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Original Article

Histopathological analysis of the association between mucosal epithelial changes and the lamina propria vascular network in irritation fibroma

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ABSTRACT

Objectives: Irritation fibroma in the oral cavity causes atrophy or squamous epithelium thickening with respect to external injury-associated factors. However, ulcers do not occur in most cases. This study aimed to elucidate the mechanism by which ulcers do not form, focusing on the vascular network in the mucosal epithelium of irritation fibroma.

Methods: Immunostaining was performed using an enzyme antibody method with primary antibodies against CD31 and Ki-67 in 17 cases of irritation fibroma in the buccal mucosa. One section was taken at three points from the margin and three points from just above the lesion for measurement. The number of blood vessels in the superficial and deep lamina propria at the measurement site were determined, and the area per blood vessel was measured.

Results: The number and area of blood vessels in the superficial lamina propria just below the lesion epithelium were smaller than those in the margin. No difference was observed in the number and area of blood vessels in the deep lamina propria between the margins and lesions.

Conclusions: Our results suggest that the vascular network in the deep lamina propria is maintained and compensates for the nutrient supply to the covering epithelium.

1. Introduction

Irritation fibroma is a fibro-hyperplastic soft tissue lesion of the oral mucosa, caused by stimulation, and classified as either traumatic, due to bites and the like, or denture, due to improper denture stimulation. Irritation fibromas are not neoplasms, but localized hyperplasia of fibrous tissue caused by local trauma or chronic irritation [1,2]. Irritation fibromas are the most common tumor-like lesions in the oral cavity and appear as asymptomatic, pedunculated, or sessile [3–5].

Manabe et al. [6] reported that peripheral dental fibers (POFs) were Bcl-2-positive and CD34-negative, while fibrous epulis was Bcl-2-negative and CD34-negative. CD34 and Bcl-2 are useful in differentiating fibroproliferative lesions. Nagasaki et al. [7] reported that irritation fibroma is caused by increased collagen synthesis by CD34-positive submucosal fibroblasts. Thus, despite increased knowledge about the properties of fibrous components, little is known about the actual lesion and its covering epithelium.

Irritation fibroma in the oral cavity shows atrophy or squamous epithelium thickening with respect to external injury factors. However, ulcers do not occur in most cases [8]. Wei et al. [9] monitored ulcers over time and reported that changes in the vascular network are closely related to ulcer development and recovery. Although it is presumed that the subepithelial vascular network of the lesion is related to ulcer formation, the underlying mechanism remains unclear.

This study aimed to elucidate the mechanism by which ulcers do not form by focusing on the vascular network in the mucosal epithelium of the irritation fibroma.

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2. Materials and methods

2.1. Case selection

In total, 17 cases of irritation fibroma of the buccal mucosa with fibroproliferative lesions were resected under a clinical diagnosis of fibroma between 2011 and 2019 at the Kanagawa Dental University Hospital. Furthermore, in one sample, the growth of collagen fibers did not extend to the lamina propria, and the margin was included. The samples were differentiated for diagnosis based on CD34 and Bcl-2 expression, which are useful indicators of irritation fibroma [6,7].

2.2. Histological examination and immunohistochemistry

Samples were fixed in a 10% neutral-buffer formalin solution, embedded in paraffin blocks, and prepared as serial sections. Hematoxylin-eosin (HE) staining was performed, and immunohistochemistry was used to detect CD31 and Ki67 expression.

Fig. 1 shows an overall section of HE-stained irritation fibroma.

CD31 and Ki-67 localization were analyzed using the enzyme-antibody method. For antigen recovery, the sections were immersed in 0.01-M citrate buffer (pH 6.0) and autoclaved at 120 °C for 5 min. Mouse anti-human CD31 and Ki-67 monoclonal antibodies (clone 2A1E2, 1:4000 dilution; Proteintech, Wuhan, China; and clone MIB-1, 1:100 dilution; MBL Co, LTD, Nagoya, Japan, respectively) were used as primary antibodies. The primary antibody was incubated at 37 °C for 1 h. After incubation with the secondary antibody, Histofine® Simple Stain MAX-PO (M) (Nichirei, Tokyo, Japan) staining was performed according to the manual instructions.

2.3. Measurement method

An Olympus BX53 microscope (BX53, Olympus, Tokyo, Japan) was used to observe the maximum cut surface of the lesions.

Fig. 2 shows a schematic diagram of the measurement site on the cut surface just above the lesion.

The spinous and basal cell layers are designated as ① and ②, respectively.

Any point immediately above the lesion was designated as point A, and points on both sides 100 μm from point A were designated as points B and C, respectively. One measurement site was defined at 200 μm between points B and C. Further, 200-μm widths were set on both outer sides on the same section, and a total of three measurement sites were set as measurement ranges.

For epithelial thickness, the thicknesses of ① to ② were measured.

The number of Ki67-positive and negative cells in the basal layer, as well as the number of blood vessels in the surface lamina propria (0–10 μm) and the deep lamina propria (10–100 μm) layers of the measurement site were counted, and the area per blood vessel was measured. In the normal mucosa, the measurement range was three places with a 200-μm margin width. The margin was used as the control group and compared with the area immediately above the lesion. Measurements were taken at three locations, and the average value was used as the numerical value for the case. Measurements were performed using Olympus cellSens Standard (Olympus, Tokyo, Japan).

2.4. Statistical analysis

Because of the non-normal distribution according to the Kolmogorov–Smirnov test, the Wilcoxon signed-rank test was used to compare the two groups.

A p-value < 0.05 was considered statistically significant.

All statistical analyses were performed using SPSS (version 23.0; IBM, Armonk, NY, USA).

3. Results

3.1. Epithelial thickness

The median epithelial thicknesses at the margin and just above the lesion were 338.74 μm (range, 192.80–473.67 μm) and 71.52 μm (39.37–380.64 μm), respectively. Atrophy was observed at the lesion coating. Significant thickness was observed at the margins (Fig. 3).

3.2. Ki67 positive cell rate

The median Ki67-positive cell rates at the margin and just above the lesion were 32.20% (range, 17.03%–66.27%)and 17.60% (range, 11.10%–47.57%), respectively. The margins showed significantly higher values (Fig. 4).

3.3. Average vascular number

The median numbers of vessels in the surface lamina propria at the margin and just above the lesion were 2.67 (range, 1.33–5.67) and 2.33 (range, 1.00–3.33), respectively. A significantly higher number of vessels was observed at the margins (Fig. 5A).

The median numbers of vessels in the deep lamina propria at the margin and just above the lesion were 4.00 (range, 2.33–8.67) and 4.00 (range, 2.67–6.33), respectively, showing no significant difference (Fig. 5B).

Comparing the median number of vessels between the surface lamina propria and deep lamina propria layers at the margin and

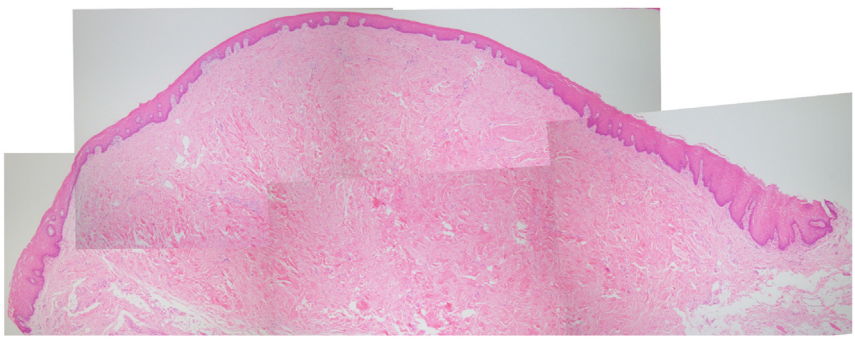

Fig. 1. Hematoxylin-eosin-stained images showing the histological findings of irritation fibroma.

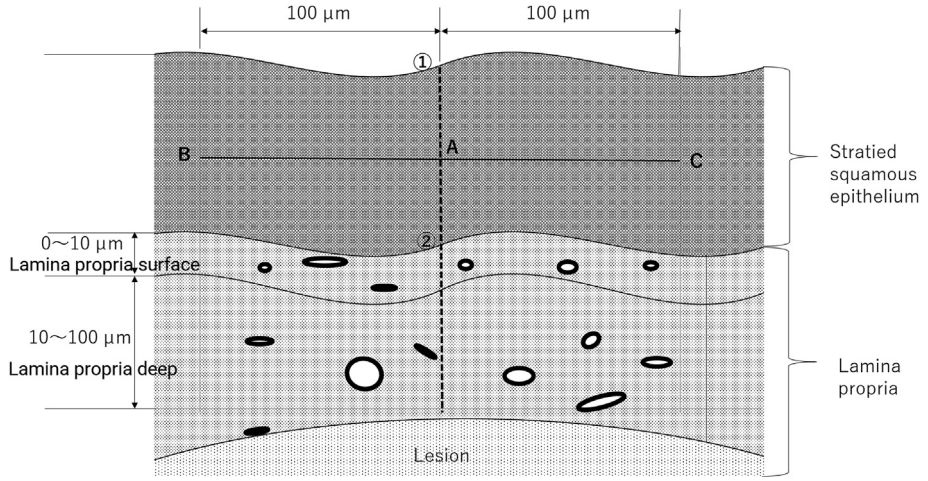

Fig. 2. Schematic diagram of the measurement site on the cut surface, just above the lesion. The spinous and basal cell layers are designated as ① and ②, respectively. Any point immediately above the lesion was designated as point A, and points on both sides 100 μm from point A were designated as points B and C, respectively. The point at 200 μm between points B and C was defined as one measurement site.

just above the lesion, the latter showed a significantly higher number of blood vessels in both areas (Fig. 5C and D).

3.4. Vascular area average

The median vascular areas in the surface lamina propria at the margin and just above the lesion were 139.29 μm^2 (range, 72.71–342.96 μm^2) and 80.65 μm^2 (23.47–141.30 μm^2), respectively. The lumen of the capillaries just above the lesion was often narrow and tended to atrophy. Between the margin and just above the lesion in the surface lamina propria, the former showed a significantly larger vascular area (Fig. 6A).

The median vascular areas in the deep lamina propria at the margin and just above the lesion were 281.46 μm^2 (range, 115.84–1142.42 μm^2) and 254.29 μm^2 (range, 59.17–533.92 μm^2), respectively, showing no significant difference (Fig. 6B).

Comparing the median vascular area between the surface lamina propria and deep lamina propria layers at the margin and just above the lesion, the latter showed a significantly larger vascular area in both areas (Fig. 6C and D).

4. Discussion

This study examined the hypothesis that ulcer formation in the coated epithelium in irritation fibroma is inhibited through maintenance of the subepithelial vascular network.

As the blood vessels that carry nutrients to the coating epithelial cells are located in the lamina propria, the corresponding capillaries have been analyzed [10–13]. We examined cases in which the lesion did not extend to the lamina propria. The lamina propria is divided into the papillary and reticular layers [10,11,13]. Murgod et al. [14] reported that the microvessels in the normal oral epithelium and lichen planus of the tongue are mainly located just below the covering epithelium [15,16]. The morphology of the epithelial papilla changes because of mechanical support between the epithelium and lamina propria [13], and because the buccal mucosa has a wide papilla and a short length [10,11]. Consequently, the superficial lamina propria was defined as 10 μm from the basement membrane. The depth of the lamina propria was determined to be 10–100 μm, with reference to the measurement distance described by Ino et al. [17] and Arima [18].

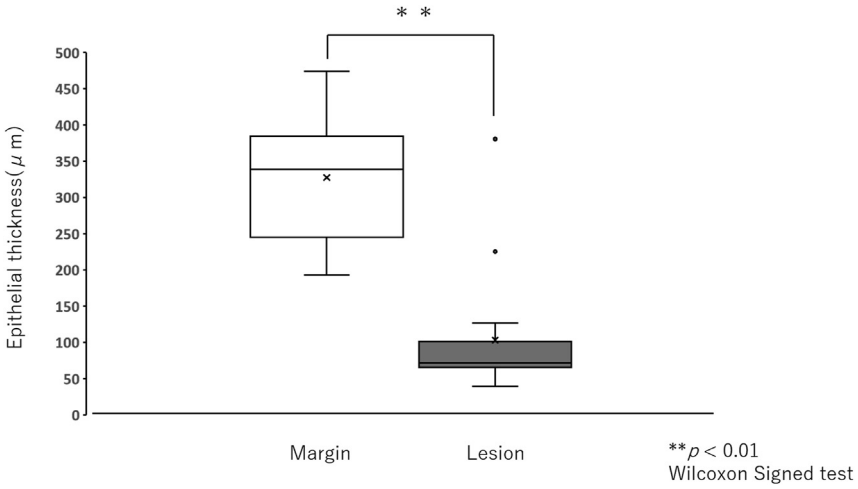

Fig. 3. Epithelial thickness. The graph shows significant differences in epithelial thickness between the margins (n = 17) and lesions (n = 17). Box plots represent the smallest observation; lower, median (horizontal bar), and upper quartiles; and the largest observation (**p < 0.01, Wilcoxon signed-rank test) are identified.

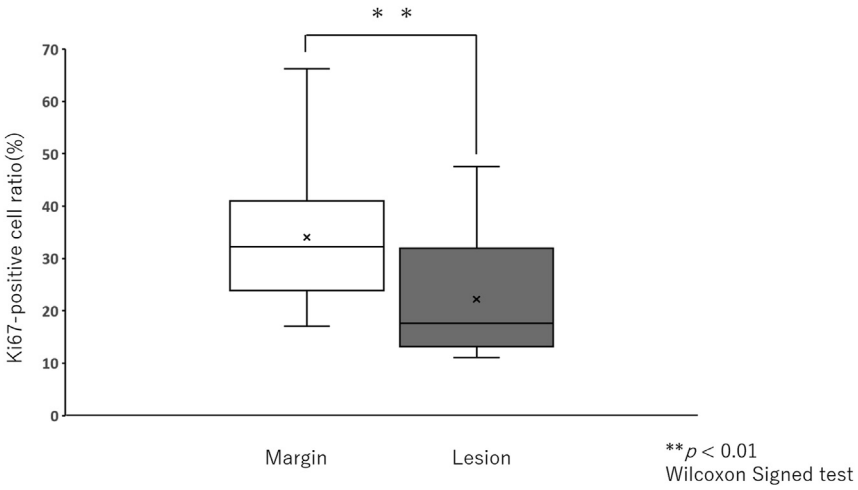

Fig. 4. Ki67-positive cell ratio. Graph showing a significant difference in the Ki67-positive cell ratio between the margin (n = 17) and lesion (n = 17). Box plots represent the smallest observation, and the lower, median (horizontal bar), and upper quartiles, and largest observation (**p < 0.01, Wilcoxon signed-rank test) are identified.

We observed that the number and area of blood vessels in the superficial lamina propria just below the lesion's covering epithelium, were smaller than those in the margin. Histologically, the capillaries of the superficial lamina propria had a narrow lumen and atrophy. These findings suggest a decrease in blood flow and a significantly thinner covering epithelium compared to the margin. Thus, atrophy was observed histologically, but no ulcers were observed. Although weaker than the margin, the coating epithelial basal cells maintained proliferative activity, suggesting slight nourishment of the covering epithelium.

Interestingly, there was no difference in the number and area of blood vessels between the margin and lesion areas in the deep lamina propria. From this, we presumed that the vascular network in the deep lamina propria was maintained and compensated for nutrient supply to the covering epithelium.

The development of pressure ulcers on the skin can be attributed to long-term ischemia due to pressure-induced vascular closure [19]. The mucous membrane may also be stimulated by various factors. Maintenance of blood flow is an important a defense mechanism against gastric mucosa irritation and is controlled and regulated by

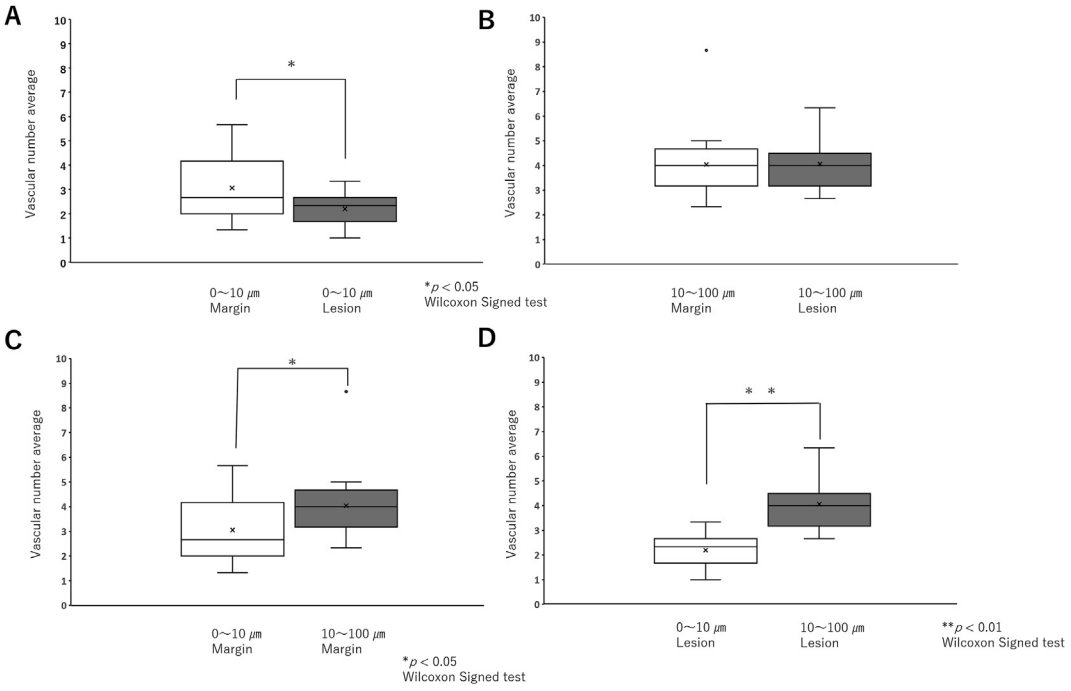

Fig. 5. Vascular number average. (A) Graph showing a significant difference in the vascular number average between the surface lamina propria (0–10 μm) margin (n = 17) and surface lamina propria (0–10 μm) lesion (n = 17). Box plots represent the smallest observation, and the lower, median (horizontal bar), and upper quartiles, and the largest observation (*p < 0.05, Wilcoxon signed-rank test) are identified. (B) Graph showing the significant difference in vascular number average between the deep lamina propria (10–100 μm) margin (n = 17) and deep lamina propria (10–100 μm) lesion (n = 17). Box plots represent the smallest observation, and the lower, median (horizontal bar) and upper quartiles, and the largest observation are identified. (C) Graph showing the significant difference in vascular number average between the surface lamina propria (0–10 μm) margin (n = 17) and deep lamina propria (10–100 μm) margin (n = 17). Box plots represent the smallest observation, and the lower, median (horizontal bar), and upper quartiles, and the largest observation (*p < 0.05, Wilcoxon signed-rank test) are identified. (D) Graph showing the significant difference in vascular number average between surface lamina propria (0–10 μm) lesions (n = 17) and deep lamina propria (10–100 μm) lesions (n = 17). Box plots represent the smallest observation, and the lower, median (horizontal bar), and upper quartiles, and the largest observation (*p < 0.01, Wilcoxon signed-rank test) are identified.

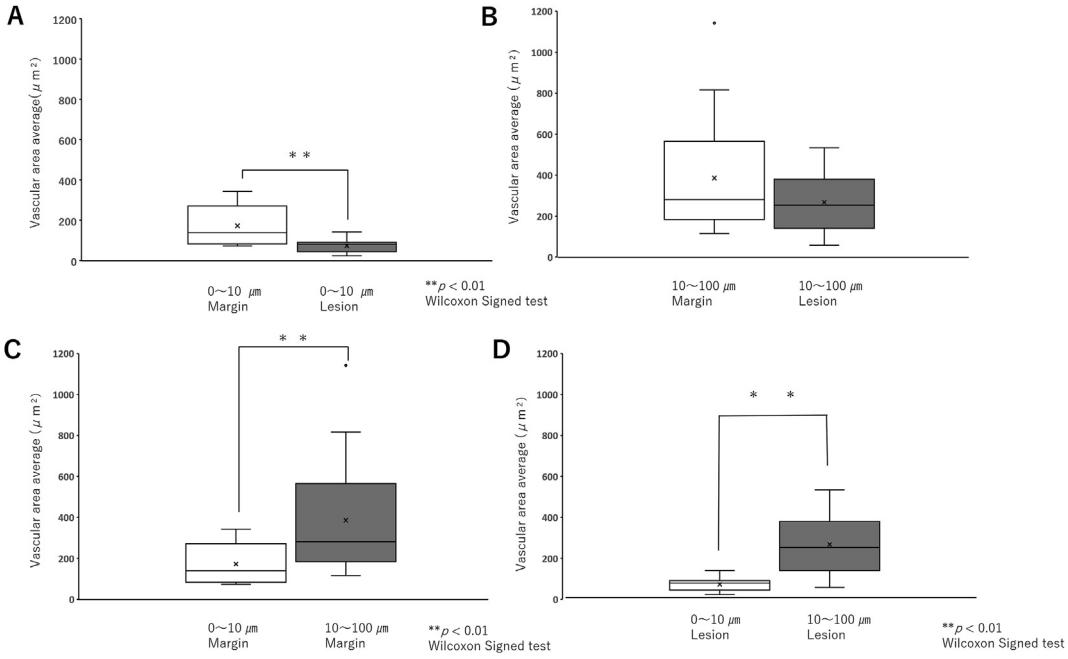

Fig. 6. Vascular area average. (A) The graph reveals a significant difference in the average vascular area between the surface lamina propria (0–10 μm) margin (n = 17) and surface lamina propria (0–10 μm) lesions (n = 17). Box plots represent the smallest observation, and the lower, median (horizontal bar), and upper quartiles, and the largest observation (*p < 0.01, Wilcoxon signed test) are identified. (B) The graph reveals a significant difference in the vascular area average between the deep lamina propria (10–100 μm) margin (n = 17) and deep lamina propria (10–100 μm) lesions (n = 17). Box plots represent the smallest observation, and the lower, median (horizontal bar), and upper quartiles, and the largest observation are identified. (C) Graph showing the significant differences in vascular area average between the surface lamina propria (0–10 μm) margin (n = 17) and deep lamina propria (10–100 μm) margin (n = 17). (d) Graph showing the significant difference in vascular area average between the surface lamina propria (0–10 μm) lesion (n = 17) and deep lamina propria (10–100 μm) lesion (n = 17). Box plots represent the smallest observation, and the lower, median (horizontal bar), and upper quartiles, and the largest observation (*p < 0.01, Wilcoxon signed test) are identified. Box plots represent the smallest observation, and the lower, median (horizontal bar), and upper quartiles, and the largest observation (*p < 0.01, Wilcoxon signed test) are identified.

many physiologically active substances [20]. Kodama et al. [21] reported that infection reduces the mucosal defense mechanism, weakening the overall organization and suggesting delayed ulcer healing. In the esophageal mucosa, a lack of blood flow delays normal wound healing [22]. Thus, the relationship between blood flow, ulceration, and tissue repair is very important. Irritation fibroma is caused by increased collagen fibers in the submucosa due to stimulation [7]. The covering epithelium is the first tissue stimulated and thus is considered as the most affected. However, resistance to irritation was suggested to be acquired by maintaining the vascular network of the deep lamina propria.

The substances contributing to vascular network maintenance in the deep lamina propria are unknown, and thus further studies are necessary to elucidate the underlying molecular mechanisms.

The thickness of the covering epithelium and the number of capillaries are reduced in the buccal mucosa near the corners of the mouth in the elderly [23]. Furthermore, the number of blood vessels in the lamina propria does not change, but the thickness tends to lessen with increasing age [24]. Early removal may thus be necessary for treating irritation fibroma in the elderly, thereby reducing the risk of ulceration.

5. Conclusions

The number and area of blood vessels in the superficial lamina propria was smaller than the number and area of blood vessels in the margin. There was no difference in the number and area of blood vessels between the marginal area and the lesion area in the deep lamina propria. In this study, in the irritation fibroma, the mechanism of not forming an ulcer formation was confirmed because the vascular network of the deep lamina propria was maintained to supply nutrients to the coated epithelium.

Ethical approval

This research was approved by the Kanagawa Dental University Research Ethics Review Board (No. 623).

CRediT authorship contribution statement

Rie Amano: Data curation, Formal analysis, Investigation, Writing – original draft, writing. **Juri Saruta:** Resources, Supervision, Validation, and review and editing. **Wakako Sakaguchi:** Resources, and validation. **Nobuhisa Kubota:** Resources. **Shinya Fuchida:** Formal analysis. **Keiichi Tsukinoki:** Conceptualization, Validation, Project administration, and review and editing.

Conflicts of interest

The authors declare no conflicts of interest.

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References

[1] Jiang M, Bu W, Chen X, Gu H. A case of irritation fibroma. Postepy Dermatol Alergol 2019;36:125–6.

[2] Esmeili T, Lozada-Nur F, Epstein J. Common benign oral soft tissue masses Dent. Clin N Am 2005;49:223–40.
[3] Mortazavi H, Safi Y, Baharvand M, Rahmani S, Jafari S. Peripheral exophytic oral lesions: a clinical decision tree. International Journal of Dentistry 2017;2017.
[4] Kinoshita H, Ogasawara T, Toya T, Makihara R, Kawahara E. Slow-growing large irritation fibroma of the anterior hard palate: a case report using immunohistochemical analysis. J Maxillofac Oral Surg 2016;15:253–7.
[5] Younwook J, Chungmin K, Seunghye K, Jaeho L. Irritation fibroma associated with ectopic eruption of the maxillary incisor. J Korean Acad Pediatr Dent 2016;43:207–12.
[6] Manabe K, Yakeishi M, Sakaguchi W, Saruta J, Tsukinoki K. Histopathological analysis of the differential diagnosis of peripheral odontogenic fibroma from fibrous epulis. J Oral Biosci 2019;61:221–5.
[7] Nagasaki M, Sakaguchi W, Fuchida W, Kubota N, Saruta J, Suzuki K, et al. Comparison of CD34 expression in fibrous reactive hyperplasia and healthy oral mucosa. J Oral Biosci 2020;62:88–92.
[8] Daddy Suradi H, Abdullah P, Pang EY. The prevalence of fibroma in oral mucosa among patient Attending USM dental clinic year 2006-2010. The Indonesian J Dent Res 2010;1:61–6.
[9] Wei W, Choi WJ, Wang RK. Microvascular imaging and monitoring of human oral cavity lesions in vivo by swept-source OCT based angiography Lasers. Med Sci 2018;33:123–34.
[10] Ten cate AR, Antonio N. Ten Cate's oral histology:development, structure, and function. St.Louis: Elsevier; 2008.
[11] Orban BJ, Bhaskar SN. Orban's oral histology and embryology. St.Louis: Mosby Year Book; 1991.
[12] Moss-Salentijn L, Hendricks-Klyvert M. Dental and oral tissues:an introduction. Philadelphia: Lea & Febiger; 1990.
[13] Avery JK. Oral development and histology. New York: Thieme; 2002.
[14] Murgod VV, Kale AD, Angadi PV, Hallikerimath S. Morphometric analysis of the mucosal vasculature in oral submucous fibrosis and its comparison with oral squamous cell carcinoma. J Oral Sci 2014;56:173–8.
[15] Tae K, El-Naggar AK, Yoo E, Feng L, Lee JJ, Hong WK, et al. Expression of vascular endothelial growth factor and microvessel density in head and neck tumorigenesis. Clin Canc Res 2000;6:2821–8.
[16] Scardina GA, Messina P. Morphological characteristics of microcirculation in oral lichen planus involving the lateral border of the tongue. J Oral Sci 2009;51:193–7.
[17] Inobe M, Ookura Y, Sakamoto A. Immunohistochemical analysis on fibroblasts of pancreatic mucinous tumors. Diagn Pathol 2010;27:273–9.
[18] Arima H. Magnified observation ation of esophageal mucosa. Gastroenterol Endosc 1998;40:1125–37.
[19] Tsuji S, Ichioka S, Sekiya N, Nakatsuka T. Analysis of ischemia-reperfusion injury in a microcirculatory model of pressure ulcers. Wound Repair Regen 2005;13:209–15.
[20] Miyake K, Kusunoki M, Shindo T, Ueki N, Kawagoe T, Futagami S, et al. Current status of gastroduodenal ulcers the medical association of nippon. Medical School 2010;6:7–12.
[21] kodama R, Fujioka T, Shuto R, Kubota T, Nasu M. Helicobacter pylori infection delays the healing of acetic acid-induced gastric ulcer in Japanese monkeys. J Gas-troenterol Hepato1 1996;11:1097–102.
[22] Miyoshi H, Shikata J, Tokura Y. A clinical study on mucosal tissue blood flow of esophagus using endoscopic laser Doppler flowmetry. Gastroenterol Endosc 1992;34:1252–7.
[23] Akimoto K. Observations on the structural changes according to aging of oral mucous membrane in the elderly-structure of buccal mucous membrane in the vicinity of angulus oris-. Kokubyo Gakkai Zasshi 2004;71:80–94.
[24] Takahashi T. Structural changes of the apex region of the tongue in the elderly. Kokubyo Gakkai Zasshi 2008;75:93–105.