

Article

Synergistic Enhancement of Peripheral Nerve Regeneration via Ibudilast-Primed Three-Dimensional Spheroid Culture of Human Adipose-Derived Stem Cells

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Abstract

Background: Peripheral nerve regeneration relies on Schwann cell activation and neurotrophic support. Although adipose-derived stem cells (ADSCs) show therapeutic potential through paracrine mechanisms, their clinical application is often limited by donor-dependent heterogeneity in therapeutic efficacy. Accordingly, strategies to standardize and potentiate their secretory function are essential. This study investigated a safety-optimized strategy to achieve this by combining three-dimensional (3D) spheroid culture with ibudilast, a clinically approved phosphodiesterase inhibitor. **Methods:** Human ADSCs were cultured in 2D or 3D conditions with varying ibudilast concentrations. Safety was confirmed via CCK-8 assays, and trophic factor secretion was quantified by RT-qPCR and ELISA. To rigorously validate functional outcomes, conditioned media were applied to a dual-model system comprising immortalized rat (RSC96) and primary human Schwann cells (HSCs), assessing migration and the expression of regeneration-associated genes. **Results:** Ibudilast demonstrated no cytotoxicity. While 3D culture alone enhanced secretion compared to 2D controls, the addition of ibudilast provided a synergistic boost, resulting in a 6- to 14-fold increase in NGF, VEGF, and IGF-1 levels compared to 3D spheroids alone. Notably, conditioned media from these primed spheroids significantly accelerated HSCs migration and induced robust upregulation of myelination-related genes (specifically *PMP22* and *EGR2*), with trophic effects sustained for up to 72 h. **Conclusions:** Ibudilast-primed 3D spheroids synergistically amplify the neuroregenerative secretome of ADSCs. By utilizing a repurposed, safe small molecule to overcome functional variability and maximize potency without genetic manipulation, this strategy represents a highly translatable candidate for peripheral nerve repair.

Keywords: adipose-derived mesenchymal stem cells; nerve regeneration; ibudilast; schwann cells; nerve growth factors



Academic Editor: Yiming Xu

Received: 4 February 2026

Revised: 13 February 2026

Accepted: 15 February 2026

Published: 20 February 2026

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1. Introduction

Peripheral nerve injuries present a significant clinical burden, often resulting in lifelong functional deficits despite advances in microsurgical techniques [1–3]. While diverse therapeutic strategies have been extensively explored over the years, Schwann cells have recently emerged as the focal point of regenerative medicine research due to their indispensable role in orchestrating the pro-regenerative microenvironment [2,4]. Notably, Schwann cells exhibit remarkable phenotypic plasticity in response to environmental cues [5]. Following

injury, they dedifferentiate into a specialized ‘repair phenotype’ to facilitate the clearance of debris and guide axonal regrowth [6–8]. Subsequently, as regeneration progresses, they re-differentiate into mature Schwann cells to form the myelin sheath essential for functional recovery [4,6,9]. In this context, adipose-derived stem cells (ADSCs), which dynamically adapt to the microenvironment to regulate anti-inflammatory responses and secrete neurotrophic factors, are anticipated to be an ideal therapeutic candidate capable of supporting the phenotypic plasticity of Schwann cells during nerve repair [2,10,11].

However, despite these therapeutic merits, the clinical translation of ADSCs faces challenges related to inherent heterogeneity. Unlike standardized chemical pharmaceuticals, ADSCs exhibit significant variability in viability and therapeutic potency depending on donor characteristics, making it difficult to maintain consistent efficacy [12,13]. To overcome these limitations, various functional enhancement strategies have been proposed [14]. For successful application in humans, it is paramount to employ methods that are not only effective but also clinically safe, avoiding risks associated with genetic manipulation [15]. In this regard, pharmacological preconditioning using clinically approved small molecules offers a compelling solution [16].

Ibuprofen, a well-established phosphodiesterase-4 (PDE4) inhibitor used for asthma and post-stroke dizziness, functions by preventing the degradation of intracellular cyclic adenosine monophosphate (cAMP) [17,18]. By elevating cAMP levels, ibuprofen activates the protein kinase A (PKA)/cAMP response element-binding protein (CREB) pathway, which is pivotal for enhancing cell survival and stimulating the secretion of neurotrophic factors [17]. Based on this rationale, we postulated that activating this pathway would potentiate the regenerative capacity of Schwann cells, thereby contributing to peripheral nerve repair. To maximize this pharmacological enhancement, we specifically engineered ADSCs into three-dimensional (3D) spheroids, creating a spatially optimized microenvironment [19]. We hypothesized that combining 3D culture with ibuprofen preconditioning would synergistically augment ADSC paracrine activity and promote Schwann cell-mediated nerve regeneration. Accordingly, this study aimed to evaluate the safety and efficacy of this hybrid cell therapy strategy *in vitro*.

2. Results

2.1. Screening for Optimal Ibuprofen Concentration to Enhance Cell Dynamics

Initial screening was conducted to determine the optimal concentration of ibuprofen (IB) for balancing cell proliferation and migration. In 2D cultures, CCK-8 analysis showed that RSC96 cells maintained stable viability across various IB concentrations. In contrast, 2D-cultured ADSCs exhibited a slight increase in proliferation at lower concentrations (3–25 μ M) but a significant reduction at 100 μ M (Figure 1A,B). BrdU incorporation assays further confirmed that IB does not impair DNA synthesis in either cell type over time (Figure 1C).

The effect of IB on cell motility was evaluated via scratch-wound assays. In ADSCs, migratory capacity was significantly attenuated at 10 μ M compared to lower doses (Figure 1D,E). For RSC96 cells, the wound closure rate peaked at 3 μ M and declined sharply at higher concentrations (Figure 1F,G). Consequently, 3 μ M was established as the optimal concentration for subsequent experiments.

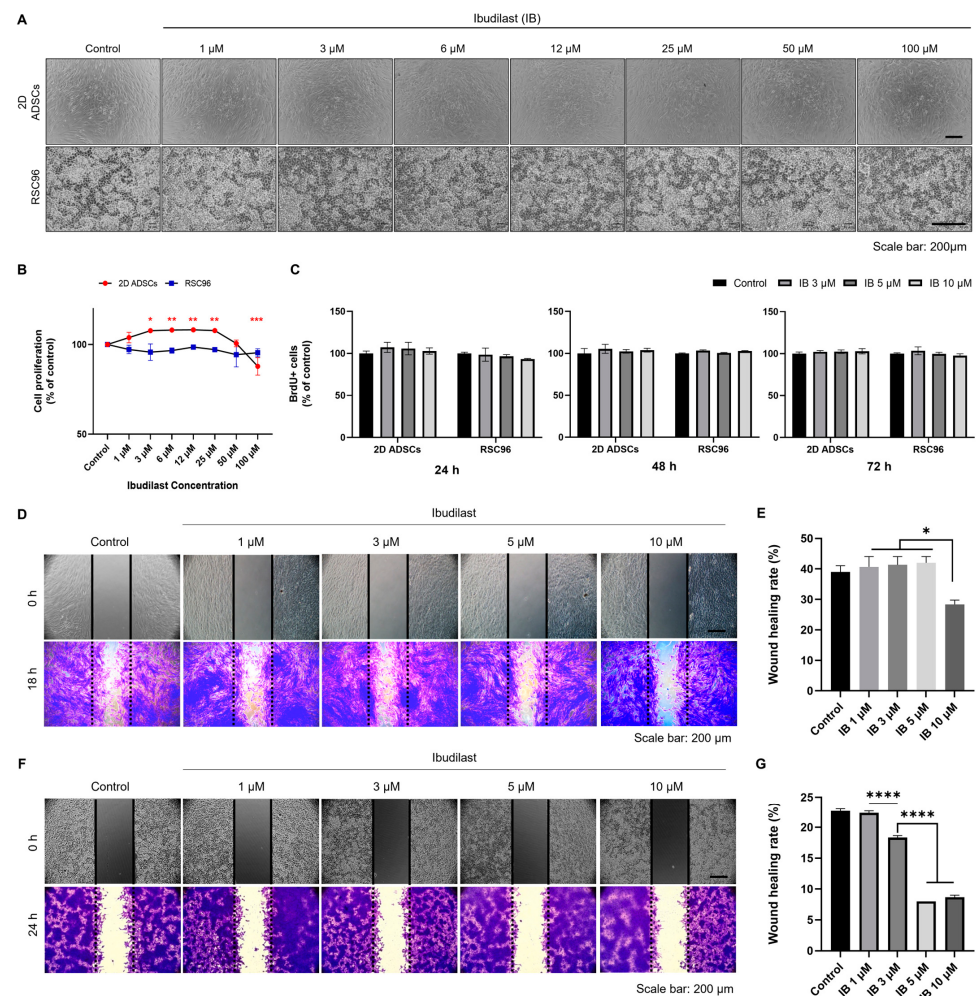


Figure 1. Effects of ibudilast (IB) on the viability, proliferation, and migratory activity of 2D-cultured ADSCs and RSC96 cells. (A) Representative phase-contrast images showing the morphological characteristics of ADSCs and RSC96 cells after treatment with the indicated concentrations of ibudilast (IB) for 72 h. Images were randomly selected from the same culture plate at 72 h post-treatment. Experiments were performed in triplicate ($n = 3$). (B) Quantitative assessment of cell viability via CCK-8 assay and (C) DNA synthesis evaluated by BrdU incorporation assay at 24, 48, and 72 h post-treatment. Cell proliferation (%) was calculated relative to the control group ($n = 3$). The percentage of BrdU+ cells at 24, 48, and 72 h was normalized to the respective control group at each time point ($n = 3$). (D) Representative scratch-wound images and (E) quantitative analysis of wound closure rates in ADSCs after 18 h of IB treatment. (F) Representative scratch-wound images and (G) quantitative analysis of wound closure rates in RSC96 cells after 24 h of IB treatment. For the scratch wound assay, a silicone insert was used to create a ~500 µm cell-free gap. The initial wound width was standardized to 500 µm in all experimental groups. Representative images at 0 h were randomly selected. Wound closure was quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA) and calculated as follows: [(initial width – final width)/initial width $\times$ 100]. Quantitative analyses were performed in triplicate ($n = 3$). Data are expressed as mean $\pm$ standard error of the mean (SEM) from at least three independent experiments. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). Scale bar = 200 µm.

2.2. 3D Spheroid Environment Enhances Stemness and Antioxidant Resilience

ADSC spheroids were successfully generated using the StemFIT 3D system or Ultra-Low Attachment (ULA) plate (Figure 2A). Briefly, 2D-expanded ADSCs were seeded onto either the StemFIT 3D system or ULA plates and incubated for 24 h to facilitate spheroid self-assembly. Following formation, the culture medium was replaced with fresh medium

supplemented with ibudilast, and the spheroids were maintained until subsequent analysis. IB treatment at 3 μ M showed high cytocompatibility with these 3D structures (Figure 2B). Notably, 3D spheroids demonstrated superior antioxidant capacity compared to 2D ADSCs, with the 3 μ M IB-treated group showing the most pronounced resistance to oxidative stress (Figure 2C,D). Additional antioxidant assays were performed to further characterize these protective effects, with detailed results provided in Supplementary Figure S1.

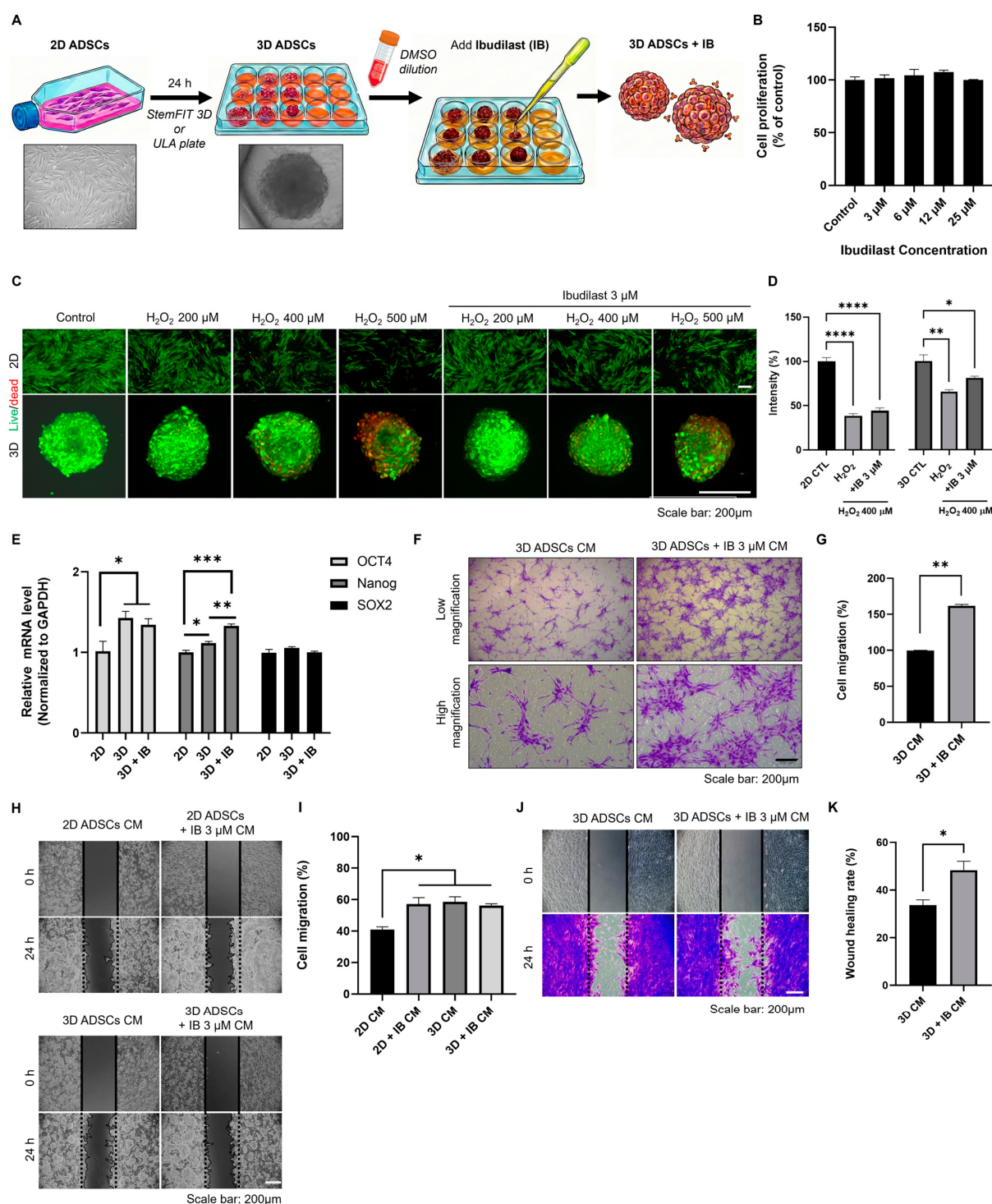


Figure 2. Characterization of ADSC spheroids and their paracrine effects on Human Schwann cell migration. (A) Schematic representation of the experimental timeline for ADSC spheroid formation using the StemFIT 3D system or Ultra-Low Attachment (ULA) plate followed by ibudilast (IB)

treatment. 2D-cultured ADSCs were seeded onto the StemFIT 3D system or ULA plate and incubated for 24 h to allow spheroid formation. Subsequently, the culture medium was replaced with fresh medium supplemented with ibudilast, and spheroids were cultured until analysis. **(B)** Cell viability of ADSC spheroids evaluated by CCK-8 assay after treatment with various concentrations of IB for 24 h. Cell proliferation (%) was calculated in comparison to the control group ($n = 8$). **(C)** Representative images and **(D)** quantitative analysis of cell viability in 2D-cultured ADSCs and 3D spheroids under oxidative stress. To evaluate the protective effects of ibudilast, 2D-cultured ADSCs and 3D spheroids were cultured with H_2O_2 (200, 400, or 500 μM) alone or concurrently treated with 3 μM ibudilast, followed by live/dead staining ($n = 12$). Quantitative analysis was performed at 400 μM H_2O_2 , where the clearest difference between 2D and 3D cultures under oxidative stress was observed ($n = 8$). **(E)** Relative mRNA expression levels of stemness-related markers (*OCT4*, *SOX2*, and *NANOG*) in 2D ADSCs, untreated spheroids, and 3 μM IB-treated spheroids, assessed by RT-qPCR. **(F)** Representative Transwell migration images and **(G)** quantitative analysis of migrated human Schwann cells (HSCs) following treatment with conditioned medium (CM) derived from ADSC spheroids. **(H)** Representative scratch-wound images and **(I)** quantitative analysis of wound closure rates in RSC96 cells treated with spheroid-derived CM. **(J)** Representative scratch-wound images and **(K)** quantitative analysis of wound closure rates in HSCs following CM treatment. Scratch wound and transwell assays were performed at multiple time points, and quantitative analyses were conducted at the time point at which the differences between experimental groups were most evident ($n = 3$). Data are presented as mean $\pm$ standard error of the mean (SEM) from at least three independent experiments. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). Scale bar = 200 μm .

The 3D microenvironment also significantly boosted the stemness of ADSCs. Expression of *OCT4* and *Nanog* was markedly higher in spheroids than in 2D cells. Specifically, *Nanog* expression in the 3D + IB group was significantly upregulated even when compared to the untreated 3D group, indicating a synergistic effect between IB and the 3D configuration (Figure 2E).

2.3. Paracrine Modulation of Human Schwann Cell Recruitment

Based on the results obtained from RSC96 cells, we next evaluated the effects of ibudilast-treated spheroids on human Schwann cells. To evaluate the therapeutic potential of the spheroid secretome, human Schwann cells (HSCs) were treated with conditioned medium (CM) derived from 3D spheroids. Transwell migration assays revealed that CM from IB-treated spheroids (3D + IB 3 μM) significantly increased the chemotactic recruitment of HSCs compared to CM from untreated spheroids (Figure 2F,G). While all CM groups (3D, 2D + IB, and 3D + IB) enhanced the migration of rat RSC96 cells compared to the 2D control, no significant difference was observed between the 3D and 3D + IB groups in these cells (Figure 2H,I). However, for HSCs, the 3D + IB CM group exhibited a significantly faster wound closure rate than the 3D CM group in scratch assays (Figure 2J,K). This demonstrates that IB treatment specifically augments the paracrine potential of spheroids to stimulate human Schwann cell dynamics.

2.4. Robust Induction of Regenerative Machinery in 3D Spheroids

Transcriptional profiling underscored the superiority of the 3D + IB model over traditional 2D cultures. In 2D ADSCs, IB treatment resulted in a transient and modest (<2-fold) increase in neurotrophic factors, which subsided after 48 h (Figure 3A). In contrast, 3D spheroids treated with 3 μM IB exhibited a massive transcriptional surge at 24 h: NGF and IGF expression increased 12–14 fold, while VEGF expression increased 6-fold (Figure 3B). Although these levels normalized by 72 h—likely due to the absence of persistent external stimuli—the initial 24 h peak is expected to provide a critical stimulus for nerve regeneration. To further validate these gene expression patterns across a

broader range of concentrations, extensive RT-qPCR analyses were conducted, as shown in Supplementary Figures S2 and S3. Furthermore, IB treatment significantly modulated the inflammatory profile of RSC96 cells, with a marked increase in anti-inflammatory cytokines observed at 72 h (Figure 3C).

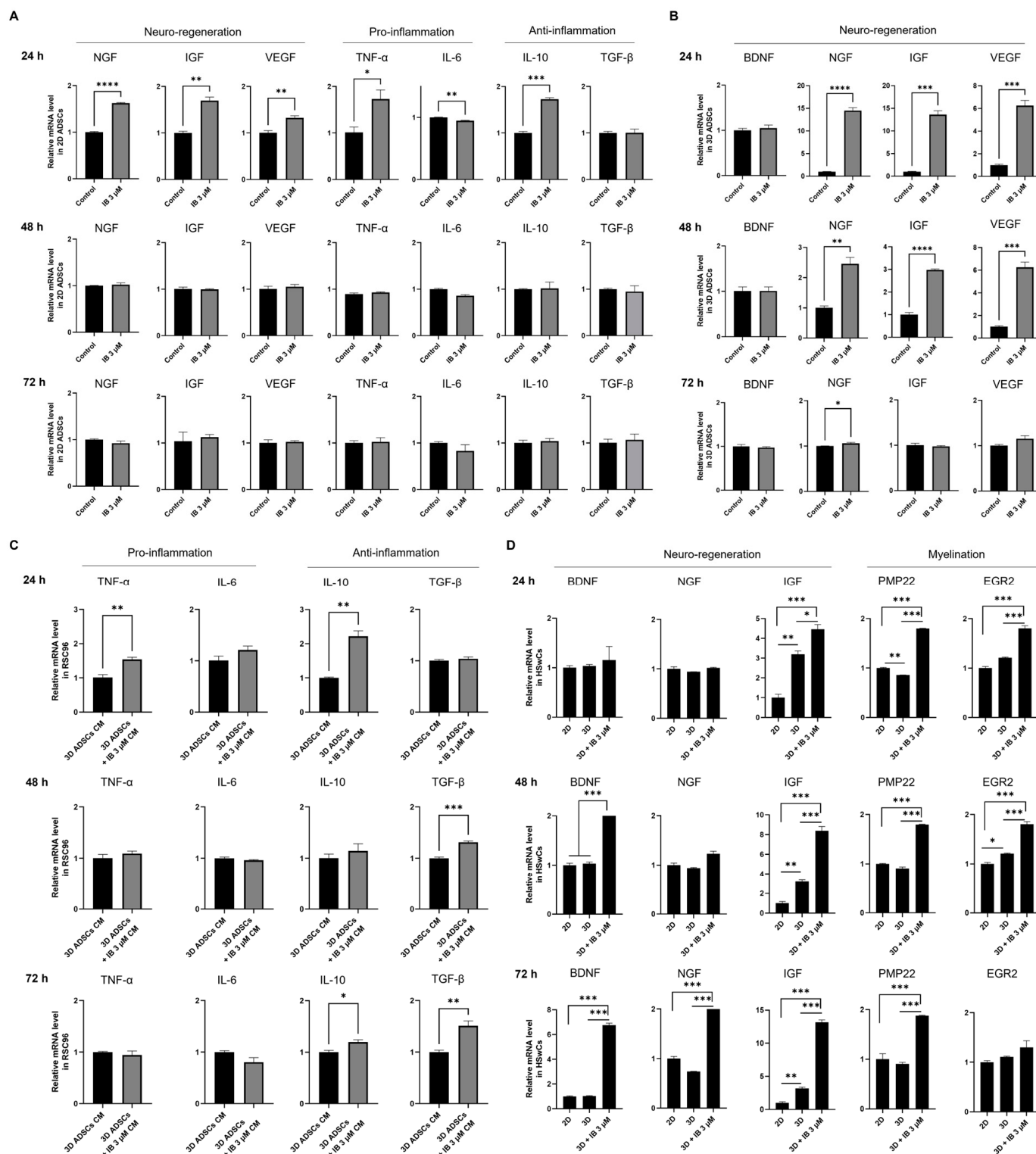


Figure 3. Assessment of the immunomodulatory effects of ibudilast on ADSCs and their supportive role for Schwann cells. To evaluate the immunomodulatory role of ibudilast in the context

of peripheral nerve regeneration, the expression of inflammation-related genes, including pro-inflammatory cytokines ($TNF-\alpha$ and $IL-6$), and anti-inflammatory mediators ($IL-10$ and $TGF-\beta$), was assessed by RT-qPCR. Furthermore, we investigated whether this favorable immunomodulatory environment subsequently promotes the expression of neuro-regenerative markers ($BDNF$, NGF , IGF , and $VEGF$) and myelination markers ($PMP22$ and $EGR2$). (A) Relative mRNA expression levels of neurotrophic factors (NGF , IGF , and $VEGF$), pro-inflammatory cytokines ($TNF-\alpha$ and $IL-6$), and anti-inflammatory cytokines ($IL-10$ and $TGF-\beta$) in 2D-cultured ADSCs treated with 3 μM ibudilast (IB) for 24, 48, and 72 h. Data are presented as fold changes relative to the 2D control group ($n = 3$). (B) Relative mRNA expression of neurotrophic factors ($BDNF$, NGF , IGF , and $VEGF$) in ADSC spheroids treated with 3 μM IB. Data are presented as fold changes relative to untreated spheroids (3D control) ($n = 3$). The most pronounced transcriptional response for most genes was observed at 24 h post-treatment, this peak serves as a reference for interpreting the expression trends at the 48 h and 72 h time points. (C) Transcriptional profiles of pro-inflammatory ($TNF-\alpha$ and $IL-6$) and anti-inflammatory cytokines ($IL-10$ and $TGF-\beta$) in rat RSC96 cells following 3 μM IB treatment. (D) Gene expression profiles in HSwCs co-cultured with ADSC spheroids. HSwCs were co-cultured with ADSC spheroids using a Transwell system (non-contact) and treated with 3 μM ibudilast (IB) for 24, 48, and 72 h. The relative mRNA expression levels of neurotrophic factors ($BDNF$, NGF , and IGF) and myelination-associated markers ($PMP22$ and $EGR2$) in HSwCs were then evaluated. All gene expression levels were normalized to $GAPDH$. Data are presented as mean $\pm$ standard error of the mean (SEM) from at least three independent experiments. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

2.5. Promotion of Myelination Markers and Growth Factor Secretion

The impact of the 3D + IB secretome on HSwC maturation was evaluated through neuro-regeneration and myelination markers. At 72 h, HSwCs treated with 3D + IB CM showed a 2–13 fold increase in neurotrophic factors compared to 2D cultures, and a 2–6 fold increase compared to the 3D control. Crucially, the key myelination indicators $PMP22$ and $EGR2$ were upregulated approximately 2-fold in the 3D + IB group compared to the untreated 3D group (Figure 3D). Finally, secretome analysis via ELISA confirmed these findings at the protein level. The secretion of IGF and VEGF protein was significantly enhanced (2–6 fold) in the 3D + IB spheroid CM compared to controls (Figure 4A,B). These data collectively demonstrate that IB treatment effectively reprograms ADSC spheroids into a potent factory for neuro-regenerative and myelination-promoting factors.

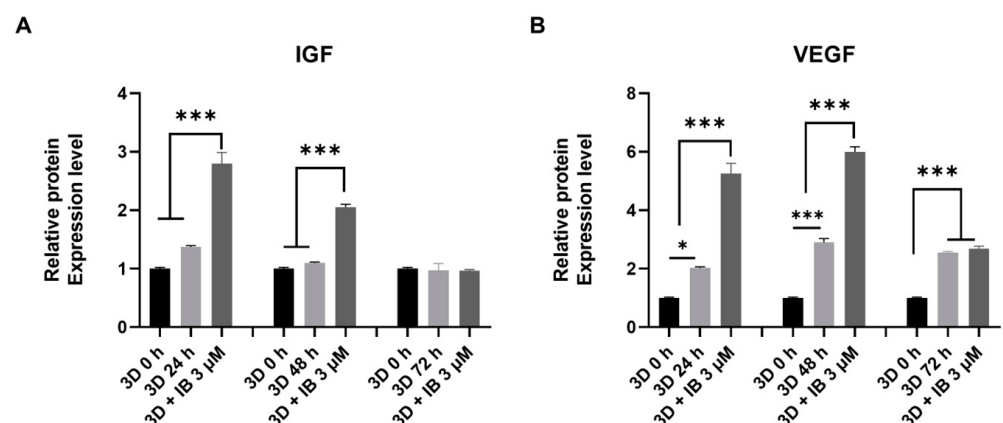


Figure 4. Enhanced secretion of growth factors from ibudilast-treated ADSC spheroids. Spheroids for gene expression analysis and conditioned media (CM) for ELISA were collected simultaneously from the same batch. The culture medium was centrifuged to remove cellular components, and the resulting supernatant was harvested and stored at $-80^{\circ}C$ until analysis. Quantitative analysis of

(A) insulin-like growth factor (IGF) and (B) vascular endothelial growth factor (VEGF) protein levels in the conditioned medium (CM) derived from ADSC spheroids. Spheroids were treated with 3 μ M ibudilast for 24, 48, and 72 h, and the protein concentrations in the supernatants were determined by enzyme-linked immunosorbent assay (ELISA) ($n = 3$). Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test (* $p < 0.05$, *** $p < 0.001$).

3. Discussion

Peripheral nerve regeneration remains clinically challenging despite advances in microsurgical repair techniques [1,2]. Functional recovery depends not only on anatomical reconstruction of the injured nerve but also on the timely establishment of a supportive biological microenvironment characterized by Schwann cell recruitment, trophic signaling, and remyelination of regenerating axons [2,4]. Therefore, therapeutic strategies that enhance this regenerative niche have emerged as critical adjuncts to surgery. Among various approaches, stem cell-based therapies acting through paracrine mechanisms have attracted increasing attention [10,11]. In the present study, we demonstrate that combining three-dimensional (3D) spheroid culture with ibudilast preconditioning creates a synergistically enhanced adipose-derived stem cells (ADSCs) population with exponentially increased neuro-regenerative potential. This superior performance surmounts a significant barrier to clinical translation by achieving a 6- to 14-fold amplification of key neurotrophic factors via a clinically safe strategy, which translated into robust Schwann cell activation.

The mechanism underlying this synergistic enhancement likely involves the convergence of physical and chemical signaling pathways. The beneficial effects observed with 3D culture alone are consistent with the concept that spheroid aggregation better recapitulates the native stem cell niche [20,21]. Three-dimensional organization enhances cell-cell communication, recreates physiologic oxygen gradients, and upregulates adhesion molecules, thereby boosting basal secretory activity [20–22]. Building upon this physical priming, the addition of ibudilast served as a pivotal pharmacological trigger. Mechanistically, the potentiating effects of ibudilast are likely driven by a multifaceted pharmacological convergence that extends beyond simple PDE inhibition [17,23]. While the canonical elevation of intracellular cAMP and subsequent activation of the PKA–CREB axis serves as the primary driver for neurotrophic gene transcription (e.g., *NGF*, *BDNF*) [23,24], ibudilast's impact is further amplified by its known role as a Toll-like receptor 4 (TLR4) antagonist. By inhibiting the TLR4 signaling cascade, ibudilast attenuates the nuclear translocation of NF- κ B, thereby suppressing pro-inflammatory cytokine production [25,26]. This immunomodulatory action is crucial, as it shifts the ADSCs secretome from a potentially reactive state to a stable, pro-regenerative phenotype. Furthermore, the sustained elevation of cAMP facilitates extensive crosstalk with the PI3K/Akt and MAPK/ERK pathways [27,28]. These cascades are pivotal for cellular survival and metabolic adaptation, particularly within the nutrient-restricted core of 3D spheroids. The concurrent activation of CREB for trophic synthesis, Akt for apoptotic resistance, and ERK for proliferation suggests that ibudilast orchestrates a comprehensive signaling network [29]. This concerted modulation not only maximizes the paracrine output but also ensures the robust viability of the spheroids, creating a synergistic platform where physical and chemical cues align to optimize neuro-regeneration.

Additionally, the role of macrophages as essential mediators of peripheral nerve repair must be considered. While direct characterization of macrophage markers was beyond the scope of this study, our data regarding RSC96 provided critical insight into the polarization niche. The significant upregulation of IL-10 and TGF- β at 72 h post-treatment is particularly noteworthy. These factors are well-established cues that drive resident macrophages from a pro-inflammatory M1 phenotype toward a pro-regenerative M2 phenotype. Considering that ibudilast acts as a macrophage migration inhibitory factor

(MIF) inhibitor, which typically prevents excessive M1-mediated inflammation, our findings suggest that the synergy between ibudilast and 3D ADSC spheroids establishes a matured M2-like niche. This temporal maturation of the secretome toward a pro-regenerative profile by day 3 appears to be a crucial step in creating an environment conducive to structural and functional nerve recovery.

It is particularly compelling to observe how this functional enhancement translates into tangible biological benefits within the regenerative microenvironment. Specifically, the marked elevation of NGF directly supports the survival of injured neurons and axonal outgrowth [30,31], while VEGF promotes angiogenesis to ensure nutrient supply [32]. Furthermore, the sustained secretion of IGF-1, observed for up to 72 h, offers a clinically relevant window of trophic support crucial for limiting Wallerian degeneration [33,34]. To rigorously evaluate how these soluble factors modulate Schwann cells, the pivotal mediators of nerve repair, we employed a strategic dual-model approach. While preliminary cellular responses were screened in immortalized rat cells (RSC96), regenerative functionality was assessed specifically using human Schwann cells (HSCs). This experimental design is advantageous, as reliance solely on rodent lines may be confounded by species-specific differences in ligand-receptor affinity [35,36]. By focusing our validation of the human ADSCs secretome directly on human target cells, we minimized artifacts arising from species mismatch and confirmed that our primed secretome is biologically active in a clinically relevant context. Indeed, this modulated secretome not only accelerated HSCs migration but also promoted a shift toward a pro-myelinating repair phenotype, evidenced by the significant upregulation of PMP22 and EGR2. Given that PMP22 is a major myelin component and EGR2 is an initial transcription factor for myelination [37–39], these findings suggest that our primed spheroids could actively facilitate remyelination, a rate-limiting step in functional recovery.

From a translational perspective, this combinatorial strategy presents a compelling alternative to complex genetic modification methods, such as viral transfection, which face high regulatory hurdles and safety concerns. A distinct advantage of our approach lies in the favorable physicochemical compatibility between the therapeutic agent and the cell source. Ibudilast is a highly lipophilic small molecule, a property that facilitates rapid transmembrane permeability and efficient intracellular uptake when dissolved in compatible vehicles such as DMSO [17,40]. This characteristic is particularly advantageous when applied to ADSCs, as their intrinsic lipid-rich nature likely enhances the affinity and retention of lipophilic compounds. Unlike hydrophilic drugs that often require specific transporters or vectors for entry, the passive loading efficiency of ibudilast creates a “bio-depot” effect within the spheroids without compromising membrane integrity [40,41]. This ensures that the regenerative signaling cascades are sustainedly activated even after the initial exposure, maximizing therapeutic potency. Building upon this biochemical advantage, our dose–response analysis further reinforces the clinical viability of this strategy. We observed that the regenerative benefits were maximized at a remarkably low concentration of 3 μM , whereas higher doses (e.g., 100 μM) began to exhibit inhibitory effects on cell viability and motility. This indicates that the synergistic effect of the 3D microenvironment allows for a significant therapeutic impact even at sub-toxic levels. Given that ibudilast is already a clinically approved drug for other indications [17,18,26,40], the demonstration of its efficacy at such a low, safe dosage significantly lowers the barrier for its clinical translation in the field of nerve repair. This low-dose efficacy minimizes the risk of systemic side effects and off-target toxicity, which are often the primary reasons for the failure of new regenerative therapies during clinical trials.

Clinically, this augmented regenerative microenvironment may be particularly beneficial in large-gap nerve defects or delayed repairs, where endogenous trophic support

is insufficient. Moreover, the robust effects of the secretome alone suggest a potential for “cell-free” therapeutics using conditioned media or exosomes, which could further resolve logistical challenges related to cell viability and immune rejection. Preconditioned ADSCs or their secretome could be readily incorporated into nerve conduits, hydrogels, or injectable delivery systems to complement existing reconstructive techniques. Additionally, given the concentration-dependent effects of ibudilast, the development of local delivery systems, such as drug-eluting scaffolds or hydrogels, will be essential to maintain the therapeutic concentration at the site of peripheral nerve injury while avoiding the inhibitory effects of higher dosages.

Despite the promising findings, several limitations of this study should be acknowledged. First, our investigation was restricted to *in vitro* analyses. While these results provide a strong mechanistic foundation, *in vivo* validation using animal models of peripheral nerve injury is essential to confirm long-term functional recovery, histological regeneration, and the persistence of the therapeutic effects within a complex physiological environment. Second, the human ADSCs used in this study were isolated from a single donor. Given that stem cell potency can be influenced by donor-specific factors such as age, sex, and metabolic status, future studies should involve a more diverse donor population to ensure the generalizability of the 3D-primed secretome’s efficacy. Furthermore, although we focused on key neurotrophic factors and myelination markers, the ADSC secretome is a highly complex mixture of proteins and microRNAs. A more comprehensive proteomic analysis would be necessary to fully characterize the synergistic changes induced by the 3D configuration and ibudilast treatment. In particular, while we characterized the cytokine profile of Schwann cells, the direct immunomodulatory impact on macrophage polarization was not validated using specific macrophage markers. Given ibudilast’s established role as a MIF inhibitor, future studies incorporating immune cell co-cultures are warranted to elucidate the interaction between the 3D-primed secretome and the local inflammatory niche. Additionally, the observed surge in gene expression was transient, peaking at 24 h and normalizing by 72 h. Crucially, ibudilast is known to exhibit concentration-dependent biphasic effects, where supra-optimal dosages may lead to inhibitory responses. While this initial “triggering” effect is robust, the long-term clinical application will require optimized localized delivery strategies, such as incorporating spheroids into drug-eluting scaffolds or bioactive hydrogels. Such systems are essential to maintain the therapeutic concentration at the injury site and provide sustained trophic support over the protracted period of nerve regeneration. Finally, although the cAMP-PKA-CREB axis is the likely mediator of ibudilast’s effects, direct investigation of the precise intracellular signaling network remains a task for future work.

4. Materials and Methods

4.1. Cell Culture and Reagents

The RSC96 cells (a spontaneously immortalized rat Schwann cell line) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in Dulbecco’s modified Eagle’s medium (DMEM; Welgene, Gyeongsan, Republic of Korea) supplemented with 10% fetal bovine serum (FBS). Human adipose-derived stem cells (ADSCs) were isolated from the abdominal adipose tissue of a 50-year-old female patient who underwent surgery at Dankook University Hospital (Cheonan, Republic of Korea). The isolation and use of human ADSCs were conducted in accordance with the guidelines and approval of the Institutional Review Board of Dankook University Hospital (IRB No. DKUH-2024-10-005; Date of Approval: 16 October 2024). ADSCs were cultured in α -minimum essential medium (α -MEM). Primary human Schwann cells (HSwCs) were purchased from iXCells Biotechnologies (San Diego, CA, USA) and maintained in Schwann

Cell Growth Medium (Cat. No. MD-0055) according to the manufacturer's instructions. All cells were cultured at 37 °C in a humidified incubator with 5% CO₂. HSwCs were used within three passages for all experiments to ensure phenotypic consistency. All culture supplements were purchased from Gibco-Invitrogen (Thermo Fisher Scientific, Waltham, MA, USA).

4.2. Ibudilast Preparation and Treatment

Ibudilast (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution and diluted with culture medium to achieve the designated working concentrations prior to treatment. RSC96 cells or ADSCs were exposed to ibudilast for 24–72 h, after which the cells were harvested. Control groups were treated with an equivalent concentration of DMSO (<0.1%).

4.3. ADSCs Spheroid Formation and Conditioned Medium (CM) Preparation

ADSC spheroids were generated using two methods. First, for large-scale production, spheroids were formed using the StemFIT 3D system (MicroFIT, Gyeonggi-do, Republic of Korea). Second, for individual analysis, spheroids were generated in 96-well ultra-low attachment (ULA) plates (Corning, Corning, NY, USA) by seeding 5×10^3 cells per well and incubating for 24 h. To prepare the conditioned medium (CM), ADSCs were seeded into the StemFIT 3D system at a density of 1×10^6 cells per well. Following spheroid formation, 1 mL of complete medium containing ibudilast was added and cultured for 24 h. The supernatant was collected, centrifuged at $1000 \times g$ for 5 min to remove cellular debris, and stored at −80 °C in aliquots.

4.4. Cell Proliferation, Viability, and Morphology Analysis

Cell proliferation was quantitatively assessed using the Cell Counting Kit-8 (CCK-8; DOJINDO, Kumamoto, Japan) and the BrdU Cell Proliferation Assay Kit (Cell Signaling Technology, Danvers, MA, USA). For both assays, cells were seeded in 96-well plates at 2×10^3 cells per well and treated with ibudilast for 24–72 h. For CCK-8, 10 µL of solution was added per well and incubated for 2 h at 37 °C. For BrdU, the incorporation was detected according to the manufacturer's protocol. Absorbance was measured at 450 nm using a microplate reader (VARIOSKAN, Thermo Fisher Scientific). Cell morphology was monitored and imaged using an inverted phase-contrast microscope (Olympus, Tokyo, Japan).

4.5. In Vitro Migration Assay

To evaluate the migratory and chemotactic capacities of the cells, both wound healing and transwell migration assays were performed. For the wound healing assay, silicone culture inserts (Ibidi, Gräfelfing, Germany) were placed in 24-well plates, and cells were seeded and cultured until reaching 90% confluence, at which point the inserts were removed to create a uniform cell-free gap. The cells were then treated with various concentrations of ibudilast or conditioned medium (for HSwCs) for 24 h. In parallel, a transwell migration assay was conducted by seeding cells at a density of 1×10^5 cells per well into the upper chamber of 24-well transwell inserts with an 8.0- µm pore size (Corning). The lower chamber was filled with culture medium supplemented with 10% FBS and ibudilast, or conditioned medium as a chemoattractant for HSwCs. After a 24 h incubation period, cells that had migrated to the lower surface of the membrane were fixed and stained with 1% crystal violet. Quantitative analysis was performed by measuring absorbance at 595 nm, and representative images were captured using the Metamorph Cell Imaging System (Olympus).

4.6. Antioxidant Activity and Oxidative Stress Assay

To evaluate antioxidant effects, cells were treated with ibudilast and 200–500 μM hydrogen peroxide (H_2O_2 ; Sigma-Aldrich). After 24–72 h, gene expression was analyzed via quantitative real-time polymerase chain reaction (RT-qPCR). For 3D conditions, ADSC spheroids formed in ULA plates were treated with H_2O_2 and ibudilast for 24 h. Spheroids were then harvested and analyzed using a Live/Dead staining kit (Thermo Fisher Scientific). Fluorescence images were obtained via confocal laser scanning microscopy (Nikon, Tokyo, Japan). Migration and wound healing assays were also conducted under the same oxidative stress conditions (200 μM H_2O_2).

4.7. Co-Culture of ADSC Spheroids and Human Schwann Cells (HSCs)

To evaluate the effects of ibudilast on the interaction between ADSC spheroids and human Schwann cells (HSCs), co-culture experiments were performed. HSCs were cultured either alone or co-cultured with ADSC spheroids, and each condition was further divided into ibudilast-treated and untreated groups. The co-culture systems were maintained at 37 °C for 24–72 h. Following incubation, HSCs were harvested and analyzed by real-time polymerase chain reaction (RT-qPCR).

4.8. Real Time-Quantitative PCR (RT-qPCR)

Total RNA was extracted using Ribospin (GeneAll, Seoul, Republic of Korea). One microgram of RNA was reverse-transcribed into cDNA using the iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). Quantitative real-time PCR was performed using the SensiMix SYBR Hi-ROX kit (Bioline, London, UK) on an ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The protocol included initial denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C (15 s) and 60 °C (1 min). Relative gene expression was calculated using the $2^{-\Delta\Delta\text{Ct}}$ method, normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Primer sequences are provided in Table 1.

Table 1. Primer sequences for the real-time polymerase chain reaction.

Species	Gene	Forward Primer (5' → 3')	Reverse Primer (5' → 3')
Human	GAPDH	CCAGAACATCATCCCTGCCTCT	GACGCCTGCTTCACCACCTT
	TNFA	CGTGGAGCTGGCCGAGGAG	AGGAAGGAGAAGAGGCTGAGGAAC
	IL6	GGTGTTCCTGCTGCCTTCC	GTTCTGAAGAGGTGAGTGGCTGTC
	IL10	TCAAGGCGCATGTGAACTCC	GATGTCAAACCTCACTCATGGCT
	BDNF	AGCTCCGGGTGGTATACTGG	CCTGGTGGAACCTCTTTGCG
	NGF	CATGCTGGACCCAAGCTCA	GACATTACGCTATGCACCTCAGTG
	IGF	AAGGAGGCTGGAGATGTATTGC	CGGACAGAGCGAGCTGACTT
	TGF- β 1	ATGACAAGTTCAAGCAGAG	CACTTGCAGTGTGTTATCC
	EGR2	AACGGAGTGGCCGAGAT	ATGGGAGATCCAACGACCTCTT
	PMP22	CTCCTCCTGTTGCTGAGTATC	GCTACAGTTCTGCCAGAGA
	SOX2	TGCGAGCGCTGCACAT	TTCTTCATGAGCGTCTTGTTT
	NANOG	GAACCTCTCCAACATCCTGAA	CCTTCTGCGTCACACCATTG
	OCT4	AGCGAACCAGTATCGAGAAA	TTACAGAACCACACTGGAC
Rat	Gapdh	GCAAGGATACTGAGAGCAAGAG	GGATGGAATTGTGAGGGAGATG
	Tnfa	CCCAATCTGTGTCCTTCTAACT	CAGCGTCTCGTGTGTTTCT
	Il6	AGGAAGGCAGTGTCACTCATTGT	CTTGGGTCCTCATCCTGGAA
	Il10	AGTGGAGCAGGTGAAGAATG	GAGTGTACGTAGGCTTCTATG
	Bdnf	GTGTGACAGTATTAGCGAGTGGG	ACGATTGGGTAGTTCGGCATT
	Ngf	CATCACTGTGGACCCCAAACTGT	GTCCGTGGCTGTGGTCTTATCTC
	IGF	GCTTTTACTTCAACAAGCCCACA	TCAGCGGAGCACAGTACATC
	TGF- β 1	CTGCTGACCCCCACTGATAC	AGCCCTGTATTCCGTCTCCT
	EGR2	GTGGAGGGCAAAAGGAGATAC	ACTGGGATTTGTCTACGGC
	PMP22	CCTGTCCCTGTTCTCTGTTT	CTGTGTCTCACTGTGTAGATGG

4.9. Secretome Analysis via Enzyme-Linked Immunosorbent Assay (ELISA)

ADSC spheroids were treated with 3 μM ibudilast for 24, 48, and 72 h. The concentrations of insulin-like growth factor (IGF) and vascular endothelial growth factor (VEGF) in the collected supernatants were quantified using ELISA kits (R&D Systems, Minneapolis, MN, USA) following the manufacturer's instructions. Absorbance was measured at 450 nm, and concentrations were determined based on standard curves.

4.10. Statistical Analysis

All data are presented as mean $\pm$ standard error of the mean (SEM) from at least three independent experiments. Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons, or an unpaired two-tailed Student's *t*-test for pairwise comparisons. Analysis was performed using GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA), and a *p*-value < 0.05 was considered statistically significant.

4.11. Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the authors used Gemini 3.0 (Google LLC, Mountain View, CA, USA) in order to generate the graphical illustration in Figure 2A and refine the English language for clarity and flow. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication. All experimental designs, wet lab data collection, analysis, and interpretation were conducted solely by the authors without the assistance of generative AI.

5. Conclusions

In conclusion, our findings demonstrate that modifying the physiological state of ADSCs through a hybrid strategy of 3D spheroid culture and low-dose ibudilast preconditioning significantly amplifies their neuro-regenerative potential. By achieving a massive, synergistic upregulation of trophic factors and promoting human Schwann cell activation at a clinically safe dosage, this approach offers a promising and translational platform for enhancing peripheral nerve repair.

Supplementary Materials: The following supporting information can be downloaded at: <https://doi.org/10.5281/zenodo.18506081>. Figures S1–S3: Supplementary Figure S1. Protective effects of ibudilast (IB) against oxidative stress-induced impairment in 2D-cultured ADSCs and RSC96 cells. To determine the optimal concentration of H_2O_2 , a dose-optimization study was initially conducted using various concentrations of H_2O_2 , and cell viability was evaluated using the CCK-8 assay. Based on these results, 200 μM H_2O_2 was selected as the optimal concentration for subsequent experiments under 2D culture conditions. (A, B) Cell viability of ADSCs and RSC96 cells measured by CCK-8 assay following treatment with indicated concentrations of IB and 200 μM hydrogen peroxide (H_2O_2) for 24, 48, and 72 h. (C) Quantitative analysis of wound closure rates and (D) representative scratch-wound images of ADSCs under oxidative stress conditions. (E) Quantitative analysis and (F) representative images of Transwell migration assays in ADSCs exposed to 200 μM H_2O_2 and IB. (G) Quantitative analysis of wound closure rates and (H) representative scratch-wound images of RSC96 cells under oxidative stress. (I) Quantitative analysis and (J) representative images of Transwell migration assays in RSC96 cells under oxidative stress. Data are presented as mean $\pm$ standard error of the mean (SEM) from three independent experiments. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). Scale bars = 200 μm . Supplementary Figure S2. Gene expression profiles of 2D-cultured ADSCs and RSC96 cells following ibudilast (IB) treatment. (A) Relative mRNA expression levels of neurotrophic factors (NGF and IGF), pro-inflammatory cytokines (TNF- α and IL-6), anti-inflammatory cytokine (IL-10), and angiogenic factor (VEGF) in 2D-cultured ADSCs treated with indicated concentrations of

IB for 24, 48, and 72 h. (B) Relative mRNA expression levels of neurotrophic factors (BDNF, NGF, and IGF), pro-inflammatory cytokines (TNF α and IL-6), anti-inflammatory cytokines (IL-10 and TGF- β), and myelination-related markers (EGR2 and PMP22) in RSC96 cells treated with IB for 24, 48, and 72 h. All gene expression levels were normalized to GAPDH and are presented as the mean $\pm$ standard error of the mean (SEM) from three independent experiments. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). Supplementary Figure S3. Gene expression alterations in 2D-cultured ADSCs and RSC96 cells under oxidative stress following ibudilast (IB) treatment. (A) Relative mRNA expression levels of neurotrophic factors, pro-inflammatory cytokines, and anti-inflammatory factors in 2D-cultured ADSCs and (B) RSC96 cells following treatment with indicated concentrations of IB in the presence of 200 μ M hydrogen peroxide (H₂O₂). All gene expression levels were normalized to GAPDH and are presented as the mean $\pm$ standard error of the mean (SEM) from three independent experiments. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

Author Contributions: Conceptualization, N.-K.L.; methodology, J.Y.B. and N.-K.L.; software, J.Y.B. and N.-K.L.; validation, N.-K.L.; formal analysis, J.Y.B. and N.-K.L.; investigation, J.Y.B. and N.-K.L.; resources, N.-K.L.; data curation, J.Y.B. and N.-K.L.; writing—original draft preparation, J.Y.B. and N.-K.L.; writing—review and editing, J.Y.B. and N.-K.L.; visualization, J.Y.B. and N.-K.L.; supervision, N.-K.L.; project administration, N.-K.L.; funding acquisition, N.-K.L. All authors have read and agreed to the published version of the manuscript.

Funding: The present research was supported by the research fund of Dankook University in 2025.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of Dankook University Hospital (IRB No. DKUH-2024-10-005; Date of Approval: 16 October 2024).

Informed Consent Statement: Written informed consent was obtained from all subjects involved in the study for participation, use of tissue samples, and publication of the data. <https://doi.org/10.5281/zenodo.18666079>.

Data Availability Statement: The authors confirm that the data supporting the findings of this study are available within the article. Raw data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments: During the preparation of this work, the authors used Gemini 3.0 (Google LLC, Mountain View, CA, USA) in order to generate the graphical illustration in Figure 2A and refine the English language for clarity and flow. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication. All experimental designs, wet lab data collection, analysis, and interpretation were conducted solely by the authors without the assistance of generative AI. No individuals were acknowledged in this section; therefore, no consent was required.

Conflicts of Interest: All authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ADSCs	Adipose-derived stem cells
BDNF	Brain-derived neurotrophic factor
BrdU	The 5-bromo-2'-deoxyuridine
cAMP	Cyclic Adenosine Monophosphate
CCK	Cell Counting Kit
CM	Conditioned medium
CREB	cAMP response element-binding protein

DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulfoxide
EGR2	Early growth response 2
ELISA	Enzyme-linked immunosorbent assay
HSwCs	Human Schwann Cells
IB	Ibuprofen
IGF	Insulin-like growth factor
IL-6	Interleukin-6
IL-10	Interleukin-10
NGF	Nerve growth factor
OCT4	Octamer-binding transcription factor 4
PDE4	Phosphodiesterase-4
PKA	Protein Kinase A
PMP22	Peripheral myelin protein 22
RT-qPCR	Quantitative real-time polymerase chain reaction
SOX2	SRY-box transcription factor 2
TGF- β	Transforming growth factor-beta
TNF- α	Tumor necrosis factor-alpha
ULA	Ultra-low attachment
VEGF	Vascular endothelial growth factor
α -MEM	Alpha-minimum essential medium
2D	Two dimensional
3D	Three dimensional

References

- Bateman, E.A.; Pripotnev, S.; Larocerie-Salgado, J.; Ross, D.C.; Miller, T.A. Assessment, management, and rehabilitation of traumatic peripheral nerve injuries for non-surgeons. *Muscle Nerve* **2025**, *71*, 696–714. [\[CrossRef\]](#)
- Wei, C.; Guo, Y.; Ci, Z.; Li, M.; Zhang, Y.; Zhou, Y. Advances of Schwann cells in peripheral nerve regeneration: From mechanism to cell therapy. *Biomed. Pharmacother.* **2024**, *175*, 116645. [\[CrossRef\]](#)
- Scheib, J.; Hoke, A. Advances in peripheral nerve regeneration. *Nat. Rev. Neurol.* **2013**, *9*, 668–676. [\[CrossRef\]](#)
- Bosch-Queralt, M.; Fledrich, R.; Stassart, R.M. Schwann cell functions in peripheral nerve development and repair. *Neurobiol. Dis.* **2023**, *176*, 105952. [\[CrossRef\]](#) [\[PubMed\]](#)
- Nocera, G.; Jacob, C. Mechanisms of Schwann cell plasticity involved in peripheral nerve repair after injury. *Cell Mol. Life Sci.* **2020**, *77*, 3977–3989. [\[CrossRef\]](#) [\[PubMed\]](#)
- Fornaro, M.; Marcus, D.; Rattin, J.; Goral, J. Dynamic Environmental Physical Cues Activate Mechanosensitive Responses in the Repair Schwann Cell Phenotype. *Cells* **2021**, *10*, 425. [\[CrossRef\]](#) [\[PubMed\]](#)
- Jessen, K.R.; Arthur-Farraj, P. Repair Schwann cell update: Adaptive reprogramming, EMT, and stemness in regenerating nerves. *Glia* **2019**, *67*, 421–437. [\[CrossRef\]](#)
- Jessen, K.R.; Mirsky, R. The repair Schwann cell and its function in regenerating nerves. *J. Physiol.* **2016**, *594*, 3521–3531. [\[CrossRef\]](#)
- Modrak, M.; Talukder, M.A.H.; Gurgenshili, K.; Noble, M.; Elfar, J.C. Peripheral nerve injury and myelination: Potential therapeutic strategies. *J. Neurosci. Res.* **2020**, *98*, 780–795. [\[CrossRef\]](#)
- Kubiak, C.A.; Grochmal, J.; Kung, T.A.; Cederna, P.S.; Midha, R.; Kemp, S.W.P. Stem-cell-based therapies to enhance peripheral nerve regeneration. *Muscle Nerve* **2020**, *61*, 449–459. [\[CrossRef\]](#)
- Rhode, S.C.; Beier, J.P.; Ruhl, T. Adipose tissue stem cells in peripheral nerve regeneration-In vitro and in vivo. *J. Neurosci. Res.* **2021**, *99*, 545–560. [\[CrossRef\]](#)
- Galipeau, J.; Sensebe, L. Mesenchymal Stromal Cells: Clinical Challenges and Therapeutic Opportunities. *Cell Stem Cell* **2018**, *22*, 824–833. [\[CrossRef\]](#) [\[PubMed\]](#)
- Choudhery, M.S.; Badowski, M.; Muise, A.; Pierce, J.; Harris, D.T. Donor age negatively impacts adipose tissue-derived mesenchymal stem cell expansion and differentiation. *J. Transl. Med.* **2014**, *12*, 8. [\[CrossRef\]](#) [\[PubMed\]](#)
- De Cássia Noronha, N.; Mizukami, A.; Caliári-Oliveira, C.; Cominal, J.G.; Rocha, J.L.M.; Covas, D.T.; Swiech, K.; Malmegrim, K.C.R. Priming approaches to improve the efficacy of mesenchymal stromal cell-based therapies. *Stem Cell Res. Ther.* **2019**, *10*, 131. [\[CrossRef\]](#) [\[PubMed\]](#)
- Herberts, C.A.; Kwa, M.S.; Hermesen, H.P. Risk factors in the development of stem cell therapy. *J. Transl. Med.* **2011**, *9*, 29. [\[CrossRef\]](#)

16. Lairson, L.L.; Lyssiotis, C.A.; Zhu, S.; Schultz, P.G. Small molecule-based approaches to adult stem cell therapies. *Annu. Rev. Pharmacol. Toxicol.* **2013**, *53*, 107–125. [\[CrossRef\]](#)
17. Rolan, P.; Hutchinson, M.; Johnson, K. Ibudilast: A review of its pharmacology, efficacy and safety in respiratory and neurological disease. *Expert Opin. Pharmacother.* **2009**, *10*, 2897–2904. [\[CrossRef\]](#)
18. Goodman, A.D.; Gyang, T.; Smith, A.D., 3rd. Ibudilast for the treatment of multiple sclerosis. *Expert Opin. Investig. Drugs* **2016**, *25*, 1231–1237. [\[CrossRef\]](#)
19. Bogers, S.H.; Barrett, J.G. Three-Dimensional Culture of Equine Bone Marrow-Derived Mesenchymal Stem Cells Enhances Anti-Inflammatory Properties in a Donor-Dependent Manner. *Stem. Cells Dev.* **2022**, *31*, 777–786. [\[CrossRef\]](#)
20. Kamprom, W.; Tangporncharoen, R.; Vongthaiwan, N.; Tragoonlugkana, P.; Phetfong, J.; Pruksapong, C.; Supokawej, A. Enhanced potent immunosuppression of intracellular adipose tissue-derived stem cell extract by priming with three-dimensional spheroid formation. *Sci. Rep.* **2024**, *14*, 9084. [\[CrossRef\]](#)
21. Lee, J.H.; Han, Y.S.; Lee, S.H. Long-Duration Three-Dimensional Spheroid Culture Promotes Angiogenic Activities of Adipose-Derived Mesenchymal Stem Cells. *Biomol. Ther.* **2016**, *24*, 260–267. [\[CrossRef\]](#)
22. He, Y.; Zhang, Z.; Li, Z.; Lin, M.; Ding, S.; Wu, H.; Yang, F.; Cai, Z.; Li, T.; Wang, J.; et al. Three-dimensional spheroid formation of adipose-derived stem cells improves the survival of fat transplantation by enhance their therapeutic effect. *Biotechnol. J.* **2023**, *18*, e2300021. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Tahvilian, R.; Amini, K.; Zhaleh, H. Signal Transduction of Improving Effects of Ibudilast on Methamphetamine Induced Cell Death. *Asian Pac. J. Cancer Prev.* **2019**, *20*, 2763–2774. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Cueva Vargas, J.L.; Belforte, N.; Di Polo, A. The glial cell modulator ibudilast attenuates neuroinflammation and enhances retinal ganglion cell viability in glaucoma through protein kinase A signaling. *Neurobiol. Dis.* **2016**, *93*, 156–171. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Wang, Q.; Chen, F.-Y.; Ling, Z.-M.; Su, W.-F.; Zhao, Y.-Y.; Chen, G.; Wei, Z.-Y. The Effect of Schwann Cells/Schwann Cell-Like Cells on Cell Therapy for Peripheral Neuropathy. *Front. Cell Neurosci.* **2022**, *16*, 836931. [\[CrossRef\]](#)
26. Oliveros, G.; Wallace, C.H.; Chaudry, O.; Liu, Q.; Qiu, Y.; Xie, L.; Rockwell, P.; Figueiredo-Pereira, M.E.; Serrano, P.A. Repurposing ibudilast to mitigate Alzheimer’s disease by targeting inflammation. *Brain* **2023**, *146*, 898–911. [\[CrossRef\]](#)
27. Nguyen, L.D.; Fischer, T.T.; Abreu, D.; Arroyo, A.; Urano, F.; Ehrlich, B.E. Calpain inhibitor and ibudilast rescue beta cell functions in a cellular model of Wolfram syndrome. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 17389–17398. [\[CrossRef\]](#)
28. Li, X.; Zou, Y.; Fu, Y.-Y.; Xing, J.; Wang, K.-Y.; Wan, P.-Z.; Wang, M.; Zhai, X.-Y. Ibudilast Attenuates Folic Acid-Induced Acute Kidney Injury by Blocking Pyroptosis Through TLR4-Mediated NF-kappaB and MAPK Signaling Pathways. *Front. Pharmacol.* **2021**, *12*, 650283.
29. Shao, J.; Yu, W.; Wei, W.; Wang, S.; Zheng, Z.; Li, L.; Sun, Y.; Zhang, J.; Li, Z.; Ren, X.; et al. MAPK-ERK-CREB signaling pathway upregulates Nav1.6 in oxaliplatin-induced neuropathic pain in the rat. *Toxicol. Lett.* **2023**, *384*, 149–160. [\[CrossRef\]](#)
30. Li, R.; Li, D.; Wu, C.; Ye, L.; Wu, Y.; Yuan, Y.; Yang, S.; Xie, L.; Mao, Y.; Jiang, T.; et al. Nerve growth factor activates autophagy in Schwann cells to enhance myelin debris clearance and to expedite nerve regeneration. *Theranostics* **2020**, *10*, 1649–1677. [\[CrossRef\]](#)
31. Baldassarro, V.A.; Lorenzini, L.; Bighinati, A.; Giuliani, A.; Alastra, G.; Pannella, M.; Fernandez, M.; Giardino, L.; Calzà, L. NGF and Endogenous Regeneration: From Embryology Toward Therapies. *Adv. Exp. Med. Biol.* **2021**, *1331*, 51–63. [\[PubMed\]](#)
32. Nikolaev, S.I.; Gallyamov, A.R.; Mamin, G.V.; Chelyshev, Y.A. Poly(epsilon-caprolactone) nerve conduit and local delivery of vegf and fgf2 genes stimulate neuroregeneration. *Bull. Exp. Biol. Med.* **2014**, *157*, 155–158. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Gorio, A.; Vergani, L.; Lesma, E.; Di Giulio, A.M. Neuroprotection, neuroregeneration, and interaction with insulin-like growth factor-I: Novel non-anticoagulant action of glycosaminoglycans. *J. Neurosci. Res.* **1998**, *51*, 559–562. [\[CrossRef\]](#)
34. Dya, G.A.; Klychnikov, O.I.; Adasheva, D.A.; Vladychenskaya, E.A.; Katrukha, A.G.; Serebryanaya, D.V. IGF-Binding Proteins and Their Proteolysis as a Mechanism of Regulated IGF Release in the Nervous Tissue. *Biochemistry* **2023**, *88*, S105–S122. [\[CrossRef\]](#)
35. Ji, Y.; Shen, M.; Wang, X.; Zhang, S.; Yu, S.; Chen, G.; Gu, X.; Ding, F. Comparative proteomic analysis of primary schwann cells and a spontaneously immortalized schwann cell line RSC 96: A comprehensive overview with a focus on cell adhesion and migration related proteins. *J. Proteome Res.* **2012**, *11*, 3186–3198. [\[CrossRef\]](#)
36. Hai, M.; Muja, N.; DeVries, G.H.; Quarles, R.H.; Patel, P.I. Comparative analysis of Schwann cell lines as model systems for myelin gene transcription studies. *J. Neurosci. Res.* **2002**, *69*, 497–508. [\[CrossRef\]](#)
37. Martinez Moreno, M.; Karambizi, D.; Hwang, H.; Fregoso, K.; Michles, M.J.; Fajardo, E.; Fiser, A.; Tapinos, N. Role of the *Egr2* Promoter Antisense RNA in Modulating the Schwann Cell Chromatin Landscape. *Biomedicines* **2024**, *12*, 2594. [\[CrossRef\]](#)
38. Stefanski, K.M.; Wilkinson, M.C.; Sanders, C.R. Roles for PMP22 in Schwann cell cholesterol homeostasis in health and disease. *Biochem. Soc. Trans.* **2024**, *52*, 1747–1756. [\[CrossRef\]](#)
39. Prior, R.; Silva, A.; Vangansewinkel, T.; Idkowiak, J.; Tharkeshwar, A.K.; Hellings, T.P.; Michailidou, I.; Vreijling, J.; Loos, M.; Koopmans, B. PMP22 duplication dysregulates lipid homeostasis and plasma membrane organization in developing human Schwann cells. *Brain* **2024**, *147*, 3113–3130. [\[CrossRef\]](#)

40. Angelopoulou, E.; Pyrgelis, E.S.; Piperi, C. Emerging Potential of the Phosphodiesterase (PDE) Inhibitor Ibudilast for Neurodegenerative Diseases: An Update on Preclinical and Clinical Evidence. *Molecules* **2022**, *27*, 8448. [[CrossRef](#)]
41. Ledeboer, A.; Hutchinson, M.R.; Watkins, L.R.; Johnson, K.W. Ibudilast (AV-411). A new class therapeutic candidate for neuropathic pain and opioid withdrawal syndromes. *Expert Opin. Investig. Drugs* **2007**, *16*, 935–950. [[CrossRef](#)]

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