Supplementary Information for Manuscript: Continuous and Quantitative Purification of T Cell Subsets for Cell Therapy Manufacturing using Magnetic Ratcheting Cytometry

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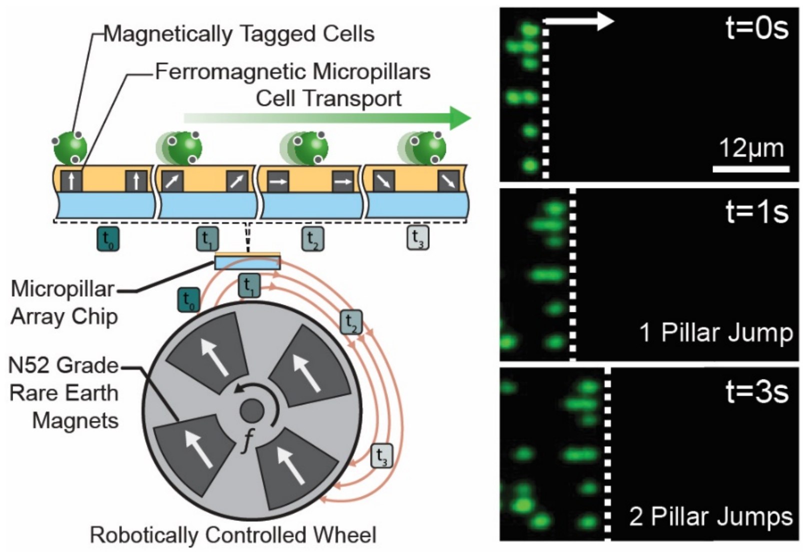
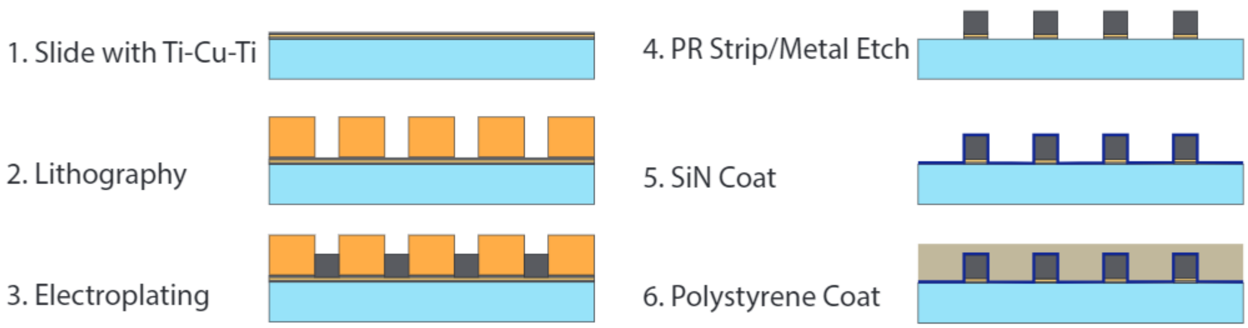
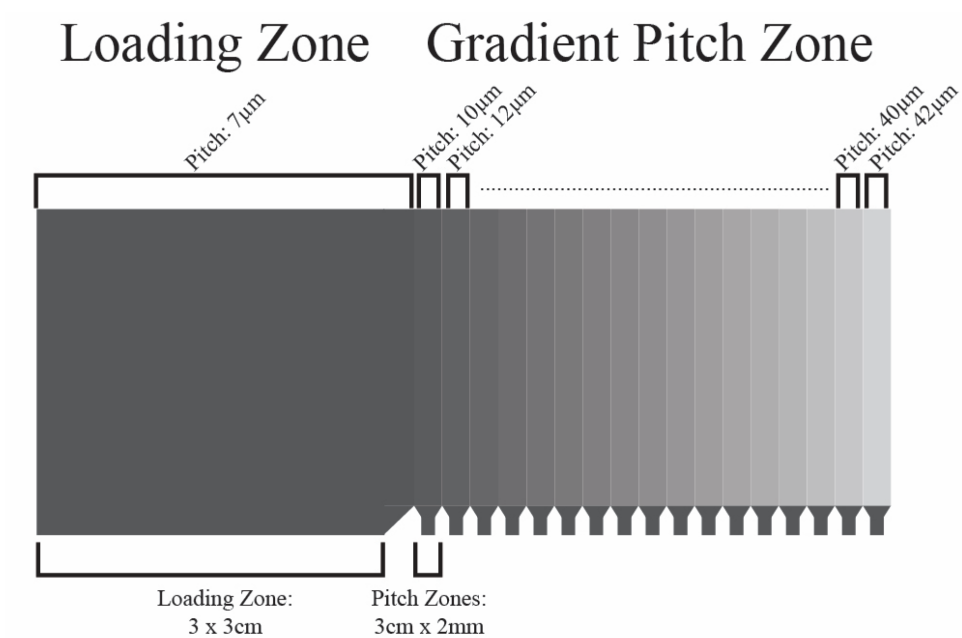


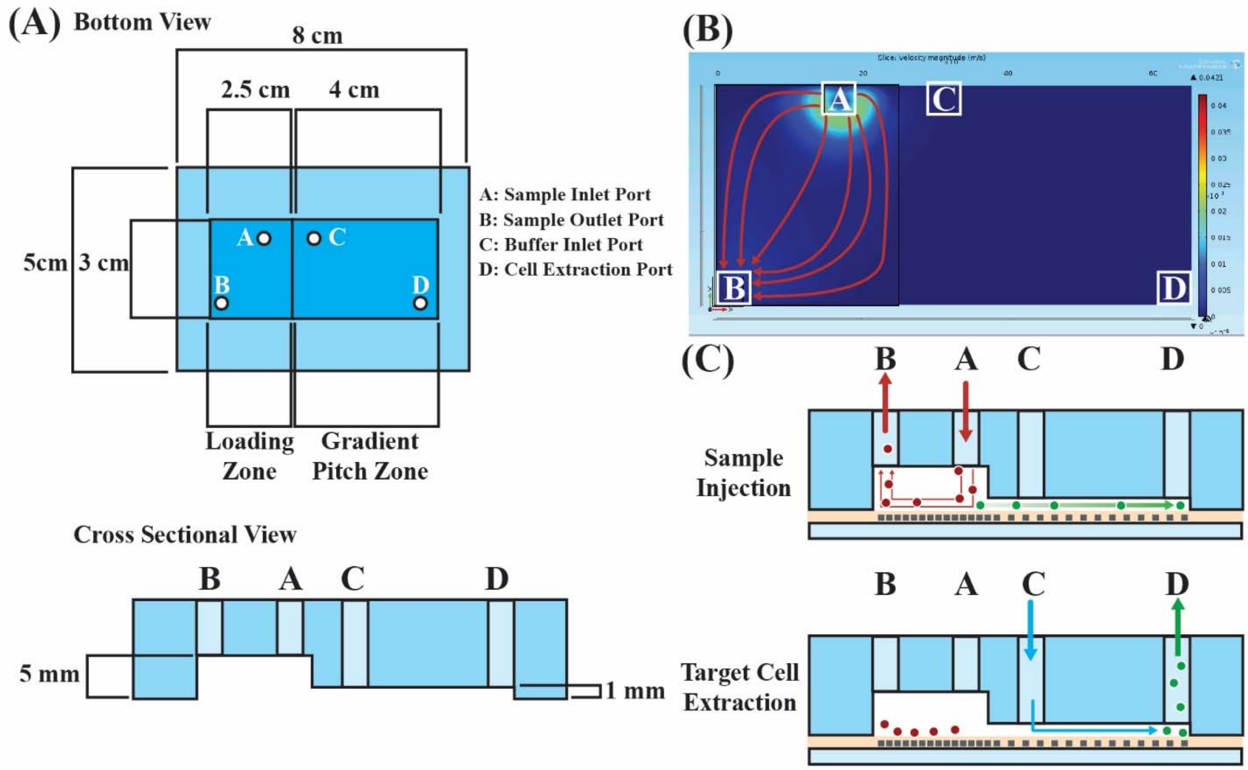
Supplementary Figure S1. Magnetic ratcheting utilizes arrays of ferromagnetic micropillars to create a micro-magnetic field landscape with shifting magnetic field maxima associated with each micro-pillar which can be used to trap and transport superparamagnetic particles. A directionally cycling magnetic field is provided by a robotically controlled wheel of N52 grade neodymium rare earth magnets which are arranged in a Halbach Array orientation. When proximal to the rotating magnetic wheel, the micro-pillars within the chip magnetize in alignment to the bulk field, introducing shifting field maxima in which superparamagnetic particles migrate at a rate proportional to the rotational frequency, *f*, of the applied field. Above a certain frequency or pitch between adjacent micro-pillars, particles fail to traverse to the next pillar, a process critically depending on the magnetic content of the particle.



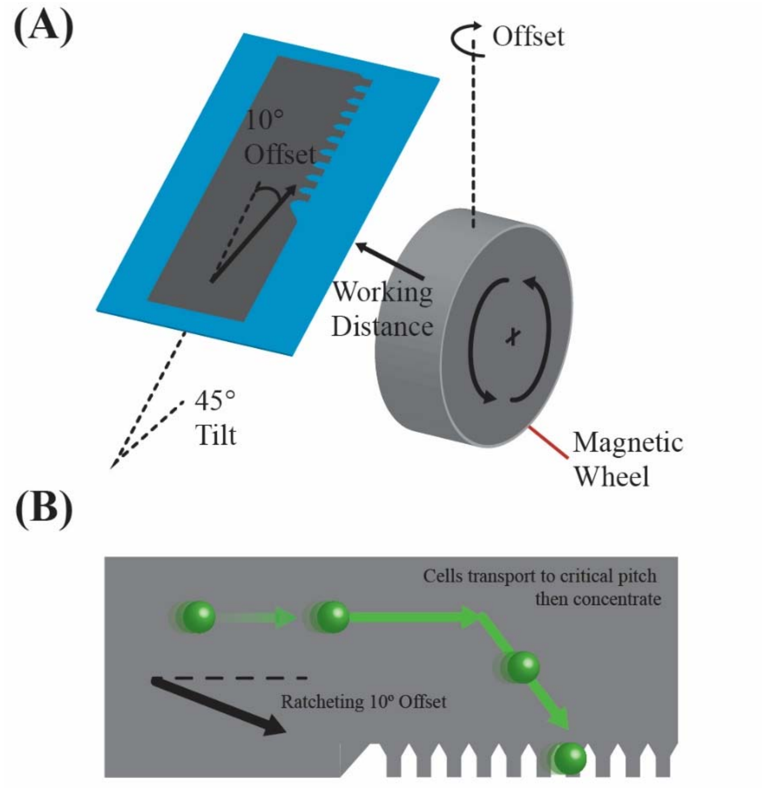
Supplementary Figure S2. Ratcheting chips are manufactured on 100mm glass wafers which are coated with a Ti-Cu-Ti layer via e-beam evaporation to form an electrode layer. Electroplating to form micro-pillar arrays are made by using lithography with SPR 220-3 photoresist. The Ti layer is then etched to expose the copper beneath where ferromagnetic pillars are then electroplated using a custom process. Pillars are electroplated to a height of 4µm. The photo resist is stripped suing acetone and the Ti-Cu-Ti layer is released using hydrofluoric acid and copper etchant to make the chip transparent. As an optional step, the elements are coated with Silicon Nitride layer (SiN) and diced to chips. The chips are then coated with spin on polystyrene (5% beight by volume polystyrene dissolved in toluene) to form a planarizing layer.



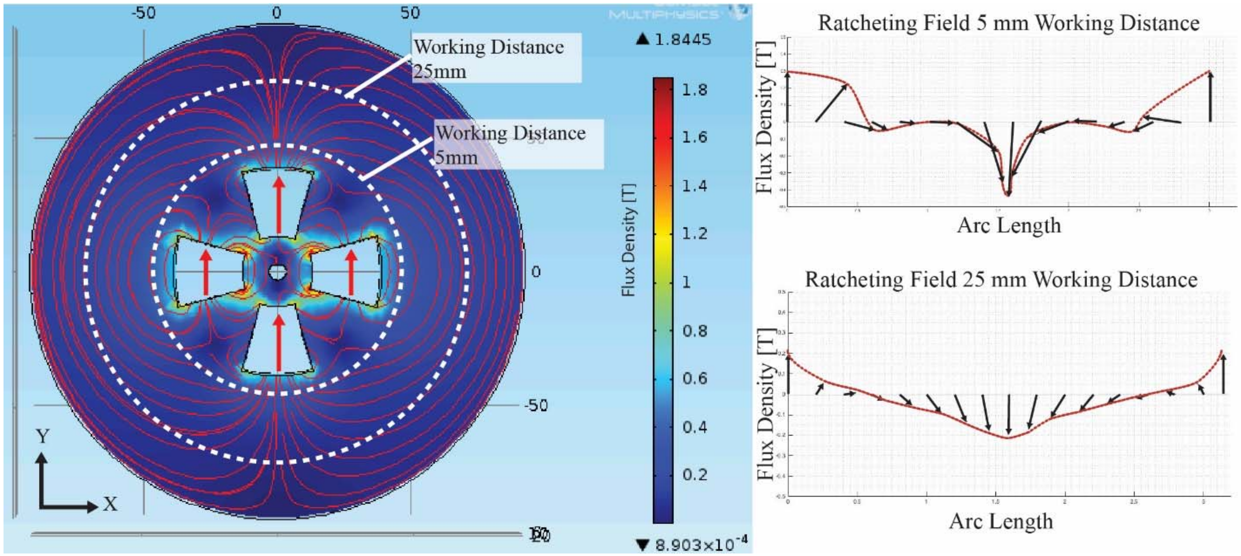
Supplementary Figure S3. Scaled ratcheting chip was 3cm in width and 6.5cm in length. The chip consists of a 3x3 cm loading zone with a 7µm pitch leading into a gradient pitch zone with sections of increasing pitch. Each pitch section is 2m long and 3cm wide and ranges in pitch from 10µm to 42µm and increments by 2µm.



Supplementary Figure S4. (A) Schematic and cross section of fluidic chamber that was clamped over the ratcheting chip to form a closed cartridge. The cartridge consists of a 3 x 2.5cm injection chamber with a 5mm depth leading into a thinner 1mm deep chamber which aligns to the gradient pitch zone. (B) Sample injection from port A to port B through the relatively deeper sample injection chamber the sample only flows over the chip loading zone and does not flow into the gradient pitch regions. (C) Once the sample has been injected and target cells separated into the gradient pitch regions, the flow is blocked in ports A and B and the target cells are extracted manually from port D using a syringe while drawing clean buffer from port C.



Supplementary Figure S5. (A) To achieve optimal ratcheting the chip must be positioned properly relative to the magnetic wheel. The chip was positioned with a 45º tilt relative to horizontal to prevent the flow of non-target cells into extraction region. The working distance defined as the tangential distance between the chip and the surface of the magnetic wheel was adjusted to balance magnetic field strength and field continuity. The ratcheting offset is defined as the angle of the ratcheting vector relative to the long axis of the ratcheting chip which was found to be optimal at 10º. (B) The ratcheting offset enabled cells to be continuously separated on the gradient pitch regions then concentrated to the edge of the chip for extraction.



Supplementary Figure S6. (A) To quantitatively asses the working distance in addition to field visualization with iron filings a Comsol simulation was performed to observe the ratcheting field magnitude and shape at different working distances. (B) At small working distances the field has a peak strength of 400mT but demonstrates abrupt changes in the magnetic field direction which is not ideal for continuous ratcheting operation. As expected a larger working distance demonstrated a lower peak strength but continuous operation. Our findings show that an optimal working distance which demonstrates and ideal and continuously directionally cycling magnetic field was between 20-25mm which was consistent with our observations of the field visualizations using iron filings.

Supplementary Video 1. Video described the concept of magnetic ratcheting transport on ferromagnetic micro-pillar arrays.

Supplementary Video 2. Video illustrates the height calibration of the cartridge saddle in reference to the magnetic wheel to obtain an optimal magnetic ratcheting field.