Original Article

An electrophysiological evaluation method for the ovine facial nerve

Hajime Matsumine [a, *], Yosuke Niimi [a], Hisami Matsumine [b]

[a] Department of Plastic and Reconstructive Surgery, Toho Women's Clinic, 5-3-10 Kiba, Koto-ku, Tokyo, 135-0042, Japan
[b] Department of Gynecology, Toho Women's Clinic, 5-3-10 Kiba, Koto-ku, Tokyo, 135-0042, Japan

A B S T R A C T

Introduction: Large-animal models such as sheep for facial nerve regeneration research have not yet been established because of the lack of methods for assessing the electrophysiological function of regenerated nerves. In this study, we developed a percutaneous measurement method for the evoked compound muscle action potential (CMAP) of the facial nerve in sheep.
Methods: Six 3-year-old castrated male Corriedale sheep were used in this study. Under general anesthesia, an anatomical exploration was performed to identify the course of the buccal branch of the facial nerve and its innervating muscles on one side, followed by the application of surface stimulating electrodes to the contralateral side of the face along the course of the buccal branch of the facial nerve to obtain CMAP measurements of the nasolabial levator muscle.
Results: Percutaneous CMAP measurements of the nasolabial levator muscle could be obtained in all animals by placing stimulating electrodes 1 cm apart on the line coinciding with the course of the buccal branch of the facial nerve revealed by the preceding anatomical exploration. Mean values for electro-physiological parameters were amplitude 4.7 ± 0.7 mV, duration 2.1 ± 0.6 ms, and latency 3.6 ± 0.4 ms.
Conclusion: We have established a percutaneous measurement method for CMAP of the buccal branch of the facial nerve in sheep. This method is expected to be very useful in future studies of facial nerve regeneration for long nerve defects in sheep.
© 2021, The Japanese Society for Regenerative Medicine. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Facial nerve palsy can have various causes, such as Bell's palsy [1], auditory nerve tumors [2], trauma [3], and malignant parotid gland tumors [3], and significantly impairs the patients' social activities and quality of life through mimetic muscle paralysis. Toward establishing new surgical treatments for facial nerve palsy, we have developed various experimental systems for facial nerve regeneration using rat models of facial nerve defects, including facial nerve regeneration in rats using a novel nerve conduit made of polylactic acid nonwoven fabric [4], facial nerve regeneration in rats using a basic fibroblast growth factor drug delivery system [5], facial nerve regeneration using

dedifferentiated fat cells [6], facial nerve regeneration using adipose-derived stem cells (ADSCs) that were induced in vitro to differentiate into Schwann cell lineages [7], facial nerve reconstruction in rats using an artificial nerve conduit containing dental pulp cells extracted from incisor teeth [8], and facial nerve regeneration using an artificial nerve conduit made of a silicone tube containing a non-cultured stromal vascular fraction as the source of ADSCs [9]. We then developed a method to measure compound muscle action potential (CMAP) in these rat models of facial nerve regeneration, for which no electrophysiological evaluation method for regenerated facial nerves had been established [10]. However, the above rat models of facial nerve defect allowed for experimental nerve transplantation into defects of up to only about 7 mm in length due to the small size of the animals, and were too small to reproduce facial nerve regeneration surgery in human patients in clinical practice. For example, at least 10 cm of nerve regeneration length is required for cross-facial nerve grafting (CFNG), where an autologous nerve graft is transplanted between the facial nerve on the healthy side and the facial nerve branch on the affected side so that the

mimetic muscles on the affected side are reinnervated by neural transmission from the facial nerve on the healthy side to restore facial movement [11]. This cannot be reproduced in rat models of facial nerve palsy. Therefore, aiming to create a large-animal model of facial nerve defect, we have conducted anatomical studies of the facial [12], hypoglossal [12] and gastrocnemius [13] nerves in sheep, describing the course of the nerves in detail. Based on the facial nerve anatomy revealed through these previous studies, in the present work we established a method for measuring CMAP of the facial nerve in sheep and describe the measurement procedure.

2. Methods

2.1. Experimental design

This animal study using sheep was approved by the animal experimentation committee at IVTeC Inc (approval number: IVT19-80) and was conducted in accordance with the Act on Welfare and Management of Animals; the Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain; the Fundamental Guidelines of the Ministry of Education, Culture, Sports, Science and Technology; the Guidelines for Proper Conduct of Animal Experiments; the Guidelines on Methods of Sacrificing Animals, and other applicable regulations. A total of 6 castrated Corriedale sheep (age 3 years, body weight 30—40 kg) were used. For all electrophysiological evaluations and surgery, the sheep were intubated, and anesthesia was induced and maintained with 2—5% isoflurane under mechanical ventilation.

2.2. Identification of the course of the buccal branch of the facial nerve and detection of muscle contraction induced by electrical stimulation

The anatomical stimulation and recording sites for CMAP measurement were determined on the right (n = 3) and left (n = 3) side of the face of the 6 sheep. On the right or left side of the sheep's face, a "modified Blair incision" was made anterior to the auricle and extended from 1.5 cm posterior to the mandibular ramus to 2 cm below the mandibular body. This flap was undermined widely to expose the platysma muscle using scissors and scalpel so that the zygomatic muscle, parotid gland, facial artery and vein, and the buccal and marginal mandibular branches of the facial nerve could be seen through the platysma fascia. An L-shaped incision was then made in the platysma overlying the mandibular area to expose the parotid gland and the buccal branch of the facial nerve emerging from the anterior margin of the parotid gland, and the course of the buccal branch was identified, which would serve as the landmark for the application of stimulating electrodes for percutaneous CMAP measurement. The buccal branch was further traced toward the most posterior molar while dissecting surrounding tissue to confirm that it was coursing behind the zygomatic muscle into the posterior aspect of the nasolabial levator muscle. The stimulating electrodes of the evoked potential testing device (Neuropack S3, Nihon Kohden, Tokyo, Japan) were applied directly to the exposed buccal branch and a single supramaximal electric stimulus (approx. 8 mA) was applied at a frequency of 0.1 Hz to visually confirm movement of the nasolabial levator muscle upon stimulation (Fig. 1). The marginal mandibular branch of the facial nerve, which

Fig. 1. Localization of the facial nerve and mimetic muscles in sheep as determined by facial anatomical exploration. When electric stimulation was applied via the stimulating electrodes applied directly to the buccal branch of the facial nerve at the site where it emerged from the anterior margin of the parotid gland and coursed toward the nasolabial levator muscle, the movement of the exposed nasolabial levator muscle upon stimulation was visually confirmed. *: buccal branch; ■: marginal mandibular branch of facial nerve; †: parotid gland; ▲: nasolabial levator muscle; ★: zygomatic muscle.

was exposed along with the buccal branch, was also electrically stimulated with the same settings to determine whether the nasolabial levator muscle was also innervated by the marginal mandibular branch (Fig. 1).

2.3. Percutaneous CMAP measurement

The side of the face contralateral to the one used for the above anatomical exploration was used to percutaneously apply electrical stimulation to the buccal branch of the facial nerve and measure CMAP of the mimetic muscles (nasolabial levator muscle) with the evoked potential testing device (Neuropack S3, Nihon Kohden). Two surface stimulating electrodes were placed 1 cm apart on the line along the course of the buccal branch of the facial nerve as revealed in the preceding anatomical exploration. The recording microelectrode (NM-450C, Nihon Kohden) was inserted to a depth of about 1 cm at the midpoint on the line connecting the nostril and the angle of the mouth, where the nasolabial levator muscle is located (Fig. 2A). A single supramaximal stimulus (approx. 8 mA) was applied at a frequency of 0.1 Hz. Ten CMAP measurements were obtained from each animal, from which amplitude, latency, and duration were determined and average values were calculated for each parameter. The method for calculating amplitude, latency, and duration was described in our previous report on measuring CMAP in rats [10]. Briefly, amplitude is the differential voltage between the baseline (zero) and positive peak potentials. Latency is the time interval between the beginning of stimulus artifacts and when the CMAP trace rises over the zero line. Duration is the time interval between the zero-crossing points of the rising and falling trace (Fig. 2B).

3. Results

The anatomical exploration performed prior to CMAP measurement revealed that the course of the buccal branch of the facial nerve was beneath the line connecting (a) the midpoint of the line connecting the outer edge of the orbit and the mandibular angle and (b) the most posterior molar. The point (a) coincided with the site where the buccal branch of the facial nerve emerged superficially from the parotid tissue. We also visually confirmed the movement of the exposed nasolabial levator muscle upon direct stimulation of the buccal branch of the facial nerve, confirming that the muscle is innervated by the buccal branch. In contrast, direct stimulation of the marginal mandibular branch of the facial nerve did not cause the nasolabial levator muscle to move, indicating that the muscle is innervated solely by the buccal branch (Figs. 1 and 3). Percutaneous CMAP measurement of the nasolabial levator muscle was feasible in all animals (No. 1 to 6) with stimulating electrodes placed 1 cm apart on the line coinciding with the course of the buccal branch of the facial nerve as determined above. Mean values of the electrophysiological parameters were amplitude 4.7 ± 0.7 mV, duration 2.1 ± 0.6 ms, and latency 3.6 ± 0.4 ms (Fig. 2B and Table 1).

4. Discussion

It is well known that rodents are very useful for facial nerve research because of their reasonable cost and high availability. Using rat models of facial nerve defects, Sasaki et al. have shown correlation between the process of facial nerve regeneration and the degree of improvement in CMAP measurements [14]. However,

in rat models, the facial nerve needs to be directly stimulated, which is made possible only by exposing the regenerated nerve and the surrounding facial nerve fibers under sedation, making it impossible to assess the electrophysiological function of the regenerated nerve at multiple time points. Moreover, placing stimulating electrodes directly on the very thin and fragile regenerated nerve could damage it. These are the two major challenges of studying facial nerve regeneration in rats.

Sheep, in which the current CMAP measurement method was established, have been widely used as a translational animal model in studies of intensive care [15] and wound healing [16]. The advantages of using sheep as an experimental model include (a) their anatomical and pathophysiological similarities to humans, and (b) their calm nature compared to other large animals commonly used as experimental models, such as pigs, enabling easier postoperative wound management. Previous studies have also shown that the anatomy of the facial nerve region in sheep is very similar to that in humans in terms of the course of the facial nerve, localization of the mimetic muscles, and the number of myelin sheaths on the facial nerve [12]. In humans, the number of myelinated fibers in the main trunk of the facial nerve was reported to be 12,929, 5980−21,800, 6,254, and 7550−8230 by Van Buskirk [17], Kullman [18], Fujii [19], and Thurner [20], respectively. In sheep, we previously found that the number of myelinated fibers in the main trunk of the facial nerve was $11,350 \pm 1,851$, which is comparable to the number in humans [12]. In contrast, our previous study found that the number of myelinated fibers in the main trunk of the rat monofaccicular nerve was 1690 ± 176 [21]. Moreover, long facial nerve defects, which cannot be created in rat models, can be stably created in sheep models. With rat models, electrophysiological evaluation was only feasible for the vibrissal muscle among the muscles innervated by the buccal branch of the facial nerve. In contrast, the use of a sheep model appears to enable functional evaluation of other muscles innervated by the buccal branch, including the zygomatic, nasolabial levator, and orbicularis oris muscles, allowing for advanced electrophysiological evaluation that more closely mimics evaluation of facial paralysis in clinical practice. Thus, this sheep model appears to be very useful for studying facial nerve regeneration for future clinical application. The present method allows for percutaneous measurement of CMAP, which can be measured only by directly stimulating the regenerated nerve in rats through invasive surgical procedures, and thus allows for evaluation of innervation status at multiple time points in a single animal. This advantage is expected to be very useful in studies of CFNG, which requires a long period of time to reinnervate the mimetic muscles. Our previous report found that the sural nerve of sheep can be harvested in lengths up to 14 cm [13], but we have not yet clarified the necessary length of an autologous nerve graft for CFNG in sheep. Further study is needed to establish a sheep CFNG model. Limitations of the present method include the need for inhalational anesthesia during measurement, the need for a wide laboratory table that can accommodate a large animal during measurement, and the need to shave the animal's fur at the site of the stimulating electrode. We are planning to use this method to evaluate the rate of axon elongation of the ovine facial nerve and also to perform chronological evaluation of electrophysiological function in studies of facial nerve regeneration using mesenchymal stem cells or other types of cells. A future research task is to investigate whether the same CMAP measurement method is applicable to branches of the facial nerve other than the buccal branch, such as the temporal and marginal mandibular branches.
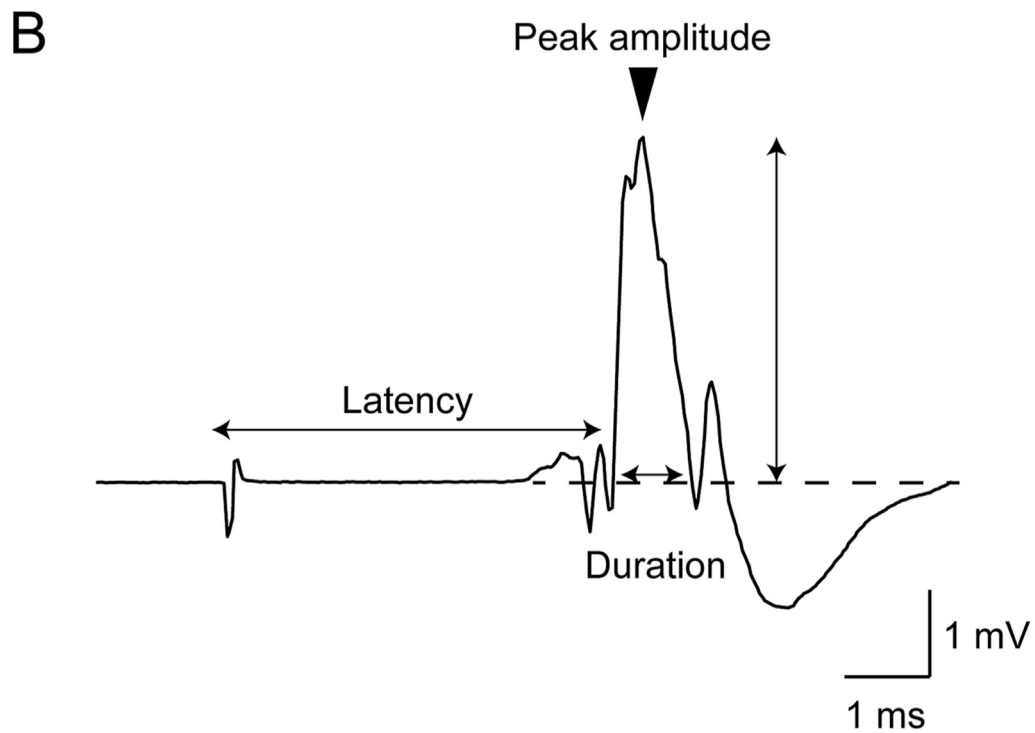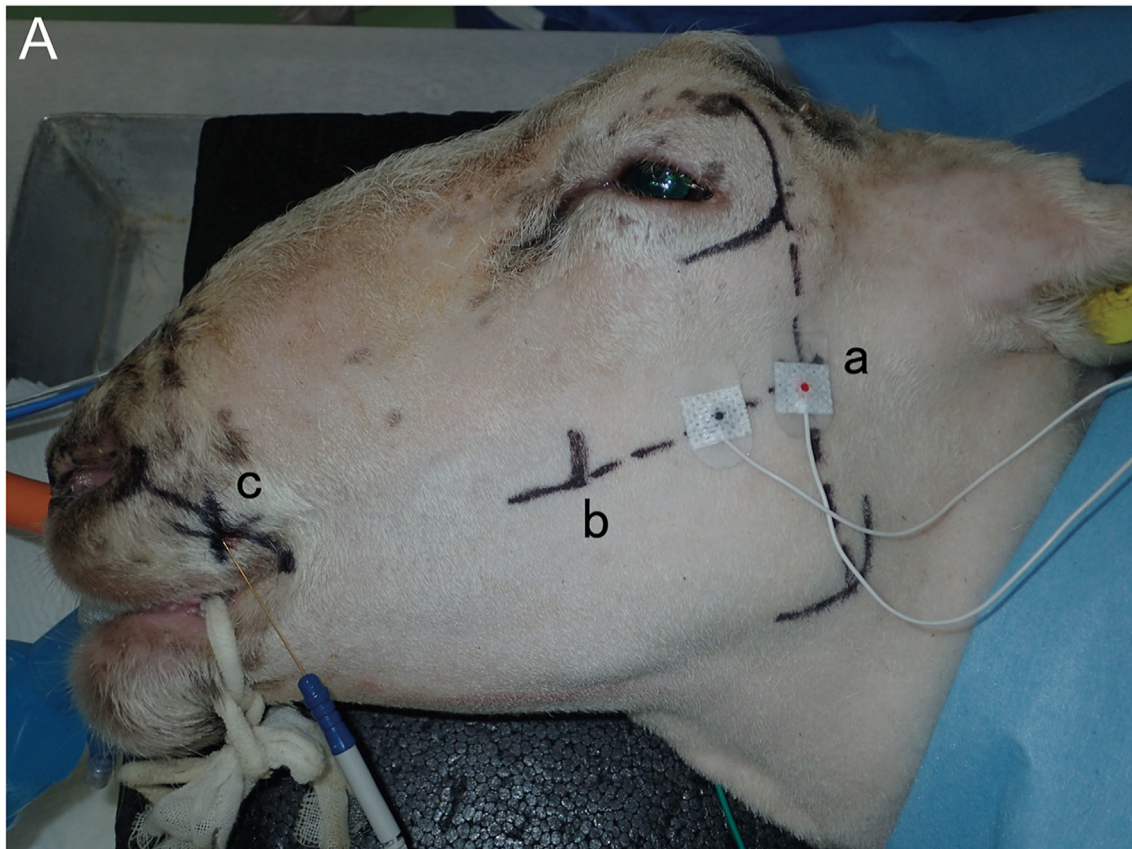
Fig. 2. Sites of percutaneous measurement of CMAP of the buccal branch of the facial nerve in sheep and CMAP wave patterns. A. Stimulating electrodes were applied to two sites on the line connecting (a) the midpoint of the line connecting the outer edge of the orbit and the mandibular angle and (b) the most posterior molar. The recording microelectrode was inserted to a depth of about 1 cm at (c) the midpoint on the line connecting the nostril and the angle of the mouth, where the nasolabial levator muscle is located. CMAP, compound muscle action potential.
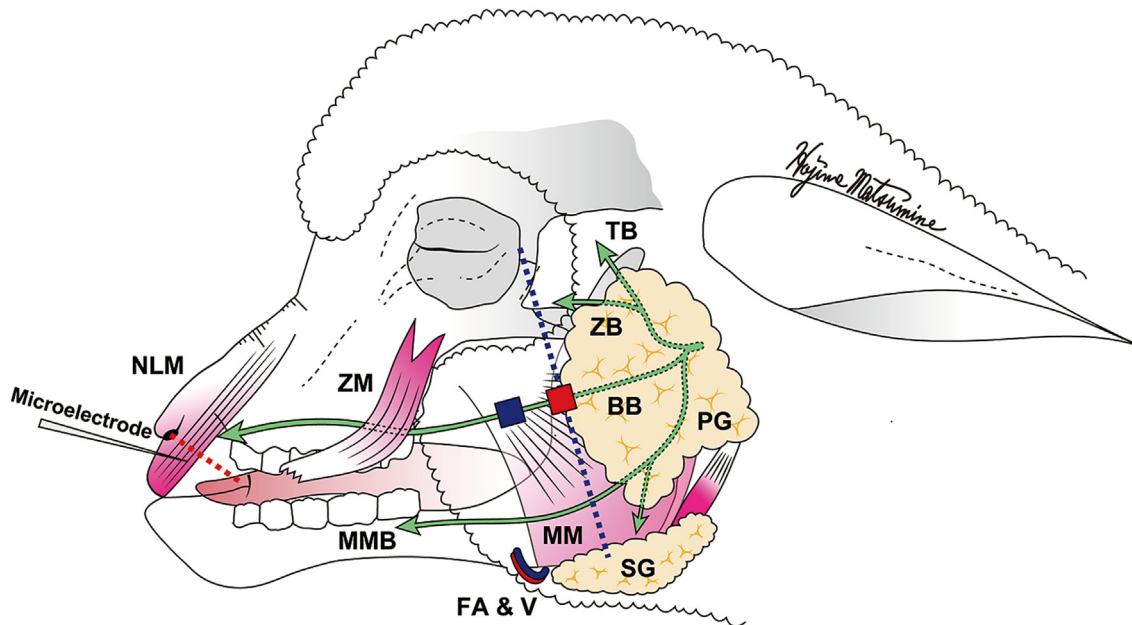
Fig. 3. Schematic diagram of the course of the buccal branch of the facial nerve and the localization of the nasolabial levator muscle in sheep. The buccal branch of the facial nerve emerged superficially from the parotid tissue at the midpoint of the blue dotted line connecting the outer edge of the orbit and the mandibular angle, coursed behind the zygomatic muscle in the posterior molar region, and entered into the nasolabial levator muscle. Red and blue squares indicate surface stimulating electrodes arranged to measure CMAP of the buccal branch of facial nerve. The recording microelectrode was inserted at the midpoint of the red dotted line connecting the nostril and the angle of the mouth. TB, temporal branch; ZB, zygomatic branch; BB, buccal branch; MMB, marginal mandibular branch of facial nerve; PG, parotid gland; SG, submandibular gland; DM, digastric posterior belly; MM, masseter muscle; FA & V, facial artery and vein; ZM: zygomatic muscle; NLM, nasolabial levator muscle.

Table 1
Compound muscle action potential in each sheep.

Animal no.	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	Ave.	SD
Amplitude (mV)	3.7	5.3	4.7	4.0	5.1	5.4	4.7	0.7
Duration (ms)	1.2	2.4	2.0	2.8	2.5	1.5	2.1	0.6
Latency (ms)	4.1	3.5	3.8	4.0	3.2	3.1	3.6	0.4

5. Conclusions

We have established a percutaneous measurement method for CMAP of the buccal branch of the facial nerve in sheep. This method is expected to be very useful in future studies of facial nerve regeneration for long nerve defects in sheep.

Declaration of competing interest

The authors declare no conflict of interest.

References

[1] Holland NJ, Weiner GM. Recent developments in Bell's palsy. BMJ 2004;329(7465):553—7.

[2] Sampath P, Holliday MJ, Brem H, Niparko JK, Long DM. Facial nerve injury in acoustic neuroma (vestibular schwannoma) surgery: etiology and prevention. J Neurosurg 1997;87(1):60—6.

[3] Matsumine H, Kamei W, Fujii K, Shimizu M, Osada A, Sakurai H. One-stage reconstruction by dual-innervated double muscle flap transplantation with the neural interconnection between the ipsilateral masseter and contralateral facial nerve for reanimating established facial paralysis: a report of 2 cases. Microsurgery 2019;39(5):457—62.

[4] Matsumine H, Sasaki R, Yamato M, Okano T, Sakurai H. A polylactic acid non-woven nerve conduit for facial nerve regeneration in rats. J Tissue Eng Regen Med 2014;8:454—62.

[5] Matsumine H, Sasaki R, Tabata Y, Matsui M, Yamato M, Okano T, et al. Facial nerve regeneration using basic fibroblast growth factor-impregnated gelatin microspheres in a rat model. J Tissue Eng Regen Med 2016;10:E559—67.

[6] Matsumine H, Takeuchi Y, Sasaki R, Kazama T, Kano K, Matsumoto T, et al. Adipocyte-derived and dedifferentiated fat cells promoting facial nerve regeneration in a rat model. Plast Reconstr Surg 2014;134:686—97.

[7] Watanabe Y, Sasaki R, Matsumine H, Yamato M, Okano T. Undifferentiated and differentiated adipose-derived stem cells improve nerve regeneration in a rat model of facial nerve defect. J Tissue Eng Regen Med 2017;11(2):362—74.

[8] Sasaki R, Aoki S, Yamato M, Uchiyama H, Wada K, Ogiuchi H, et al. PLGA artificial nerve conduits with dental pulp cells promote facial nerve regen-eration. J Tissue Eng Regen Med 2011;5(10):823—30.

[9] Shimizu M, Matsumine H, Osaki H, Ueta Y, Tsunoda S, Kamei W, et al. Adipose-derived stem cells and the stromal vascular fraction in polyglycolic acid-collagen nerve conduits promote rat facial nerve regeneration: rat facial nerve regeneration with ADSCs or SVF. Wound Repair Regen 2018;26(6):446—55.

[10] Takeuchi Y, Osaki H, Matsumine H, Niimi Y, Sasaki R, Miyata M. A method package for electrophysiological evaluation of reconstructed or regenerated facial nerves in rodents. Methods (Duluth) 2018;5:283—98.

[11] Fujii K, Matsumine H, Osaki H, Ueta Y, Kamei W, Niimi Y, et al. Accelerated outgrowth in cross-facial nerve grafts wrapped with adipose-derived stem cell sheets. J Tissue Eng Regen Med 2020;14(8):1087—99.

[12] Niimi Y, Matsumine H, Fukuda S, Salsbury JR, Niimi Y, Herndon DN, et al. Surgical anatomy of ovine facial and hypoglossal nerves for facial nerve reconstruction and regeneration research: an experimental study in sheep. Microsurgery 2020;40(1):51—8.

[13] Niimi Y, Fukuda S, Gilbert RS, Baljinnyam T, Niimi Y, Matsumine H, et al. Surgical anatomy of the ovine sural nerve for facial nerve regeneration and reconstruction research. Sci Rep 2019;9:10564.

[14] Sasaki R, Matsumine H, Watanabe Y, Takeuchi Y, Yamato M, Okano T, et al. Electrophysiologic and functional evaluations of regenerated facial nerve defects with a tube containing dental pulp cells in rats. Plast Reconstr Surg 2014;134:970—8.

[15] Fujiwara O, Fukuda S, Lopez E, Zeng Y, Niimi Y, DeWitt DS, et al. Peroxynitrite decomposition catalyst reduces vasopressin requirement in ovine MRSA sepsis. Intensive Care Med Exp 2019;7:12.

[16] Niimi Y, Fukuda S, Alharbi S, Prough DS, Enkhbaatar P. Polyurethane foam for skin graft fixation in clinical-relevant ovine burn wound model for wound repair and regeneration research. Regen Ther 2020;14:341–3.

[17] Van Buskirk PC. The seventh nerve complex. J comp Neural 1945;82:303–26.

[18] Kullman GL, Dyck PJ, Cody DT. Anatomy of the mastoid portion of the facial nerve. Arch Otolaryngol 1971;93:29–33.

[19] Fujii M, Goto N. Nerve fiber analysis of the facial nerve. Ann Otol Rhinol Laryngol 1989;98:732–6.

[20] Thurner KH, Egg G, Spoendlin H, Schrott-Fischer A. A quantitative study of nerve fibers in the human facial nerve. Eur Arch Oto-Rhino-Laryngol 1993;250:161–7.

[21] Matsumine H, Sasaki R, Takeuchi Y, Watanabe Y, Niimi Y, Sakurai H, et al. Unilateral multiple facial-nerve branch reconstruction using "end-to-side loop graft" supercharged by hypoglossal nerve. Plast Reconstr Surg Glob Open 2014;2(10):e240.