Hydrogel Magnetomechanical Actuator Nanoparticles for CAR T cell therapy

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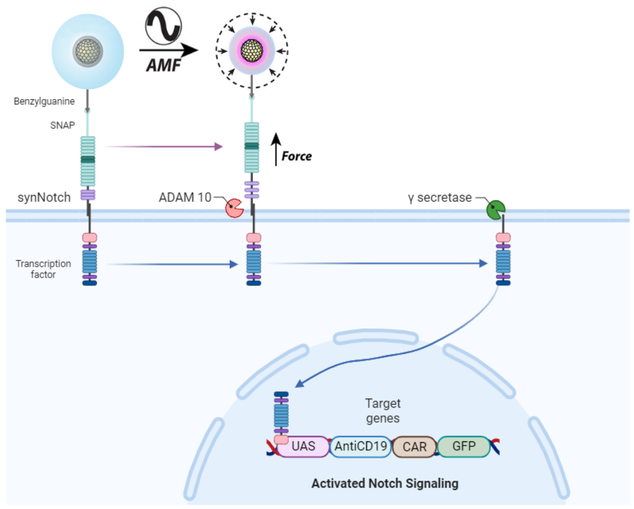
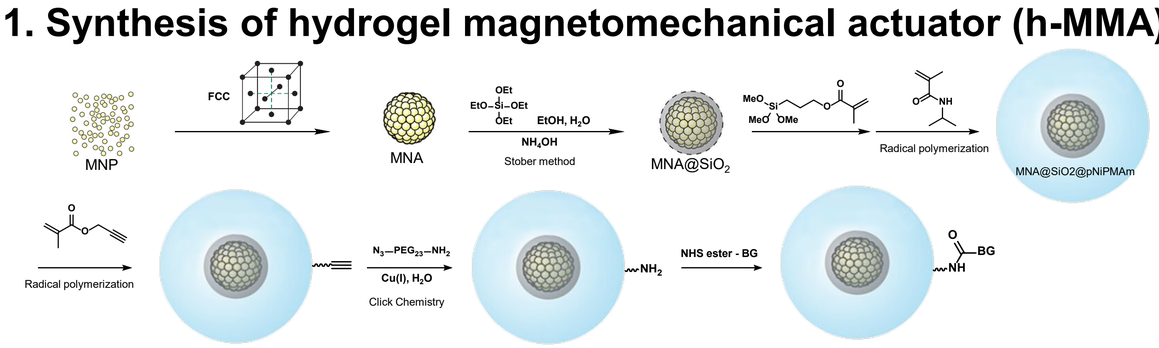
Introduction



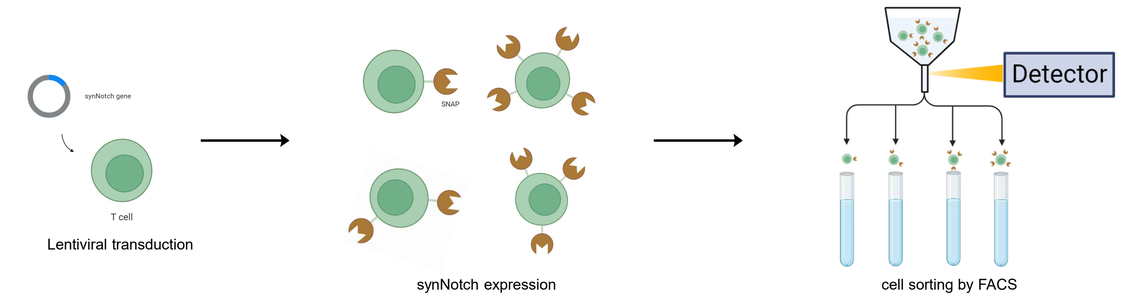
As a new enabling nanotechnology tool for wireless, target-specific, and long-distance stimulation of mechanoreceptors in vivo, here we present a hydrogel magnetomechanical actuator (h-MMA) nanoparticle. To allow efficient force generation, h-MMA integrates a two-step transduction mechanism that converts magnetic anisotropic energy to thermal energy within its magnetic core and then to mechanical energy to induce the surrounding polymer shell contraction, finally delivering forces to activate targeted mechanoreceptors. We show that h-MMAs enable on-demand modulation of Notch signaling in both fluorescence reporter cell lines and a xenograft mouse model, demonstrating its utility as a powerful *in vivo* perturbation approach for mechanobiology interrogation in a non-invasive and untethered manner.

Experimental Methods

1. Synthesis of hydrogel magnetomechanical actuator (h-MMA)



2. Cell sorting



Results

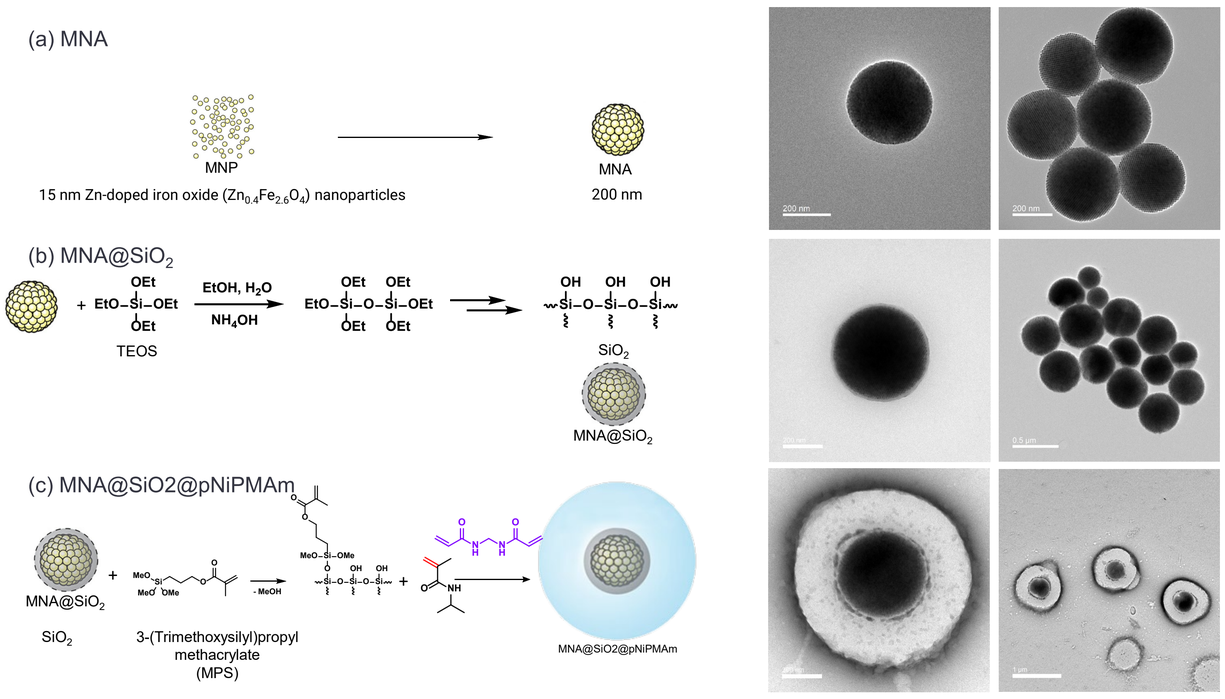


Fig. 1. Schematics and TEM images of (a) magnetic nanoparticle assembly (MNA) in a face-centered cubic (FCC) superlattice structure, (b) MNC@SiO_2, and (c) MNC@SiO_2@pNiPMAm. (left) a single particle, (right) a set of particles.

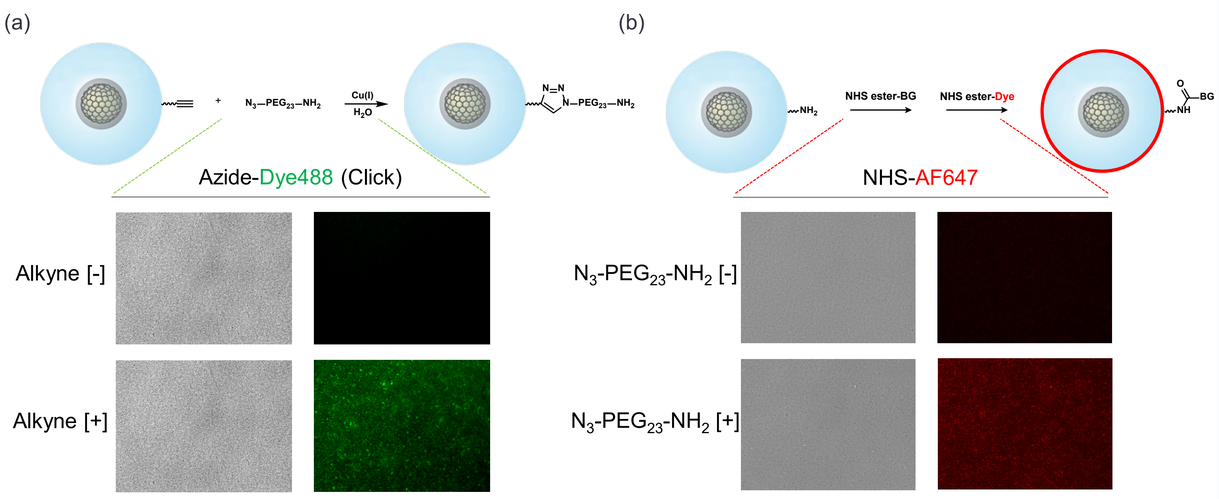


Fig. 2. EVOS image of (a) azide-alkyne cycloaddition click chemistry with dye488 to functionalize to amine group and (b) nucleophilic attack of amine to NHS ester to functionalize with benzylguanine and to label with AF647 dye.

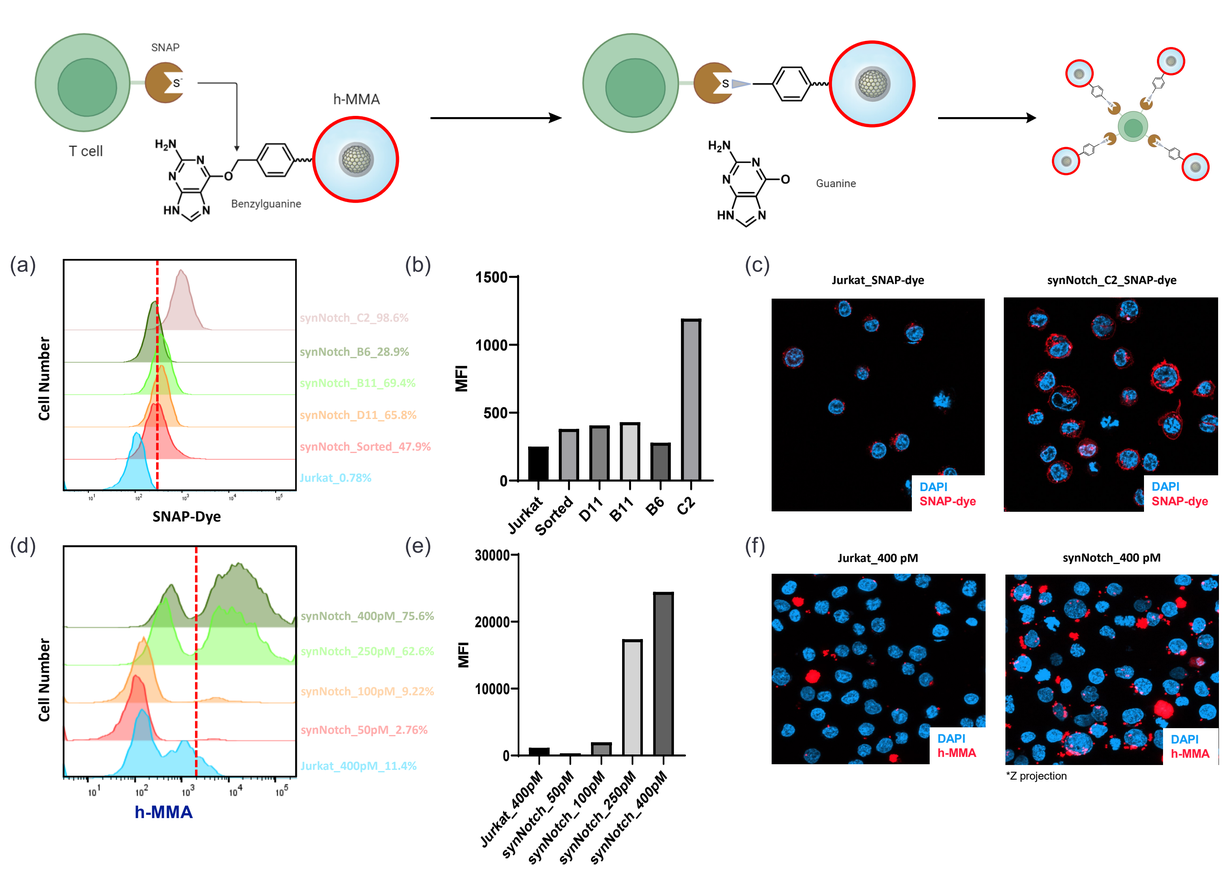


Fig. 3. Schematics of interaction between SNAP of a T cell and benzylguanine of a h-MMA particle and visualize the cells with fluorescent h-MMA particles with its fluorescence intensity proportional to the extent of synNotch expression. (a,b) FACS data showing the C2 single-cell line is expressing the synNotch the most. (c) confocal images of cells with SNAP-dye indicating the expression level of synNotch. (d,e) FACS data showing the fluorescence intensity depending on the concentration of the h-MMA particles. (e) confocal images of C2 single-cell line cells with fluorescent dyed h-MMA particles indicating the the expression level of synNotch and the extent of interaction between cells and h-MMA particles depending on the concentration of particles.

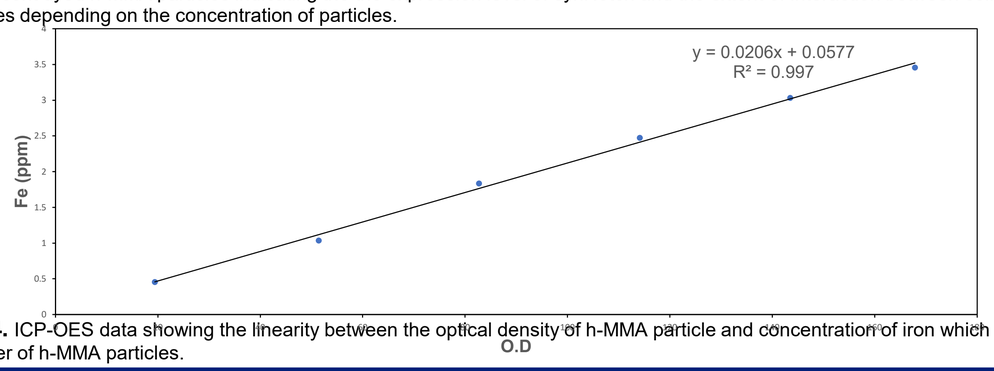


Fig. 4. ICP-OES data showing the linearity between the optical density of h-MMA particle and concentration of iron which is indicating the number of h-MMA particles.

Conclusion & Further Study

[Conclusion]

We successfully achieved the creation of h-MMA particles by designing and optimizing reaction conditions, ultimately functionalizing them with benzylguanine to interact with SNAP of synNotch. After expressing synNotch gene by lentiviral transduction to Jurkat cells, we sort out the single cell line that exhibited the highest level of synNotch expression. Subsequently, using the Jurkat cells with the highest level of synNotch expression, we determined the optimal particle concentration for sufficient mechanical force to stimulate the synNotch. To quantitatively control the concentration of h-MMAs relative to cells, we developed a protocol utilizing ICP-OES analysis to determine the number of particle based on the optical density (OD) of the h-MMA solution. This enabled us to establish a method for adjusting the concentration of h-MMA particles effectively.

[Further Study]

We optimized the concentration of h-MMA particles to induce gene expression successfully within the cells. Furthermore, we are planning to quantitatively screen the extent of UAS-tBFP expression within cells by optimizing factors such as the intensity and frequency of AMF applied *in vitro*. We also aim to validate whether activation of the CAR gene in primary T cells using h-MMA particles effectively leads to cancer cell therapy *in vivo*. To achieve this, we plan to utilize xenograft mouse models and employ IVIS imaging to visualize and monitor tumor progression.

Reference

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