Master's Thesis of Veterinary Medicine

The nerve regeneration effect of an adipose-derived stem cell and reduced graphene oxide layered hydrolyzed polycaprolactone nanofibril sheet in a rat sciatic nerve defect model

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The nerve regeneration effect of an adipose-derived stem cell and reduced graphene oxide layered hydrolyzed polycaprolactone nanofibril sheet in a rat sciatic nerve defect model

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Abstract

Peripheral nerve injury in small animals can be caused by trauma or iatrogenic damage. If the nerve is completely transected, the prognosis is poor, and a salvage procedure can be considered. Use of autograft is considered the gold standard for nerve transplantation, but this technique has some disadvantages, such as the necessity for additional surgery and limited supply. Thus, a nerve guidance conduit (NGC) should be created to replace the autograft. This study investigated the nerve regeneration effect of NGCs applied with human adipose-derived mesenchymal stem cells (ADSCs) and polycaprolactone (PCL) nanofibrils (NFs) with or without a reduced graphene oxide (rGO) coating in a rat sciatic nerve defect model.

Twenty-four rats were divided into four groups as follows: the autograft, PCL NGC, ADSC+NF (ADSCs with NFs in a PCL NGC), and ADSC+rGO/NF (ADSCs with rGO-coated NFs in a PCL NGC) groups. Analysis of the ankle angle and grading of autotomy severity were performed for eight weeks to evaluate functional recovery. After euthanasia at eight weeks postoperatively, muscles and nerves were harvested to measure the muscle mass recovery ratio and perform immunofluorescence staining.

The mean ankle angles of ADSC+NF and ADSC+rGO/NF groups

were significantly larger than those of the PCL NGC group eight weeks after injury. The ankle angles tended to be larger in the ADSC+rGO/NF group than in the ADSC+NF group. In contrast to the autograft and PCL NGC groups, autotomy was not detected in the ADSC+NF and ADSC+rGO/NF groups until the second week. The severity of autotomy in the ADSC+rGO/NF group was less than that in the other groups. Immunofluorescence staining showed more positive NF200 signals in the ADSC+NF and ADSC+rGO/NF groups than in the PCL NGC group but less positive NF200 signals than in the autograft group.

These results showed that ADSCs with PCL NFs could promote peripheral nerve regeneration and that rGO could enhance functional recovery and pain relief. An NGC using ADSC+rGO/NF could be a treatment option for peripheral nerve defects in small animal patients.

Keywords: Adipose-derived stem cell, reduced graphene oxide, hydrolyzed polycaprolactone nanofibril, nerve regeneration, sciatic defect model

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Introduction

In small animals, peripheral nerve injury (PNI) can be caused by trauma or iatrogenic damage. Depending on the degree of damage, PNI can be classified into neurapraxia, axonotmesis, and neurotmesis. Neurapraxia is a transient, physiological failure of nerve transmission without structural damage. Axonotmesis is a condition in which the axons are damaged while the internal structure of the nerve remains intact. Neurotmesis is the complete transection of all nerve structures and has a worse prognosis than neurapraxia and axonotmesis (Welch 1996, Platt and Olby 2014). A salvage procedure may be considered for a patient with severe PNI due to abrasion and self-mutilation (Platt and Olby 2014).

Transplantation is required if the peripheral nerve defect is large, and a nerve autograft is used as the gold standard transplantation method. However, use of an autograft has many disadvantages, including insufficient functional recovery, necessity for additional surgical sites, damage to donor sites, and limited supply (Hadlock, Sundback et al. 2000, Rodr ı ´guez, Verdú et al. 2000). Various nerve guidance conduits (NGCs) have been investigated for utilization in large deficits when an autograft is unavailable or as an alternative to an autograft (Daly, Yao et al. 2012). NGCs have been

developed mainly in the form of hollow cylinders made from various artificial materials (Ijpma, Van De Graaf et al. 2008). They have recently been modified to create a conducive microenvironment such as by altering the structure of the conduit or adding substances to improve neural regeneration (Daly, Yao et al. 2012).

Electrospun nanofibers are commonly used in producing NGCs to mimic the natural structure of the extracellular matrix (ECM), thereby facilitating cell adhesion or proliferation (Yang, Murugan et al. 2005). However, an electrospun nanofiber scaffold has very small pores; thus, it is difficult for cells to infiltrate between scaffolds (Wang, Xu et al. 2013). A method for producing electrospun nanofiber fragments known as nanofibrils (NFs) was previously reported as a means of overcoming these disadvantages (Kim and Yoo 2015). Since NFs are comparable to cells in size, they promote cell infiltration and improve cell–matrix interaction. Furthermore, when NFs are combined with adipose–derived mesenchymal stem cells (ADSCs), ECM protein is released from the cells, resulting in a clay–like texture of the ADSC and NF mixture. This texture is advantageous because it facilitates transplantation into tissues. (Lee, Kim et al. 2017, Kim, Mandakhbayar et al. 2021). Numerous studies have been conducted to investigate the effect of ADSC on nerve regeneration. When ADSCs are transplanted into an area of PNI, they

secrete various neurotrophic factors, aid in re-myelination, inhibit inflammatory reactions, and stimulate the growth of axons (Chamberlain, Fox et al. 2007, Rodríguez Sánchez, de Lima Resende et al. 2019, Jiang, Mee et al. 2021). Thus, combining ADSCs and NFs would be a potential strategy for nerve regeneration by simultaneously creating ECM-like structures and molecular microenvironments.

Graphene is a two-dimensional structure consisting of a monolayer of carbon atoms that has recently attracted much attention as it can promote the adhesion, growth, proliferation, and differentiation of various cells (Park, Park et al. 2011). Reduced graphene oxide (rGO) has properties suitable for neural regeneration such as a flexible nature, good electrical conductivity, biocompatibility, neural interfacing, and drug release applications. (Reddy, Xu et al. 2018). Some studies have reported that rGO-based NGCs can aid in nerve regeneration *in vitro* and *in vivo* by enhancing Schwann cell migration, proliferation, and myelination (Wang, Cheng et al. 2019, Park, Jeon et al. 2020). If rGO, an electrically conductive material, is applied to insulator polycaprolactone (PCL) NFs, the effect of neural regeneration could be improved.

This experiment aims to evaluate the peripheral nerve regeneration efficacy of the human ADSCs with PCL NFs or rGO-

coated PCL NFs through functional evaluation, muscle mass comparison, and immunofluorescence in the rat sciatic nerve defect model. The aim of this study was to confirm whether ADSCs with PCL NFs (with or without an rGO coating) are suitable as a treatment for small animal PNI.

Materials and Methods

Preparation of implants

PCL (20%, w/v, Polysciences, Warrington, PA) dissolved in chloroform/MeOH (1/3, v/v) was fabricated into a hollow cylindrical shape with an inner diameter of 2 mm through an electrospinning process (15 kV, 1 h) and then was cut to a length of 1.5 cm. The conduit was sterilized by UV irritation and prepared for cultivation. Hydrolyzed PCL NFs were made by grinding the PCL mesh produced through electrospinning and hydrolyzing it using NaOH solution. To confirm the effect of rGO, some PCL NFs were coated 30 times with rGO (Graphite powder, Daejung Chemicals & Metals, Siheung, Korea) by an electrical interaction-mediated layer-by-layer coating technique (rGO/NFs), and others were not coated (NFs). PCL NFs and human ADSCs (Cefobio, Seoul, Korea) were cultured together in the prepared PCL conduit. A pipet was used to insert ADSCs (3×10^6 cells) and PCL NFs (3.0 mg) into the central area of the conduit, and both ends of the conduit were filled with cylindrical polydimethylsiloxane (diameter=2 mm, length=7 mm) to block the potential loss of ADSCs with NFs from the conduit. The conduit containing ADSCs+NFs or ADSCs+rGO/NFs was cultured for four days in 4 mL of DMEM in a 6-well plate before surgery.

Experimental animals and surgical procedure

Animal experiments were approved by the Seoul National University Institutional Animal Care and Use Committee (SNU-210303-4). Twenty-four adult male Sprague-Dawley (SD) rats (260 to 330 g, Han-Lym Lab. Animal Co., Hwaseong, Korea) were used in this study. Animals were randomly divided into four groups as follows: autograft group (n=6), PCL NGC group (n=6), ADSC+NF group (n=6), and ADSC+rGO/NF group (n=6) (Figure 1).

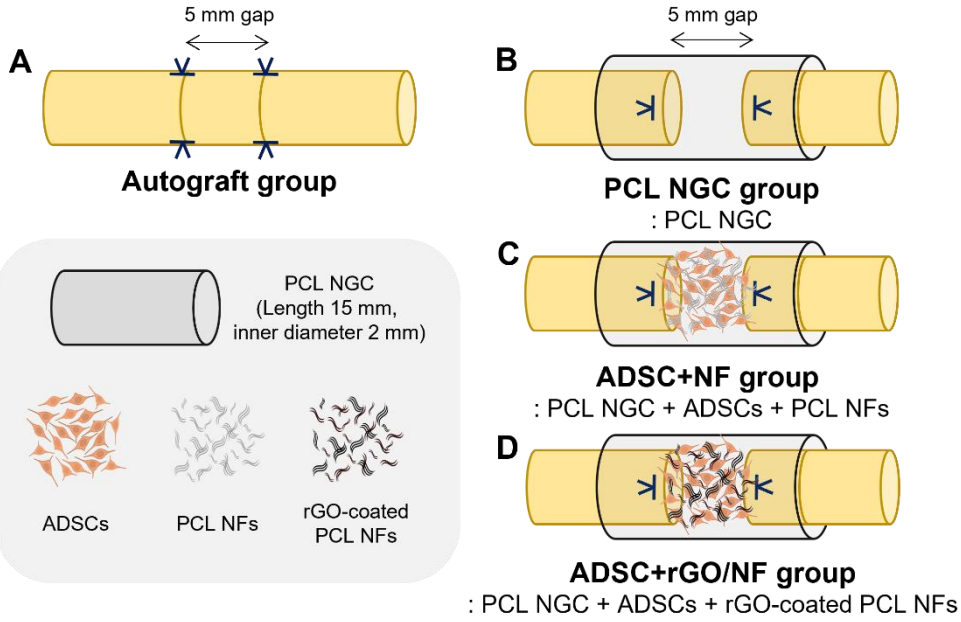

Figure 1. Experimental group. (A) Autograft group. (B) The PCL NGC group was repaired with PCL NGC. (C) The ADSC+NF group had a PCL NGC including ADSCs and NFs applied. (D) ADSC+rGO/NF group underwent transplantation with a PCL NGC including ADSCs and rGO-coated NFs.

Surgical procedure

The animals were anesthetized with alfaxalone (10 mg/kg, IP; Alfaxan; Jurox) and isoflurane (2–3 MAC, inhalation, Ifran; Hana Pharm. Co., Seoul, Korea). A lateral skin incision was made in the femoral region, and the sciatic nerve was exposed by retracting the biceps femoris muscle caudally. In the autograft group, we excised a 5 mm segment of the sciatic nerve in the middle and repositioned it at the same site. The nerve segment was repaired with 8–0 nylon epineural sutures (Ailee, Busan, Korea). In groups using a 15–mm cylindrical PCL conduit, the sciatic nerve was transected in the middle. The contraction of the proximal and distal nerve ends caused a 5–mm gap. The distal and proximal nerve stumps were inserted by 5 mm at both ends of the conduit, keeping a 5–mm gap between the two nerve stumps by suturing the nerve to the conduit using 8–0 nylon suture. Both conduit ends were sealed using fibrin glue (Greenplast®; Green Cross Young–in, Korea). After implant placement, the muscles and subcutaneous tissues were sutured with a 4–0 PDS suture (Ethicon Inc., Somerville, NJ, USA), and the skin was closed with a surgical stapler (Appose™ skin stapler®, Covidien Inc, Mansfield, MA) (Figure 2).

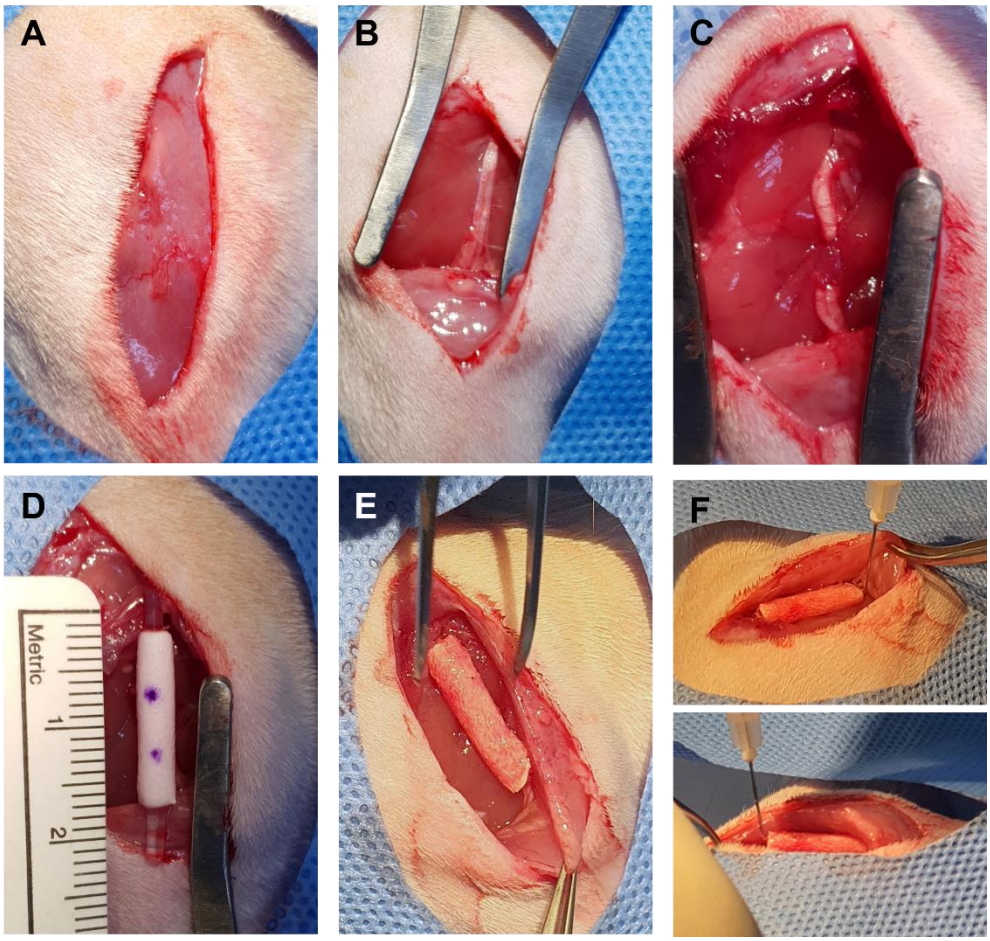

Figure 2. Nerve bridging surgery using conduit. (A) A lateral skin incision was made in the femoral region. (B) The sciatic nerve was exposed by retracting the biceps femoris muscle. (C) Neural resection was performed to make a 5-mm gap. (D) A 5-mm gap is marked in the center of the conduit. (E) The conduit was sutured to maintain a 5-mm nerve gap using 8-0 nylon suture. (F) Fibrin glue was applied to both ends of the conduit.

Functional evaluation (Ankle angle analysis)

The ankle stance angle (ASA) and toe-off ankle angle (TOAA) were measured and analyzed for functional evaluation at 2, 4, 6, and 8 weeks after surgery. The ankle angle at the mid-stance, termed the ASA, is commonly used as a functional evaluation parameter in rat sciatic defect models (Lin, Pan et al. 1996). The TOAA is measured the moment when the foot is off the ground and the ankle is in maximal plantar flexion. In addition, the TOAA has been confirmed as a valid parameter that can reflect functional recovery (Lee, Giusti et al. 2013). The rat walked through a 15×15×100 cm track for ankle angle analysis, and the movement was recorded on the left side of the rat with a camera. The ankle angle was analyzed by manually identifying the leg and foot segments in the video frame using Kinovea software (Version 0.8.15, Kinovea Org., France). As guidelines, the front side of the tibia and the line connecting the fourth metatarsal and the lateral malleolus were used (Figure 3).

After sciatic nerve transection in rats, autotomy of the foot may occur by licking, scratching, and chewing. Autotomy may be related to abnormal nerve sensation and neuropathic pain. The severity of autotomy was observed at 2-week intervals and evaluated using a grading scale (Table 1) (Park, Ki et al. 2015).

Table 1. Autotomy grading scale

Autotomy grade	Range of autotomy
0	No autotomy at the paw
1 (mild)	Autotomy at the claw of the paw
2 (moderate)	Autotomy from the claw to the toe
3 (severe)	Autotomy from the claw to the sole

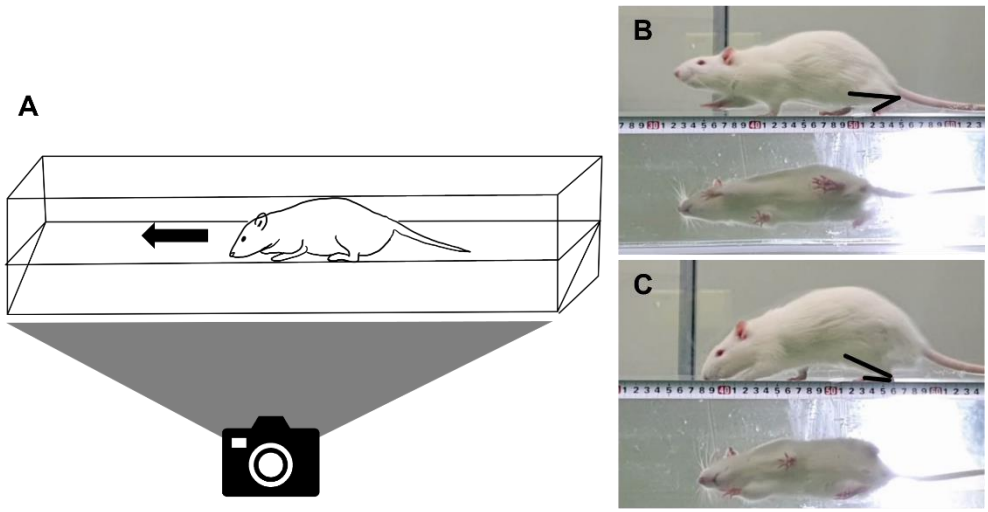

Figure 3. Monitoring method for ankle angle analysis. The rat walked through a 15 × 15 × 100 cm track while being recorded (A). The ankle angles when the foot was off the ground (B, toe-off ankle angle) and at the mid-stance (C, ankle stance angle) were measured using the recorded video.

Muscle recovery ratio

At eight weeks after surgery, the bilateral triceps surae muscles (TSMs) and tibialis anterior (TA) muscles were harvested from the bone attachments. The moist weights of muscles were instantly weighed on an analytic scale. The wet muscle weight ratio for the experimental and contralateral sides was calculated as follows:

$$Wet\ muscle\ weight\ ratio\ (\%) = \frac{Wet\ muscle\ (experimental\ leg)}{Wet\ muscle\ (normal\ leg)} \times 100$$

Immunofluorescence

The sciatic nerves from the experimental sites were harvested eight weeks following surgery. In the conduit groups (PCL NGC, ADSC+NF, and ADSC+rGO/NF groups), nerve samples were resected to contain more than approximately 2–3 mm of normal nerves at the end of the implant, and more than 10 mm of the sciatic nerve was resected centered on the autograft sites in the autograft group. The harvested nerves were fixed with 4% paraformaldehyde, treated with sucrose, and embedded in the OCT compound. The nerve specimens were sectioned into 10–μm sections and stained with immunofluorescence. The slide was incubated with rabbit anti–rat NF200 antibody and stained with Alexa Fluor 488 labeled–goat anti–rabbit IgG (H+L). Moreover, DAPI was used to stain the nuclei of the cells.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.01 (GraphPad Software, La Jolla, CA, USA). The muscle recovery ratio between groups was compared using an unpaired t-test. When comparing the change in the ankle angle over time and the difference between groups, one-way ANOVA was used. Statistical significance was considered at $P < 0.05$.

Results

Functional evaluation (ankle angle analysis)

A rat in the ADSC+NF group died one week after surgery. The other rats survived the 8-week postoperative study period, and the experimental results from five rats in the ADSC+NF group and six in each of the other three groups were obtained.

The ankle angle was measured and averaged using images from the recorded videos, and measurements from each rat were made at least three times. The TOAA of the ADSC+NF group was significantly larger than that of the PCL NGC group and smaller than that of the autograft and ADSC+rGO/NF groups during the experimental period. In the ADSC+rGO/NF group, the TOAA was significantly larger than that of the PCL NGC group for 8 weeks and significantly smaller than that of the autograft group in weeks 6 and 8 (Figure 4A). There was no statistical difference in the ASA between the ADSC+NF and ADSC+rGO/NF groups. The ASA of the ADSC+NF group was smaller than that of autograft group for eight weeks and larger than that of the PCL NGC group at weeks 4, 6, and 8. In the ADSC+rGO/NF group, the ASA was significantly larger than that of the PCL NGC group, and a significant difference from the autograft group was confirmed in weeks 4 and 6 (Figure 4B).

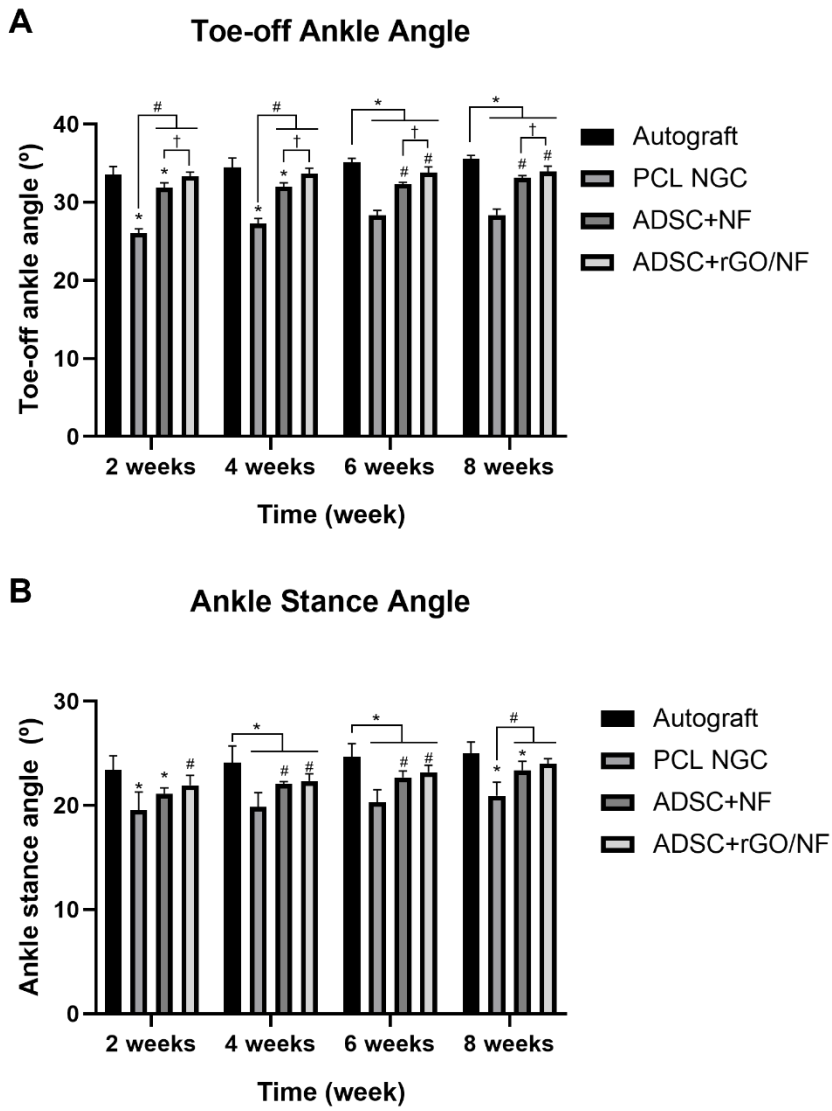

Figure 4. Ankle angle analysis (A) Toe-off ankle angle and (B) ankle stance angle of the experimental hindleg at 2, 4, 6, and 8 weeks postoperatively (n = 5 in the ADSC+NF group, n = 6 in the rest of the groups). $^{*}P < 0.05$ compared with the autograft group; $^{\#}P < 0.05$ compared with the PCL NGC group; $^{\dagger}P < 0.05$ indicates a significant difference between the indicated groups.

Autotomy

When monitored for eight weeks, the number of rats that showed autotomy was the smallest in the autograft group. In the case of the PCL NGC group, grade 2–3 (moderate to severe) autotomy was commonly confirmed. Unlike the autograft and PCL NGC groups, autotomy was not identified until week 2 in the ADSC+NF and ADSC+rGO/NF groups. Over time, the ADSC+NF group showed moderate to severe autotomy, similar to the PCL NGC group. In the ADSC+rGO/NF group, mild autotomy was mainly observed, and severe autotomy (grade 3) was the least common in this group compared with the other groups (Figure 5).

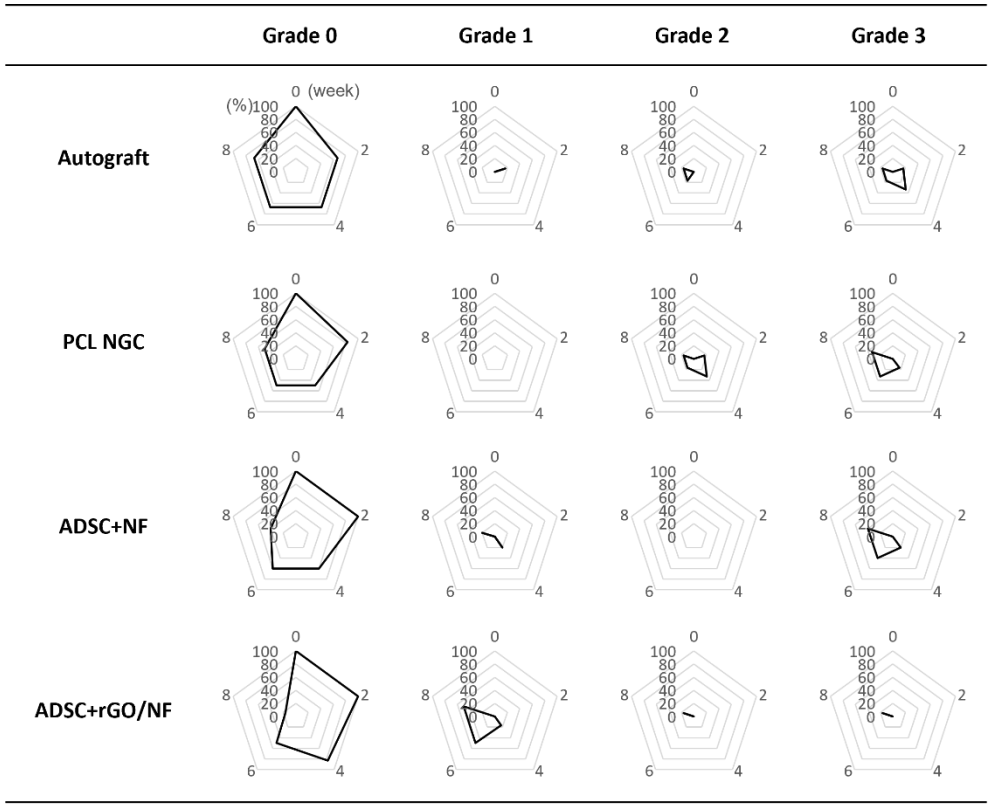

Figure 5. Monitoring of autotomy severity. The occurrences of autotomy monitored in weeks 2, 4, 6, and 8 are expressed as a percentage in pentagons of each grade. (n = 5 in the ADSC+NF group, n = 6 in the rest of the groups).

Muscle recovery ratio

The eight weeks postoperatively, the TSM and TA muscles of the experimental legs were atrophied enough to be grossly observed in all groups. The muscle recovery ratios of the TSM and TA muscles, which show the degree of muscle atrophy, were significantly higher in the autograft group than in other groups. No significant difference in the muscle recovery ratio was identified among the PCL NGC, ADSC+NF, and ADSC+rGO/NF groups (Figure 6).

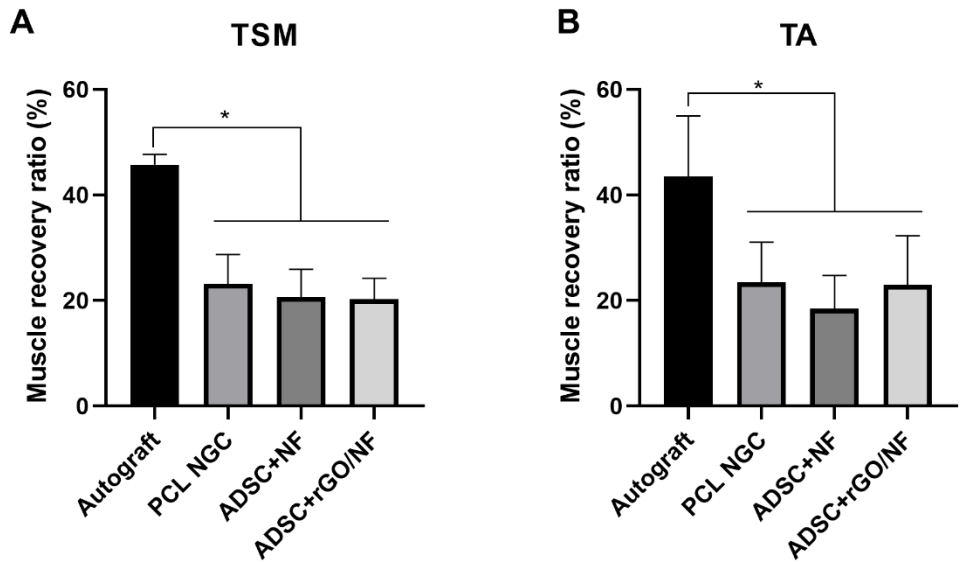

Figure 6. Muscle recovery ratio. Recovery of the triceps surae muscle (TSM, A) and tibialis anterior (TA, B) muscle was calculated by the weight ratio of the experimental leg muscle to the normal leg muscle (n = 5 in the ADSC+NF group, n = 6 in the rest of the groups). * Significant difference between groups ($P < 0.05$).

Immunofluorescence

Immunofluorescence staining was performed in all groups, and a representative photograph of the central portion of a longitudinal section from each group is shown in Figure 7. The cross-section of the autografted portion was confirmed to be NF-positive and showed a more precise NF 200 signal than that of other groups. The NF 200 staining was weakly confirmed in the part where only the hollow PCL conduit was applied. In the ADSC+NF and ADSC+rGO/NF groups, overall NF 200-positive regions were identified.

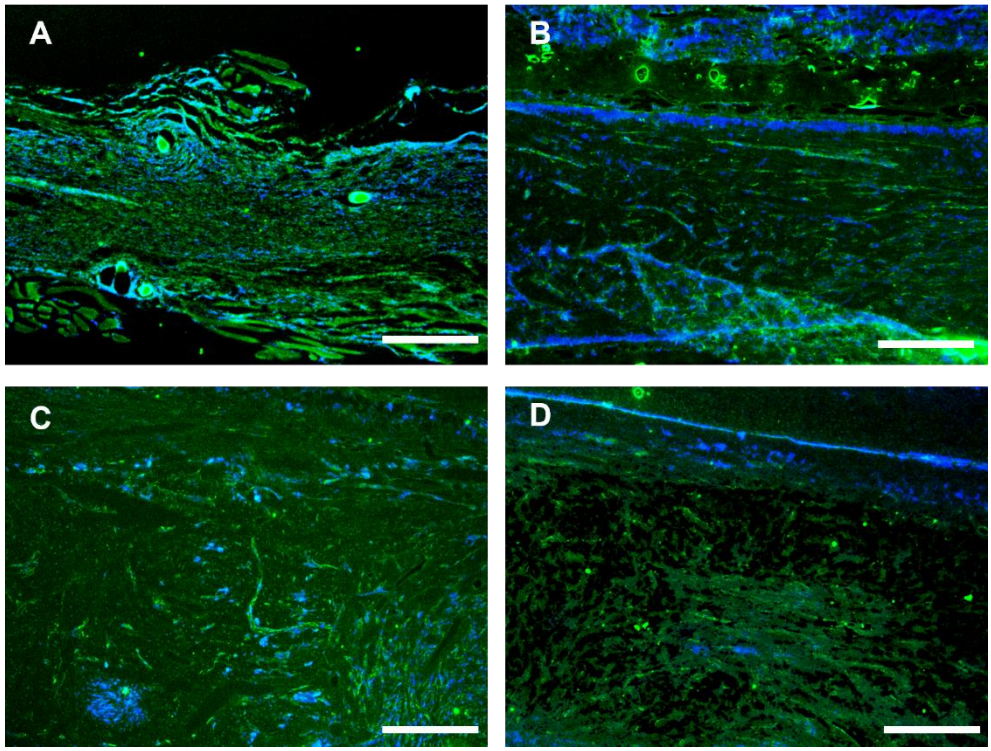

Figure 7. Immunofluorescence staining. Longitudinal sections of implants stained with NF-200 (green) and DAPI (blue) in the autograft (A), PCL NGC (B), ADSC+NF (C), and ADSC+rGO/NF groups. Scale bar = 200 ㎛

Discussion

Transplantation of ADSC+NF and ADSC+rGO/NF was confirmed to facilitate nerve regeneration in a sciatic nerve defect model using functional and pain-related evaluation and immunofluorescence staining. No alternative method for autograft usage in PNI has been proposed; thus, confirming the effect of ADSCs with NFs or rGO/NFs in this study is meaningful in that it suggests novel nerve guidance conduit options.

Both the TOAA and ASA of the ADSC+NF and ADSC+rGO/NF groups were significantly larger than that in the PCL NGC group. These results may be because ADSCs with NFs facilitate nerve regeneration and functional recovery by creating a microenvironment that assists in nerve regeneration and making a structure similar to the ECM inside the conduit. In particular, the ADSC+rGO/NF group showed similar results to the autograft group in weeks 2 and 4 for the TOAA and in weeks 2 and 8 for the ASA. In addition, the TOAA was significantly larger in the ADSC+rGO/NF group than in the ADSC+NF group for eight weeks. As a similar result, two studies reported that the sciatic function index (SFI) of the group using an rGO-coated NGC was similar to the autograft result and was significantly better than that of the non-coated NGC

group (Wang, Cheng et al. 2019, Park, Jeon et al. 2020). From these results, the electrical conductivity of rGO may have played a critical role in recovering nerve function, and rGO enhanced the effect of ADSCs with NFs.

Autotomy could occur in animals with nerve injury and is known to be associated with neurological pain and neural recovery (Park, Ki et al. 2015). In this study, autotomy was utilized as an assessment of neurogenic pain. A neuroma is formed as the axonal fibers sprout out in the radial direction on the transected nerve surface, and pain is caused when the axonal fiber extends to chemically and mechanically damaged tissues in the surrounding area (Hama, Uemura et al. 2021). NGCs have been demonstrated to reduce pain by preventing the formation of neuromas and scars (Onode, Uemura et al. 2019, Zhou, Zhao et al. 2019). In this study, autotomy was found to occur later in the ADSC+NF and ADSC+rGO/NF groups than in the autograft group and PCL NGC group, and the severity of autotomy in PCL NGC group at 2 weeks showed less severe than that of autograft group. These finding may be related to the fact that NGCs can relieve pain. The autotomy was less severe in the ADSC+NF and ADSC+rGO/NF groups than in the PCL NGC group and was the mildest in the ADSC+rGO/NF group. These results may be due to the paracrine effect of ADSCs and the

conductivity and ECM-mimicking structure of nanofibers, facilitating the pain relief. In small animals, sensory abnormalities or pain can lead to autotomy, and salvage procedures may be considered in severe cases. Therefore, the mild occurrence of autotomy means that ADSC+rGO/NF can be a treatment option for PNI in small animals.

The TA muscles and TSMs recovered significantly more in the autograft group than in the other groups, but the other three groups showed similar recovery results. This result contradicts the hypothesis that that muscle recovery in the ADSC+NF and ADSC+rGO/NF groups would be superior to that in the PCL NGC group, as shown by the ankle angle data. Nerves may be randomly innervated to an inappropriate target muscle during nerve regeneration and reinnervation into muscles and then could be removed and remodeled to the appropriate nerve over time. Functional recovery is possible through proper reinnervation in this process, but muscle mass recovery may not correspond (Brushart, Tarlov et al. 1983, Landegren, Risling et al. 2011). As a corresponding result, two studies showed that the differences in the muscle mass recovery ratio between groups except the autograft group were not apparent at approximately two months, but a significant difference was confirmed at four months (Alberti, Neufeld et al. 2016, Wang, Yang et al. 2017). However, when compared to

functional recovery, the difference in the muscle recovery ratio revealed in the fourth month was less positive. In this recent study, a recovery time of eight weeks may have been insufficient to show the difference in muscle weights between groups or that the difference in functional recovery between groups was not significant enough to be reflected in muscle weights.

Immunofluorescence staining results were similar to other evaluations. The NF200 signal, a marker of myelinated A－β fiber neurons, was the strongest in the autograft group and was more positive in the ADSC+NF and ADSC+rGO/NF groups than in the PCL NGC group. These findings demonstrate that applying ADSCs with NFs improves nerve regeneration *in vivo*, although not as effectively as an autograft. When comparing the ADSC+NF and ADSC+rGO/NF groups, NF 200 stained broadly in the ADSC+NF group, while the slide in the ADSC+rGO/NF group showed relatively weaker staining for NF 200 with black heterogeneous parts. The heterogeneous black area is presumed to be the rGO－coated NFs. rGO could absorb the surrounding light more than GO (Podolska, Barras et al. 2020); thus, it might relatively attenuate the fluorescence. For this reason, it is difficult to say that the results of the ADSC+NF group is better than those of ADSC+rGO/NF group or to evaluate which result was more beneficial.

One limitation of this experiment is that an electrophysiology assessment, which could objectively evaluate functional recovery, was not conducted with the gait analysis. Although the TOAA utilized in this study is an analytic method that correlates with the isometric mechanical force (Lee, Giusti et al. 2013), it would have been advantageous to proceed with an electrophysiology assessment to investigate the influence of the applied implants on functional recovery more precisely. Another limitation is the short experimental period of eight weeks. Considering the results of the muscle recovery ratio, a more extended monitoring period may have helped to identify the differences between groups and long-term effects. However, since stem cells usually die within a month when applied to nerve defects (Jiang, Mee et al. 2021), eight weeks is considered sufficient to evaluate the efficacy of ADSCs with NFs implants on early nerve regeneration.

Conclusion

In conclusion, while applying these ADSCs with NFs to the internal space of a PCL conduit does not replace an autograft, it supports nerve regeneration and functional recovery and reduces neuropathic pain. In addition, more satisfactory results were obtained when rGO was coated on NFs. These results suggest that these ADSC+NF and ADSC+rGO/NF could be promising materials as nerve regeneration conduits. In addition, this implant may be considered a treatment option for small animal patients with severe PNI.

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국문 초록

지방유래 줄기세포와 환원 그래핀옥사이드 적층 가수분해 폴리카프로락톤 나노피브릴 시트의 쥐 좌골신경 결손 모델에서의 신경 재생 효과

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소동물에서의 말초 신경 손상은 외상이나 의인성 손상으로 발생할 수 있다. 신경이 완전히 끊어졌을 때 예후가 좋지 않으며, 절단술까지도 고려가 된다. 자가이식이 최적의 이식 방법으로 사용되고 있지만, 추가적인 수술의 필요성이나 제한된 공급과 같은 단점들을 가지고 있다. 따라서 자가이식의 대안으로 쓸 수 있는 신경 유도 도관(NGC)을 개발하는 것이 필요하다. 이 연구는 사람 지방유래 줄기세포(ADSC)와 환원 그래핀

옥사이드(rGO)를 코팅하거나 하지 않은 폴리카프로락톤(PCL) 나노피브릴(NFs)을 사용한 신경 유도 도관의 신경 재생 효과를 쥐 신경 결손 모델에서 조사하기 위해 진행되었다.

스물네 마리의 쥐가 다음과 같이 네 그룹으로 나뉘었다: 자가이식군, PCL NGC 군, ADSC+NF 군 (PCL NGC에 줄기세포와 나노피브릴 적용), 그리고 ADSC+rGO/NF 군 (PCL NGC에 줄기세포와 환원 그래핀 옥사이드 코팅 나노피브릴 적용). 발목 각도 분석과 자절 중증도 평가가 기능적 회복을 평가하기 위해서 8주 동안 수행되었다. 수술 후 8주 차에 안락사를 진행한 다음, 근육과 신경을 채취하여 근육량 회복 비율을 구하고, 면역형광염색을 진행하였다.

ADSC+NF와 ADSC+rGO/NF 그룹의 평균 발목 각도는 수술 후 8주 동안 PCL NGC 군의 발목 각도보다 유의미하게 높았다. ADSC+rGO/NF 군의 발목 각도는 ADSC+NF 군의 발목 각도 보다 높은 경향이 있었다. 자가이식 군과 PCL NGC 군과 달리, ADSC+NF 군과 ADSC+rGO/NF 군에서는 자절이 2주 차까지 확인되지 않았다. ADSC+rGO/NF 군의 자절 정도가 다른 군의 자절 정도보다 심각하지 않게 확인되었다.

이러한 결과는 나노 피브릴과 줄기세포가 말초신경재생을 촉진하고, 환원 그래핀 옥사이드가 기능 회복과 통증 완화를 도와준다는 것을 보여주었다. 또한, 줄기세포와 환원 그래핀 옥사이드 코팅을 한 나노피브릴을 사용한 신경 유도 도관을 소동물 환자에서 말초 신경 결손을 치료하는 방법의 하나로 생각해 볼 수 있다.

주요어 : 지방유래줄기세포, 환원 그래핀 옥사이드, 가수분해 폴리카프로락톤 나노피브릴, 신경 재생, 좌골 신경 결손 모델

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