

METAL-POLYMER HYBRID MICROCHANNELS FOR MICROFLUIDIC HIGH GRADIENT SEPARATIONS

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INTRODUCTION: Microfluidic devices have long promised to revolutionize the diagnostic world by providing high throughput small volume automated assays at an affordable price. To date however, microfluidic technology has not fully delivered on its promise. It is still difficult to integrate individually successful microfluidic devices into a single automated sample to answer device.

One difficult integration to achieve is front end sample processing such as cell or nucleic acid purification with back end amplification or analysis. Central to this problem is that typical microfluidic devices operate with fluid volumes of less than 10 microliters and unprocessed sample volumes are in the range of milliliters. This discrepancy cannot be ignored for many clinically important rare targets such as bacteria during sepsis or disseminated tumor cells in peripheral blood. Typical microfluidic sample volumes are unlikely to contain extremely rare targets (1). Efficient processing of large sample volumes (>1ml) is required to capture enough of these rare targets for analysis. Immunomagnetic separation is a very attractive solution to this problem because of its ability to rapidly, efficiently and selectively separate targets present in complex biological fluids.

Many magnetic label manufacturers now offer products that can bind to important targets or to primary antibodies to these targets. Large particulate super paramagnetic particles on the order of ~1-5 μm diameter can be easily manipulated in relatively weak magnetic fields, but smaller sub-micron particles require strong gradient fields. The smaller particles offer important advantages in speed and efficiency of target labeling (2), but they complicate device design. In order to fully take advantage of magnetic separation in the microfluidic environment, inexpensive, easily integrated devices capable of forming high magnetic gradients must be developed.

The ability of such devices to replace conventional biological protocols has already been demonstrated by Immunicon's innovative CPAS (formerly Celltracks) system (3). The system uses

sputtered micron sized magnetic lines and CD-ROM technology for optical detection and is designed to replace the functions of much more expensive cell analysis such as flow cytometry.

The devices we present here can also be used for direct optical analysis of rare targets, but they were designed to act as a sample preparation unit for further microfluidic processing by genetic amplification and hybridization devices. They consist of sub-millimeter iron or nickel-iron matrix elements fabricated into acrylic and polycarbonate substrates that geometrically concentrate external magnetic flux to form the necessary magnetic gradients. Initial prototypes used randomly distributed iron beads as matrix elements (Fig. 1).

Although results achieved in these structures were encouraging, difficulties in the manufacture of reproducible devices prompted the exploration of alternative more controllable and more easily manufactured designs. Preliminary results for laser machined electroplated devices are promising. For these devices magnetic gradients can be precisely controlled by manipulating the size and or shape of the matrix elements.

In both designs, targets are collected at the walls of the channel where they can be washed or otherwise processed in a flow stream. The channels can concentrate milliliter volumes greater than 200 fold in just a few minutes and can be integrated with other polymer based downstream microfluidic amplification and analysis devices. Trapping of bacteria, mammalian cells and target antibodies are shown.

METHODS: *Device fabrication.* Iron sphere device prototypes consisted of 50-300 μm diameter iron spheres (Starmet, Concord, MA) imbedded by compression molding into acrylic or polycarbonate channels. Channels in both acrylic and polycarbonate structures were formed by laser cutting desired channel shapes in 100 μm thick double sided tape (3M, St. Paul, MN). Acrylic channels were covered with microscope cover glass to facilitate epi-fluorescent microscope viewing. For polycarbonate structures two iron sphere containing sheets were taped together.

Plated prototype devices were made using a 40 W CO₂ laser engraving system (Universal Laser

Systems, Scottsdale, AZ). Laser machined acrylic sheets were sputtered with a gold seed layer prior to plating in a low temperature nickel-iron bath (4). Plating thickness was typically 100 μm . Channels were formed using cut tape and cover slips. Samples were pumped through all devices using a micro peristaltic pump (Instech, Plymouth Meeting, PA) fitted with silicon tubing and barbed connectors. 10mm x 10mm x 5 mm thick Neodymium magnets (magnetized through the thickness), were placed on the device surface to generate the external magnetic field. Devices with matrix elements on both top and bottom were fitted with N to S facing magnets on either side of the device. Glass covered channels were fitted with magnets only on the matrix (opaque) side. A substantially uniform magnetic field of ~ 0.7 T was observed in channels with magnets on both sides and a variable field of ~ 0.2 - 0.4 T was observed at the surface of channels with a single magnet.

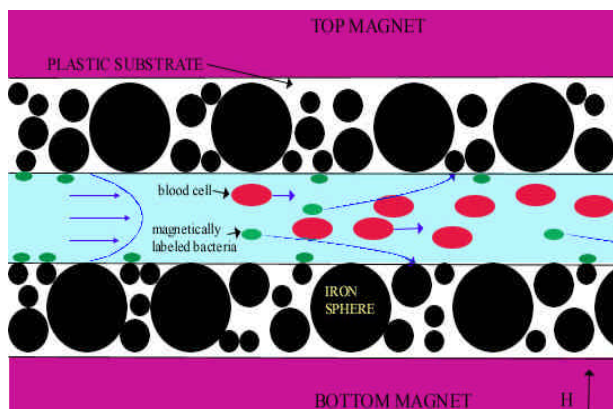


Fig. 1: Schematic of the structure containing spherical matrix elements on both top and bottom of the channel.

Target capture protocols. Citrated sheep blood (Colorado Serum Company) was diluted 1:1 with phosphate buffered saline (PBS)/5mM EDTA pH 7.4 and inoculated with 100-500 cfu of E.coli K12 per ml. 10ul of anti-E. coli biotinylated polyclonal antibody (Virostat, Portland Maine) was incubated with the sample for 15 minutes at room temperature on a rotary mixer. 20 ul of colloidal super paramagnetic streptavidin microbeads (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) were added and another 15 minute incubation was performed. Samples were pumped through the devices at various flow rates. Fractions were collected and plated overnight on LB agar. Capture efficiency was determined by comparing the number of colonies in a given volume pumped through the device with the magnets alternately off and on.

A GFP expressing line of E. coli K12 was used to generate fluorescent images. In imaging experiments a much higher density of $\sim 100,000$ cfu per ml was used.

Rabbit white blood cells were captured using anti-CD45 monoclonal antibody conjugated to FITC (Molecular Probes, Eugene, OR) and anti-mouse IgG1 super paramagnetic microbeads (Miltenyi Biotec). Rabbit blood was diluted 1:1 with PBS/EDTA and incubated with primary antibody for 15 min as before. 40 μl of microbeads were incubated as before for magnetic labeling. For capture of IgG the anti-CD45-FITC antibody was incubated with anti-mouse IgG1 microbeads for 5 minutes in PBS/EDTA.

RESULTS: Capture of bacteria. Plating experiments of E.coli inoculated blood demonstrated that large sample volumes could be concentrated in minutes with relatively high recovery rates. About 50% recovery was obtained at the maximum tested average flow rate of ~ 19 mm/s. This corresponded to ~ 350 $\mu\text{l}/\text{min}$ in the device tested. Epi-fluorescent video of magnetically labeled GFP expressing E. coli was used to visually confirm the capture of E.coli at the surface of imbedded matrix elements.

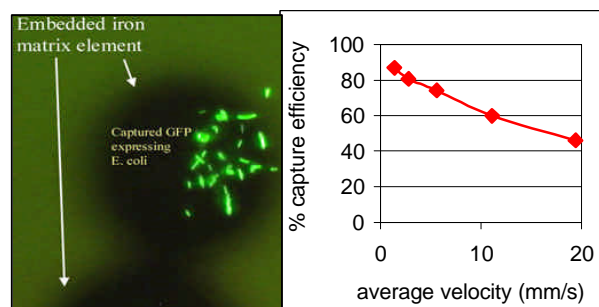


Fig. 2: Epi-fluorescent image of magnetically labeled GFP expressing E. coli captured at the surface of an imaging device (left). Graph showing capture efficiency vs. average flow velocity in polycarbonate devices with 100 μm channel height (right).

Capture of Mammalian cells and IgG. Preliminary studies of capture of these targets in ridge structures demonstrated that they could be captured in high velocity flow streams and localized onto the tips of the saw-tooth ridge elements. Although capture rates were not quantified, sample flow at the inlet and outlet were observed through the epi-fluorescent microscope. The majority of labeled cells were depleted from the flow stream at flow velocities up to 4mm/s. Similar results were observed in both sphere imbedded and plated ridge structures.

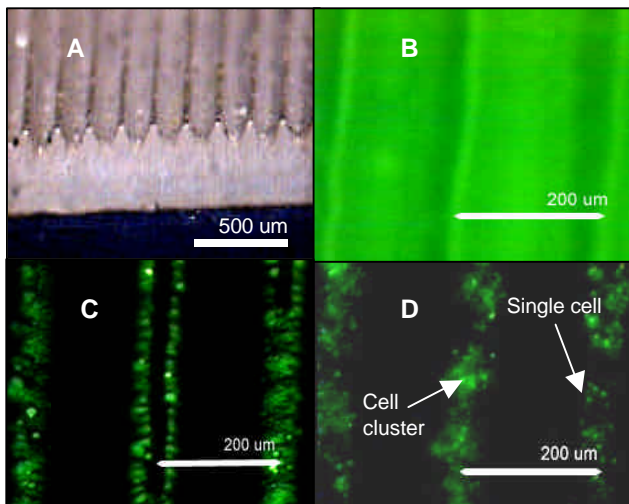


Fig 3: Laser machined acrylic ridge structure prior to electroplating (A). Ni-Fe ridge device with fluorescein labeled CD45 antibody coupled to IgG1 specific magnetic beads prior to separation (B). Same device after separation. Fluorescence is focused on the ridges (C). Similar device after separation of white blood cells using the fluorescent anti-CD45 antibodies (D).

DISCUSSION & CONCLUSIONS: The efficient capture and concentration of rare targets for downstream analysis is an important problem facing the advancement of microfluidic diagnostics. The devices presented here demonstrate that this problem can be effectively and inexpensively addressed with metal-polymer hybrid microchannels. The ability to selectively capture labeled targets in a relatively high velocity flow stream permits the rapid concentration and purification necessary to analyze rare targets on a microfluidic scale.

While initial prototypes using imbedded iron spheres demonstrated efficient separation of bacteria from whole blood, the desire for highly reproducible and manufacturable structures led to the design of Ni-Fe plated ridge devices that promise to replace the initial prototypes. The plated prototypes shown here are limited by the laser used. It has low resolution (~100μm) and the features sculpted tend to be somewhat rough. With the application of more sophisticated fabrication technology however, the features can be resolved to the lithographical level. This kind of resolution would enable precise control of magnetic gradient properties. Larger features can produce more global gradients that can attract targets from further distances while smaller features can produce extremely high local gradients. Combining these features could result in devices capable of capturing very weakly labeled targets in high flow rate

devices. It would also permit high flexibility in designing for capture of different targets e.g. large and small cells viruses or macromolecules. Although a direct quantitative comparison of the capture rate for bacteria vs. mammalian cells was not done, it was obvious from the microscope studies that mammalian cells are more difficult to capture. Although mammalian cells are larger particles, their smaller surface to volume ratio gives them a lower overall volume magnetic susceptibility upon labeling. Their larger size also makes them more subject to drag forces from the velocity profile of the channel flow. Channels with greater heights can reduce this susceptibility but the matrix magnetic gradients tend to fall off very rapidly with distance from the channel surface.

Future studies will focus on integration with downstream components, optimization of ridge structures and quantitative testing with relevant models and actual clinical samples.

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