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Research Article – Basic and Applied Anatomy

Study of human adult parotid duct in the area of penetration through buccinator muscle and their functional relationship as a sphincter

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Summary

The adult human parotid duct is roughly 6-8 cm long. From the parotid gland, parotid duct traverses through masseter muscle, penetrates through buccinator muscle, and opens into the oral cavity. This unique form of the parotid duct is likely correlated with the function of the duct, directly affected by the movement of the buccinator muscle during mastication and swallowing. Histological structure of the duct is known to be different in each region, and details of smooth muscle present in the parotid duct are mostly unclear. In this study, we conducted SEM and histological observations of the area where the parotid duct penetrates the buccinator muscle, and an observation of smooth muscle to investigate its existence using α-smooth muscle antibody. We confirmed the presence of an abundance of skeletal muscle bundles likely originating from the buccinator muscle under the epithelium of the parotid duct wall in the region where it penetrates the buccinator muscle. We also observed that some of the muscle fibers were completely attached to the epithelium. We observed a lack of smooth muscle in this region of the duct wall. From these results, we suggest that the area of the duct penetrating buccinator muscle plays a role in regulating the salivary passage through the contraction of the surrounding buccinator muscle fibers.

Key words

Parotid duct; orifice; buccinator muscle; sphincter; human adult; α-smooth muscle actin (SMA).

Introduction

Many studies have been reported regarding the human parotid gland, but only a few reports on the parotid duct; the structure and function of the region where the main duct penetrates buccinator muscle are mostly unknown. Kang, et al (2006) reported that buccinator muscle fibers at the terminal portion of the human parotid gland play a functional role in saliva secretion, suggesting that they act as a dilator of the duct. Their report focused on observations of the duct's outer surface, leaving unclear the relationship between the duct and buccinator muscle with regards to their functional roles in salivary secretion.

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From observing the area of the parotid duct penetrating buccinator muscle in 4-10 month old fetuses, we reported in 2010 that buccinator muscle fibers invaded the duct wall in the area of penetration in 7 month old fetuses and older. We suggested that these muscle fibers act similar to a sphincter muscle and play a role in regulating salivary flow during secretion. Takeda (1987) also made histological observations of the duct's structure, reporting that the duct wall is composed of mucosa, smooth muscular layer and adventitia, suggesting that the duct contributes to control salivary secretion due to the structural similarity of its wall to other excretory passages with known peristaltic activity. Overall, studies showing details of the duct's smooth muscle are scarce.

In this study, we conducted scanning electron microscopic (SEM) and histological observations of the parotid duct inner wall in the area where it penetrates buccinator muscle to examine the reciprocal structural relationship, and an immunohistological staining of smooth muscle to investigate its existence using α-smooth muscle actin (SMA).

Material and Methods

Fifteen parotid ducts were obtained from human adults ranging from age 55 to 86 from the Japanese cadaver collection at the Department of Anatomy, Kyorin University School of Medicine, Japan. Twelve specimens were embalmed with 10% formaldehyde fixative. After removing facial skin, the parotid duct from buccinator muscle to its opening into the oral cavity was removed en bloc (Fig. 1). Six specimens were cut open from the entrance of the buccinator muscle towards the opening of the oral

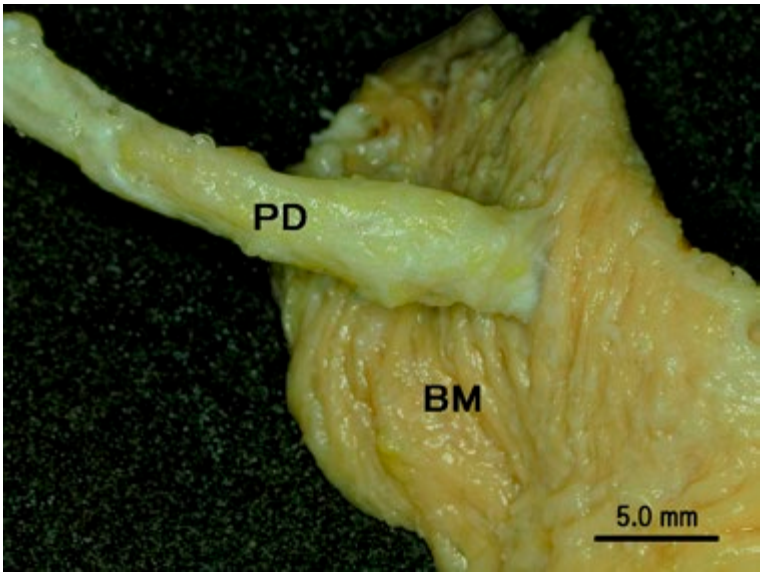


Figure 1 – Parotid duct (PD) of an adult human removed *en bloc* and penetrating buccinator muscle (BM**).**

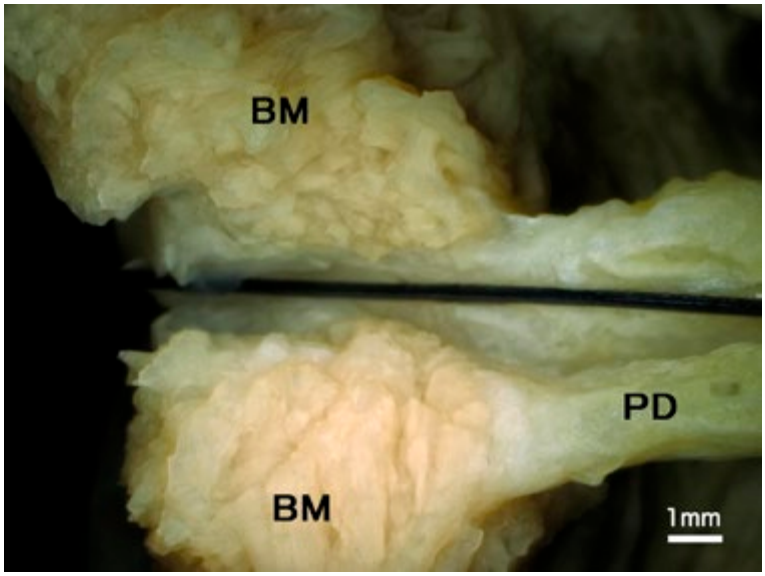


Figure 2–- Internal view of the parotid duct (PD) of an adult dissected from the entrance of the buccinator muscle (BM) to the opening into the oral cavity (to the left).

cavity under a dissection light microscope for SEM (Hitachi S-2250N, Tokyo, Japan; Fig. 2). After staining with tannin-osmium (1%) solution, specimens were dehydrated with alcohol and freeze dried with *t*-butyl alcohol. For histological examination, six specimens were prepared as paraffin sections and stained with hematoxylin and eosin (HE), with several of these slides stained with Van Gieson for observation of elastic fibers. All slides were examined under a light microscope, and photographs were taken with a digital camera (Keyence VHX-9000, Osaka, Japan).

Three cadavers were dissected and the same areas were removed en bloc. These specimens were fixed in 4% paraformaldehyde in 0.01 mol/L phosphate buffered saline, pH 7.2-7.4 (PBS; from Wako Pure Chemical Industries, Osaka, Japan), for over 24 hours. After fixation, the specimens were rinsed in PBS with 30% sucrose for at least 24 hours. Cryostat sections 20 μm thick were cut and thawed onto gelatin coated slides. These sections were stained with a monoclonal antibody against alpha-smooth muscle actin (SMA Molecular Probes, Carlsbad, CA, USA), 1:50, overnight at 4°C, washed in PBS, incubated in the secondary antibody, *i.e.* Cy3-labeled goat anti mouse IgG (Invitrogen, Carlsbad, CA, USA), 1:400, 1 hour at 37°C, washed in PBS again and mounted in antifade reagent (Invitrogen, Carlsbad, CA, USA). They were examined under a fluorescence microscope, and photographs were taken with a digital camera (Keyence VHX-9000, Japan).

Results

SEM observation of the parotid duct wall

Using SEM, we observed the inside of adult parotid duct wall from where it penetrates buccinator muscle and opens into the oral cavity (Fig. 3). In the connective tissue of the duct wall near the opening area, there was an abundance of buccinator muscle fiber bundles. Some of the muscle fibers ran immediately adjacent to the epithelium of the duct wall (Figs. 4, 5). Structurally, there were distinct bends of the duct towards inside the mouth; one where it enters buccinator muscle and the other where it opens into the oral cavity (Figs. 6, 7).

Histological and immunohistochemical examination of the parotid duct wall

Observation of HE stained longitudinal section of the duct confirmed the presence of mature buccinator muscle bundles surrounding the area of the duct where it penetrates buccinator muscle, consistent with results from morphological examination of the same area using SEM (Fig. 8). A part of the muscle fibers surrounding the duct wall was adjacent to the duct epithelium (Figs. 9, 10). Also, an abundance of blood vessels was present within the connective tissue of the duct wall (Fig. 11). Likewise, Van Gieson stain revealed an abundance of elastic fibers running along with connective tissue (Fig. 12). There was no smooth muscle surrounding the duct wall in the region where it penetrates buccinator muscle. From SMA immunohistochemical staining only blood

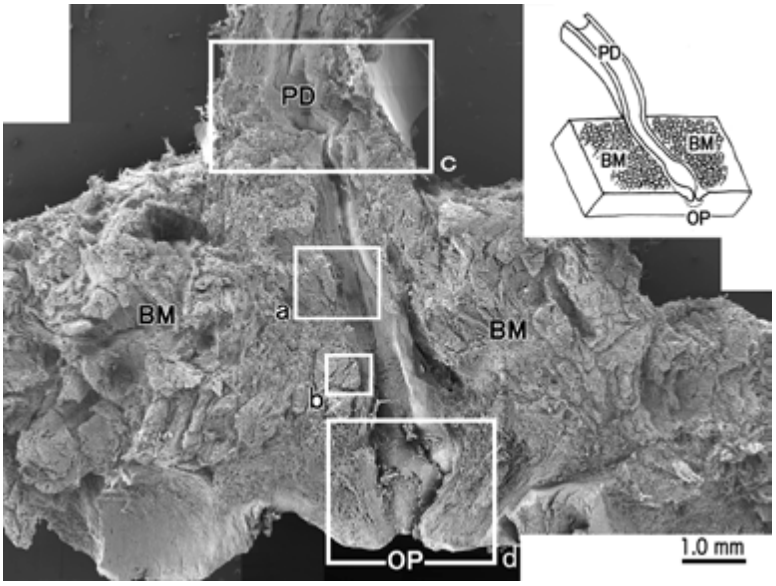


Figure 3 – Internal view of the parotid duct (PD) of an adult dissected from the entrance of the buccinator muscle (BM) to the opening (OP) into the oral cavity and viewed by SEM.

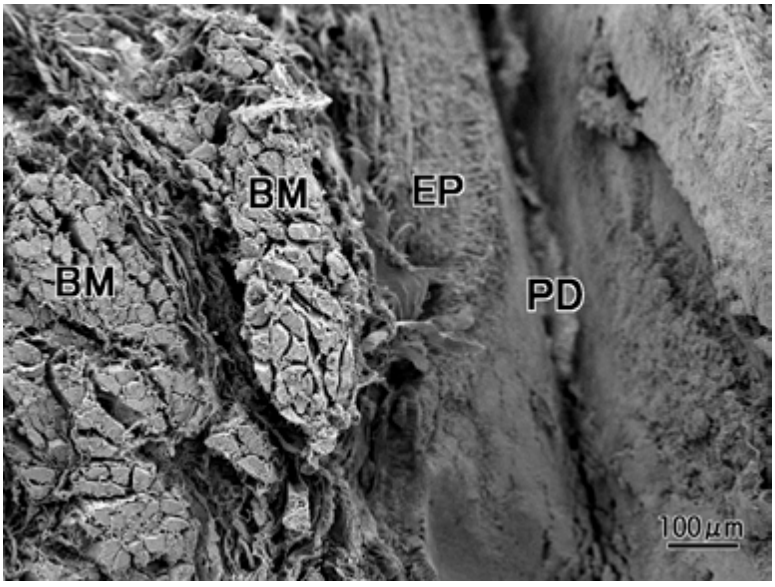


Figure 4 – Magnified view of the boxed area (a) of figure 3, showing buccinator muscle fibers surrounding the duct wall. EP: epithelium; BM: buccinator muscle; PD:parotid duct.

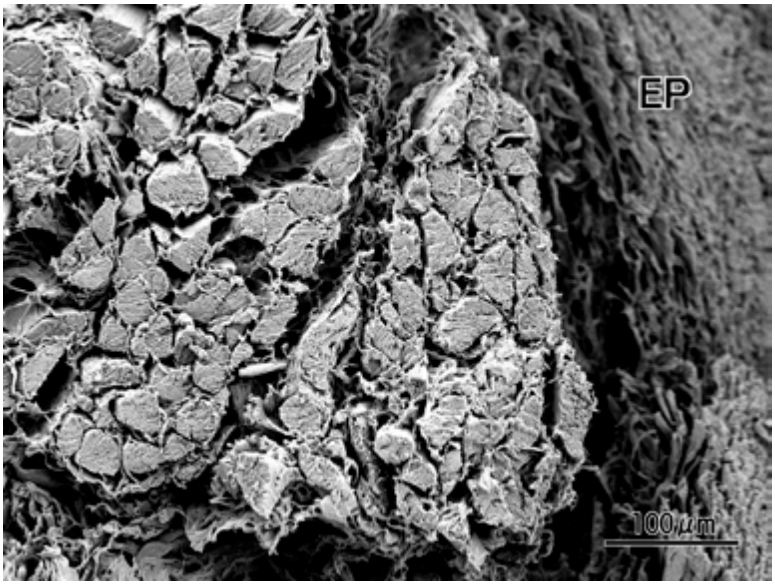


Figure 5 – Magnified view of buccinator muscle fibers in the boxed area (b) of figure 3. EP: epithelium.

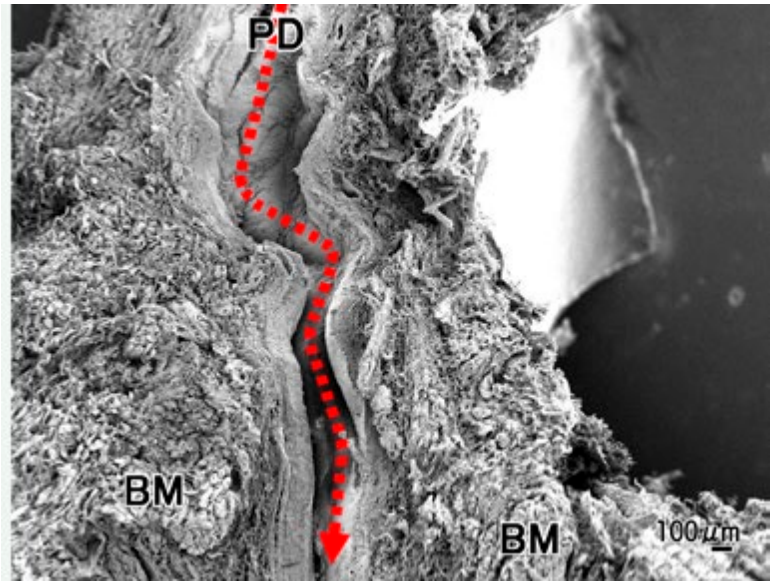


Figure 6 – Magnified view of the parotid duct at the entrance into buccinator muscle in the boxed area (c) of figure 3. EP: epithelium; the red line marks the inside of the duct and the direction of salivary flow.

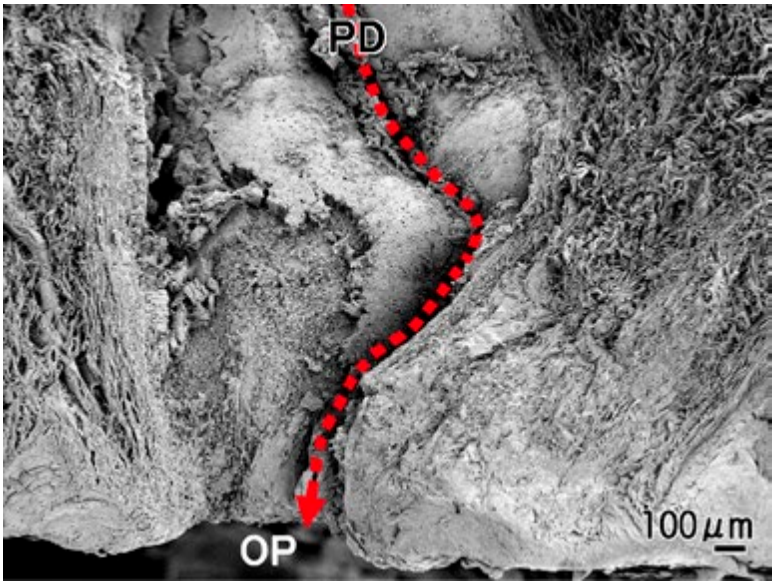


Figure 7 – Magnified view of the parotid duct (PD) opening (OP) shown in the boxed area (d) of figure 3. The red line marks the inside of the duct and the direction of salivary flow.

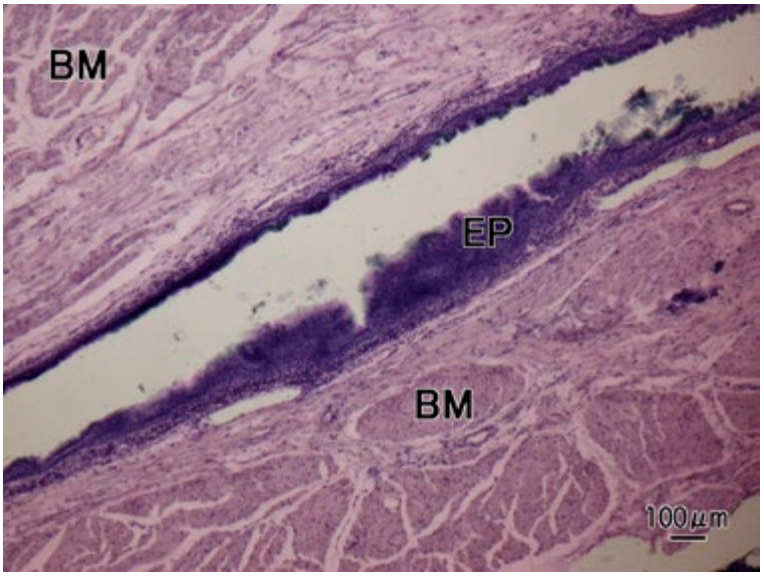


Figure 8 – Longitudinal section of the parotid duct of an adult penetrating buccinator muscle (BM). EP: epithelium. Hematoxylin and eosin staining.

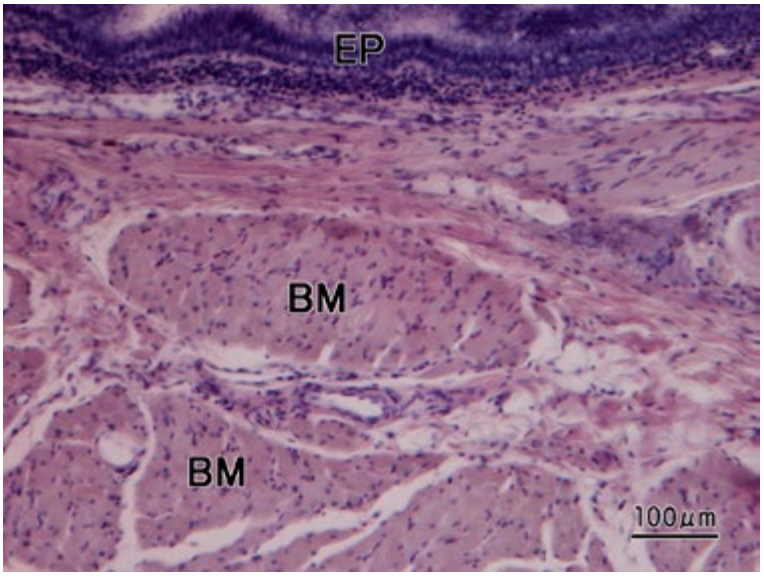


Figure 9 – Magnified view of figure 8 showing buccinator muscle (BM) fibers inside the duct wall. EP: epithelium.

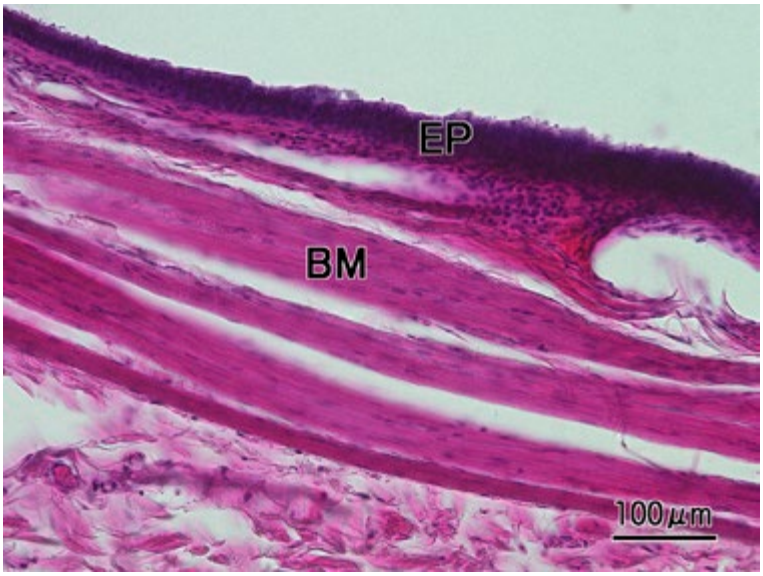


Figure 10 – Highly magnified view of figure 8 shows distinct striated fibers originating from buccinator muscle (BM), in contact with the duct wall. EP: epithelium.

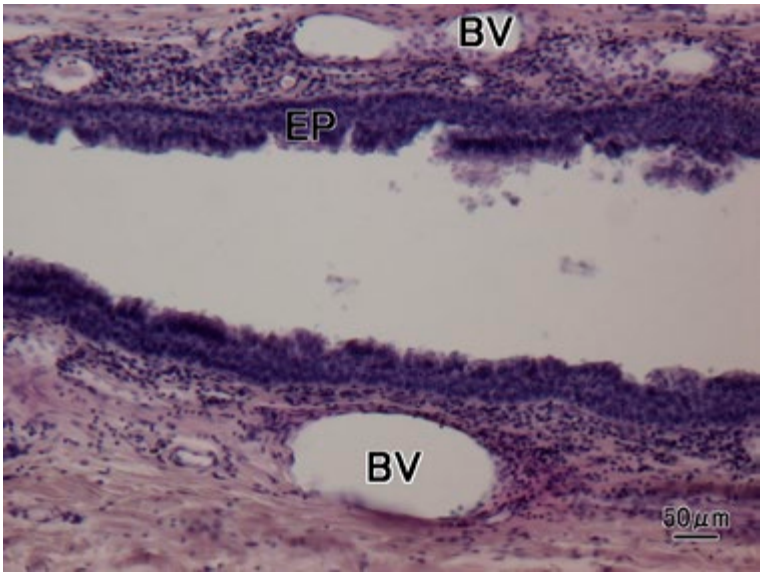


Figure 11 – Longitudinal section of the parotid duct of an adult where it traverses over the masseter, proximal to where it penetrates the buccinator muscle. BV: blood vessels; EP: epithelium.

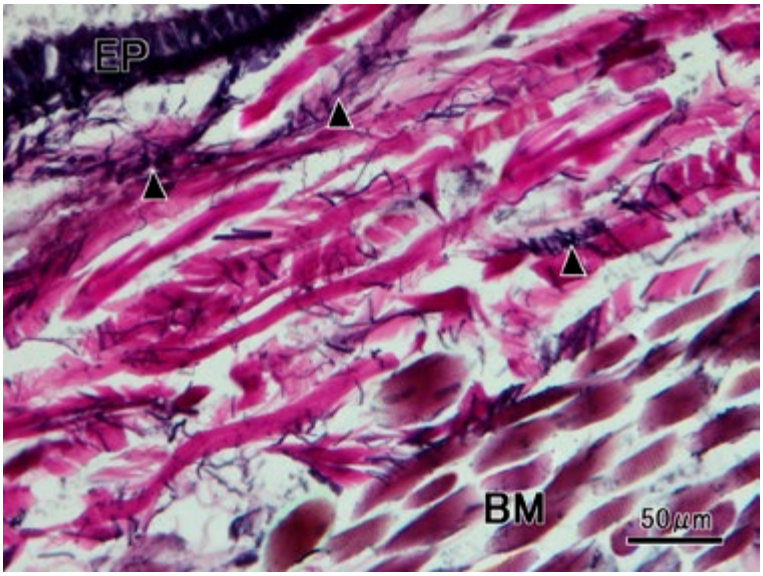


Figure 12 – Magnified longitudinal section of the parotid duct of an adult penetrating buccinator muscle (BM). Arrowhead = elastic fibers; EP-epithelium. Van Gieson's elastica stain.

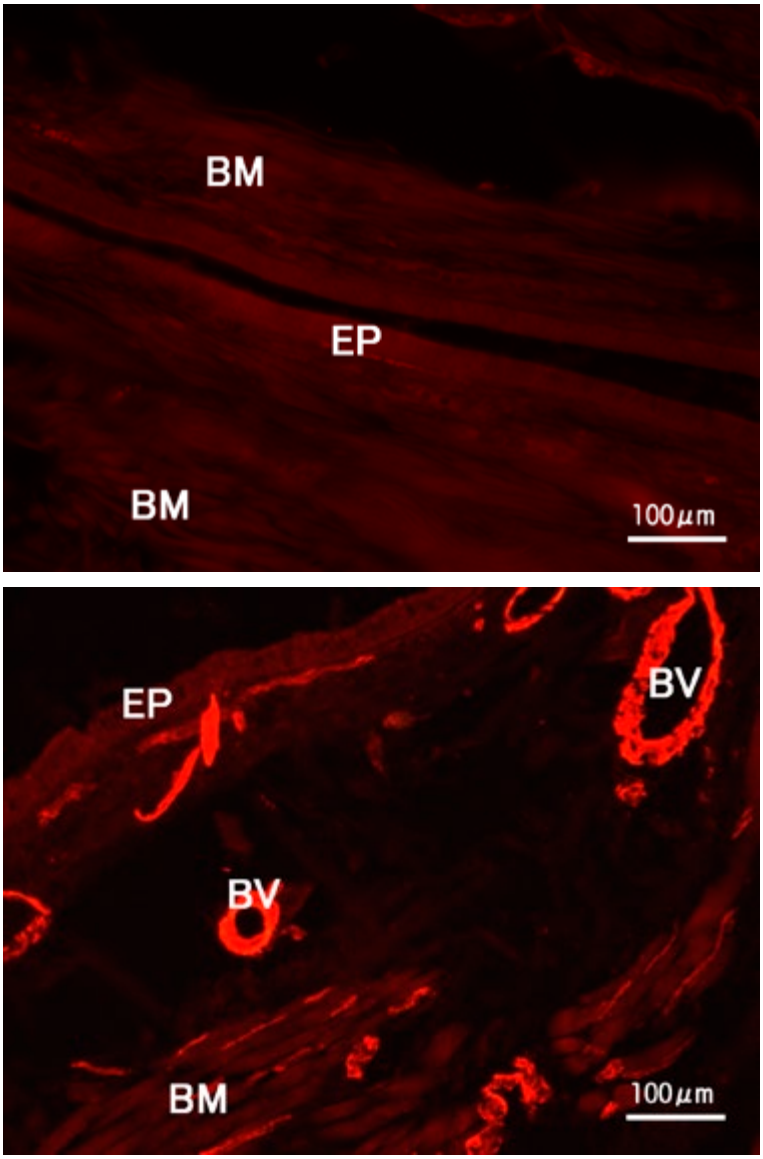
vessels, which were a positive control, showed labeling. There was no presence of smooth muscle inside the inner wall of the parotid duct between the area where it penetrates buccinator muscle and where it traverses masseter muscle (Figs. 13, 14, 15).

Discussion

Parotid gland secretes serous saliva containing enzymes such as amylase, proline-rich proteins, and glycoproteins. Saliva with high viscosity, including mucin- containing saliva secreted from submandibular and sublingual glands, is involved in a number of important functions such as oral cavity protection, buffering, pellicle formation, maintenance of tooth integrity, antimicrobial action, tissue repair, digestion and tasting. The parotid duct serves as the conduit for roughly 23% of all saliva produced at rest, and over 50% of all stimulated saliva which is triggered by degustation, olfaction, and other mechanical stimulation in the oral cavity (Antonio, 2003).

Odajima (1972) conducted a morphological observation of the human adult parotid duct and suggested that the spiral shape of the duct is an indication of the duct function as a sphincter in regulating salivary secretion. Upon leaving the parotid gland, the parotid duct forms a unique structure by traversing over the masseter and penetrating the buccinator immediately prior to opening into the oral cavity. We speculate that the duct's inner structure is similar to the "Sphincter of Oddi" of the major duodenal papilla, another structure with a sphincter muscle.

As the parotid duct opens into the oral cavity, buccinator muscle fibers cross over one another and eventually join the orbicularis oris muscle at its insertion (DiDio,



Figures 13, 14 – Longitudinal section of the parotid duct of an adult penetrating the buccinator muscle (BM). Only blood vessels (BV), which were a positive control, show intensely stained smooth muscle cells, while there was no reaction in the duct wall. EP: epithelium. Alpha-smooth muscle actin immunostaining.

1968). The buccinator muscle, located in the lateral wall of the oral cavity, is positioned in the deepest layer of all facial muscles between the maxilla and the mandible in the cheek. It is a thin quadrilateral muscle, whose fibers converge towards the modiolus near the angle of the mouth where the central fibers intersect each other. There

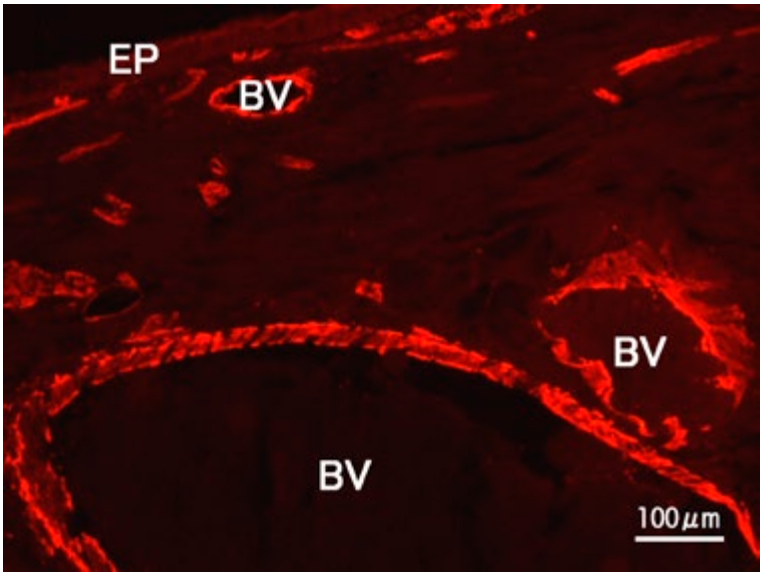


Figure 15 – Longitudinal section of the parotid duct of an adult where it traverses over the masseter, proximal to where it penetrates the buccinator muscle. Only blood vessels (BV), which were a positive control, show intensely stained smooth muscle cells, while there was no reaction in the duct wall. EP: epithelium. Alpha-smooth muscle actin immunostaining.

is good evidence that a substantial number of buccinator muscle fibers is diverted internally to attach to the submucosa as it courses through the cheek (Standring, 2005). Shimada et al. (1989) suggested that the buccinator muscle converges with the zygomaticus major and minor and orbicularis oris muscles lateral to the angle of the mouth, together functioning as the mouth sphincter muscle. Lang (1995) reported that the buccinator, along with masseter and pterygoid muscles, plays a role during mastication. According to Casey (1983), the superior pharyngeal constrictor muscle forms a sphincter muscle with the buccinator and orbicularis oris muscles, altogether performing important functions in the first phase of swallowing. It has been reported that a series of smooth movements from mastication to swallowing seemed to be resulting from a group of muscles, the superior pharyngeal constrictor, buccinator, and orbicularis oris muscles, working together as a single sphincter muscle (Tsumori et al., 2007, Shimada and Gasser,1989). Morphologically, the fact direct penetration of the parotid duct through the buccinator muscle supports our speculation that the duct plays a rather significant functional role during salivary passage.

Parotid duct's histological structure is known to vary based on its location. Takeda (1987) reported that there is a smooth muscle layer in the parotid duct wall; however, his report did not specify the area observed, and his observation definitely did not include the region penetrating buccinator muscle. Our histological examination of the duct where it penetrates buccinators muscle reveals that the duct wall consists of two layers; a thin, dense connective tissue layer adjacent to the epithelium, surrounded by a thick, sparse connective tissue layer, encompassed

by many blood vessels. Additionally, an abundance of elastic fibers in the connective tissue inside the duct wall may indicate strengthening, as well as increasing elasticity and flexibility of the wall against external force caused by oral movement such as mastication.

Furthermore, our previous study reporting that the structure of the parotid duct region penetrating buccinator is fully mature in 7 month old fetuses (Amano, 2010) also supports the notion that salivary secretion is an important function that is established relatively early during human development. Our findings from both SEM and histological examinations revealed numerous muscle bundles from the buccinator surrounding the portion of the duct where it penetrates through the buccinator itself, and some of the muscle fibers completely invading the duct wall. These results are consistent with our proposal that contraction of these buccinator muscle fibers during oral movement such as mastication and swallowing directly causes the compression of the duct, thereby playing a sphincter like function on the parotid duct.

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