

RADIATION-HYPERTHERMIA TREATMENT OF CANCER VIA IRON OXIDE NANOPARTICLES

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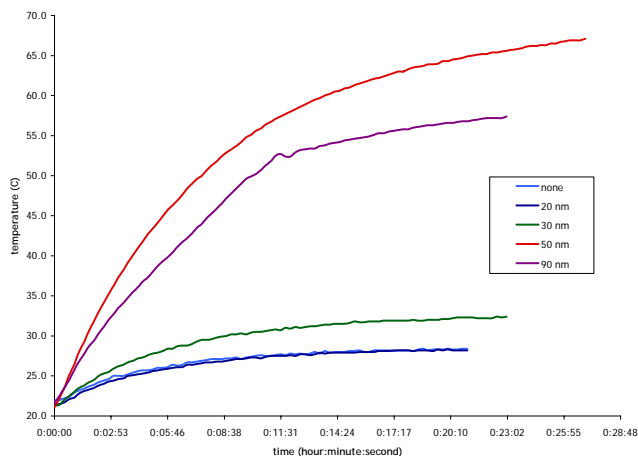
Introduction

Throughout the 10 week program several tests were conducted and results gathered which have aided in the larger research effort by Dr. Jack Hoopes and his lab. As the weeks went by methods were modified and enhanced. Initially three cell lines were considered, MCF-7 (human breast cancer), MDA-231 (human breast cancer) and MTGB (mouse breast cancer) but difficulties were encountered and only MCF-7 cells were found to grow colonies which could be properly stained and counted. MDA-231 cells require a special type of agar medium for cell culture, while MTGB cells were expected to grow in the alpha MEM medium alone. Although the exact reason for the inability of these cells to grow in culture is unknown, future tests will hopefully be more promising as new cells are harvested. Here we will discuss the tests conducted, as close to chronological order as possible.

All in vitro AMF heating experiments were conducted using a 17-turn coil with a capacitor load resulting in about 148 – 160 kHz frequencies. Field output may vary depending on the experiment, but remains in the range of 400 ± 50 Oe.

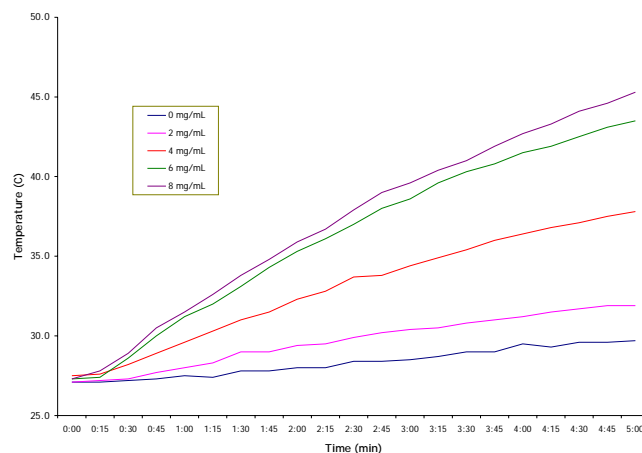
Comparison of particle heating by particle size

One of the first tests conducted was to determine which particle size would be best for our uses in in-vitro and in-vivo studies. From the gathered data we observed that, not only were the 20 and 30 nm ineffectual, but also that the 50 nm size particles produced the best heating. The 90 nm also produced a reasonable curve. Below is the graph depicting the various nanoparticle sizes and their temperature profiles. For this experiment, each eppendorph tube was filled with 200,000 MCF-7 cells with a concentration of 6 mg/mL of nanoparticles. The remaining volume, 300 microliters total, was made of media specific to the MCF-7 cell line.



Comparison of particle heating by concentration

For the sake of consistency and convenience, we needed to decide the optimal concentration for our studies. Thus, one of the first tests conducted involved the treatment of 200,000 MCF-7 cells with 0 mg/mL, 2 mg/mL, 4 mg/mL, 6 mg/mL and 8 mg/mL concentrations of 50 nm nanoparticles. Again only 300 microliters of volume was placed in the eppendorph tubes, diluting with media. Based on this data, as seen below, 6 mg/mL became the concentration of choice, since it produced a workable temperature curve with moderate killing.

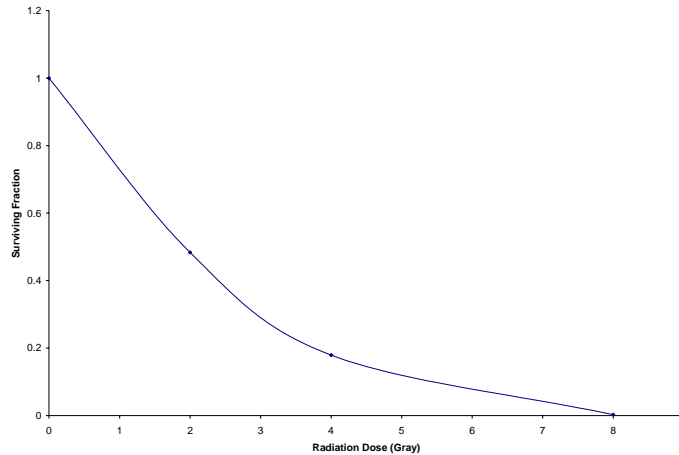


Isotherm development by manual power adjustment confirmation

Several phantom runs were conducted with 300 microliters of only media to determine a procedure for maintaining a relatively stable isotherm. The investigation showed that, by manually adjusting the power, temperatures can be stabilized to within $\pm 2^\circ\text{C}$ of target isotherms of 37, 41, 43, 45, and 47°C.

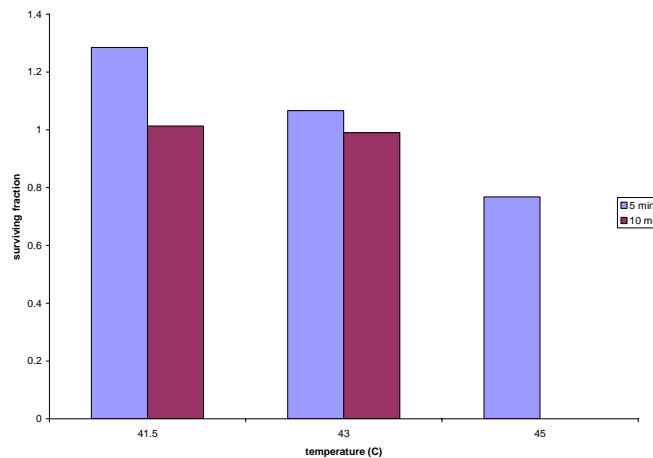
Radiation Test

It had previously been shown that radiation and heat treatment to cancer cells produces the most effective killing. Because of this finding, we looked at the cell death due to radiation alone. Below is the data obtained from exposing 200,000 MCF-7 cells to 0 Gy, 2 Gy, 4 Gy and 8 Gy. From the results we decided 4 Gy was the best radiation dose to administer, as 8 Gy killed almost all the cells, while 2 Gy did not kill enough.



Isothermal treatments

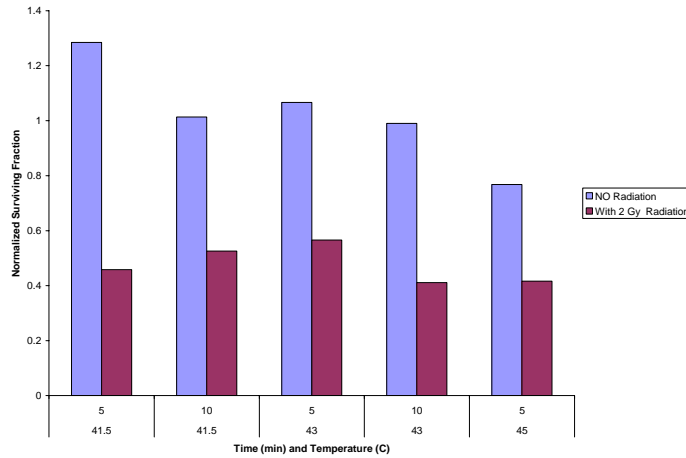
Using the standard concoction of 200,000 cells, 6 mg/mL of 50 nm particles, and total volume of 300 microliters, isotherm experiments with 41.5, 43, and 45°C for 5 and 10 minutes were conducted in an effort to produce a dose response curve. Previous studies performed using 8 mg/mL and 6 mg/mL of 50 nm particles yielded a reasonable curve for the former, but a questionable result for the later. Retests, however, generated the chart below, which shows an excellent curve for the 5 minute duration. Although the 10 minute tests killed more cells as expected, it exhibits a far shallower curve that is more difficult to analyze given the scarcity of temperature data points.



Isotherms in conjunction with radiation

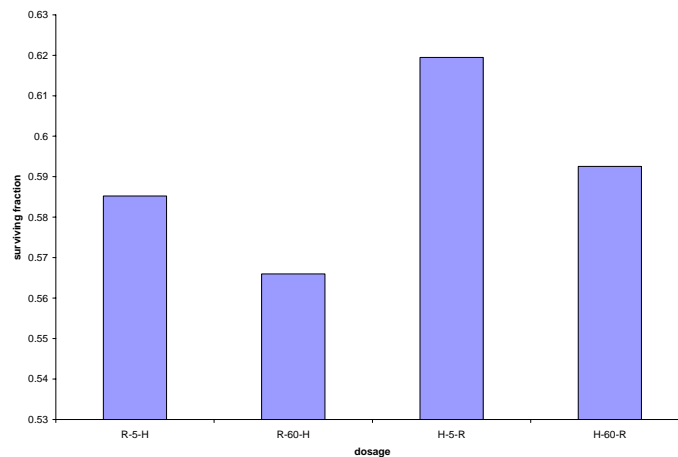
Comparison tests were conducted, set at the same conditions as the Isothermal treatments, with the added condition of 2 Gy radiation for all samples. Across the board,

the combination treatment was more effective in killing cells than just isothermal heating alone, as the following bar graph suggests.



Radiation Sequence Timing

To determine if the time of heat and radiation administered would make a difference in results we conducted a timing test in which radiation was administered 5 minutes prior to heating, 60 minutes prior to heating, 5 minutes after heating and 60 minutes after heating. We gave 2 Gy radiation and 5 minutes of heat at 43 degrees. The results of this test proved inconclusive; while all the numbers were very close and roughly the same, possibly indicating that timing plays no role in treatment, there is a slight trend for more survival for the runs where heat was administered first.



Direct toxicity of particles in media to in-vitro cultures

MCF-7 cells were first harvested, plated, and allowed to incubate overnight so that they adhere to the plates. The wells were then exposed to varying concentrations (0, 1, 6, 8

mg/mL) of 50 nm or 90 nm particles and co-incubated for 30 min, 4 hours, and overnight (between 18 – 24 hours). The wells were washed three times with PBS after exposure.

The in vitro toxicity tests were conducted twice, both instances yielding spurious results. The control plate for both the initial experiment and its retest were almost devoid of colonies, indicating a possible error in the procedure, probably in plating.

Washed Exposure Testing

Another experiment involves particle exposure procedure similar to the Direct Toxicity studies, but cells were additionally treated with AMF heating afterwards. Both the MCF-7 and MTGB cell lines were used, plating 300,000 cells per well. The cells were exposed to 1 mg/mL of 50 nm particles with co-incubation times of 30 min, 4, and 24 hours. The cells were washed 3 times with PBS, harvested, then exposed to AMF at 100% power for 5 minutes followed by 85% power for 10 minutes. For each time point, two samples were plated: one with particles and AMF, and another with particles but no AMF. Overall controls with no particles and no AMF and no particles and AMF were also prepared.

The colony counting results for these tests are still pending, but TEM images of the particle-exposed cells were taken both before and after AMF heating. These images indicate that the cells have taken up the particles, as they are found in vacuoles within the cell membrane. No particles were found outside the cell membrane, but this is probably due to the trypsinization it experienced when it was harvested for AMF exposure.

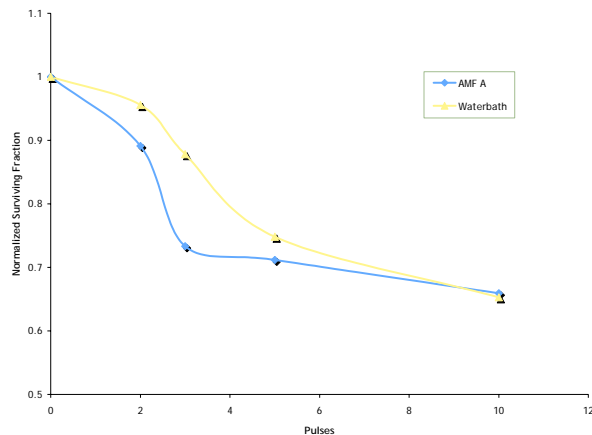
SUBGLOBAL HEATING:

Comparison of isothermal nanoparticle heating versus isothermal water bath heating

Wide pulse full-power exposures

Subglobal heating (below 39°C) was accomplished using both AMF and waterbath heating methods. Cell samples were exposed to 3 minutes of full power heating followed by a 12-minute cool-down time. The number of pulses was varied from 1 pulse to 2, 3, 5, and 10 pulses.

Overall pulsing produces much less cell death than does persistent power treatment. Moreover studies conducted also indicate that AMF heating is much more effective than waterbath heating; this is supported by comparison experiments conducted in the waterbath for the isotherm AMF heating tests. Other subglobal AMF heating experiments also indicate 41.5°C as the thermal dose near the threshold for significant cell killing; anything below 40°C only results in minimal cell death.

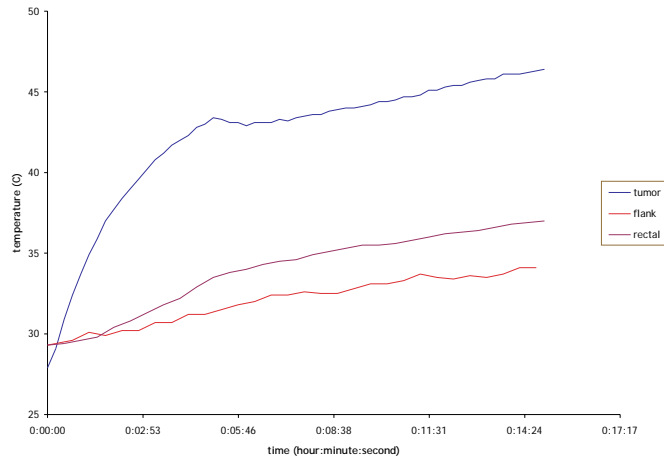


Mouse in vivo studies

Both MDA-231 and MTGB cells were injected into nudemice, but the tumors were not as robust as expected. From monitoring the tumor sizes daily, it is observed that the tumors generally tend to grow, but then regress after a few days; measureable MDA-231 tumors emerge after 5 days, while MTGB tumors remain negligible even after a week. The exact reason for this phenomenon is still unknown, and thus, most of the scheduled tests were abandoned.

Two preliminary in vivo studies were conducted. The first involved male nude mice without tumors: one mouse was simply placed in the coil without particles, while another was injected intravenously with 0.5 mL of 50 mg/mL concentration of 50 nm particles. The first mouse, with no particles, received full power (over 900 Oe) and died after 14 minutes. The second mouse again had no particles, but only received 40% power (about 450 Oe). Finally, the last mouse received a dose of particles, enough to turn him a shade of black; even his eyes turned dark. Both mice had normal vital signs throughout the treatment, and survived the procedure. After about a day, the blackened mouse also began to clear up.

In another mouse study, 10 million MDA-231 cells were injected into the right flank and injected with roughly 100 microliters of 50 nm particles. The mouse was then heated for 5 minutes at 450 Oersted and 10 minutes at 400 Oersted. Three temperature probes were placed in the mouse. One in the tumor, one on the other flank of the animal, and one in the rectum to measure core temperature. Below is the temperature graph and it is clear to see that the tumor temperature was 10 degrees higher than the core body temperature.



Problems encountered

In general problems were those concerning lab technique. Initially plating efficiencies were seen to be very high but in some tests were below 50%. More care should be taken to develop good technique and remove areas of potential error. For example, taking care to use sterile tips and properly discarding and replacing dirty tips each time pipetting from a different solution. Another problem noticed during our technique was that the amount of solution pipetted in the well needed to be large enough so the cell solution would not bubble.

Conclusion

Information collected from this summer's project can be summarized in the following bullet points:

- The alternating magnetic field heats particles better than the waterbath, killing more cells.
- Heat + Radiation kills more cells than heat alone or radiation alone.
- 50 nm size particles are best for heating.
- Isothermic heating is better than pulsing.
- Particles are uptaken by cell.
- When the cell divides both daughter cells contain particles.
- Particles are not toxic in vivo.
- Alternating Magnetic Field of 400 – 450 Oe is non-lethal.
- Tumor heating with particles is 10 C above core temperature.

Future endeavors include:

- Radiation/Heat timing retesting
- Direct in vitro toxicity retesting
- In vivo toxicity testing
- Washed exposure retesting (without trypsinization)
- Antibody conjugation to particles
- Antibody vs. non-antibody comparison testing
- More comprehensive in vivo testing