Biosynthesis of Superparamagnetic Iron Oxide Nanoparticles (SPIONs) and Investigation of Their Cytotoxicity Effects on Human Mesenchymal Stem Cells (hMSCs)

M. Darroudi[1,2], A. Ghafarpour[3], N. Samandarinejad[3], D. Taherzadeh[4], S.S. Tabrizi Hafez moghaddas[5]

[1]Nuclear Medicine Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[2]Department of Modern Sciences and Technologies, Mashhad University of Medical Sciences, Mashhad, Iran
[3]R & D Unit, Abnoos Skin & Hair Clinic, Mashhad, Iran
[4]Neurogenic Inflammation Research Centre and Department of Clinical Biochemistry, Mashhad University of Medical Sciences, Mashhad 9177948564, Iran
[5]Biology Department, Payame Noor University, 19395-4697 Tehran, Iran

Abstract

Superparamagnetic iron oxide nanoparticles (SPIONs; Fe_3O_4) are one of the most versatile and safe nanoparticles in a wide variety of biomedical applications, for example, magnetic resonance imaging (MRI), targeted delivery of drugs or genes, and in hyperthermia. Although, the potential benefits of SPIONs are considerable, there is a distinct need to identify any potential cellular damage associated with these nanoparticles. In this work, the SPIONs were synthesized by the covalent binding of starch onto the surface of SPIONs, by a "green" co–precipitation method. The field emission scanning electron microscopy (FESEM) illustrated the regular spheres of SPIONs that were coated by starch molecules with a mean diameter of about 30 nm. The X–ray diffraction (XRD) patterns indicated that the biosynthesized samples were pure Fe_3O_4 with a spinel structure while the coating of starch did not result in a phase change. The starch coating onto the SPIONs was also demonstrated by Fourier transform infrared (FTIR) spectra and magnetic measurements revealed that the saturated magnetization of the starch – SPIONs reached 38.4 emu/g, having nanoparticles with the characteristics of superparamagnetism. *In vitro* cytotoxicity studies on human mesenchymal stem cells (hMSCs) showed a dose dependent toxicity with non-toxic effect of concentration below 31.25 µg/mL. In this study, we have focused on biosynthesis of SPIONs and their induced toxic biological responses for a better toxicological understanding of SPIONs.

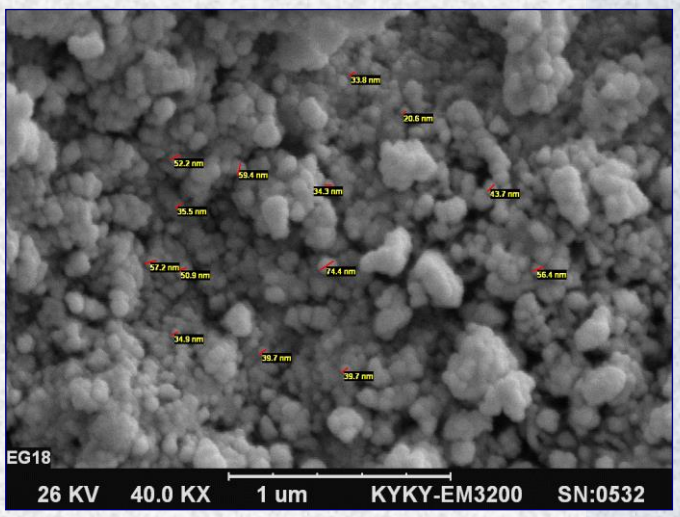

Figure 1: FESEM image of obtained SPIONs.

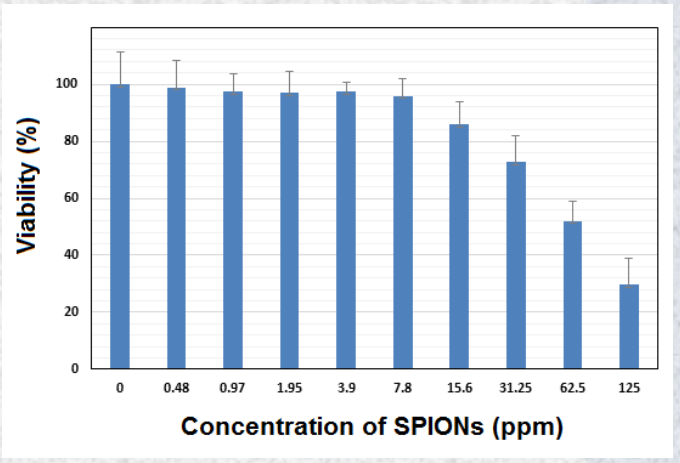

Figure 2: MTT assay of obtained SPIONs.

1. Introduction

Magnetic nanoparticle is an attractive research material not only in the field of magnetic recording but also in a variety of topics such as biomedical, optical, electronics, chemical and mechanical applications [1]. As a member of magnetic nanoparticles family, SPIONs can avail in different fields such as pigments, catalysts, ferrofluids, cancer therapy, drug delivery system, tissue engineering, magnetic resonance imaging (MRI), and hyperthermia [2-5].

2. Experimental

Briefly, 1.00 g of $FeCl_2.4H_2O$ was dissolved in 40 ml of double distilled water (DDW); then, hydrochloric acid was added (Until pH=3(±0.01)) and stirred at room temperature for 30 min. The pH of precipitation process was increased to about 9.1(±0.01) after adding 20% aqueous ammonia plus being digested for 60 min at 70˚C. Afterwards, an aqueous gelatin solution (40 ml; 2% w/w) was added to the initial solution as a stabilizing agent and thus had the final solution stirred for 90 min at 50°C. The precipitate was then washed with DDW and ethanol twice to remove the excess of ammonia remaining in the precipitate. The SPIONs were finally collected as dark brown nanopowders after a drying process at 80˚C.

3. Results and discussion

The broadening of peaks indicates nanocrystalline behavior of the particles. The size of the particles has been computed from the width of first peak using Debye scherrer formula. where K is constant, λ is the wavelenght of X-rays, β is the full width at half maximum and θ is Bragg angle.

$$D = K\lambda / \beta COS\,\theta$$

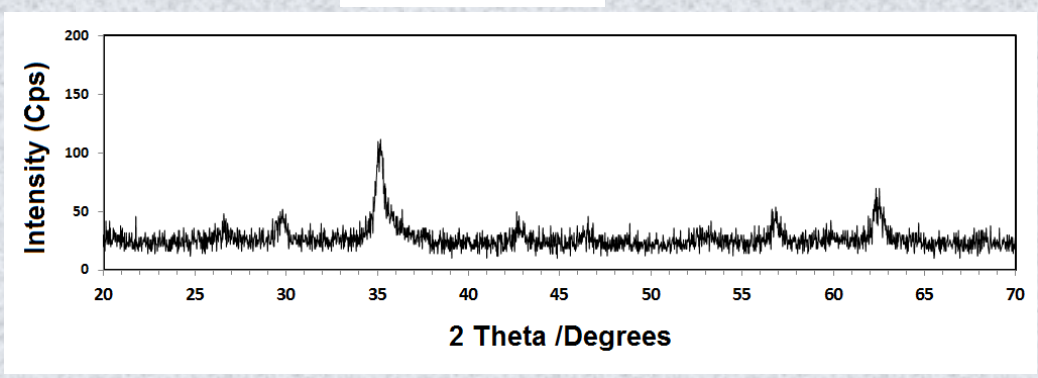

Figure 3: X-ray diffraction of obtained SPIONs.

The results of *in vitro* cytotoxicity studies after 24 h of incubation with different concentrations of nanoparticles, ranging from 0 to 125 µg/mL, are shown in Fig. 2. As the results showed, for concentration above 31.25 µg/mL the metabolic activity was decreased in a concentration dependent manner meaning that metabolic activity was started to decrease from 31.25 µg/mL and in 125 µg/mL maximal decreasing was observed.

4. Conclusion

In this work, a facile, fast, homogeneous, eco-friendly, and modified "green" co–precipitation method was applied for preparation of SPIONs in gelatinous solutions. On the other hand, the synthesis of SPIONs using gelatin as a bio-template is a novel and versatile route that has not yet been reported. *In vitro* cytotoxicity studies on human mesenchymal stem cells (hMSCs) showed a dose dependent toxicity with non-toxic effect of concentration below 31.25 µg/mL.

References

[1] V.H.B. Ho, M.J. Smith, N.K.H. Slater, Ultrasound in Medicine and Biology 37 (2011), 169–175.
[2] J. Meng, G. Yang, L. Yan, X. Wang, Dyes and Pigments 66 (2005) 109–113.
[3] L.M. Rossi, F.P. Silva, L.L.R. Vono, P.K. Kiyohara, E.L. Duarte, R. Itri, R. Landers, G. Machado, Green Chemistry 9 (2007) 379–385.
[4] N.M. Nor, K.A. Razak, S.C. Tan, R. Noordin, Journal of Alloys and Compounds 538 (2012) 100–106.
[5] M.T. Basel, S. Balivada, H. Wang, T.B. Shrestha, G.M. Seo, M. Pyle, G. Abayaweera, R. Dani, O.B. Koper, M. Tamura, V. Chikan, S.H. Bossmann, D.L. Troyer, International Journal of Nanomedicine 7 (2012) 297–306

***Corresponding author:**
Email: darroudim@mums.ac.ir, majiddarroudi@gmail.com (M. Darroudi)

Tel.: 0098 513 8002286
Fax: 0098 513 8002287