Track Etched Magnetic Micropores to Efficiently Sort Rare Pathogens from Large Volume, Unprocessed Clinical and Environmental Samples

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We developed a microfluidic chip to selectively isolate magnetically tagged cells from heterogeneous suspensions, the track-etched magnetic micropore (TEMPO) filter. The TEMPO consists of an ion track-etched polycarbonate membrane coated with soft magnetic film ($Ni_{20}Fe_{80}$). In the presence of an applied field, provided by a small external magnet, the filter becomes magnetized and strong magnetic traps are created along the edges of the pores. In contrast to conventional microfluidics, fluid flows vertically through the porous membrane allowing large flow rates while keeping the capture rate high and the chip compact. By utilizing track-etching instead of conventional semiconductor fabrication, TEMPOs can be fabricated with microscale pores over large areas $A > 1$ cm^2 at little cost (< 5 Cents/cm^2). The large density of micropores ($\rho = 10^6$ cm^{-2}) allows sorting directly from unprocessed environmental and clinical samples, as the blockage of a few pores does not significantly change the behavior of the device. The utility of this approach is demonstrated by sorting rare E. coli from unprocessed, large volume clinical (mouth wash, 10 mL) and environmental (river water, 10 mL) samples. Furthermore, an assay was developed using this chip to isolate rare Tuberculosis (TB) bacteria from mouth wash samples, for the sensitive diagnosis of Tuberculosis in HIV co-infected patients.

Introduction: The isolation of biological targets, such as circulating tumor cells (CTCs), pathogenic bacteria, or circulating microvesicles (CμVs) from easily accessible biological fluids is of great relevance for medical diagnostics, as well as agricultural and environmental monitoring. Detection platforms that utilize micro- and nanoscale structures, where dimensions can be designed to match those of the targeted object, have been utilized for highly selective sorting. However, the limited throughput and susceptibility to clogging of microscale devices, make these approaches unsuitable for many practical applications. Here, we report the development of a new approach to magnetic separation that maintains microfluidic's high enrichment and selectivity, but can sort cells from unprocessed large volume samples, and can be implemented on a chip that costs little to manufacture.

Materials and Methods: The TEMPO filter consists of a track-etched polycarbonate membrane coated with a thin layer of soft magnetic material (**Fig. 1a,b**). The micropores create large gradients ∇B (**Fig. 1c**), which imparts strong magnetic forces $F \sim (B\cdot\nabla)B$ on magnetic nanoparticle (MNP)-labeled cells as they pass through the pores. Targeted cells are trapped and isolated from the unlabeled cells, which flow through the filter unimpeded. The chip sits in a large uniform magnetic field $|B| = 0.2$ T, provided by a small external NdFeB magnet. This field magnetizes both the MNP-labeled cells and the soft magnetic material on the TEMPO filter. When the NdFeB magnet is removed, the force disappears and the trapped cells can be released.

Results and Discussion: To demonstrate the utility of our platform, a microfluidic chip with a 5 µm pore size filter was used to selectively and rapidly isolate *E. coli* from clinical (*i.e.* mouthwash) and environmental (*i.e.* river water) samples.(**Fig. 1d**) This chip is also being applied to the isolation of rare mycobacterium Tuberculosis (mTB) from the mouth wash of patients. A micro-magnetic labeling strategy has been developed that labels mTB with 200 nm magnetofluorescent particles. This labeling has been demonstrated to be specific to mTB, and mTB enrichment data from clinical samples will be presented.

Conclusions: The TEMPO filter uses the following elements to maximize sorting selectivity at very high flow rates in unprocessed samples. 1) Strong magnetic field B and gradients ∇B, to create strong magnetic traps, 2) A large area flow channel to obtain low hydrodynamic drag forces F_d at high flow rates Φ, and 3) Close proximity for each cell to the region of strong magnetic forces via its micropore geometry. 4) Many pores for robustness against clogging.

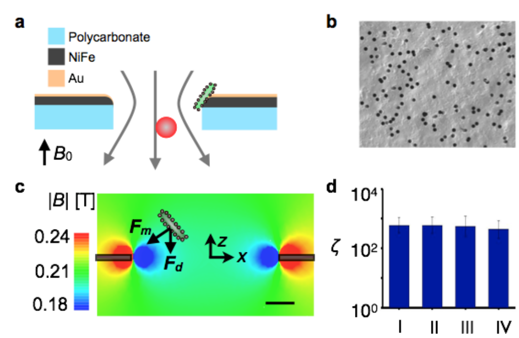

Fig. 1. **a.** A cross sectional schematic of the TEMPO, **b.** An SEM micrograph. Each pore has a diameter of 5 µm. **c.** Finite element simulation of magnetic field in a micropore. **d.** High enrichment of E. coil in I. PBS, II. With excess of MNPs, III. in mouth wash, IV. In river water.