PhD Thesis Title: Effects of magnetic hyperthermia using magnetic iron oxide nanoparticles coated with PAMAM dendrimer on cancer cells in vitro and in animal models of breast cancer
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ABSTRACT:

Breast cancer is the most common cancer in women worldwide with over two million newly diagnosed female cases in 2018. The traditional treatments for breast cancer e.g., radiation therapy and chemotherapy, possess several side effects. Therefore, it is still challenging to find an efficient method to eradicate breast cancer cells, specifically and locally, with using ionizing radiation. Magnetic hyperthermia has recently attracted much attention in the field of cancer treatment. In this method, magnetic nanoparticles (MNPs) are injected into the tumor and subsequently exposed to an alternating magnetic field (AMF) to convert the magnetic energy to heat through several physical mechanisms, of which, the Néel and Brownian relaxation losses are the main mechanisms involved in superparamagnetic nanoparticles' heating.

The main challenge in magnetic hyperthermia is developing a biocompatible and efficient nanostructure to generate enough heat. The current study aimed to develop iron oxide nanoparticles (IONPs) coated with the fourth generation (G4) of polyamidoamine (PAMAM) dendrimer (G_4@IONPs) for magnetic hyperthermia treatment of breast cancer. The cytotoxicity assessments in mice breast carcinoma cells (MC_4L_2) indicated that G_4@IONPs had no significant toxicity in concentrations below 500 mg/ml. Furthermore, the biodistribution of G_4@IONPs in Balb/c mice showed that these nanoparticles were mainly excreted from the kidney after 24 hours due to their small size (10±4 nm). The radiofrequency (RF) absorption of G_4@IONPs was determined by AMF specific absorption rate (SAR), which was defined as the amount of induced heat per unit mass of magnetic nanoparticles. A magnetic hyperthermia system (LABA, HT-1000W, NATSYCO) with a frequency range of 100–400 kHz was used for magnetic hyperthermia experiments. The temperature of the nanoparticles was measured with a digital thermometer using a thermocouple inserted into the sample. Subsequently, temperature-time curves were plotted at frequencies of 200 and 300 kHz in AMF amplitude of 12 kA/m. The SAR values were calculated using the formula: $SAR=(1/m_{Fe})C(\Delta T/\Delta t)$, where m_{Fe} is the mass of iron in the sample, C is the specific heat capacity of the sample, and $\frac{\Delta T}{\Delta t}$ is the initial slope of the temperature-time curves. The SAR values of the G_4@IONPs sample at 200 and 300 kHz were 49.8 and 129.3 W/g_{Fe}, respectively.

Additionally, the COMSOL multiphysics software (AC/DC and bioheat transfer modules) was used to simulate the temperature rise of G_4@IONPs at the same frequencies; the results showed a good agreement between the measured and simulated temperature-time curves. To assess the capability of magnetic hyperthermia to eradicate breast cancer cells, MC_4L_2 cells incubated with G_4@IONPs were exposed to AMF (300 kHz and12 kA/m) for 2 hours.

The cell survival ratio significantly decreased to 41.7 ± 2.3% after the magnetic hyperthermia treatment due to increased cellular apoptosis and Bax/Bcl-2 ratio. Furthermore, tumor-bearing Bagg albino strain C (BALB/c) mice were exposed to AMF for 20 minutes (three times every other day) after intratumoral G_4@IONPs injection. After the last treatment, tumor size was measured every three days. Histopathological and Immunohistochemical studies were performed on the liver, lung, and tumor tissues in treated and control mice. The histopathological results did not indicate any metastatic cells in the liver and lung tissues in the treated mice, while the control tissues contained metastatic breast cancer cells. Furthermore, magnetic hyperthermia treatment significantly inhibited tumor growth by increasing cancer cell apoptosis as well as reducing the tumor angiogenesis.

Finally, to study the capability of G_4@IONPs to enhance the contrast in magnetic resonance imaging (MRI), relaxometry experiments were performed with different concentrations of G_4@IONPs in T_1 and T_2-weighted MR imaging. The results showed that G_4@IONPs could significantly improve transverse relaxivity in MRI. All in all, G_4@IONPs seem to be efficient nanostructures for magnetic hyperthermia treatment of breast cancer and a capable MRI contrast agent for T_2-weighted imaging. Future studies are needed to load the chemotherapy drugs into the dendrimer structure for multidisciplinary cancer theranostic approaches.

References to author publications that relate specifically to the dissertation:

1. **Salimi, Marzieh**, et al. "Biodistribution, pharmacokinetics, and toxicity of dendrimer-coated iron oxide nanoparticles in BALB/c mice." *International Journal of Nanomedicine* 13 (2018): 1483-1493. DOI: 10.2147/IJN.S157293

2. **Salimi, Marzieh**, et al. "Magnetic hyperthermia of breast cancer cells and MRI relaxometry with dendrimer-coated iron-oxide nanoparticles." *Cancer Nanotechnology* 9.1 (2018): 1-19. DOI: 10.1186/s12645-018-0042-8

3. **Salimi, Marzieh**, et al. "Treatment of Breast Cancer-Bearing BALB/c Mice with Magnetic Hyperthermia using Dendrimer Functionalized Iron-Oxide Nanoparticles." *Nanomaterials* 10.11 (2020): 2310. DOI: 10.3390/nano10112310

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1. **Salimi, M**; Sarkar, S; Saber, R. “Magnetic hyperthermia and MR imaging using G_4 PAMAM dendrimer coated Fe3O4 nanoparticles.” *34th Annual Meeting of the Society for Thermal Medicine*, Cancun, Mexico 2017.

2. **Salimi, M**; Sarkar, S; Saber, R. “Application of G_4 dendrimer-coated iron oxide nanoparticles in magnetic hyperthermia.” *6th International Conference on Nanotechnology*, Dubai, Emirates 2017.

3. **Salimi, M**; Sarkar, S; Saber, R. “Effect of magnetic fluid hyperthermia with dendrimer coated iron oxide nanoparticles on breast cancer in BALB/c mice.” *12th Iranian Congress of Medical Physics*, Tehran, Iran 2018. Abstract appears in Iranian Journal of Medical Physics. DOI: 10.22038/ijmp.2018.12621