Preliminary Biocompatibility Assessment of Stimuli-responsive Magnetic Nanoparticle-loaded Chitosan Microbeads

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Statement of Purpose: Local drug delivery systems are particularly useful for delivering antibiotics, growth factors, or other compounds directly to affected tissue. Many local drug delivery systems are limited in their ability to release drugs consistently over a long period of time. Current research is exploring the application of stimuli-responsive "smart" biomaterials, to release active concentrations of drugs at clinician-determined intervals, on-demand. Magnetic nanoparticles provide a stimulus response to magnetic energy that could enable this "smart" biomaterial response. Before further research is invested in the drug loading potential of biomaterials containing these magnetic nanoparticles, biocompatibility should be evaluated first. The objective of this study is to investigate the effects of magnetic nanoparticles and nanoparticle-loaded chitosan microbeads on cell viability.

Methods: Monodisperse MNPs of iron oxide (Fe_3O_4) were formed by reacting Iron(II) chloride ($FeCl_2$) with Iron(III) chloride ($FeCl_3$) at a molar ratio of 0.5 dissolved in hydrochloric acid (HCl) by dropping into a basic solution of sodium hydroxide (NaOH) between pH 11 and 12.[1] MNPs were washed with HCl and deionized water several times.

Chitosan microbeads were formulated using a water-in-oil emulsion technique. Liquid paraffin in a 50:50 mix of heavy and light chains was mixed with 0.3% Span 80 and stirred with an impeller stirrer. Chitosan solutions in acetic acid at 2 weight% were added dropwise from a syringe and blunt 21 gauge needles using a syringe pump. For incorporation of MNPs, Fe_3O_4 particles were suspended in acetic acid at 50 weight% prior to the addition of chitosan and dispersed in an ultrasonicator for 2 hours. Emulsions were stirred with an impeller for 5-15 minutes and then a 1mM genipin solution was added to crosslink microspheres. Cross-linked beads were recovered by centrifugation and dried overnight. Chitosan microbeads and iron oxide nanoparticles were sterilized by low-dose gamma irradiation.

NIH3T3 fibroblast cells were seeded at $1x10^4$ cells/cm^2 in 96 well plates in DMEM media with 10% Fetal Bovine Serum (FBS) and antibiotics (100 U/mL penicillin,100 μg/mL streptomycin and 0.25 μg/mL amphotericin B). Iron oxide nanoparticles were suspended in media by ultrasonication and added to cultures at concentrations ranging from 5 mg/ml to 0.02 mg/ml. Chitosan microbeads were suspended in solutions at concentrations ranging from 20 mg/ml to 1.25 mg/ml. Cultures at 1 and 3 days after addition of nanoparticles or microbeads were imaged under a microscope and then cells were lysed with 25 mM Tris and 0.5% Triton X-100 and stored at -80°C until analysis. Viability was assessed by comparing DNA quantity determined using Quant-it™ PicoGreen (Invitrogen) to controls.

Results: The nanoparticles tended to aggregate in solution, but were dispersed easily by ultrasonication.

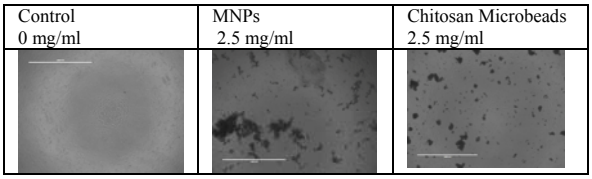

Figure 1. Photomicrographs of NIH3T3 cultures 1 day after addition of MNPs and chitosan microbeads

Chitosan microbeads were very difficult to evenly distribute in the solution and to make precise dilutions. Both types of nanoparticles had clusters that were large enough to clog the pipette tip during transfer of the solution. In cultures, particles tended to aggregate around cell clusters (Figure 1). Concentrations of iron oxide particles up to 0.31 mg/ml supported viability and proliferation of fibroblast cells (Figure 2A.). Unloaded chitosan microbeads supported proliferation at all concentrations studies, but proliferation was inhibited by increasing concentrations of chitosan microbeads containing MNPs (Figure 2B).

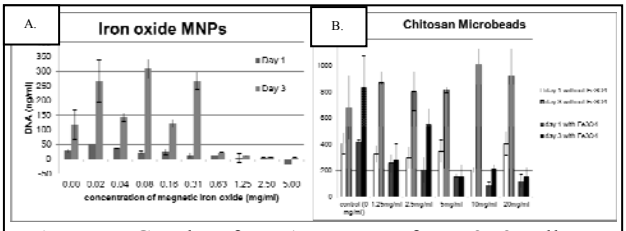

Figure 2. Graphs of DNA content of NIH3T3 cells seeded wells 1 day and 3 days after exposure to various concentrations of A.) Iron oxide MNPs and B.) chitosan microbeads loaded with 50 wt % MNPs.

Conclusions: In conclusion, this preliminary study shows that there are biocompatible ranges of MNPs, chitosan microbeads, and MNP-loaded chitosan microbeads. Loading percent of MNPs in chitosan beads may be lowered from 50% to 40% and 20% to improve biocompatibility. Further, lower concentrations of chitosan microbeads should also be evaluated. Longer sonication times for chitosan microbead solutions may improve their suspension in cell culture media and improve viability of cells exposed to microbeads. Future biocompatibility studies will include microbeads loaded with lower weight percent MNPs and a wider concentration range of chitosan microbeads. In vivo evaluations of biocompatibility are also needed prior to clinical use of these stimuli-responsive biomaterials containing nanomaterials.

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References:

1. Kang YS et al. Chemistry of Materials 1998; 10(6):1733-173