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Glioma Selectivity of Magnetically Targeted Nanoparticles: A Role of Abnormal Tumor Hydrodynamics

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Abstract

Magnetic targeting is a promising strategy for achieving localized drug delivery. Application of this strategy to treat brain tumors, however, is complicated by their deep intracranial location, since magnetic field density cannot be focused at a distance from an externally applied magnet. This study intended to examine whether, with magnetic targeting, pathological alteration in brain tumor flow dynamics could be of value in discriminating the diseased site from healthy brain. To address this question, the capture of magnetic nanoparticles was first assessed *in vitro* using a simple flow system under theoretically estimated glioma and normal brain flow conditions. Secondly, accumulation of nanoparticles via magnetic targeting was evaluated *in vivo* using 9L-glioma bearing rats. *In vitro* results that predicted a 7.6-fold increase in nanoparticle capture at glioma-versus contralateral brain-relevant flow rates were relatively consistent with the 9.6-fold glioma selectivity of nanoparticle accumulation over the contralateral brain observed *in vivo*. Based on these findings, the *in vitro* ratio of nanoparticle capture can be viewed as a plausible indicator of *in vivo* glioma selectivity. Overall, it can be concluded that the decreased blood flow rate in glioma, reflecting tumor vascular abnormalities, is an important contributor to glioma-selective nanoparticle accumulation with magnetic targeting.

Keywords

magnetic nanoparticles; brain tumor targeting; magnetic targeting; glioma vascular abnormalities; tumor selectivity

1. Introduction

Delivery of therapeutic agents to brain tumors is one of the most challenging goals of chemotherapy. Extensive research is currently underway to explore the applicability of various delivery vehicles (e.g. liposomes, nanoparticles) to target brain tumor lesions [1]. Magnetic nanoparticles are a particularly interesting platform, since their magnetic responsiveness renders them useful for magnetic targeting.

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Magnetic targeting is a promising strategy for achieving localized drug delivery to tumor tissue. The accumulation and retention of drug-loaded magnetic nanoparticles in tumors can be enhanced by the attraction of nanoparticles to the tumor location using an externally applied magnetic field. Several studies have already demonstrated the feasibility of magnetic targeting in rodents bearing subcutaneous tumors [2-4]. In these studies, magnetic nanoparticles were administered locoregionally into the afferent blood vessels supplying the tumor and a magnetic field source was positioned directly towards the tumor. Typically, magnetic field density is maximal at the magnet pole face and cannot be focused at a distance from the magnet [5]. It has been mathematically derived that the force of magnetic attraction, imposed by an external magnet on a magnetic nanoparticle within the body, is maximal at the edge of the body part that is in direct contact with the magnet and there are no other maxima at deeper body locations [6]. Thus, the superficial tumor position, and their consequently closer physical proximity to the external magnet, played a major role in achieving higher nanoparticle accumulation in the tumor over deeper located tissues.

Brain tumors present a more complex situation. Based on considerations of magnetics alone, brain tumors would seem to be a doomed goal for magnetic drug targeting since their deep, intracranial location would preclude selective accumulation of the magnetic carrier without simultaneous deposition within the normal brain regions. However, since nanoparticles administered intravascularly are carried to the tumor target by circulation, hydrodynamics is as important a parameter, as magnetics, in governing the localization of nanoparticles by magnetic targeting [7]. Conceptually, the magnetic targeting situation is analogous to a magnetic separation process, where magnetic nanoparticles are extracted from their carrier fluid as they flow through a region of magnetic field gradient [8]. Theoretical analysis of the magnetic separation system demonstrated that the capture efficiencies of nanoparticles by the magnet were inversely related to the flow rates of the carrier medium [9].

The flow rates of blood, functioning as a physiological carrier fluid, vary profoundly in different tissues and can also be altered by pathological conditions. Brain tumors are known to be pronouncedly distinct from the intact brain in their vascularization and hemodynamic characteristics [10]. The structural and functional abnormalities of brain tumor microvasculature were shown to include reduced vascular density [11], increased capillary diameter [12] and markedly decreased blood flow [13,14].

The purpose of this study was to evaluate whether pathological alteration in the flow dynamics of a brain tumor could be of value in discriminating the diseased site from healthy brain with magnetic targeting. To thoroughly address this issue, our investigation was designed in two parts. In the first part, *in vitro* capture of magnetic nanoparticles by a magnetic field gradient was carried out under flow conditions that were theoretically estimated to approximate the physiological situation of a glioma and normal brain. In the second part, *in vivo* brain accumulation of nanoparticles by magnetic targeting was studied in rats harboring 9L-glioma.

2. Theoretical Considerations

2.1 Significance of capillary blood flow for nanoparticle capture by magnetic targeting

The underlying principles of magnetic targeting of brain tumors can be conceptually illustrated using the scheme of Figure 1. As seen, the rat has a brain tumor confined to the right hemisphere and its head is positioned in the air gap between the poles of an electromagnet. The magnetic field density is homogeneous within the air gap and declines with distance from the air gap, generating a gradient of magnetic field density in the y-direction. Due to system symmetry, the tumor lesion and corresponding region of the contralateral hemisphere are exposed to the same magnetic field gradient.

Magnetic nanoparticles are administered intravenously and passively distributed throughout the animal body by systemic circulation. A fraction of the nanoparticles, carried by cerebral blood flow, reaches the brain microvasculature [15]. The physical situation experienced by the nanoparticles in a single capillary is analogous to that previously described for the magnetic separator [16] and is schematically depicted in the inset of Figure 1. Magnetic capture of nanoparticles is governed mainly by two processes: magnetic attraction and hydrodynamic drag. The magnetic attraction force (F_m) acts on a nanoparticle in the direction of steepest increase in magnetic energy density and is described by:

$$\vec{F}_m = \frac{\chi}{2\mu_0} \nabla B^2 \quad [1]$$

where χ is the nanoparticle's magnetic susceptibility, V - the nanoparticle volume, μ_0 - the magnetic permeability of free space and B is the magnetic field density.

The hydrodynamic drag force (F_d) acts on the particle in the axial direction of the flow and is given by Stokes' law:

$$\vec{F}_d = 6\pi R\eta(\vec{v} - \vec{v}_p) \quad [2]$$

where R is the magnetic nanoparticle's radius, η - the medium viscosity, v - the streamline (bulk flow) velocity and v_p is the nanoparticle velocity.

The vector sum of the magnetic and drag forces acting on a nanoparticle determines its trajectory inside the capillary (Figure 1: inset). Outside the magnetic field gradient, nanoparticles are carried by blood flow and their velocity (v_p) is equal to that of the flow (v). Thus, according to equation [2], the drag force acting on the particles in the axial (z) direction is zero. Within the region of the magnetic field gradient, the force of magnetic attraction causes particles to deflect from the streamline, in the transverse (x) direction, towards the magnet and also changes nanoparticle velocity in the z-direction. The consequently increased difference in velocity between particles and bulk flow results in an increased drag force. When a particle's axial velocity is reduced to zero, the drag force, striving to sweep the particle with the flow, reaches its maximal value and becomes directly proportional to the bulk flow velocity. To capture magnetic nanoparticles, the force of magnetic attraction must overcome the drag force:

$$|\vec{F}_m| \geq |\vec{F}_d| \propto v \quad [3]$$

Equation [3] suggests that blood flow rate is an important parameter in determining the likelihood and extent of nanoparticle retention by magnetic targeting.

2.2 Calculation of linear blood velocities in cerebral capillaries

The average linear blood velocity in a single cerebral capillary was estimated by simplistically modeling the tumor (and contralateral normal brain) tissue as a spherical mass of volume V impenetrated by N parallel, cylindrical capillaries of diameter d . It was also assumed that all capillaries are equally perfused. Capillary flow rate is then derived from the following analysis:

1. Due to the assumed spherical geometry, the tumor radius (r) and tumor cross-sectional area (c) can be calculated as:

$$r = \sqrt[3]{3V/4\pi} \quad [4]$$

$$c = \pi r^2 = \pi \left(\frac{3V}{4\pi} \right)^{2/3} \quad [5]$$

2. The total number of capillaries (N) per tumor lesion is given by the product of tumor microvessel density (n) and cross-sectional area (c):

$$N = nc \quad [6]$$

3. Knowing the capillary diameter (d), the total cross-sectional area of tumor capillaries (C_T) can be calculated as:

$$C_T = \left(\frac{d}{2} \right)^2 \pi N \quad [7]$$

4. Total blood flow (F) to a tumor of mass (m) can be calculated from the blood perfusion rate of the tumor (P) as:

$$F = Pm \quad [8]$$

5. The average linear blood velocity (v_L) in a single capillary is then given by:

$$v_L = \frac{F}{C_T} \quad [9]$$

Substituting Equations [5], [6], [7] and [8] into Equation [9] and assuming tissue specific gravity(s) of 1 (i.e. $m = V$), the following equation is obtained:

$$v_L = \frac{PV}{n} \left(\frac{\pi d}{2} \right)^{-2} \left(\frac{3V}{4\pi} \right)^{-2/3} \quad [10]$$

According to Equation [10], the average linear blood velocity in a single capillary (v_L) is a function of four parameters: blood perfusion of the tumor (P), tumor volume (V), microvessel density (n), and capillary diameter (d).

3. Material and Methods

3.1. Materials

Magnetic nanoparticles (G100) were generously contributed by Chemicell ® (Berlin, Germany).

3.2. *In Vitro* studies

3.2.1 Zero Field Cooled (ZFC) magnetization—Magnetization measurements were performed using a MPMS-XL superconducting quantum interference device (SQUID) magnetometer (Quantum Design Inc. San Diego, CA). The zero-field-cooled (ZFC) magnetization experiment was conducted by cooling the lyophilized nanoparticle sample to 5° K in zero field and monitoring the magnetization of the sample as it was warmed up to 300°K in a constant magnetic field of 100 Oe.

3.2.2 Kinetic analysis of magnetic entrapment under physiologically relevant flow conditions—Thin tubing with an internal diameter of 0.058 cm was positioned in the air gap between the poles of an electromagnet. An aqueous nanoparticle suspension, at a concentration of 0.2 mg Fe/mL, was continuously infused through one end of the tubing and collected at the other end. Linear flow rates were controlled with a syringe pump. The magnetic field within the air gap was initially turned off while the tubing was primed with nanoparticle suspension and was then set to 0.4 T at time 0 of the experiment. Images of the tubing segment located between the poles of the electromagnet were acquired with a digital camera before and serially after the initiation of the magnetic field, at 2 minute intervals for 20 minutes and subsequently at 5 minute intervals for an additional 10 minutes.

Quantitative information was derived from the collected images using Jasc Paint Shop Pro 8 software. All images were converted to grayscale. A series of images collected at a given flow rate were visually inspected to determine a region of the tubing exhibiting a progressive decrease in light intensity (i.e. a dark region), representative of nanoparticle accumulation. This manually defined region of interest (ROI) was consistently used for all images collected at the same flow rate. The average pixel intensity (I) was calculated within the ROI. To standardize the ROI intensity values for each image, average pixel intensity was also calculated for the brightest (B) and the darkest (D) regions of the image. The weighted ROI intensity $W(t)$ for an image taken at time t was determined as:

$$W(t) = \frac{I - D}{B - D} \quad [11]$$

For a given flow rate, the reduction in weighted ROI intensity due to the presence of magnetic nanoparticles $dW(t)$ was calculated as:

$$dW(t) = W(0) - W(t) \quad [12]$$

where $W(0)$ is the weighted ROI intensity at $t = 0$.

3.3. In Vivo studies

All animal experiments were conducted according to protocols approved by the University of Michigan Committee on Use and Care of Animals (UCUCA).

3.3.1 Induction of brain tumors—Intracerebral 9L tumors were induced in male Fisher 344 rats, weighing 125-150 g, according to a previously described procedure [17]. Briefly, rat 9L-glioma cells (Brain Tumor Research Center, University of California, San Francisco) were cultured at 37°C, in a humidified atmosphere of 5% CO₂, in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 100 IU/mL penicillin, 100 µg/mL streptomycin and 0.29 mg of L-glutamine. Prior to implantation, cells were grown to confluency in 100 mm culture dishes, harvested and resuspended in serum free DMEM at a concentration of ~10⁵ cells/µL. The cell suspension (10 µL) was implanted in the right forebrain of the animals at a depth of 3 mm beneath the skull through a 1 mm diameter burr hole. The surgical field was cleaned with 70% ethanol and the burr hole filled with bone wax (Ethicon Inc., Summerfield, NJ) to prevent extracerebral extension of the tumor.

3.3.2 MRI imaging—MRI experiments were performed on a 12-cm horizontal-bore, 7 Tesla Varian Unity Inova imaging system (Varian, Palo Alto, CA). Animals were anesthetized with 1.5% isoflurane/air mixture and imaged using a 35 mm diameter quadrature RF head coil (USA Instruments Inc, OH). MRI of the rat brain was initiated on day 10 after tumor cell implantation, and was repeated approximately every 2 days to monitor tumor volume in order to select tumors of sizes between 50 and 70 µL. Thirteen axial sections of the rat brain were acquired with a T₂-weighted fast spin echo sequence using the following parameters: repetition time (TR) = 4 s, echo time (TE) = 60 ms, field of view = 30 × 30 over 128 × 128 matrix, slice thickness = 1mm, slice separation = 1.5 mm, four signal averages per phase encoding step. Tumor volumes were calculated from the collected images as previously described [17].

T₂-weighted images were inspected to determine the position of the slice having the best cross-sectional visualization of the tumor lesion. Gradient echo (GE) single slice scan was acquired at this position to provide qualitative information on nanoparticle accumulation in the tumor. GE images were acquired with the following parameters: TR = 20 ms, TE = 5 ms, field of view = 30 × 30 over 128 × 128 matrix, slice thickness = 1mm.

3.3.3 Magnetic targeting—Animals were anesthetized with an inhaled 1.5% isoflurane/air mixture and tail veins cannulated using a 26-gauge angiocatheter. During the targeting study, rats were placed ventrally on a platform with the head positioned between the poles of an electromagnet. Magnetic field density at the poles was adjusted to 0 T (control) or 0.4 T (experimental). Nanoparticle suspension, in PBS, was then injected at a dose of 12 mg Fe/kg through the catheter and the animal retained in the magnetic field for 30 minutes.

The rats were imaged with MRI before the administration of nanoparticles and after the magnetic targeting as described above. Immediately following MRI, the animals were sacrificed and dissected. The isolated brain was divided into right and left hemispheres and the tumor carefully separated from normal tissue of the right hemisphere. The left hemisphere and the tumor tissues were frozen and kept at -80°C .

3.4. *Ex vivo* analysis of tissue samples

3.4.1 Nanoparticle quantification by Electron Spin Resonance (ESR)

spectroscopy—Nanoparticle concentrations in tissue samples were determined using ESR according to a previously described procedure [18]. Briefly, ESR spectra of samples were acquired using an EMX ESR spectrometer (Bruker Instruments Inc., Billerica, MA) equipped with a liquid nitrogen cryostat. The acquisition parameters were: $\sim 9.2\text{GHz}$ resonant frequency, 20mW microwave power, 145K temperature set-point, 5G modulation amplitude and 5×10^4 receiver gain. Due to the 100 kHz modulation of the magnetic field, the measured signal was the derivative $\delta P / \delta H$ of the absorbed microwave power with respect to the external static field, H . The integral $\int P(H) dH$ is known to be proportional to the amount of resonating electronic spins present in the sample. Hence, the double integral of the ESR spectra of tissue samples was calculated to quantify the accumulation of nanoparticles. Data were corrected for background tissue absorption using control tissue samples from animals not exposed to nanoparticles. Calibration curves were constructed using control tissue samples spiked with nanoparticle solutions of known iron concentrations.

3.4.2 Transmission electron microscopy—For TEM analysis, freshly dissected tumor and contralateral brain tissue samples were sectioned into 1 mm cubes with a sharp razor blade and incubated overnight at 4°C with a fixative solution (2.5% gluteraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4). Samples were then rinsed with buffer and post fixed with 1% osmium tetroxide for 1 hr, dehydrated with a series of ethanol solutions of 50% - 100%, cleared with propylene oxide, and then gradually infiltrated with Epon resin. The samples were arranged in molds filled with Epon and the resin was allowed to polymerize at 60°C for 24 hours. The tissue blocks were sectioned with a diamond knife mounted on a RMC MT-7 ultramicrotome. The ultrathin sections were supported on a copper grid. For positive control, a few drops of diluted nanoparticle preparation were deposited onto carbon-coated copper grids, and the grids were allowed to air-dry. Samples were examined with a CM-100 electron microscope (Philips Electron Optics, The Netherlands) at 60 kV.

3.5. Statistical analysis

Data are presented as mean $\pm$ SD.

Nanoparticle concentrations in excised tumor and contralateral brain tissues were compared using the unpaired t test. A p -value of <0.05 was considered to be statistically significant.

Non-linear curve-fitting module of OriginPro 7.5 software was used to fit kinetic profiles of nanoparticle entrapment in the flow system to a $w = a_1 * t / (a_2 + t)$ model.

4. Results

4.1 *In vitro* magnetic entrapment of nanoparticles under physiologically relevant flow conditions

Cerebral hydrodynamic parameters reported in literature for 9L glioma bearing rats are summarized in Table 1. As seen, these parameters clearly illustrate a pronounced difference in vascular architecture and blood perfusion rate between brain tumors and contralateral normal brain. The potential impact of these differences on efficiency of magnetic nanoparticle capture, under a given magnetic field gradient, was first examined using the simple flow system described in Section 3.2.2. Experimental conditions, including nanoparticle concentration, strength of the applied magnetic field and flow rates in the tubing, were carefully selected to approximate the physical situation encountered in subsequent animal studies.

4.1.1 Establishment of physiologically relevant *in vitro* experimental conditions

Concentration of magnetic nanoparticles Magnetic nanoparticles, G100, used in both *in vitro* and *in vivo* studies were composed of an iron oxide core and a starch shell. The physical properties of G100 were previously characterized [19] and are summarized in Table 2. The nanoparticle concentration used for flow experiments was based on the estimate of concentration in the blood of a 200-gram rat following intravenous administration. Assuming that initial distribution of nanoparticles is limited to the vasculature and, thus, the initial volume of distribution is equal to the total blood volume, nanoparticle concentration can be calculated as a ratio of the amount of injected nanoparticles to the total blood volume. Total blood volume in rats is typically about 6% of their body weight and was estimated to be approximately 12 mL. At the dose of 12mg Fe/kg body weight, the total amount of injected nanoparticles for a 200-gram rat would be 2.4 mg Fe. Accordingly, the estimated nanoparticle concentration in rat blood was 0.2 mg Fe/mL.

Magnetic field gradient To expose the nanoparticle suspension, flowing through a tubing, to the magnetic field gradient similar to that acting on the tumor lesion of a rat, the magnet configuration to be employed in the *in vivo* experimental setup was adopted. Tubing was positioned 8 mm away from the right pole of the electromagnet to approximate the estimated tumor location within the 25 mm air gap, adjusted to accommodate the size of a rat head. This position was also representative of a corresponding contralateral brain region due to symmetry of magnet configuration.

Capillary flow rates Linear capillary flow rates were calculated according to Equation [10] using the parameters presented in Table 1. Estimated linear flow rates for the tumor and contralateral brain regions of equal volume were 0.074 ± 0.051 cm/s and 0.31 ± 0.16 cm/s, respectively.

4.1.2. Self-agglomeration of magnetic nanoparticles in the absence of magnetic field—To rule out the possibility that magnetic nanoparticles might aggregate due to interparticle magnetic attraction even in the absence of an external magnetic field, the magnetization of the nanoparticles under zero-field-cooled (ZFC) conditions was examined. The ZFC magnetization profile (Figure 2) displayed a broad peak centered at about 160 K, corresponding to the blocking temperature (T_B) of the nanoparticles. Above T_B , nanoparticles exhibit a typical superparamagnetic behavior, i.e. have no net magnetization in the absence of a magnetic field [8]. Thus, magnetically mediated nanoparticle agglomeration without exposure to a magnetic field would not be likely for G100 at temperatures well above 160 K. In agreement with this prediction, no agglomeration of magnetic nanoparticles was seen in the tubing perfused at the slowest flow rate of 0.05 cm/s in zero magnetic field (Figure 3A: 0 min).

In contrast, 10 minutes after the initiation of 0.4 T magnetic field at the same flow rate, significant accumulation of nanoparticles was observed (Figure 3B: 10 min).

4.1.3. Kinetic analysis of in vitro magnetic entrapment of nanoparticles—Kinetic analysis of magnetic nanoparticle entrapment in a flow system is shown in Figure 4. As seen, at a given linear flow rate, the extent of nanoparticle capture, reflected by a reduction in weighted pixel intensity (dW), exhibited a hyperbolic dependence on time. Both the rate and extent of nanoparticle accumulation were significantly affected by flow conditions; higher accumulation rates and greater saturation extents were observed with decreasing linear flow rates. For example, after 10 minutes of tube perfusion with nanoparticle suspension, the dW values corresponding to linear flow rates of 0.05, 0.08, 0.1 and 0.2 cm/s were 0.74, 0.53, 0.36 and 0.07, respectively. As can be seen in the inset of Figure 4, the extent of nanoparticle entrapment at 10 minutes decayed exponentially with the increase in linear flow rate. An interesting finding of this experiment was that the kinetic profile obtained at the flow rate of 0.08 cm/s, corresponding closely to the mean flow rate estimated for brain tumors (0.074 cm/s), exhibited a significant enhancement of nanoparticle entrapment at any given time point comparing to that at 0.2 cm/s flow rate, which was approximately the mean flow rate estimated to be encountered in the contralateral brain (0.31 cm/s). For example, after 10 minutes of infusion, nanoparticle entrapment at the flow rate of 0.08 cm/s was 7.6-fold higher than that obtained at 0.2 cm/s.

4.2 In vivo magnetic targeting studies

Figure 5A shows representative GE MRI images of control and experimental animals after magnetic targeting. The region of pronounced hypointensity observed in the brain of the targeted animal reflected the presence of magnetic nanoparticles. This region corresponded to the tumor location that was clearly visualized on T2-weighted MRI scans due to positive contrast (data not shown). Interestingly, no detectable hypointensity was observed in the contralateral brain of the targeted animal. This finding implicated tumor selectivity in nanoparticle accumulation, which was in good agreement with the *in vitro* results.

Also of significance was the lack of clear contrast enhancement of the tumor area on the GE scan of the control animal brain, suggesting that nanoparticle accumulation was indeed caused by the magnetic field. These results were consistent with findings of the *in vitro* flow experiments, where no nanoparticle aggregation was observed in the tubing in the absence of a magnetic field (see Figure 3A).

Quantitative tissue analysis of nanoparticle concentration by ESR method following magnetic targeting (see Figure 5B) revealed that nanoparticle accumulation in the tumor tissue of targeted animals (29.8 ± 7.9 nmol Fe/g tissue, $n=6$) was 9.6-fold higher ($p<0.005$) than that found in the contralateral brain tissue (3.1 ± 2.0 nmol Fe/g tissue, $n=6$).

The presence of nanoparticles within the glioma lesion of targeted animals could also be observed by electron microscopy. A representative TEM micrograph obtained from glioma section of a targeted rat is shown in Figure 6A. The presence of magnetic nanoparticles in tissue sections was confirmed by structural comparison to images of control G100 nanoparticles (see Figure 6B). Nanoparticles could not be detected in the contralateral brain sections of targeted animals or in control animals using TEM.

4.3 In vitro – in vivo correlation

The *in vivo* plasma elimination of biopolymer-coated iron oxide magnetic nanoparticles of hydrodynamic diameters above 50 nm is known to be rapid due to clearance by the reticulo-endothelial system [20]. Indeed, a half-life of 7 ± 1 minutes was reported for a monoexponential

elimination profile of dextran-coated nanoparticles in rats after intravenous administration [21]. The total area under the monoexponentially decaying concentration versus time curve (AUC_T), with an initial concentration of C_0 and plasma half-life of $t_{1/2}$ can be calculated as:

$$AUC_T = \int_0^{\infty} C_0 e^{-\frac{\ln 2}{t_{1/2}} t} dt = \frac{C_0}{\ln 2} t_{1/2} \quad [13]$$

Assuming that the concentration of nanoparticles, intravenously injected to rats, is decaying exponentially from the initial concentration of 0.2 mg Fe/mL (see Section 4.1.1) with a half-life of 7 minutes, the AUC_T calculated according to Equation [13] is 2 mg Fe×min/mL. This value reflects the total exposure of the cerebral vasculature to blood-carried nanoparticles.

During the *in vitro* experiments conducted in the present study, the tubing was perfused continuously with a nanoparticle suspension at a constant concentration of 0.2 mg Fe/mL. In order to expose the tubing to the same total amount of nanoparticles as that estimated above for the *in vivo* situation (2 mg Fe×min/mL), the tubing would have to be perfused for 10 minutes. Therefore, the 10 minute time point was selected for comparison of *in vivo* and *in vitro* results, using 0.08 cm/s and 0.2 cm/s to approximate glioma and normal brain flow rates, respectively. After 10 minutes perfusion, the *in vitro* ratio of nanoparticle entrapment at 0.08 cm/s to that at 0.2 cm/s was 7.6. As noticed, the corresponding *in vivo* glioma selectivity of nanoparticle accumulation over the contralateral brain tissue was 9.6. Based on these findings, the *in vitro* results appeared to be in a reasonable agreement with the *in vivo* data.

5. Discussion

Target selectivity is a factor of critical importance in the design of drug delivery approaches for brain tumor treatment, since any damage to the delicate and highly functional normal brain parenchyma can pose a serious threat of severe neurological and neurocognitive sequelae [22,23]. To this regard, the interaction between magnetically responsive drug carriers and externally applied magnetic field appears to be an attractive means of targeting and subsequently retaining the drug within the tumor lesion. However, non-invasively applied external magnetic fields cannot confer magnetic force-induced selectivity between the tumor lesion and normal brain tissue. This study was designed to explore whether the hydrodynamic component of magnetic targeting contributes to selective accumulation of magnetic nanoparticles in tumors due to the pronounced pathophysiological alterations of brain tumor vasculature.

Mathematical simulation would have been helpful to understand the role of such alterations in nanoparticle capture within tumor tissue. However, magnetic targeting of tumors is a complex multivariate process, accurate theoretical description of which is extremely challenging and computationally demanding [24,25]. In this study, we utilized a simple flow system which, although it did not accurately mimic the *in vivo* situation, adequately represented the interplay of the two major factors governing nanoparticle capture: magnetics and hydrodynamics. The experimental conditions adopted in this investigation were set to effectively decouple the hydrodynamic component from the magnetic component, assuming that changes in the vascular architecture and functionality would affect only the flow dynamics but not the strength of magnetic interaction. This should be a reasonable assumption, since the magnetic interaction is primarily determined by the magnetic field gradient and nanoparticle characteristics, according to Equation [1]. Thus, magnet configuration and tube positioning were set to provide an *in vivo* equivalent of the magnetic field gradient, equally representing both the glioma and contra-lateral brain. In addition, nanoparticle concentration might also contribute to the magnetic holding via inter-particle magnetic interactions [26]. Nanoparticles, magnetized in

an external magnetic field, could generate local small magnetic fields, which might be sufficient to deflect the neighboring particles from their original trajectory in the direction of magnet source, thereby enhancing magnetic holding. To account for this effect, the nanoparticle concentration selected for the *in vitro* experiments was in accordance with the estimated *in vivo* blood concentration. The parameters defining the magnetic component of nanoparticle capture were maintained constant throughout the experiment, allowing for isolated and independent testing of the effect of flow dynamics on nanoparticle capture.

Calculation of the capillary flow rates was made under a simplifying assumption that both the tumor and intact cerebral vasculature had a homogeneous and aligned geometry. In reality, cerebral capillaries are arranged in complex branched networks, characterized by especially tortuous, sinusoidal and irregular branching patterns in gliomas [27]. Varied spatial distribution of nanoparticles confined by the branched capillary network would result in altered nanoparticles-magnetic field interaction, and along with the multi-directional flow could potentially affect the extent of magnetic retention. However, the average impact of these effects should be relatively minor, due to the random branching directions of the capillaries with respect to the magnetic field gradient. On the other hand, the estimates of flow rates seemed to embody the most important determinants of average vascular functionality, including perfusion and vascular morphological characteristics such as microvessel density and capillary diameter.

Magnetic targeting experiments in 9L-glioma bearing rats demonstrated the feasibility of enhancing selectivity of nanoparticle accumulation in glioma over contra-lateral normal brain with the application of an external magnetic field. An interesting finding of this study was that the *in vitro* predicted increase in nanoparticle capture in glioma at a pathophysiologically relevant flow rate over that of the contralateral brain was relatively consistent with the *in vivo* results. As shown, the *in vitro* predicted and *in vivo* determined ratios of nanoparticle capture in glioma versus contralateral brain were 7.6 and 9.6, respectively. The difference between these two values could be attributed to physiological factors that were not accounted for by the model, such as the complex capillary-bed geometry, interactions between blood constituents and magnetic nanoparticles, and the increased vascular permeability in tumors. Nevertheless, the *in vitro* model, within a reasonable degree of accuracy, reflected the roles of the two major driving forces, hydrodynamics and magnetics, affecting nanoparticle targeting selectivity of tumors over normal brain. To this end, the *in vitro* ratio of nanoparticle capture at the estimated glioma flow rate to that of the contra-lateral brain can be viewed as a valid indicator of *in vivo* glioma selectivity. It was, therefore, concluded that decreased average capillary flow rates in glioma, reflecting glioma vascular abnormalities, should be an important mediator in achieving glioma selectivity of nanoparticle accumulation with magnetic targeting.

The role of hydrodynamics in magnetic targeting, elucidated in the present study, implies the possibility of augmenting tumor selectivity via pharmacological modulation of the blood flow. A number of vasoactive agents, (e.g. isoproterenol, vasopressin, serotonin, bradykinin etc.), have been shown to induce significantly different and sometimes opposing changes in blood flow in solid tumors and corresponding normal tissues [28]. In addition, alteration of blood flow in glioma and normal brain by nitric oxide modulators, has been observed in glioma-bearing rats [29]. A favorable pharmacological modulation of the blood flow rates in conjunction with magnetic targeting could therefore be adopted to obtain enhancement in both the extent and selectivity of nanoparticle accumulation in gliomas. Hence, understanding the impact of hydrodynamics on magnetic targeting to improve the magnetically mediated drug delivery to brain tumors warrants further investigation.

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Figure 1.

Schematic illustration of brain tumor magnetic targeting following the systemic administration of magnetic nanoparticles. Targeting can presumably be achieved due to the combination of several phenomena including passive biodistribution of the administered nanoparticles, pathophysiological peculiarities of tumor vasculature and the principles of magnetic entrapment.

Figure 2.

Zero field cooled (ZFC) magnetization curve of freeze-dried G100, measured at 100 Oe. The curve exhibits a broad maximum corresponding to the blocking temperature, $T_B \sim 160\text{K}$. The decay of magnetization above T_B is an indication of superparamagnetic behavior of the nanoparticles at room temperature.

Figure 3. Images illustrating extraction of nanoparticles from a stable colloidal fluid, pumped at a linear velocity of 0.05 cm/s, (A) before and (B) 10 minutes after the initiation of a 0.4T magnetic field.

Figure 4.

In Vitro kinetic analysis of magnetic nanoparticle entrapment with the magnetic field ($B=0.4T$) at physiologically relevant linear flow velocities: ■ 0.05, △ 0.08, ★ 0.1, and ▼ 0.2 cm/s. Solid lines represent nonlinear least squares regression fits of the data sets to the model $w=a_1 \cdot t / [a_2 + t]$ (R^2 s are 0.97, 0.96, 0.98 and 0.71 for 0.05, 0.08, 0.1 and 0.2 cm/s, respectively). The inset illustrates the extent of nanoparticle capture, represented by dW , after 10 minutes of tube perfusion as a function of linear flow velocities.

Figure 5.

In Vivo magnetic targeting in 9L-glioma bearing rats. (A) Typical MRI images obtained from experimental and control animals following intravenous nanoparticle administration and magnetic targeting. Hypointense region in the brain of the targeted animal reflects nanoparticle accumulation within glioma lesion. (B) Nanoparticle concentrations in excised glioma and contra-lateral brain tissues of targeted and control rats quantified by ESR spectroscopy. Data are taken from reference (19).

Figure 6.
(A) Representative TEM micrograph taken of a tumor section dissected from a targeted animal. The image demonstrates the presence of entrapped magnetic nanoparticles within the glioma lesion. (B) TEM micrograph of nanoparticles obtained from a standard G100 preparation, shown for comparison.

Parameters used for theoretical estimation of capillary blood flow in tumor and contralateral brain tissue of 9L glioma bearing rats.

Parameter	Symbol	Units	Tumor	Contralateral brain	Reference
Tissue perfusion	F	ml/100g/min	85.3 ± 26.9	147.7 ± 31.1	(1)
Capillary diameter	d	μm	21.04 ± 5.87	7.44 ± 1.44	(2)
Microvessel density	n	Vessels/mm ²	170.9 ± 13.8	534.9 ± 127.2	(3)
Tissue volume	V	μL	53 ± 5		Present study

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Table 2

Physical properties of G100 magnetic nanoparticles.

Property	Value	Units	Reference
Hydrodynamic diameter	110 ± 22	nm	(¹)
Saturation magnetization (Ms)	94	Emu/g Fe	(¹)
R ₂ relaxivity	43.8 ± 2.6	s ⁻¹ mM ⁻¹	(¹)
Blocking temperature (T _B)	160	K	Present study

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