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# In Vitro Analysis on The Cytotoxicity of Super paramagnetic Iron Oxide Nano particle (SPION) Mediated Anticancer Drug Delivery System.

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**KEYWORDS :** Super paramagnetic iron oxide nano particles (SPIONs), In vitro cytotoxicity assay, LDH assay, MTT assay.

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## ABSTRACT

*In this study, we present in vitro cytotoxicity of bare and surface modified iron oxide (Fe3O4) nanoparticles) using live cell assays, LDH, and MTT assays with variation of the concentration of nanoparticles and incubation time. The surface of nanoparticles is modified with 3-aminopropyl triethoxy silane to enhance the biocompatibility. While the cytotoxic effect was negligible for 24 h incubation even at highest concentration of 100 µg/ml. Bare nanoparticle represented higher level of toxicity than those of silanised nanoparticles. Current results confirmed the predictions and demonstrated that Magnetic nanoparticle (MNP)-silane system functionalised with silane has potential as new targeting strategy in cancer therapy.*

## INTRODUCTION

Parameters determining biocompatibility and toxicity are the nature of the magnetically responsive component and the final size of the particles including their core and the coatings (shell). Iron oxides both magnetite ($Fe_3O_4$) and maghemite ($\gamma$-$Fe_2O_3$) occur naturally as nano-sized crystals in the earth's crust generated by various environmental sources such as volcanoes and fires, it would seem that there is no intrinsic risk associated with these nanoparticles (NPs). However, the major concern is the increased exposure (via different routes) level to humans and the ecosystem as more and more NPs are being manufactured to meet the demands of the rapidly proliferating field of nanomedicine. The dramatic growth and the therapeutic benefits that SPION have to offer, accompanies the risks and concerns associated with their exposure. Therefore, there is a considerable need to address biocompatibility and biosafety concerns associated with their usage in a variety of applications. Several studies have examined the cytotoxic potential of several different types of SPION with a range of surface coatings and have generally found low or no cytotoxicity associated with these NPs until high exposure levels (>100 µg/ml).(Elias and Tsourkas,2009) The toxicity was also found to be dependent on various factors such as type of surface- coating or its breakdown products, chemical composition of cell-medium, oxidation state of iron in SPION and proteinSPION interaction.(Maynard et al.,2006)

## 1-MATERIALS AND METHODS:

Vero cells were cultured on tissue culture polystyrene falcon flasks in DMEM media supplemented by 10% fetal calf serum and 1% penicillin/streptomycin (1000 units/10,000 µg/ml) at 37°C in 5% $CO_2$ atmosphere. When the cells attained 80% growth, they were trypsinized and counted on hemocytometer. Seeded cells in a 96 well plate at a density of $1.0 \times 10^4$ in 100 µL of culture medium. Cytotoxicity of both bare and surface modified MNPs at different analyte concentrations were assessed by carrying out MTT and LDH assay.

## 2-RESULTS AND DISCUSSION

### Cytotoxicity of the MNPs:

### 2.1. MTT Assay

The MTT cell proliferation assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability. The yellow tetrazolium MTT (3-4,5-dimethyl thiazolyl-2)-2,5-diphenyl tetrazolium bromide) is reduced by metabolically active cells, in part by the action of dehydrogenese enzymes,to generate reducing equivalents such NADH and NADPH. The resulting intracellular purple formazan was solubilised and quantified by taking OD at 570nm using the ELISA plate reader.

**Survivability of the cells with the test compound were determined as follows.**

Cell viability (%) = $(OD_{570nm,test\ sample}) - (OD_{570nm,negative\ control}) / (OD_{570nm,positive\ control}) - (OD_{570nm,negative\ control})$.

The result of MTT assay showed (Fig.1) that after 24 hour exposure to both bare and silane coated MNPs exhibited no significant toxicity to the cells from 12.5-200µg/ml concentration.It was previously reported that iron oxide nanopartcles have no significant toxicity upto 100 µg/ml concentration(Sonvico et al.,2005). The experiment has proven that there is no significant difference in the cell viability of the MNP-Silane treated cells as compared to the cells cells without any treatment with the nanoparticle. Hence these NPs are safe as such and can be used for drug delivery purposes.

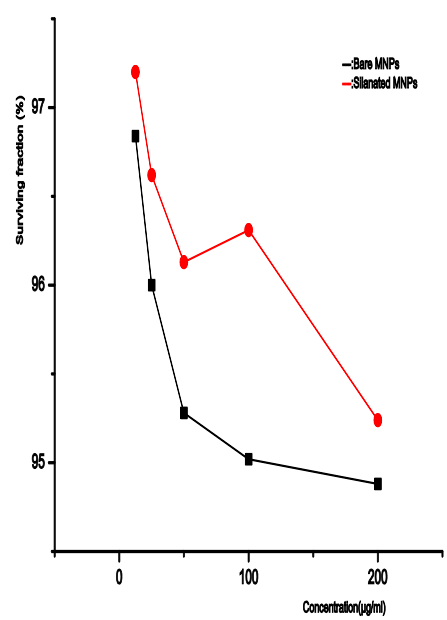


**Fig.1: MTT assay showing viability of Vero cells after 24 hour incubation with bare and surface silanated MNPs.**

**2-2.LDH Assay**

Cell membrane damage caused by nanotoxicity leads to the release of cytoplasmic enzymes, and the measurement of LDH release is a well accepted assay to estimate cell membrane integrity and quantify cell cytotoxicity. Any LDH released into cell culture supernatent will reduce the tetrazolium salt INT to formazan by a coupled enzymatic reaction(Korzeniewski and Callewaert.,1983;Arechabala et al.,1999). The formazan dye formed is water soluble and is quantitatively measured by taking OD at 490nm with an ELISA plate reader.

**Relative cytotoxicity of the test compound were calculated based on the $OD_{490nm}$ valuesas follows:**

Cytotoxicity(%)= $(OD_{490nm,testsample})-(OD_{490nm,negative\ control})$ / $(OD_{490nm,positive\ control})-(OD_{490nm,negative\ control})$

The leakage of LDH upon adding the MNPs were determined in vero cell line using the LDH cytotoxicity assay.The results demonstrated that exposure to MNPs for 24 hours resulted in concentration dependent increase in LDH leakage (Fig.2). Compared to the MTT assay, LDH has proven as more sensitive

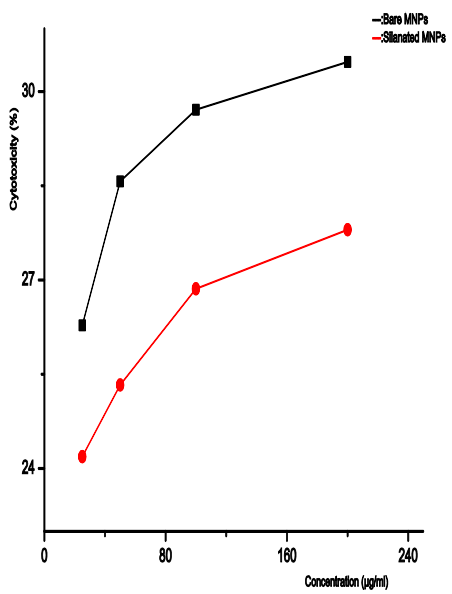


**Fig.2: LDH assay showing the cytotoxicity to Vero cells after 24 hour incubation with bare and surface silanated MNPs.**

Studies comprising metal oxide NPs invitro demonstrated that iron oxide NPs to be safe and non-cytotoxic below 100µg/ml concentration which approves its usage for in vivo application. Also superparamagnetic iron oxide nanoparticles are considered to be biodegradable with iron being reused/recycled by cells using normal biochemical pathways for iron metabolism(Bulte and Kraitchman.,2004).

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