Magneto-mechano-genetic (MMG) modulation of piezo1 ion channel

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Introduction

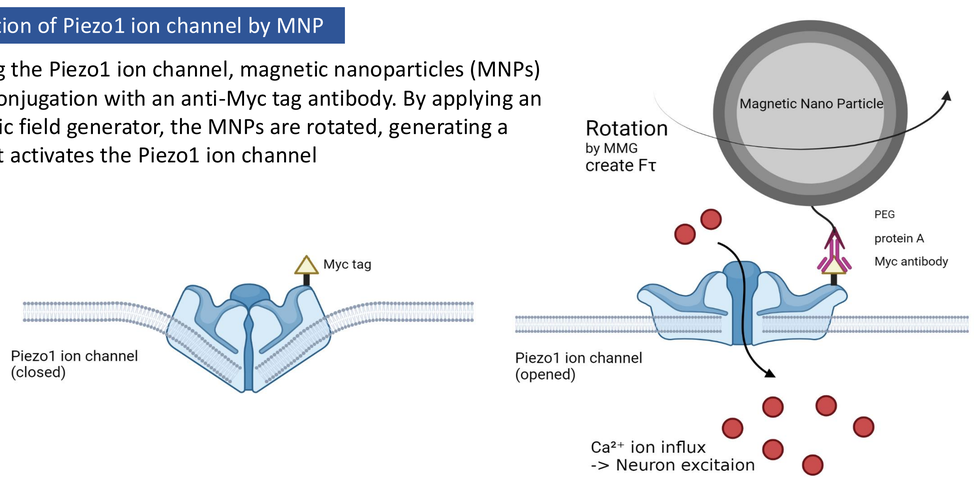
Neuromodulation technologies play a vital role in advancing our understanding of the complexities of neuronal connectivity and brain function, serving as indispensable tools for both fundamental research and therapeutic applications. While traditional methods, such as optogenetics and electrical deep brain stimulation (DBS), have demonstrated significant potential, they often necessitate invasive devices that may disrupt the natural behaviors of animals.

The Magnetic-Mechano-Genetics (MMG) approach we have studied offers a novel solution to these limitations. MMG utilizes magnetic fields that has long-range working distance with minimal interference, enabling wireless and remote activation of neuronal populations. This technology is particularly advantageous for long-term studies in freely moving animals, as it eliminates the need for invasive implants and allows for large-area and deep-brain stimulation without the risk of tissue heating or damage.

MMG technology utilizes nanoscale magnetic particle to convert rotating magnetic field into mechanical torque force, to activate the mechanosensitive piezo1 ion channel. The strategy includes genetic expression of piezo1 ion channel into desired target cell, specific labelling of magnetic nanoparticle (MNP), which rotates in response to rotating magnetic field (RMF) generated through the magnetic force generator (MFG). In this study, we have effectively conjugated anti-Myc antibodies to magnetic nanoparticles (MNPs) and targeted genetically encoded Piezo1 ion channels, demonstrating precise neuronal activation. Furthermore, biophysical properties of MMG technology, such as neuronal activation efficiency at different magnetic field strengths was investigated

Figure 1) Activation of Piezo1 ion channel by MNP

In cells exhibiting the Piezo1 ion channel, magnetic nanoparticles (MNPs) are labeled via conjugation with an anti-Myc tag antibody. By applying an external magnetic field generator, the MNPs are rotated, generating a torque force that activates the Piezo1 ion channel



Surface functionalization

[1] Experimental Methods

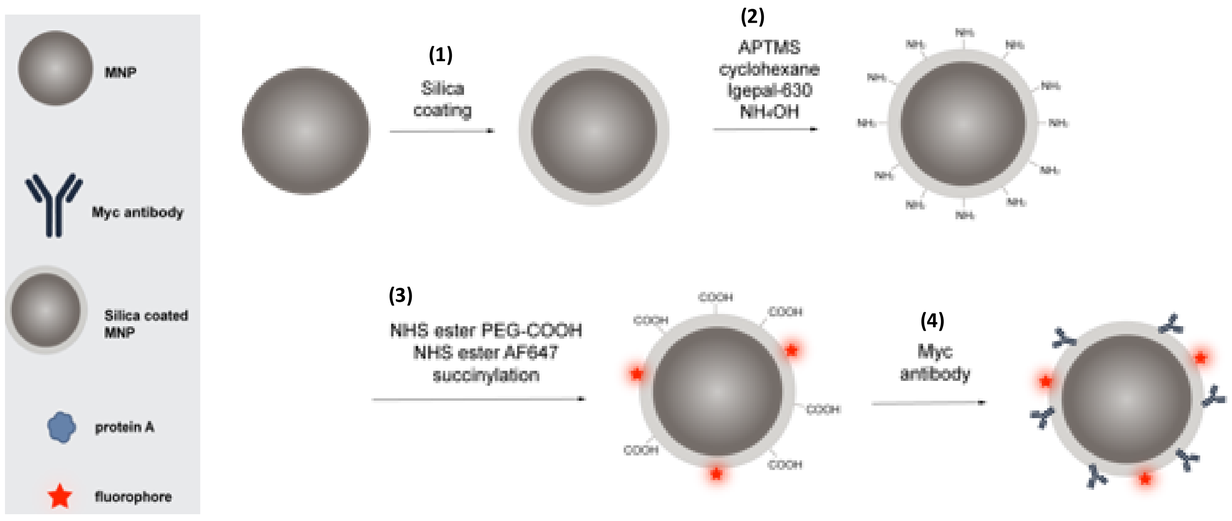


Figure 2) Sequence of MNP surface functionalization

(1) the Stober method for coating MNP with Silica (0.1% TEOS for silica source) (2) Reverse micelle method for coating silica coating of MNP with APTMS (3-Aminopropyl)-trimethoxy silane) (3) Succinylation of MNP with NHS-Ester-PEG3400-COOH (100nmol/ml), Succinic anhydride (1ng/ml) (4) Using "EDC-NHS coupling" for conjugation of Myc Antibody onto the carboxylated MNP.

Figure 3) Schematic of SDS-PAGE

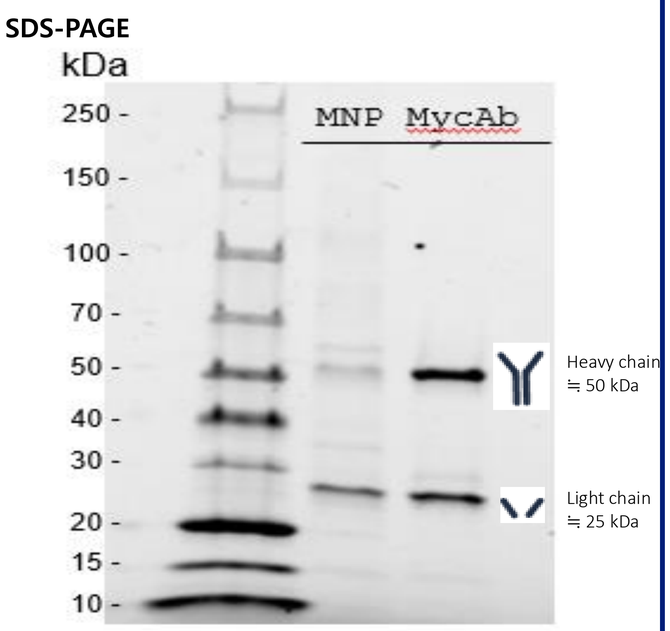
Myc Ab-conjugated MNP particles are heated above 95°C to denature the antibody, separating it into Heavy and Light chains, which are then subjected to electrophoresis; the differential migration of each protein based on molecular weight(kDa) is used to confirm the presence of Myc Ab in the sample.

[2] Result

Figure 4) Result of SDS-PAGE

The following SDS-PAGE results show, from left to right, the ladder, sample, and Myc-Ab lanes. As seen in the image, the experiment with MNPs revealed proteins at approximately 25 kDa and 50 kDa. When compared with the Myc-Ab lane, these bands correspond to the heavy and light chains of Myc-Ab.

This confirms that the experiment aimed at conjugating Myc-Ab to MNPs through surface functionalization was successful.



Reference

•**Jung-uk Lee, Wookjin Shin, Yongjun Lim, Jungsil Kim, Woon Ryoung Kim, Heehun Kim, Jae-Hyun Lee, Jinwoo Cheon**. (2021). Non-contact long-range magnetic stimulation of mechanosensitive ion channels in freely moving animals. *Nature Materials, 20*(7), 1029–1036. https://doi.org/10.1038/s41563-020-00896-y .

•**Wookjin Shin, Yeongdo Lee, Jueun Lim, Youbin Lee, Jungsu David Lah, Somin Lee, Jung-uk Lee, Ri Yu, Phil Hyu Lee, Jae-Hyun Lee, Minsuk Kwak, Jinwoo Cheon**. (2024). Nanoscale Magneto-mechanical-genetics of Deep Brain Neurons: Reversing Motor Deficits in Parkinsonian Mice. *Nano Letters, 24*(2), 270–278. https://doi.org/10.1021/acs.nanolett.3c03899 .

•**Seo-Hyun Choi, Jihye Shin, Chanhyun Park, Jung-uk Lee, Jaegyeong Lee, Yuko Ambo, Wookjin Shin, Ri Yu, Ju-Young Kim, Jungsu David Lah, Donghun Shin, Gooreum Kim, Kunwoo Noh, Wuhyun Koh, C. Justin Lee, Jae-Hyun Lee, Minsuk Kwak, Jinwoo Cheon**. (2024). In vivo magnetogenetics for cell-type-specific targeting and modulation of brain circuits. *Nature Nanotechnology, 16*(9), 4393–4416. https://doi.org/10.1038/s41565-024-01694-2

Piezo1 activation by various MMG stimulation of different magnetic field strengths

[1] Experimental Methods

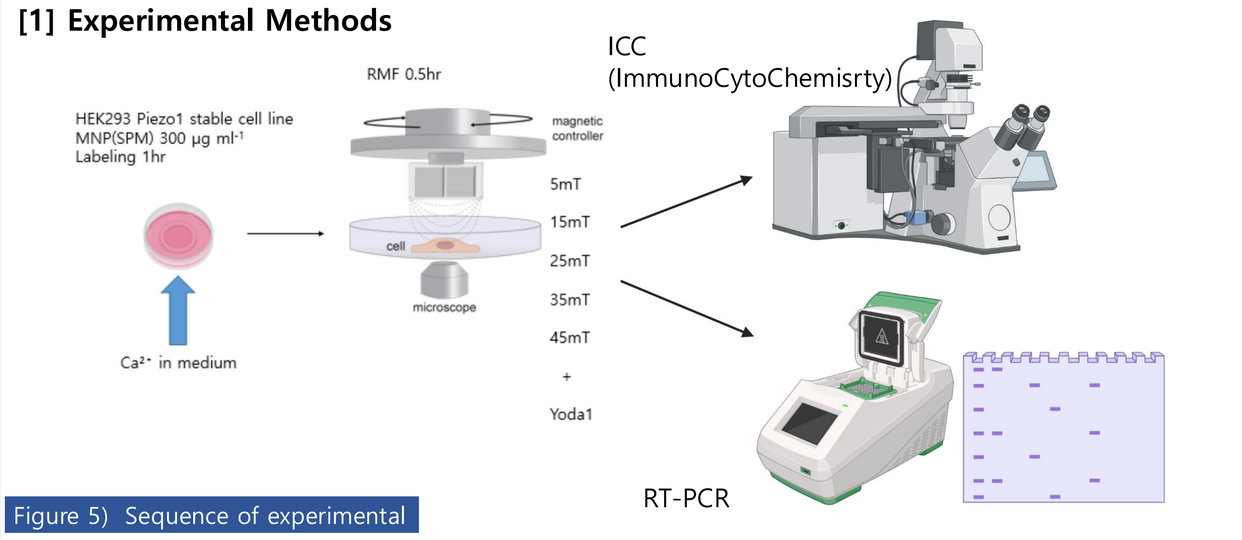


Figure 5) Sequence of experimental

(1) Label the HEK293 Piezo1 stable cells with surface-functionalized MNPs (SPMs) for 1 hour. To check if the Piezo1 ion channel is activated, (2) replace the medium with one containing Ca^{2+}. (3) Expose the cells, which have been treated with the particles and placed in a confocal dish, to different magnetic field strengths of 5, 15, 25, 35, and 45 mT for (RT-PCR) 30 minutes each. (ICC) 2hr each.

(4-1-1) For **Immunocytochemistry** (ICC): Following fixation, permeabilization, and blocking, treat the cells with a primary antibody specific to c-Fos (hosted in rabbit), followed by a secondary antibody conjugated with a fluorescent marker (Alexa 594) that specifically binds to the primary antibody (Anti-Rabbit). (4-1-2) Stain the cells with DAPI (Nuclear Blue) and observe c-Fos expression using a Zeiss microscope.

(4-2-1) For **RT-PCR**: Extract mRNA from the cells and reverse transcribe it into cDNA. (4-2-2) Amplify the cDNA using β-actin primers (to ensure experimental integrity) and c-Fos primers (to assess the experimental outcome). (4-2-3) Analyze the amplified samples by running them alongside a ladder in electrophoresis to verify the results.

[2] Results

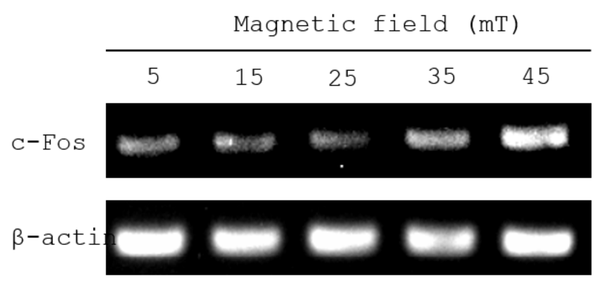


Figure 6) Result of RT-PCR

The RT-PCR results yielded two key observations. Firstly, the use of the beta-actin primer as a control effectively verified that there were no experimental issues with the RT-PCR procedure across all treated samples. Secondly, the c-Fos primer was utilized to determine the level of c-Fos expression in each sample. The consistently clear PCR results for beta-actin across all magnetic field strengths confirm the reliability of the RT-PCR experiment. Regarding c-Fos expression levels, the results demonstrated a linear increase in c-Fos expression with increasing magnetic field strength, culminating in the highest band intensity observed at 45 mT.

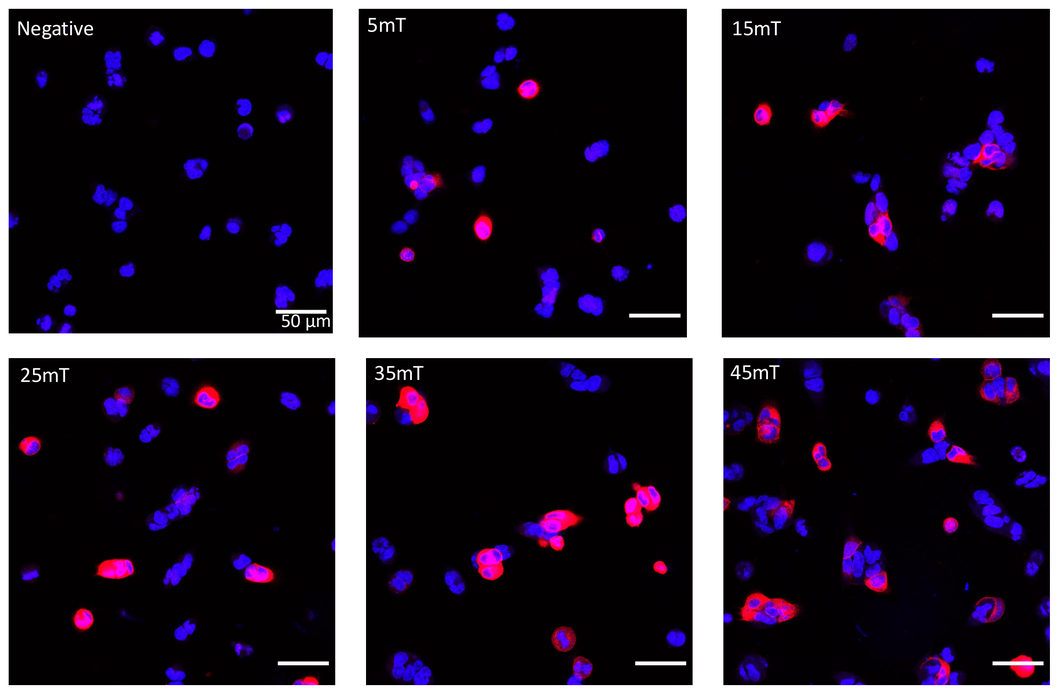


Figure 7) Result of ICC

The ICC results showed significant increase in the level of c-Fos positive cells (%) compared to the negative sample, which showed negligible expression of c-Fos. Also, the level of c-Fos expressing cells (%) increases in correlation with increasing magnetic field strength (mT). The results showed highest level of c-Fos at 45 mT, which is in correlation with the results from above RT-PCR experiment. Also, in the abovementioned RT-PCR experiment, noticeable increase in c-Fos was observed between 25 to 35 mT. This phenomenon is also simultaneously observed in the ICC experiment results.

Conclusion & Further Study

In the first experiment, we successfully conjugated the Myc antibody to the surface of magnetic nanoparticles (MNPs) through a surface functionalization process. This experiment is of great importance, as it directly influences the precision and effectiveness of magnetic-mechano-genetics (MMG) technology. The stable conjugation of the Myc antibody on the MNP surface is crucial for ensuring specific interactions with ion channels via Myc-tag targeting. This specific interaction guarantees that the MNPs are accurately attached to mechanosensitive ion channels and are capable of effectively transmitting the necessary force to activate these channels. The specific labeling of the MNPs is achieved through the high specificity of the Myc-tag and Myc-tag antibody interaction.

In the second experiment, we explored the activation of the Piezo1 ion channel under various magnetic field strengths. This experiment was essential for determining the optimal magnetic field strength required to activate Piezo1 ion channels. The activation of the Piezo1 ion channel was confirmed by assessing c-Fos protein expression levels through both RT-PCR and immunocytochemistry analyses. Our findings indicated a linear increase in c-Fos expression with increasing magnetic field strength, up to 45 mT. Unfortunately, we were unable to determine the maximum threshold force for c-Fos expression. However, we observed a significant increase in c-Fos expression at 35 mT compared to lower magnetic field strengths (5, 15, and 25 mT). Future experiments that focus on the torque force generated by the magnetic nanoparticles (MNPs) could provide deeper insights into the threshold force required for Piezo1 ion channel activation.

MMG technology presents significant promise as a next-generation tool in neuroscience, offering numerous advantages such as relatively non-invasive neural modulation, a reduced risk of tissue heating and damage, and the capability for deep brain stimulation. At present, the Piezo1 ion channel is primarily employed to excite specific neurons through the influx of Ca^{2+} ions. However, in the field of neuroscience, it is crucial to have both activation and inhibitory tools to fully elucidate the roles of specific neurons. By selectively silencing neurons, researchers can gain a deeper understanding of how different regions of the brain contribute to various processes, which is also vital for developing targeted therapies for neurological disorders characterized by excessive neural activity. Therefore, neural modulation using various types of ion channels, particularly those that utilize Cl^- and K^+ ions, is essential. As each of these ion channels requires different optimal force strengths for activation, it is imperative to conduct parallel investigations into a variety of MNPs and the forces they generate.