Cytotoxic effects of magnetic field gradients on iron oxide labeled cancer cells

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Target audience: MR physicists, biologists, and physicians who are interested in cancer imaging and therapy, specifically in minimally invasive image-guided tumor treatment.

Purpose: Minimally invasive cancer therapy is an important clinical challenge and several groups have demonstrated use of superparamagnetic nanoparticles for hyperthermia treatment [1]. Here we demonstrated that SPIO nanoparticles could be used for non-thermal destruction of cells by exposing the magnetically labeled cells to variable gradients of magnetic field, which is called GIFT (gradient-induced Fe therapy), in a standard MRI system. SPIO nanoparticles can also serve as MRI contrast agent thus enabling image-guided delivery of therapy.

Method: Bruker Biospec 9.4T MRI system equipped with G060 gradient system was used in the study. A pulse sequence with square waveform gradients in x,y,z channels with G_{max} = ±93.5 G/cm and a period of 185 µs was applied for ~30 min with a duty cycle of ~12% (1000 cycles followed by 1.5 s delay) as shown in Fig. 1. A water-cooled chamber was used to stabilize the sample temperature during the experiments. Cultured cell study: Human breast cancer HER2/neu positive BT-474 cells were grown to 60-80% confluency in 4-well chamber slides. Specific cell surface labeling was achieved by treating the cells with biotinylated trastuzumab antibody followed by streptavidin-conjugated SPIO nanoparticles (MiltenyiBiotec) at 4 °C to prevent internalization. An alternative protocol included cell transfection with BNF nanoparticles (Micromod Partikeltechnologie) in the presence of poly-L-lysine transfection agent [2]. Both protocols resulted in efficient cell labeling with iron oxide confirmed by Prussian blue staining. Animal study: Female athymic nude mice were orthotopically inoculated in the mammary fat pads with human breast cancer cells MDA-MB-231/luc stably transfected to express firefly luciferase enzyme. Each mouse was inoculated with two tumors using injection of 5e6 control unlabeled cells and 5e6 cells prelabeled with BNF nanoparticles before the inoculation. GIFT and MR imaging were initiated starting from 48h after the tumor inoculation. T2 weighted RARE MRI (TE/TR = 30/2000ms) was performed using a standard volume coil and GIFT therapy was delivered to the animal positioned in the cylindrical water-cooled chamber to stabilize the body temperature. Bioluminescence imaging (BLI) of MDA-MB-231/luc xenografts was performed with a Xenogen IVIS 200 system approximately 10 min after i.p. administration of D-luciferin solution to detect tumor viability before and after the treatment. After completing therapy and imaging studies animals were sacrificed and tumors excised and fixed for histopathological examination.

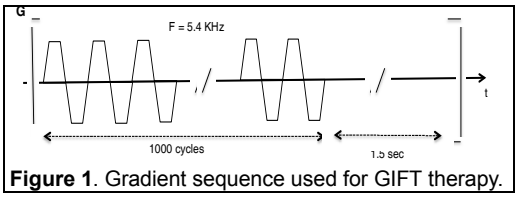

Figure 1. Gradient sequence used for GIFT therapy.

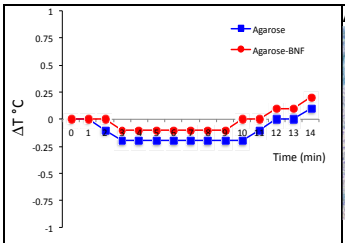

Figure 2. Temperature changes detected in the phantom during GIFT were less than ±0.2 °C.

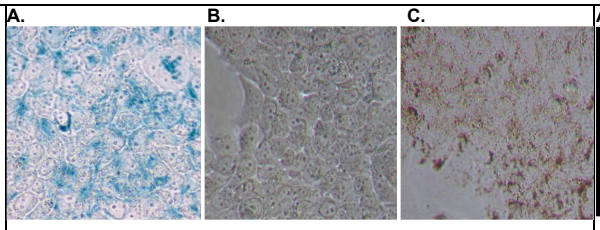

Figure 3. **A**. Prussian blue staining of BT-474 breast cancer cells labeled with BNF nanoparticles. **B & C**. Unlabeled and BNF-labeled BT-474 cells imaged 24h after GIFT treatment, respectively.

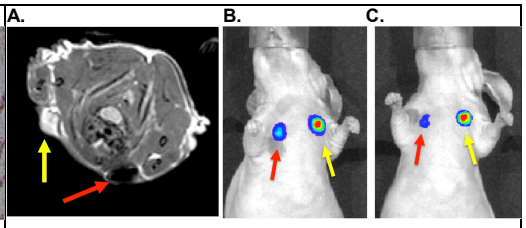

Figure 4. **A**: T2w MRI of the control (yellow) and BNF prelabeled (red) tumors. **B&C**: BLI of unlabeled and BNF-labeled BT-474 cells imaged before and 24h after GIFT, respectively.

Results and discussion: Temperature changes during the course of GIFT were assessed using agarose phantoms prepared with 100 µg/ml of BNF particles using an insertable temperature probe. No significant increase in the temperature of the BNF-containing sample was detected over 15 min period as shown in Fig. 2. There was also no measurable increase in the animal body temperature during the treatment. Prussian blue staining of the labeled cells and results of GIFT for cultured BT-474 cells are shown in Fig. 3. Almost complete destruction and detachment of cells by cycling gradients of the magnetic field was achieved at 24h after the procedure for all iron-oxide labeling protocols. MR images of tumor xenografts measured 48h after the implantation are shown in Fig. 4A and demonstrate strong and uniform SPIO labeling of the tumor cells post-implantation. BLI images of animals recorded before and at 24h after GIFT are shown in Fig. 4B. Significantly reduced BLI intensities from the iron oxide prelabeled tumor were detected after GIFT (Fig. 4C), whereas no significant differences were observed when no oscillating gradients of magnetic field was applied, indicating cytotoxic effects of GIFT therapy.

Conclusion: In conclusion, we hypothesize that variable gradients of the magnetic field induce damage to iron-oxide labeled cells and that the efficient killing of SPIO labeled cancer cells and significant reduction in the viable tumor volume following GIFT observed in our studies are likely due to the mechanical destruction of cell membranes and/or organelles [3] by magnetically polarized nanoparticles or their aggregates and not related to radiofrequency induced hyperthermia [1].

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References: 1. Ivkov, R et al., Clin Cancer Res, 2005; 11: 7093s-7103s. 2. Berman SC et al., Magn Reson Med, 2011; 65: 564-74. 3. Mitsunaga M et al., Nature Med, 2011; 17: 1685-91.