MICROFLUIDIC MAGNETOPHORETIC DEVICE AND METHODS FOR USING THE SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. application Ser. No. 11/583,989 filed on Oct. 18, 2006 now U.S. Pat. No. 7,807,454, which application is incorporated herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

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BACKGROUND

Sorting cells based on their surface markers is an important capability in biology and medicine. Magnetic Activated Cell Sorting (MACS) has become widely used as a cell sorting technique because it allows the rapid selection of a large number of target cells. The applications of MACS span a broad spectrum, ranging from protein purification to cell based therapies. Typically, target cells are labeled through a superparamagnetic particle that is conjugated to a molecular recognition element (e.g. a monoclonal antibody) which recognizes a particular cell surface marker.

Application of MACS has typically been limited to pre-enrichment before fluorescence-based cytometry. Nevertheless, due to its high throughput compared to other methods such as Fluorescence Activated Cell Sorting (FACS), MACS is still a widely used technology.

Current MACS systems are capable of high-purity selection of the labeled cells. However, they operate in a "batch mode" where the non-target and target cells are sequentially eluted after the application of the external magnetic field. In other words, the cells attached to magnetic particles are held in place while the unattached cells are eluted. Then, after this first elution step is completed, the magnetic field that prevented the magnetic particles from being eluted is removed and the magnetic particles can be eluted and recovered to recover target cells.

In order to achieve higher throughput and higher recovery of the rare cells (or other target components), improvements on existing MACS systems are needed.

SUMMARY

Embodiments of the invention provide a microfluidic device employing one or more sorting stations for separating target species from other species in a sample. The separation is driven by magnetophoresis. A sorting station generally includes separate buffer and sample streams. A magnetic field gradient applied to the sorting station deflects the flow path of magnetic particles (which selectively label the target species) from a sample stream into a buffer stream. The buffer stream leaving the sorting station is used to detect or further process purified target species labeled with the magnetic particles.

One aspect of the invention pertains to microfluidic sorting devices having the following features: (a) at least one inlet channel configured to provide separate streams of a sample and a buffer; (b) a sorting station fluidly coupled to the at least

one inlet and located in a path of the sample stream; (c) a magnetic field gradient generator; and (d) at least one outlet channel configured to separately receive the buffer stream with deflected magnetic particles and a waste stream containing the sample at least partially depleted of the target species. The sample may include at least magnetic and non-magnetic particles. In some cases it includes a target species, non target species, and magnetic particles having an affinity for the target species in the sample. The buffer is generally substantially free of the sample. The magnetic field gradient generator is designed or configured to interact with an external magnetic field to produce a change in magnetic field gradient in the sorting station and thereby deflect the magnetic particles toward the buffer stream.

The sorting devices may have various flow inlet and outlet configurations. For example, the at least one inlet channel may include a first inlet channel for providing at least a portion of the buffer stream and a second inlet channel for providing at least a portion of the sample stream. In certain embodiments, the device may have (i) a first inlet channel for providing the buffer stream, and (ii) a second and third inlet channels for providing separate streams of the sample. The second and third inlet channels may be located on opposite sides of the first inlet channel, such that, during operation, the buffer stream enters a sorting station straddled by two sample streams. In certain embodiments, the reverse is true: the sample stream enters a sorting chamber straddled by two buffer streams.

In some embodiments, the outlet of sorting device includes (i) a first outlet channel for collecting at least a portion of the buffer stream containing purified target species; and (ii) a second outlet channel for collecting at least a portion of the sample stream. The second outlet channel may be sized and positioned to collect a separate portion of the buffer stream. In certain embodiments, the device includes (i) a first outlet channel for collecting at least a portion of the buffer stream containing purified target species; and (ii) a second outlet channel and a third outlet channel for collecting separate streams of the sample. In other embodiments, the first outlet channel is for collecting sample and the second and third outlet channels are for collecting buffer. In either case, the second and third outlet channels may be located on opposite sides of the first outlet channel.

The magnetic field gradient generator may also have various configurations. In certain embodiments, it includes a plurality of ferromagnetic elements (e.g., strips or pins) patterned on the sorting device proximate sorting station. These shape the magnetic field from an external source to provide a desired magnetic field gradient. The external magnetic field may be provided by a permanent magnet or an electromagnet proximate the plurality of ferromagnetic elements. In certain embodiments, the magnetic field gradient generator comprises two permanent magnets located on opposite sides of the plurality of ferromagnetic elements. In some embodiments, the plurality of ferromagnetic elements is disposed within a fluid pathway of the sorting station to allow fluid contact between the ferromagnetic elements and the sample stream.

The sorting device may include one, two, or more magnetic field gradient generators, each imposing a magnetic field gradient on flowing magnetic particles. In some designs, at least two magnetic field gradient generators are located in fluid paths for two separate sample streams, which may be provided on opposite sides of a fluid path for a buffer stream.

In some designs, the sorting device allows magnetic particles to continuously flow past the magnetic field gradient generator without being captured. In other designs, the mag-