Neuroprotective effects of methylcobalamin-containing nanofiber sheets in a rat sciatic nerve adhesion model
- A comparative study with neuroprotective sheets derived from small intestinal submucosa -

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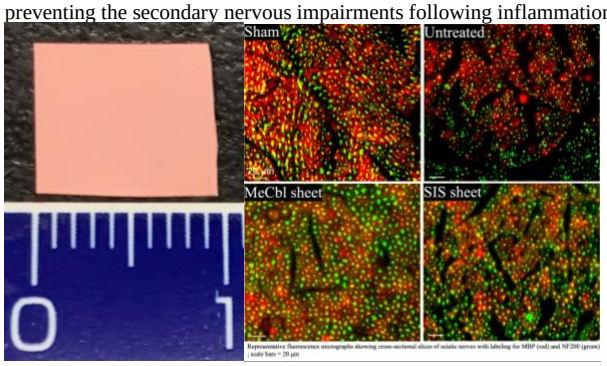
INTRODUCTION: Methylcobalamin (MeCbl) is the active form of vitamin B12, which is reported to have both an anti-inflammatory effect as well as a neuroregenerative effect. We have engineered a nano-fiber sheet containing MeCbl, termed MeCbl sheet, allowing locally sustained release of MeCbl. Using a rat sciatic nerve crush injury model and a sciatic nerve transection model, we have reported the efficacy of MeCbl sheet for the promotion of peripheral nerve regeneration. However, the neuroprotective effect of MeCbl sheet against post-inflammatory scarring and adhesions around peripheral nerve and its superiority over competing implants remain unknown. Hence, the purpose of this study is to evaluate the neuroprotective effects of the MeCbl sheet against post-surgical adhesions and to compare the effect of MeCbl sheet with that of porcine small intestinal submucosa (SIS) sheet, which has already been reported to be useful and used in clinical practice.

METHODS: We used thirty male Wistar rats (6 weeks old, 200g). The left sciatic nerve was exposed and the perineural soft tissues were cauterized by using a bipolar cautery within an 8mm range from the bifurcation of the nerve, aiming to induce inflammation and scar formation, following dissection of the neural epineurium. The rats were then randomly allocated into four groups for the surgical interventions, as follows: the Sham group (n=4), in which only sciatic nerve exposure was performed; the Untreated group (n=10), in which no treatment was performed after cautery and epineurium dissection; the MeCbl sheet group (n=8), in which the sciatic nerve was wrapped with MeCbl sheet (10mm×7mm); and the SIS sheet group (n=8), in which the sciatic nerve was wrapped with SIS sheet (10mm×7mm).At the 2-week postoperative day, we performed the von Frey filament test and the Hargreaves test to assess the sensory function. For evaluation of motor function, we measured the muscle wet weight of the tibialis anterior muscle of the ipsilateral side, and performed the isometric tetanic force test of tibialis anterior. Furthermore, electrophysiological examination was performed on the ipsilateral sciatic nerves, yielding recordings of compound muscle action potential (CMAP), nerve conduction velocity (NCV), and terminal latency (TL). To evaluate postoperative scar formation, the infiltration of inflammatory cells, and their impact on nerve fibers, the left sciatic nerves were harvested and masson's trichrome staining and immunohistochemistry staining were undertaken. Statistical analysis was performed using one-way ANOVA with Tukey – Kramer's multiple comparison test.

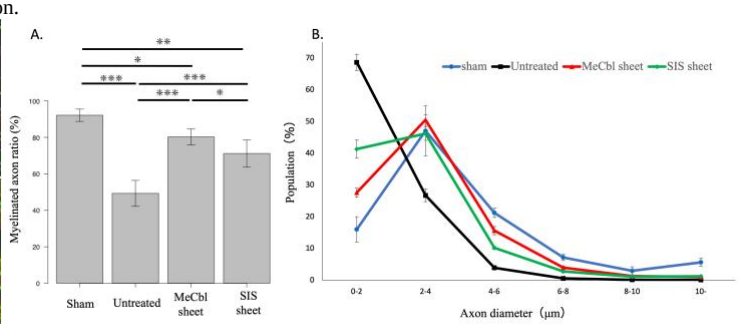
RESULTS SECTION: The MeCbl sheet group demonstrated significantly favorable outcomes compared to the Untreated group in terms of the von Frey filament test, Hargraves test, anterior tibial muscle wet weight, CMAP, and NCV. There was no significant difference observed between the MeCbl sheet and SIS sheet in any of these parameters. In the isometric tetanic force test, the Untreated group, MeCbl sheet group, and SIS sheet group all exhibited significantly reduced values compared to the sham group; however, no significant differences were noted among these three groups. Furthermore, no significant differences were observed among the four groups regarding TL. Quantitative assessment of collagen infiltration within nerve bundles through Masson's trichrome staining revealed that the MeCbl sheet group significantly mitigated collagen infiltration compared to the untreated group. No significant difference was noted between the MeCbl sheet group and SIS sheet group. Immunohistochemistry staining revealed that the MeCbl sheet group had fewer infiltrating inflammatory cells within nerve bundles compared to the Untreated group. Additionally, the MeCbl sheet group demonstrated a higher count of remaining axons and a greater degree of myelination compared to the Untreated group. Furthermore, MeCbl sheet group showed a propensity for the preservation of axons with larger diameters than those observed in the SIS sheet group. Notably, for the number of axons with diameters of 4 to 6μm, MeCbl sheet group showed a statistically higher compared to the SIS sheet group. Particularly in the evaluation of infiltrating macrophages' count and myelination ratio, the MeCbl sheet group yielded significantly superior results compared to the SIS sheet group.

DISCUSSION: SIS sheet, which was used as a comparison group in this study, has been reported to improve the postoperative myelination rate and the number of axons in a rabbit sciatic nerve transection model, as well as the results of electrophysiological examination, and its efficacy was similarly confirmed in our study. SIS sheet has also been used clinically, and its safety and efficacy have already been reported. Our results showed that MeCbl sheet was non-inferior to SIS sheet in many parameters, including postoperative sensory and motor function, and significantly better in some parameters. This indicates that MeCbl sheet may be useful as a new neuroprotective material against inflammation and scar formation. Regarding the sheet made of polycaprolactone, employed as a constituent of the MeCbl sheet, its beneficial effectiveness as a barrier has been previously reported. Furthermore, several studies reported a transformation of macrophage polarization from proinflammatory(M1) to anti-inflammatory(M2) facilitated by vitamin B, including vitamin B12, in a rat femoral nerve transection model, leading to diminished perineural inflammation. In this study, the MeCbl sheet group has demonstrated significantly fewer macrophages within the nerve parenchyma compared to both the Untreated and SIS sheet groups. This observation could be attributed not only to the barrier function of the sheet, but also to the anti-inflammatory effects inherent to MeCbl. Furthermore, by inhibiting infiltration of elements like collagen, macrophages, and fibroblasts within the nerve, subsequent formation of scar tissue and adhesions has been mitigated. This has potentially contributed to the superior outcomes in terms of residual axon count, axon diameter, myelination ratio, sensory and motor function assessments, as well as electrophysiolosical evaluations when compared to the Untreated and SIS sheet groups.

SIGNIFICANCE/CLINICAL RELEVANCE: This is the first study to investigate the neuroprotective effects of MeCbl sheet comparing with other neuroprotective materials within the rat sciatic nerve adhesion model. Our findings indicate that the MeCbl sheet may represent a novel approach to preventing the secondary nervous impairments following inflammation.



Representative fluorescence micrographs showing cross-sectional slices of sciatic nerves with labeling for MBP (red) and NF200 (green) ; scale bars = 20 μm



(A) MeCbl sheet group showed a significantly higher myelinated axon ratio than Untreated and SIS sheet group. (B) MeCbl sheet group showed a significantly higher population of axon diameter at 4-6 μm than Untreated and SIS sheet group. *P < 0.05, **P < 0.01 and ***p < 0.001