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A Study on the Distribution and Morphology of Lingual Papillae by *in vivo* Observation of Lingual Mucosa Using a New Diagnostic Device for Oral Mucoscopy

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Abstract

To investigate the *in vivo* morphological characteristics of the lingual mucosa, 34 healthy subjects (17 males and 17 females) between the ages of 19 and 20 were selected. After obtaining informed consent, macroscopic images of the tongue mucosa and serial images of the lingual mucosa were obtained using an oral mucoscope. The sequential images were montaged and simultaneously magnified images of six regions of the tongue mucosa were acquired. The morphology of the tongue papillae distributed in the mucosa was observed and compared between males and females. With the tongues of the subjects, not only was there a difference in the density of distribution of fungiform papillae between the tongue apex and the central part of the tongue body, but there also seemed to be a clear difference in the shape and size of the filiform papillae. However, both filiform papillae and fungiform papillae showed no clear difference in morphology. We were not able to find any clear differences in morphology, size, or density of distribution between the sexes.

Key Words：Oral mucosa, Lingual papillae, Oral-mucoscopy, *In-vivo* imaging

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Introduction

The lingual mucosa is located at the entrance of the digestive tract and contributes to mastication and food intake, and is formed by numerous lingual papillae, such as filiform, fungiform, foliate and vallate papillae, which are densely distributed on the lingual dorsal surface[1]. The filiform papillae are also known as "mechanical papillae" that contribute to food mastication[2], while the fungiform papillae, foliate papillae, and vallate papillae are also known as “gustatory papillae” that contain dense taste buds for taste perception[3]. Although there have been numerous studies regarding the autopsy-based morphology of the above-mentioned lingual papillae[4-10], there have been few studies regarding the observation of non-invasive intravital morphological features *in viv*o, such as those by Tsuchida et al.[11] and Just et al.[12] This method enables image acquisition of the surface morphology of the examinee's oral mucosa by sliding the sensor surface of the image acquisition device. In addition, it is possible to obtain a wide range of mucosal surface morphology images by reconstructing the images (unlike biomedical image acquisition by dermoscopy, which is often used).

Therefore, the purpose of this study was to investigate the *in vivo* morphological characteristics of the tongue mucosa in clinically healthy male and female subjects.

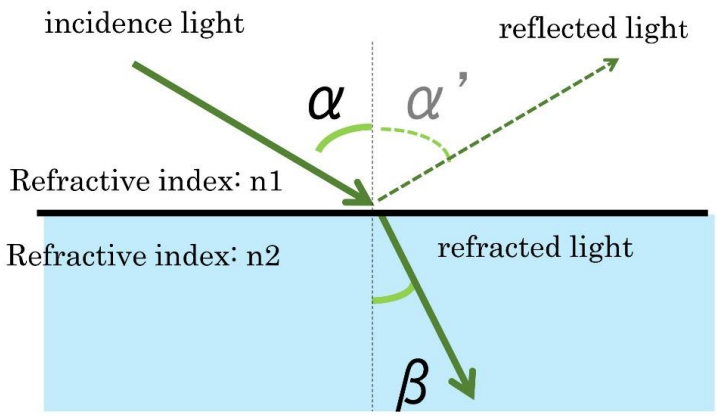
Materials and Methods

For this study, 34 subjects consisting of 17 male and 17 female adolescents aged between 19 and 20 years, were randomly selected at the Faculty of Health and Medical Sciences, Hokuriku University, and a new type of oral mucosa examination device, a contact-type oral mucoscope, was used. Images of tongue mucosa and tongue papillae were collected from the subjects, and the lingual mucosa and lingual papillae examined for their distribution density and morphological differences. Furthermore, by comparing an equal number of males and females (17 each), we examined the presence or absence of differences relating to gender.

Image acquisition and image processing using an oral mucosal speculum followed the method described in the study of Tsuchida et al.[11] For observation of each subjects, macroscopic photographs of the tongue were taken, and images collection of the lingual mucosa was performed using a device with an infection-preventing sleeve.

The outline and features of the device used in this study are the same as those described in detail by Tsuchida et al.[11] To capture the images, the device was plugged into a USB port of a PC and use the PC's software was used to save the images. Fig. 1a shows the Snell-Descartes law, which is the basic principle of the optical path of this device. Since it is too

dark in the oral cavity to collect photographic images with natural light, it was necessary to acquire images while “reflecting” (shining) light onto the mucosal surface. For the image acquisition, we used a small LED to irradiate light onto the surface of the lingual mucosa. The incident light (α) is divided into two light paths: light (α') that is reflected from the liquid surface (consisting mainly of saliva) covering the mucosa and transmitted light that is refracted (Fig. 1a). Because the irradiated multiple light paths are reflected by the liquid surface of saliva covering the mucosa, and interference can sometimes occur (Fig. 1b). Also, the liquid surface of saliva and the like covering the observation surface is not necessarily flat. As a result, light is reflection by the non-flat liquid surface, and consequently scatters in various directions (Fig. 1c). The imaging system developed this time incorporates a method to eliminate, to the greatest extent possible, blurry images caused by these problems, and as shown in the comparison image on the right, it is able to obtain images that are quite clear. As we are currently in the process of applying for a patent for the device used for mucoscopy in this study, we will refrain from describing it here.



Snell's Law: (refractive index of air)×sin α = (refractive index of saliva)×sin ß

Fig. 1a. Reflection due to differences in refraction

Refractive index medium 1 (air): n1,

Refraction index of medium 2 (saliva): n2

Angle of incidence from medium 1 to medium 2: α

Angle of incidence from medium B to medium A: β

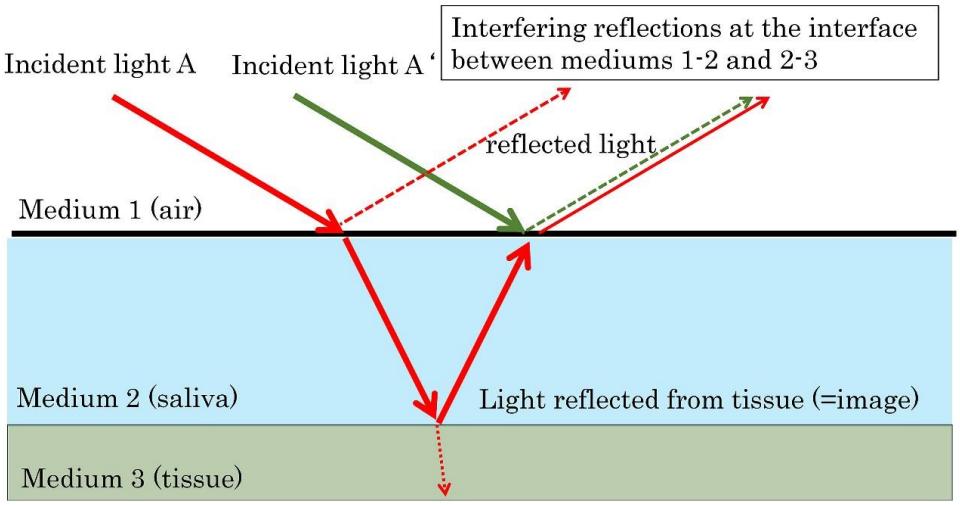


Fig. 1b. Interference due to differences in reflective interfaces
(partially modified from http://hooktail.sub.jp/wave/interfernce1/)

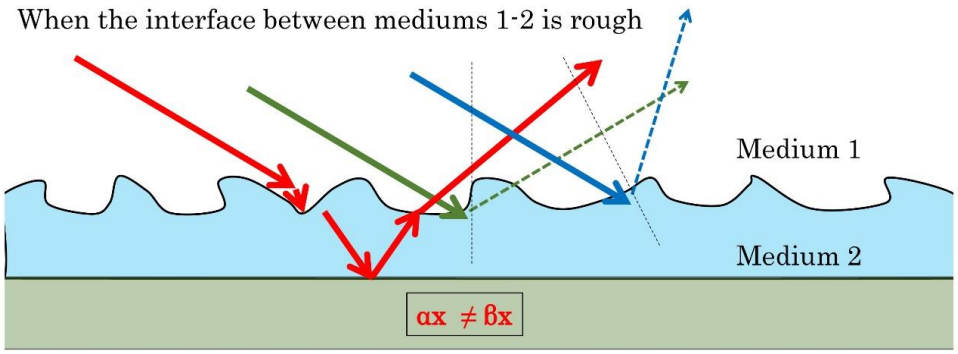


Fig. 1c. Diffuse reflection (properties of the interface)
(refractive index of air)*sin α1=(refractive index of saliva)*sin β1
(refractive index of air)*sin α2=(refractive index of saliva)*sin β2
(refractive index of air)*sin α3=(refractive index of saliva)*sin β3
(refractive index of air)*sin α4=(refractive index of saliva)*sin β4
Apparently due to the displacement of the axis of the "dotted line"

The actual procedure for taking an image of the tongue mucosa was as follows: First, a naked-eye image of the entire tongue was taken, and at that time, a CASMATCH® (DNP, Japan) was used for color matching and as an indicator when inserting the scale (Fig. 2). Next, a red line was drawn across the body of the tongue at 1 cm intervals from the tongue apex to a line 5 cm from the tongue apex to the left and right tongue margins using a HyDent marker Stick (Pascal International, Bellevue, WA, USA). The dorsal surface of the tongue along the midline was then initially scanned with a "contact oral mucoscope" from the tongue root side to the tongue apex side, and a series of static images were taken. Next, the dorsal surface of the tongue on the right side (facing left) was scanned from the root of the tongue to the apex of the tongue and a series of still images were taken. Finally, the dorsal surface of the tongue on the left side was scanned from the root of the tongue to the apex of the tongue, and a series of still images were taken.[11] Each still image was taken in such a way that the peripheral areas overlapped as much as possible, so that when the images were later combined, they could be connected so that no two areas were far apart. After that, the commercial panorama image stitch software (PTgui PRO, New House Internet Services B.V., Rotterdam, The Netherlands) was used for the montage reconstruction of the acquired images. As shown in Fig. 3, the regional still image capture of the lingual mucosa were divided into six areas according to the method used by Shimizu et al.[13] to assess the tongue coat index (Fig. 3).

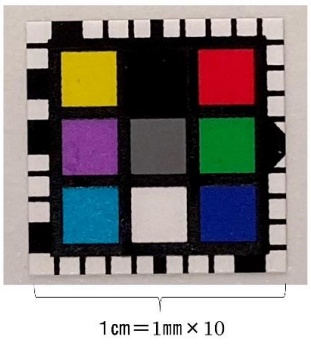


Fig. 2. Color correction and scale with CASMATCH® correction system

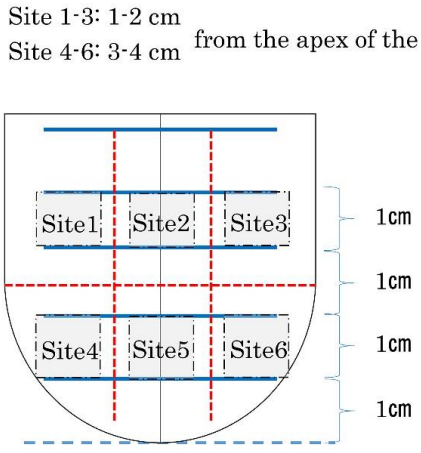


Fig. 3. Regional still image capture

Results

In this study, we used a newly developed oral mucoscopy device and we were able to obtain *in vivo* images of a wide range of tongue mucosal surfaces. Using this device, we were able to obtain suitable magnified images of filiform papillae and fungiform papillae distributed on the surface of the dorsal mucosa of the tongue.

We have presented composite images of each row of the dorsum of the tongue of three male subjects in Figs. 4-1, 2, 3 (A, B) and three female subjects in Figs.5-1, 2, 3(A, B), along with still images of each sites of the lingual dorsum acquired using a contact-type magnifying oral mucosal mucoscope. We have also represented comparative images of six sites among a male subject (Figs. 6-1, 2) and a female subject (Figs. 7-1, 2). Using this device, we were able to obtain relatively suitable magnified images of filiform papillae and fungiform papillae distributed on the surface of the dorsal mucosa of the tongue.

With the tongues of the individuals, not only was there a difference in the density of distribution of fungiform papillae between the tongue apex and the central part of the tongue body, but there also seemed to be a clear difference in the shape and size of the filiform papillae. The morphological observations *in vivo* show that the filiform papillae distributed in the central part of the tongue tend to have many secondary elongated projections, while those in the apex of the tongue tend to have shorter projections and smaller filiform papillae on the whole. However, it is necessary to clarify the difference objectively by quantifying the size and distribution density in the future. In addition, as mentioned above, the filiform papillae were observed to have quite long secondary processes *in vivo*, but when the same area in the center or on both sides of the tongue were compared among individuals, it was found that some were shorter than others and some were extremely long. Furthermore, in terms of overall size, those distributed on the body of the tongue tend to be larger than those distributed on the apex of the tongue, and it is thought that there is a difference in the frequency of distribution per unit area.

As for the fungiform papillae, the shape is almost dome-shaped. There was a slight difference in diameter between large and small (500 μm to 1 mm) with no significant difference, but this difference was not found to be due to individual or regional differences. However, as mentioned earlier, there was a clear difference in distribution frequency between the tongue apex and the tongue body, with the tongue apex tending to have a clearly higher distribution frequency than the tongue body. As for the lingual body, the distribution frequency tends to be slightly higher at the lateral margin than at the midline.

Fig. 4-1. (A) Macroscopic and magnified montage images of the dorsal mucosa of the tongue in a male subject (Code: M001). (B) Site-specific enlarged mucosal images of the six regions (Site 1-6) shown in Fig. 3.

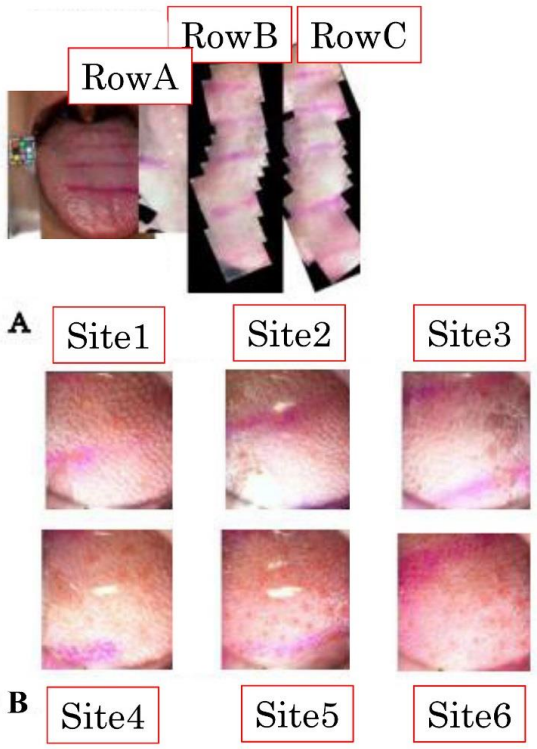


Fig. 4-2. (A) Macroscopic and magnified montage images of the dorsal mucosa of the tongue in a male subject (Code: M002). (B) Site-specific enlarged mucosal images of the six regions (Site 1-6) shown in Fig. 3.

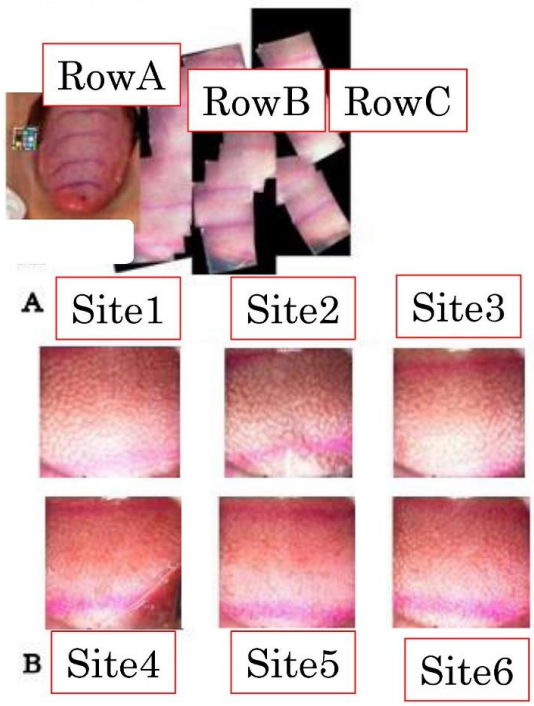


Fig. 4-3. (A) Macroscopic and magnified montage images of the dorsal mucosa of the tongue in a male subject (Code: M003). (B) Site-specific enlarged mucosal images of the six regions (Site 1-6) shown in Fig. 3.

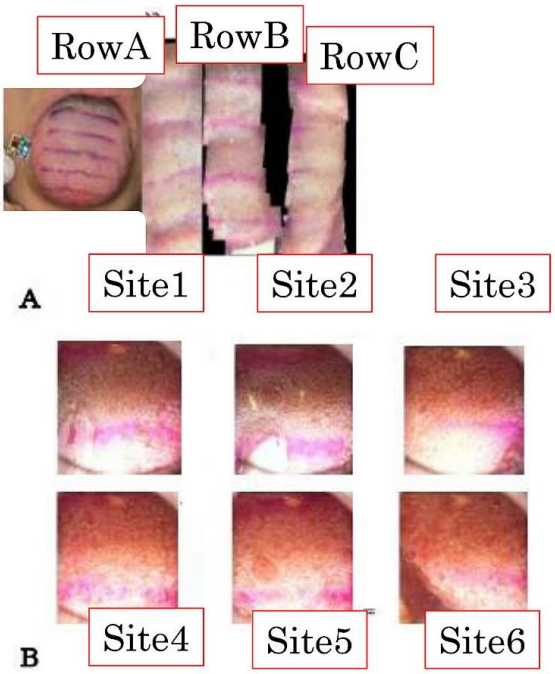


Fig. 5-1. (A) Macroscopic and magnified montage images of the dorsal mucosa of the tongue in a female subject (Code: F001). (B) Site-specific enlarged mucosal images of the six regions (Site 1-6) shown in Fig. 3

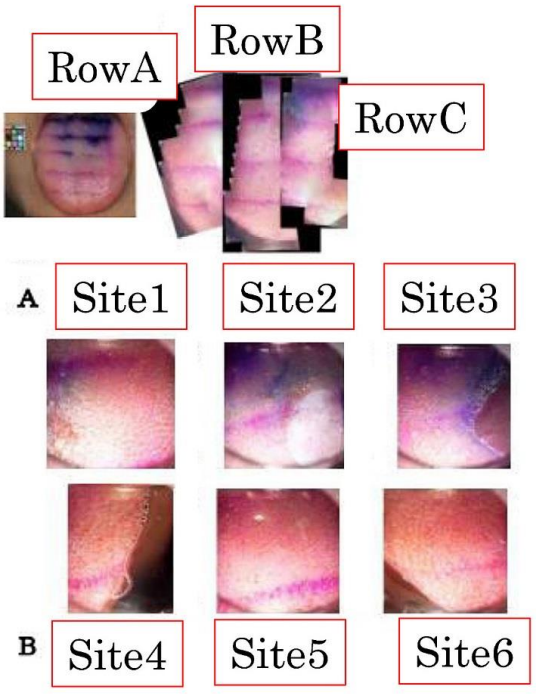


Fig. 5-2. (A) Macroscopic and magnified montage images of the dorsal mucosa of the tongue in a female subject (Code: F002). (B) Site-specific enlarged mucosal images of the six regions (Site 1-6) shown in Fig. 3

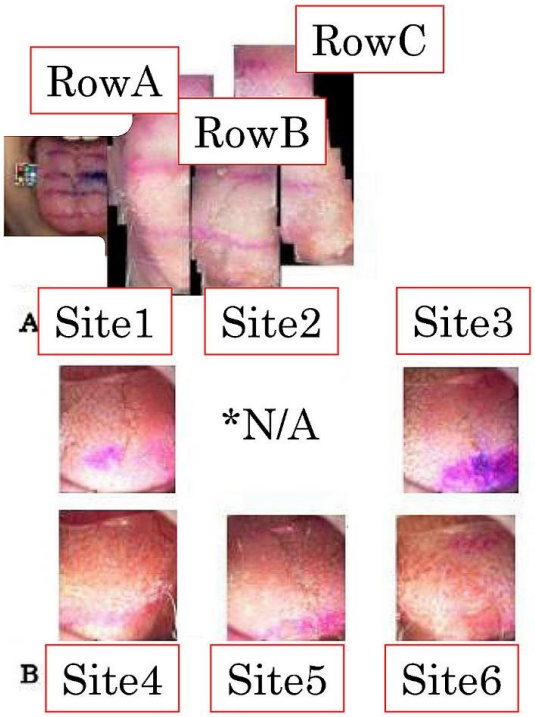
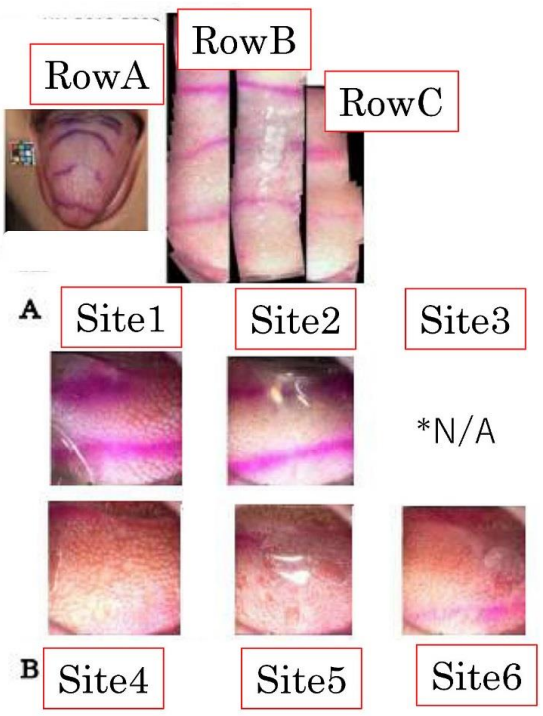


Fig. 5-3. (A) Macroscopic and magnified montage images of the dorsal mucosa of the tongue in a female subject (Code: F003). (B) Site-specific enlarged mucosal images of the six regions (Site 1-6) shown in Fig. 3



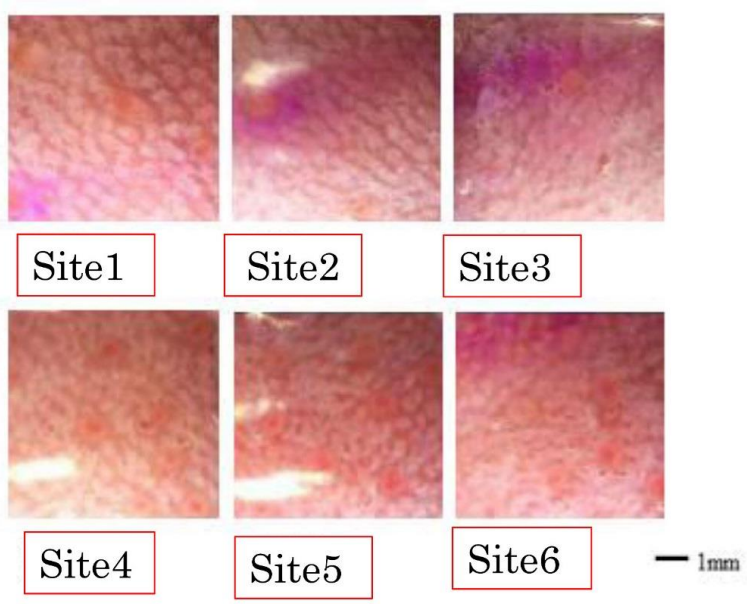


Fig. 6-1. Comparative images of six sites by the same male subject (same magnification): Code M001

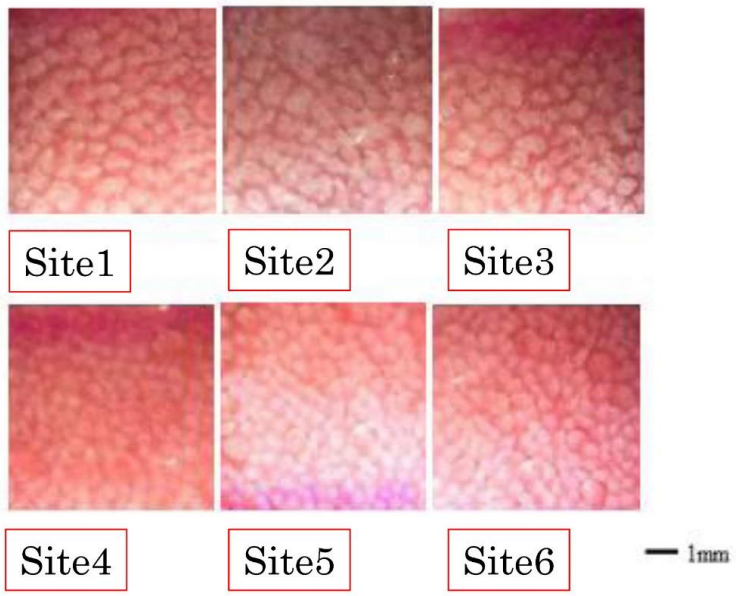


Fig. 6-2. Comparative images of six sites by the same male subject (same magnification): Code M002

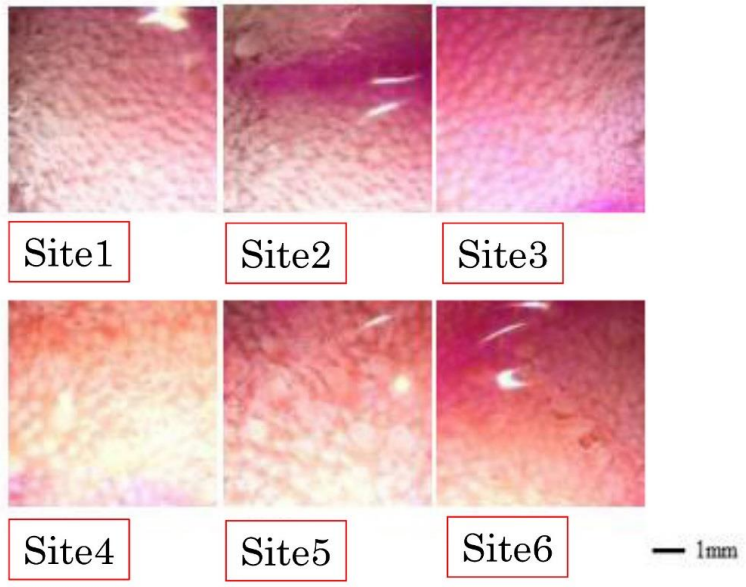


Fig. 7-1. Comparative images of six sites by the same male subject (same magnification): Code M001

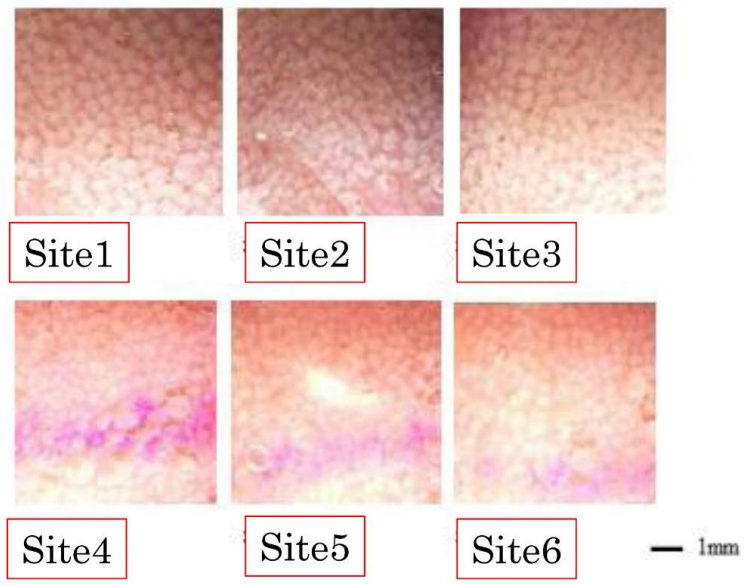


Fig. 7-2. Comparative images of six sites by the same female subject (same magnification): Code F004

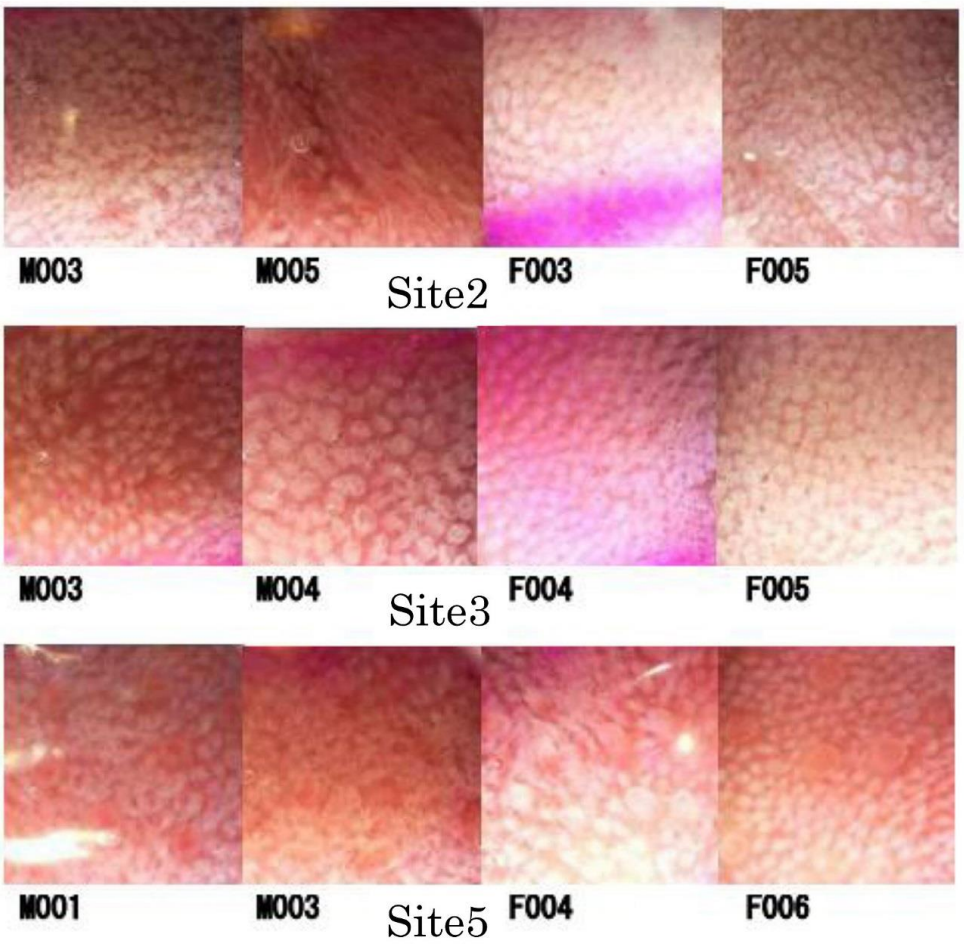


Fig. 8. Morphological comparison images between subjects by sites

We have presented comparison of magnified images of the tongue mucosa of the same area among male and female subjects in Fig. 8. Both filiform papillae and fungiform papillae showed no clear difference in morphology in the tongue among the subjects.

Discussion

In this study, we were able to obtain relatively clear *in vivo* images of the distribution and shape of filiform papillae, which exist densely on the lingual surface, and fungiform papillae, which are scattered between them, as well as their morphology *in vivo*. Since the subjects were all healthy, no significant biofilm adhesion or other abnormalities were

recognized in this observation. Tsuchida et al.[11] measured the morphology of filiform papillae and fungiform papillae on the images of oral mucosa examination among young female subjects, and found that the length of filiform papillae in healthy subjects was about 0.3 mm to 0.5 mm. These classified into short, medium, and long types depending on the subject.

However, in our study, there seemed to be a clear difference in the shape and size of filiform papillae as well as in the density of distribution of fungiform papillae between the apex and center of the tongue in the same individual. Our *in vivo* observations also revealed that the filiform papillae distributed in the central part of the tongue tended to have a large number of elongated secondary processes, while the processes at the apex of the tongue tended to be shorter and generally smaller. In the future, it will be necessary to objectively clarify the differences by quantifying the size and distribution frequency.

As previously mentioned, filiform papillae were observed to have quite long secondary projections *in vivo*, but when the same area in the middle or on both sides of the tongue was compared between individuals, it was found that some were varied e.g. some of them had short and others were extremely long secondary processes. It has been reported that through observation of the lingual mucosa and distribution of filiform papillae in Sjogren's syndrome patients using dermoscopy[14, 15], and it was found that the filiform papillae were attenuated, and in severe cases of attenuation, the mucosa was flattened. Other factors associated with attenuation of filiform papillae include diabetes mellitus[16] and vitamin E deficiency[17]. Short filiform papillae were also reported by Tsuchida et al.[11], suggesting a preliminary stage of degeneration, although this may vary among female individuals.

In contrast, long filiform papillae have been reported by Tsuchida et al.[11] to be present in healthy young female individuals, while other studies have linked black hairy tongue to long filiform papillae[18, 19, 20]. Kobayashi et al.[21] observed black hair tongue and elongated filiform papillae using dermoscopy. Furthermore, when we observed the overall size of the lesions, those distributed in the body of the tongue tend to be larger than those distributed in the apex of the tongue. It is thought that there is a difference in the frequency of distribution per unit area.

However, we have not yet reached the stage where we can present a statistical comparison of the actual length, size, and frequency of distribution of these lesions, so it is necessary to obtain such data in the future to evaluate the exact differences in details.

The shape of the fungiform papillae is almost dome-shaped, with no significant difference, though there is a slight difference in diameter between large and small (500 μm to 1 mm). We also found that the variation of diameter of fungiform papillae was not due to individual or regional differences in the lingual dorsum. However, as mentioned earlier, there was a

clear difference in the distribution density of fungiform papillae between the tongue apex and the lingual body, with the tongue apex tending to have a clearly higher distribution density than the lingual body. Also in regards to the fungiform papillae density in the lingual body, the distribution frequency of fungiform papillae tended to be slightly higher at the lateral margin than at the midline. However, because there is no statistical data quantifying the distribution density (frequency) in our present study, therefore, a statistical survey is needed to conduct a statistical study to compare the distribution density in the future.

In our present study, the morphology, size, and distribution frequency were not clear regarding the both filiform papillae and fungiform papillae. We were also unable to find any clear differences in the morphology, size, and distribution density between men and women. In the future, the observation method and device used in this study could be used for clinical examination to detect various abnormalities in the oral cavity, especially the tongue. In addition, the distribution of filiform papillae tends to be more common at the apex and margin of the tongue than at the center of the tongue (Tsuchida et al.[11]).

Therefore, it is necessary to quantify the distribution density per unit area, and then examine the degree of difference between the center of the tongue and the apex or margin of the tongue.

Another interesting question is whether the morphology of the filiform papillae with long secondary processes observed in this study changes with age.

Conclusions

The conclusions of this study can be summarized as follows:

1. In this study, we used the newly developed oral mucoscopy device and we were able to obtain *in vivo* images of a wide range of tongue mucosal surfaces.

2. Using this device, we were able to obtain relatively suitable magnified images of filiform papillae and fungiform papillae distributed on the surface of the dorsal mucosa of the tongue.

3. For the tongue of the same individual, not only is there a difference in the density of distribution of fungiform papillae between the tongue apex and the central part of the tongue body, but there also seems to be a clear difference in the shape and size of the filiform papillae. The morphological observations in vivo show that the filiform papillae distributed in the central part of the tongue tend to have many secondary elongated projections, while those in the apex of the tongue tend to have shorter projections and smaller filiform papillae as a whole.

4. In addition, as mentioned above, the filiform papillae were observed to have quite long secondary processes in vivo, but when the same area in the center or both sides of the tongue was compared among individuals, it was found that some were shorter than others and some were extremely long.

5. As for the fungiform papillae, the shape is almost dome-shaped and there is no significant difference, but there is a slight difference in diameter between large and small (500 μm to 1 mm). However, it was not found to be due to individual or regional differences.

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This work was approved by the ethical committees of Hokuriku University (H30-04). All subjects were given a detailed explanation of this study and provided written informed consent in accordance with the ethical guidelines of the ethical committees of Hokuriku University.

The authors declare that they have no conflict of interest.

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