TERM

Effectiveness and Biocompatibility of Decellularized Nerve Graft Using an *In Vivo* Rat Sciatic Nerve Model

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Abstract

BACKGROUND: Decellularized nerve allografting is one of promising treatment options for nerve defect. As an effort to develop more efficient nerve graft, recently we have developed a new decellularization method for nerve allograft. The aim of this study was to evaluate the effectiveness and biocompatibility of nerve graft decellularized by our newly developed method.

METHODS: Forty-eight inbred male Lewis rats were divided into two groups, Group I (autograft group, n = 25), Group II (decellularized isograft group, n = 23). Decellularized nerve grafts were prepared with our newly developed methods using amphoteric detergent and nuclease treatment. Serum cytokine level measurements at 0, 2, and 4 weeks and histologic evaluation for inflammatory cell infiltration at 6 and 16 weeks after nerve graft.

RESULTS: There was no significant difference in mean maximum isometric tetanic force and weight of tibialis anterior muscle or ankle angle at toe-off phase between two groups at 6 and 16 weeks survival time points ($p > 0.05$). There was no inflammatory cell infiltration in either group and histomorphometric assessments of 6- and 16-week specimens of the isograft group did not differ from those in the autograft group with regard to number of fascicle, cross sectional area, fascicle area ratio, and number of regenerated nerve cells.

CONCLUSION: Based on inflammatory reaction, axonal regeneration, and functional outcomes, our newly developed decellularized nerve grafts were fairly biocompatible and had comparable effectiveness to autografts for nerve regeneration, which suggested it would be suitable for nerve reconstruction as an alternative to autograft.

Keywords Decellularization · Nerve graft · Biocompatibility · Amphoteric detergent

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1 Introduction

Management of defects of peripheral nerves is still an unsolved clinical challenge. Despite the development of surgical techniques and the introduction of various graft materials, outcomes of functional restoration remain insufficient. Various methods have been applied to reconstruct segmental nerve defects. Reported meaning functional recoveries using autografts are at best 85% in sensory nerves and 86% in motor nerves [1]. Autologous nerve grafting as the standard method of nerve reconstruction has limitations, including donor site morbidity, limited amount of available donor tissue, and size

mismatches [2–4]. Alternatives such as conduits using vein, muscle, synthetic polymers, or collagens and cold preserved allogenic nerves have been developed [5–7]. However, those alternatives show limited nerve regeneration potential [7, 8].

Using decellularized nerve allograft from donor nerve tissue is another treatment option for reconstruction of nerve defects. The rationale of performing decellularization is that removing cellular elements can reduce immunologic response while preserving extracellular matrix (ECM) components for guidance of axonal ingrowth [9–12]. Although decellularized nerve graft does not retain Schwann cells, it is still a good scaffold for new axonal ingrowth[8, 13]. Advantages of using decellularized nerve allograft include its non-immunogenic nature, no donor site morbidity, unlimited supply of donor nerve theoretically, and shorter surgical time. Several nerve decellularization methods including physical, chemical, and biological methods have been utilized alone or in combination. However, which one is the most efficient remains unclear. Our group has recently developed a new decellularization method with combined amphoteric detergent and nuclease treatment and reported the result of decellularization using our new method, in which it revealed adequate cell removal with sufficient preservation of the ECM [14]. The aim of this study was to verify the efficacy and biocompatibility of nerve graft decellularized with our combined amphoteric detergent-nuclease method using an *in vivo* rat sciatic nerve defect model.

2 Materials and methods

2.1 Study design

Among fifty rats planned to be studied, two rats in decellularized nerve isograft group died before designated survival time points. So forty-eight rats were included in this study. Forty-eight genetically identical inbred male Lewis rats weighing 200 to 300 g at 49 to 56 days old were divided into two groups. In group I (autograft, control group, n = 25), a unilateral 10-mm sciatic nerve gap was repaired with an ipsilateral reversed autologous graft. In group II (decellularized nerve isograft, experimental group, n = 23), the same gap was reconstructed with a decellularized nerve isograft. Twelve rats weighing 200 to 300 g were used as nerve donors.

The biocompatibility of decellularized nerve graft was evaluated by comparing inflammatory reactions based on serum cytokine level (0, 2, 4 weeks) and inflammatory cell infiltration (6, 16 weeks) after grafting with autograft and assured secondarily by final functional recovery. The effectiveness was evaluated based on functional outcomes

such as maximum isometric tetanic force (MITF), wet weight of the tibialis anterior muscle, and ankle angle at toe-off phase after 6 weeks (n = 22) and 16 weeks (n = 26). Functional results of the two groups were compered statistically. Histomorphometric evaluations for number of fascicle, cross sectional area, fascicle area ratio, and number of regenerated cells were also performed.

2.2 Nerve graft processing and decellularization

Genetically identical twelve inbred male Lewis rats weighing 200 to 300 g at 49 to 56 days old were used as nerve donors. The animal studies were performed after receiving same approval number of nerve graft study (-IACUC approval No. CUMC-2019-0108-04). Under inhalation anesthesia, through a gluteal splitting incision, sciatic nerves were fully exposed from the inferior margin of the piriformis muscle to a point 5 mm distal to the bifurcation point. Then 15 to 17-mm segment of sciatic nerves were harvested bilaterally and prepared using the decellularization method combining amphoteric detergent and nuclease described by Shin et al.[14]. The decellularization process was initiated immediately after nerve harvest. Nerve segments were placed in a 15-ml conical tube filled with deionized distilled water (DW) for 7 h under continuous shaking at room temperature. Amphoteric detergent was added and stirred for 24 h followed by rinsing for 15 h in 1 M NaCl at room temperature. Nerve fragments were then agitated with 10-μg/ml deoxyribonuclease I and 200-μg/ml ribonuclease A in Tris–HCl buffer at 37 °C for 24 h. Finally, these decellularized nerve segments were washed with PBS at 4 °C for 72 h to remove residual reagents and then stored at -70 °C in a deep freezer prior to implantation (Table 1).

Table 1 Summary of nerve allograft decellularization process. All processes proceed with agitation

Nerve allograft processing
Amphoteric detergent + nuclease
DW wash for 7 h (room temperature)
CHAPS for 24 h (room temperature)
1-M NaCl for 15 h (room temperature)
Nuclease for 24 h (37 °C)
PBS wash for 72 h (4 °C)

Nuclease, 10-μg/ml DNase + 200-μg/ml RNase in Tris–HCl buffer. CHAPS with aprotinin in PBS. Abbreviation: DW, distilled water

2.3 Surgical procedure

Surgical procedures were performed under isoflurane gas inhalation anesthesia. After shaving and skin preparation, the sciatic nerve at randomly selected side was fully exposed from the inferior margin of the piriformis muscle to a point 5 mm distal from the bifurcation of peroneal and tibial nerves through a transgluteal approach. In group I, a 10-mm segment in the mid-portion of the sciatic nerve was resected and replaced at the original site in a reversed direction as an interposition autograft. The nerve graft was repaired using 10–0 nylon epineural sutures under a surgical microscope. In groups II, a 10-mm nerve segmental defect was reconstructed with previously prepared decellularized nerve isograft which had been thawed overnight and soaked in normal saline at room temperature for 5 min before implantation. After grafting, the incision wound was closed in layers and 0.1 mL/kg of buprenorphine hydrochloride was administered subcutaneously for pain control. Rats were allowed to move freely in the cage. During the survival period, rats were given food and water with 12 h/12 h of light/dark cycle.

2.4 Inflammatory reaction

Inflammatory or immune reactions to decellularized nerve graft were evaluated based on serum levels of IFN-r, IL-12p70, IL-1a, IL-1B, IL-2, IL-4, and TNF-α just before surgery and at 2 and 4 weeks after surgery. Any histologic evidence of inflammatory reaction was assessed using H&E stained specimens from 6 week- and 16 week-survival groups by one independent pathologist (Chung CK, M.D., Ph.D.).

2.5 Functional testing

2.5.1 Ankle angle at toe-off phase

To evaluate functional recovery, ankle angle at toe-off phase was measured at postoperative 6 and 16 weeks using Lee's method [15]. Briefly, rats were placed in walking tract (length, 1 m; width, 10 cm; height, 10 cm) made of Plexiglass. During walking, a video was acquired with a digital camera at a distance of 1 m and calibrated to prevent optical distortion. Lines of leg and foot segments were identified and ankle angle at toe-off phase was measured at the moment of maximal plantar flexion of ankle joint and expressed in degrees.

2.6 Maximum isometric tetanic force (MITF)

To evaluate sciatic nerve regeneration, MITF of tibialis anterior (TA) muscle was measured at 6 and 16 weeks after surgery using the method described by Shin et al. [13]. Under inhalation anesthesia, the sciatic nerve was exposed fully 5 mm distal to the bifurcation of peroneal nerve through gluteal incision of the hind limb. The peroneal nerve was dissected to connect the electrode. Another skin incision on anterior to the ankle joint and foot was made to expose tibialis anterior muscle–tendon unit distally. After detachment of the TA tendon from bone insertion, distal femur and distal tibia were fixed to the wood block of the platform with K-wires. The TA tendon was secured with a force transducer (MDB-2.5, Transducer Techniques, Temecula, CA, USA) (Fig. 1). The TA muscle–tendon unit was placed in its original anatomical orientation. Force transducer signals were displayed by a Smart Sensor Indicator (Transducer Techniques, Temecula, CA, USA). The electrode was connected to peroneal nerve and a bipolar stimulator. Normal saline was dropped onto the TA muscle to prevent tissue from drying out. The bipolar stimulator was used to deliver supra-maximal stimuli at a frequency of 100 Hz with 0.2 ms duration at different intensity levels (2.8, 3.2, and 3.6 V). It was repeated with duration of 0.3 and 0.4 ms at an intensity level from which the highest isometric tetanic force was obtained with a duration of 0.2 ms. The highest force value was recorded as the MITF of the case. There were 3 min intervals between each stimulus to prevent muscle fatigue. These same evaluations were performed for the contralateral normal side. The MITF was normalized using data of the contralateral side and reported as a percentage of the normal side.
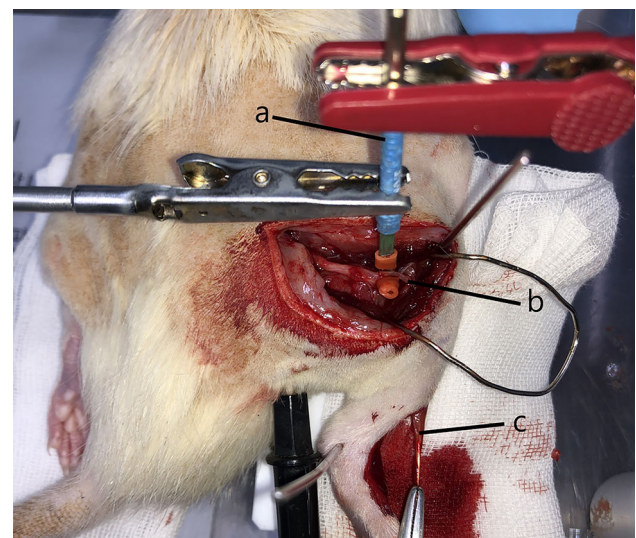

Fig. 1 Maximum isometric tetanic force measurement. The femoral condyle and heel are attached to the testing block with two Kirschner wires. The force transducer is attached to the distal portion of the tibialis anterior muscle with a custom clamp. An electrode (**a**) is connected to the peroneal branch of the sciatic nerve (**b**). Tibialis anterior tendon was secured to a force transducer (**c**)

2.7 Tibialis anterior wet muscle weight

Following completion of MIFT testing, the entire TA muscle was carefully dissected from surrounding tissues and harvested. The wet weight of TA muscle was measured in grams. Muscle weight of TA was compared to that of the contralateral normal side and reported as a percentage of the normal side.

2.8 Histomorphometric analysis

At 6 and 16 weeks, after all other measurements were completed, implanted nerves were dissected and one 4-μm-thick cross section was taken from each repaired nerve at the middle of the graft. Hematoxylin–eosin staining of sectioned nerves was undertaken for histoarchitectural analysis. Images were obtained at $100 \times$ magnification using a microscope with a mounted camera (3DHistech, panoramic midi, Budapest, Hungary) and an image acquisition software (Panoramic viewer Ver. 1.15.3, 3DHistech). Sections at mid-portion of nerve grafts were analyzed for the number of fascicles, cross sectional area, and fascicle area ratio at additional $6 \times$ magnification. The number of regenerated cell nuclei was analyzed at additional $8 \times$ magnification using Image J (NIH, Bethesda, MD USA).

2.9 Statistical analysis

All quantitative data are presented as mean ± standard derivation. Mean values for the maximum isometric tetanic muscle force and muscle weight were summarized for left and right sides separately. The percentage of the left to the right side per rat was taken for contralateral comparison.

To evaluate functional recovery following reconstruction for segmental nerve defects using autografts and decellularized nerve isografts, the maximum isometric tetanic forces and muscle weight of tibialis anterior muscle of isograft group at 6 and 16 weeks after grafting were compared to those of the autograft control group using an independent t-test. Results of histomorphometric parameters (number of fascicles, cross sectional area, fascicle area ratio, and number of regenerated cell nuclei) were also analyzed using independent t-test. All statistical analyses were performed using SPSS Statistics version 24 (IBM Corp., Armonk, NY, USA). Results were considered statistically significant at p value < 0.05.

3 Results

3.1 Inflammatory reaction outcomes

There was no significant difference in serum levels of inflammatory cytokines between the two groups except TNF-α right just before surgery ($p = 0.04$) and IL-4 at 4 weeks after surgery ($p = 0.03$, favorable for isograft group). At 2 and 4 weeks after grafting, serum levels of all inflammatory and immune markers measured in both groups were similar (mostly $p > 0.05$) (Table 2). So compare to biocompatible autograft, our processed decellularized isograft did not cause any elevation of serum levels of inflammatory cytokines after grafting. In histologic evaluation, both groups showed regenerated nerves at the mid-segment section of grafts without substantial inflammatory cell infiltration. Few specimens of grafts in both groups showed scanty mast cell and/or lymphocyte infiltration. Three of 23 isografts and 3 of 25 autografts showed focal fibrosis.

3.2 Ankle angle at toe-off phase

Mean ankle angle at toe-off phase was $51.0 \pm 10.8\%$ in group I (reverse autograft) and $51.1 \pm 11.1\%$ in group II (decellularized nerve isograft) compared to the opposite normal side at 6 weeks after surgery. At 16 weeks, it was $51.6 \pm 16.8\%$ in group I and $52.1 \pm 16.4\%$ in group II. Compared to the normal side, there was about a 50% reduction in toe-off angle. However, mean ankle angle at toe-off phase showed no significant difference between the autograft group and the isograft group or between the two time points (6 and 16 weeks after surgery) ($p > 0.05$) (Fig. 2).

3.3 Functional outcomes

The recovery of tibialis anterior MIFT at 6 weeks was $16.4 \pm 8.9\%$ in group I (reverse autograft) and $14.0 \pm 5.9\%$ in group II (decellularized nerve isograft). At 16 weeks, it was $57.6 \pm 22.6\%$ in group I and $49.5 \pm 20.1\%$ in group II (Fig. 3). The recovery of tibialis anterior MIFTs in the same group showed significant differences between 6 and 16 weeks ($p < 0.05$). Higher % recoveries of MITFs were observed at longer survival time point (16 weeks). However, there was no significant difference in the recovery between group I and group II at the same survival time point (6 weeks, $p = 0.47$; 16 weeks, $p = 0.34$).
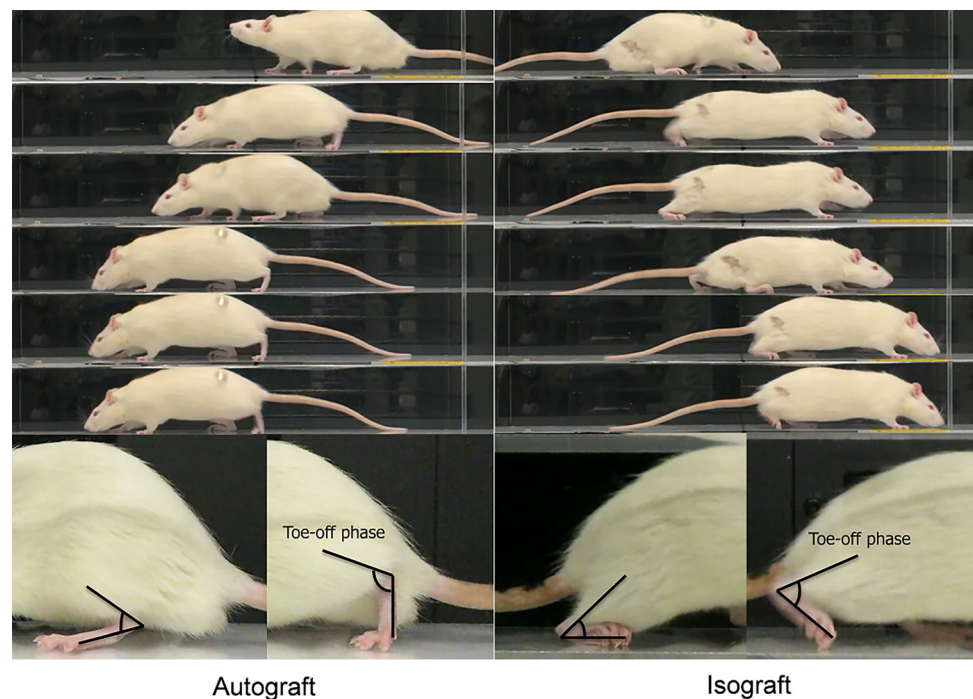
The recovery of TA muscle weight at 6 weeks was $41.0 \pm 4.6\%$ in group I (reverse autograft) and $44.2 \pm 8.8\%$ in group II (decellularized nerve isograft)

Table 2 Serum levels of inflammatory and immune markers

Markers	0 weeks		p value	2 weeks		p value	4 weeks		p value
	AUTO	ISO		AUTO	ISO		AUTO	ISO	
IFN-r	22.0 ± 11.9	32.0 ± 21.2	0.05	24.9 ± 13.1	29.1 ± 16.9	0.35	30.5 ± 14.3	27.4 ± 15.2	0.47
IL-12p70	23.1 ± 8.3	29.2 ± 19.9	0.17	25.0 ± 6.9	26.2 ± 8.3	0.62	29.8 ± 10.3	25.0 ± 9.3	0.10
IL-1α	12.1 ± 5.5	17.24 ± 12.1	0.07	13.4 ± 5.1	15.2 ± 7.4	0.33	16.2 ± 6.4	14.2 ± 5.7	0.27
IL-1β	27.5 ± 22.8	33.8 ± 19.7	0.32	28.3 ± 15.6	31.8 ± 19.8	0.61	31.6 ± 24.2	24.8 ± 11.6	0.23
IL-2	22.8 ± 9.3	29.9 ± 20.6	0.13	26.0 ± 10.1	27.7 ± 11.3	0.60	30.1 ± 9.1	25.7 ± 11.6	0.14
IL-4	14.2 ± 5.5	18.1 ± 13.5	0.19	16.2 ± 5.8	15.6 ± 6.0	0.72	20.4 ± 9.9	15.2 ± 5.9	**0.03**
TNF-α	20.9 ± 8.3	28.7 ± 15.2	**0.04**	23.2 ± 8.5	26.7 ± 12.3	0.25	26.8 ± 10.5	26.4 ± 10.4	0.88

There was no significant difference in serum levels of inflammatory cytokines between the two groups except TNF-α just before surgery ($p = 0.04$) and IL-4 at 4 weeks after surgery ($p = 0.03$, favorable for isograft group)

Fig. 2 Ankle angle at toe-off phase was measured by video analysis. Compared to the normal side, there was about a 50% reduction in toe-off angle, showing no significant difference between the autograft group and the isograft group at either time point (6 or 16 weeks after surgery) ($p < 0.05$)

Toe-off phase

Autograft   Isograft

($p = 0.30$). It was 69.2 ± 10.4% in group I and 61.7 ± 13.2% in group II ($p = 0.10$) at 16 weeks (Fig. 4).

Results of TA muscle weight were the same as those of MITF. There were significant differences in the recovery of TA muscle weight between 6 and 16 weeks for both group I and group II ($p < 0.05$). However, there was no difference in the recovery of TA muscle weight between group I and group II at the same survival time point (6 or 16 weeks).

3.4 Histomorphometric outcomes

Histomorphometric results of the isograft group (group II) did not differ significantly from those of the autograft group (group I) with regard to histomorphometric parameters such as fascicle number, fascicle area ratio, cross section area, and regenerated nerve cell number (Table 3).

4 Discussion

The concept of nerve defect reconstruction with nerve allograft is attractive. However, possible immune reaction and hindrance of axonal ingrowth by occupied nerve material in the endoneurial tube might reduce nerve regeneration potential [16, 17]. To promote nerve regeneration without such drawbacks, decellularization of the nerve graft has been attempted. Recent studies have shown

Fig. 3 Recovery of maximum isometric tetanic force (MITF) in each treatment group at 6 and 16 weeks after surgery. There was no significant difference in tibialis anterior MIFT between group I and group II at the same survival time points (6 or 16 weeks after surgery) ($p > 0.05$). Data are expressed as percentage of the normal, contralateral side as the mean force. *, significant at $p < 0.05$
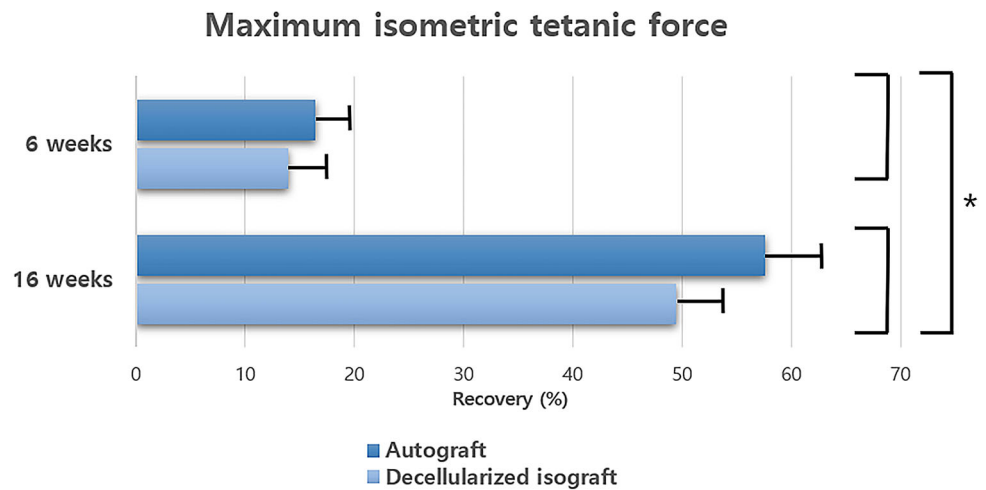
Maximum isometric tetanic force

Fig. 4 Recovery of tibialis anterior muscle weight between 6 and 16 weeks after surgery. There was no significant difference in tibialis anterior muscle weight between group I and group II at the same survival time points (6 or 16 weeks after surgery) ($p > 0.05$). Data are expressed as a percentage of the normal, contralateral side as the mean weight. *, significant at $p < 0.05$
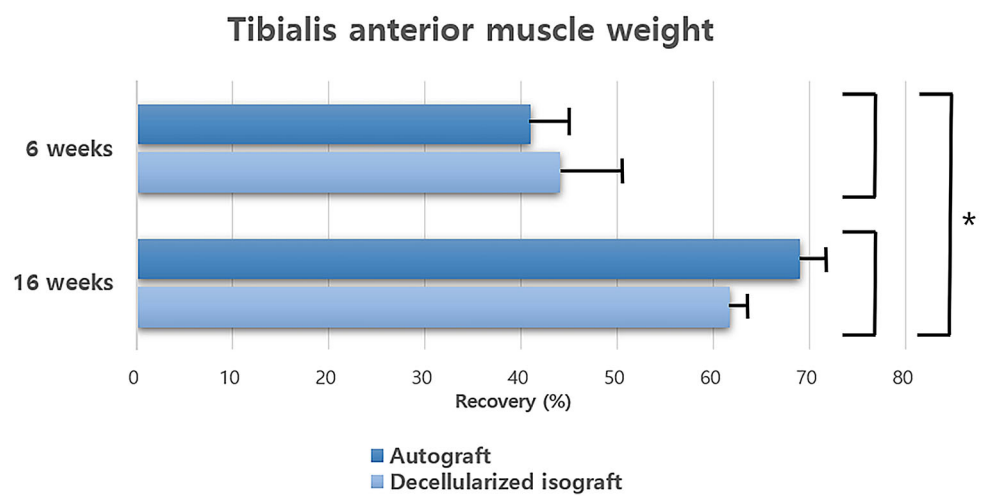
Tibialis anterior muscle weight

Table 3 Summary of histomorphotometric analysis of section at mid-portion of nerve graft

Parameters	Autograft	Isograft	*p* value
Fascicle number			
6 weeks	2.4 ± 1.1	2.8 ± 1.1	0.61
16 weeks	3.1 ± 1.2	2.7 ± 0.9	0.23
Fascicle area ratio (%)			
6 weeks	$38\% \pm 5\%$	$36\% \pm 8\%$	0.55
16 weeks	$35\% \pm 10\%$	$36\% \pm 7\%$	0.85
Cross section area (mm^2)			
6 weeks	3.3 ± 0.4	3.4 ± 0.4	0.46
16 weeks	3.5 ± 0.6	3.2 ± 0.6	0.36
Cell nucleus number			
6 weeks	1854 ± 572	1683 ± 447	0.48
16 weeks	2082 ± 556	2116 ± 613	0.89

good results in neurotransplant experiments using decelluarized nerve allografts [10, 12, 18, 19].

Fresh nerve allografts normally induce a strong immune reaction which compromises nerve regeneration [18]. A number of chemical and physical processes have been developed to reduce immune rejection [19–22]. Avance (decellularized, AxoGen, Alachua, FL, USA), a commercialized processed nerve allograft, has demonstrated greatly diminished immune rejection response. By measuring levels of interferon gamma (IFN-γ) in implanted rats, Whitlock et al. have proven the validity of the proprietary processing for reducing immune-rejection response of Avance allograft nerves [8].

To pursue more efficient nerve allograft, we have developed a new decellularization method of nerve graft using amphoteric detergent with nuclease. It is currently the most suitable for nerve decellularization in terms of adequate cell removal and sufficient preservation of the ECM [14]. To exclude any possible minor effect of immune reaction in allograft, we used isograft rather than

allograft in the present study. Biocompatibility was evaluated by inflammatory reaction, histological evaluation, and functional outcome as a final index of biocompatibility on nerve regeneration. The effectiveness was evaluated based on functional evaluation, maximum isometric tetanic force (MITF), muscle weight, ankle angle at toe-off phase, and histomorphometric analysis. Results of our study revealed similar biocompatibility and functional outcome between decellularized nerve allograft processed with our new method and autograft control.

Ankle contracture angle in toe-off phase is an important measurement for evaluating muscle recovery. Ankle angle in toe-off phase measured from video gait analysis is a useful parameter that reflects functional recovery of the muscle force [15]. In the present study, the ankle angle at toe-off phase showed about 50% of the normal side. However, it was not significantly different between autograft and isograft groups or between 6 and 16 weeks. Although some previously published studies have reported that rat walking tracks do not reflect maximal muscle force capacity [23], video gait analysis has shown a gradual improvement in ankle angle and performance compared to the immediate postoperative period. The reason why there was no significant difference in ankle angle at toe-off phase between 6- and 16-week survival groups despite recovering muscle tetanic force and muscle weight was thought to be due to ankle joint contracture after open surgery.

Direct muscle strength measurements are needed to evaluate the recovery of motor function of nerves. Giusti et al. [24] have described isometric tetanic force recovery in a 10-mm Lewis-rat sciatic nerve defect repaired with a reverse autograft, a rodent decellularized nerve allograft, or a collagen conduit. In their study, the isometric tetanic force recovery was similar between the reverse autograft and decellularized nerve allograft groups at 12 weeks. However, decellularized nerve allografts were found to be inferior to autografts at 16 weeks. Isometric tetanic force was decreased in the decelluar nerve allograft group at 16 weeks whereas autograft group was improved. Thus 16 weeks instead of 12 weeks were chosen as the end point in this study and muscle functional analysis was implemented at 6 and 16 weeks.

At 6 and 16 weeks, decellularized nerve isograft was slightly inferior to reverse autograft groups in terms of isometric tetanic force and muscle weight recoveries. However, differences between the two groups were not statistically significant. We could conclude that results of the isograft group were comparable to those of autograft group. Outcomes of both groups were significantly improved from 6 to 16 weeks. Tang et al. [25] have reported that muscle tetanic force percent recovery for decellularized nerve allograft processed by Avance method is about 56% of normal control side at 12 weeks and about 72% at 20 weeks. The present study using our newly developed decellularization method showed similar results.

Histomorphometric analysis for fascicle number, fascicle area ratio, cross section area and regenerated nerve cell number did not show significant differences between the two groups. Substantial nerve regeneration was observed for all tissues. Foreign body reactions due to suture materials were observed in some cases. A few lymphocyte infiltrations were observed in both autograft and isograft groups at 16 weeks. However, both groups were histologically similar, showing no or little inflammation reaction or immune-rejection profile (Fig. 5).

Compared to autograft group, there was no significant inflammatory or immune-rejection reaction in the processed isograft group. Serum levels of TNF-α just before surgery were higher in the decelluarized nerve isograft group than in the autograft group. However, 2 and 4 weeks later, they were decreased to similar levels to those of autograft group. IL-4 stimulates activated B cells and T cells to play an important role in congenital immunity and acquired immunity. IL-4 level at 4 weeks was rather significantly lower in the isograft group. The low level of IL-4 at 4 weeks after surgery was thought to be a no-immunological rejection, suggesting that the biocompatibility of processed decellularized isograft was at least comparable to that of autograft. When results of serum markers, histological evaluation, and histomorphometric analysis were combined, it could be concluded that the new method of our decellularization processing was immunologically safe and biocompatible.

One limitation of this study was that there was no comparison between our new method and existing nerve processing methods for Avance nerve allograft as an in-vivo study or a clinical trial. Such comparative studies are needed in the future. However, our previous *in vitro* study performed by another subgroup has demonstrated at least similar decellularization efficiency and ECM preservation in our newly developed method. Thus, it is not absolutely necessary to compare to results of Avance method to demonstrate the biocompatibility and effectiveness of our method. A small sample size of 48 was another limitation. Further studies in a larger scale will be helpful to support our method. Furthermore, despite the need for pain behavior analysis in injured nerve restoration studies, unfortunately, we didn't additionally analyze the pain behavior in this study. Finally, clinical trials with properly designed protocols will verify the effectiveness and biocompatibility of decellularized nerve allograft processed with our newly developed method.

In summary, nerve isografts processed with our newly developed decellularization method were fairly biocompatible and had effectiveness comparable to autografts in nerve regeneration based on inflammatory reaction, axonal
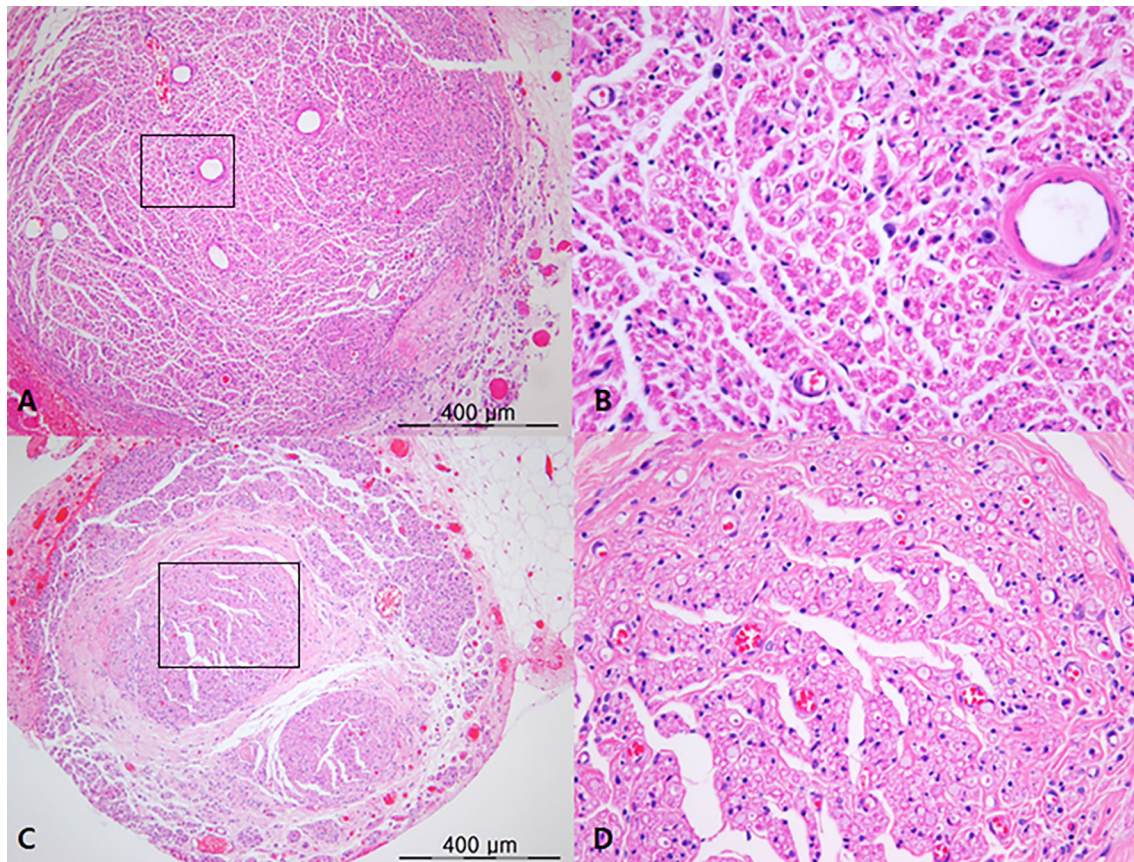
Fig. 5 Transverse sections of mid-portion of the graft nerve. **A, B** Autograft group at 16 weeks showed excellent nerve regeneration (**B**, H–E staining: 400×). **C, D** Isograft group at 16 weeks showed comparable nerve regeneration (**D**, H–E staining: 400×). There was no substantial inflammatory cell infiltration (**D**)

regeneration, and functional outcomes. Results of this study suggest that nerve allografts processed with our new method will be suitable alternatives to autografts for nerve reconstruction.

Compliance with ethical standards

Conflict of interest The authors have declared that there is no conflict of interest.

Ethical statement The animal studies were performed after receiving approval of the Institutional Animal Care and Use Committee (IACUC) in University (IACUC approval No. CUMC-2019-0108-04).

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