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Antibody-Conjugated Nontoxic Arginine-Doped Fe_3O_4 Nanoparticles for Magnetic Circulating Tumor Cells Separation

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***Abstract*—**Nano-sized materials present new opportunities in biology and medicine and they are used as biomedical tools for investigation, separation of molecules and cells. To achieve more effective cancer therapy, it is essential to select cancer cells exactly. This research suggests that using the antibody-functionalized nontoxic Arginine-doped magnetic nanoparticles (A-MNPs), has been prosperous in detection, capture, and magnetic separation of circulating tumor cells (CTCs) in tumor tissue. In this study, A-MNPs were synthesized via a simple precipitation reaction and directly immobilized Ep-CAM EBA-1 antibodies over superparamagnetic A-MNPs for Mucin BCA-225 in breast cancer cell. The samples were characterized by vibrating sample magnetometer (VSM), FT-IR spectroscopy, Tunneling Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM). These antibody-functionalized nontoxic A-MNPs were used to capture breast cancer cell. Through employing a strong permanent magnet, the magnetic separation was achieved within a few seconds. Antibody-Conjugated nontoxic Arginine-doped Fe_3O_4 nanoparticles have the potential for the future study to capture CTCs which are released from tumor tissue and for drug delivery, and these results demonstrate that the antibody-conjugated A-MNPs can be used in magnetic hyperthermia techniques for cancer treatment.

***Keywords*—**Tumor tissue, antibody, magnetic nanoparticle, CTCs capturing.

I. Introduction

NANO-SIZED materials hold great potential for use within a wide range of studies and applications in various areas including biology and medicine [1], [2]. Magnetic iron oxide nanoparticles are widely used in laboratory diagnostics, cell sorting, and analysis, medical drug targeting, tumor therapy, or cardiovascular disease. They can be coupled to biological entities, e.g. cells. Once their surface is functionalized with the appropriate bioligands, they can bind and interact with biological entities, thus providing a key method of labeling. Another major advantage is their magnetic nature; they can be controlled and manipulated by an external magnetic field gradient. The impressive developments in nanobiotechnology enabled the modulation and tailoring of their composition, size, surface functionalization, and magnetic properties [3], [4].

Synthesis and recognition of nanometer-sized particles have been intensively studied recently because of their technological and fundamental scientific importance [5]. In several previous works, MNPs were reported as an effective tool for magnetically assisted biomolecule separation [6]. In addition to this, separation of molecules and cells using magnetic force is very simple, fast, efficient, and a low-cost method in medical laboratory and research activities.

Iron-oxide nanoparticles with biocompatible coatings are the only nanomaterials which have been approved by the Food and Drug Administration (FDA) for clinical application. General biocompatible coatings have great impacts on critical features of MNPs. Newly, amino acids were advanced as a unique biocompatible coating [7], [8].

To achieve more effective cancer therapy, it is essential to select cancer cells [9], [10]. The nanoparticles as nanoparticles drug delivery systems, which contain anticancer agents, have received a great deal of attention due to their novel growth responses in tumor sites. An example of the useful and perfect solutions for the most of the severe difficulties in chemotherapy is the use of drug-loaded NPs that have targeting roles [11], [12].

In this work, the Fe_3O_4 NPs were synthesized by arginine as the pH agent and employed for grafting of Mucin BCA-225.

II. Experimental

A. Materials and Characterization

All the chemicals were of reagent grade and used without further purification. All used solvents as ethanol and methanol were of analytical grade and the used aqueous solvents purchased from Merck (Darmstadt, Germany).

SEM images were taken on LEO-1455VP provided with an energy dispersive X-ray spectroscopy. The magnetic measurement of the sample was carried out in a VSM (Meghnatis Daghigh Kavir Co.; Kashan Kavir; Iran) at room temperature. Fourier transform infrared (FT-IR) spectra were recorded on Magna-IR, spectrometer 550 Nicolet with 0.125 cm^{-1} resolution in KBr pellets in the range of 400-4000 cm^{-1}. Transmission electron microscope (TEM) images were taken on a JEM-2100 with an accelerating voltage of 200 kV.

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B. Arginine-Doped MNPs Synthesized

In a typical synthesis procedure, $FeCl_2.4H_2O$ (2.237 g) and $FeCl_3.6H_2O$ (6.875 g) were dissolved in 20 ml of distilled water under constant stirring for 30 min in an Argon atmosphere. Afterward, 1.5 g of arginine (the pH control agent) was dissolved in 10 ml distilled water and was added to the above solution under constant stirring to reach pH 9. Then, the reaction was allowed to proceed for 30 min with constant stirring under argon atmosphere. Finally, the precipitates were washed several times with ethanol and distilled water and dried at 80 °C for 6h.

C. Directly Immobilized Arginine-Doped MNPs

Nontoxic Arginine-Doped Fe_3O_4 Nanoparticles (A-MNPs) were directly immobilized Ep-CAM EBA-1 antibodies over A-MNPs without an addition of any linker for Mucin BCA-225 in breast cancer cell. The covalent attachment of superparamagnetic A-MNPs onto antibody via EDC and NHS is done according to the last work [12].

III. Characterization Techniques

A. FT-IR

FT-IR spectra provide a direct proof for the synthetic process of A-MNPs. The results were shown in Fig. 1. The Fe-O characteristic peaks of magnetite Nanoparticles appear at about 640 cm^{-1}, split into two peaks, 577 and 500 cm^{-1}. In aqueous medium, the surface of the magnetite nanoparticles is modified by OH groups, due to coordination of unsaturated surface Fe atoms with hydroxyl ions or water molecules. These OH groups absorb IR waves at about 1630 cm^{-1} (deforming) and 3400 cm^{-1} (stretching) [13]. In amino acid coated nanoparticles (NMA), the C=O and C-O stretching vibrations can be seen at ~1170 cm^{-1} and ~1711 cm^{-1}, respectively. The peak at 2921 cm^{-1} is due to CH stretching vibration, and N-H stretching vibration overlaps with OH stretching at 3373 cm^{-1}, which indicates that the surface of the A-MNPs was successfully coated with an arginine.

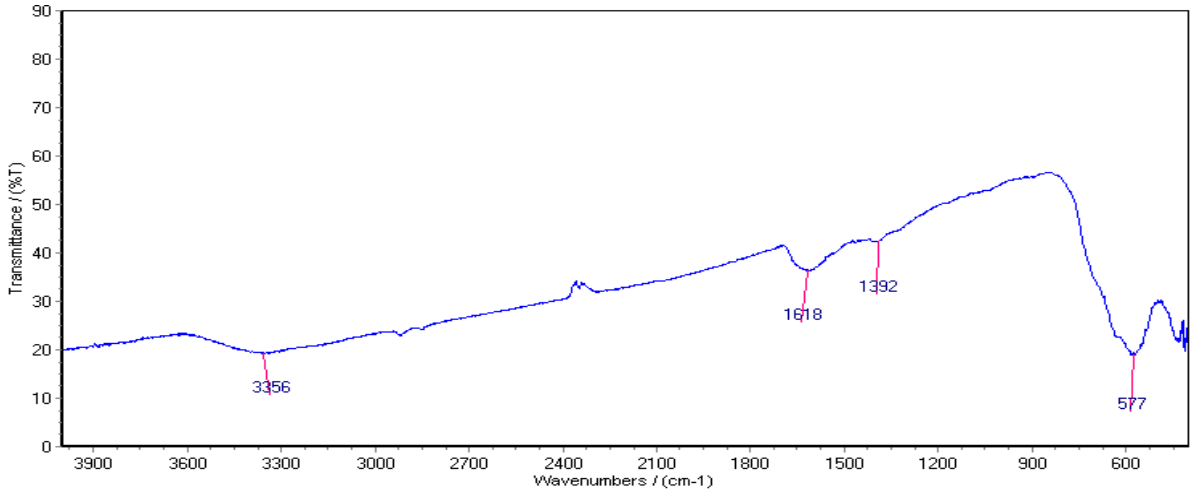

Fig. 1 FT-IR spectra of Arginine-doped MNPs

B. Vibrating Sample Magnetometer (VSM)

VSM in the magnetic properties of the synthesized magnetic NPs, at room temperature, is no hysteresis (illustrated in Fig. 2), both remanence and coercivity are zero, suggesting that the samples are superparamagnetic. The saturation magnetization values obtained at room temperature were mentioned to be about 45 emu g^{-1}. It is strongly magnetic and allows for effective magnetic separation. Notably, one finds that A-MNPs have superparamagnetic properties at room temperature, which implies that no remanence remains when the applied magnetic field is removed. In this novel synthesis, in addition to reducing nanoparticle production processes, we were able to increase the saturation magnetization of immobilized Arginine-doped MNPs.

C. TEM and SEM

The average size of the nanoparticles was obtained to be 40 nm using SEM (Fig. 3) and TEM (Fig. 4) micrograph.

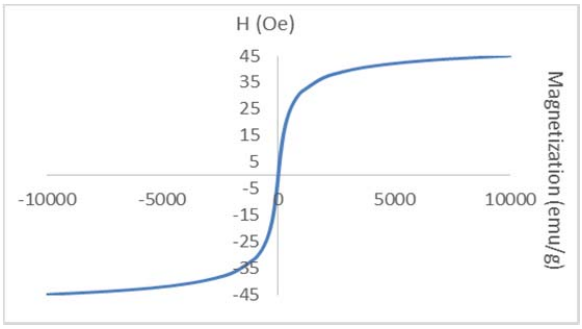

Fig. 2 Magnetization curve at 298 K with Antibody-Conjugated Arginine-Doped Fe_3O_4 Nanoparticles

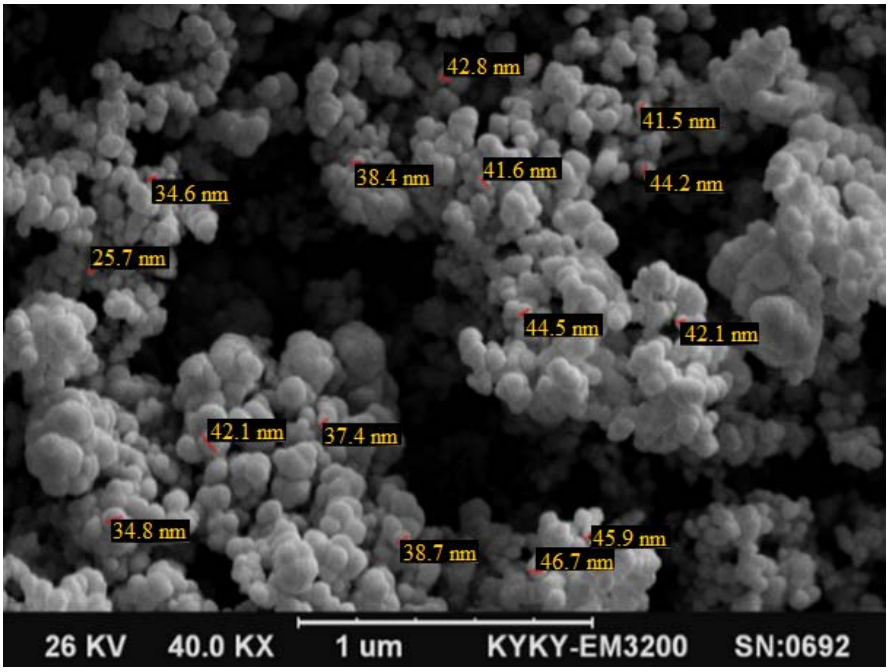

Fig. 3 SEM images of A-MNPs

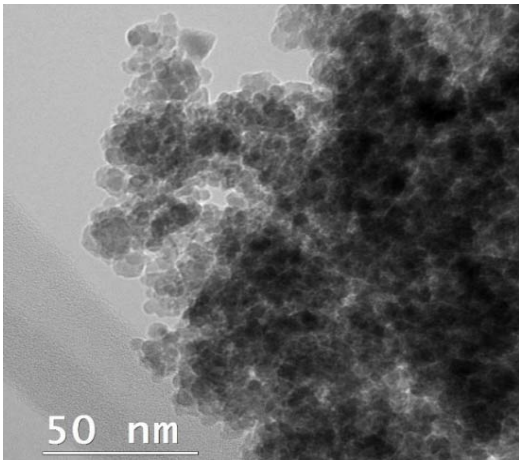

Fig. 4 Transmission electron microscopy (TEM) images of A-MNPs

IV. Capture and Detection Result

A-MNPs, after being functionalized by the antibody, are selectively interred to target, then arginine-doping into MNPs offers direct binding sites for antibodies without prior modifications with costly noble metals or molecular linkers. In the presence of a permanent magnet, the magnetic separation of A-MNPs in water was completed within 15 s. Fig. 5 shows the separation and redispersion process of A-MNPs. In the absence of an external magnetic field, a dark homogeneous dispersion exists. When an external magnetic field was applied, the black particles agglomerated at the wall of the vial, and the dispersion became clear and transparent.

SEM and microscopic images showed that antibody-conjugated A-MNPs were observed on the surface of CTCs. According to these SEM images, white dots on the surface of CTCs are antibody-conjugated A-MNPs. For comparison, a microscope image is given in Fig. 7. The picture is composed of the image that shows Antibody-Conjugated Arginine-Doped Fe_3O_4 nanoparticles that appear black dots on the surfaces of the cells.

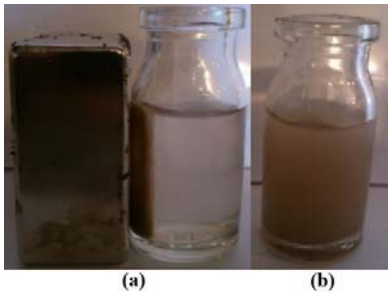

Fig. 5 (a) Separation of Antibody-Conjugated Arginine-Doped Fe_3O_4 Nanoparticles by a magnet, (b) redispersion of it after removal of the magnet

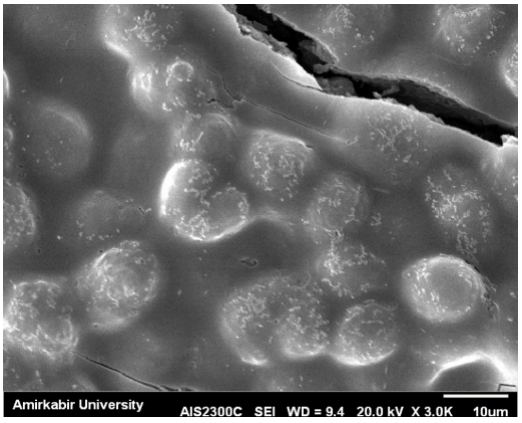

Fig. 6 SEM image of antibody-conjugated A-MNPs (white dots) on the surface of CTCs

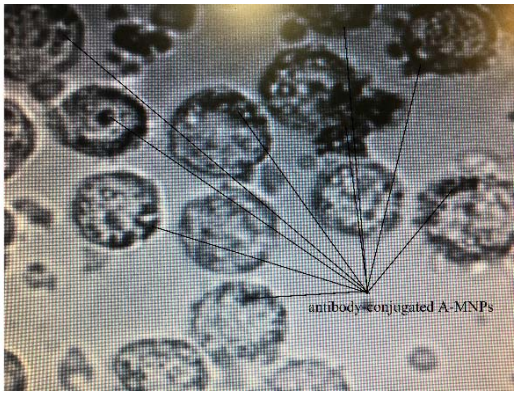

Fig. 7 Microscopic image of antibody-conjugated A-MNPs (black dots) on the surface of CTCs

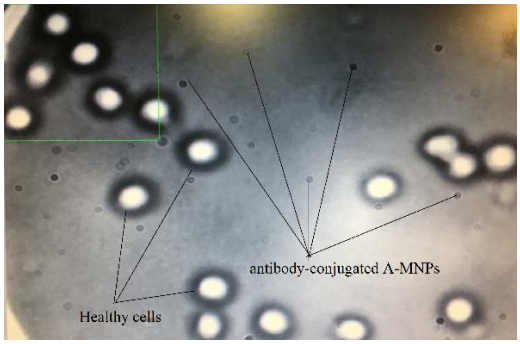

Fig. 8 Microscopic image of antibody-conjugated A-MNPs free from the surface of healthy cells. Note: black circle around the cells are not MNPs

Fig. 9 SEM image of antibody-conjugated A-MNPs free from the surface of healthy cells. Black circles show aggregated nanoparticles

The control test shows that Antibody-conjugated A-MNPs did not attach to the surface of normal cells, yet they bind to their specific target i.e. breast cancer cells according to Figs. 6 and 7. Thus, demonstrating Ep-CAM EBA-1 antibodies over A-MNPs has a very high affinity for connecting to the Mucin BCA-225 in breast cancer cell.

V. Conclusion

This study is focused on synthesizing A-MNPs by a single-step facile co-precipitation method by arginine as the pH agent and employed for grafting of Mucin BCA-225. This research suggests that using the antibody-functionalized nontoxic Arginine-doped MNPs, has been successful for detecting CTCs in tumor tissue, capturing and magnetic separation of CTCs. Therefore, choosing an efficient immobilization method, which can produce binding to a specific target, is essential to achieve satisfactory diagnostic results.

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