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Tessellated permanent magnet circuits for flow-through, open gradient separations of weakly magnetic materials

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ABSTRACT

Emerging microfluidic-based cell assays favor label-free red blood cell (RBC) depletion. Magnetic separation of RBC is possible because of the paramagnetism of deoxygenated hemoglobin but the process is slow for open-gradient field configurations. In order to increase the throughput, periodic arrangements of the unit magnets were considered, consisting of commercially available Nd-Fe-B permanent magnets and soft steel flux return pieces. The magnet design is uniquely suitable for multiplexing by magnet tessellation, here meaning the tiling of the magnet assembly cross-sectional plane by periodic repetition of the magnet and the flow channel shapes. The periodic pattern of magnet magnetizations allows a reduction of the magnetic material per channel with minimal distortion of the field cylindrical symmetry inside the magnet apertures. A number of such magnet patterns are investigated for separator performance, size and economy with the goal of designing an open-gradient magnetic separator capable of reducing the RBC number concentration a hundred-fold in 1 mL whole blood per hour.

1. Introduction

Magnetic cell separation relies on high magnetic susceptibility contrast between cells tagged with magnetic beads and untagged cells [1]. Conjugation of magnetic beads with cell targeting ligands, typically monoclonal antibodies against cell surface receptors, makes the magnetic cell tagging highly specific to the target cell population. The availability of inexpensive permanent magnets and commercial magnetic cell separators combined with the need for highly sensitive and specific cell separation methods on a preparative scale for diagnostic and therapeutic applications contributed to the wide adoption of the magnetic cell separation method for biomedical and clinical applications [2]. The potential drawback of the method is the reliance on the magnetic labeling reagents that may have an undesirable effect on the target cell biology and may adversely affect downstream cell product processing and molecular analysis for diagnostic applications [3]. In particular, the presence of iron in the analyte is known to interfere with the polymerase chain reaction (PCR) [4]. The issue is even more acute in case of the cell product being intended for therapeutic applications. Here an additional concern is the potential toxicity of unbound, residual magnetic beads in the cell suspension, and in the case of a required untagged cell fraction, the presence of non-specifically bound material in the cell product [5].

The availability of high magnetization saturation ($M_s \approx 1$ T) and high coercive force ($\mu_0 H_c \approx 1$ T) permanent magnets creates opportunities for label-less magnetic cell separation, whereby weak magnetic susceptibility contrast between cell fractions can be compensated by the high magnetic field and high magnetic field gradient of a suitable permanent magnet separator [6]. A prime candidate for such an investigation is the separation of red blood cells (RBCs) from white blood cells (WBCs) because of the high concentration of endogenous iron in the hemoglobin of the RBCs. In the absence of bound oxygen molecules, the hemoglobin is paramagnetic which increases the RBC magnetic susceptibility to a sufficient degree that its magnetic field-induced motion can be distinguished from that of the WBC [7]. The separation of RBCs from WBCs has important practical applications because it is a first step in many analytical and diagnostic applications that require access to pure WBC populations without the RBC interference. Currently, it is accomplished quickly and efficiently by centrifugation or by flash RBC lysis when small blood sample volumes are involved, but those methods have their own disadvantages that make their use problematic in a number of specialized applications [8]. In particular, centrifugation is incompatible with the microfluidic architecture used for lab on a chip fabrication and the flash lysis

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procedure contaminates the sample with RBC ghosts and free hemoglobin, interfering with downstream molecular analysis. Thus, label-less magnetic RBC and WBC separation may find practical applications in new diagnostic instruments designed for blood analysis [9]. In this study we have concentrated on the question of throughput achievable with the use of commercial permanent magnets for RBC separation from blood.

2. RBC throughput model

The separation may be achieved using the quadrupole magnetic field and thin annular channel geometry of the quadrupole magnetic sorter (QMS) [10,11]. The quadrupole field results in a high magnetic field gradient across the thickness of the annular channel. The cell suspension is introduced on a continuous basis adjacent to the inner channel wall with flow rate $Q(a')$ and cell-free fluid introduced to the rest of the channel cross section with flow rate $Q(b')$. The two fluid streams enter the channel on opposite sides of a thin cylinder mounted axisymmetrically within the annular channel inlet. The cylinder serves to smoothly merge the two streams. Under the influence of the magnetic field gradient, deoxygenated RBCs are driven across the channel thickness toward the outer wall as they are carried along the channel length. At the channel outlet the fluid is divided into two streams by a second thin cylinder. One stream is collected from the region close to the inner channel wall and this carries the WBCs that are not influenced by the magnetic field. The other is collected from the region adjacent to the outer wall and this carries the RBCs. By the convention of QMS, these have flow rates of $Q(a)$ and $Q(b)$, respectively.

The merging of the fluid streams at the channel inlet results in a virtual cylindrical surface within the channel dividing fluid elements originating at the two different sources, the position across the channel thickness depending on the ratio of the flow rates. Similarly, the position of the virtual cylindrical surface between fluid elements exiting to each side of the outlet flow splitter depends on the ratio of the outlet flow rates. The positions of these inlet and outlet splitting cylindrical surfaces are easily calculated, and with knowledge of the magnetophoretic mobility of the RBCs as well as the magnetic field and field gradient across the channel, the flow rates for cell separation may be optimized [12]. This theory for optimization shows that the total throughput TP, in terms of the number of RBCs removed from a cell suspension, for an array of N_a QMS channels is given by

$$TP = cN_aQ(a') = 2\pi cN_aL\frac{\langle B_o^2\rangle}{\mu_0}\frac{I_2[\rho_i, \rho_{ISS}]}{I_1[\rho_i, \rho_{OSS}]}m_1 \tag{1}$$

where c is the RBC number concentration, $Q(a')$ is the volumetric flow rate of the cell suspension introduced to a single annular channel, L is the axial length of the magnetic field (taken as the vertical dimension of the magnet assembly), $\langle B_o^2\rangle$ the magnitude of the magnetic flux density squared at the inner surface of the outer channel wall (averaged over the array), and $\rho = r/r_o$ is the dimensionless radial coordinate scaled to the annular flow channel outer wall radius, r_o (Table 1). Characteristically, the dependence of throughput on the field gradient does not appear explicitly in Eq. (1), because the quadrupole field is a linear function of the radial coordinate. For a given $\langle B_o^2\rangle$ the field gradient and therefore RBC migration velocity across the channel thickness is inversely dependent on r_o. However, for a fixed channel thickness w, the channel volume increases withr_o, resulting in a slower fluid flow velocity along the channel for fixed volumetric flow rates, almost exactly compensating for the reduced RBC migration velocity. Here $\mu_0 = 4\pi \times 10^{-7}$ Tm/A, and m_1 is the critical mobility for an RBC to migrate from the inner channel wall to the outlet virtual splitting surface before exiting the channel. It follows that all RBCs with mobilities greater than or equal to m_1 are predicted to exit at outlet b in the $Q(b)$ outlet stream. There are two more terms included in Eq. (1),

Table 1
Magnet, magnet array, flow and RBC parameters.

Parameter	Unit	Value
Magnet aperture diameter	mm	9.65
Magnet block dimensions:	mm	
width, b		Variable, see text
length, L		203.2
thickness		4.75
Annulus inner radius, r_i	mm	3.97
Cylinder outer radius	mm	4.76
Annulus outer radius, r_o	mm	4.36
Annular width, $w = r_o - r_i$	μm	390
NdFeB N42, H_c	A/m	9.79×10^5
NdFeB N42, B_r	T	1.30
Total flow rate, Q	mL/min	0.833
Inlet flow rate ratio, $Q(a')/Q$	–	0.20
$\rho_{ISS} = r_{ISS}/r_o$	–	0.937
Outlet flow rate ratio, $Q(a)/Q$	–	0.25
$\rho_{OSS} = r_{OSS}/r_o$	–	0.940
Deoxy RBC magnetophoretic mobility	mm^3/TAs	$(4.17 \pm 2.08)\times10^{-6}$
RBC number concentration	N/mL	5.0×10^8

the meaning and origin of which may be fully understood by referring to the original derivation of the theory [12]. The term $I_1[\rho_i, \rho_{OSS}]$ is the solution to an integral associated with RBC trajectory within the annular channel from initial to final radial positions (both rendered dimensionless by division by the radius of the outer wall r_o), in this case from the inner channel wall $\rho_i = r_i/r_o$ to the outlet splitting surface $\rho_{OSS} = r_{OSS}/r_o$. The term $I_2[\rho_i, \rho_{ISS}]$ is the solution to an integral associated with calculation of volumetric flow rate between two limits in ρ, in this case for the $Q(a')$ flow stream between ρ_i and the inlet splitting surface at $\rho_{ISS} = r_{ISS}/r_o$. These are given by

$$\begin{aligned} I_1[\rho_i, \rho_{OSS}] &= [4\ln\rho - 2\rho^2 + 2A_2(\ln\rho)^2]_{\rho_i}^{\rho_{OSS}} \\ I_2[\rho_i, \rho_{ISS}] &= [2\rho^2 - \rho^4 + 2A_2\rho^2\ln\rho - A_2\rho^2]_{\rho_i}^{\rho_{ISS}} \end{aligned} \tag{2}$$

in which $A_2 = (1 - \rho_i^2)/\ln(1/\rho_i)$.

The tessellation study was designed to explore the influence of both the number of flow channels of fixed dimensions (arranged in regular square arrays) and the dimensions of the magnets between the channels on the predicted RBC sorting throughput. The decrease of magnet size increases the density of the channels in the cross sectional area of the magnet array but may adversely affect throughput because of the expected decrease in value of the parameter $\langle B_o^2\rangle$. This is illustrated in Fig. 1 which shows four different flow channel arrays (N_a=1, 4, 9, and 16) and three different magnet block widths (b=25.4 mm, 12.7 mm and 6.35 mm, with the other two dimensions held constant, see Table 1, designated Quad 1, Quad 2, and Quad 3, respectively). The concomitant decrease of the magnetic field B_o at the outer channel wall with decreasing size of the magnet blocks (with the flow channel diameter held constant) is illustrated in Fig. 2. To simplify the analysis, the inlet and outlet flow rate ratios were held constant, fixing ρ_{ISS} and ρ_{OSS}, and the other, unrelated parameters, including cell parameters (m_1, c) and the channel geometry (L, ρ_i) were grouped into a constant K, and the throughput, TP, was calculated by varying only parameters N_a and $\langle B_0^2\rangle$ The results are reported as the normalized throughput, TP' with respect to parameter K:

$$\begin{aligned} K &\equiv \frac{2\pi cLm_1}{\mu_0}\frac{I_2[\rho_i,\rho_{ISS}]}{I_1[\rho_i,\rho_{OSS}]} \\ TP &= N_a\langle B_o^2\rangle K \\ TP' &= TP/K = N_a\langle B_o^2\rangle \end{aligned} \tag{3}$$

where the normalized throughput, TP', has dimensions of tesla^2 (T^2). The results are also reported as the ratio of TP' to the surface, S tessellated by the magnets, TP'/S (in T^2/m^2) that is a measure of efficiency of such a tessellated magnet design.

The distribution of the RBC at the sorter outlet was calculated by