Inducing Phagocytosis on Magnetic Nanoparticles to Bypass Magnetic Hyperthermia Treatment Complications in Glioblastoma

Wafiza Julkipli

Abstract

Glioblastoma is an aggressive form of brain cancer that forms on astrocytes in the brain. It accounts for 52% of all primary brain tumors. Current treatments include radiation therapy, chemotherapy, and surgery, all of which have major side effects. Magnetic Hyperthermia Treatment(MHT) is a new approach to cause tumor apoptosis by heating tumor cells with Magnetic Nanoparticles(MNPs). MNPs arrive at the site of the tumor and induce heat which causes misfolded proteins, clumping and ultimately triggers cell apoptosis. Though MHT has high efficacy, there are two complications that lead to its poor acceptance: the toxicity of MNPs after treatment and that healthy cell tissue may be damaged. While MNPs do surround the tumor, leaks and refluxes can occur which damages cells outside of the tumor environment. Then, after treatment, MNPs remain in the body and can cause toxicity or harmful side effects. One strategy to circumvent these issues is the use of induced phagocytosis on MNPs by engineered microglia from stem cells and additional stem cell transplants in the treatment area. The use of engineered microglia allows for MNP removal to remain after the treatment, as inducing existing microglia to attack MNPs would interfere with treatment. Adding additional stem cells in the area will allow for replacement of destroyed tissue. The use of these modifications on MHT can overcome its complications.

The study will be conducted on mice engineered to have glioblastoma formation. Three groups of mice will be formed: mice with MHT treatment and modifications as the experimental group, mice with MHT treatment with no modifications as the control group, and normal healthy mice as the blank group. MNPs will be delivered to the tumor sites of experimental and control groups through convection-enhanced delivery(CED). MHT will be conducted on the experimental and control groups during a 40 minute period using alternation magnetic fields(AMF) to activate the MNPs previously injected. Then, the experimental group will receive stem cell transplants including the engineered microglia through the carotid artery, as this will lead them directly to the brain. A week after modifications have been made, the control and experimental groups will be scanned to identify the prevalence of MNPs using CT scans. While both experimental and control groups have glioblastoma, the success of the experiment will be determined by the amount of MNPs remaining in the mice brains of both the experimental and control groups compared to Day 1 of the experiment and the side effects present among the groups. Overall, the study will require 80 days to prepare for and to conduct. This study will require various tools and machinery mainly found in a laboratory setting, therefore it will be conducted in a lab.

This study is designed to assess the hypothesis that the removal of MNPs and addition of stem cells will reduce the side effects associated with MHT in the experimental group as opposed to the mice in the control group. The use of stem cells in the brain to repair tissue has been tested before, specifically on traumatic brain injury. These studies have found that the use of stem cells is beneficial to healing the area of injuries. Using that principle, the use of stem cells should be beneficial after MHT. Since using phagocytosis is a new approach to remove MNPs from the brain, it has not been tested yet. However, since cell phagocytosis lowers the pH of its contents, and metals are destroyed in low pH, the MNPs should be destroyed during phagocytosis. Using such evidence, the use of induced phagocytosis and transplanted stem cells should bypass the complications of MHT.

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Glioblastoma is a form of fatal cancer that occurs on astrocytes, a type of glial cell in the brain. It affects 2-3 adults out of a 100,000 adults every year and ninety percent of adults with glioblastoma die within 24 months of diagnosis. Though it has such a high prevalence, there is no cure. However, many treatments for glioblastoma are in preclinical trials, such as MHT. MHT is a treatment that uses MNPs to increase the heat of tumor cells and cause cell apoptosis. The efficacy of the treatment is very high, with various studies proving complete tumor cell death after MHT. But MHT comes with complications such as leaks of the MNPs into other areas of the brain and the toxicity of MNPs after treatment. The goal of this study is to bypass these complications for a more promising treatment. If MHT is proven as a viable treatment, it could help glioblastoma patients as well as tumor patients, in general. This study aims to induce microglia phagocytosis on MNPs, which has not been conducted, therefore it could open up a new avenue of research.

Magnetic Nanoparticle

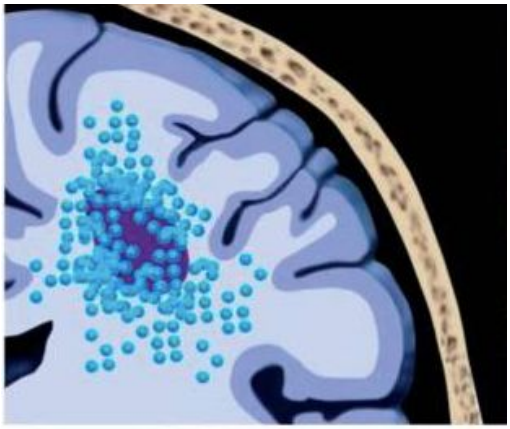
MNPs are nanoparticles that have a magnetic charge and work together to induce heat on cells. The heat causes problems within the cells and triggers cell apoptosis. Their heat is produced through a hysteresis loss, which is where the magnets produce energy that is lost in the form of heat (Mahmoudi et al., 2018).The most common type of MNPs are iron oxide nanoparticles, which are made of Fe(11) and Fe(111), with an alkaline base.

Magnetic Hyperthermia Therapy

MHT is a localised form of hyperthermia therapy, which uses intracellular MNPs

Figure 1

Magnetic Nanoparticles at Tumor



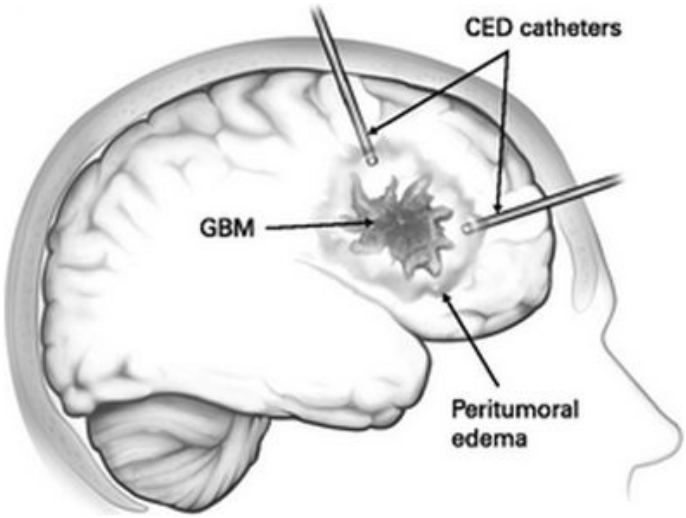
Note. The MNPs(blue circles) cluster at the site of a tumor(purple mass). CED not pictured.

activated by an external alternating magnetic field(AMF) to create heat. These MNPs are delivered at the tumor site using a CED. The MNPs cluster around the tumor site as shown in Figure 1 and become activated when the AMFs are started. Then they heat the tumor site and destroy cells (Mahmoudi et al., 2018).

Convection Enhanced Delivery

Figure 2

CED Catheter Placement



Note. The catheters will be filled with MNPs.

CED is a delivery method to deliver MNPs at a specific location in the brain. CED involves the use of a minimally invasive surgery to create two burr holes in the brain. These burr holes will be near the tumor and through these holes, two catheters will be inserted as shown in Figure 2. The catheters will be filled with MNPs and require an external infusion pump to allow for an even rate of infusion of the MNPs into the tumor site. This process occurs before the activation of the AMFs (Convection Enhanced, n.d.).

MHT Complications

There are a few complications with MHT that this study is designed to resolve. The main issue that faces MHT is the toxicity of MNPs after MHT. These MNPs remain in the body and though inactive, could disrupt bodily functions. Another issue with MHT lies in the potential reflux of the MNPs into healthy cells(Jiang et al., 2019). MNP exposure on healthy cells could cause damage to the heart, eyes, development as more that are detailed in Figure 5.

Stem Cells

Stem cells are cells that have the potential to differentiate into a variety of cell types. They can be found in the bloodstream, bone marrow, and embryos. When they are exposed to certain chemicals, it triggers their transformation into certain types of cells. They are used in treatments to repair damaged tissue in the body.

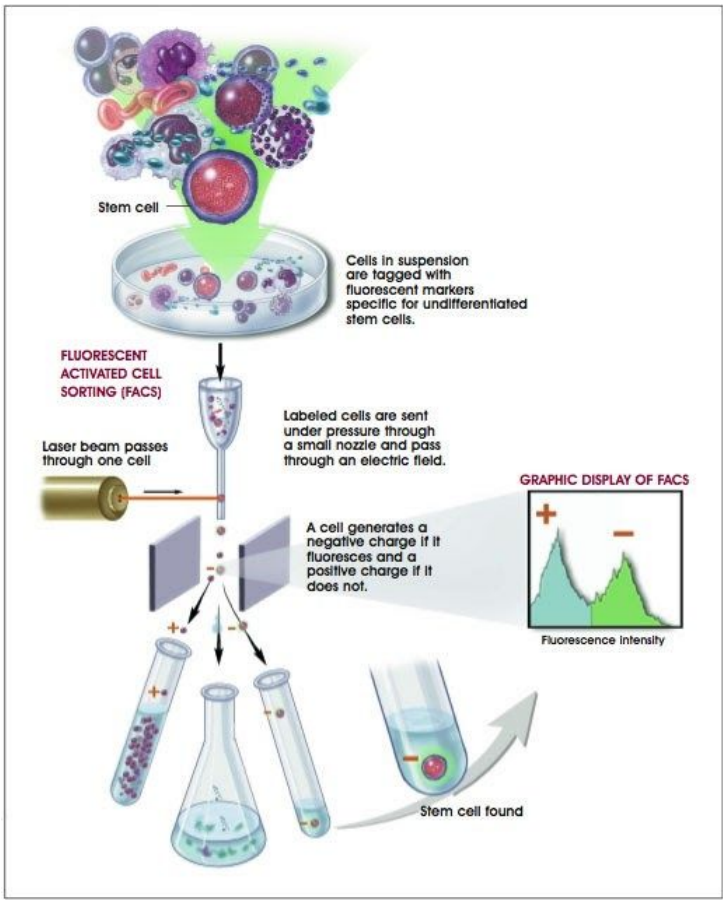
Microglia from Stem Cells

In order to differentiate stem cells into microglia, they need to be exposed to two proteins: TMEM119 and DHDHD. These proteins will cause the stem cells to become microglia(Microglia Differentiation, n.d.). Some stem cells don’t differentiate or fully

differentiate even when exposed to these proteins, therefore the culture of stem cells need to be purified. Fluorescent-activated cell sorting(FACS) is a method used to separate fully differentiated microglia from partially differentiated stem cells. The undifferentiated cells first need to be tagged with fluorescent markers as shown in Figure 3. Then, they are sent through a pressurized nozzle and a laser beam shines on each cell before they enter an electrical field. Here, the undifferentiated cells become negatively charged and the differentiated cells become positively charged. Depending on their charges, they are separated into two test tubes for further use (Winslow, 2001).

Figure 3

FACS Process

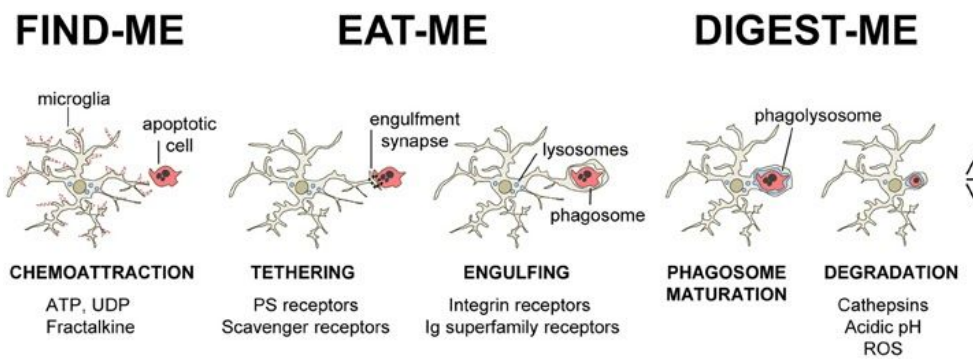


Quantum Dots

Quantum dots are semiconductor nanoparticles that are used for various purposes, including labelling stem cells. Stem cells that are tagged with a quantum dot can be tracked through the ultrasound waves these dots emit. Researchers have used such dots to track the location of transplanted stem cells and to identify their integration into the body(Ratteu, 2012).

Phagocytosis

Figure 4
Microglial Phagocytosis



Note. Phagocytosis shown in microglia, but occurs in all immune cells.

Microglia are helper cells in the nervous system that attack pathogens using phagocytosis. Phagocytosis is a process the immune system uses to engulf pathogens and destroy them. It is triggered when a receptor on a microglia binds to a pathogen, identified by its coating or molecules it secretes. Then, the pathogen is engulfed into the cell, and forms a bubble in the cell called a phagosome as shown in Figure 3. This phagosome combines with lysosomes in the cell to lower the pH of the pathogen and kills it. The contents of the pathogen, that are not dissolved, are then emptied outside of the cell as waste material(Khan Academy, n.d.).

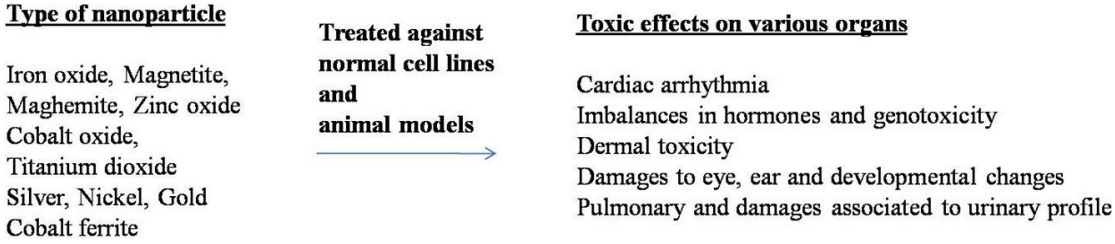
Predictions

Mice with induced phagocytosis on MNPs and transplanted stem cells after MHT

will have reduced side effects compared to mice that undergo MHT without any modifications. The side effects of MNPs on normal cells, described by Jiang et al. (2019) in Figure 5 above, are mainly due to prolonged circulation of MNPs in the body. Since the removal of MNPs is prompt in this study, these side effects should not appear. Though no other studies have induced

Figure 5

Magnetic Nanoparticle Toxicity on Healthy Cells



Note. Picture depicts graphical abstract created by Jiang et al. (2019)

phagocytosis on MNPs, Zhang et al. (2018) discovered that low PH encourages the solubility of heavy metals. This means that the MNPs should be destroyed during phagocytosis due the low pH it induces. As for the stem cell transplants, these should heal any leaks or refluxes of the MNPs as brain stem cell transplants have been conducted successfully in various studies. In one particular study, Osanai et al. (2012) transplanted stem cells to the brain via the carotid artery forming a non-invasive way to deliver stem cells rapidly and directly to the brain after a traumatic brain injury. The rats used in the study had complete recovery after the transplants. The same method will be used in this study to allow the stem cells to reach the area of the tumor site after MHT rapidly.

Materials and Methods

This study consists of a preparation period and an experimental period.

Preparation

For this study, there is a large amount of preparation needed beforehand. This includes the purchase of 45 *Mus Musculus,* or house mice, which will be split evenly between the three mice groups: experimental, control, and blank. Preparation also includes stem cell derivation from the mice, microglia creation and regramming, inducing glioblastoma in mice, and MNP synthesis. The preparation should take 60 days, which is the sum of the time each step of the preparation will take.

Stem Cell Derivation

Peripheral blood stem cells will be taken from mice prior to tumor growth to ensure that there is an ample amount. Mice will be given G-CSF 4 days before the stem cell harvest. On the day of the derivation, blood will be taken from the mice using a tube to an apheresis machine and returned via another tube after the stem cells have been taken by the machine. The procedure will be repeated on 15 mice and after collection, each stem cell will be labelled with a quantum dot.

Stem Cell Differentiation

After collection of stem cells, half of them will be put into petri dishes to begin their differentiation into microglia. Then, they will be exposed to two proteins TMEM119 and P2RY12 and given 2 weeks to differentiate into microglia. Then, they will be purified through the FACS method(refer to Figure 3) and stored until the experiment date.

Microglial Reprogramming

The new microglia will be reprogrammed to add a receptor that detects the Fe(111)

nitrate on the MNPs. This will act like an opsonin receptor, in that it will induce phagocytosis on all particles coated with Fe(111) nitrate. The addition of the receptor will be made through editing the opsonin receptor protein and replacing its detection of immunoglobulin with Fe(111) nitrate.

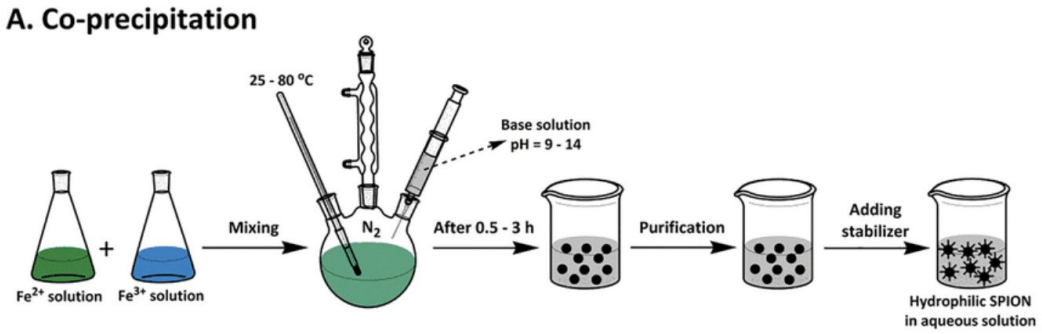
Genetic Modifications to Mice

30 of the mice, which are part of the experimental and control groups need to be engineered to have glioblastoma. This will occur through the delivery of Cre Recombinase through the TVA system. Mice will be given 40 days to develop glioblastoma and grow tumors larger than 5 mm, and then divided between the experiment and control groups.

Magnetic Nanoparticle Synthesis

Figure 6

MNP Co-precipitation



Note. Base solution is alkaline. Solution is purified and stabilized with Fe(III) nitrate. Iron oxide particles will be synthesized using co-precipitation.

The process, as shown in Figure 6, will begin with Fe(II) and Fe(III) having a simultaneous precipitation through the addition of an alkaline base for uniform MNPs. After an hour of precipitation, the solution will be purified and then stabilized through the addition of

Fe(III) nitrate. They will also be coated with Pluronic F127 coating. 640 grams of this aqueous solution should be made to suffice for 30 mice.

Experiment

The goal of this experiment is to treat the control and experimental groups with MHT and then transplant microglia and stem cells into the experimental group. The side effects that all groups of mice show will be observed and recorded. The levels of MNPs will also be recorded through a CT scan. At the end of the observation period, an analysis will be made comparing the side effects and levels on MNPs between the mice groups. This will take 40 days to conduct.

CED Implantation

CED implantation calls for a minimally invasive procedure to insert 2 catheters near the tumor site stereotactically.

Figure 7
Infusion Pump

Note. Above is a standard infusion pump used.

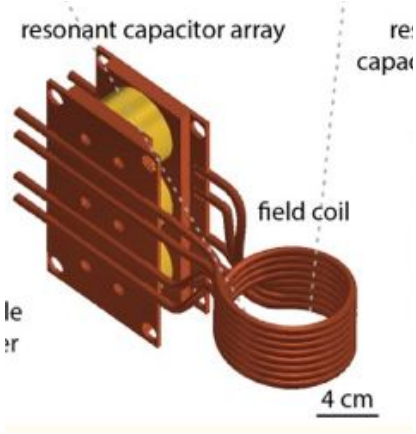
These catheters will contain the MNPs and will infuse them into the tumor site on the command of an external infusion pump shown in Figure 7. This procedure will occur on the day of MHT therapy for the mice.

MHT Therapy

The therapy will begin with the infusion of the MNPs into the tumor site through the CED.

Figure 8

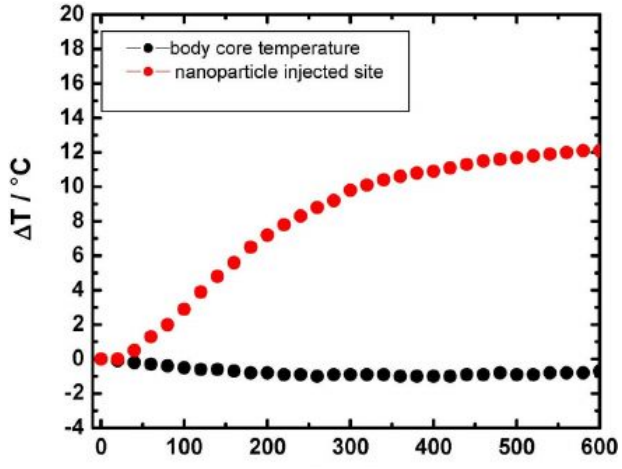
Field Coil Size For Animal Models



Then, a small resonant capacitor array with a field coil, pictured in Figure 8, will activate the MNPs at the site of the tumor. A CT scan will be used to ensure proper placement of the MNPs and the estimated heat of the MNPs is shown in Figure 8. After 40 minutes of activation, the coil will be removed and the MNPs will become inactive.

Figure 8

Body Core Temperature vs. Heating Site Temperature



Note. Graph was recorded in degrees Celsius.

Microglial and Stem Cell Transplantation

The stem cells and microglia will be sent through the carotid artery, which will lead them directly to the brain. The small deficit of healthy cells will attract the stem cells to the destroyed cells and repair them. The microglia will alert the immune system of the MNPs and begin to destroy them.

Observation Period

For 30 days after the treatment, all groups of mice will be observed for any possible side effects. Data collection will occur on a daily basis about the conditions of the mice. They will also be screened using an CT scan every 5 days to find the amount of MNPs in their system. The quantum dots will be used to track progress of stem cells every week.

Timeline

In total, the study will take 105 days, including preparations used for the study. This timeline is a combination of the total time needed for each mouse in each step of preparation and each step of the experiment. Tumor growth requires 40 days and the rest of the preparation takes 20 days. The experiment requires 5 days to induce MHT and add modification to all mice, a 30 day observation period, with 10 days for analysis of data.

Location

This study requires CT scans, areas to hold mice, AMFs, and surgical tools. These can only be found in a lab or hospital setting, therefore the study will be conducted in a certified lab.

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