Synthesis and Characterization of PEG-Iron Oxide Core-shell Nanoparticles for Cancer Therapy

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Statement of Purpose: Hyperthermia, the heating of tissue in the 42-46°C range, can induce cellular death on its own or work in conjunction with chemotherapy for improved cancer therapy. In many cases, synergistic effects have been observed in combined thermal and chemotherapies.[1] In this study, core-shell nanocomposites were prepared with the intent of co-delivery of a chemotherapeutic (paclitaxel – PTX) and heat by utilizing the heating effects of magnetic nanoparticles. The core-shell nanoparticles were prepared using atomic transfer radical polymerization (ATRP) to coat iron oxide (Fe_3O_4) nanoparticles with a poly(ethylene glycol) (PEG) based polymer shell. Thermal therapy application feasibility was demonstrated *in vitro* with a thermoablation (50°C+) study on A549 lung carcinoma cells. Combinational therapy of PTX and hyperthermia on A549 was investigated to demonstrate a synergistic effect.

Methods: Citric acid coated Fe_3O_4 nanoparticles were synthesized using a one-pot co-precipitation technique. $FeCl_2{\cdot}4H_2O$ and $FeCl_3{\cdot}6H_2O$ were combined in a 1:2 molar ratio followed by the dropwise addition of NH_4OH and 2M citric acid. The polymer coating was synthesized using a surface initiated polymerization (i.e., ATRP). Through a ligand exchange, the citric acid coating was replaced with a silane initiator, 3-bromopropyltrimethoxysilane (BPTS). BPTS initiator coated particles and poly(ethylene glycol) 400 dimethacrylate (PEG400DMA) were combined with 2,2 bipyridine and copper (I) bromide in ethanol and reacted for 24 hours. The particles were characterized using Fourier transform infrared (FTIR) spectroscopy, to verify surface functionalization; thermal gravimetric analysis (TGA), to quantify mass percent of coating; dynamic light scattering, to determine particle size; and transmission electron microscopy, to image particles. Cell studies were carried out using A549 lung carcinoma cells obtained from ATCC. Dry nanoparticles were UV sterilized and diluted with media for direct exposure. Thermal therapy studies were carried out on citrate coated and PEG400DMA coated nanoparticles at 10 and 15 mg/ml concentration. Systems were exposed to an alternating magnetic field (AMF) for 10 minute at 31.5 kA/m and 289kHz. Initial dual therapy studies investigated combinations of 50μM PTX and 10 mg/ml citrate coated iron oxide. Systems were exposed to the AMF for 30 minutes at varying field intensity to maintain a hyperthermia (44±2°C) temperature range. Live/Dead Viability Assay (Molecular Probes) was used to assess toxicity.

Results: FTIR measurements verified the PEG coating by observing peaks at 1715cm^{-1} and 1105cm^{-1} which represent the carbonyl group (C=O) and ether group (C-O-C) respectively. TGA indicated different mass loss profiles and slight differences in overall mass loss between the core citrate coated particles and the polymer coated particles. The results of a thermal therapy study using 15 mg/ml PEG400DMA coated nanoparticles can be seen below in figures 1 and 2. Control indicates fresh media and F indicates 10 minute field exposure. Figure 1 displays the cell viability showing there is no toxic effect from the AMF coil (Control F) or particles (15 mg/ml NF) in solution over that time span. When the particles were exposed to the field (15 mg/ml F) there was near complete death at the center of the well due to heating. Representative images can be seen in figure 2 where live cells are stained green and dead cells stained red. Similar results were observed in the citrate coated particle studies. Initial work with the dual therapy studies have proven inconclusive. A thermal toxic effect was observed in the systems with particles despite lowered field strength and PTX stunted cell proliferation at the concentration chosen. The procedure will be altered lowering the concentrations of both PTX and magnetic nanoparticles.

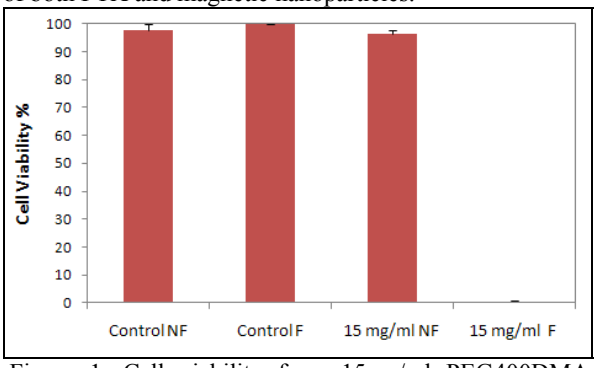

Figure 1. Cell viability from 15mg/ml PEG400DMA coated particle study. NF/F indicates no/field exposure.

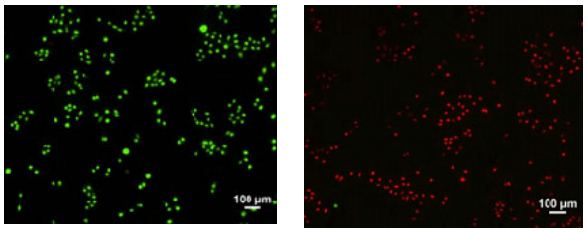

Figure 2. Live-dead images from the control and PEG400DMA coated particles with field.

Conclusions: ATRP was successfully utilized to coat iron oxide nanoparticles with a PEG based polymer shell. For the time frame of the thermal therapy experiments, there is a minimal toxic effect observed in A549 cells for the 15 mg/ml concentration of PEG-coated Fe_3O_4 nanoparticles. Thermoablation of A549 demonstrates the potential use of polymer coated particles for thermal therapy. Future work involves the refinement of the dual therapy study to demonstrate a synergistic effect of a co-delivery of paclitaxel and hyperthermia from magnetic nanoparticles.

References:

[1]Issels RD. Eur J Cancer. 2008:44:2546-2554.