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ORIGINAL ARTICLE

Facial nerve dissection in parotid surgery: a microscopic investigation study

Tsuyoshi Jinnin[1] · Sugiko Futaki[2] · Azumi Hirata[2] · Hiroko Kuwabara[3] · Masaaki Higashino[1] · Yoichi Kondo[2] · Ryo Kawata[1]

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Abstract

In parotid surgery, it is crucial to identify and preserve the facial nerve, which runs through the parotid gland. The purpose of this study was to histologically clarify two clinical questions: whether "superficial" and "deep" lobes exist anatomically and what are the structures surrounding facial nerve. Parotid gland tissues were obtained from dissection of donated cadavers. The gland was cut perpendicular to the facial nerve plane at 5 mm intervals, and the pieces were embedded in paraffin, thinly sliced, and stained. The morphology of the nerve was observed at each site, and the relationships between the thickness of the perineural tissue (defined as the tissue between the groups of nerve fasciculi and the glandular parenchyma), nerve diameter, and distance from the proximal end of the nerve were examined. In addition, the dissection layer was examined histologically in isolated parotid tissues. The interlobular connective tissue was spread like a mesh within the parotid gland and subdivided the glandular parenchyma. The facial nerve was located in the interlobular connective tissue, and its course was not restricted to the boundary plane between the superficial and deep lobes. The thickness of the perineural tissue decreased with increasing distance from the proximal end of the nerve. The dissection layer was clarified that located in the perineural tissue. The perineural tissue is thinner in more distal regions, which may make dissection more difficult there. No particular anatomical structure appears to separate the superficial and deep lobes.

Keywords Facial nerve · Parotid gland · Surgery · Nerve dissection · Parotid lobe

Introduction

The facial nerve runs through the parotid gland in a complex manner that reflects embryonic development. In a developing fetus, the facial nerve forms first, and the parotid gland anlage subsequently forms from the oral epithelium. The course of the nerve through the gland becomes complex, because the gland develops posteriorly around the facial nerve (Gasser 1970; Guizetti and Radlanski 1996). This complex structure makes parotid surgery difficult.

In parotid surgery, surgeons focus on identifying and preserving the facial nerve. Although the general surgical approach for benign parotid tumors is to preserve the nerve, it has to be sacrificed in surgery for some malignant parotid tumors. Even if surgeons are able to preserve the nerve in benign parotid surgery, temporary postoperative paralysis occurs in approximately 20% of cases, and patients with such outcomes experience decreased quality of life until recovery from paralysis (Kawata et al. 2021). Avoiding this complication requires an accurate understanding of the relevant anatomy and careful surgical manipulation.

Several anatomical questions remain unanswered regarding the spatial relationship between the facial nerve and the parotid gland. The first is, whether the superficial and deep lobes exist anatomically, as is commonly assumed in clinical practice, and the second is, which structure surrounds the facial nerve.

The terms "superficial" and "deep" are commonly used in parotid gland surgery. For example, a common type of surgery is "superficial parotidectomy." For practical reasons,

✉ Tsuyoshi Jinnin
tsuyoshi.jinnin@ompu.ac.jp

[1] Department of Otorhinolaryngology-Head and Neck Surgery, Osaka Medical Pharmaceutical University, 2-7 Daigaku-machi, Takatsuki, Osaka 569-8686, Japan

[2] Department of Anatomy and Cell Biology, Osaka Medical and Pharmaceutical University, Takatsuki, Osaka, Japan

[3] Department of Pathology, Osaka Medical and Pharmaceutical University, Takatsuki, Osaka, Japan

the "superficial lobe" is defined as the portion of the parotid gland over the facial nerve, and the "deep lobe" is defined as the portion below the nerve. Consequently, many clinicians assume that superficial and deep lobes exist anatomically; however, this is a topic of much discussion. For example, McWhorter (1917) described the parotid gland as consisting of a superficial and a deep lobe, with the facial nerve running between the lobes. In contrast, McKenzie (1948) reported that the parotid gland does not have a simple two-lobe structure but rather is intertwined with the facial nerve in a complex fashion (Dobrosielski-Vergona 1993). Although both of these descriptions were based on gross anatomy, it is unclear why these findings were different, even though the authors used similar methods.

The second question is relevant for nerve dissection in parotid gland surgery, because the difficulty of the operation depends on the course of the facial nerve through the gland. Although the main trunk and proximal (posterior) part of the nerve can be dissected relatively easily from the surrounding tissue, dissection becomes increasingly difficult in more distal (anterior) regions. The perineural structure is assumed to differ over the course of the nerve, but a detailed explanation is lacking.

To obtain useful information for parotid surgery, this study examined the histological structure of the tissue surrounding the facial nerve within the parotid gland and the relationship between the nerve and the tissue.

Materials and methods

Consent for cadaver use

The cadavers used in this study were donated to Osaka Medical and Pharmaceutical University for anatomical education, research, and clinical skills training. Written informed consent was obtained from donors before donation and from their families before and at donation. The research protocol was developed in strict accordance with the Guidelines for Cadaver Dissection in Education and Research of Clinical Medicine from the Japan Surgical Society and the Japanese Association of Anatomists. The study was approved by the ethics committee of Osaka Medical and Pharmaceutical University (Approval No. 2506).

Cadaver parotid tissue

To examine the tissue surrounding the facial nerve, we used ten parotid glands (six from the right side and four from the left) obtained from nine cadavers. Eight cadavers were male, and one was female; the median age of the donors at death was 76 years (range, 42–104 years). Total parotidectomy was performed on each cadaver, i.e., the right or left parotid gland was harvested en bloc, including the facial nerve. The facial nerve was cut at the stylomastoid foramen, and that end was defined as the proximal end of the nerve. Six parotid glands were used to study the facial nerve structure, and four were used to identify the dissecting layer of the facial nerve.

Facial nerve in the parotid gland

Parotid glands ($n=6$) were cut perpendicular to the main trunk of the facial nerve at 5 mm intervals from the proximal end (Fig. 1). Each sliced piece was embedded in paraffin, cut into 5 μm sections, and stained with Masson's trichrome stain. To distinguish the facial nerve branches from the branches of other nerves, such as the glossopharyngeal nerve, we compared each tissue section with those from the adjacent 5 mm slices and predicted the branching patterns of the facial nerve. In addition, we examined the facial nerve branches and their surrounding structure. Some sections were subjected to silver impregnation to observe reticular and collagen fibers in the peripheral tissue.

Relationships between the thickness of the perineural tissue, nerve diameter, and distance from the proximal end of the nerve

Figure 2 shows the histological structure of the facial nerve. The nerve was seen as either a single fasciculus or a group of fasciculi; each fasciculus was covered by the perineurium. The single fasciculus/group of fasciculi was each surrounded by perineural connective tissue. The perineural tissue was defined as the connective tissue between the single fasciculus/group of fasciculi and the glandular parenchyma and included the epineurium. Thus, the nerve was defined as consisting of a single fasciculus/group of fasciculi together with the perineural tissue. We manually defined the outline of the nerve, which included a fasciculus or a group of fasciculi and the perineural tissue. The outline was almost oval, and the intersection of the major and minor axes was manually defined as the center point. We drew a line through the center point to measure the diameter, which could be approximated as a minor axis, and defined the length of the line as the nerve diameter.

The thickness of the perineural tissue was measured at both ends of the diameter (T1 and T2 in Fig. 2), and the mean value was used for further analysis. To assess the relationships between the thickness of the perineural tissue, nerve diameter, and distance from the proximal end of the nerve, we used four sections at 5, 15, 25, and 35 mm from the proximal end of the nerve. We examined three relationships, i.e., those between (1) the thickness of the perineural tissue and the nerve diameter, (2) the thickness of the perineural tissue and the distance from the proximal end of the

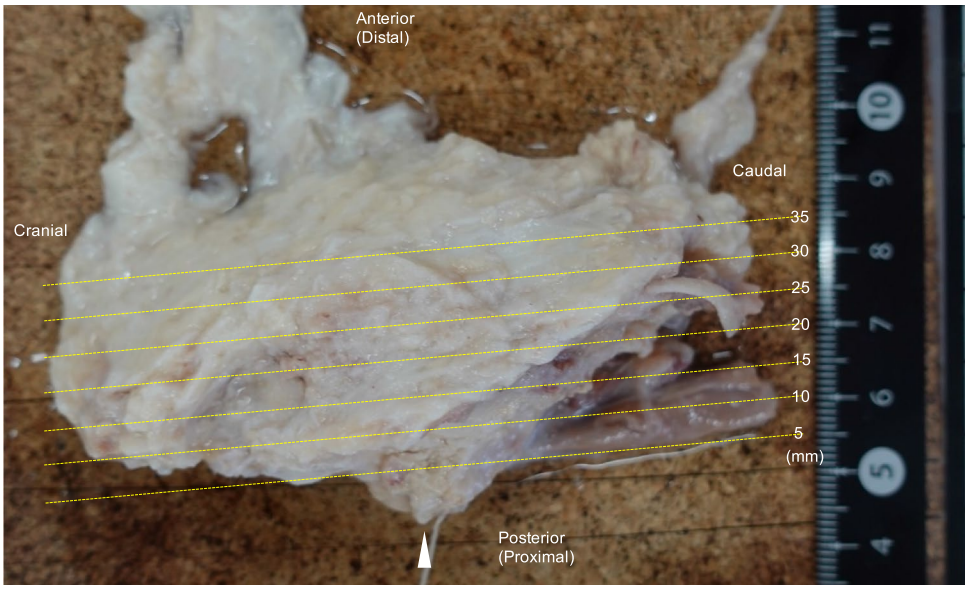

Fig. 1 Parotid gland sections for the microscopic study of facial nerve. Parotid glands were harvested en bloc and cut into slices at 5 mm intervals from the proximal (posterior) end to the distal (anterior) end (at 5 to 35 mm). The proximal end of the facial nerve was located at the proximal side of the gland (arrowhead)

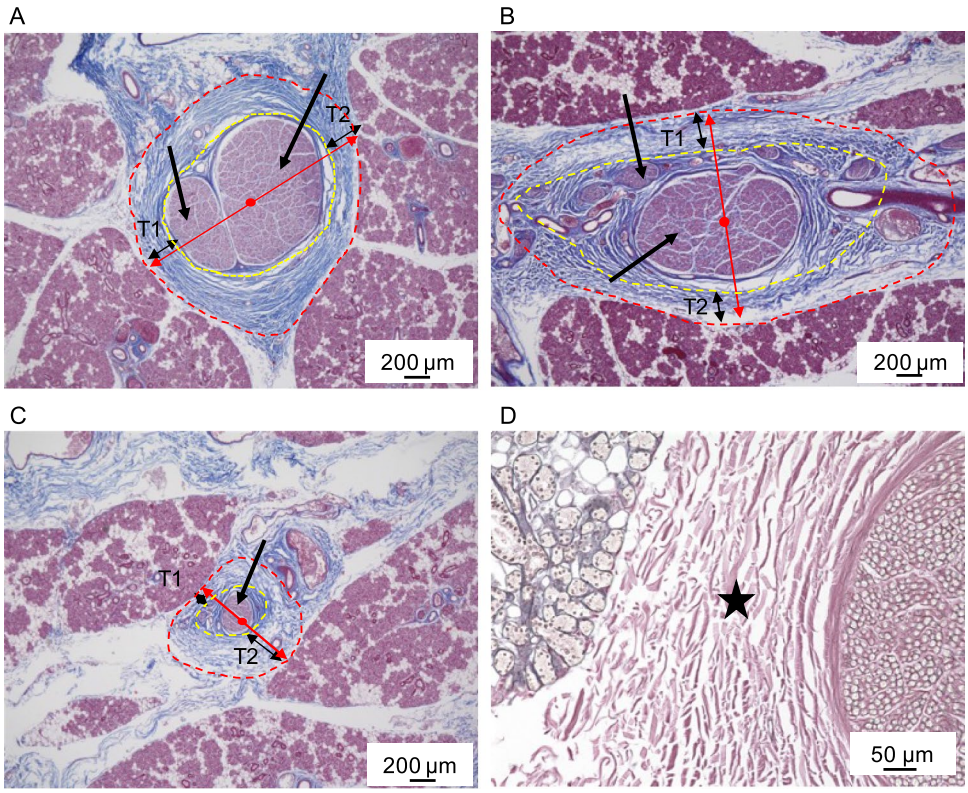

Fig. 2 Nerve tissues in the parotid gland stained with Masson's trichrome or silver impregnation stain. **A**, **B**, and **C** Images of facial nerves in a harvested parotid gland sliced at 5 mm (**A**), 15 mm (**B**), and 25 mm (**C**) from the proximal end and stained with Masson's trichrome stain. The black arrows show nerve fasciculi. The yellow and red circles (drawn with dashed lines) show the group of fasciculi and the perineural tissue, respectively. The red dot and associated arrows show the center and diameters of the nerve, respectively. Lines T1 and T2 show the thickness of the perineural tissue. (**D**) A magnified image of the connective tissue around a facial nerve stained with silver impregnation (the section was adjacent to that shown in panel **A**). Collagen fibers are seen uniformly distributed throughout the perineural tissue (**D**, star)

nerve, and (3) the nerve diameter and the distance from the proximal end of the nerve.

The data were analyzed by multivariate analysis of variance (MANOVA) and Tukey–Kramer post hoc tests. Furthermore, a linear mixed model analysis was performed to clarify whether the nerve diameter is associated with the thickness of the perineural tissue.

Structure of the dissection layer

In the other glands ($n = 4$), the layer in which the nerve was surgically dissected from the surrounding tissue was examined histologically. A thin probe with a tip as thin as the tip of the scissors used for parotid grand surgery was inserted from the proximal end of the nerve, approximately

20 mm along the upper surface of the nerve. The probe was removed, and dental agar impression (CORNET; Clark Corporation, Saitama, Japan) was injected into the resulting gap at 60 ℃ using a dental syringe (Agar Syringe, Youdent Corporation, Chiba, Japan). Once the agar had solidified, the parotid gland was cut perpendicular to the nerve at a site 10 mm from the proximal end of the nerve. After confirming the presence of the nerve stump at the cut surface of the remaining parotid tissue, a thin probe was inserted approximately 20 mm along the upper surface of the nerve in a similar fashion. The probe was removed, and agar was infused as described above. The parotid gland was then cut perpendicular to the nerve at a site 30 mm from the proximal end of the nerve. Sections prepared from each cross-section were embedded in paraffin, sliced, and stained with Masson's trichrome stain.

Results

Structure of the facial nerve in the parotid gland

Images of the stained sections at 5, 15, and 25 mm from the proximal end of the parotid gland are presented in Fig. 3; these sections correspond to the lines at 5, 15, and 25 mm, respectively, in Fig. 1. In relation to the course of the facial nerve, these three sections corresponded to the main trunk, the site immediately after the bifurcation (superior temporofacial branch and inferior cervicofacial branch), and the peripheral branch, respectively.

The interlobular connective tissue (arrows in Fig. 3) was spread like a mesh within the parotid gland and varied in thickness. It appeared to not only separate the superficial and deep lobes but also to subdivide the glandular parenchyma lobules throughout the gland. The facial nerve (arrowheads in Fig. 3) was found within the interlobular connective tissue. The nerve was seen as a single main trunk in proximal sections (Fig. 3A), and the number of nerves increased in subsequent sections as the facial nerve branched toward the distal region (Fig. 3C, D). Both the nerve and the perineural tissue became thinner as they became more distal.

When observed at a high magnification (Fig. 2A–C), connective tissue rich in collagen fibers, defined as the perineural tissue, was seen between the nerve fasciculus or group of fasciculi and the glandular parenchyma. The perineural tissue connected almost seamlessly to the interlobular connective tissue. We focused on these connective tissues, because we presumed that they would be dissected during surgery. They were formed by many layers of collagen fibers and appeared to be loose and easy to detach.

To observe the fibers of the perineural tissue in more detail, we stained the section with silver impregnation (Fig. 2D). Although reticular fibers were stained black

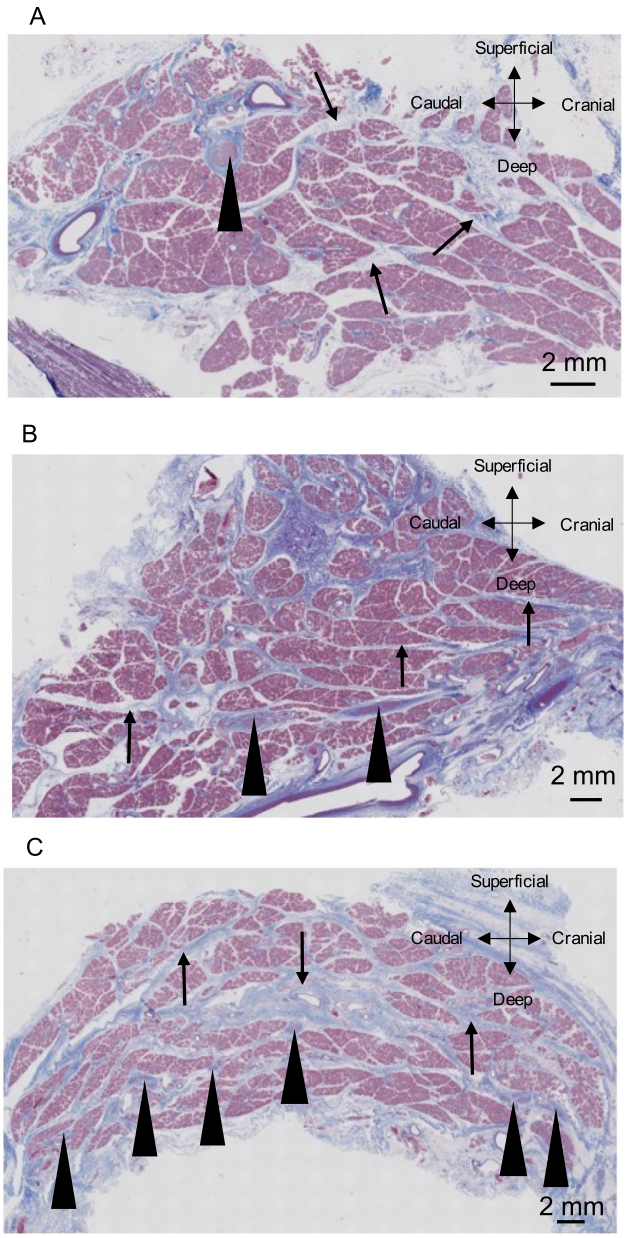

Fig. 3 Distribution of the facial nerve branches in the parotid gland. Images of entire tissue sections of a parotid gland (Masson's trichrome stain) sliced at 5 mm (**A**), 15 mm (**B**), and 25 mm (**C**) from the proximal end. The arrowheads show nerve branches. The black arrows indicate interlobular connective tissues, which are spread like a mesh inside the parotid gland and subdivide the glandular parenchyma

around the acinus of the glands, the perineural tissue contained only collagen fibers (indicated as a black star in Fig. 2D) and no layers of reticular fibers. The perineural tissue contained uniform collagen fibers and no specific gaps or layers that could indicate a layer for dissecting the nerve. These observations suggested that nerve dissection

could be performed in every layer of the collagen fibers in the perineural tissue.

Relationships between the thickness of the perineural tissue, nerve diameter, and distance from the proximal end of the nerve

We focused on the perineural tissue as the space for nerve dissection and investigated whether the nerve diameter and the distance from the proximal end of the nerve are related to the thickness of the perineural tissue. To do so, we analyzed the parotid gland sections at 5, 15, 25, and 35 mm from the proximal end of the nerve (Fig. 1). The nerve diameter and thickness of the perineural tissue were defined as described in the Materials and methods (Sect. "Relationships between the thickness of the perineural tissue, nerve diameter, and distance from the proximal end of the nerve") and Fig. 2.

A correlation analysis revealed that all three factors (i.e., the thickness of the perineural tissue, the nerve diameter, and the distance from the proximal end of the nerve) were significantly correlated with each other (each $p < 0.01$) (Table 1). Each measured value is shown in the scatter plot in Fig. 4.

MANOVA showed a significant difference between the groups sorted by distance from the proximal end of the nerve (Pillai's trace = 0.474, $p < 0.001$); the result of the Tukey–Kramer post hoc test is shown in Fig. 5. The nerve diameter tended to be smaller in the distal region, and the post hoc test showed a significant difference between each group of two measurements except for the two most distal groups (25 and 35 mm; Fig. 5A). The thickness of the perineural tissue also showed a similar tendency, with a significant difference between each group of two measurements except among the three most distal groups (15, 25, and 35 mm) in the post hoc test (Fig. 5B). The linear mixed model analysis showed that nerve diameter was significantly related to the thickness of the perineural tissue (correlation coefficient, 0.17; 95% CI, 0.13 to 0.21; $p < 0.001$) (Table 2).

Layer in which surgical nerve dissection is performed

To confirm the histological area for nerve dissection, we injected agar gel into the space created by dissecting the nerve. Figures 6A, B shows the site of the facial nerve dissection (with Masson's trichrome stain) at 10 and 30 mm from the proximal end of the harvested parotid gland; the two parts of the figure show the main trunk and the distal portion of the facial nerve, respectively. The agar injected into the dissection site was seen in the perineural tissue, where a space had been made by partial destruction of the tissue. Around the agar, connective tissue remained on both the neural and the parenchymal side.

Table 1 Correlations between thickness of the perineural tissue, distance from the proximal edge of the nerve, and nerve diameter

Variables	Coefficient	Correlations 95% CI	*P* value
Thickness of perineural tissue; distance from the proximal edge of the nerve	−0.45	−0.29 to −0.59	< 0.01
Thickness of perineural tissue; nerve diameter	0.79	0.71 to 0.85	< 0.01
Nerve diameter; distance from the proximal edge of the nerve	−0.64	−0.51 to −0.74	< 0.01

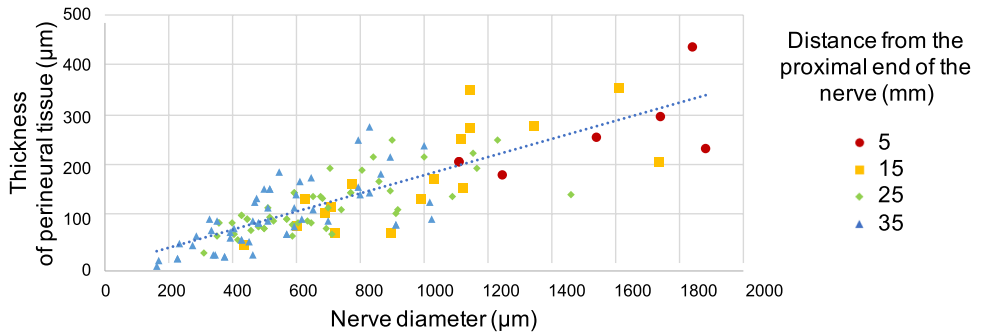

Fig. 4 Relationship between nerve diameter, thickness of perineural tissue, and distance from the proximal end of the nerve. The plot indicates the measurements obtained in slices of parotid gland tissue. The red circles, yellow squares, green rhombuses, and blue triangles show the value from the slice at 5, 15, 25, and 35 mm from the proximal end, respectively. The broken line is the trendline, and the r-square value is 0.6364

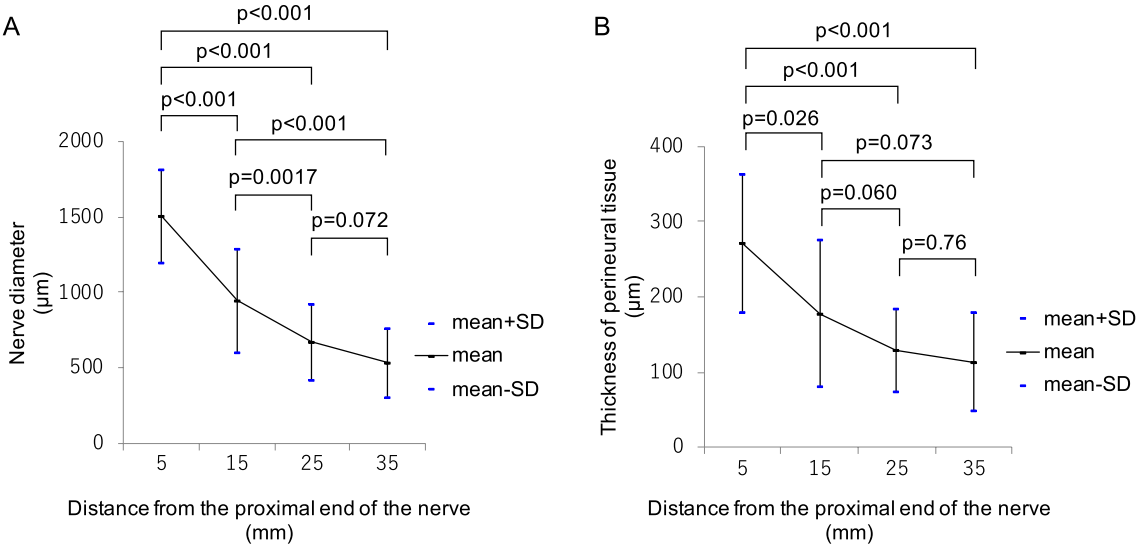

Fig. 5 Nerve diameters and thickness of perineural tissues according to the distance from the proximal end of the nerve. Nerve diameters (**A**) and thickness of perineural tissues (**B**) according to the distance from the proximal end of the nerve were analyzed by Tukey–Kramer post hoc tests. The p values from the post hoc test are shown at the top of each graph. Error bars indicate standard deviations

Table 2 Partial regression coefficients of the thickness of the perineural tissue, calculated by linear mixed model analysis

	Partial regression coefficients			Significance testing		
	Coefficient	SE	95% CI	*t* value	Degrees of freedom	*P* value
Nerve diameter	0.17	0.012	0.13 to 0.21	14.41	3	< 0.001
Constant term	138.35	2.97	128.90 to 147.81	46.56	3	< 0.001

The estimated variance of the residual error (level 1) was 2259.4, and that of the constant term (level 2) was −32.4

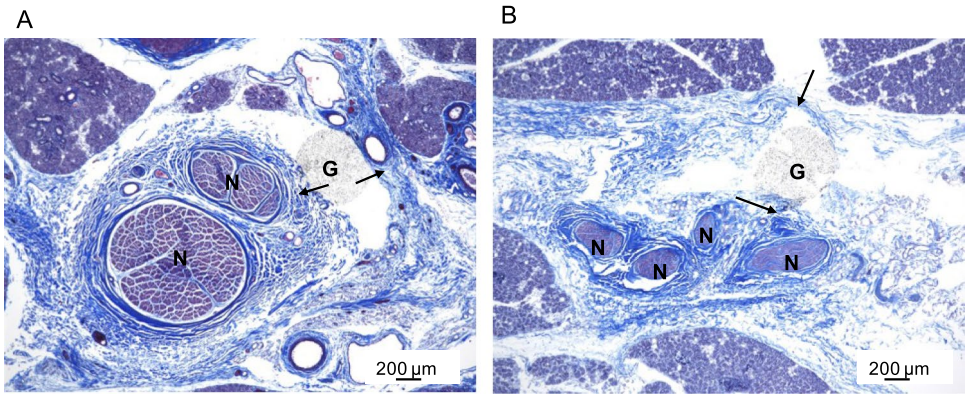

Fig. 6 Site of facial nerve dissection. **A** and **B** Sections of harvested parotid grand at 10 and 30 mm from the proximal end of the nerve with Masson's trichrome stain, respectively. The main trunk of the facial nerve (**A**) and the distal portion (**B**) of the nerve are shown. Agar injected into the dissection site was present in perineural tissue (**A** and **B**, arrows). *N* facial nerve, *G* agar

Discussion

Clinically, the parotid gland is divided into a superficial lobe, which is above the facial nerve, and a deep lobe, which is below it. The classification of tumors according to location in the superficial or deep lobe is critical, because the level of difficulty in successfully preserving the facial nerve during surgery depends on tumor location. However, the question of whether the parotid gland is composed of the two lobes anatomically has been a topic of discussion, and many conflicting reports have been published. Gasser observed the developmental process of the parotid gland in human embryos and fetuses (Gasser 1970), and the

findings supported those of McKenzie, i.e., that there is no clear superficial and deep lobe. Nevertheless, it remains unknown why different conclusions were drawn in studies that used almost the same method of gross autopsies.

In the present study, we showed that the facial nerve courses through the interlobular connective tissue, which spreads across the entire area of the parotid gland. The interlobular connective tissue is looser than the glandular parenchyma, so the former seemed suitable as a feature for separating the parotid gland artificially. However, the connective tissue of the separating plane did not represent a specific partition between the superficial and deep lobes but was found at many locations throughout the parotid gland. In other words, it may be possible to divide the parotid gland into two arbitrary lobes by dissecting along the interlobular connective tissue, but this surface of separation is just an artificial surface. Therefore, we concluded that the superficial and deep lobes do not exist anatomically in the parotid gland.

The tissues of the neck region include fascia, which has a complex structure, and the basic technique used in head and neck surgery involves dissection along the fascial layer. The arrangement of the fascia in the neck is widely accepted (Grodinsky and Holyoke 1938; Guidera et al. 2014). However, there are no recognized structures in the parotid gland such as the fascial layers for surgery. Nevertheless, surgeons have had the impression that there is a kind of "layer" between the nerve and the parotid parenchyma. Although this structure was suggested to be potentially helpful during surgery (Conley 1978), it has not been described in detail. This study identified the nerve dissection layer in the parotid gland as "perineural tissue" (Fig. 6). This tissue is contiguous with the interlobular connective tissue, as described above, and extends like a meshwork throughout the parotid gland, separating the many lobules. Dissecting along the perineural and interlobular connective tissue facilitates surgery. This finding is highly relevant, because it demonstrates that dissecting the nerve along a kind of "layer" is anatomically feasible during parotid surgery, even if the layer is not as clear as the fascia in other neck surgeries. We suggest that it is helpful to keep the layers of connective tissue in mind during surgery and that it is undesirable to operate only by roughly cutting the gland parenchyma. In addition, this suggestion is supported by two kinds of experiences from actual parotid gland surgeries.

First, the surgical procedure for nerve dissection sometimes does not progress when the point of dissection is deviated from the nerve for fear of damaging the nerve. In addition, if a lot of excess connective tissue is left on the surface or the dissection wrongly detach an adjacent layer across the lobule, it is difficult to see the course of the nerve. The surgeon should insert the dissection instrument close to the nerve, i.e., in the perineural tissue. Although it is difficult to recognize the dissection layer macroscopically during surgery, the layer is clearly demonstrated in the microscopic images of our study (Fig. 6). These images can be helpful in understanding the logic of the operation procedure.

Second, during surgery, surgeons often experience more difficulty dissecting the more peripheral nerve branches, whereas they find it relatively easy to dissect the nerve near the main trunk (i.e., the proximal side).

The perineural tissue, i.e., the dissection layer identified in this study, was proportional to the nerve diameter (Fig. 4) and was thinner in the distal region, because the nerve diameter was smaller. The thinner the perineural tissue, the narrower the space for inserting a surgical instrument, making dissection more difficult. We hypothesized that this finding explains why it is more difficult to dissect nerve branches in more distal regions, where the perineural tissue is thinner. In actual surgery, dissection near the main trunk is often achieved without exposing the glandular parenchyma on the side of the separated parenchyma, perhaps because the perineural tissue is thicker near the main trunk, so some of the perineural tissue remains next to the glandular parenchyma where the nerve is being dissected. The results of the present study support this speculation.

This study showed that both the nerve and the perineural tissue tended to be thinner in the distal region (Table 1, Fig. 5A, B); however, between the most distal sections, the post hoc test showed no significant differences (Fig. 5A, B). The perineural tissue was significantly thinner in distal sections (15 mm or more) than in the most proximal Sect. (5 mm). Thus, in the more distal regions, dissection should be performed more carefully and closer to the nerve. In other words, our findings indicate that nerve dissection is relatively easy only in the main trunk, which is consistent with clinical experience. On the other hand, the linear mixed model analysis showed that the thickness of the perineural tissue was related to the nerve diameter, except for the effect of the distance from the proximal end of the nerve (Table 2). Therefore, in surgery, even if the nerve branch being dissected is less than 15 mm from the proximal end of the nerve, dissection should be performed carefully in case the nerve is small.

Regarding the perineural tissue, we defined it as including the epineurium and interlobular connective tissue and found that collagen fibers were distributed evenly within it. The perineural tissue can be considered to be uniform connective tissue, and it is unlikely that dissection through the perineural tissue always occurs at a particular location. Of relevance in this context is that the epineurium of the median, ulnar, and radial nerves through the elbow or wrist joint is regarded as loose connective tissue that ensures mobility of the nerves as the joints move (Sunderland and Bradley 1949; Sunderland 1965); thus, it is reasonable to suggest that such loose

connective tissue in the neck is a suitable layer for dissection also in parotid gland surgery.

The limitations of this study include the use of formalin-embedded tissue and the possibility of postmortem degeneration of the parotid parenchyma or neural tissue. Furthermore, it was not possible to perform dissection exactly as it is performed in actual surgery.

Conclusions

This study shows that interlobular connective tissue of varying thicknesses divides the glandular parenchyma of the parotid gland. Although the interlobular connective tissue is spread over the gland, it does not form a clear partition that divides the gland into superficial and deep lobes. The facial nerve courses through the interlobular connective tissue, and the thickness of the perineural tissue decreases with increasing distance from the proximal end of the nerve. Our findings suggest that in parotid gland surgery, dissection of the nerve from the glandular parenchyma occurs in the perineural tissue. The more distal the nerve, the thinner the perineural tissue and, presumably, the more difficult it is to perform the dissection. This conclusion reflects the general clinical principle that the difficulty of separating the facial nerve from glandular parenchyma increases as the nerve becomes more distal.

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Author contributions All authors contributed to the study conception and design. Material preparation and data collection and analysis were performed by TJ, SF, and AH. The first draft of the manuscript was written by TJ, and all authors commented on all versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The data that support the findings of this study are available from the corresponding author, Tsuyoshi Jinnin, upon reasonable request.

Declarations

Conflict of interest The authors have no conflicts of interest to disclose.

Ethical approval The research protocol was developed in strict accordance with the Guidelines for Cadaver Dissection in Education and Research of Clinical Medicine. This study was approved by the ethics committee of Osaka Medical and Pharmaceutical University (Approval No. 2506).

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