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Summary

The use of peripheral nerves as donor nerves for peripheral nerve regeneration studies, which can provide a long peripheral nerve with intact physiological functions, for autologous nerve grafts was unknown in swine. This study investigated the surgical anatomy of sural nerves (*nervus suralis*) of cadavers and anesthetized miniature pigs. A loose-S shape incision line was made from the border of the biceps femoris muscle (*musculus biceps femoris*) to 2 cm above the calx in the leg of anesthetized miniature pigs. The sural nerve was found to branch from the sciatic nerve (*nervus ischiadicus)* under the biceps femoris muscle and run along the small saphenous vein (*vena saphena parva*). After being isolated and stimulated using a nerve stimulator, the sural nerve innervated no muscles and tissues in the leg. The sural nerve (14.5 ± 0.5 cm) was obtained from between the sciatic nerve and peripheral branches in anesthetized miniature pigs. Toluidine-blue staining of the obtained sural nerve indicated that the numbers of myelinated fibers in the distal and proximal portions were 2271 ± 639 and 2639 ± 622, respectively. It is concluded that the sural nerve of miniature pigs can be used for peripheral nerve regeneration studies.

Introduction

Miniature pigs are a suitable experimental model in translational research, because their anatomical structure is similar to humans and, compared with rats and mice, their body size is closer to humans (*Swindle et al., 1994; Vodicka et al., 2005; Wang et al., 2007; Sasaki et al., 2010*). However, the use of peripheral nerves as donor nerves for peripheral nerve regeneration studies, which can provide a long peripheral nerve with intact physiological functions, for autologous nerve grafts was unknown in swine. The sural nerve (*nervus suralis*), a sensory nerve of the leg, is the most common peripheral nerve donor in human facial nerve reconstruction, because a long nerve can be easily obtained without functional defects (*Doi et al., 1984; Kim & Seo, 2001*). Therefore, in this study, the use of the sural nerve as a donor for autologous nerve grafts was investigated in miniature swine cadavers and anaesthetized miniature swine.

Materials and Methods

The cadavers of 3 healthy 8-month-old NIBS miniature pigs (25.8-27.4 kg) were used for this study (Nisseiken, Ome, Japan). Prior to their use in this study, the three pigs were used in a cardiology or general surgery study and were euthanized at the end of that project by intravenous injection of KCl under deep sevoflurane-induced anesthesia. After the confirmation of death, the three cadavers were used for the present study. One cadaver was frozen at -15°C until used. Before the experiment, the frozen cadaver was kept overnight at room temperature.

For the anesthetized pig experiment, healthy 9- to 11-month-old male NIBS miniature pigs (Nisseiken) (26-30 kg) and a Clawn miniature pig (Japan Farm Clawn Institute, Kagoshima) were used. The pigs were first pre-medicated using a combined intramuscular injection of medetomidine (80 μg/kg), ketamine (5 mg/kg), and butorphanol (0.2 mg/kg) before being intubated and anesthetized by sevoflurane-induced anesthesia. The sural nerve was dissected. One week after surgery, functional defect, such as dragging the leg was evaluated.

Animal care and handling procedures were performed in accordance with the "Principles of Laboratory Animal Care" of the Tokyo Women's Medical University Animal Experimentation Committee.

The distal and proximal portions (2 cm from the distal and proximal end) of the obtained sural nerve were subsequently examined. Samples were prefixed with 2% glutaraldehyde (distilled EM grade, EM Science, Gibbstown, NJ, USA), 2% paraformaldehyde (Wako Pure Chemical, Osaka) and 0.1 mol/L cacodylate buffer (Wako), and postfixed with 2% osmium tetroxide (Wako) and 0.1 mol/L cacodylate buffer (Wako) before being embedded in Quetol-812 resin (Nisshin EM, Tokyo, Japan) at 60°C for 2 days. The embedded specimens were cut into approximately 1.5 mm sections with a glass knife and heat-stained with 0.5% toluidine blue. The numbers of myelinated fibers in the distal and proximal portion of the sural nerve specimens were manually counted on photographs taken with a conventional microscope.

Results

Sural nerve dissection in miniature pig cadavers

The sural nerve was found to branch from the sciatic nerve under the biceps femoris muscle (*musculus biceps femoris*) and to run along the small saphenous vein (*vena saphena parva*). The sural nerve branched into approximately 3 branches at the calx. The mean length of the obtained sural nerve between the sciatic nerve and peripheral branches in the miniature pig cadavers was 12.33 ± 1.2 cm (range: 10 to 14 cm, n = 3, mean ± S.E.M.).

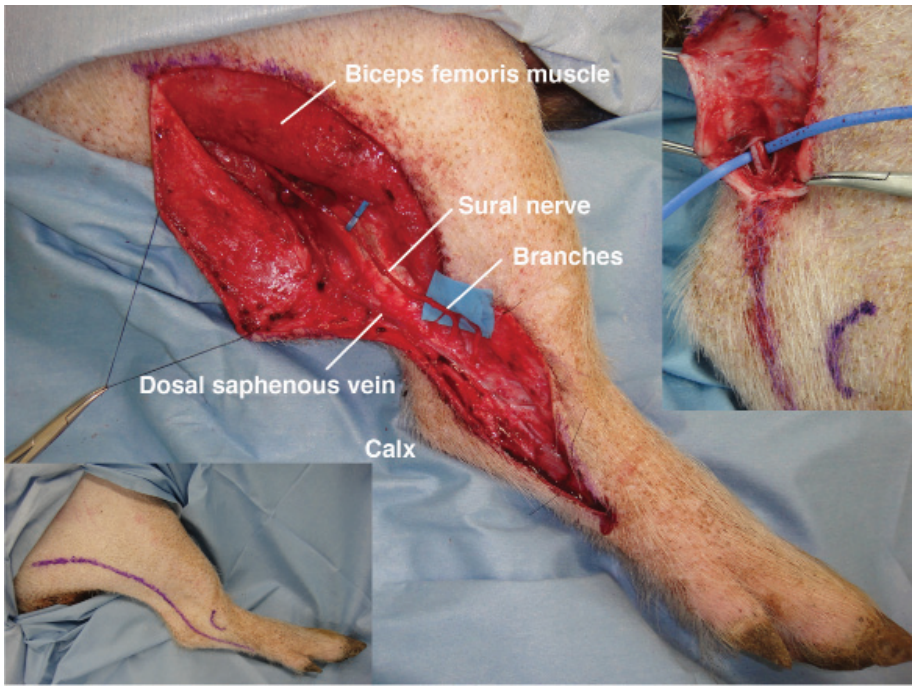

Figure 1. The surgical anatomy of the miniature pig sural nerve. The incision line for obtaining the sural nerve (left below). The sural nerve was found to run along the small saphenous vein (right above). The sural nerve branched from the sciatic nerve under the biceps femoris muscle into 3 branches at the calx (central).

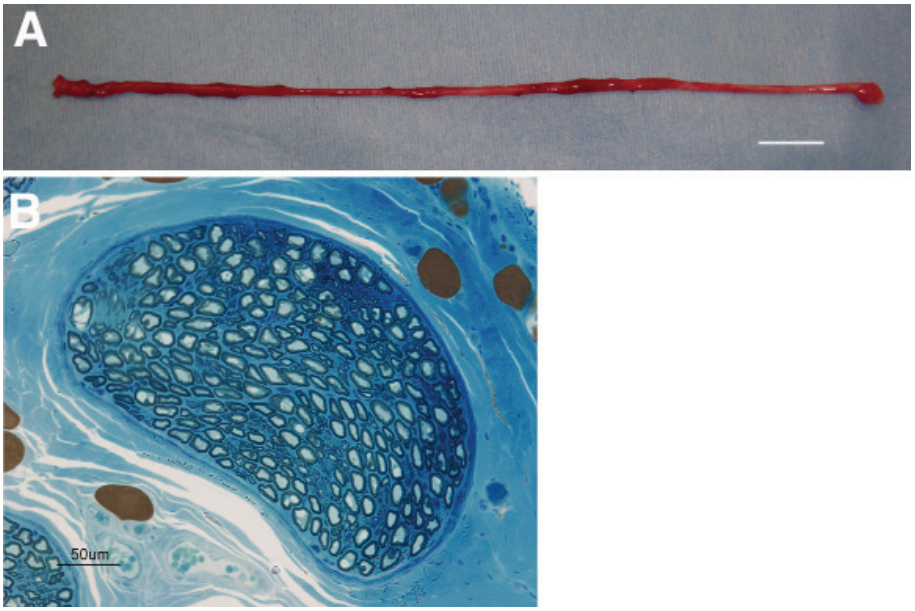

Figure 2. (A) A sural nerve obtained from an anesthetized miniature pig. The sural nerve (13.5 cm) was obtained from between the sciatic nerve branch and the peripheral branches of an anesthetized miniature pig. **(B)** Toluidine blue staining of the section of nerve bundle at the distal portion (2 cm from the distal end) of a sural nerve obtained from an anesthetized miniature pig.

Sural nerve dissection in anesthetized miniature pigs

Based on the anatomy of the cadaver sural nerves, a loose-S shape incision line was performed from the border of the biceps femoris muscle to 2 cm above the calx in the leg of anesthetized miniature pigs (Fig. 1, left below). The sural nerve in the pigs branched from the sciatic nerve under the biceps femoris muscle and ran along the small saphenous vein (Fig. 1, right above). The sural nerve branched into approximately 3 branches at the calx (Fig. 1, central). The sural nerve was isolated and then stimulated using a nerve stimulator (Vari-stim® III, Medtronic, Jacksonville, FL). The nerve stimulation results indicated that the sciatic nerve (*nervus ischiadicus)* innervated muscles and tissues in the leg. However, the sural nerve innervated no muscles and tissues. The sural nerve (14.5 ± 0.5 cm, range: 13.5 to 15 cm, n = 3, mean ± S.E.M.) was obtained between the sciatic nerve branch and peripheral branches in the anesthetized miniature pigs (Fig. 2-A). One week after surgery, no functional defect, such as dragging the leg was observed. Moreover, no plantar decubitus ulcer was observed at 3 months after surgery. Toluidine-blue staining of the distal and proximal portions of the obtained sural nerve indicated 5.33 ± 2.1 and 5.00 ± 1.5 distal and proximal stumps, respectively. There were 2271 ± 639.5 and 2639 ± 622.7 myelinated fibers in the distal and proximal portions, respectively (Fig. 2-B).

Discussion

Miniature swine are used as an experimental or pre-clinical model in maxillofacial surgery, including mandibular reconstruction (*Terheyden et al., 1999*) and distraction osteogenesis (*Goldwaser et al., 2013*), dental implantology (*Eom et al., 2012*), bisphosphonate-related jaw osteonecrosis (*Li et al., 2013*) and face transplantation (*Kuo et al., 2012*) because the anatomical structure of the maxillofacial region is similar to humans and, compared with rats and mice, their facial size is closer to humans (*Swindle et al., 1994; Vodicka et al., 2005; Wang et al., 2007; Sasaki et al., 2010; Sasaki et al., 2014a; Sasaki et al., 2015*). Miniature pigs are also suitable for surgical training and teaching for newly innovated transplantation methods (*Swindle et al., 1994; Swindle and Smith, 2013*). Although facial nerve regeneration studies using a tissue-engineered nerve guide are reported in rats (*Sasaki et al., 2008; Sasaki et al., 2011; Matsumine et al., 2012; Sasaki et al., 2014b*), no facial nerve regeneration study using miniature swine has been reported. Validation of an animal model more closely related to humans is needed in translational research (*Vodicka et al., 2005*). Therefore, the surgical anatomy of the face of miniature swine, including the buccal branch and marginal mandibular branch of the facial nerve, was investigated for facial nerve regeneration research in our previous study (*Sasaki et al., 2010*). Miniature pigs are a suitable experimental model for facial nerve regeneration surgery (*Sasaki et al., 2010; Sasaki et al., 2014a*). However the use of peripheral nerves as donor nerves, which can provide a long peripheral nerve without functional defects, for autologous nerve grafts as the

control against novel tissue-engineered nerve guide was unknown in miniature pigs. The human sural nerve is the most common donor peripheral nerve in facial nerve reconstruction, because a long nerve can be easily obtained with intact physiological functions (*Doi et al., 1984; Kim & Seo, 2001*). Therefore, this study investigated use of the sural nerve as a donor for autologous nerve grafts in miniature swine. Interestingly, the sural nerve in pigs was found to be similar to the human sural nerve in that it is located parallel to the small saphenous vein (*Doi et al., 1984; Kim & Seo, 2001*). Although a maximum of 40 cm of sural nerve can be obtained in human clinical studies (*Kim & Seo, 2001*), the obtained sural nerves from cadavers and live pigs were 12.33 ± 1.2 cm and 14.5 ± 0.5 cm, respectively. The removal of the sural nerve from miniature pigs caused no functional defect. It is concluded that the sural nerve of the miniature pig can be used for peripheral nerve regeneration studies, including the examination of autologus nerve grafts with intact physiological functions. The surgical anatomy of the sural nerve of the miniature pig described in this paper would be expected to contribute to peripheral nerve reconstructive research.

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