Dual Modality Nano-Particles for Tumor Uptake

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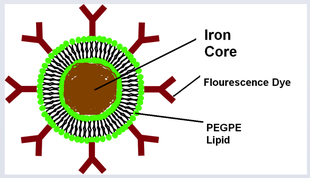
Abstract

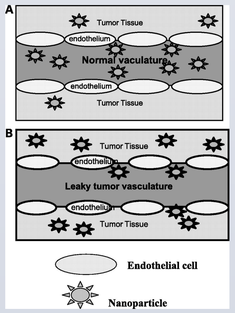
Nanoparticles can be used as effective carriers of drugs and imaging agents for tumors. To measure the uptake in tumors, fluorophores and MR contrast agents can both be incorporated into the same nanoparticles. The goal of our project was to prepare new super paramagnetic iron oxide (SPIO) nanoparticles that are also labeled with a near infrared fluorescence dye (DiIC18(5)-Dioctadecyl-3,3,3,3-tetramethylindodicarbocyanine-5,5-disulfonic acid) which would then be measurable using MR and fluorescence microscopy. The experimental design consisted of injecting the nanoparticles into mice via tail vein followed by uptake evaluation in situ by the aforementioned methods. The super-paramagnetic iron oxide particles are used as a T2 contrast agent. The nanoparticles had an average size of 22 nanometers as measured by dynamic light scattering and transmission electron microscopy. The concentration of iron oxide in the nanoparticles was 1 mg/mL. The nanoparticles in the tumors were visualized 24 hours post injection by MR and then by fluorescence microscopy using a Cy5 filter. By using dual-labeled nanoparticles, we expect to obtain more detailed information on tumor uptake and distribution of nanometer sized drug formulations. Also, these dual labeled nanoparticles will allow for a more accurate analysis of tumor vasculature and changes post heating.

Background

Iron oxide nanoparticles are composed of an iron core surrounded by lipids. The lipid can then have other molecules attached to it. Moieoties, as they are called, can be attached which allow for the nanoparticle to target certain cells. A fluorescing Dye can be attached to the outside allowing for the particle to Be looked at under a Flourescence microsope.



The reason nanoparticles are used to carry drugs and agents is because they collect in tumors. This is known as enhanced permeability and retention (EPR), and occurs because of the leaky vasculature inside tumors. The nanoparticles are able to escape, but become trapped once inside the tumor. They are to large to escape elsewhere in the body, which effectively targets them to tumors.



Objective

The objective was to see if subjecting mice to heat treatment could help increase tumor vasculature. Being that nanoparticles can be made with a dye and MR contrast agent, they could be used to help look at tumor vasculature. Their uptake and perfusion into the tumor is a good marker of blood flow. The MR agent being used is Super Paramagnetic Iron Oxide because of it's relatively high contrast and because of the body's ability to expel iron through natural processes.

Materials and Methods

The nanoparticles we made consisted of a monolayer of lipid with super paramagnetic iron oxide particles on the inside and a fluorescing dye. It took multiple trials to figure out what ratio of these ingredients would work best. The final Iron Oxide nano-particles consisted of;

-200uL of SPIO at the concentration of 5mg of iron per mL

-500uL of PEGPE (the lipid suspended in chloroform)

-50ul of fluorescence dye

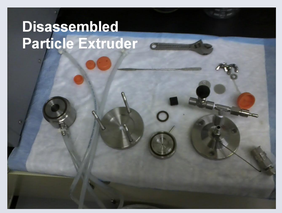
Procedure for making SPIO nanoparticles:

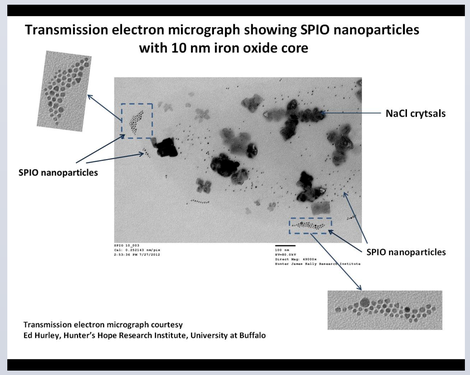
-First, the ingredients above are all measured out and mixed in a round bottom flask (vortexing is not necessary)

-Next, the solvent is evaporated out of the solution. This was done using a rotary evaporator machine. This takes approximately 40 minutes

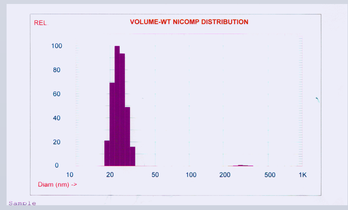
-After all the solvent is evaporated, the thin film of lipids left on the side of the flask is re-suspended with 1 mL of saline. This was done using a combination of heating, and sonication.

-From here, the nanoparticles may need to be extruded and sized to ensure their effectiveness. This can be done using an extruder. The size distribution can be checked using a laser.





-The nanoparticles from here are complete, and can be stored until their use in a fridge and with foil Protecting the fluorescing nanoparticles from light to ensure maximum fluorescence.



Imaging the Nanoparticles:

-The 200 uL of nanoparticles were injected into each of three tumor bearing mice to measure perfusion. Two of the mice were heated to see if heat would effect the penetration of the nanoparticles.

-The liposome's perfusion into the tumor, and other parts of the body, was first measured through magnetic resonance imaging (MRI). Three sets of scans were taken for each of the three mice. The scans were taken pre-injection, immediately post injection, and 24 hours post injection.

-after these scans were taken, the mice were optically imaged. After this the tumors were excised, imaged, sectioned, and fluorescence microscopy performed on the sections to measure fluorescence.

Results

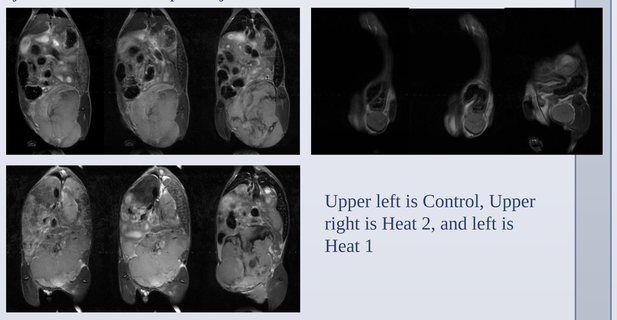
Tumor Volumes:

The tumor volumes were calculated on the computer and are as follows;

Control:	Heat 1:	Heat 2:
3366.34 mm^3	4432.94 mm^3	186.98 mm^3

Tumor Images:

The Images show coronal scans of a mouse pre injection, immediately post injection and 24 hours post injection:



Upper left is Control, Upper right is Heat 2, and left is Heat 1

Image Analysis:

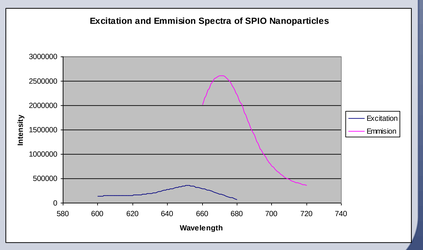
-The scans that were thought would most accurately measure the effect of the iron would be a T2 echo gradient scan. These scans were taken axially on the mice. The change in T2 relaxation values were looked at in the tumor, liver, and kidneys. Below are the changes in T2 relaxations.

Relaxation (Hz)

Mouse:	Scan Name:	Noise	Tumor	Liver	Kidney's	Phantom
Control	Base	37.7573	16.3198	33.3032	11.4545	3.242
Control	Post injection	41.6344	15.1284	45.3904	15.8026	3.6859
Control	24 hours post	71.8976	17.2792	36.4	21.9602	3.4978
Heat 1	Base	63.2099	17.6587	25.0219	13.6011	3.5511
Heat 1	Post Injection	23.4737	18.3983	37.5124	19.0287	3.7285
Heat 1	24 hours post lower Tumor	67.7367	18.0851	22.3385	15.1031	3.3025
Heat 1	24 hours post upper Tumor	25.4339	18.4719	17.0801	15.4281	3.3242
Heat 2	Base	24.7964	16.5708	32.6073	17.9381	3.7936
Heat 2	Post Injection	24.3044	17.859	43.2439	26.1567	3.2192
Heat 2	24 hours post	28.5548	18.0476	31.1922	18.7407	3.0875

Reflective Fluorescence Imagining:

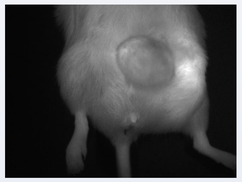
-The SPIO nanoparticles were measured for their excitation and emmision peaks



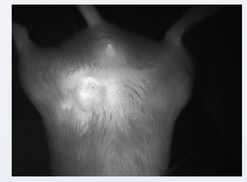
Optical Analysis of Tumor:

Through reflective fluorescence imaging, the tumors were looked at In Vivo;

Control

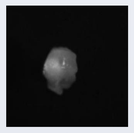


Heated



The tumors were also excised and imaged as shown below:

Control



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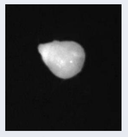
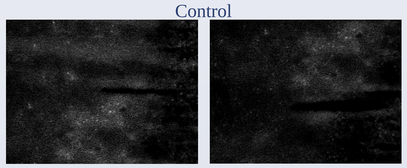


Fluorescence microscopy was also done on the tumors:

Control



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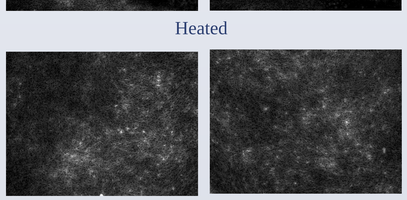

Conclusions

Through our experiments, we were able to show that dually labeled nanoparticles could be produced at a consistent size through our procedure. These nanoparticles collected in tumors, but it also seems they collected in other organs of the mouse. Heat also seems to have had an effect, but to what extent is still unknown. Future experiments will test larger size nanoparticles to what effect size has for increasing contrast in the MR scans. Hopefully future experiments will lead to clearer results with regard to iron oxide uptake in tumors and the effect of heat treatment in uptake of these nanoparticles.

References

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