Imaging the delivery of brain-penetrating PLGA nanoparticles in the brain using magnetic resonance

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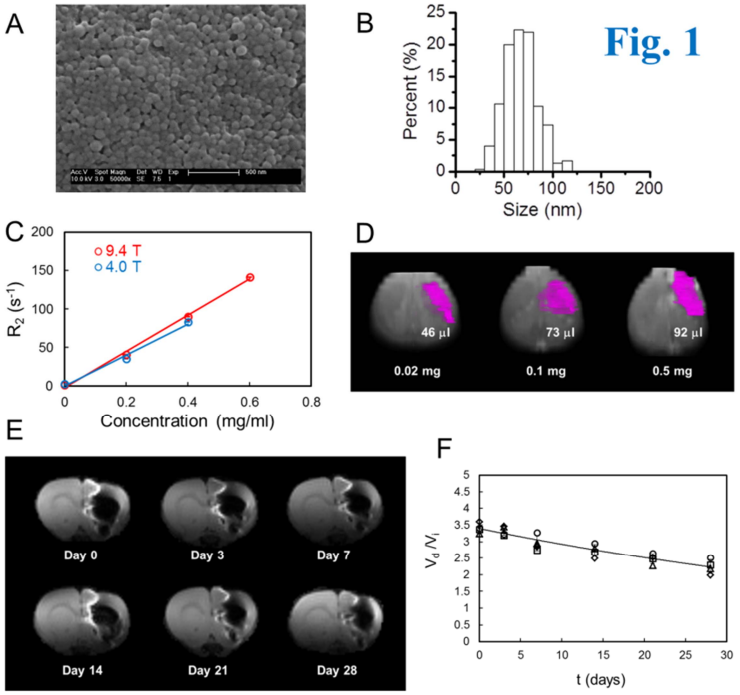
TARGET AUDIENCE Neuroscientists and clinicians interested in novel therapies for glioblastoma treatment.

PURPOSE Glioblastoma is the most common malignant tumor of the CNS, with an incidence of approximately 18,500 cases per year [1]. The difficulty in producing effective therapies is thought to be secondary to two factors: poor drug delivery and inadequate tumor sensitivity. An ideal therapy for glioblastoma, therefore, must be highly penetrative—to reach cells that have migrated into regions far from the site of tumor initiation—and must carry a payload that is effective against the cell subpopulation most responsible for tumor initiation, growth, vascularization, and therapeutic resistance. To address drug delivery challenges, we have developed brain-penetrating nanoparticles (NPs), a delivery vehicle comprised of the FDA-approved copolymer poly(*lactic-co-glycolic acid*) (PLGA) [2]. These NPs disperse over a clinically relevant volume in the brain when administered via convection-enhanced delivery (CED). When loaded with drugs with *in vitro* efficacy against brain cancer stem cells (BCSCs), brain-penetrating NPs produce unprecedented survival in a BCSC-derived xenograft model of glioblastoma. Magnetic Resonance Imaging (MRI) of drug-loaded NPs makes possible the intraoperative monitoring of their distribution in the brain to ensure that they reach dispersed tumor cells. Moreover, MR provides non-invasive monitoring of the therapeutic benefit with time and evaluation of tumor relapse. Therefore, functionalization of NPs to allow visualization by MRI will facilitate clinical translation of brain-penetrating nanoparticles. This was achieved by encapsulation of superparamagnetic iron oxide (SPIO) inside the NPs without altering their physical properties [3]. In this study, we demonstrated that the NPs are efficient T_2 contrast agents, allow detection at low concentration by MRI after CED to the brain and over a long time period (relevant to release of chemotherapy drugs) and have minimal toxicity.



MATERIALS AND METHODS ***Synthesis of SPIO***. Iron acetylacetonate (Fe(acac)3) (0.396 g, 1.56 mmol), oleic acid (1.47 mL, 4.64 mmol), oleylamine (1.02 mL, 3.09 mmol), 1,2-hexadecanediol (2.005 g, 7.76 mmol) and benzyl ether (10 mL) were added to a single-neck round bottom flask equipped with a magnetic stir bar and a condenser and deoxygenated for an hour. The reaction was gradually heated to 200°C and held at that temperature for 3 hours and then allowed to cool room temperature. The reaction mixture was precipitated in ethanol and centrifuged twice. Ethanol was decanted and the product was dried via nitrogen purge leaving a black powder. ***Synthesis of SPIO NPs.*** 100 mg of PLGA and 20 mg SPIO were dissolved in 2 mL of chloroform. The polymer/drug solution was then added dropwise to 4 mL of 2.5% polyvinyl alcohol (PVA) as the outer aqueous phase and sonicated to form an emulsion. The emulsion was poured into a beaker containing aqueous 0.3% (v/v) PVA and stirred at room temperature for 3 h to allow the solvent to evaporate and particles to harden. Then, the nanoparticle solution was first centrifuged at low speed (8,000 x g for 10 min) to pellet the large particles. The supernatant was decanted and small nanoparticles were collected through high-speed ultracentrifugation (100,000 x g for 30 min, x 2). To prevent nanoparticle aggregation during lyophilization, trehalose was added to the final aqueous solution at a ratio of 0.5:1 (trehalose:nanoparticles) by mass immediately prior to lyophilization. ***CED of SPIO NPs.*** Sprague-Dawley rats were anesthetized with ketamine/xylazine. Animals were prepped with betadine and alcohol and placed in a stereotactic frame. A linear midline incision was made and a burr hole was drilled in the skull 3mm lateral and 0.5 mm anterior to bregma. A 26G Hamilton syringe with 28G stepdown inner cannula, was inserted to a depth of 5mm. The tissue was allowed to equilibrate mechanically for 5 minutes. SPIO-loaded nanoparticles in PBS were infused continuously at a rate of 0.667µL/min. Following infusion, the syringe was left in place for 5 min, after which it was removed. The burr hole was filled with bone wax (Lukens, Reading PA), the scalp was closed with surgical staples, and the rat was removed to a clean cage with free access to food and water mixed with ibuprofen for analgesia. ***In vivo MRI.*** The spin-echo MRI datasets were obtained on a 4.0T Bruker horizontal-bore spectrometer (Bruker, Billerica, MA, USA) with a ^{1}H RF surface coil of 2.5 cm diameter, positioned on top of the animal head. 16 coronal slices of 128×128 resolution and 1 mm thickness were acquired using a FOV of 32×32 mm^2, a TR of 6s and 8 different TE values in the range from 15 to 115 ms, resulting in an in-plane resolution of 250×250 μm^2. Measurement of SPIO volume, V_d, was obtained using BioImage Suite (http://www.bioimagesuite.org/). Each spin-echo image was normalized to its maximum intensity to ensure similar intensity threshold for each dataset. Next, the 3D volume occupied by the SPIO nanoparticles, V_d, was calculated at each TE using four intensity threshold values (15, 20, 25 and 30%). The lifetime τ of the SPIO nanoparticles in the rat brain was estimated from the exponential decrease of V_d/V_i over time (t) according to $\mathbf{V_d/V_i = (V_d/V_i)_0\ exp(-t/\tau)}$ where $(V_d/V_i)_0$ represents the ratio between the volume V_d immediately after the CED of SPIO-containing brain-penetrating nanoparticles (day 0) and the injected volume V_i.

RESULTS AND DISCUSSION SEM of SPIO-loaded brain-penetrating nanoparticles showed that nanoparticles were approximately 68 ± 6 nm in diameter and morphologically spherical **(Fig.1A-B)**. Their r_2 relaxivities are high, 236 ± 10 $s^{-1}mg^{-1}ml$ at 9.4T and 202 ± 21 $s^{-1}mg^{-1}ml$ at 4.0T **(Fig.1C)** demonstrating that these SPIO-loaded brain-penetrating NPs are excellent transverse (T_2) relaxation agents. Examples of 3D distributions of the SPIO nanoparticles at 4.0T are shown in **(Fig.1D)** for the three doses investigated, at 30% intensity threshold and a TE of 72 ms. The brain volume occupied by the NPs, V_d, depends on the dose injected **(Fig. 1D)** but also on the TE and the intensity threshold value used (data not shown). V_d increases with the SPIO dose at the same TE and threshold value. At the same TE, V_d increases with increased threshold value. However, V_d increases with TE (at the same threshold value) only at 0.02 mg and 0.1mg doses, while at 0.5 mg the volume remained almost unchanged with increasing TE. The V_d/V_i values obtained at TE=72 ms and 30% intensity threshold were 2.3, 3.7 and 4.6 for SPIO doses of 0.02 mg, 0.1 mg and 0.5 mg, respectively. The lifetime of the NPs in the brain (τ = 65 ± 11 days) was estimated over a four-week period **(Fig.1E)** in 4 animals using two different doses (0.5mg in 20µl and 0.25mg in 10µl). Interestingly, the V_d/V_i values from all animals investigated were very similar at all time-points (less than 10% standard deviation for each time-point) and were independent of injected volume V_i **(Fig.1F)**. The average V_d/V_i value immediately after the infusion (day 0) was 3.41 ± 0.15. In summary, we demonstrated that these NPs have excellent transverse relaxivity T_2 (higher than 200 $s^{-1}mg^{-1}ml$ at both 4.0T and 9.4T) and that the MRI contrast from the SPIO-loaded NPs lasted over a long period suggesting that a single administration of PLGA brain-penetrating NPs allows controlled release of cargo agents over long time periods. Therefore, this novel drug delivery platform can have an immediate impact on monitoring of treatment in patients with glioblastoma.

REFERENCES [1] Mrugala MM, Chamberlain MC *Nat Clin Pract Oncol.***5**: 476-486 (2008); [2] Zhou J, et.al. *Proc Natl Acad Sci* **110**: 11751-11756 (2013); [3] Ragheb RR, et.al. *Magn Reson Med.* 70: 1748-1760 (2013).

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