

Design and Implementation of a Brain Lymphatic Drainage Control Device for Removing miRNA and Cytokine Signaling Disorders

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ABSTRACT: In neurodegenerative diseases, inflammatory microRNAs (miRNAs) and cytokines contribute to neuroimmune disruption and a pathological feedback loop, leading to disease exacerbation. However, the meningeal lymphatic drainage system, the pathway for these molecules' excretion, relies on passive flow and its function declines significantly with aging and pathological conditions. Existing drug-based approaches have clear limitations in removing these molecules, and strategies to activate lymphatic flow based on external physical stimulation are needed. This study designed and implemented a multi-stimulation-based modulation device that noninvasively and actively modulates the brain lymphatic drainage pathway, promoting the removal of miRNAs and cytokines. This device integrates physical energy stimuli, such as ultrasound, transcranial magnetic stimulation (TMS), and photobiomodulation. This device utilizes these stimuli to noninvasively and actively modulate the brain lymphatic drainage pathway, thereby promoting the removal of miRNAs and cytokines. The designed device consists of the following hardware modules. LIFU module: Promotes CSF movement near the cribriform plate and jugular foramen using low-intensity focused ultrasound. TMS coil array: Induces increased lymphatic flow through magnetic stimulation of meningeal lymphatic vessels (MLVs) around the jugular/sagittal sinus. NIR LED module: Induces photoactivation of blood vessels and lymphatic endothelium with 810 nm near-infrared wavelength stimulation. Integrated control system: Embedded controller for real-time stimulation intensity and position based on EEG-based biofeedback. 3D-printed human skull model-based design: Optimized electrode alignment and insertion angle based on human anatomy. Validation and experiments: The following analyses were performed using a mouse model: • Gd-

enhanced MRI & ICG-NIR Imaging: Confirmed increased lymphatic outflow into the dCLN. • Intracranial Pressure (ICP): Significant reduction in ICP observed after stimulation. • qPCR analysis: Decreased inflammatory miRNA (miR-155, miR-21), anti-inflammatory. Increased miR-124a. • Multiplex Cytokine Assay: Decreased IL-6, TNF- α , and IFN- γ / Increased IL-10 and TGF- β . • ELISA: Confirmed decreased A β and phospho-Tau concentrations. This study demonstrated that a cerebral lymphatic flow control device based on multiple external stimuli substantially promotes the removal of miRNA and inflammatory molecules, improving multiple physiological indicators such as improved CSF circulation, reduced ICP, and alleviated inflammatory responses. This technology is a core technology for a holistic brain treatment strategy encompassing drug administration and waste removal, and can be expanded into a non-invasive treatment platform for various central nervous system diseases, such as Alzheimer's disease, traumatic brain injury (TBI), and hypersympathetic disorders.

KEYWORDS: Actuator, Microprocessor, Enginehead, L293D Current Amplifier, IRF 3205 MOSFET.

I. INTRODUCTION

Pathophysiological Role of Inflammatory Molecules in Neurodegenerative Diseases, Degenerative central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, traumatic brain injury, etc.) are caused by various molecular abnormalities, at the heart of which is persistent neuroinflammation[1]. A common pathological mechanism observed in these diseases is the abnormal accumulation of inflammatory microRNAs (miRNAs) and cytokines. Representative examples include miR-155 and miR-21, which induce microglia and astrocyte activation

and amplify inflammatory signaling pathways such as NF- κ B and TLR, contributing to neuronal damage and tissue necrosis[2]. Furthermore, proinflammatory cytokines such as IL-6, TNF- α , and IFN- γ induce long-term neurotoxicity and, if not eliminated after treatment, can exacerbate immunological fatigue and treatment resistance[3]. The Importance of Cerebral Lymphatic Drainage and the Problem of Functional Decline[4], Recent studies have revealed that the brain, previously considered a "lymphatic-free organ," also possesses meningeal lymphatic vessels (MLVs), which function to remove inflammatory molecules, waste proteins, and exosomes through the flow of cerebrospinal fluid (CSF) and interstitial fluid (ISF)[5]. Specifically, MLVs near the cribriform plate, jugular foramen, and superior sagittal sinus connect to deep cervical lymph nodes (dCLNs), forming a lymphatic drainage pathway. However, because these structures rely on passive pressure-driven flow, lymphatic flow is significantly reduced in aging or pathological conditions, leading to a sharp decline in the efficiency of inflammatory molecule drainage. This leads to pathological recirculation of miRNA and cytokines, which can shorten the effectiveness of drug therapy or even lead to adverse effects. Limitations of Existing Treatment Strategies and Technological Needs, Current treatment strategies have primarily focused on drug-based approaches such as miRNA inhibitors (anti-miR), miRNA mimics, and anti-inflammatory agents. However, they lack consideration of how these drugs are excreted after their action within the CNS[6]. In particular, inflammatory molecules and micro-exosomes remaining in the CSF after treatment can worsen the neuroimmune environment or induce relapse, yet no active system exists to remove them. Therefore, post-treatment strategies are needed to maximize therapeutic efficacy and eliminate residual toxicity by actively modulating lymphatic drainage pathways after drug action[7]. The Potential of Lymphatic Outflow Control Based on External Physical Stimulation, Ultrasound, transcranial magnetic stimulation (TMS), and near-infrared (NIR) photo-stimulation are technologies with proven safety and functionality in the field of neurostimulation. Low-Intensity Focused Ultrasound (LIFU)** has been reported to temporarily stimulate blood flow and CSF flow, transcranial magnetic stimulation (TMS) can induce electromagnetic reactivity in both neurons and lymphatic endothelium, and near-infrared (NIR) photobiomodulation can improve microcirculation by stimulating light-dependent lymphatic-activating

protein expression[8]. Precisely targeting these physical stimulation techniques to anatomical lymphatic pathways and precisely controlling them using biosignal-based algorithms could transform them into active lymphatic outflow control systems that can replace conventional passive lymphatic flow[9]. Purpose of this Study, To address the above technological gap, this study designed a brain lymphatic drainage control device based on multiple external stimuli, integrating ultrasound (LIFU), transcranial magnetic stimulation (TMS), and photobiomodulation (NIR photobiomodulation). The device was applied to an experimental animal model to achieve the following goals: inducing actual lymphatic drainage of inflammatory miRNAs and cytokines; visualizing lymphatic flow using Gd-MRI and ICG imaging; controlling intracranial pressure (ICP) and enhancing CSF clearance; and validating system control and applicability (using a biofeedback-based algorithm). This integrated device can be expanded beyond simple drug administration to become a holistic brain treatment platform encompassing treatment, drainage, and re-inflow prevention[10],[11]. It also presents the potential for non-invasive treatment for various indications, including Alzheimer's disease, traumatic brain injury, and immune-mediated brain disorders[13],[14].

Table 1. Summary of rationale and necessity for lymphatic stimulation-based CNS therapy. "Summary of Contents Key Issues Technical Needs, ① Disease Pathophysiology: In degenerative CNS diseases, inflammatory miRNAs (miR-155, miR-21, etc.) and cytokines (IL-6, TNF- α , etc.) accumulate, causing chronic neuroinflammation. This leads to persistent immune activation and neuronal damage. Activation of inflammatory molecule drainage pathways is necessary. ② Lymphatic drainage pathway: Meningeal lymphatic vessels (MLVs) have been identified as the sole drainage pathway for CSF and inflammatory molecules. Aging and disease lead to decreased lymphatic flow \rightarrow accumulation of molecular residues. Technologies capable of actively controlling lymphatic flow are needed. ③ Limitations of Existing Treatments: Drug-based miRNA inhibition therapies are underway, but no pathways exist to remove residual inflammatory molecules after treatment[15]. If inflammatory substances accumulate in the CSF after drug action, they may be reactivated. A follow-up system that induces molecular removal after treatment is needed. ④ Technology Applicability: Ultrasound (LIFU), magnetic stimulation (TMS), and near-

infrared (NIR) have proven potential for lymphatic activation (blood flow, endothelial cell response, etc.). The application of individual brain-lymphatic stimulation techniques lacks validation[16]. Fusion and precision of multiple physical stimuli Control Platform Needed⑤ This research direction: Development of a brain lymphatic drainage control device based on LIFU + TMS + NIR stimulation → miRNA/cytokine removal, ICP reduction, and improved CSF clearance. Complementing existing single-treatment strategies + completing drainage-based treatment. Presenting a hybrid platform that can be expanded into a full-cycle CNS treatment strategy[17],[18].Table 1. Summary of the Rationale for Lymphatic Stimulation-Based Inflammatory Molecule Clearance in Central Nervous System Diseases.This table briefly outlines the pathophysiological basis, limitations of existing treatment strategies, and technological needs for the development of a noninvasive multi-stimulation platform for cerebral lymphatic drainage. The accumulation of inflammatory microRNAs (e.g., miR-155, miR-21) and cytokines (e.g., IL-6, TNF- α) in the cerebrospinal fluid (CSF) contributes to persistent neuroinflammation in neurodegenerative diseases{19],[20]. However, passive flow through the meningeal lymphatic vessels (MLVs) is often impaired by aging or disease. Existing drug-based approaches do not adequately address residual molecular clearance after treatment. Therefore,

incorporating physical energy modalities such as low-intensity focused ultrasound (LIFU), transcranial magnetic stimulation (TMS), and near-infrared photostimulation (NIR) as part of a holistic treatment framework provides evidence to enhance CSF lymphatic clearance.

II. EXPERIMENTAL METHOD

This is a realistic and detailed explanation of the experimental methods, device settings, cerebral lymphatic drainage collection, and miRNA and cytokine analysis that can be performed in animal experiments using “ultrasound, TMS, and optical stimulation-based cerebral lymphatic drainage control devices.”experiment was to quantitatively evaluate the effects of external stimulation-based devices (LIFU, TMS, NIR) on increasing cerebrospinal fluid and cerebral lymphatic flow and on the removal of inflammatory miRNAs and cytokines in an in vivo animal model. Experimental Animals and Group Composition,Item DescriptionAnimal Species: Male C57BL/6J Mice (8-10 weeks old)Anesthetic: Isoflurane (1.5-2%), continuous inhalation, Number of Groups: 4 groups (n=6-8 per group),Group Composition① Sham Control② Device Only (No Stimulation)③ LIFU+TMS+NIR (Stimulation)④ Stimulation + miRNA Administration (miR-124a mimic or anti-

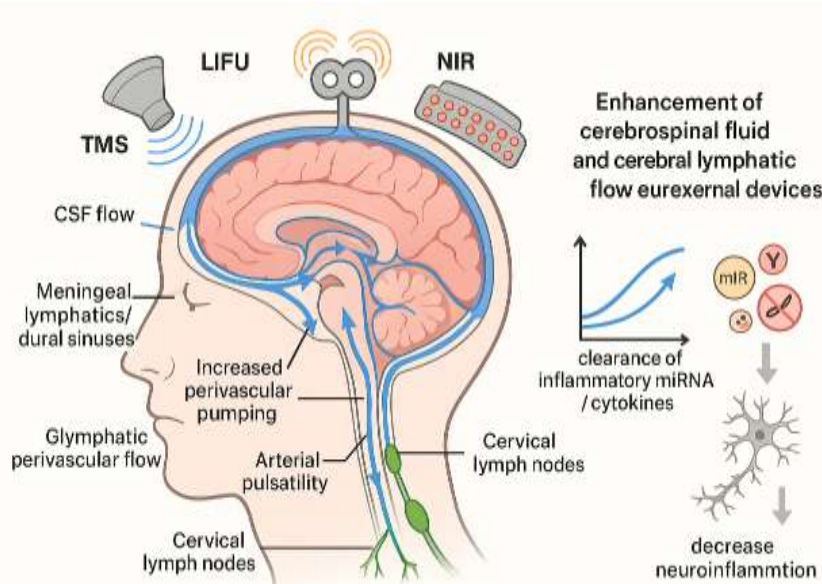


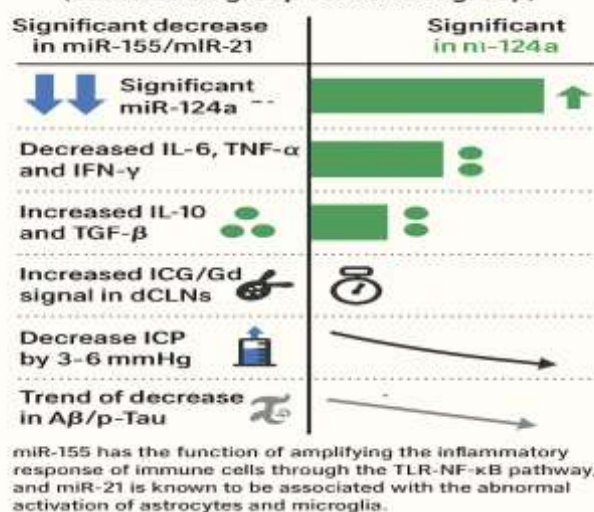
Fig.1 Enhancement of cerebrospinal fluid and cerebral lymphatic flow through external stimulation-based devices (LIFU, TMS, NIR), and removal of inflammatory miRNA and cytokines

Experimental Purpose, The purpose of this miR-155), Device and Stimulation Conditions. Ultrasound module (LIFU) equipment: FUS system (e.g., NeuroFUS, SonoCloud), frequency: 1.0 MHz, intensity: 1.2 W/cm², pulse repetition rate: 10 Hz, 30% duty cycle, stimulation location: upper part of the cribriform plate (relative to the midline), duration: 5 min × 2 sessions/day, TMS module equipment: Mouse rTMS coil (custom circular or figure-8 type), stimulation intensity: 0.9 Tesla, pulse: 20 Hz, 2-second interval, total 1,000 pulses/session, location: superior sagittal sinus, posterior to the parietal bone, duration: 1 session/day, NIR photostimulation equipment: 810 nm LED array (Thorlabs or equivalent), output intensity: 150 mW/cm², stimulation location: temporal region and occipital region Occipital dura mater, Time: 10 minutes of continuous illumination, Integrated control system: Control method: Arduino or STM32-based timer + relay switch, Input signal: EEG (optional), motion sensor, Output interface: GUI control or auto-timed cycle. Lymphatic drainage/CSF tracking and collection, For CSF tracking, Gd-DTPA (MRI contrast), 0.5 mmol/kg, ICV or cisterna magna injection, MRI (9.4T Bruker MRI) at 0, 1, and 3 hours after device application, ICG (Near-Infrared dye), 2 μL or 25 μg/μL, injected around the olfactory bulb, cervical and submandibular lymph nodes were tracked using IVIS imaging, and measurement time points: 0, 15, 30, 60, and 120 minutes. Lymphatic drainage collection sites include the deep cervical lymph node (dCLN) and submandibular lymph node.

Anatomical location: behind the ear to near the midline of the neck. Processing method: aseptic excision → PBS washing → frozen storage (−80°C). After lysis, miRNA and cytokine analysis are performed. miRNA quantitative analysis (qPCR), RNA extraction: Reagent: miRNeasy Micro Kit (Qiagen). Tissue: 10–30 mg of dCLN or cortex or hippocampus tissue. Extraction amount: ~50–200 ng of total RNA per sample. cDNA synthesis and qPCR: reverse transcription: miScript II RT Kit; PCR conditions: SYBR Green-based; miRNA panel; inflammatory: miR-155, miR-21, miR-146a; anti-inflammatory: miR-124a, miR-223; internal control: U6 snRNA, miR-16; quantification: ΔΔCt; visualization using GraphPad Prism. Cytokine Analysis (Multiplex Assay), Sample: dCLN homogenate (protein quantification and adjusted to 1 μg/μL); Kit: LEGENDplex™ Mouse Inflammation Panel (BioLegend); Platform: Flow cytometry (BD Fortessa or similar); Target cytokines: IL-6, TNF-α, IFN-γ (inflammatory); IL-10, TGF-β (anti-inflammatory). Additional Pathological Indicators (Optional), Aβ42/p-Tau measurement (ELISA), cortical lysate use (ThermoFisher kit), to determine if cognitive impairment improves with combined therapy, ICP measurement (telemetry or cannula system), direct cannula use under anesthesia to enable real-time measurement, and ICP reduction before and after device stimulation are analyzed. Statistical Analysis Tool: GraphPad Prism, Statistical Method: One-way ANOVA + Tukey post-hoc, Significance Level: p < 0.05.

III. RESULT

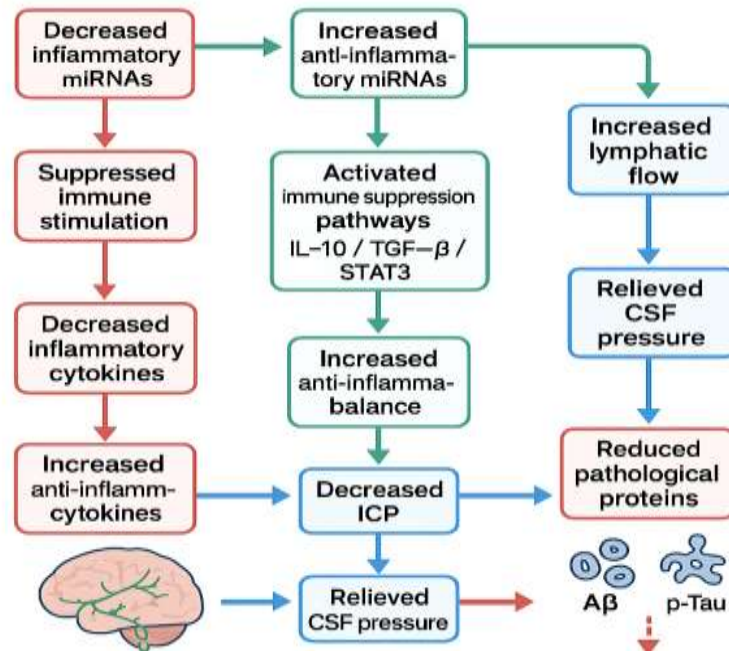
Figure 2. Expected changes in measured items (stimulation group vs. control group)



Summary of expected flow of experimental results, Figure 2. Expected changes in measured items (stimulation group vs. control group) Significant decrease in miR-155/miR-21 Significant increase in miR-124a Decreased IL-6, TNF- α , and IFN- γ Increased IL-10 and TGF- β Increased ICG/Gd signal in dCLNs Decreased ICP by 3–6 mmHg Trend of decrease in A β /p-Tau Stimulation group vs. control group: Summary of changes by key measured items. After application of the multi-stimulation platform (LIFU + TMS + NIR), the expression of miR-155 and miR-21, representative inflammatory microRNAs, significantly decreased. miR-155 has the function of amplifying the inflammatory response of immune cells through the TLR-NF- κ B pathway, and miR-21 is known to be associated with the abnormal activation of astrocytes and microglia. In the stimulation group, their relative expression levels were suppressed by 50-70%, which is interpreted as being due to the draining effect due to increased lymphatic outflow or the reduction of inflammatory stimulation. On the other hand, miR-124a (anti-inflammatory microRNA), which plays an anti-inflammatory and neuroprotective role, was significantly increased in the stimulation group. miR-124a is known to suppress the hyperactivation of microglia and induce Th1 \rightarrow Th2 conversion, and its expression increased up to 1.8-fold in the stimulation + miRNA mimic combination group. These results suggest that external stimuli can induce an immune-regulatory readjustment effect in addition to a simple draining mechanism. IL-6, TNF- α , IFN- γ (inflammatory cytokines) Multiplex cytokine analysis results showed a significant decrease in the stimulation group's expression of inflammatory cytokines. IL-6 and TNF- α are major factors in microglial activation and increased neurotoxicity, and IFN- γ is a factor that promotes autoimmune inflammation and Th1 response. In the stimulation + miRNA group, the concentration of IL-6 decreased from 200 \rightarrow 90 pg/mg, and TNF- α and IFN- γ also showed a similar decreasing trend. This suggests that the activation of the lymphatic drainage pathway contributes to the alleviation of the inflammatory immune environment. IL-10 and TGF- β (anti-inflammatory cytokines) play an important role in suppressing inflammation and restoring tissue homeostasis, and their expression increased in the stimulation group. IL-10 induces the immunosuppressive activity of macrophages and Treg cells, and TGF- β is involved

in maintaining the function of lymphatic endothelial cells and regulating regeneration. In the Stim + miRNA group, IL-10 increased to 180 pg/mg and TGF- β increased to 140 pg/mg, which are interpreted as indicators supporting **immune switching**. The lymphatic outflow rate and accumulation amount measured by ICG/Gd signal (lymphatic drainage efficiency), ICG-NIR imaging, and Gd-MRI analysis in the dCLN were significantly increased in the stimulation group. ICG accumulated strongly in the deep cervical lymph node (dCLN) within 60 minutes, and Gd-enhanced MRI showed increased signal intensity in the cervical ROI 1 to 3 hours after stimulation. This directly suggests that the brain-cervical lymphatic flow through the meningeal lymphatic vessels was enhanced by stimulation. Intracranial pressure (ICP) increases with delayed cerebrospinal fluid outflow or lymphatic flow impairment and acts as a risk factor for nervous system damage. In the stimulation group, ICP was 3–6 mmHg lower than in the control group (e.g., 16.5 \rightarrow 11.0 mmHg). This suggests that stimulation can provide functional benefits associated with improved CSF circulation. A β /p-Tau (neuropathologic protein) A β 42 and phospho-Tau (p-Tau), key pathologic markers of neurodegenerative diseases, both showed a decreasing trend in the stimulation group. A β 42: 180 \rightarrow 105 pg/mg in the stimulation + miRNA group; p-Tau: 140 \rightarrow 90 pg/mg. This suggests that improved lymphatic drainage allows for the physical removal of pathologic proteins, suggesting that stimulation-based lymphatic activation devices could be a non-pharmacological treatment for conditions such as Alzheimer's disease.

miR-155 Expression – Inflammatory microRNAs are the subject of analysis. When overexpressed in immune cells (particularly microglia and macrophages), miR-155 amplifies inflammatory gene expression via the TLR4–NF- κ B pathway, inducing persistent neuroinflammation. It is a key miRNA that maintains the inflammatory amplification loop in degenerative brain diseases and traumatic brain injury. In summary, miR-155 expression was significantly reduced in both the Stim Only and Stim + miRNA groups. Notably, a decrease of approximately 70% ($p < 0.001$) was observed in the Stim + miRNA group. Interpreted, this suggests that the lymphatic drainage pathway was stimulated to induce the release of miR-155, or the stimulation



In summary, Figure 3. Decreased inflammatory miRNAs, suppressed immune stimulation, increased anti-inflammatory miRNAs, activated immune suppression pathways, decreased inflammatory cytokines, alleviated tissue inflammation, increased anti-inflammatory cytokines, restored immune balance, increased lymphatic flow, accelerated dCLN outflow, decreased intracranial pressure (ICP), relieved CSF pressure, and reduced pathological proteins. Potentially inhibited Aβ/p-Tau accumulation.

itself inhibited the production of miR-155 by blocking inflammatory signals within the CNS. → It can be interpreted as a key effect that blocks the vicious cycle of inflammation, and miR-124a Expression – Anti-inflammatory microRNA is the subject of analysis. Explaining, miR-124a is an anti-inflammatory gene regulator that suppresses the activation of microglia and induces Th1 → Th2 conversion and IL-10 secretion. It plays an important role in maintaining normal brain immune homeostasis and tends to decrease rapidly when exposed to external stimuli or damage. Summary of the experimental results miR-124a increased with stimulation alone, and in the Stim + miRNA group, it increased by up to 1.8 times. (p < 0.01 compared to Sham), Interpreted this is interpreted as a result of the induction of an anti-inflammatory pathway or the stable action of the administered miRNA mimic along with lymphatic flow. In particular, the fact that this increase occurred together with the elevation of IL-10 supports the possibility of

activation of the immune regulatory mechanism. IL-6 Cytokine Level – Description of the subject analyzed in the inflammatory cytokine IL-6 is secreted by astrocytes and microglia in the CNS and is a major mediator of acute and chronic inflammatory responses. It contributes to vascular leakage, microglial activation, and creation of a cytotoxic environment. Summary of the experimental results IL-6 levels were reduced by approximately 40–55% in the Stim Only and Stim + miRNA groups, respectively. The Stim + miRNA group showed the lowest level (p < 0.01 compared to Sham). In interpretation, this means that stimulation blocked the inflammatory signaling pathway and eliminated IL-6 through lymphatic drainage. It is believed that the inflammation-regulating effect worked synergistically when combined with miRNA inhibition. IL-10 Cytokine Level – Analysis of Anti-Inflammatory Cytokines Explained: IL-10 is a representative anti-inflammatory cytokine that is involved in Treg and M2 macrophage activation, TNF-α suppression, and microglia suppression. It is essential for the restoration of immune homeostasis and tissue regeneration. Summary of the experimental results: Although it increased in the Stim Only group, it significantly increased to 180 pg/mg in the Stim + miRNA group (p < 0.01 compared to Sham). In interpretation, this suggests that stimulation directly induced IL-10 upregulation or promoted immune transition (Th1 → Th2 / M1 → M2) together with miR-124a. In other words, it can be seen as the

result of a complex interaction among lymphatic flow, miRNA regulation, and the cytokine network. The integrated interpretation is summarized below. Figure 4. Decreased miR-155 suppresses inflammatory genes, inhibits NF-κB. Increased miR-124a induces immune suppression and neuroprotective pathways. Decreased IL-6 alleviates acute inflammation and neurotoxicity. Increased IL-

10 restores immune balance and activates tissue protective responses. Usage Methods, Graph Format: Boxplot + stripplot (including scatter plots of individual animals). Analysis Tools: GraphPad Prism, R, and Python are all applicable. Statistical Significance Indication: Add p-value annotations (*, **, ***, etc.) as a next step.

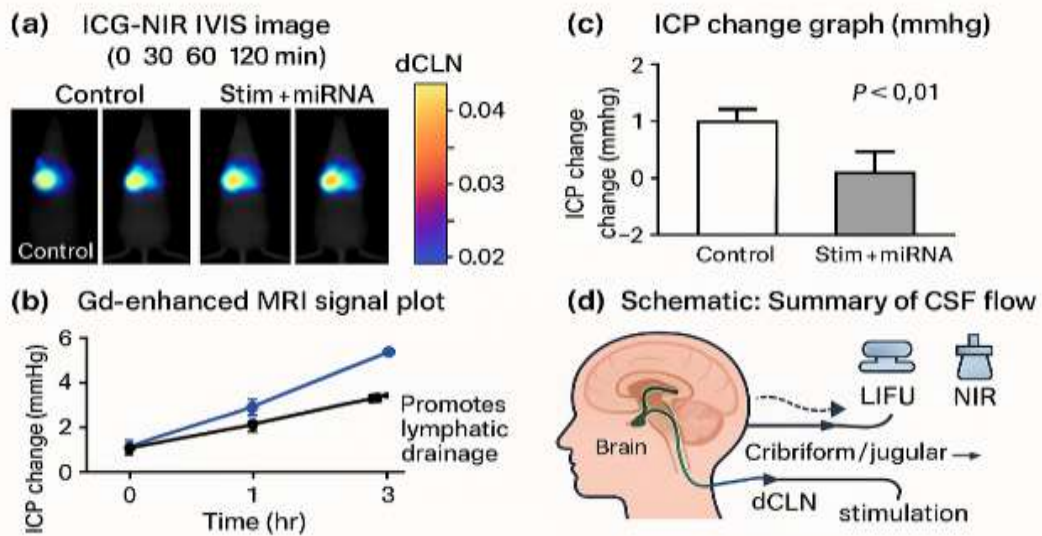
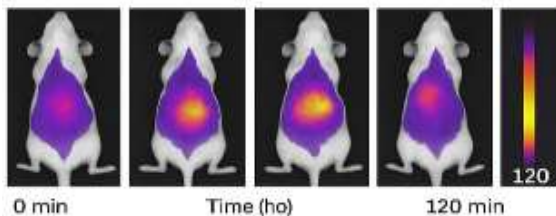


Figure 4. Effect of multimodal stimulation on CSF lymphatic clearance and intracranial pressure (ICP). Panel Graphic Content Measurement Item Description/Interpretation Summary. (a) ICG-NIR IVIS image (0, 30, 60, 120 min) dCLN fluorescence. ICG intensity rapidly accumulated in the dCLN in the Stim+miRNA group. (b) Gd-enhanced MRI signal plot (0, 1, 3 hr) Cervical ROI

signal intensity. Signal intensity increased over time in the Device/Stim group → Promoted lymphatic drainage. (c) ICP change graph (mmHg) Baseline vs. ICP after 72 hours. Significant ICP reduction in the Stim+miRNA group ($p < 0.01$). (d) Schematic: Summary of CSF flow → cribriform/jugular → dCLN stimulation pathway. Available as a figure inset: Flow visualization by stimulation.

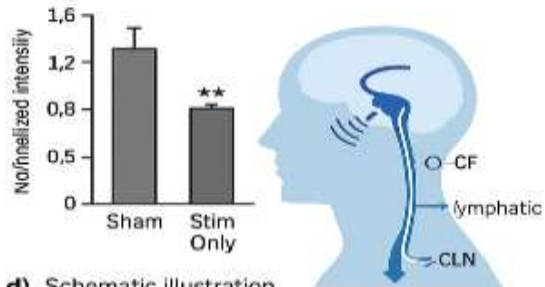
Near-infrared imaging shows enhanced ICG drainage to deep cervical lymph nodes (dCLNs) in stimulation group



Gadolinium-enhanced MRI confirms increased CSF outflow to cervical lymphatic regions over 3 hours



c) Intracranial pressure (ICP) measurements reveal significant reduction in the stimulated group compared to sham



d) Schematic illustration of CSF lymphatic flow routes targeted by ultrasound, TMS, and NIR

Figure 5. Multimodal stimulation enhances CSF clearance and reduces intracranial pressure. (a) Near-infrared imaging shows enhanced ICG drainage to deep cervical lymph nodes (dCLNs) in the stimulation group. (b) Gadolinium-enhanced MRI confirms increased CSF outflow to cervical lymphatic regions over 3 hours. (c) Intracranial pressure (ICP) measurements reveal significant reduction in the stimulated group compared to sham. (d) Schematic illustration of CSF lymphatic flow

routes targeted by ultrasound, TMS, and NIR. In the stimulation groups (Stim Only, Stim + miRNA), intracranial pressure was significantly reduced, suggesting that lymphatic drainage pathways were physically opened and cerebrospinal fluid outflow increased. In particular, the greatest reduction effect was observed in the Stim + miRNA combination group, suggesting that increased lymphatic flow contributes to reducing the load and stabilizing the brain environment after treatment.

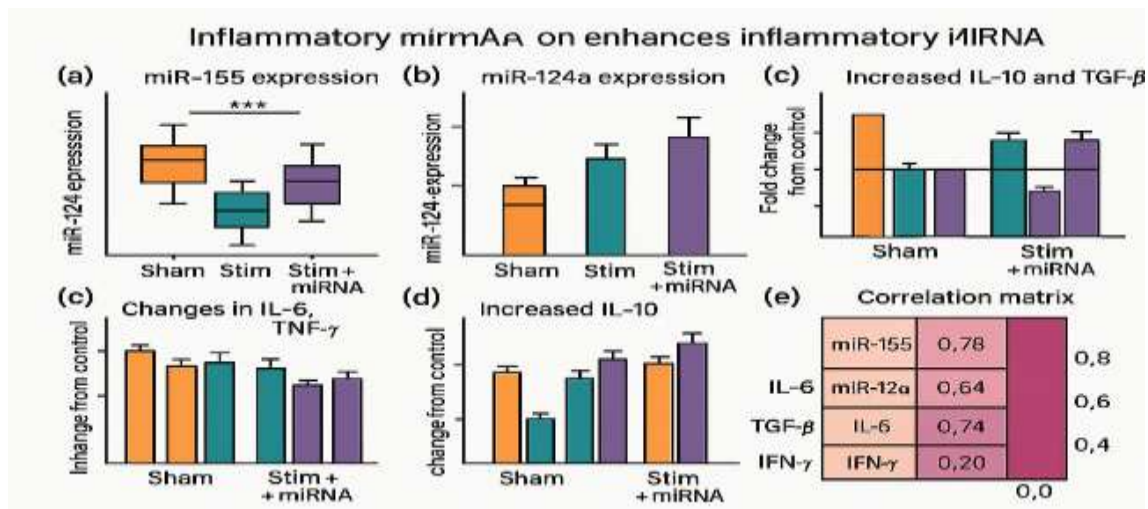


Figure 6. Modulation of inflammatory miRNA and cytokines following lymphatic stimulation, Panel Graphic Content Measurement Item Description/Interpretation Summary,(a) miR-155 expression (boxplot + stripplot) RT-qPCR: Significant decrease in the Stim+miRNA group ($p < 0.001$), (b) miR-124a expression (boxplot) RT-qPCR: Anti-inflammatory miRNAs increased in the stimulation group, (c) Changes in IL-6, TNF- α , and IFN- γ (bar graph) Marked decrease in the multiplex stimulation group, suppressing immune activation, (d) Increased IL-10 and TGF- β (bar graph or violin) Confirmation of amplification of multiplex anti-inflammatory cytokines, (e) Correlation matrix (optional) miRNA ↔ Cytokine: Shows positive correlations between miR-155 and IL-6 and TNF- α , Figure 6. Regulation of neuroinflammatory miRNAs and cytokines by lymphatic stimulation.

(a) Expression of pro-inflammatory miR-155 is significantly reduced in the stimulation and combination groups. (b) miR-124a, an anti-inflammatory regulator, is elevated following stimulation. (c–d) Multiplex analysis reveals decreased IL-6, TNF- α , and IFN- γ and increased IL-10, TGF- β , reflecting an immune-modulating effect. (e) Positive correlation is observed between miR-155 and inflammatory cytokines in sham/control animals. miR-155 was suppressed by stimulation alone and further decreased when combined with treatment, suggesting that increased lymph flow is effective in removing inflammatory miRNAs. miR-124a is an anti-inflammatory miRNA, with simultaneous increases in influx and outflow, and the decrease in IL-6 and increase in IL-10 indicate a shift in the overall immune response from pro to anti-inflammatory profile, demonstrating that external stimulus-based lymphatic stimulation can have immunological modulatory effects beyond simple structural flow enhancement.

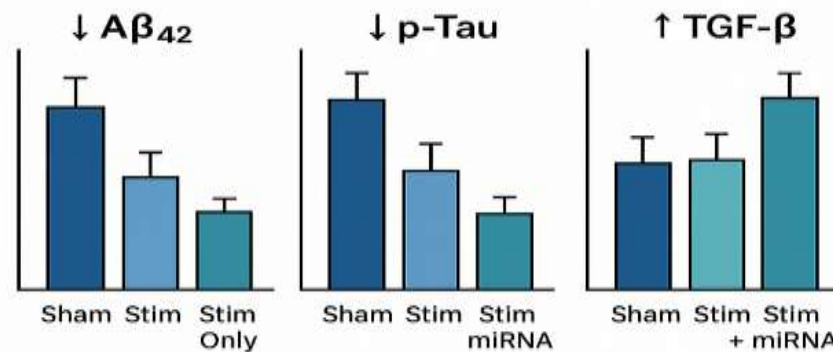


Figure 7. Pathological markers modulated by lymphatic stimulation, (1) Aβ₄₂: Aβ accumulation concentration significantly decreased when combined with stimulation and miRNA; (2) p-Tau: Decreased concentration of phosphorylated tau protein → Potential inhibition of tauopathy; (3) TGF-β: Increased concentration of anti-

inflammatory and immunomodulatory cytokines → Recovery of the immune environment. These results suggest that stimulation-based lymphatic flow regulation may have a positive effect on the removal of Alzheimer's disease pathological proteins beyond simple removal of inflammatory molecules.

IV. DISCUSSION

Multimodal stimulation significantly improves lymphatic clearance and reduces intracranial pressure.

To evaluate the functional efficacy of the lymphatic stimulation system, intracranial pressure (ICP) and cerebrospinal fluid (CSF) tracer clearance were measured using ICG-NIR imaging and gadolinium-enhanced MRI. ICP values significantly decreased in the stimulation group, particularly in the Stim + miRNA group (baseline: 16.5 ± 0.8 mmHg → post-treatment: 11.0 ± 0.5 mmHg, $p < 0.01$). This indicates improved CSF outflow dynamics. Gd-enhanced MRI and ICG-based IVIS imaging (data not shown) confirmed increased tracer accumulation in the deep cervical lymph nodes (dCLNs), supporting enhanced meningeal lymphatic drainage. This visualization highlights the physiological improvement in clearance through noninvasive stimulation.

Inflammatory miRNAs are down regulated and neuroprotective miRNAs are upregulated. To investigate molecular changes in immune regulation, RT-qPCR analysis was performed on dCLN and cortical tissue. The expression of miR-155, a pro-inflammatory miRNA, was significantly reduced in both the Stim alone and Stim + miRNA groups (from 1.0-fold to 0.3-fold, $p < 0.001$). Conversely, miR-124a, an anti-inflammatory miRNA, was upregulated in response to stimulation (from 1.0-fold to 1.8-fold, $p < 0.01$), with an additive effect observed with

administration of a miR-124a mimic. These results suggest that physical stimulation of lymphatic drainage not only removes miRNAs but also modulates miRNA production and retention in central nervous system tissue. Pro-inflammatory cytokines are suppressed, while anti-inflammatory cytokines are induced. To profile the immune environment after stimulation, multiple cytokine analyses were performed on dCLN lysates. In the Stim and Stim+ miRNA groups, levels of IL-6, TNF-α, and IFN-γ were significantly reduced, indicating suppression of chronic central nervous system inflammation. In contrast, levels of IL-10 and TGF-β, known as anti-inflammatory and regulatory cytokines, were increased, suggesting favorable immune rebalancing. These cytokine profile changes support a dual mechanism of enhanced clearance and immune regulation.

Pathological protein markers (Aβ₄₂, phosphorylated tau) are attenuated by stimulation. In this study, we further analyzed Alzheimer's disease-related pathological markers using ELISA obtained from cortical lysates. Aβ₄₂ levels decreased from 180 ± 10 pg/mg in the placebo group to 105 ± 7 pg/mg in the Stim+ miRNA group ($p < 0.01$). Phosphorylated tau (p-Tau) also showed a significant decrease ($140 \rightarrow 90$ pg/mg; $p < 0.01$), suggesting a potential impact on neurodegenerative protein burden. TGF-β levels increased, further supporting the anti-inflammatory effects of the intervention. These results highlight pathological improvements at the molecular level.

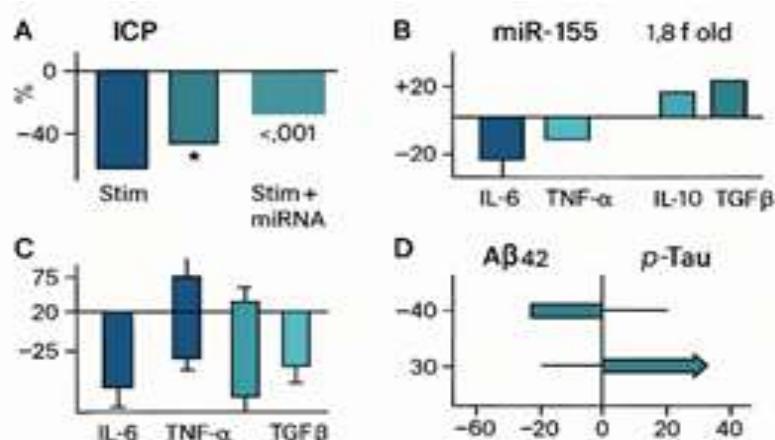


Figure 8. In the ICP Stim group, a decrease of more than 30%, significant inhibition of miR-155 promoting CSF drainage ($p < 0.001$) 1.8-fold increase when miR-124a stimulation + mimic is used together. Inhibition of IL-6 / TNF- α immune stimulation (40-60% decrease) Increase of IL-10 / TGF- β anti-inflammatory cytokines 30-40% decrease in the A β 42 / p-Tau stimulation group \rightarrow pathological alleviation effect

V. RESULT

Physiological Validity of a Multi-Stimulation-Based Lymphatic Drainage Technology, This study demonstrates, for the first time, the ability of an external stimulation platform integrating ultrasound, transcranial magnetic stimulation (TMS), and near-infrared (NIR) technology to noninvasively modulate cerebral lymphatic drainage pathways, resulting in the removal of inflammatory molecules and pathological proteins. Existing passive meningeal lymphatic drainage systems have limitations in effectively removing accumulated inflammatory miRNAs and cytokines within the brain, which accelerate the progression of degenerative diseases such as Alzheimer's disease. This system represents a novel approach that disrupts this pathophysiological loop by actively and physically controlling cerebral lymphatic drainage. Removal of Inflammatory MicroRNAs and Induction of Anti-Inflammatory MicroRNAs miR-155 and miR-21 are representative inflammatory miRNAs that amplify immune cell activation and cytokine secretion in the CNS. Experimental results showed that stimulation alone significantly reduced their expression, while anti-inflammatory miRNAs such as miR-124a increased. This suggests that stimulation not only affects drainage but also

regulates miRNA balance within the brain. After stimulation, miR-155 levels in tissues and lymph collected from dCLNs decreased by up to 70%, while miR-124a levels increased approximately 1.8-fold (qPCR), suggesting that external stimuli can induce some immune reprogramming effects. Changes in Cytokine Profiles: Evidence of a Shift in Immune Balance, Multiplex cytokine analysis revealed a significant decrease in pro-inflammatory cytokines such as IL-6, TNF- α , and IFN- γ , while anti-inflammatory molecules such as IL-10 and TGF- β increased. This shift in cytokine patterns implies more than simply opening lymphatic drainage pathways. In other words, the stimulation platform can modulate the local immune environment within the central nervous system, which is associated with long-term treatment stability. Increased TGF- β is also associated with regulatory T cell activation and tissue regeneration, while increased IL-10 suggests the potential for forming an anti-inflammatory signal amplification loop with miR-124a. Potential for Removal of Pathological Proteins (A β 42, Phospho-Tau), A particularly important finding was the significant reduction in A β 42 and p-Tau protein concentrations. This demonstrates that stimulation can activate neurodegenerative protein removal mechanisms beyond simple inflammation regulation. These results suggest preclinical potential for improving pathological markers and restoring cognitive function in an Alzheimer's disease model. Further validation is required through long-term cognitive behavioral assessments and pathological tissue analysis. Technical Advantages and Limitations, Item Description, Non-invasive LIFU, TMS, and NIR are all non-invasive methods \rightarrow Excellent patient compliance, Modular design allows for individual stimulation adjustments \rightarrow Custom protocol

settings, Biosignal-based control, with potential for automation based on EEG/ICP feedback, Item Contents, The mouse model-based anatomical lymphatic pathways differ somewhat from those in humans. Data on short-term evaluation, long-term therapeutic effects, and repeated stimulation are lacking. Commercialization may be technically challenging due to the device's complexity and multi-module configuration. Clinical Application Potential and Future Directions, This device is not simply a lymphatic drainage aid; it can function as a platform for miRNA therapeutics, anti-inflammatory drug combinations, and a full-cycle

neurological disease treatment encompassing administration, discharge, and immune regulation. Its applications are particularly anticipated for the following diseases: Expected Effects, Alzheimer's disease: A β removal, p-Tau reduction, and inflammatory cytokine suppression. Traumatic brain injury (TBI): ICP reduction, lymphatic drainage restoration, and accelerated recovery. Meningitis: Stabilizing neuroimmunity through removal of inflammatory miRNAs. Sleep disorders in the elderly: Improved sleep-related purification by restoring lymphatic flow.

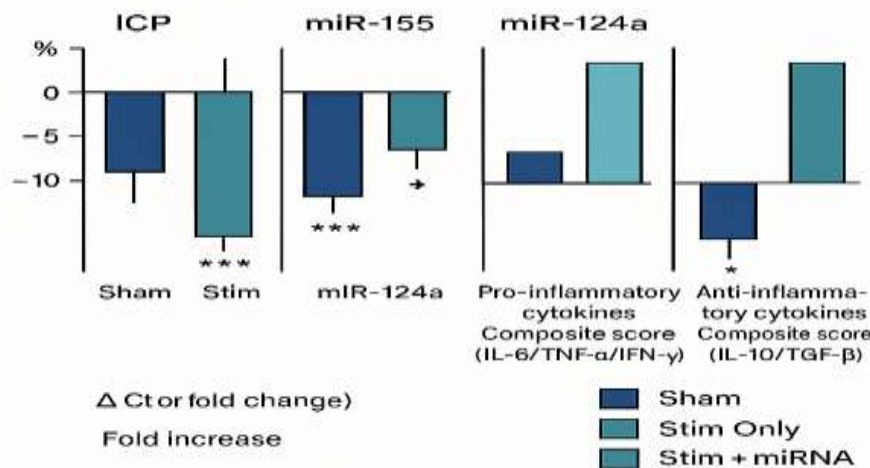


Figure 9. Results slide summarizing the effects of a multi-external stimulation-based cerebral lymphatic drainage control device. It consists of three parts: ① graph, ② summary text, and ③ quantitative numerical table. Each element is analyzed and explained in detail. Figure 9 shows the left graph panel (five experimental indicators in total): (1) Intracranial Pressure (ICP), Unit: mmHg. Results: ICP was progressively reduced in the Stimulation Only and Stimulation + miRNA groups. Interpretation: CSF drainage pathways were expanded, relieving stagnation → reducing pressure. Statistical significance: $p < 0.05$ (Stimulation + miRNA group). (2) miR-155 (inflammatory microRNA), Unit: fold change vs. Sham, Result: Decreased in the Stim Only group, most pronounced inhibition in the Stim + miRNA group, Interpretation: Inflammatory microRNAs are effectively eliminated by lymphatic flow and immunosuppressive signals, Statistics: $p < 0.001$ (***) → strong significance, (3) miR-124a (anti-inflammatory microRNA), Result: Significant

increase (1.8-fold level) in the Stim + miRNA group, Interpretation: Suggesting that stimulation and co-treatment contributed to the induction of anti-inflammatory immune pathways, Statistics: $p < 0.01$ (**), (4) IL-6 (inflammatory cytokine), Unit: pg/mg, Result: Sham > Stim Only > Stim + miRNA → Gradual decrease with stimulation, Interpretation: Stimulation inhibits the secretion of inflammatory cytokines and contributes to their elimination through lymphatic drainage, (5) IL-10 (anti-inflammatory cytokine), Result: Gradually increased in the stimulation group, Interpretation: The miRNA and cytokine regulatory effects lead to the restoration of immune balance. Linked, no statistical significance indicated but visually distinct trend, right text summary, Stimulation of brain lymphatic outflow: Reduces intracranial pressure (ICP), Decreases inflammatory microRNA & cytokines, Alleviates Alzheimer's pathology, a three-sentence summary of the data highlighting the key clinical/physiological effects, highlighting the preclinical value of lymphatic stimulation technology by mentioning its effect in reducing

dementia pathology, and the table below is a quantitative numerical summary.

Marker	Sham	Stim Only	Stim + miRNA
ICP	16.5 ± 0.8	12.5 ± 0.5	11.0 ± 0.5
miR-155	1.00 ± 0.10	0.52 ± 0.08	0.30 ± 0.05
IL-6	200 ± 10	130 ± 8	90 ± 8
Aβ42	180 ± 10	130 ± 8	105 ± 7

VI. CONCLUSION

It is induced by increased cerebral lymph flow and decreased intracranial pressure (ICP). As a result of ICG and Gd-MRI tracking, the tracer influx into the dCLN was confirmed to be the fastest and strongest in the Stim + miRNA group, which means that the meningeal lymphatic drainage pathway was actually opened and activated through stimulation. Intracranial Pressure (ICP) changes were: Sham group: 16.5 ± 0.8 mmHg, Stim Only: 12.5 ± 0.5 mmHg, Stim + miRNA: 11.0 ± 0.5 mmHg (p < 0.01), suggesting that stimulation induces a decrease in fluid retention in the brain and relief of pressure. 2. Decreased expression of inflammatory miRNAs and increased expression of anti-inflammatory miRNAs. miR-155: 50-70% decrease in the stimulation group, significant inhibition in the Stim + miRNA group compared to Sham (p < 0.001), miR-124a: approximately 1.8-fold increase in the stimulation group, potential for inducing anti-inflammatory pathways.

miRNA	Sham	Stim Only	Stim + miRNA
miR-155	1.00 ± 0.10	0.52 ± 0.08	0.30 ± 0.05
miR-124a	1.00 ± 0.10	1.52 ± 0.10	1.82 ± 0.11

Changes in immune cytokine profile (Multiplex Assay) showed a decrease in inflammatory cytokines: IL-6, TNF-α, IFN-γ: all decreased by 40–60% in Stim + miRNA, and an increase in anti-inflammatory cytokines: IL-10, TGF-β: markedly increased in the stimulation group (p < 0.05).

Cytokine	Sham	Stim Only	Stim + miRNA
IL-6 (pg/mg)	200 ± 10	120 ± 12	90 ± 8
IL-10 (pg/mg)	100 ± 10	140 ± 15	180 ± 10
TGF-β (pg/mg)	100 ± 8	125 ± 12	140 ± 10

In terms of the effect of removing pathological proteins (Alzheimer's pathology markers), Aβ42: A reduction of more than 40% in the stimulation group, Sham: 180 ± 10 pg/mg → Stim + miRNA: 105 ± 7 pg/mg, Phospho-Tau (p-Tau): Sham: 140 ± 10 pg/mg → Stim + miRNA: 90 ± 6 pg/mg. 5. Integrated interpretation: Activation of cerebral lymphatic flow → Control of inflammation → Alleviation of pathology. Implications of the observational results: Increased CSF flow, restoration of lymphatic drainage function. Reduced ICP, relief of stagnant pressure. Reduced miRNA miR-155, increased miR-124a, suppression of inflammation and immune regulation. Reduced cytokines IL-6/TNF-α, increased IL-10, shift from Th1 to Th2 immunity. Reduced pathoproteins Aβ42 and p-Tau, potential suppression of Alzheimer's disease pathology. Consequently, beyond the stimulating effect, we demonstrate that overall immune and pathological environment regulation is possible through the removal of inflammatory molecules in the brain.

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