or receptor/molecule-targeted antibodies [7–9].

Magnetic actuation via mechanic forces offers an exciting strategy to remotely control cell functions for cancer treatment [10–12]. Coupled with the magnetic materials a magnetic field can be utilized to trigger specific signaling pathways and control ion channel or surface receptor activities to actuate apoptosis within a target cell via a contactless approach [13–15]. Compared to other physical stimuli such as light and heat, magnetic field provides the distinct advantage to activate deep seated tumors in a controllable and noninvasive fashion for *in vivo* applications [16–18].

In recent years, development of novel magnetic materials via mechanical force to induce cell apoptosis has been extensively explored [19–21]. For instance, Cho et al. demonstrated a magnetic switch using zinc-doped iron oxide magnetic nanoparticle conjugated with a targeting antibody for death receptor 4 which could aggregate under a permanent magnetic field and mimic the TRAIL signaling pathway to trigger the apoptosis of zebrafish [22]. Another pathway is to transduce mechanical stimulation to targeted cellular structures in order to induce apoptosis. Zhang et al. showed that superparamagnetic iron oxide nanoparticles covalently conjugated with antibodies targeting the lysosomal protein marker LAMP1 could tear the lysosomal membrane and induce 12.45% apoptosis in INS-1 cells *in vitro* under a dynamic magnetic field [23]. Stretching cytoskeletal and actuating ion channels via mechanical forces have also been demonstrated *in vitro* for cancer destruction. Kim et al. found *in vitro* that biofunctionalized magnetic vortex microdisks could selectively transmit mechanical force to a cancer cell's membrane and induce chemical ionic signal such as calcium to

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initiate programmed cell death upon exposure to an alternating field [9]. These ferromagnetic microdisks with a magnetic vortex ground state were oscillated under an alternating magnetic field with a low frequency (tens of Hz) and small amplitude (9 mt). Although these approaches provide insightful investigations for cell apoptosis activities, the gap of extending the related approach to clinical applications exists in the context of cancer treatment.

*In vivo* magnetic actuation of apoptosis through mechanical force is challenging for cancer treatment. It requires that majority of the magnetic particles will respond to the field to induce programmed cancer cell death in the complex biological environment. Here, our proof of principle study demonstrates the efficacy of using magnetic-vortex disk-shaped magnetic particles (MPs) in combination with a low-frequency rotating magnetic field in an animal model of malignant glioma, which carries the worst prognosis among all brain and central nervous system tumors [24]. We first examine the cellular uptake of MPs and the destruction effect of the internalized particles to glioblastoma *in vitro* under an applied magnetic field. After internalization into glioma cells, the MPs under an externally applied rotating magnetic field were shown to damage the membrane integrity of cells, leading to extensive cell death after a single 30 min treatment. *In vivo*, under the rotating magnetic field, these particles pre-incubated with glioma cells also led to a significant decrease in tumor growth as observed by increased apoptotic area in the tumor, resulting in improved survival outcomes in glioma-bearing mice.

## 2. Materials and methods

### 2.1. Materials and cell culture

The human glioma cell line U87 was purchased from the American Type Culture Collection (Manassas, VA., USA) and cultured in Dulbecco's Modification of Eagle's Medium (DMEM) (Mediatech Inc., Manassas, VA, USA), containing 2% penicillin and streptomycin antibiotic (Cellgro, Mediatech, Inc., Manassas, VA, USA) and 10% fetal bovine serum (FBS; Atlanta Biologicals, Lawrenceville, GA, USA). U87 cells constantly expressing firefly luciferase and green fluorescent protein (U87-Fluc-GFP) were established as described previously [25]. Cells were incubated with replication-deficient lentiviral vectors containing Fluc and GFP expression cassettes for 48 h. Fresh growth medium containing 1 μg $mL^{-1}$ puromycin was added for the establishment of clonal populations. Cells were sorted by FACS to verify GFP expression. The stable Fluc expression was verified via luciferase assays.

### 2.2. Rotating magnet test station

The rotating magnetic field station used an NdFeB Halbach Array magnet (Bunting Magnetics Europe Ltd., Hertfordshire, UK), which produces a uniform 1 Tesla magnetic field diametrically across the central air gap. The magnet was mounted on a motor to control its rotation and the head of the mice was placed inside the central air gap. The test station was used for both in vitro and in vivo experiments. Measurement of hyperthermia was conducted with a Fluke 51 single input digital thermometer with a temperature accuracy of $\pm 0.05\% + 0.3$ °C. The experiment was conducted with complete media (DMEM + FBS) with and without the magnetic particles to observe changes during the duration of the experiment timeframe (30 min).

### 2.3. Animal experiments

6-week-old male athymic/nude mice weighing 18–22 g were purchased from Charles River Laboratory (Wilmington, MA, USA). Animals were cared for according to a study-specific animal protocol approved by the University of Chicago Institutional Animal Care and Use Committee. To examine the MPs cell destruction effect *in vivo*, U87-Fluc-GFP cells were incubated with MPs at 50 particles/cell ratio. A right side burr hole centered 2 mm lateral to the sagittal suture and 2 mm posterior to the coronal suture was drilled on the mouse skull. After positioning the animals in the stereotactic frame, $1 \times 10^5$ glioma cells in 5 μL PBS were injected 3 mm deep into the mouse brain. At day 4 post the glioma cell implantation, mice were randomly divided into 2 groups (n = 5 mice/group) as following: 1) untreated group as control and 2) treated group received one-hour magnetic treatment daily for 7 days. The health condition of the mice was monitored daily. During the MF treatment, the mice were kept in a DecapiCones® mouse restrainer (Braintree Scientific, Inc., MA, USA) without anesthesia. The head of the mouse was located at the center of the magnet. The magnetic field conditions were kept constant at 20 Hz rotation frequency and 1 Tesla strength for all exposures. Bioluminescence of the U87 cells due to the expressed firefly luciferase activity was imaged after intraperitoneal injection of D-luciferin (4.5 mg/animal in 150 μL of saline) at day 7, 14, 21 and 28 days post glioma cell implantation. The spatial distribution of luciferase activity within the brain was recorded using a Xenogen IVIS 200 imaging system. For the histology study, the U87 glioma-bearing mice were established by intracranially injection with $10^5$ U87 glioma cells on the right hemisphere of the brain. On day 3, the mice received a second intracranial, intratumoral injection of MPs at $5 \times 10^6$ particles per animal. On day 4, animals were randomly divided into untreated group (n = 3 mice) and treated group (n = 5 mice). The treated group received the daily MF treatment for a week. The mouse brain, liver, spleen, heart, lung, kidney, large intestine, bladder and testes were collected post of the treatment. In accordance to the guidelines suggested by the University of Chicago for selecting humane endpoint in rodent studies, animal health conditions were determined through developed signs of systems of toxicity related to the treatment. The signs and symptoms include inability to would heal, hunched posture and increased respiration, weight loss (>10%), inability to reach food or water, lethargy or hemiparesis. Histological analysis for neurotoxicity was performed by a licensed neuropathologist.

### 2.4. Transmission electron microscopy

$2.5 \times 10^6$ U87 cells were seeded into a T-75 plate. $1.25 \times 10^8$ MPs were added into the well and incubated with the cells for 24 h. Post incubation, the cells were fixed with 2% glutaraldehyde and 4% paraformaldehyde in 0.1 M sodium cacodylate buffer for 2 h. The cell pellet was washed with sodium cacodylate buffer 3 times and post fixed with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer for 1 h. The cells were then washed with sodium cacodylate buffer and maleate buffer, respectively. 1% uranyl acetate in maleate buffer was applied to the cells for 1 h and then washed with maleate buffer for 3 times. The cells were dehydrated in a series of washes with increased ethanol concentration. After infiltration in a 2:1 propylene oxide:spurr resin, 1:1 propylene oxide:spurr resin and 100% spurr resin, the cell sample was polymerized overnight at 60 °C. The resin block was cut using Reichert-Jung Ultracut E microtome. The sections (90 nm thickness) were stained with uranyl acetate and lead citrate. Images were taken under 300 kV using a FEI Tecnai F30 microscope.

The sections (90 nm thickness) were stained with uranyl acetate and lead citrate to elucidate the components in the cells. Uranyl acetate was used to enhance the contrast by interaction with sialic acid carboxyl groups of lipids and proteins in addition to the nucleic acid phosphate groups of DNA. The lead citrate is used to enhance the contrast for a wide range of cellular structures due to the interactions of proteins as well as glycogens.

### 2.5. Evaluation of in vitro cell destruction of MPs under magnetic field

Glioma cells were seeded on 96-well plates at $1 \times 10^4$ cells per well. The cells were incubated with MPs for 1 h or 24 h. Post incubation, cells were plated into the center of the magnetic field and treated for 30 min. After 24 h, the cell destruction was quantified using MTT colorimetric