Cytotoxicity of Synthesized Iron Oxide Nanoparticles: Toward Novel Biomarkers of Colon Cancer

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***Abstract*— *In* this paper we present the preliminary results of a novel biological analysis platform for early colon cancer detection using magnetic separation of magnetized markers. The platform consists of a microfluidic structure integrated with biosensors. Super-Paramagnetic Iron Oxide nanoparticles (SPIO-NPs) were functionalized with purified DNA Aptamer and their synthesis is described. In this paper, we also present the physicochemical results of the synthesized SPIO/Au-NPs characterized by TEM and XRD. Toxicity of our synthesized biomarkers on HCT116 cell line is discussed. Based on our findings, a concentration of 1mg/ml of our biomarkers added to 5 x 105 cells per well has no effect the viability of the human cells even after 24 hours.**

I. INTRODUCTION

Recent advances in microfabrication and nanotechnology has greatly attracted the attentions of life science researchers for the diagnosis and treatment of deadly diseases such as colon cancer. Among these, the synthesis of Core-shell structures based on Super-Paramagnetic Iron Oxide Nano-Particles SPIO-NPs was proven to have great advantages for a variety of applications such as cells labeling [1], targeted drug delivery [2], tumor treatment [3] using hyperthermia [4], magnetic resonance imaging (MRI) [5-7], bio-sensing [8,9] and magnetic separation[10-11]. In this paper, we employ SPIO-NPs as biomarkers for colon cancer in a high-throughput biosensing platform to achieve early detection in subjects with high risk or genetic dispositions. As shown in (Fig. 1), our platform consists of a microfluidic structure to deliver samples toward a large number of sensing sites. The SPIO-NPs and sensor's surfaces are functionalized with different Aptamers including the KCHA10a, in order to detect various cells (e.g. HCT 116 cell line) associated with colon cancer [12-13]. The sensor is an interdigitated electrode connected to an impedometric readout system that measures the concentration of cancer cells attached to the surface of electrodes via a highly sensitive electric impedance measurement [14]. The role of SPOI-NPs coated with the same Aptamer is to intercept cancer cells during the mixing phase and direct them toward the electrodes upon application of a magnetic gradient. This SPIO-NPs assisted process can significantly increase sensitivity.

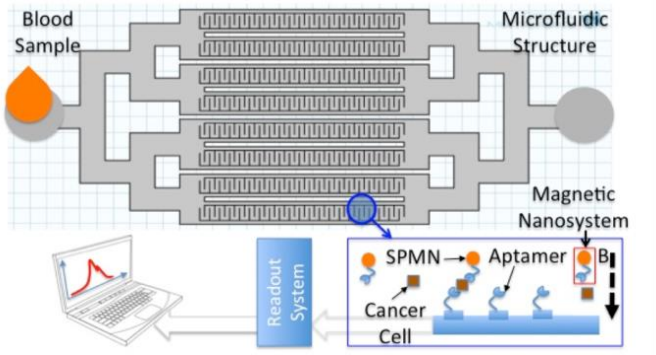

Figure 1. Automated Biological Analysis Platform for the detection of colon cancer. This platform consists of a microfluidic structure (8 channels), microelectrodes coated by Aptamer, readout system to computer. SPIO-NPs are used to detect the colon cancer cells and manipulate them toward the surface by applying the permanent magnetic field.

It is noteworthy that SPIO-NPs are selected for their ability to become magnetized only in presence of magnetic field, so as to avoid aggregation that might affect the measurement. SPIO-NPs are widely used in drug delivery and nano- medicine applications due to their biocompatibility and absent cytotoxicity. For this reason, toxicity study of our synthesized magnetic tags is crucial for bio-sensing purposes. Furthermore, as seen in Fig. 1, cancer cells are sandwiched between the proposed biomarkers (SPIO-NPs and DNA Aptamer) and electrode-bonded DNA Aptamer. In as much, cancer-cell viability is a key to a strong and stable binding between cell walls and Aptamer at the sensing site. In the remaining sections of this paper, we describe the materials and methods, in section II. Synthesis of SPIO-NPs is put forward in this section along with a short description of microelectrode and microfluidic devices.

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We also present the biological protocol of investigating the toxicity responses of cells to SPIO-NPs in the same section. In section III, the preliminary experimental results are presented. Section IV concludes this work.

II. MATERIALS AND METHODS

A. Synthesis of Magnetic Nanoparticles

Successful Synthesis of ultra-small magnetite (Fe_3O_4)-based Superparamagnetic nanoparticles is reported in this section. Ferrous and ferric ions are mixed by adding NaOH and by resorting to the co-precipitation assisted hydrothermal synthesis method. Iron (II) chloride and iron (III) are first dissolved in distilled water including iron ions, with a concentration of 0.25 M. This process is performed in argon gas (Ar) at atmospheric pressure and room temperature (T = 25 °C). NaOH solution is also added to obtain a precipitate in the solution. Then the solution is heated up to 80 °C in a water bath, for 35 minutes to trigger nucleation. Concomitantly, the solution is stirred using at 350 RPM in a non-magnetic stirring device. With reduce RPM and time reaction, the monodipersed NPs obtained. The generated nanoparticles are subsequently washed several times using water and ethanol under the effect of a 1.4 Tesla static magnetic field. The resulting nanoparticles are dried freeze- dryer for 24 hours [15].

B. Cell Culture

Epithelial and cancer cell lines; HCT116 (NCBI code: C570) associated with human colon cancer, were cultured with McCoy`s 5A modified medium (Catalogue number: 30- 2007 from Gibco) containing L-glutamine, Penicillin, streptomycin, Amphotericin B with these concentrations respectively300mg/l, 100µg/ml, 100IU/ml, 2.5 µg/ml provided by the Pasteur Institute of Iran. Incubation took place in a SANYO device (model MCO-17AI) at 37$^{\circ C}$ and carbon dioxide concentration was set to 5%. Furthermore 10% fetal bovine serum and epidermal growth factor (Nano Zist Arrayeh Company) was added to the culture medium which contains growth and adhesion factors and also antitrypsin activity to promote cell proliferation and cell attachment. When cells are confluent (25 cm^2 flasks, JET BIOFIL) the passage of cells is performed by discarding the culture medium and trypsinizing the cells using EDTA solution containing 0.25% Trypsin /EDTA [16].

C. MTT assay

MTT is a well-established cell viability assay. We use this test to assess toxicity of our homemade SPIO-NPs through survival curves. The cells are detached from surfaces using Trypsin-EDTA solution as mentioned above. The suspended HCT 116 cells with various dilutions degrees of $5x10^5$, $2.5x10^4$and $1.25x10^4$ are positioned in the 96-wells plate.

Different concentrations of SPIO-NPs are then added to each well (Fig. 2). The cells/SPIO-NPs are incubated for 24 hours. The supernatant was removed and 100 µl of media mixed to 20 µl of 5mg/ml MTT solution and incubated at 37 $^{\circ C}$ for 3 hours. Viable cells uptake MTT into their mitochondria and metabolized it to Formazan crystals. After discard of the supernatant, 150µl Ethanol is mixed with DMSO (50% V/V) added to each well and shaken at 37$^{\circ C}$ to dissolve all crystals. Elisa reader was used for reading samples in 540 nm and cell viability was compared with control cells. When cells at high concentrations internalize SPIO-NPs, the color change to a dark brown that shows clearly on spectro-photometry readings [17].

III. RESULTS

This section is divided into two parts. The first part is about the iron oxide characterization results of Fe_3O_4 nanoparticles. The second part is dedicated to the MTT assay result.

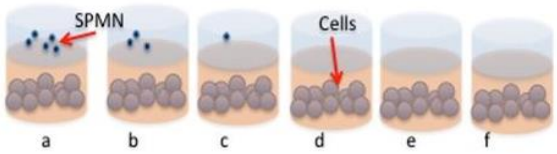

Figure 2. Serial dilution process. The Number of cells/well in (a,d) , (b,e) and (c,f) is 5 x 10^5, 2.5 x 10^4 and 1.25 x 10^4 Cells/ml respectively. (d,e,f) are control cells

A. *SPIO-NPs characterization*

1) 1 Morphology NPs size and its distribution

The synthesized SPIO-NPs were characterized with a transmission electron microscopy (TEM). The images were taken under a Zeiss-EM10C electron microscope operating at acceleration voltage of 80 KV. The samples for TEM measurements were prepared by dropping the reaction solutions onto formvar/carbon-coated grid Cu Mesh 300. A very small amount of synthesized nanoparticles were mixed with the appropriate dispersant (ethanol 96 %) in a glass vial.

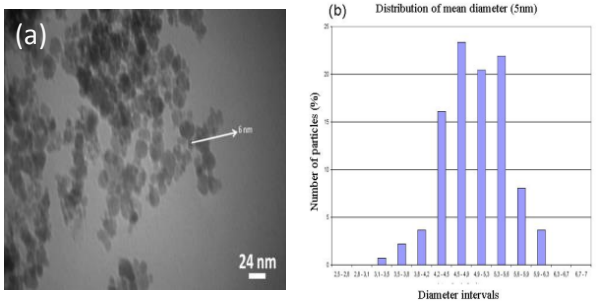

Figure 3. Size distribution of nanoparticles a) Low magnification TEM image of synthesized Fe_3O_4. b). Estimated average size: 5 ± 0.6 nm.

The dispersant used showed no reaction with the nanoparticles and successfully prevented their aggregation under ultrasonic waves (Misonix-S3000). TEM analysis indicated that the synthesized Iron oxide (Fe-O) magnetic NPs are highly mono- dispersed (Fig.

2) 2 Surface chemistry and composition

The SPIO-NPs were dried and pressured manually on Si sample holder. The weak Fe2p signal (Fig. 4) indicates that the oxide surface is covered with residues from synthesis phase confirmed by the very strong C1s signal originating from ethanol (Fig. 5).

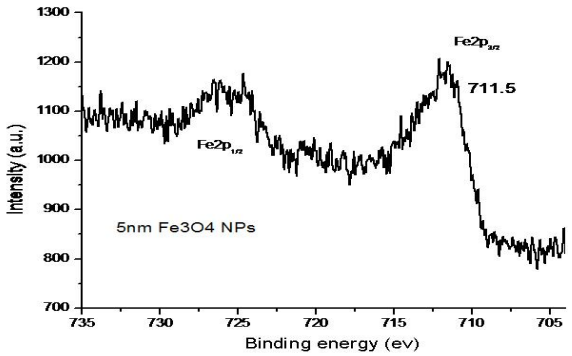

Figure 4. XPS spectrum of 5nm Fe_3O_4 NPs. The peak at ~715 eV corresponds to Fe2P3/2 of Fe3+ and a small peak at ~723 eV corresponds to Fe2P1/2 confirming formation of magnetite [18, 19].

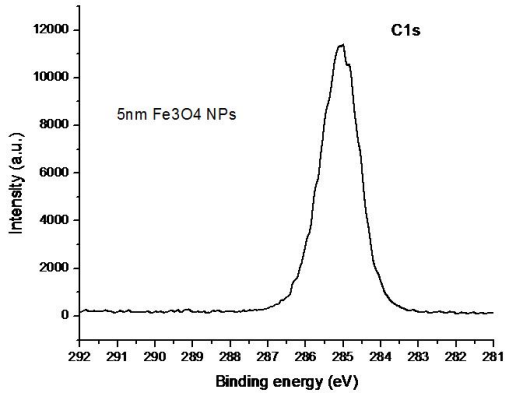

Figure 5. High resolution XPS spectra of C1s for 5nm size Fe_3O_4 NPs. C1s peak position is located at 285.0eV from C-H bond.

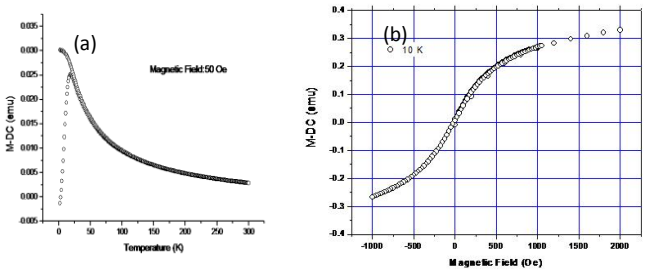

Figure 6. Magnetic response of 5nm-size NPs Fe_3O_4 as a function of (a) temperature and (b) magnetic field, respectively.

3) 3 Magnetic properties and Hysterisis

Dried samples were prepared for magnetometric measurements in a SQUID. Notice the faint magnetization at room temperature from (Fig. 6a) that proves the dominance of *KT* over atomic magnetization vectors causing them to cancel one another. This behavior is typical to single domain Superparamagnetism. Our synthesized NPs were tested for Hysterisis by measuring the coercive field. A follow up of the magnetization over a wide range of changing magnetizing field in an increasing order was followed by the opposite cycle (Fig. 6b). The two cycles match, proving the absence of Hysterisis or any latent magnetic energy.Furthermore, we observed the quick aggregation of nanoparticles under the magnetic field. SPIO-NPs disaggregated by removing the magnetic field. This simple experiment show another proves for the magnetic properties of synthesized SPIO-NPs.

4) 4 Crystallography

Structural characterization of the synthesized nanoparticles was also performed using an X-Ray powder diffraction (XRD) method. XRD patterns were obtained at λ=1 .54 A°. XRD patterns (Fig. 7) revealed that all SPIO-NPs are highly crystalline materials.

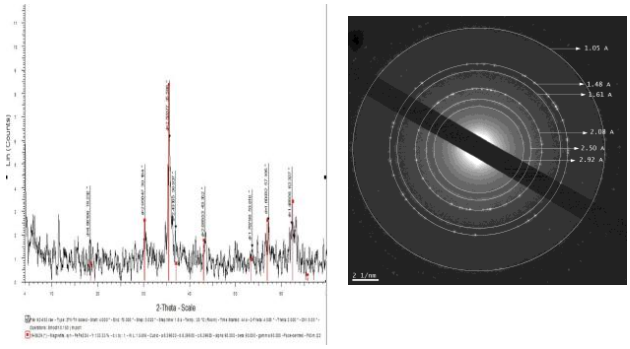

Figure 7. Synthesized nanoparticles XRD Pattern based X-Ray powder diffraction. (left) absence of broad base-line. (right) sample XRD image.

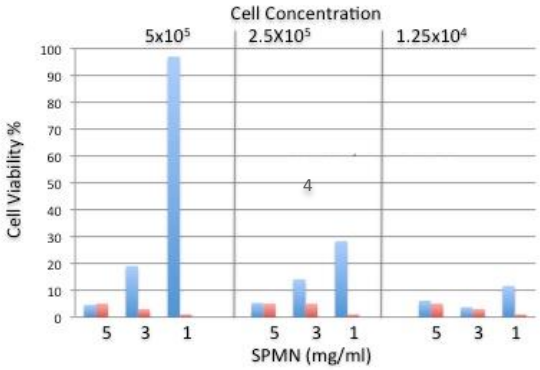

Figure 8. Comparison of the viability of the HCT116 cell line exposed to different amount of Magnetic nanoparticles. Blue and Red columns show the viability and SPIO-NPs concentration (mg/ml) respectively.

A. MTT assay results

The result of MTT assays (Fig. 8) shows the effect of SPIO-NPs concentrations (1, 3 and 5 mg/ml added to 5x 10^5, $2.5x10^4$) on cells at $1.25x10^4$ concentration after incubation for 24 hours. The assessment of cell viability in comparison with control (cell culture with 0mg/ml SPIO-NPs) can prove non-toxicity effect of HCT 116 cell line. Based on these results, the lower SPNMs, the higher viability is expected for high cellular concentration.

IV. CONCLUSION

This paper presents a new approach toward an automated biological analysis platform for the detection of colon cancer. The proposed method can be used for the detection of various biomarkers such as epidermal growth factor receptor (EGFR) associated with colon cancer or other biomarkers such as Prostate Specific Antigen (PSA) for the detection of different types of cancers. In this direction we synthesized SPIO-NPs as a method of enhancing the sensitivity. In this paper we demonstrated the non-toxicity property of SPIO-NPs. A low complexity procedure was developed to reveal the advantage of SPIO-NPs as a non-toxic material.

This is very important advantage of SPIO-NPs for drug delivery purposes; however the non-toxicity of SPIO-NPs may offer another advantage. This assures that cancer cells exhibit unaltered cytoskeleton and cell wall mechanical properties. Consequently, cell viability during incubation and trapping phase by the SPIO-NPs/Aptamer nanosystem, prior to and during sensing is preserved for accurate measurement. Future work will pertain to the development of the sensing platform featuring a large number of micro-channels for high throughput assays.

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