Synthesis and Characterization of CREKA-conjugated Iron Oxide Nanoparticles for Hyperthermia Applications

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Statement of Purpose: One of the current challenges in the delivery of systemic nanoparticle systems in cancer therapy applications is the lack of effective tumor penetration. In this research, a nanoparticle system composed of an iron oxide core with a crosslinked dextran coating functionalized with the tumor homing peptide, CREKA, has been developed to overcome this limitation by homing to tumor tissue. CREKA binds to fibrinogen complexes within the tumor extracellular matrix, making the peptide applicable for targeting various tumor types[1]. The iron oxide core allows for particle heating upon exposure to an alternating magnetic field (AMF) while the dextran coating stabilizes the particles in suspension and decreases the cytotoxicity. The overall goal of this study was to develop and optimize these systems for effective magnetically mediated hyperthermia treatments (i.e., the heating of tissue between 42 and 45°C using magnetic nanoparticles)[2]. Magnetically mediated hyperthermia provides the opportunity for localized heating, and it is hypothesized that this specific particle system can enhance particle accumulation at the tumor site, providing a high enough concentration to induce localized hyperthermia conditions upon exposure to an AMF.

Methods: Dextran coated iron oxide nanoparticles (IONPs) were synthesized using a one-pot co-precipitation method. $FeCl_2 \cdot 4H_2O$ and $FeCl_3 \cdot 6H_2O$ were combined in a 1:2 molar ratio in the presence of dextran, followed by the drop-wise addition of NH_4OH. The IONP coating was then crosslinked with epichlorohydrin to stabilize the dextran. Primary amines were then functionalized to the surface to provide the seeds for further functionalization with the CREKA peptide through a 4A AMAS linker. The nanoparticles were characterized using dynamic light scattering (DLS) to determine the hydrated diameter, transmission electron microscopy to image the core iron oxide crystals, UV-visible spectroscopy to examine the stability of the IONPs in cell media, and thermogravimetric analysis (TGA) to determine the mass percent of the dextran coating. Heating profiles of the IONPs in the presence on an AMF were completed to define the specific absorption rate (SAR) of the nanoparticles. To evaluate the binding affinity of CREKA-conjugated IONPs to fibrinogen complexes, fibrinogen clots were synthesized and exposed to CREKA-conjugated IONPs, CREKA alone, or a fluorescently tagged FITC-conjugated IONP as a control. After one hour, the clots were washed to remove any unbound nanoparticles. A bound-to-free ratio was taken to compare the binding affinity of the particle systems and free CREKA. *In vitro* cell studies were completed on A549 lung carcinoma in order to evaluate the cytocompatibility of the IONPs. Magnetically mediated hyperthermia (MMH) studies were also completed on A549 lung cancer cells. A549 cells were treated for 30 minutes with MMH in combination with 100μM cisplatin (CDDP). Controls for this study included no treatment, cisplatin only, IONPs only, MMH without cisplatin, and cisplatin and IONPs. After treatment, the cells were washed, reseeded into 48 well plates and then analyzed using a calcein AM live stain 3 days post treatment.

Results: The particles were stable in PBS and media over at least twelve hours, had a hydrated diameter of 50 nm as determined by DLS, and generated enough heat to raise solution temperatures well into the hyperthermia range when exposed to an AMF. The cytocompatibility of the IONPs was analyzed through cytotoxicity studies on A549 lung cancer cells. These studies were completed for low particle concentrations with high exposure time, and the results determined that the particles have low cytotoxicity over 48 hours. Fibrinogen clots were used to determine the binding affinity of CREKA-conjugated IONPs. The bound-to-free ratio CREKA-conjugated IONPs was found to be 0.62 ± 0.03 while the control IONP with a fluorescent tag had a significantly lower bound-to-free ratio of 0.42 ± 0.04. The combined MMH and CDDP study shows that a greater toxic effect is seen 72 hours post treatment when CDDP is combined with MMH than either treatment alone.

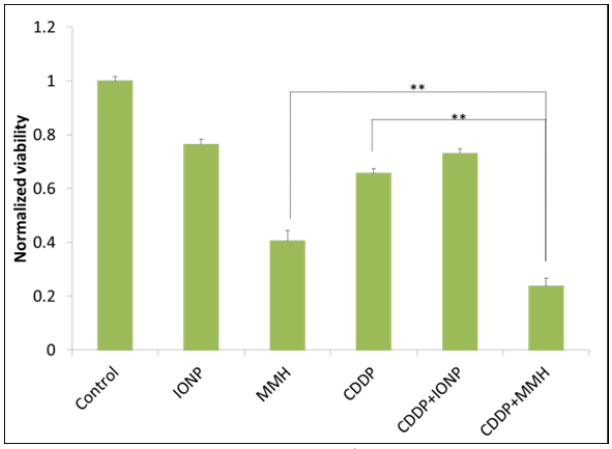

Figure 1. Normalized viability of A549 lung cancer cells 72 hours post treatment. Error bars represent standard error (n=3) and ** indicates a significant difference ($p<0.01$) by a 1-way ANOVA test.

Conclusions: CREKA-conjugated IONPs were successfully synthesized and characterized with optimal properties for tumor localization and magnetically mediated hyperthermia applications. CREKA-conjugated IONPs bind to fibrinogen complexes with a greater affinity than unconjugated IONPs. This novel nanoparticle system also has the ability to generate heat upon exposure to an AMF, which can be used to induce hyperthermia. This MMH can be used in combination with cisplatin to enhance its efficacy.

References:

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