# Manipulation of Iron Nanoparticles and Their Effects on Human Colon Carcinoma Cells

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## Abstract

**Magnetic nanoparticles (MNPs) were synthesized to evaluate the influence of various polymer coatings on particle size, zeta potential, and cellular uptake. Stock solutions of gum arabic (GA), chitosan, and polyethylenimine (PEI) at 2% by weight were added to the MNP solutions. The particle size was obtained with the vibrating sample magnetometer and Malvern Zetasizer. The coated particles were on average approximately 100 nm. The zeta potential of the particles was acquired with the Malvern Zetasizer. Results revealed a negative surface charge for each of the coatings at low concentrations. Increased amounts of PEI demonstrated the ability to alter the surface charge from negative to positive. The live/dead (LD) assay showed that PEI coated particles demonstrated toxic effects with a LD 50 ≈ 100 $\mu$g iron/millions cells. In contrast, GA and chitosan-coated MNPs were the least toxic to the human colon carcinoma cells (HCT-166) with a LD 50 > 1000 $\mu$g iron/millions cells. Being non-toxic is important because it relates the biocompatibility of the nanoparticles with the body system.**

## Introduction

Colon cancer is one of the most prevalent and fatal types of carcinoma disease [1]. There are several studies dedicated to optimizing cancer therapy through the use of nanotechnology applications [2]. Magnetic field hyperthermia (MFH) makes use of MNPs, such as magnetite. When coated in glycomolecules, like GA and chitosan, a crab shell derivative, it is thought that MNPs are more likely to be taken up by cancer cells than by healthy cells because cancer cells require more energy to proliferate. Also, because cancer cells are negatively charged, it's interesting to note whether surface charge affects particle uptake across the cell membrane's phospholipid bilayer. With an accumulation of MNPs in or around cancerous cells, an alternating magnetic field (AMF) causes hysteresis heating. Hysteresis heating around 42º-45ºC leads to the apoptosis of cancerous cells while leaving healthy cells unharmed [3].

However, MFH is most effective if the MNPs are at an optimal size, concentration, adhesiveness, and strength. The goals of this experiment were to manipulate the physical and chemical surface properties of magnetic nanoparticles (MNPs) by coating them with polymer and to evaluate how these polymers influence surface charge. Additionally, we sought to determine the cell viability when exposed to polymer-coated MNPs.

## Methods/Materials

The MNPs were synthesized via wet chemistry techniques. A 2:1 molar solution of iron (III) chloride and ferrous chloride was added to 20 mL of deionized water. An alkaline solution was prepared by adding 15 mL of reagent $NH_4OH$ to 65 mL of deionized water. The latter solution was stirred for approximately 5 minutes on a magnetic stir plate. The $Fe^{2+}$, $Fe^{3+}$ solution was added drop-wise to the alkaline solution. It is believed that pouring the iron solution into the alkaline solution would result in smaller particles; however a slower reaction rate correlates with more uniform particles. The MNP solution was stirred for 1 hour and washed via centrifugation. The MNPs were redispersed with deionized water via ultrasonication. This process was repeated about six times or until the pH of the suspension was comparable to that of the deionized water. The polymer-coated MNPs were synthesized via the same method with the addition of 2% polymer solution after ultrasonication.

Overall, four types of MNPs were used: naked, chitosan-coated, GA-coated, and PEI-coated. The Malvern Zetasizer was used to measure size and zeta potential. Size measurements were verified via magnetic susceptibility analysis. A live/dead cell assay was performed with each particle type at concentrations from 31-8000 $\mu$g iron/million cells.

## Results and Discussion

Size analysis via vibrating sample magnetometer and dynamic light scattering (Malvern Zetasizer) showed that the naked particles were 3.84-60.4 nm. The smaller sizes represent primary particles, and the larger sizes represent agglomerates. The coated particles were about 100 nm. Zeta potential results, which correlate with electrophoretic mobility, showed that the nutrient medium for cells (DMEM) in which the particles were suspended for testing rendered all the particles negatively charged. PEI had

the strongest negative charge at -6.9 mV. It was interesting to note that PEI and chitosan-coated MNP's exhibited a negative charge in DMEM even though PEI and chitosan are cationic polymers. The live/dead cell assay showed that the LD 50 results for GA, chitosan, and PEI were 6700, 1450, and 106 $\mu$g iron/ millions cells, respectively (see Figure 1).

The particle size retrieved via the two size analysis techniques gave size measurements which were consistent with predicted and literature results. The MNPs were tested in DMEM solution to more accurately simulate the body system. In this solution, all the coatings, even those which are typically positively charged, rendered a negative surface charge. The PEI coated MNPs were very toxic to the cells at higher concentrations, which could cause rejection if used in the human body. Although chitosan particles were promising in relation to their biocompatibility, GA best reflected the live/dead cell assay results of particles with no coatings.

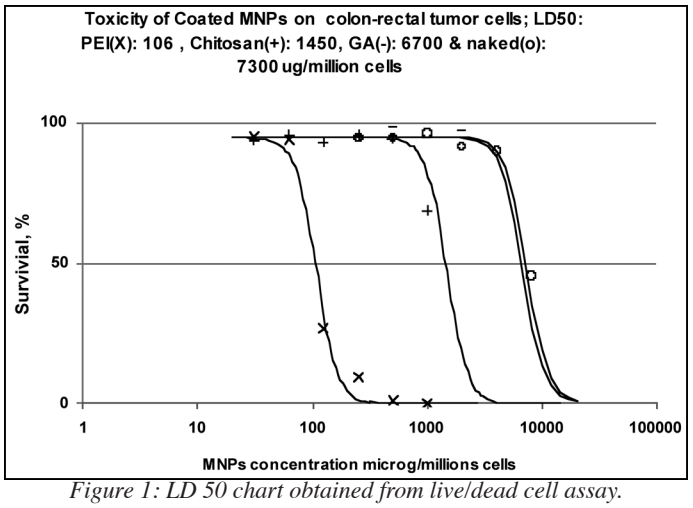

*Figure 1: LD 50 chart obtained from live/dead cell assay.*

## Summary

Polymer-coated MNPs imparted an overall negative charge. The live/dead cell assay showed that overall GA was the least toxic to the colon carcinoma cells meaning that it would be the most biocompatible. Further studies need to be done to identify and optimize surface coatings that could render a positive surface charge. Additionally, particle uptake of the coated MNPs needs to be observed via TEM imaging.

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## References

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