International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2014): 5.611

Magnetic Nanoparticles Synthesized with Different Precursor Stoichiometry Induced Differential Toxicity in Multiple Cell Lines

Koyeli Girigoswami[1], Sanjay K. Metkar[2]

Faculty of Allied Health Sciences, Chettinad Hospital and Research Institute, Chettinad Academy of Research & Education (CARE), Kelambakkam, Chennai 603 103, India

Running Title: Toxicity of magnetic nanoparticles in different cell lines.

Abstract: ***Superparamagnetic nanoparticles are extensively used in biomedical applications, targeted drug delivery, magnetic imaging contrast agents etc. Along with its potent application it becomes necessary to exploit the biological implications of these profoundly used iron oxide nanoparticles. Iron oxide nanoparticles can have toxic effect depending on its size and cellular uptake at various extents on different types of cell lines. It is in critical need to scrutinize the possible toxicity of different magnetic nanoparticles at different types of cell lines. In the present study we have compared the toxicity of two types of iron oxide nanoparticles synthesized by using the precursors at different stoichiometry. The characterization of the as synthesized nanoparticles was done by using UV visible spectrophotometry, DLS and FTIR. The toxicity was assayed for cell lines like – 3T3, PC12 and MCF 7 cells. The results revealed that the two types of iron oxide nanoparticles exert differential toxicity in these cell lines. The outcome of this study may enlighten the safer mode and dose of application of such nanoparticles for various purposes in future.***

Keywords: iron oxide nanoparticles; 3T3; MCF 7; PC12; nanoparticle toxicity

1. Introduction

Nanoparticles have versatile applications exploiting their ultra-small size, large surface-area-to-volume ratio along with high reactivity that differ from those of bulk materials with the same composition (1). In biomedical applications high potentials have been found for super paramagnetic nanoparticles, because of their unique capabilities of magnetic interactions over space and physical barriers (2-4). The paramagnetic properties of such nanoparticles direct them to a assigned location or heated in the presence of an AC magnetic field applied externally (5-6). The unique property of magnetic nanoparticles (MNPs) has enabled them for applications as *in vitro* and *in vivo* medical diagnostic tools. Recently, different sizes of magnetic nanoparticles with polymeric coatings have been exploited as contrast agents in magnetic resonance imaging (MRI), drug delivery and therapy (7-9). These MNPs used for biomedical applications must have optimized size and functionalized surface for different applications (10, 11). However, careful toxicity assessments are essential to ensure the bio safety and biocompatibility of MNPs, as different types of MNPs exert differential responses on various cell lines. Magnetic nanoparticles have been found to have potent toxicity besides its biomedical applications (12, 13). Polyvethyleneoxide (PEO) triblock coated magnetic nanoparticles were also found to be toxic in cell lines which are further related inversely to its tail length (14). The toxicity of different coatings on iron oxide nanoparticles on various cell lines have also been established (15). Thus, it can be seen that iron oxide nanoparticles in both bare and coated form are toxic to cell lines.

In the present study we have synthesized magnetic nanoparticle by using different ratio of Fe^{3+} to Fe^{2+} ions and exploited their toxicity on different cell lines. The magnetic nanoparticles were synthesized and characterized by using UV visible spectrophotometer, dynamic light scattering (DLS) and FTIR spectroscopy. The toxicity of these nanoparticles was assayed by MTT assay on different types of cell lines like, PC12, MCF7 and 3T3 cells. Our results can give insight into the properties of these nanoparticles towards the different types of cells.

2. Materials and Methods

2.1 Materials

$FeCl_3.6H_2O$, $FeCl_2.4H_2O$, NH_4OH were purchased locally. MTT assay kit (EZ Count MTT Assay kit) DMEM, FBS, antibiotics, trypsin were purchased from Himedia.

2.2 Synthesis of Magnetic Nanoparticles

The magnetic nanoparticles were synthesized according to Hariani et. al. (16). Briefly we dissolve $FeCl_3.6H_2O$ and $FeCl_2.4H_2O$ in 30 ml H_2O (in the ratio given below) and stir it with magnetic stirrer for 10 min. 2 ml of 5 M NH_4OH was added drop wise for 1 min with continuous stirring for 30 min at room temperature. The solution was centrifuged and the pellet was dried at 80 °C for 3 h after washing 3 times with distilled water. The pellet was crushed with mortar pestle and stored in 2.0 ml tubes for characterization.

Name of MNP	Fe^{3+}	Fe^{2+}	Fe	Fe^{3+} : Fe^{2+}
B.	$FeCl_3.6H_2O$	$FeCl_2.4H_2O$	0.1 M	2:1
C.	$FeCl_3.6H_2O$	$FeCl_2.4H_2O$	0.1 M	1:1

2.3 Characterization of the synthesized MNPs

The magnetic nanoparticles synthesized were visualized by exploring the magnetic attraction property using a magnet. The UV visible spectra were recorded by dissolving the MNP B and MNP C in distilled water, sonicating at bath sonicator for 20 min, respectively and recording the UV visible spectra using (Shimadzu (Japan) UV-1800). The hydrodynamic diameter of the synthesized MNP B and MNP C were measured using particle size analyzer (Malvern Nano ZS90) respectively. The IR spectrum of MNP B and MNP C were recorded by IR spectroscopy (Bruker, Alpha –T FTIR). The MNP B and MNP C were used to make KBr pellet and their IR spectra was measured taking the one for only KBr pellet as background (17).

2.4 Cell Culture

The cell lines PC12, MCF 7 and 3T3 were maintained in DMEM with 10 % FBS and 1 % antibiotics at 37 °C CO_2 enriched incubator as done by Girigoswami et al (18). The cells were maintained in exponential phase and subcultured three times a week to keep them in the logarithmic phase of their growth.

2.5 MTT Assay

EZcount MTT Cell Assay Kit from Himedia is used for the assay as done by Girigoswami et.al (18). The cell lines PC12, MCF 7 and 3T3 were cultured in 24 well plate at 10^4 cells/ well and allowed to adhere for 24 h. After 24 h the cells were treated with MNP B and MNP C respectively at different concentrations (50 µg/ml, 100 µg/ml, 500 µg/ml, 100 µg/ml). For control cells 3 wells were kept untreated. 48 h post treatments MTT assay was done by adding MTT reagent (5 mg/ml) to a final concentration of 10 % of total volume and replaced in the incubator. The plates were incubated for 4 h for the formation of blue coloured Formazan. After 4 h 100 µl of solubilization solution (supplied in the kit) was added to each well and the formazan crystals were completely dissolved by gentle stirring in a gyratory shaker with gentle pipetting. The absorbance was measured at 570 nm using a spectrophotometer. The same experiment is repeated without the cells for the standardization of MNP B and MNP C respectively at appropriate concentrations (50 µg/ml, 100 µg/ml, 500 µg/ml, 1000 µg/ml). The corresponding absorbance values at 570 nm were subtracted from the values of the samples and recorded as $(O.D.)_{test}$ to calculate the percent cell viability. The absorbance of untreated control cells was taken as $(O.D.)_{control}$. The percent cell viability is calculated using the formula;

$$\% \text{ cell viability} = (O.D)_{test} / (O.D.)_{control} \times 100$$

3. Results and Discussion

3.1: The synthesized nanoparticles MNP B (Fe^{3+}: Fe^{2+} = 2:1) and MNP C (Fe^{3+}: Fe^{2+} = 1:1) were visualized for the magnetic properties using a magnet (figure 1). The figure clearly shows that both the nanopartilces (MNP B and MNP C) have strong magnetic properties.

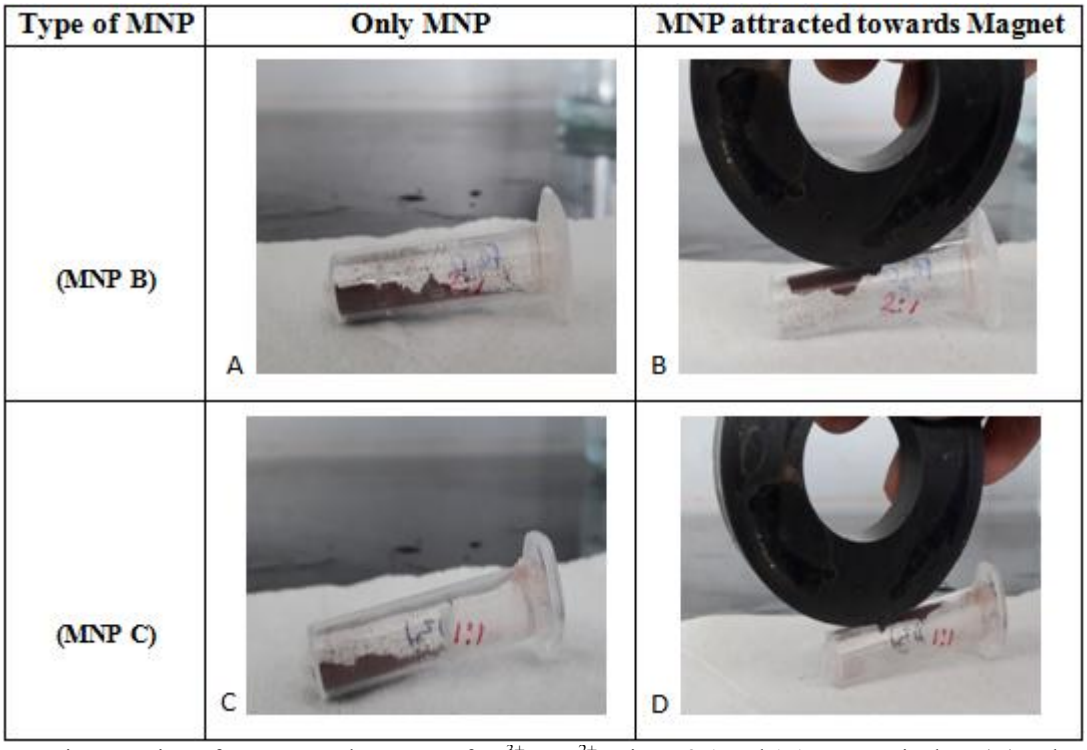

Figure 1: Magnetic attraction of MNP B and MNP C of $Fe^{3+}$to Fe^{2+} ratio as 2:1 and 1:1 respectively. (A) only MNP B; (B) MNP B attracted by magnet; (C) only MNP C; (D) MNP C attracted by magnet

3.2: The hydrodynamic diameter was measure by dynamic light scattering using particle size analyzer (figure 3). For MNP B and MNP C the hydrodynamic diameter was found to be 309 nm and 346 nm respectively. Thus the particle size is found to be within the nanoparticle dimensions for both MNP B and MNP C.

3.3: The absorbance spectrum of MNP B and MNP C is shown in figure 2. It is visible that both the nanoparticles absorb UV light characteristic of iron oxide nanoparticles.

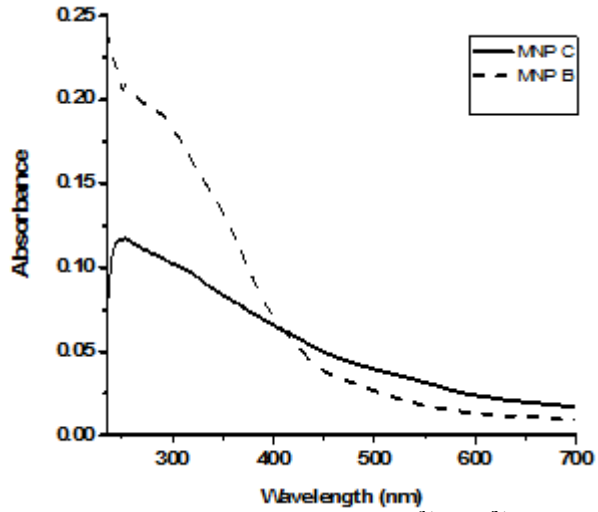

Figure 2: UV-visible absorbance of MNP B and MNP C of $Fe^{3+}$to Fe^{2+} ratio as 2:1 and 1:1 respectively

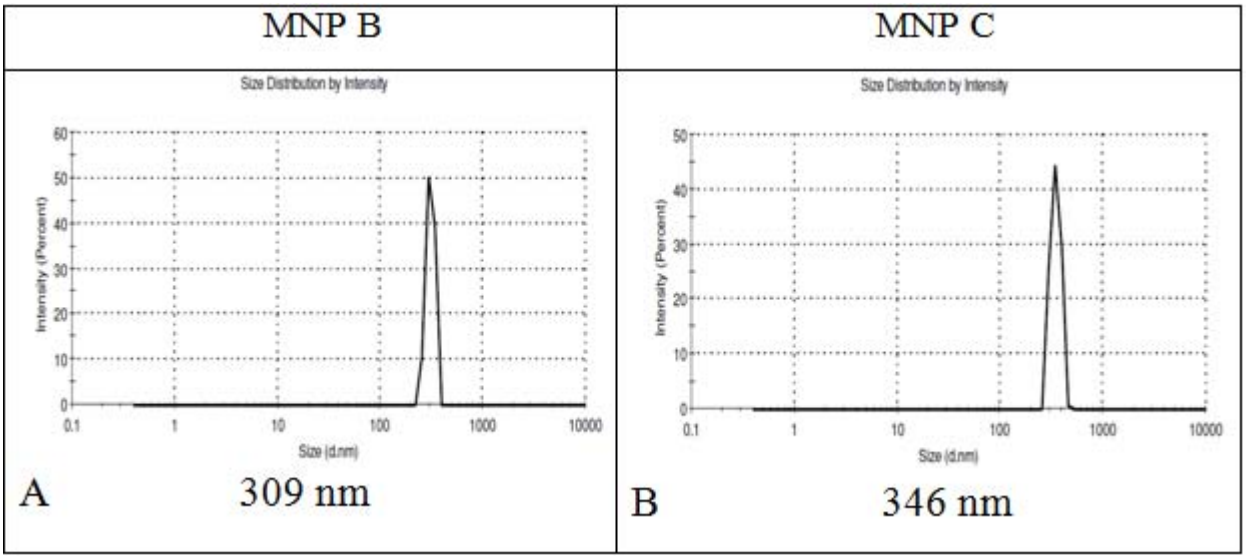

Figure 3: Hydrodynamic diameters of MNP B and MNP C respectively

3.4: The FTIR spectrum of MNP B and MNP C is shown in figure 4 and figure 5 respectively. Fe_3O_4 shows strong IR absorption in the region of 500–600 cm^{-1} due to the presence of iron oxide skeleton for both MNP B and MNP C respectively. The characteristic band at 576 cm^{-1} for MNPB and 578 cm^{-1} for MNP C respectively corresponds to Fe - O bonds in the IR spectra of Fe_3O_4 (19). The peaks at 1021cm^{-1} and 1023 cm^{-1} for MNP B and MNP C respectively corresponds to Lepidocrocite peaks of iron oxide. The peaks at 1629cm^{-1} and 1636 cm^{-1} for MNP B and MNP C respectively corresponds to the amide I peaks. The peak at 3406 cm^{-1} for MNP B and 3407 cm^{-1} and 3160 cm^{-1} corresponds to the hydroxyl bond stretching. The FTIR spectrum of MNP B and MNP C thus clearly states that they have all the characteristics of iron oxide.

3.5: The cell viability was measured for both MNP B and MNP C in different cell lines at different concentrations. The standardization of MNP B and MNP C for the MTT assay is given in S1. This data gives the background absorbance corresponding to the nanoparticles only (without cells) which is further subtracted from the values obtained with the cells. Figure 6, 7 and 8 shows the cell viability on PC12, MCF7 and 3T3 cells assayed after 48 h upon treatment with different doses (50µg/ml, 100 µg/ml, 500 µg/ml, 1000 µg/ml) of MNP B and MNP C respectively. The results show that MNP B is not having any effect on cell viability for PC 12 and MCF 7 cells whereas it exhibits a decrease in cell viability for 3T3 cells at all the doses exposed. MNP C exhibit toxicity to some extent on PC12 and MCF 7 cells at higher concentrations (1000 µg/ml) whereas it has toxicity towards 3T3 cells at all doses used.

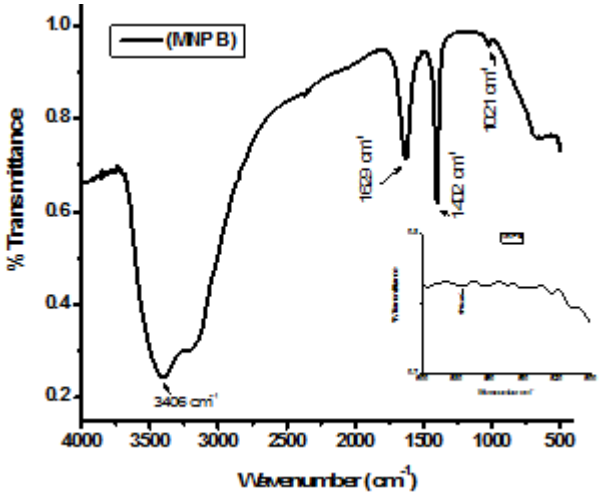

Figure 4: The FTIR spectra of MNP B nanoparticles.

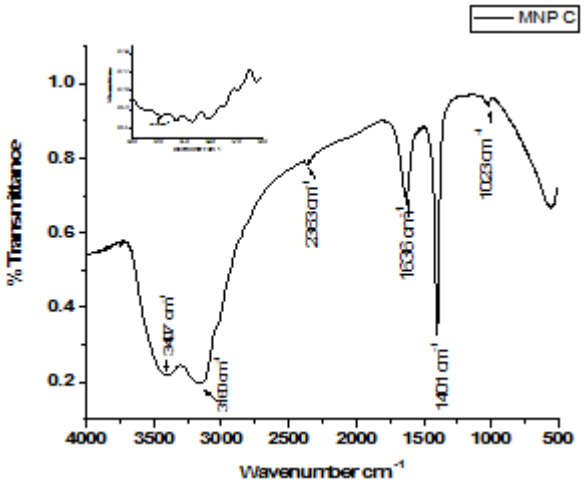

Figure 5: The FTIR spectra of MNP C nanoparticles

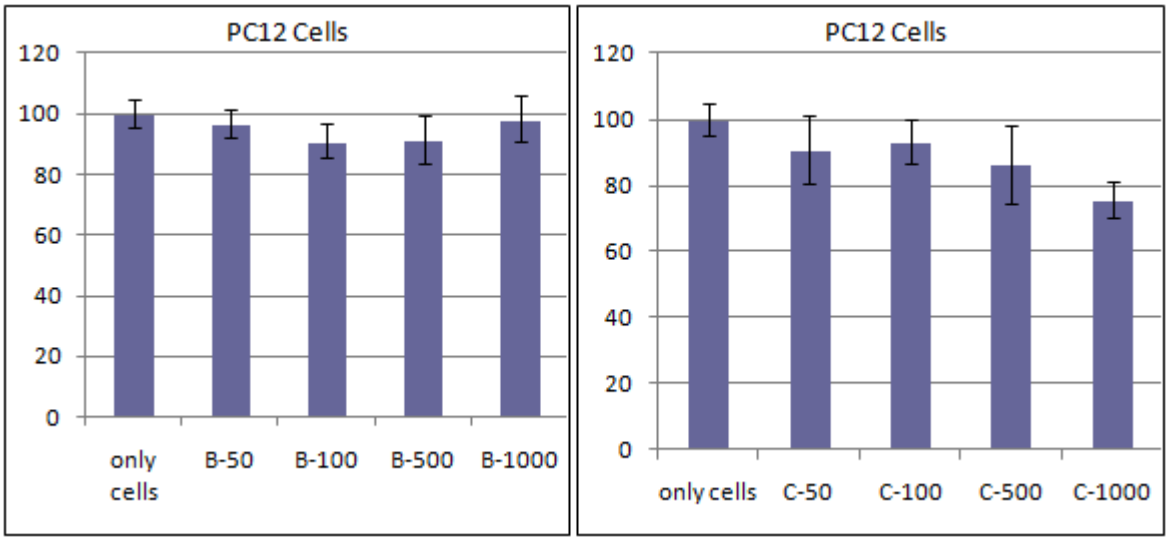

Figure 6: (A) cell viability on PC12 cells assayed after 48 h upon treatment with different doses (50 µg/ml, 100 µg/ml, 500 µg/ml, 1000 µg/ml) of MNP B treatment compared with control cells. (B) cell viability on PC12 cells assayed after 48 h upon treatment with different doses (50 µg/ml, 100 µg/ml, 500 µg/ml, 1000 µg/ml) of MNP C treatment compared with control cells.

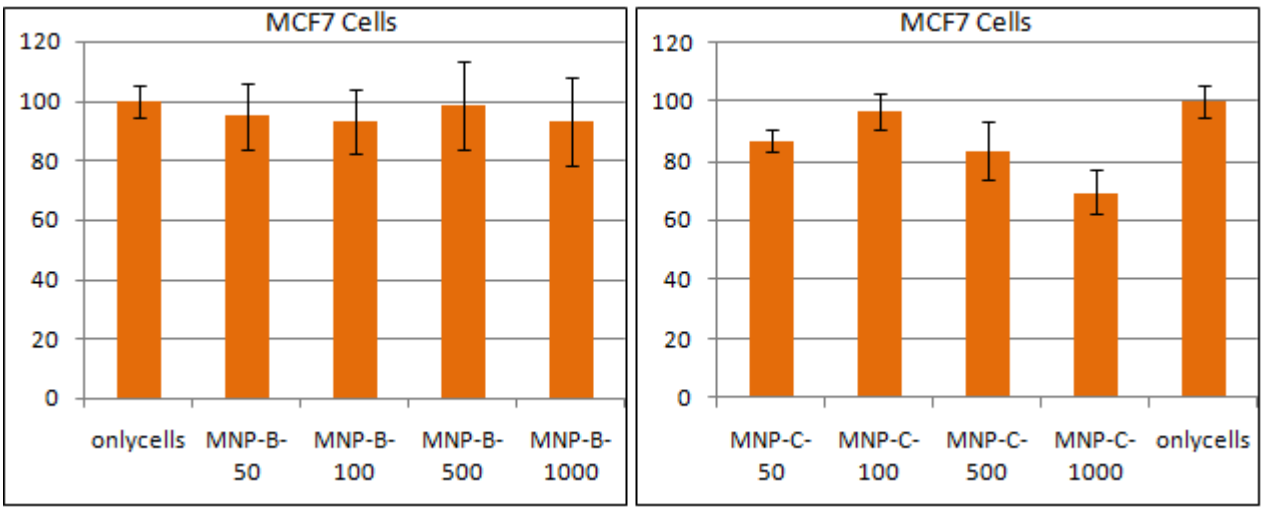

Figure 7: (A) cell viability on MCF 7 cells assayed after 48 h upon treatment with different doses (50 µg/ml, 100 µg/ml, 500 µg/ml, 1000 µg/ml) of MNP B treatment compared with control cells. (B) cell viability on MCF 7 cells assayed after 48 h upon treatment with different doses (50 µg/ml, 100 µg/ml, 500 µg/ml, 1000 µg/ml) of MNP C treatment compared with control cells.

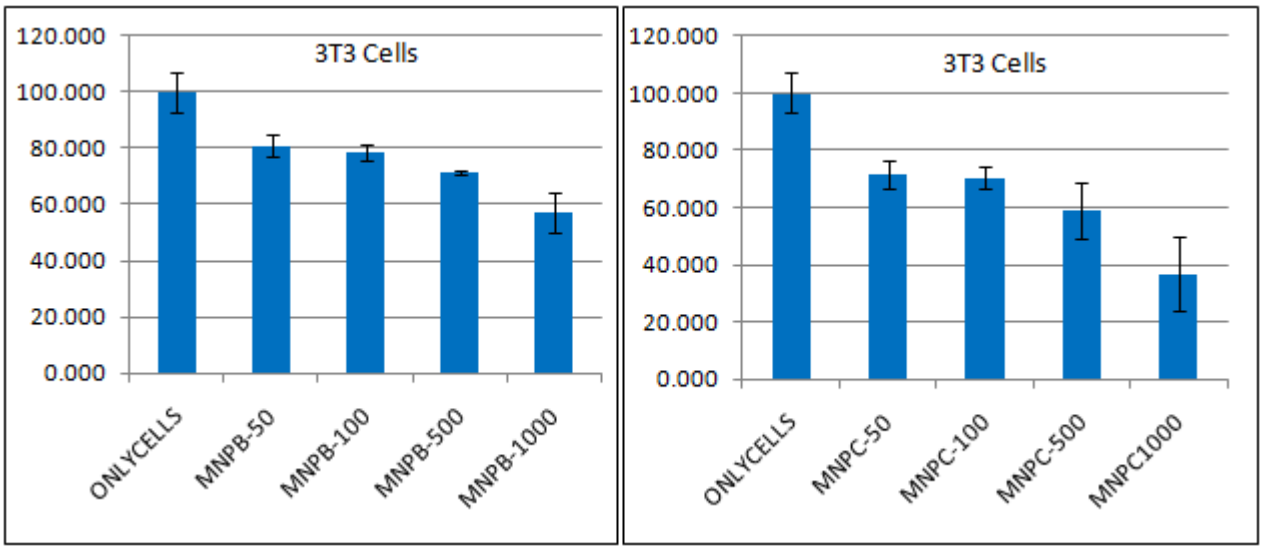

Figure 8: (A) cell viability on 3T3 cells assayed after 48 h upon treatment with different doses (50 µg/ml, 100 µg/ml, 500 µg/ml, 1000 µg/ml) of MNP B treatment compared with control cells. (B) Cell viability on 3T3 cells assayed after 48 h upon treatment with different doses (50 µg/ml, 100 µg/ml, 500 µg/ml, 1000 µg/ml) of MNP C treatment compared with control cells.

4. Conclusion

From the above study it can be concluded that magnetic nanoparticles can be synthesized using different stoichiometry of Fe^{+3} to Fe^{+2} ions. The synthesized nanoparticles exhibit all the properties of superparamagnetic iron oxide nanoparticles and can thus be widely used for different biomedical applications. The cellular toxicity seen also renders the magnetic nanoparticles to be safe for using at lower concentrations for PC12 and MCF7 cells. But our study reveals that 3T3 cells are sensitive to magnetic nanoparticles. Thus, different cell lines exhibits different types of response towards magnetic nanoparticles.

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Author Profile

Koyeli Girigoswami* and **Sanjay K. Metkar** is Faculty of Allied Health Sciences, Chettinad Hospital and Research Institute, Chettinad Academy of Research & Education (CARE), Kelambakkam, Chennai 603 103, India

Figure S1

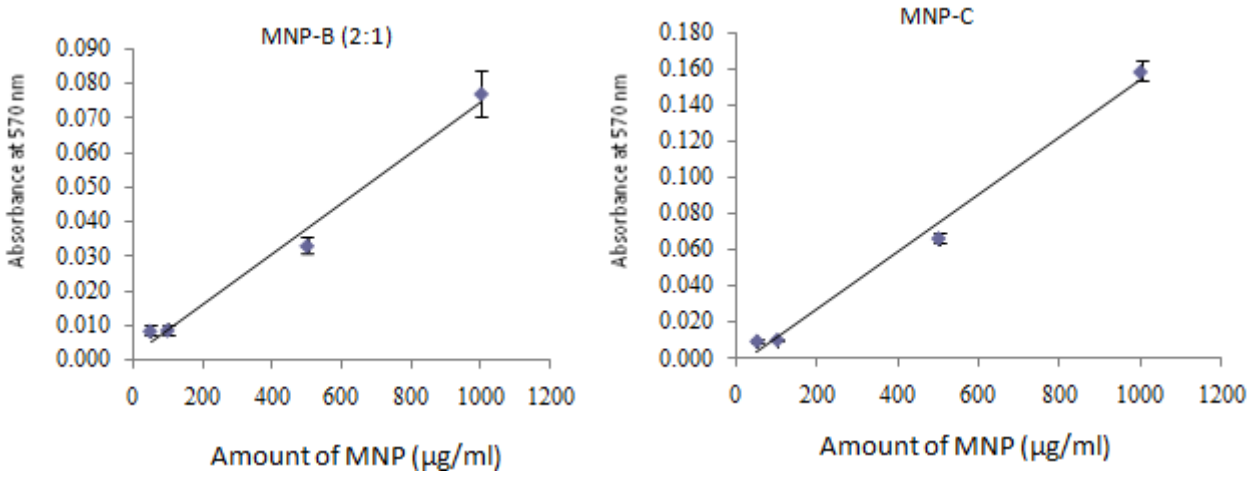

Figure S1: Standardization of the magnetic nanoparticles for MTT Assay. The MNP B and MNP C were added at different concentrations in a 24 well plate with 1 ml DMEM (with 10 % FBS) in each well. The plates were incubated for 48 h at 37 °C and MTT reagent (5 mg/ml) was added at 10 % to the volume of the medium. The plates were incubated for further 4 h at 37 °C and the absorbance at 570 nm was recorded. The experiment was repeated thrice and a standard curve was plotted

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