Review

Ibrahim Yildiz*

Applications of magnetic nanoparticles in biomedical separation and purification

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Abstract: In recent years, nanoparticle formulations utilizing magnetic materials have started gaining more consideration in the biomedical arena due to their inherent superparamagnetic/paramagnetic nature paving ways to develop assays and sensors benefitting mostly magnetic separation technique. Coupled with the developments in the synthetic field in obtaining controlled-sized particles and surface functionalities, a variety of sensitive and selective assays have been developed ranging from detection/separation/enrichment of antibodies, enzymes, proteins, and nucleic acids to whole cells, virus, and pathogens utilizing a variety of signal transduction mechanisms including fluorescence, surface plasmon resonance, and magnetic resonance. In addition to ongoing research in this field, there are already commercialized products engineered to detect and quantify biomolecules with proper surface formulations. Owing to the immensity of applications of magnetic particles, in this article, recent trends in the design and applications of iron oxide nanoparticles will be reviewed.

Keywords: biosensor; iron oxide; magnetic separation; nanoparticles; superparamagnetic particles.

1 Introduction

Nanomaterials having magnetic properties have found widespread use in various fields such as electronics, material sciences, and biomedical sciences stemming from many benefits they provide in comparison with their bulk counterparts [1]. In general, magnetic nanoparticles are made up of an inorganic core having paramagnetic/superparamagnetic features such as magnetite (Fe_3O_4) or maghemite (Fe_2O_3) with a protecting layer composed mostly of polymers providing colloidal stability and solubility [2]. Furthermore, composite materials having magnetic core(s) and shells with inorganic compositions such as silica, gold, and various other materials were reported for different applications [3]. Synthesis, passivation, and functionalization of various magnetic nanoparticles (MNPs) for various applications have been reviewed extensively in the literature, and readers may be referred to those publications [2, 4–7]. Additionally, for biomedical applications, MNPs need to be encapsulated by biocompatible materials, mostly by polymeric ligands carrying chemical functional groups to conjugate biomolecules and ligands in order to impart imaging, therapeutics, and targeting modalities. (Figure 1) [6, 8, 9].

Over the last decades, the use of magnetic particles, especially superparamagnetic MNPs [10], in biomedical research has gained an incredible momentum due to unique features and modalities they offer in various areas such as imaging [11], drug delivery [12], magnetic separation, and purification [13]. Being an inherent T2 magnetic resonance imaging (MRI) contrast agent, superparamagnetic iron oxide nanoparticles (SPIONs) have been utilized in tumor imaging [14], detection of stem cells [15], metastatic breast cancer imaging [16], detection of Alzheimer's disease [17], lymph node imaging [18], and for many other imaging applications, readers may be referred to comprehensive reviews [4, 11, 19, 20] in this field. SPIONs have found applications in drug delivery field such as chemotherapeutics [21], gene delivery [22], and as well as in hyperthermia [23, 24].

Magnetic separation, in principle, offers unprecedented opportunities in biomedical research with selective, sensitive, and controlled target capture. As opposed to complex and time-consuming chromatographic separations and purifications, magnetic separation could provide efficient separation and purification in terms of time, labor, and yield by utilizing an external magnetic field to capture target molecules with MNPs utilizing affinity interaction. In order to design an efficient platform for magnetic separation, a superparamagnetic – magnetically responsive only in the presence of an

***Corresponding author: Ibrahim Yildiz,** Applied Mathematics and Sciences, Khalifa University, PO Box 127788, Abu Dhabi, UAE, e-mail: ibrahim.yildiz@kustar.ac.ae

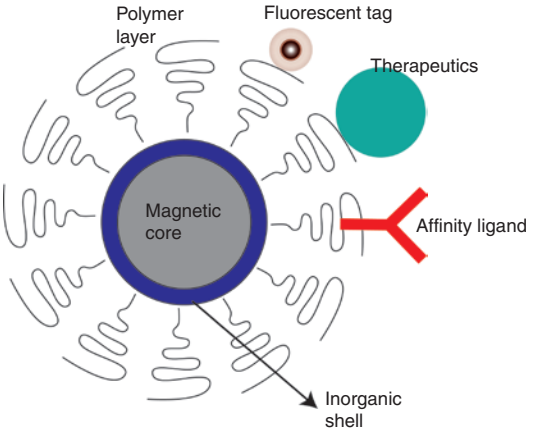

Figure 1: Design of magnetic nanoparticles for biomedical applications. MNPs mostly consist of a magnetic core encapsulated by a protective inorganic shell bearing polymeric ligands displaying fluorescent tags for imaging, therapeutics, and affinity ligands for targeting and separation of biomolecules and cells.

external magnetic field – adsorbent or catcher with high magnetization needs to be employed with proper features such as stability, biocompatibility, having binding units – affinity ligands – to capture the target of interest with high specificity and selectivity, and it should be noted that after target capture with MNPs, the platform should be compatible with assays or methods for detection and quantification.

Applications of magnetic separation and purification based on MNPs in biomedical field can be broadly categorized into several subclasses, which include separation and purification of cells, proteins, which could be subdivided into antibodies and biomarkers, pathogens such as bacteria and viruses, peptides, and nucleic acids. In this review, recent studies for each category will be reviewed.

2 Applications

2.1 Cell separation

Separation of a specific type of cell in a mixed population of cells and other biomolecules could be realized with immunomagnetic separation based on immobilization of specific antibodies against the target cell(s) on the MNPs keeping in mind that the target cell(s) should have overexpressed receptors on the cell membrane against antibodies with high specificity. In addition, MNPs displaying specific biomolecules, apart from antibodies, against cell membrane receptors such as lectins, carbohydrates, and peptides could also exhibit high potential in this area [25]. Most of the applications have been directed to the separation of islet cells [26–28], T cells [29–31], stem cells [15, 32–34], and cancer cells [35–42].

Efficient islet cell separation is a crucial step in isolating intact cells for transplantation, which is a promising treatment option for type 1 diabetes. The ability of heparin, dextran, or siloxane-coated MNPs to separate/purify and reduce the volume of islet cells has been tested on the cells originating from Wistar rats [26]. Heparin-coated MNPs were found more efficient in terms of recovery, cell viability, and most of all separated cells were able to secrete insulin after a glucose challenge test.

In another application, MNPs were encapsulated with poly(glycidyl methacrylate) (PGMA) polymer, and the resultant assembly was conjugated with an anti-CD4 monoclonal antibody, which is specific to a glycoprotein, cluster of differentiation 4 (CD4), overexpressed on $CD4^+$ cells – a special type of T cells helping in adaptive immunity and whose enumeration is crucial in immunodeficiency and certain types of viral infections [30]. This assembly was used to magnetically separate $CD4^+$ cells from a whole blood sample with 95% purity.

Facile extraction and separation of neural stem cells may bring about novel therapies in regenerative medicine due to their potent nature to replicate and differentiate into neural cells, and MNPs might circumvent the existing challenges in isolating and enriching neural stem cells such as risks of brain surgery and difficulties in locating their positions. In this respect, MNPs made up of Fe_3O_4 core with a silica shell having amine terminal groups have been conjugated with anti-CD133 antibody, which is specific to cluster of differentiation 133 biomarker (CD133) in neural stem cells [34]. The preformed particles were injected into the cerebrospinal fluid region in a rat brain followed by the application of an external spinning magnetic field, which caused release of stem cells with MNPs from the endothelial lining; then, the released cells were collected either with syringe or a magnet probe (Figure 2). The ability of extracted cells to differentiate was validated *in vitro* in culture medium, and above all, stem cell extraction had been done while the rat was alive and had remained healthy after the procedure.

Another area in which MNPs have been used extensively is cancer research, especially MRI imaging [11], drug delivery [12], and – the most appealing one – cancer cell separation and detection [36, 43]. MNPs having an amphiphilic polymer surface bearing carboxylic acid functional groups were conjugated with antibodies against human epithelial growth factor receptor 2 (HER2), which is overexpressed in certain cancer types, in order to evaluate the potency of particles to capture and separate cancer cells

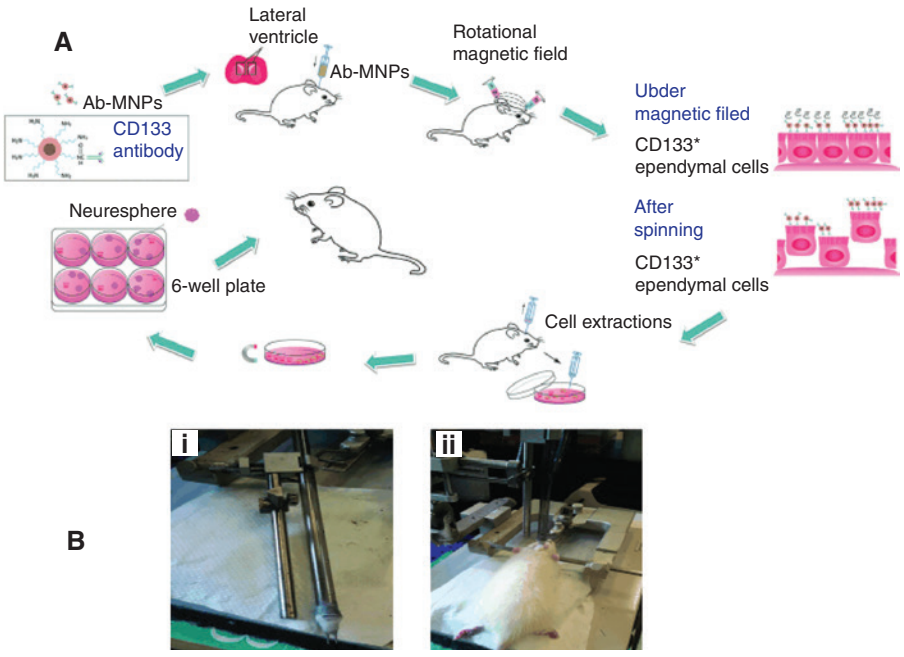

Figure 2: Extraction of neural stem cells from live rat brain by MNPs. (A) Neural stem cells (NSCs) could be isolated from an adult rat after injecting the CD133-modified MNPs into the subventricular zone of the brain. NSCs bound to MNPs on the endothelial lines dissociate and enters the cerebrospinal fluid under an external spinning magnetic field. NSCs bound to MNPs can be extracted either using a microsyringe or NdFeB magnetic probe needle. (B) Photographic images of (i) magnetic probe and (ii) micro-surgery (Reproduced with the permission of the publisher, John Wiley and Sons, Copyright 2013, from Ref. [34]).

out of a blood sample spiked with human breast cancer cell line, SK-BR3, as a model system [36]. Experiments showed that 73.6% of cancer cells were separated with a magnet with a 1/10,000,000 selectivity toward cancer cells compared to normal cells.

Detection of circulating tumor cells (CTCs) in the early stages of cancer progression is an important step in the treatment process, and MNPs are holding great promise in the detection and separation of CTCs [37, 38, 44–46]. Magnetic nanobeads with antiepithelial cell adhesive molecule (EpCAM) antibodies against epithelial cancer cells have been used to separate CTCs in a microfluidic channel attached to a high-gradient magnetic field [37] (Figure 3). The microfluidic device was capable of separating 90%

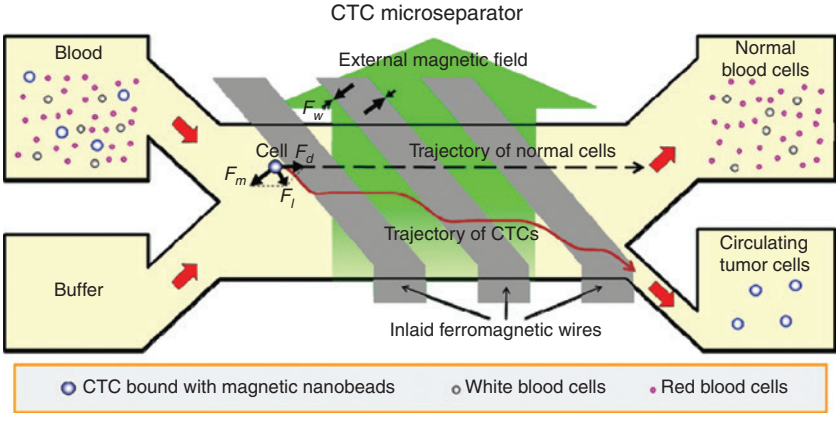

Figure 3: Separation of CTCs based on antibody-immobilized magnetic nanobeads and a microfluidic device. A model CTC line – breast cancer cell lines (SKBR-3) – were spiked into human peripheral blood. The sample was mixed with anti-EpCAM antibody-modified magnetic nanobeads. Two syringe pumps were used to inject the blood sample and PBS buffer into the two inlets. Application of magnetic field through inlaid ferromagnetic wires caused deviation of CTCs from trajectory of normal cells, and CTCs could be directed into another channel (Reproduced with the permission of the publisher, American Chemical Society, Copyright 2013, from Ref. [37]).

of the spiked CTCs from blood sample with 97% purity with a flow rate up to 5 ml/h. In a recent study [42], MNPs were used to capture CTCs with a covalently bound synthetic EpCAM recognition peptide instead of anti-EpCAM antibodies, and studies showed that breast, prostate, and liver cancer cells from spiked human blood could be separated with a high selectivity and yield as comparable to antibody-based separation.

2.2 Protein separation

Protein separation/purification is a major field of biochemistry, and there are already a great variety of established methods comprising chromatographic and electrophoretic techniques such as size exclusion, ion exchange, affinity, and so on [47]. Magnetic particle-based separation of proteins – including antibodies, biomarker, and enzymes – in general rely on the immobilization of affinity ligands on the surface of particles toward the target proteins, and a comprehensive list of applications including home-made and commercial magnetic particles up to 2004 had been reviewed elsewhere [13]. However, here we aim to review the recent studies in this field.

In principle, histidine-tagged (His-tag) proteins could be purified magnetically with the particles having nitrolotriacetic acid (NTA) or Nα,Nα-Bis(carboxymethyl)-L-lysine hydrate (ANTA) ligands in a similar manner in which affinity chromatography exploits NTA or ANTA ligands [48–52]. MNPs with silica shell bearing NTA ligands were used to separate His-tag-fused recombinant Chi-A (human chitinase, acidic) protein in *Escherichia coli* (*E. coli*) crude cell lysate [49]. His-tagged fatty acid-binding protein 1 (FABP1) was successfully separated from *E. coli* lysate with MNPs bearing gold shell having ANTA ligands complexed with Co^{2+} ions [50]. Furthermore, strong affinities between different molecules have been employed to generate magnetic separation platforms such as between imidazole and heme groups to separate hemoglobin [53], between maltose and maltose-binding proteins [54], between aminophenylboronic acid (APBA) and glycoproteins [55].

Owing to the fact that bacterial-based proteins such as Protein A, Protein G, Protein L, and recombinant forms of protein A and B, have high affinities toward antibodies, they were mostly employed as affinity ligands immobilized on a chromatography matrix for antibody purification purposes [56]. Employment of magnetic beads carrying affinity ligands in antibody purification has an attractive feature such that magnetic separation could circumvent lengthy purification and separation processes. To this end, Protein A-conjugated superparamagnetic MNPs efficiently purified immunoglobulin G 2a (IgG2a) from mouse ascites by magnetic separation such that 1-mg particles were able to produce 22 mg of IgG2a [57]. Furthermore, MNPs coated with tin(IV) hydroxide shell bearing Protein A was tested to separate IgGs from rabbit blood serum, and the efficiency of system was validated by the surface plasmon resonance (SPR) technique [58]. In a similar fashion, MNPs consisting of magnetite and silane coating were covalently modified with Protein A, and the ability of the resultant assembly in preparative purification of IgGs from cell culture supernatant were compared with conventional chromatography techniques [59]. With respect to yield and purity of up to 100 l of cell culture supernatant, the magnetic system produced similar results; however, purification time considerably was shortened compared to conventional methods.

Synthetic affinity ligands started to appear in antibody purification as an alternative to protein-based affinity ligands due to several advantages associated with stability, cost, and scalability [60]. In this regard, dextran-coated MNPs were modified with Protein A mimetic ligands, 22/8, and this formulation yielded purification of human IgG with 95% purity and 568±33 mg IgG yield per 1 g of formulation [61]. Boronic acid – which can form reversible selective interactions with antibodies – modified MNPs have been utilized to purify antibodies, and it has been shown that 95% purity could be obtained using CHO cell supernatants [62]. In another study, testing the feasibility of charge interaction to separate antibodies with MNPs, negatively charged MNPs having thermo-responsive polymeric surfaces have been evaluated to purify monoclonal antibodies from a Chinese hamster ovary (CHO) cell culture supernatant previously diafiltered with cation exchange method. In the end, it has been found that by using magnetic separation and employing an elution method exploiting thermo-responsive surface to wash away antibodies from MNPs, a recovery of 94% of the antibodies could be realized in a relatively short period [63]. Antibodies raised against certain biomolecules could also be separated, in principle, selectively from a mixture by immobilizing those biomolecules on MNP. Toward this end, a magnetic platform consisting of superparamagnetic MNPs displaying M13 bacteriophages on the surface was tested to separate antibodies raised against major coat protein of M13, thus, resulting in an efficient magnetic affinity separation with a high yield in a single step process [64].

Magnetic particles play a promising role in constructing biosensors exploiting magnetic separation, thereby, facilitating enrichment of target biomolecules for sensing applications. In this sense, thermo-responsive polymer-coated iron oxide and gold (Au) nanoparticles have been

used to construct a biosensor to detect malarial biomarker protein (PfHRP2) [65]. Antibody – against PfHRP2 – labeled Au nanoparticles were used to fish PfHRP2 biomarker in a spiked serum sample following the addition of iron oxide with a thermal stimulus, which caused the aggregation of particles together, hence, providing an efficient magnetic separation (Figure 4). By this methodology, the target biomarker was concentrated 50 times and used directly for a lateral flow strip test.

2.3 Peptide separation

Recent developments in proteomics studies have led to identification and profiling of many proteins, playing crucial roles in various biological pathways, thanks to mostly peptide mass fingerprinting (PMS) and mass spectroscopy (MS) techniques [66]. In particular, posttranslational protein modifications through glycosylation and phosphorylation have important consequences in cellular mechanisms, and identification of these modifications in protein structures has crucial importance in the understanding of these mechanisms [67]. Now that the general methods in protein identification rely mostly on MS analysis of peptide fragments generated by means of tryptic digestion of target protein, MNPs have found two potential applications in this process in that, first, the target protein could be enriched by magnetic affinity separation [68, 69], and second, peptide fragments could be enriched by magnetic separation for further analysis with MS [70–77]. One example of the first class applications is that an NTA-derivative coated MNP was complexed with Ni^{2+} ion in order to selectively bind to a His-tag protein, streptopain, from cell lysates, so that enrichment of protein could be achieved via magnetic separation, which followed tryptic digestion of protein and characterization with MS analysis [68]. In the same study, it was shown that complexation of Zr(IV) or Ga (III) ions with an NTA derivative has the potential of selectively enriching phosphorylated peptides. Peptide fragments of serum biomarkers could be enriched by MNPs having antibodies against target peptides. To this end, polyclonal antibodies against tumor necrosis factor α (TNFα) peptide has been conjugated to MNPs, and the resulting particles were used to capture TNFα from a serum sample spiked with TNFα followed by MS analysis [71]. It has been found that employment of peptide enrichment through MNPs has brought about 1000 times signal enhancement in the MS analysis with high accuracy and precision, and the results showed that this technique could be applied to the detection of serum biomarkers with high sensitivity and selectivity. Because of the fact that phosphate groups have affinities to adsorb

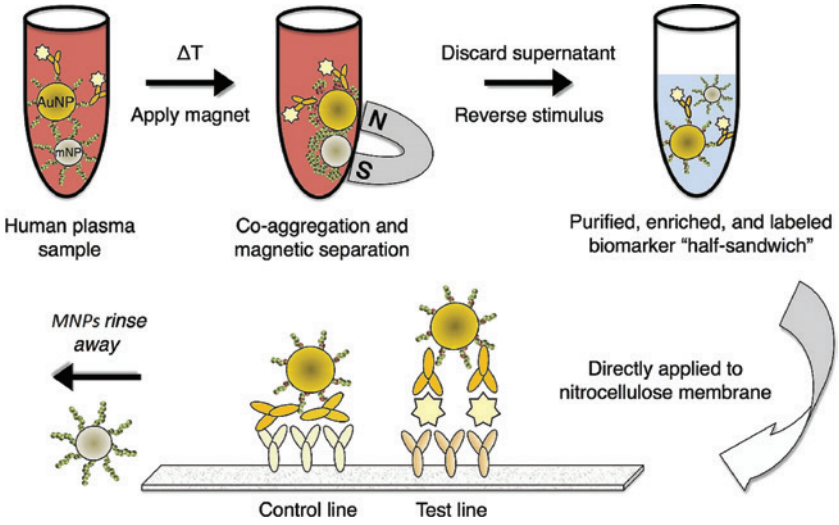

Figure 4: Separation and enrichment of malarial biomarker protein based on antibody-immobilized Au nanoparticles and thermo-responsive MNPs. Antibody-labeled Au nanoparticles with thermo-responsive polymer surface coating – poly(N-isopropylacrylamide) (pNIPAm) – were used to capture malarial biomarker protein in plasma. Subsequently, MNPs having pNIPAm coating and free pNIPAm were added to plasma following a temperature increase, which causes AuNPs/MNPs to aggregate, and they were separated by a magnet. By removing the supernatant, magnetically separated aggregates were reconstituted with a smaller volume of buffer having lower temperature, which caused disaggregation of particles. An immunochromatographic membrane assay with test and control lines of antibodies could detect sensitively the enriched biomarker protein (Reproduced with the permission of the publisher, American Chemical Society, Copyright 2012, from Ref. [65]).

on titanium oxide (TiO_4) surfaces, phosphopeptides could be enriched for MS analyses using magnetic particles having a TiO_4 shell [75, 77]. Pyridyldithiol ligand immobilized MNPs have been used to enrich cysteinyl peptides as a result of ensuing disulfide bond formation between ligands and sulfhydryl of cysteine groups [74]. In general, glycopeptides have affinities toward some hydrophilic molecules, and this feature could be harnessed to enrich these peptides specifically with MNPs carrying required hydrophilic moieties [72, 76, 78].

2.4 Nucleic acid separation

Separation and detection of nucleic acids play major roles in biomedical sciences especially in diagnostics and therapy. In general, separation of nucleic acid from a bio-sample requires complex and multistep procedures [79]. MNPs, both home-made and commercial ones, having proper surface functionalities, which have affinities toward nucleic acids were extensively utilized in the purification and detection of various DNAs and RNAs from crude cell extracts by means of magnetic separation, thus, provided many benefits compared to conventional methods such as fluid phase methods, which involve cell lysis followed by multiple precipitation, washing, and extraction steps [80]. There are several types of affinity interactions for magnetic separation of nucleic acids with MNPs such as electrostatic charge interaction [81–83], complementary base pair interaction [84–86], inherent binding interaction of nucleic acids with bare iron oxide [87] or iron oxide with silane shell [88].

2.5 Pathogen separation

Detection of pathogens including viruses and bacteria bears utmost importance for clinical and environmental applications. Furthermore, samples containing low levels of pathogens pose significant challenges for detection; in essence, magnetic separation could offer a considerable benefit for analysis by concentrating the sample manifold into small sample volumes and eliminating other materials so that reliable and sensitive detections could be carried out. The most frequently utilized strategy to separate and enrich pathogens from samples involves antibodies against target pathogens on the surface of MNPs. To this end, antibody-conjugated MNPs have been used to separate human immunodeficiency virus (HIV-1) [89], *Salmonella typhimurium* [90], *Vibrio cholerae* [91], and type 2 dengue virus [92]. In addition to antibody-pathogen affinity, there are several other affinity interactions utilized to magnetically separate pathogens from samples. Antibiotic-conjugated MNPs have shown great utility to separate Gram-positive and Gram-negative bacteria from aqueous solution based on binding affinity of antibiotics to the cell wall of bacteria [93]. Peptides could be suitable alternatives to antibodies in affinity-based separation of pathogens now that through phage display technology highly selective and specific peptides could be identified against target pathogens. In this manner, multiple antigenic peptides were covalently immobilized on MNPs to capture herpes simplex virus-1 resulting in highly sensitive and fast detection of viruses [94]. MNPs carrying cation exchanger surface functionality have been studied to separate *E. coli* and *Agrobacterium tumefaciens* from water samples based on the negative charge of the bacterial cell wall surface, and it was shown that detection of both pathogens via multiplexed PCR was possible [95]. The high density of anionic phospholipids present on the membrane of bacteria both for Gram negative and positive were targeted via a high-affinity synthetic ligand, zinc-coordinated bis(dipicolylamine) (bis-Zn-DPA), to capture bacteria from bovine whole blood through a microfluidic device utilizing MNPs carrying multiple copies of ligands on their surfaces (Figure 5) [96]. It was shown that

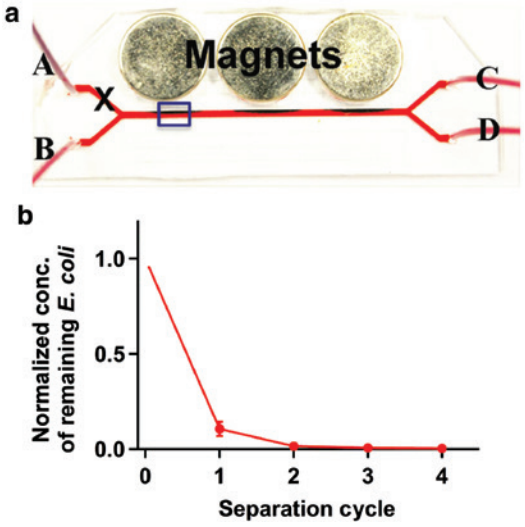

Figure 5: Magnetic separation of bacteria from whole blood utilizing synthetic ligand-coated MNPs. Blood sample spiked with fluorescently labeled *E. coli* was incubated with MNPs having synthetic affinity ligand and then infused through inlet B, while A is closed and exited through outlets C and D. Placement of three permanent magnet caused *E. coli* bound with MNPs to exit from inlet C (b). Separation efficiency of MNPs on the concentration of *E. coli* in blood sample after repeated cycle could be observed utilizing fluorescence measurement. (Reproduced with the permission of the publisher, American Chemical Society, Copyright 2014, from Ref. [96]).

complete removal of *E. coli* was achieved at a flow rate of 60 ml/h in two cycles of purification. Affinity of bacteria toward carbohydrates present on the cell membrane of mammalians has led to the design of a magnetic affinity probe consisting of MNPs displaying mannose, and the resultant platform was tested to separate *E. coli* from buffer samples [97]. It was found out that 88% of bacteria can be separated from a sample within a short time of incubation.

3 Conclusion

MNP-based separation techniques in biomedical applications have become routine qualitative and quantitative tools for diagnostics. Coupled with microscopy and other detection methods, magnetic separation has started to furnish fast, convenient, selective, and sensitive purification/enrichment/detection of biomolecules, and cells, and it has been proven that they can be engineered to be an integral part of systems such as microfluidic devices and chromatography columns. In this area, it seems that the main focus for the future studies will likely involve optimization of MNPs in terms of surface chemistry, engineering shell components, and controlled or stimuli-responsive aggregation/de-aggregation dynamics to modulate a magnetic response. It is not a hypothetical idea that MNPs will find ways for *in vivo* separations of biomolecules and cells in near future for diagnosis and, if not, interventions such as removal of pathogens, CTCs, antigens locally or systemically.

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Ibrahim Yildiz
Applied Mathematics and Sciences, Khalifa University, PO Box 127788, Abu Dhabi, UAE,
ibrahim.yildiz@kustar.ac.ae

Ibrahim Yildiz Prior to joining Khalifa University as an assistant professor, Dr. Yildiz worked as a Research Associate in the Department of Biomedical Engineering at Case Western Reserve University, Cleveland, OH, USA. He obtained his PhD in Organic Chemistry from the University of Miami in 2008. His research focuses mainly on nanomaterial synthesis, functionalization, characterization for biomedical applications. In addition, he has experience in organic synthesis and computational modeling. Dr. Yildiz has co-authored 33 research articles and multiple book chapters in the area of organic, physical, and material chemistry.