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Microchip-based detection of magnetically labeled cancer biomarkers ☆

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ABSTRACT

Micro-magnetic sensing and actuation have emerged as powerful tools for the diagnosis and monitoring of cancer. These technologies can be miniaturized and integrated onto compact, microfluidic platforms, enabling molecular diagnostics to be performed in practical clinical settings. Molecular targets tagged with magnetic nanoparticles can be detected with high sensitivity directly in unprocessed clinical samples (e.g. blood, sputum) due to the inherently negligible magnetic susceptibility of biological material. As a result, magnetic microchip-based diagnostics have been applied with great success to the isolation and detection of rare cells and the measurement of sparse soluble proteins. In this paper, we review recent advances in microchip-based detection of magnetically labeled biomarkers and their translation to clinical applications in cancer.

Contents

1. Introduction

In the last several decades, our ability to measure the molecular signals associated with cancer has advanced dramatically. Techniques such as genetic sequencing, high-throughput molecular screening, and flow cytometry have enabled sophisticated measurements that promise improvements in early diagnosis, personalized tailoring of treatment, and understanding of the underlying causes and mechanisms of cancer [1–3]. Unfortunately, the realization of tangible improvements in patient care from these molecular measurements has been constrained by significant engineering challenges in their translation to clinical applications. These challenges stem from the small concentration of cancer biomarkers in clinical samples, the heterogeneity of biomarker expression, and the extensive sample preparation that is often necessary prior to these measurement techniques [4,5]. Here, we review the use of magnetic actuation and sensing on microfluidic chips as a modality that is uniquely well suited to address these challenges.

The fundamental benefit of using magnetic fields to measure and control biological systems, rather than alternatives such as optical, acoustic, or electrical fields, is the negligible intrinsic magnetic susceptibility of biological systems. Magnetic sensing and sorting are based on the selective labeling of biological targets with magnetic nanoparticles (MNPs) conjugated with appropriate affinity ligands. The lack of magnetic background enables sensing and sorting to be performed on magnetically labeled cells in unprocessed clinical samples without interference from host cells or variations in pH, salinity, or turbidity [6,7]. By eliminating sample processing, magnetic detection minimizes the loss of precious sample and simplifies clinical use.

Magnetic sensors and particles can be scaled down to the micro- and nano-levels, enabling measurements to be made on biologically relevant length-scales, such as that of circulating tumor cells (~10 μm) [6], circulating microvesicles (~100 nm), and soluble proteins (~1 nm) [8].

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The ability to measure clinical samples on these length-scales enables rare cells to be resolved and sparse molecular signals to be detected. Furthermore, magnetic sensing and sorting can be integrated onto microchips for automated, portable use in practical clinical settings [4].

The invention of new techniques to measure molecular biomarkers with magnetic microchips promises enormous impact on many applications in cancer diagnostics and monitoring. One example is the measurement of soluble blood-borne cancer biomarkers, which currently suffers from a lack of predictive value [9,10]. It is hypothesized that these diagnostics can be improved by increasing detector sensitivity and specificity, by expanding the number of proteins that are measured, and by measuring these biomarkers as a function of treatment progression [9,10]. Magnetic sensing, with its ability for ultra-sensitive, multiplexed detection on low-cost, portable microchips has proven uniquely well suited to meet these goals [11]. Another example where magnetic detection can address an important challenge in cancer diagnostics and monitoring, is the detection of circulating tumor cells (CTCs). Monitoring cancer progression with CTCs is an emerging technology that has shown great potential for observing the complex molecular state of a tumor, via a non-invasive blood test [12]. However, the low concentrations of CTCs versus the vast backgrounds of host cells make it challenging to efficiently isolate and profile these cells. The detection of magnetic nanoparticle labeled cells, with its inherent insensitivity to background and minimum sample processing, has been demonstrated as an effective tool to improve resolution of these rare cells [6].

In this paper, the relative utility of magnetic sensing and actuation is outlined, and recently reported technologies that harness these approaches for applications in cancer are reviewed. The review is organized by the three basic elements of magnetic detection: the labeling of molecular markers with magnetic nanoparticles (MNPs) (Fig. 1a) and the quantitative sensing (Fig. 1b) and the magnetic isolation (Fig. 1c) of these labeled biomarkers.

2. Recent developments in magnetic sensing and actuation

2.1. Magnetic nanoparticle labeling

Magnetic nanoparticles have physical properties than are qualitatively different than that of the bulk. These properties are controlled by the geometry of the particle and can be finely engineered for specific tasks [13]. The super-paramagnetic nature of very small ($d < 20$ nm) magnetic nanoparticles (MNPs) (Fig. 2a) has many advantages for biological sensing and actuation applications. At this size-scale, particles consisting of most magnetic materials (ferrites, iron) will contain only a single magnetic domain with an orientation defined by the magnetic anisotropy of the particle [5]. When these particles are suspended in fluid, thermal fluctuations at room temperature overcome this anisotropy barrier, causing the magnetic moment to spontaneously and randomly flip. An ensemble of MNPs displays a negligible net remnant magnetic moment in the absence of an external magnetic field, but become strongly magnetized in the presence of an applied field. As such, superparamagnetic particles may be described with a high magnetic susceptibility χ at fields lower than their saturation value B_s and, above B_s, by a constant magnetization M_s (Fig. 2b).

These superparamagnetic MNPs offer several important advantages for diagnostic applications:

1. MNPs conjugated with the appropriate affinity ligands can be made to selectively bind to a molecular target of interest. Highly efficient two-step bio-orthogonal magnetic labeling strategies may be utilized which enable the use of generic nanoparticles, the efficient utilization of valuable affinity ligands, and amplified magnetic labeling ($>10^5$ MNP/cell) [5] (Fig. 2d).
2. MNPs facilitate molecular-specific mechanical actuation of intended targets. Because biological objects have negligible intrinsic magnetic moments, only magnetically-labeled targets will respond to external magnetic field gradients and experience a mechanical force [14]. Due to the superparamagnetic nature of MNPs, the magnetization of the MNPS vanishes when the external field gradient is removed, enabling stable long-term storage of these reagents.
3. Biological targets labeled with MNPs assume a magnetic moment proportional to their expression of a specific biomarker, enabling quantitative measurements of molecular signals.[7,15]

Ferrite particles are among the most widely utilized MNP. In particular, cross-linked iron oxide (CLIO) nanoparticles have found wide application due to their stability and biocompatibility [16]. CLIO nanoparticles contain a superparamagnetic iron oxide core (3–5 nm monocrystalline iron oxide) composed of ferrimagnetic magnetite (Fe_3O_4). The metallic core is coated with biocompatible dextran, cross-linked, and functionalized with primary amine, resulting in an average hydrodynamic diameter of 25–40 nm [17].

Much work has been done to enhance the magnetization of MNPs. Highly magnetic particles become increasingly important when labeling cells with weakly expressing biomarkers[5] or when trying to detect small objects, such as a bacteria [18]. Doping of ferrite MNPs with elements such as manganese (Mn), cobalt (Co) or nickel (Ni) has been

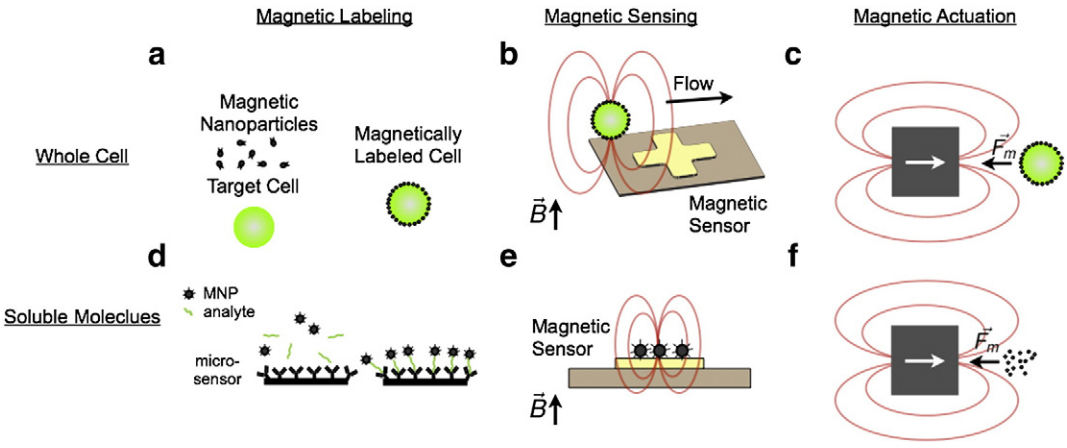

Fig. 1. Magnetic sensing and actuation. a. Molecular markers of interest on cells can be labeled with magnetic nanoparticles (MNPs). A cell labeled with MNPs assumes a magnetic moment proportional to the expression of the targeted biomarker. b. Magnetic sensors can be used to quantitatively detect them. c. External magnetic field gradients can be used to apply forces to these cells and d. soluble biomarkers, such as proteins or nucleic acid, can be captured onto magnetic beads for isolation or detection. For example, shown here a sandwich assay is used to capture an analyte onto a surface, and then label that analyte with MNPs. e. Magnetic sensors can be used to quantify soluble biomarkers labeled with MNPs. f. External magnetic field gradients can be used to isolate magnetic beads that have captured soluble biomarkers.