Magneto-Mechanical Force-Induced T Cell Activation for Efficient *ex vivo* T Cell Expansion

Jaehee Seo[1], Sujin Lee[2,3], Minsuk Kwak[3]

1. Department of Biochemistry, Yonsei University, Seoul 03722, Korea
2. Graduate Program of Nano Biomedical Engineering (NanoBME), Advanced Science Institute, Yonsei University, Seoul 03722, Korea
3. Institute for Basic Science (IBS), Center for Nanomedicine, Seoul 03722, Korea

Introduction

Adoptive T cell therapy (ACT) using tumor-infiltrating lymphocytes reinfuses tumor-cytotoxic T cells, activated and expanded *ex vivo*, to immunity-deprived patients, reinforcing the patients' immune response against tumor (**Figure 1**). Studies on ACT are set to continue as many cancer types persist the lack of antitumor activity-present effector T cells on tumor sites and is time-consuming.

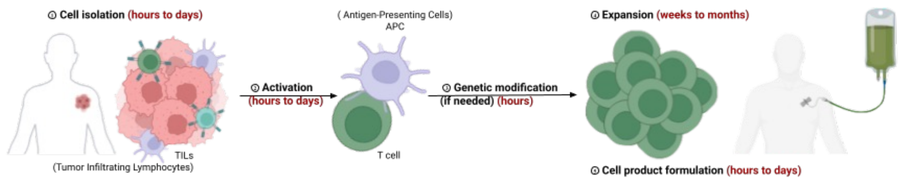


Figure 1. Adoptive cell therapy (ACT) using tumor infiltrating lymphocytes (TIL)

When T cells attach to antigen-presenting cells, they form MHC-TCR complexes that, through a cascade of reactions, lead to T cell activation. MHC-TCR complexes are known to amplify signaling and bond lifetime upon applied moderate mechanical force. Utilizing this, magneto-mechanical force-transductive superparamagnetic antigen-presenting cells, MNA nanoparticles, we opt to identify its performance when applied external rotating magnetic field-induced mechanical force. Activated T cells increase intracellular calcium levels. This property is used for the observation of T cell activation under the confocal microscope (**Figure 2**).

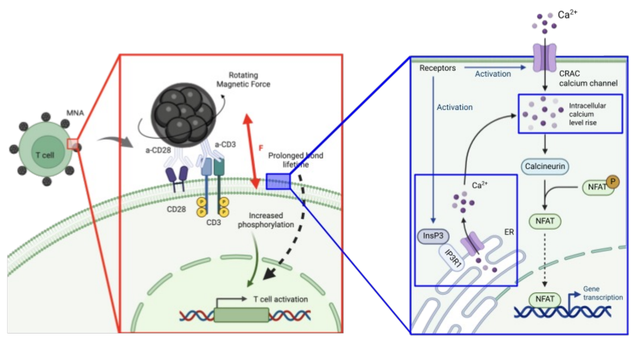


Figure 2. Overall scheme of magneto-mechanical force-induced T cell activation

Hypothesis

Research by Wauters, et al. reveal that size of APCs matter in T cell activation. Because commercial antigen-presenting cells (positive control) is 11.25 folds greater than that of MNA nanoparticles, it is likelier that MNA nanoparticles will activate less (**Figure 3**). However, TCR bonds stabilized by moderate mechanical force, forming catch bonds, have greater bond lifetime (**Figure 4**). Therefore, we hypothesize that T cells, attached to artificial antigen-presenting cells (aAPCs) with superparamagnetic characteristics, MNA, are asserted mechanical force through rotating magnetic fields (RMF), will rotate in coordination with mechanical force, leading to increments in bond lifetime, hence increased T cell activation (**Figure 5**).

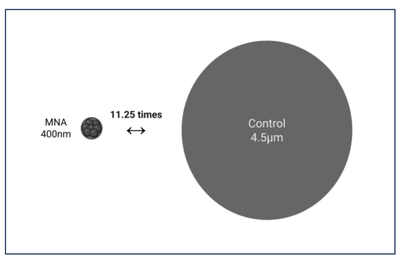


Figure 3. Size difference of MNA nanoparticles and Dynabeads (control)

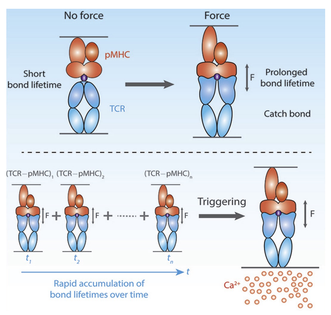


Figure 4. TCR catch bonds increase bond lifetime

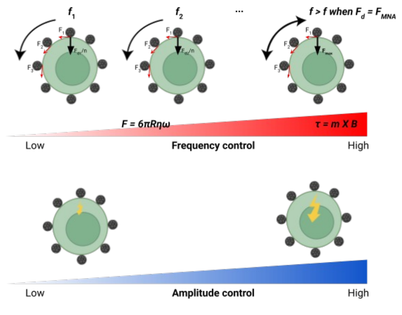
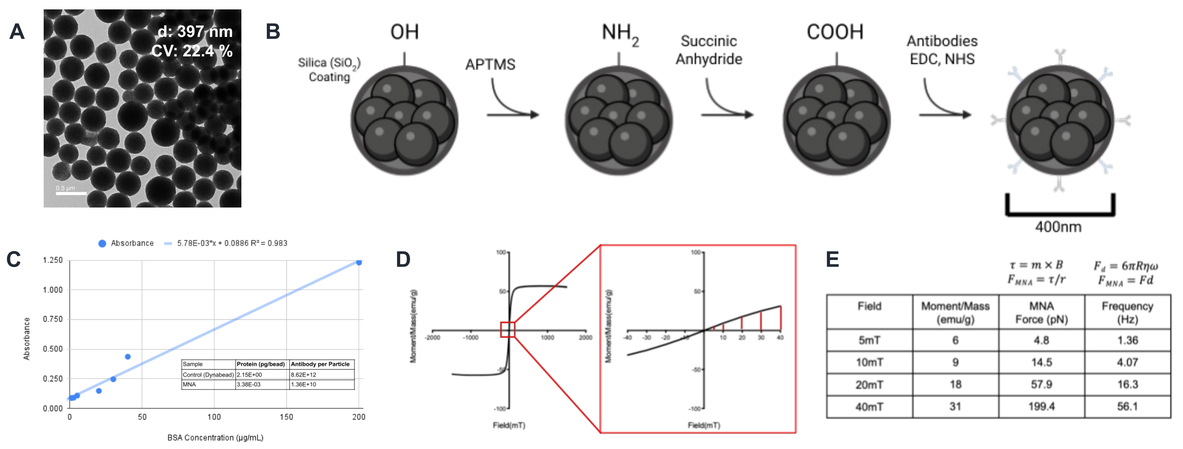


Figure 5. Effect of RMF frequency and amplitude

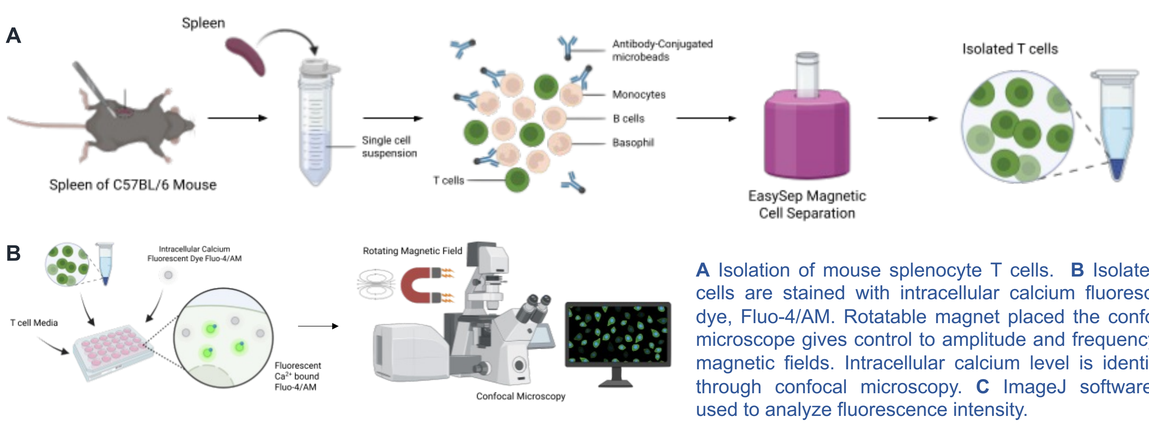
Experimental Methods

Figure 6. Fabrication and Characterization of MNA T Cell Activator



A SEM image of MNA nanoparticles. Diameter of each particle measures about 400nm. **B** Sequential chemical reactions attach anti-CD3 and anti-CD28 antibodies to superparamagnetic iron oxide nanoparticles (SPION). **C** Each MNA particle has 3.38 X 10^3 pg antibodies, measured with BCA assay. **D** Magnetic moment values of MNA nanoparticles, measured with VSM. **E** Optimal mechanical force is converted to magnetic force.

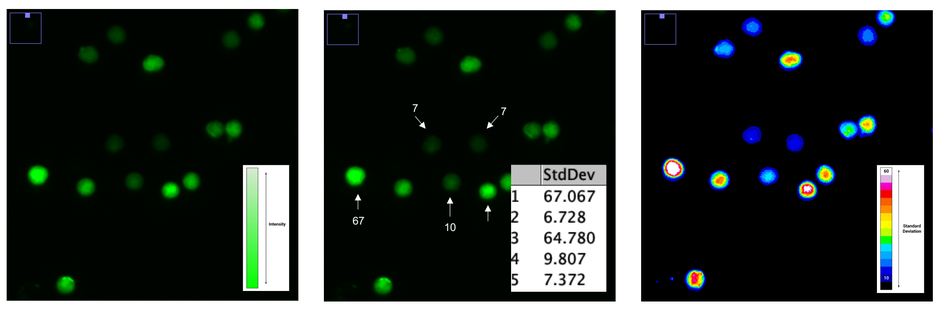
Figure 7. Calcium Flux Assay with Intracellular Calcium Indicator (Fluo-4/AM)



A Isolation of mouse splenocyte T cells. **B** Isolated T cells are stained with intracellular calcium fluorescent dye, Fluo-4/AM. Rotatable magnet placed the confocal microscope gives control to amplitude and frequency of magnetic fields. Intracellular calcium level is identified through confocal microscopy. **C** ImageJ software is used to analyze fluorescence intensity.

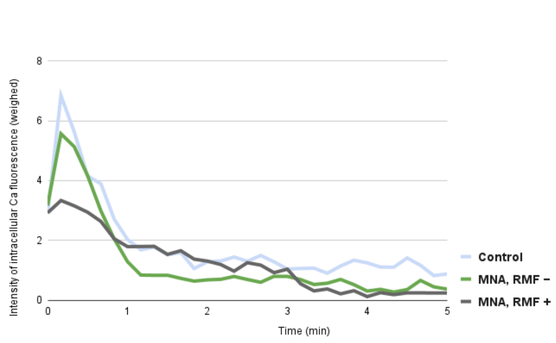
Results

Figure 8. Selection and Calculation of Activated T Cells



Activated T cells obtain high fluorescence intensity due to increased intracellular calcium levels. An imaging software is used to calculate standard deviation values of each cell. Values that surpass 60, we have identified as activated T cells.

Figure 9. Effect of Rotating Magnetic Force on MNA-Induced T Cell Activation



Graph 1. Effect of Rotating Magnetic Force on MNA-Induced T Cell Activation

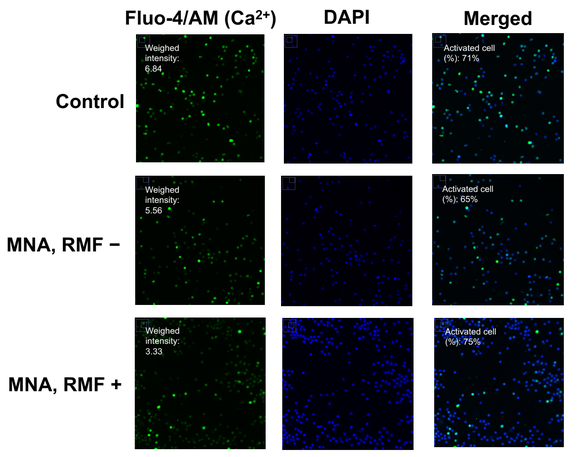
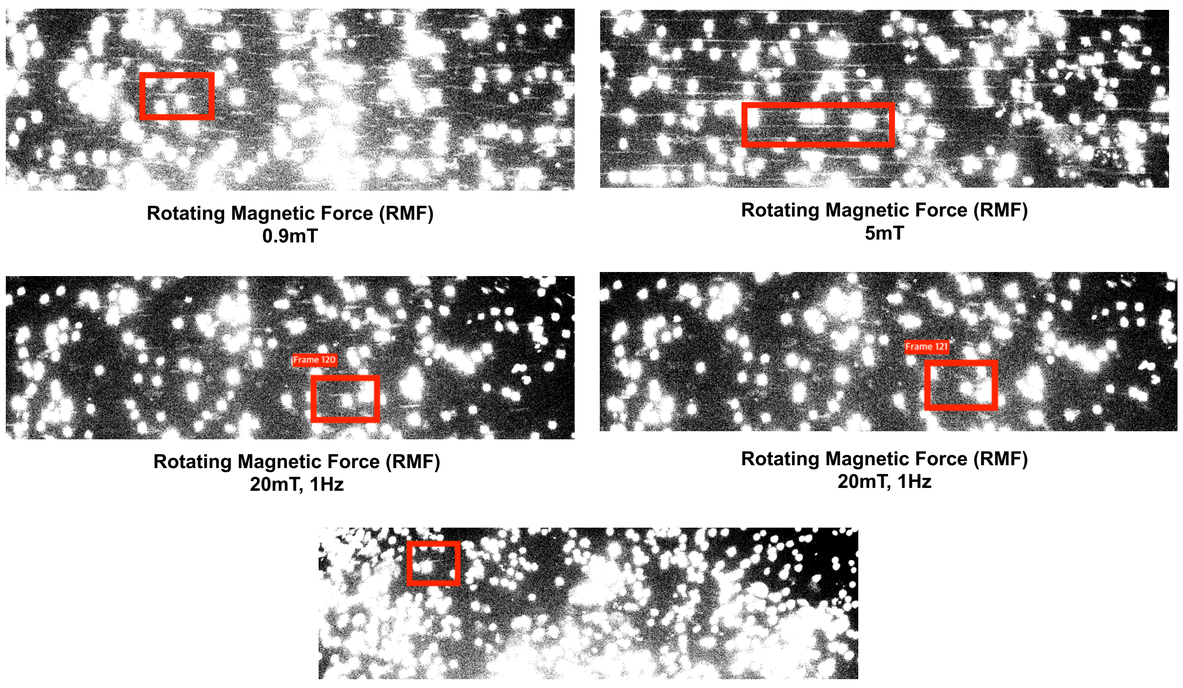


Figure 10. Observation of MNA Nanoparticle Alignment



Conclusion & Further Study

MNA nanoparticles applied no magneto-mechanical force activated 23% less T cells than Dynabeads. The difference of cell size between MNA and Dynabeads may contribute and explain this, as force applied by artificial antigen-presenting cells alone affects T cell activation (Wauters et al.). MNA nanoparticles applied magneto-mechanical force activated 67% less T cells than those without rotating magnetic force (RMF) (**Figure 9**). Reasoning upon this result is presumptuous, yet we were able to identify that MNA nanoparticles, unlike our hypothesis, instead of attachment on T cells during free-float in cell media, showed that it formed rod-like aggregates that align along magnetic fields (**Figure 10**). As amplitude of the magnetic field increases, the length of MNA nanoparticle rod-like alignment lengthens. When rotating magnetic fields are applied, rod-like MNA nanoparticle alignments rotate. As existing research reveal that shape of artificial antigen-presenting cell affects T cell activation, further studies on MNA nanoparticle-induced T cell initiation deems necessary.

The level of T cell activation, quantified indirectly through fluorescence calcium imaging (**Figure 8, Graph 1**), takes consideration of two factors: (1) the percentage of T cells with intracellular calcium and (2) the intensity of increased calcium levels weighed on total number of cells per frame. This calculation, however, cannot take consideration of the effect of T cell density on its activation. Although T cell density is assigned equal densities at confocal plate preparation stage, the number of cells shown on each frame changes due to cell movement. Therefore, it is necessary to observe the effect of MNA nanoparticles on T cell activation on single-cell scale.

Reference

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