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Targeting of systemically-delivered magnetic nanoparticle hyperthermia using a noninvasive, static, external magnetic field

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Abstract

One of the greatest challenges of nanoparticle cancer therapy is the delivery of adequate numbers of nanoparticles to the tumor site. Iron oxide nanoparticles (IONPs) have many favorable qualities, including their nontoxic composition, the wide range of diameters in which they can be produced, the cell-specific cytotoxic heating that results from their absorption of energy from a nontoxic, external alternating magnetic field (AMF), and the wide variety of functional coatings that can be applied. Although IONPs can be delivered via an intra-tumoral injection to some tumors, the resulting tumor IONP distribution is generally inadequate; additionally, local tumor injections do not allow for the treatment of systemic or multifocal disease. Consequently, the ultimate success of nanoparticle based cancer therapy likely rests with successful systemic, tumor-targeted IONP delivery.

In this study, we used a surface-based, bilateral, noninvasive static magnetic field gradient produced by neodymium-boron-iron magnets (80 T/m to 130 T/m in central plane between magnets), a rabbit ear model, and systemically-delivered starch-coated 100 nm magnetic (iron oxide) nanoparticles to demonstrate a spatially-defined increase in the local tissue accumulation of IONPs. In this non-tumor model, the IONPs remained within the local vascular space. It is anticipated that this technique can be used to enhance IONP delivery significantly to the tumor parenchyma/cells.

Keywords

iron oxide nanoparticles; magnetic capture; *in vivo*; rabbit ear model; hyperthermia

1. INTRODUCTION

One of the greatest challenges of nanoparticle cancer therapy is the delivery of adequate numbers of nanoparticles to the tumor site. Iron oxide nanoparticles (IONPs) have many favorable qualities, including their nontoxic composition, the wide range of diameters in which they can be produced, the cell-specific cytotoxic heating that results from their absorption of energy from a nontoxic, external alternating magnetic field (AMF), and the wide variety of functional coatings that can be applied. Although IONPs can be delivered via an intra-tumoral injection to some tumors, the resulting tumor IONP distribution is generally inadequate; additionally, local tumor injections do not allow for the treatment of systemic or multifocal disease. Consequently, the ultimate success of nanoparticle based cancer therapy likely rests with successful systemic, tumor-targeted IONP delivery.

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Researchers previously have applied external, permanent magnets to attract nanoparticles or micron-sized particles to sites in living tissue to deliver chemotherapy or cause tumor vessel occlusion. Driscoll *et al*¹ reported fixation of 1 micron diameter magnetic particles in a rat tail model after intra-arterial injection, using permanent magnets with a field gradient of 50 T/m in tissue. Goodwin *et al*² applied permanent magnets to attract 500 to 5000 nm iron particles to liver sites in a porcine model after intra-arterial injection to deliver chemotherapy agents. Their magnets produced fields of 0.025 to 0.1 T, but they did not report the value of field gradient. They reported a ten-fold increase of iron particles in the targeted liver lobe, as compared to the control lobe; histology showed particles located outside the liver arteries in the interstitium.

Lübbe *et al*³ injected magnetite particles of diameter 50 to 100 nm intravenously into mice implanted with human tumors. Rare earth magnets of strength 0.2 to 0.5 T (field gradient not stated) external to the tumors caused particle occlusion of tumor vessels when the injection volume equaled 10% of the blood volume; injection volume equal to 0.5% of blood volume did not cause tumor vessel occlusion. The injected concentration equaled 6 mg Fe/ml. The tumors with vessel occlusion showed a complete response at 14 days. Magnetic targeting with chemotherapy agent epirubicin bound to the magnetic nanoparticles caused tumor shrinkage and complete response in 7 days (1 mg epirubicin/kg BW, 6 mgFe/ml injection concentration 0.5% of blood volume). Lübbe *et al*⁴ conducted a Phase I study in humans of magnetic attraction of drug-bearing magnetic nanoparticles of 100 nm diameter, which were injected intravenously. Permanent magnets in various, tumor-specific configurations within 5 mm of the tumor surface produced fields of 0.5–0.8 T; field gradient strength was not reported. They reported evidence of effective concentration of nanoparticles, such as discoloration of skin over the tumor, reduction in the circulating drug concentration compared to the case of no magnets applied, and histological evidence of iron in the tumor of one patient.

Fortin-Ripoche *et al*⁵ applied external neodymium-iron-boron magnets to attract magnetoliposomes of diameter 150 nm to 250 nm to a mouse tumor site after intravenous injection. The magnetic field equaled about 0.3 T and the maximum field gradient equaled about 30 T/m. They did not report the maghemite content of each liposome. They observed intraluminal accumulation in the targeted tumor capillaries, where some magnetoliposomes remained 16 hours after removal of the magnet. The magnetoliposomes could be used to deliver chemotherapy agents. Intravenous injection of unencapsulated 7.5 nm maghemite particles produced no significant accumulation in the magnetically-targeted tumors.

These past studies show that external static magnets can concentrate magnetic particles at superficial anatomical sites after injection into a vessel. Quantification of the magnetic force on particles is not possible if the gradient of the external, static magnetic field is not published. In the present study, we designed a static magnetic array to produce a specified magnetic field gradient, and we demonstrated its ability to concentrate iron oxide nanoparticles in living normal tissue after injection into both arteries and veins. In contrast to previous studies, the IONPs we injected have been used for hyperthermia therapy in animal models.⁶

2. METHODS

2.1. Magnet Array

We designed a static magnet array to produce a specified magnetic field gradient in target tissue, specifically normal tissue in the ear of a rabbit. This animal model is suitable for demonstrating the feasibility of IONP magnetic capture, because both sides of the tissue are accessible, the 2 mm thickness of the ear increases the value of magnetic field gradient, its

area allows more than one magnetic holding experiment to be performed with magnets of reasonable size, its translucence allows immediate gross visual confirmation of nanoparticle fixation, and injection into a single, central artery gives access to most of the tissue in the ear. We designed the magnet array to produce a magnetic field gradient in tissue of three times the maximum value of 30 T/m reported by Fortin-Ripoche *et al*⁶. In their study, 30 T/m produced accumulation of magnetoliposomes in tumor capillaries. However, it is not possible to compute the force on their magnetoliposomes, because they did not state the quantity of maghemite within each. To be surer of capture of the IONPs, we over-designed the magnet array as stated.

The magnetic scalar potential produced by a magnet with magnetization $\mathbf{M}_Q$ at a point, P, is given by⁷:

$$\phi_p = \frac{1}{4\pi} \oint_s \frac{\mathbf{n} \cdot \mathbf{M}_Q}{r_{pQ}} ds_Q \quad (1)$$

where ds_Q is an infinitesimal area on the magnet surface, $\mathbf{n}$ is a unit vector normal to the surface, r_{pQ} is the distance between the infinitesimal area and the point P, and the integral is computed over the entire surface of the magnet, S. The vector product $\mathbf{n} \cdot \mathbf{M}_Q$ vanishes on the sides of the magnets, because the vectors are perpendicular. The negative contribution from the back side of the magnet becomes negligible for a magnet several times higher than its diameter; however, we included this contribution for the sake of accuracy. With an array of two separated magnets, a second integral is evaluated, in which the second vector $\mathbf{M}_Q$ may be parallel or anti-parallel to the first, according to the chosen orientations of the two magnets.

The magnetic field, H, is computed from $H = -\phi$, i.e., the negative gradient (spatial derivative) of the potential. Next, the gradient of the magnetic field itself must be computed to yield a quantity proportional to the force on the magnetic particle⁷. This force also is proportional to the effective dipole moment of the particle, which is proportional to the product of its volume and the magnetization of the material. The magnetization is in turn proportional to the applied magnetic field, if below the saturation value; above saturation, the magnetization is a known constant value for a given material.⁸

We computed the gradient of the magnetic field between two stack of magnets separated by 2.0 mm and with anti-parallel magnetization vectors (North-North configuration) using numerical integration (100 cells along the radius of the magnet) first to find the magnetic scalar potential. We used numerical differentiation then to find the magnetic field and the gradient of the magnetic field. We included the magnet back-side contributions to magnetic scalar potential, which changed results by less than 3%. Analytical solutions were derived along the central magnet axis to validate the numerical results. Each stack consisted of several axially-magnetized neodymium-iron-boron (NdFeB) discs of diameter 0.5 inch and total height of 11/16 inches (K&J Magnetics, www.kjmagnetics.com/). Furlani⁹ gives magnitude of the magnetization of NdFeB as 8×10^5 A/m.

Figure 1 shows lines of constant magnitude of the magnetic field gradient in the space between magnets stacks, i.e., in tissue. In the plane parallel to both magnet faces and centered between them (central plane), the magnetic field gradient varies between 80 and 130 T/m, exceeding 80 T/m from the array center out to a radius of 5.5 mm. A deep local minimum of field gradient appears at a radius of 6 mm, the edge of the magnets, where the gradient is close to zero (nearby, the gradient is above 240 T/m in a very small region by the magnet corners). At larger radius values, from 6.2 to 8.5 mm, the field gradient rises again above 80 T/m in the central plane. We considered this distribution adequate, even though the

minimum field gradient near the center of the central plane was not quite three times the value used by Fortin-Ripoche *et al.* For comparison, in the same plane, the North-South magnet configuration has a complementary distribution, producing 80 T/m or greater only between radius values of 4.5 and 7.5 mm, and attaining a peak of 280 T/m at radius 6 mm. We chose the North-North configuration to produce the larger tissue volume with a field gradient magnitude in the intended range defined above.

The magnet stacks defined above were mounted in opposition (North to North) on the arms of a C-shaped steel yoke. This assembly could be slid onto the side of the rabbit ear, positioned where desired, then fixed to the operating room table. Before the experiment, we confirmed that calipers set to 2.0 mm could slide over both ears of the subject without binding and thus affecting tissue blood perfusion.

We evaluated magnetic capture with magnetic nanoparticles that we have used previously for hyperthermia cancer therapy in animal model.⁶ (MicroMod #10-00-102, starch-coated, iron oxide (Fe₃O₄), saturation magnetization >76 emu/gFe, 100 nm particle diameter, 66 nm iron oxide core diameter, 15 mg Fe/mL aqueous solution).

2.2. Animal Model

The experimental subject was a 4.4 kg female New Zealand white rabbit maintained under surgical plane anesthesia according to a protocol approved by the Dartmouth College Institutional Animal Care and Use Committee. After shaving and site preparation, a 0.9 mL catheter was placed intra-arterially in the central artery at the base of the left ear. The magnet array was positioned over the distal third of the left ear, about 4.5 cm from the injection site.

Once vessel access was established, 1.6 cc of IONP solution was delivered into the primary base artery of the left ear with the intention of capturing IONPs during their first pass through the magnet array. This injection contained 24.5 mg Fe; it was followed by a 2 cc injection of saline solution. We estimate that the iron concentration in the ear on the first pass was 1660 mg Fe/kg, based on the approximate volume of the ear. After dilution by the full blood volume of the rabbit, the concentration was computed to be 5.6 mg Fe/kg BW during subsequent passes through the ear. This does not account for uptake by the liver, spleen, and other organs or the capture of IONPs in the first pass. After injection, the distal half of the ear was grossly dark for about 12 minutes. After 20 minutes in place, the magnet array was shifted to other side of the ear centerline, where it dwelled for 20 minutes with the intent of capturing IONPs now at the lower, systemic value of concentration.

Next, the magnet array was removed while the right main ear vein was catheterized. Then, the magnet array was positioned over the distal third of the right ear, and the same injection made into the right main ear vein. No gross darkening was observed. After 20 minutes, the magnet array was removed from the ear, and the major artery of the right ear was catheterized. The magnet array was repositioned on the opposite side of the right ear midline, then the third and final injection of 24.5 mg Fe was made, as before. Grossly visible darkening of the distal half of the ear followed the second injection, as was the case after the first injection. The magnet array remained in place for 20 minutes to capture IONPs during the first pass at very high concentration and at the lower systemic concentration during subsequent passes. After the three IONP injections, we estimate the systemic concentration to be 16.8 mg Fe/kg BW, not accounting for uptake by the magnet array in the first pass or subsequent uptake by other organs. Tissues were saved for microscopic examination following the termination of the experiment.

3. RESULTS

After the intra-arterial injection in the left ear, a dark ring was grossly visible at the location of the magnet array. This was distinct from the temporary, uniform darkening that had occurred in the distal half of the ear immediately after injection. The ring radius was 1–2 mm larger than the magnet radius of 6 mm. Fig. 2 shows the ring in the left ear 29 minutes after removal of the magnet array. A similar ring was grossly visible after the intra-arterial injection in the right ear. Both rings persisted until the end of the experiment, 134 minutes after the first injection. No such rings were grossly visible at the two locations where the magnet array dwelt in place with the intent to attract IONPs circulating after dilution by the full blood volume of the subject.

After intra-arterial injection into the right ear, in regions at the edge of the magnet array nanoparticles occupied 9 to 13% of tissue area, as shown by quantitative histological analysis of 40× microscopy (Fig. 4a); the same area was 1 to 3% in areas under the center of the array (Fig 4b). Fig. 3 shows the fractions of tissue area occupied by IONPS at five locations with respect to the magnet array. Fig. 4 shows that the IONPs remained predominantly in the vasculature.

Fig. 5 shows less aggregation of IONPS in vessels after intra-venous injection than after intra-arterial injection.

4. DISCUSSION

The dark flushing of the distal half of the rabbit ear suggests that this half is a suitable target for magnetic capture after injection in to the central ear artery; the proximal half appears not to be supplied only by that artery.

Although the quantification of iron is accurately represented in the quantitative histological analysis in Fig. 3, it should be noted that the experimental information is derived from a single animal experiment. Nonetheless, the plots show that the iron accumulated preferentially at the edge of the magnet array.

Fig. 4 illustrates vessels packed with iron after the favorable conditions enforced for this part of the experiment: IONP injection into the artery supplying the tissue adjacent to the magnet array and only 1 minute of washout time. Upon future quantification and analysis, this IONP distribution might prove capable of producing therapeutic temperatures in surrounding tissue when energized by an alternating magnetic field. However, the case of intravenous injection is of greater interest clinically, as we discuss below. We note that the IONPs have not extravasated under the influence of magnetic forces, but have remained in the vessels. Future work will determine whether a magnet array will drive extravasation from tumor vessels, as opposed to the normal vessels studied here.

Figs. 3 & 4 show significantly greater accumulation of iron at the edge of the magnet array than at the center of the magnets. The computed magnetic field gradient varies from 80 T/m to 130 T/m from center to edge, a smaller proportional change than the ratio of IONP accumulation, about 11:1. We speculate that few IONPs reached the center of the magnet, there to be captured, because most were captured when first exposed to the large magnetic field gradient at the edge of the magnet. In the two-dimensional structure of the rabbit ear, we believe it was not possible to reach the center of a circular magnet without first crossing its edge. In retrospect, the North-South orientation of magnets would have served equally well in this experiment, because that array produces a high magnetic field gradient only at the magnet edges. In a hyperthermia treatment, it possibly could be advantageous to deposit IONPs preferentially at the margin of a tumor, because in theory this yields the sharpest

transition from a therapeutic temperature in tumor tissue to a non-toxic temperature in surrounding normal tissue¹⁰. The North-South configuration would be advantageous in anatomical sites that require larger magnet separation than 2 mm, because it produces a higher peak field gradient (280 T/m) than the North-North configuration (130 T/m); greater separation inherently would reduce the magnetic field gradient of any given array.

The histology in Fig. 5 is similar to that reported by Fortin-Ripoche *et al*, Fig. 7⁵, in that iron particles line the vessel wall after intra-venous injection and capture by a static magnet. The systemic concentrations after injection were similar: 4.3 to 14.7 mg/kg BW for Fortin-Ripoche *et al* and 16.8 mg/kg BW in this work. Other experimental conditions were different: magnet dwell time was 1 to 24 hours for Fortin-Ripoche *et al* and 20 minutes in this work; magnetic field gradient was 30 T/m for Fortin-Ripoche *et al* and 80–130 T/m for this work; captured magnetic particles were liposomes containing an unspecified quantity of 7.5 nm maghemite particles for Fortin-Ripoche *et al* and magnetite particles of 66 nm core diameter for this work; the washout time after removal of the magnet was unspecified for Fortin-Ripoche *et al* and 39 minutes for this work; tissue was implanted human tumor tissue for Fortin-Ripoche *et al* and normal rabbit ear tissue in this work.

With the intent of making magnetic capture of IONPs clinically relevant, our future studies will use intra-venous injection, will continue to use IONPs suitable for hyperthermia therapy, will study IONP capture in implanted human tumor tissue, will reduce the magnetic field gradient to the value used by Fortin-Ripoche *et al*, and will study the effect of magnet dwell time and washout time after removal of the magnet array. We note that our washout time after magnetic capture in response to intra-venous injection (39 minutes) is sufficient for a hypothetical follow-on hyperthermia treatment. However, the iron content in the target tissue was not quantified as a concentration value, and iron content may have decreased steadily as washout progressed after removal of the magnet array. In future work, we will quantify the tissue uptake of iron by mass spectroscopy in order to predict tumor temperatures by solution of the bioheat equation with a spatially-distributed input from IONP heating¹¹.

5. CONCLUSIONS

In this preliminary study, an external array of rare earth (NdFeB) magnets concentrated iron oxide nanoparticles (IONPs) of 100 nm diameter suitable for hyperthermia therapy that were injected intra-arterially into the ear of a rabbit, with a concentration of 1660 mg Fe/kg in the ear itself. Quantitative histological analysis showed IONPs in regions under the edges of the magnet area occupied six times the area that was occupied by IONPs outside the magnet array. The nanoparticles did not enter the interstitium, but remained in the vasculature in this normal tissue. The same magnet array captured these particles to a lesser degree when circulating at a systemic concentration of about 17 mg Fe/kg.

In future studies, we will adapt the magnetic capture technique described here to concentrate IONPs suitable for hyperthermia therapy in pre-clinical rodent tumors after intravenous injection, with quantification of iron uptake by mass spectroscopy. We will also study vascular disrupting agents with the intent of enhancing the effect of the static magnetic field gradient on nanoparticle concentration in a tumor.

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Figure 1.

Lines of constant magnetic field gradient magnitude between magnets arranged anti-parallel (North-to-North). Each NdBF_e magnet is 0.5 inch in diameter and 11/16 inches long, and they are separated by 2.0 mm. The magnetization of NdBF_e is 8×10^5 A/m.

Figure 2.

The left ear of the rabbit 79 minutes after intra-arterial injection of IONPs into the central ear artery and 59 minutes after removal of the magnet array. IONPs accumulated in vessels form a dark ring with a diameter 2–4 mm larger than the 12 mm diameter of the magnet array.

Figure 3.

Plot of quantitative histological analysis of sections from right ear of rabbit after intra-arterial injection of IONPs situated at five locations with respect to the magnet. Tissue was harvested 21 minutes after injection and 1 minute after removal of the magnet array. The fraction of IONP area with respect to total area (300 micron square sections) is plotted against location of the sections with respect to the magnet. The magnet center is at 0 mm, and the edges of the magnet are at ± 6 mm. The tissue in Fig. 4a) is at a radius of 6 mm, and that of Fig. 4b) is at a radius of 0 mm. (Prussian Blue stain, 40 $\times$ magnification)

Figure 4.

High magnification photomicrographs in a section of the right rabbit ear. IONPs were delivered to the tissue via an intra-arterial injection, and a low concentration was present in the system circulation at the time of the direct intra-arterial injection. Tissue was harvested 21 minutes after injection and one minute after removal of the magnet array. (Prussian Blue stain, 40× magnification)

- a) Section situated at the edge of the magnet array demonstrating vessels packed with iron.
- b) Section situated in the center of the magnet array. The lumens of several arteries are filled with IONPs. However, the engaged number of vessels is small compared to the edge of the array.

Figure 5. High magnification photomicrographs in a section of the right rabbit ear. IONPs were delivered systemically via an intra-venous injection. Tissue was harvested 59 minutes after intra-venous injection and 39 minutes after removal of the magnet array. Section situated at the edge of the magnet array demonstrating IONPs appearing in vessels, especially on the vessel walls, but in lower concentration than after intra-arterial injection. (Prussian Blue stain, 40× magnification)