Histopathological and Immunohistochemical Studies of the Distribution of Elastic Fibers in Oral Fibrous Hyperplasia

（口腔線維過形成病変における弾性線維の分布に関する病理組織学的および免疫組織化学的研究）

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Abstract

Oral fibrous hyperplasias (OFH) are thought to result from hyperplasia of collagen fibers. However, details regarding the presence of elastic fibers and reticular fibers other than collagen fibers in OFH are unclear.

Therefore, this study focused on elastic fibers in the connective tissue with regard to OFH, and assessed the histopathological, histochemical, and immunohistochemical distribution of the elastic fibers.

All cases of OFH (120 cases) were performed Elastica van Gieson (EvG) staining, and the distribution of elastic fibers was assessed using image analysis (binarization). Cases were classified into 2 groups; one group with elastic fibers (EF+ group) and one group without elastic fibers (EF− group).

Elastic fibers were observed in 20 cases of fibroma of the buccal mucosa, 20 cases of fibroma of the labial mucosa, 19 cases of fibroma of the dorsal surface of tongue, 8 cases of fibroma of the gingiva, 1 case of fibrous epulis, and 1 case of fibromatous epulis. Histopathologically, elastic fibers were observed with mingled hyperplastic collagen fiber bundles and extended in the lesion. The distribution quantity of elastic fibers was fibroma of labial mucosa and buccal mucosa, and there were fewer elastic

fibers in fibrous epulis and fibromatous epulis. Immunohistochemically, spindle cells in all cases of OFH were diffusely positive for Vimentin and negative for Actin, and CD34-positive spindle cells were interspersed into the connective tissue in EF+ group.

In conclusion, elastic fibers were observed in 57.5% of OFH cases. The distribution of these fibers was site-specific, and differed from the collagen fibers that constituted OFH. CD34 positivity was observed in the spindle cells constituting OFH accompanied by elastic fibers, and undifferentiated mesenchymal cells around myogenic blood vessels near the lesion were related to the formation of a part of elastic fibers that constituted OFH.

Keywords

oral fibrous hyperplasia, elastic fibers, binarization, CD34, site-specific localization

Introduction

Connective tissue lesions of the oral region, particularly oral fibrous hyperplasia (OFH), which includes traumatic fibroma, irritation fibroma, denture hyperplasia, and fibrous epulis, are thought to result from hyperplasia of collagen fibers (1). However, details regarding the presence of elastic fibers and reticular fibers other than collagen fibers in OFH are unclear.

On the other hand, elastofibroma, which is a lesion caused by hyperplasia of elastic fibers, occurs in the subscapular region (2-8). Furthermore, several cases presenting elastofibromatous changes in the oral mucosa have been reported in recent years (9-14). As mentioned above, elastic fibers are also distributed around the vascular wall in the oral mucosal tissues and are speculated to be involved in the pathogenesis of OFH.

Therefore, this study focused on elastic fibers in the connective tissue which constituted OFH, and performed histopathological, histochemical, and image analyses of the distribution of elastic fibers in OFH. Furthermore, we performed an immunohistochemical study to explore the origins of elastic fibers in OFH.

Materials and Methods

1. Materials

Subjects comprised 120 cases of OFH including fibroma (sites were buccal mucosa, labial mucosa, dorsal surface of tongue, and gingiva), fibrous epulis, and fibromatous epulis. Samples were surgically excised at the Nihon University Hospital at Matsudo, Japan, from 1995 to 2013, and were histopathologically diagnosed by hematoxylin-eosin (HE) staining (Fig. 1). Normal tissues of the same sites as subjects were used as controls to determine the distribution quantity of existing elastic fibers. Exclusion criteria for subjects were inflammatory reaction, ulcer formation, and cases containing hard tissue.

This study was approved by the ethics committee of Nihon University School of Dentistry at Matsudo (approval number: EC 11-029).

2. Methods

2.1. Histopathological and histochemical staining

Specimens were immediately fixed in 10% neutral formalin solution for 24-48 h at room temperature, and paraffin blocks were prepared according to conventional

methods. Paraffin blocks sliced at a thickness of 4 μm were performed HE and Elastica van Gieson (EvG) staining.

In addition, all cases were classified into 2 groups based on EvG staining results; one group with elastic fibers (EF+ group) and one group without elastic fibers (EF− group).

2.2. Image analysis (binarization) of elastic fibers

The lamina propria of OFH was classified into 2 layers according to collagen fiber orientation in specimens stained by EvG under an optical microscope (×400) (Fig. 2). The collagen fibers run parallel to the free surface of the epithelium at the layer of ≤100μm below the basal lamina, but intertwined with each other at the deeper layer.

For image analysis, the lamina propria of OFH was divided into 3 areas. The superficial area was defined as the epithelial side (≤ 100μm below the basal lamina). The deepest area was defined as the base (clinical base of the mass), and the remaining area was defined as the central area. As the control cases have no clinical base, the lamina propria was divided into 2 areas.

For each area (epithelial side, central area, and base), the hot spot (15) was

defined as the 100 μm × 100 μm area with the greatest distribution of elastic fibers (Fig. 2). The images of 3 spots in each area were recorded to accurately determine the hot spots. However, the elastic fibers that constituted the elastic lamina of myogenic blood vessels were excluded. After images were binarized using Image J (ver. 1.46r; National Institutes of Health; Bethesda, MD) (16), elastic fibers were extracted, and the largest pixel counts in each area were recorded (Fig. 2).

2.3. Statistical tests for quantity of elastic fibers

"Quantity" express the value of elastic fibers of the hot spot in this study. Hot spot pixel counts were used to perform the following nonparametric tests in each area using IBM SPSS statistical software (ver. 22.0; IBM, Chicago, IL).

1) Sex differences in quantity of elastic fibers in OFH: Mann-Whitney U test (two-independent samples test)

2) Correlations between quantity of elastic fibers and age, and macrosize in OFH: Spearman's rank correlation

3) Comparison of quantity of elastic fibers between control and OFH: Mann-Whitney

U test (two-independent samples test)

4) Comparison of quantity of elastic fibers among OFH in the same area: Kruskal-Wallis H test (several-independent samples test)

5) Comparison of quantity of elastic fibers among 3 areas in the same case: Friedman test (several-related samples test)

2.4. Immunohistochemical staining

Subjects for immunohistochemical staining were the EF+ and EF− groups. Specimens were prepared using standard techniques. After deparaffinization in a xylene-alcohol series, specimens were treated with 3% hydrogen peroxide to block endogenous peroxidase activity. The primary antibodies used in the present study were monoclonal mouse anti-Vimentin (Vimentin) (Clone V9, IgG1, dilution 1: 100; Dako Cytomation, Glostrup, Denmark), monoclonal mouse anti-human Actin (Actin) (clone HHF35, IgG1, dilution 1: 100; Dako Cytomation), and monoclonal mouse anti-human CD34 Class II (CD34) (clone QBEnd10, IgG1, dilution 1: 100; Dako Cytomation). For antigen activation, Vimentin, Actin, and CD34 were treated by using 10 mM/l citrate buffer solution (pH 6.0) in a microwave for 13 min. Secondary antibody was

ChemMate Envision (Dako Cytomation). The chromogenic substrate was liquid DAB+ (Dako Cytomation), and counterstaining was performed by using Mayer's hematoxylin.

Mouse IgG1-negative control (Dako Cytomation) was used as a negative control and tissue containing healthy oral mucosa was used as a positive control.

Results

1. Histopathological and histochemical findings

1) HE findings

Specimens showed relatively dense hyperplasia with irregular bundles of eosinophilic collagen fibers in the lamina propria under stratified squamous epithelium with hyperkeratosis and acanthosis.

2) EvG findings

In all cases of OFH, collagen fibers in the lesion that were stained red with acid fuchsin showed irregular bundles.

The EF+ group, which included elastic fibers stained black-purple with resorcin

and fuchsin in the lesion, consisted of 69 OFH cases; 20 cases of fibroma of the buccal mucosa, 20 cases of fibroma of the labial mucosa, 19 cases of fibroma of the dorsal surface of tongue, 8 cases of fibroma of the gingiva, 1 case of fibrous epulis, and 1 case of fibromatous epulis (Table 1) (Fig. 4).

Elastic fibers were observed in the EF+ group and either paralleled or intertwined collagen fibers (Fig. 2). Elastic fibers showed a fine granular appearance (i.e., cross-sections of elastic fibers were frequently observed) on the epithelial side, and elongated filamentous morphology in the central area and base. Elastic fibers were observed into the elastic lamina, which constitutes the vascular wall of myogenic blood vessels stained yellow with picric acid, in the submucosal tissues (Fig. 3). These elastic fibers were comparatively isolated, intermingled with hyperplastic collagen fibers bundles, and extended toward the epithelial side in the lesion.

Two cases of fibroma of the labial mucosa showed different patterns of elastic fibers (Fig. 2). On the epithelial side in these 2 cases, conspicuous quantities of elastic fibers showed thick irregular fascicular bundles or globules.

2. Statistical tests for quantity of elastic fibers

1) Sex differences in quantity of elastic fibers in OFH: Mann-Whitney U test (two-independent samples test)

Clinical data for 120 cases are summarized in Table 2. Significant differences were observed in the quantity of elastic fibers in the central area of fibroma of the labial mucosa between males and females ($p < 0.05$) (Table 3). However, sex differences were not considered for further statistical tests because there were very few males with fibroma of the labial mucosa (male to female ratio, 4: 16; Table 2).

2) Correlations between quantity of elastic fibers and age, and macrosize in OFH: Spearman's rank correlation

No significant correlations were observed in quantity of elastic fibers and age, and macrosize ($-0.39 \leq r \leq 0.43$). Therefore, further comparisons were performed without considering sex, age, or macrosize.

3) Comparison of quantity of elastic fibers between control and OFH: Mann-Whitney U test (two-independent samples test)

There were significantly greater quantities of elastic fibers in the central areas of

the dorsal surface of tongue in control than in fibroma ($p < 0.05$) (Table 3) (Fig. 6).

4) Comparison of quantity of elastic fibers among OFH in the same area: Kruskal-Wallis H test (several-independent samples test)

Significant differences were observed in each area, and quantities of elastic fibers were particularly marked in fibroma of the labial mucosa (Table 4) (Fig. 7). Two cases of fibroma of the labial mucosa showed particularly high quantities of elastic fibers on the epithelial side (Fig. 2). Moreover, the lesion with the second highest quantity of elastic fibers was fibroma of the buccal mucosa, while fewer elastic fibers were observed in fibrous epulis and fibromatous epulis.

5) Comparison of quantity of elastic fibers among 3 areas in the same case: Friedman test (several-related samples test)

Significant differences were observed in fibroma of buccal mucosa ($p < 0.001$) and fibroma of labial mucosa ($p < 0.01$). Thereafter, each area between fibroma of buccal mucosa and fibroma of labial mucosa was compared, elastic fibers were mostly distributed in the base ($p < 0.01$) (Fig. 8).

3. Immunohistochemical findings

Immunohistochemically, cytoplasm of spindle cells consisted in all OFH cases showed diffusely positive reactions for Vimentin, and negative for Actin except for cells composed of the vascular wall, respectively (Table 1) (Figs. 4, 5). In addition, positive reactions for CD34 were observed in spindle cells and undifferentiated mesenchymal cells around myogenic blood vessels, which were interspersed in the connective tissue in the EF+ group.

Discussion

This study focused on elastic fibers in the connective tissue with regard to OFH, and performed histopathological and histochemical studies of the distribution of elastic fibers. In addition, the quantity of elastic fibers in OFH was investigated using image analysis. To clarify the role of elastic fibers in OFH, lesions were classified into 3 areas and the quantity of elastic fibers was measured and compared among these.

Significant differences were observed only in the tongue, as compared to the quantity of elastic fibers in control and OFH (Fig. 6). This indicated that the quantity of

elastic fibers in OFH was reflected the quantity of elastic fibers in each existing tissue.

The quantity of elastic fibers was particularly high in fibroma of the labial mucosa (Fig. 7), and 2 OFH cases showed markedly higher quantities of elastic fibers on the epithelial side (Fig. 2). The second highest quantities of elastic fibers were seen in fibroma of the buccal mucosa, and there were fewer elastic fibers in fibrous epulis and fibromatous epulis.

On functional classification, the oral mucosa can be divided into 3 main types: lining; specialized; and masticatory. For example, the buccal and labial mucosa are lining mucosa, the dorsal surface of tongue is specialized mucosa, and the gingiva is masticatory mucosa. Elastic fibers are more abundant in flexible lining mucosa, and can be seen in most regions of the oral mucosal tissue. Therefore, the quantity of elastic fibers is higher in the buccal and labial mucosa than on the dorsal surface of tongue and gingiva (17). This indicated that existing elastic fibers, which have site-specific localization, in contrast to collagen fibers in each tissue, were influenced by the distribution of elastic fibers in OFH.

Elastic fibers were commonly concentrated at the base of lesions, near the submucosal tissue in both fibroma of the buccal and labial mucosa wherein elastic

fibers were significantly abundant (Fig. 8). The buccal and labial mucosa accompany submucosal tissue. The capillary plexus in normal buccal and labial mucosa is derived from well-developed myogenic blood vessels present in the submucosal tissue, forming loops while entering the lamina propria, and elastic lamina comprising elastic fibers is present in these vascular walls.

On the other hand, the dorsal surface of tongue and gingiva lack submucosal tissue. However, the deep layer of lamina propria on the dorsal surface of tongue has myogenic blood vessels, similarly to the buccal and labial mucosa. Blood vessels distributed in the gingiva are thought to pass through the surrounding periodontal ligament, alveolar bone, and alveolar mucosa (17). In other words, myogenic tissue is absent near the gingival mucosa, while myogenic tissues such as the buccinator, orbicularis oris muscle, and tongue muscle are present near the mucosal tissue of the buccal mucosa, labial mucosa, and dorsal surface of tongue. This suggested that the presence of myogenic blood vessels with elastic lamina greatly influenced site-specific localization.

We also performed an immunohistochemical study to explore the origin of elastic fibers in OFH. Based on the results of immunohistochemical staining (Table 1) (Figs. 4,

5), spindle cells in all cases of OFH were positive for Vimentin and negative for Actin, respectively. This suggested that OFH was of mesenchymal cell origin, not myofibroblastic origin (5-8). With regard to CD34, spindle cells were positive in all cases in the EF+ group and negative in all cases in the EF– group. This suggested that no correlations existed between collagen fibers and CD34, but that correlations existed between elastic fibers and CD34 (6-8). Therefore, it was a possible that CD34 was involved in the elastic fibers observed in OFH.

It has been reported that CD34-positive spindle cells participate in the formation of elastofibroma (6-8). CD34 is a single-chain transmembrane protein of approximately 116 kDa and is expressed on immature haematopoietic stem/progenitor cells, capillary endothelial cells, and embryonic fibroblasts (18). Consequently, it has been suggested that undifferentiated mesenchymal cells around myogenic blood vessels are related to the formation of the elastic fibers that constitute OFH. In addition, histochemical findings suggested that elastic fibers were derived from the elastic lamina, constituting the vascular wall, and entered the lesion in a continuous or transitional pattern in the upper parts of the lesion (Fig. 3). These results suggested that elastic fibers in OFH were derived from the elastic lamina of myogenic blood vessels in the submucosa or

deep layer of the lamina propria.

CD34-positive cases in the EF+ group were mainly fibroma of the buccal mucosa, labial mucosa, and dorsal surface of tongue (Table 1) (Fig. 4). However, there were also cases with no CD34 expression in the gingiva (Table 1) (Figs. 4, 5). We compared OFH cases occurring in the gingiva, and found 8 CD34-positive cases in the EF+ group with fibroma of the gingiva, and 2 cases with epulis, including fibrous and fibromatous (Table 1). The difference in the number of positive cases may be attributed to the differences in pathogenesis of fibroma and epulis.

There is much discussion over whether fibroma is a real tumor, but it is classified as a nonepithelial benign tumor, whereas epulis is classified as a lesion that synthesizes localized masses in the gingiva, and that originates from connective tissues such as gingiva, periodontal ligament, and alveolar bone periosteum (1). No detailed information is available on the pathogenesis of fibroma of gingiva, but it is commonly accepted that both gingiva and periodontal ligament are involved in epulis.

The elastic system fibers are constituted by fibers of three different types; oxytalan, elaunin, and elastic fibers. The microfibrils consist of the fundamental framework of the elastic system fibers, and elastogenesis is the process of deposition of

elastin. Oxytalan fibers are the bundles of microfibrils that first appear during elastogenesis. The elaunin fibers are considered to be a transitional pattern between oxytalan fibers and elastic fibers (19, 20). All elastic system fibers are observed in the gingival lamina propria, but only oxytalan fibers are observed in the periodontal ligament (21-25). In other words, periodontal ligament fibroblasts with the possibility of the pathogenesis of epulis are not related to elastin secretion. Elastin and microfibrils are produced as well as collagen proteins by fibroblasts (19-25). Therefore, variations in the proteins secreted by the fibroblast in OFH lead to site-specific localization.

In conclusion, elastic fibers were observed in 57.5% of OFH cases. The distribution of these fibers was site-specific, and differed from the collagen fibers that constituted OFH. CD34 positivity was observed in the spindle cells constituting OFH accompanied by elastic fibers, and undifferentiated mesenchymal cells around myogenic blood vessels near the lesion were related to the formation of a part of elastic fibers that constituted OFH.

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Table 1. Number of positive cases by immunohistochemical staining

	Groups (*n*)	Vimentin	Actin	CD34
Fibroma of the buccal mucosa	EF+ (20)	20	0	20
	EF- (0)	—	—	—
Fibroma of the labial mucosa	EF+ (20)	20	0	20
	EF- (0)	—	—	—
Fibroma of the dorsal surface of tongue	EF+ (19)	19	0	19
	EF- (1)	1	0	0
Fibroma of the gingiva	EF+ (8)	8	0	8
	EF- (12)	12	0	0
Fibrous epulis	EF+ (1)	1	0	1
	EF- (19)	19	0	0
Fibromatous epulis	EF+ (1)	1	0	1
	EF- (19)	19	0	0

(cases)

All of cases (n = 120) are confirmed collagen fibers.

EF+: Cases with elastic fibers (n = 69), EF—: Cases without elastic fibers (n = 51)

Table 2. Demographic and clinical characteristics of oral fibrous hyperplasia (OFH) cases (n = 120)

		Age			Size (mm^3)		
	Male : Female	*Med*	*Min*	*Max*	*Med*	*Min*	*Max*
Fibroma of the buccal mucosa	10 : 10	63	14	81	452	100	4500
Fibroma of the labial mucosa	4 : 16	47	17	77	184	36	420
Fibroma of the dorsal surface of tongue	8 : 12	53	37	79	116	12	528
Fibroma of the gingiva	10 : 10	62	27	80	440	1	7000
Fibrous epulis	10 : 10	48	8	74	144	4	2520
Fibromatous epulis	8 : 12	39	15	78	298	30	4480

Med : Median, *Min* : Minimum, *Max* : Maximum

Table 3. Comparison of quantity of elastic fibers between control and OFH

		Epithelial side			Central area				Base		
	n	*Med*	*Min*	*Max*	*Med*	*Min*	*Max*		*Med*	*Min*	*Max*
Control of the buccal mucosa	5	60.9	22.9	70.1	92.6	76.2	110.5				
Fibroma of the buccal mucosa	20	37.1	0.0	159.9	76.4	18.4	145.1		85.8	18.2	214.0
Control of the labial mucosa	5	74.2	13.8	116.4	119.7	64.6	230.4				
Fibroma of the labial mucosa	20	67.4	6.1	535.2	83.1	12.9	222.8	+	132.8	37.9	404.5
Control of the dorsal surface of tongue	5	55.0	34.5	98.6	69.6	54.0	90.0	*			
Fibroma of the dorsal surface of tongue	20	30.2	0.0	139.4	33.3	0.0	83.6		28.4	0.0	87.0
Control of the gingiva	5	0.0	0.0	32.1	0.0	0.0	5.8				
Fibroma of the gingiva	20	0.0	0.0	130.7	0.0	0.0	81.0		0.0	0.0	242.4
Fibrous epulis	20	0.0	0.0	0.0	0.0	0.0	15.4		0.0	0.0	103.5
Fibromatous epulis	20	0.0	0.0	0.0	0.0	0.0	16.3		0.0	0.0	8.7

($\times 10^3$ pixel count)

Minimum (*Min*), median (*Med*), and maximum (*Max*) are listed as representative values in the table, because nonparametric test is performed.

Sex differences: $+p < 0.05$ (Male < Female) (Mann-Whitney U test)

Comparison of OFH: $*p < 0.05$ (Mann-Whitney U test)

Table 4. Comparison of quantity of elastic fibers among OFH in the same area

Epithelial side	Fibroma of the buccal mucosa	Fibroma of the labial mucosa	Fibroma of the dorsal surface of tongue	Fibroma of the gingiva	Fibrous epulis	Fibromatous epulis
Fibroma of the buccal mucosa						
Fibroma of the labial mucosa	*ns*					
Fibroma of the dorsal surface of tongue	*ns*	*ns*				
Fibroma of the gingiva	***	***	*			
Fibrous epulis	***	***	***	*ns*		
Fibromatous epulis	***	***	***	*ns*	*ns*	

Central area	Fibroma of the buccal mucosa	Fibroma of the labial mucosa	Fibroma of the dorsal surface of tongue	Fibroma of the gingiva	Fibrous epulis	Fibromatous epulis
Fibroma of the buccal mucosa						
Fibroma of the labial mucosa	*ns*					
Fibroma of the dorsal surface of tongue	*ns*	*ns*				
Fibroma of the gingiva	***	***	*ns*			
Fibrous epulis	***	***	***	*ns*		
Fibromatous epulis	***	***	***	*ns*	*ns*	

Base	Fibroma of the buccal mucosa	Fibroma of the labial mucosa	Fibroma of the dorsal surface of tongue	Fibroma of the gingiva	Fibrous epulis	Fibromatous epulis
Fibroma of the buccal mucosa						
Fibroma of the labial mucosa	*ns*					
Fibroma of the dorsal surface of tongue	*ns*	**				
Fibroma of the gingiva	*	***	*ns*			
Fibrous epulis	***	***	**	*ns*		
Fibromatous epulis	***	***	**	*ns*	*ns*	

ns : Not statistically significant

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (Kruskal-Wallis H test)

Figure legends

Fig. 1 Case of oral fibrous hyperplasia (OFH)

Before excision (a), Excised specimen (b), Hematoxylin-eosin staining (c: ×40)

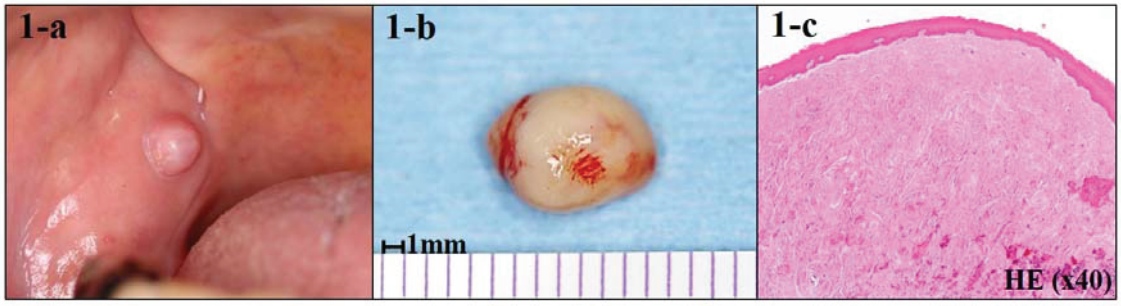
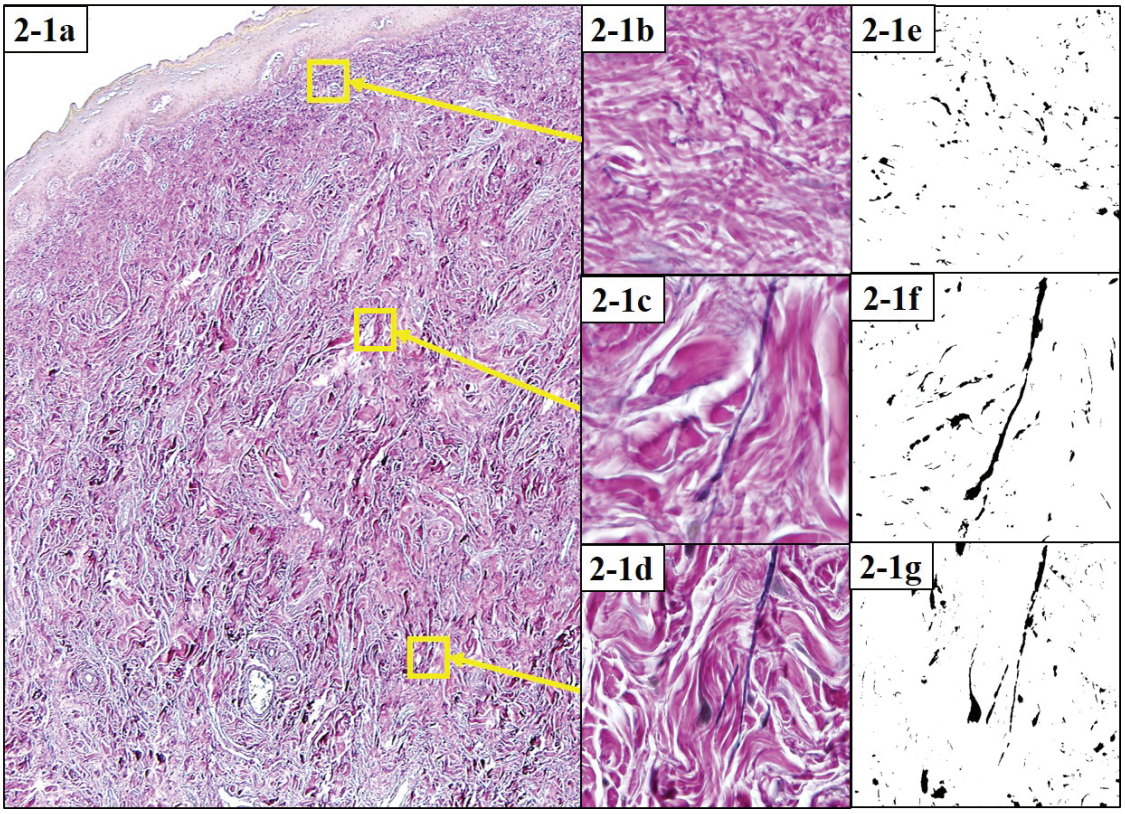


Fig. 2 Image analysis method (binarization), and Elastica van Gieson (EvG) finding of OFH

The lamina propria is classified into 3 areas based on collagen fibers orientation (1a, 2a; ×40) (1b-g, 2b-g; ×400). The epithelial side is defined the area of ≤100μm below the basal lamina (1b, 2b). The central area is below the epithelial side (1c, 2c). The base is defined as the clinical base of the mass (1d, 2d). The hot spot (yellow squares; 1a, 2a) is defined as the 100μm × 100μm area with the greatest quantity of elastic fibers (1b-d, 2b-d). After images are binarized using Image J, elastic fibers are extracted (1e-g, 2e-g).

Elastic fibers show a fine granular appearance on the epithelial side (1b), and elongated filamentous morphology in the central area and base (1c, 1d). Two cases of fibroma of the labial mucosa show different patterns of elastic fibers, which appear thick irregular fascicular bundles or globules on the epithelial side (2b).


2-1a
2-1b
2-1e
2-1c
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2-1d
2-1g

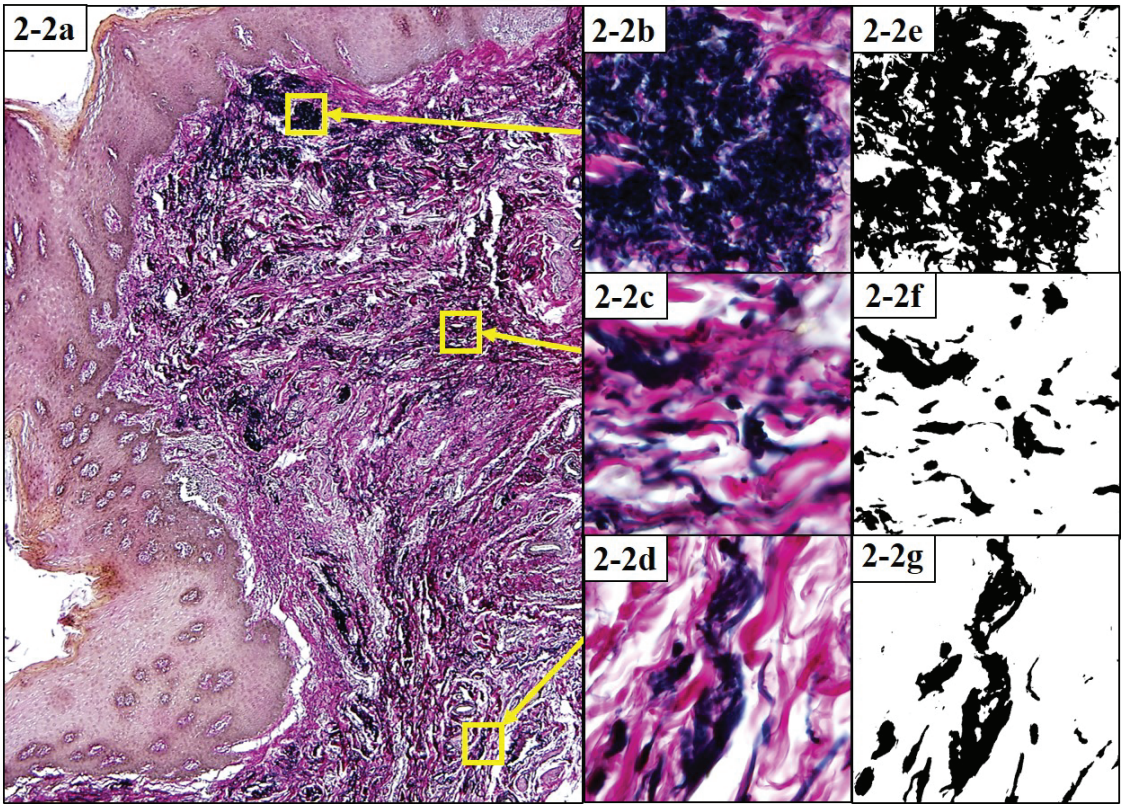
2-2a
2-2b
2-2e
2-2c
2-2f
2-2d
2-2g

Fig. 3 EvG finding in deep layer of lesion

Elastic fibers are observed into the elastic lamina, which constitutes the vascular wall of myogenic blood vessels, in the submucosal tissues. These elastic fibers are comparatively isolated, intermingled with hyperplastic collagen fibers bundles, and extend toward the epithelial side in the lesion (arrow) (×200).

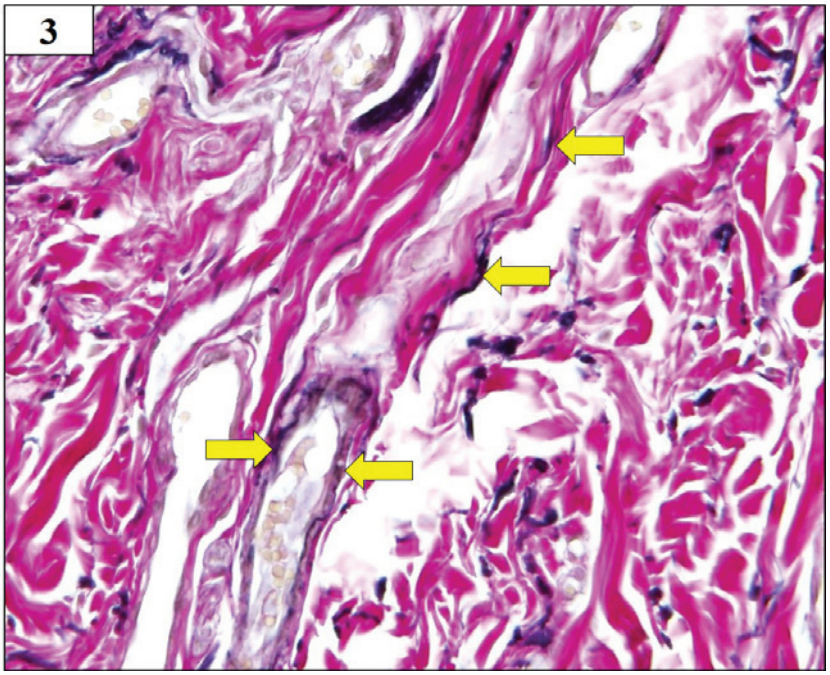

Fig. 4 EvG and immunohistochemical staining of cases in EF+ group

Cases with elastic fibers (EF+ group) include fibroma of the buccal mucosa (1a-d), fibroma of the labial mucosa (2a-d), fibroma of the dorsal surface of tongue (3a-d), and fibroma of the gingiva (4a-d). Cytoplasm of spindle cells in all of cases show diffusely positive reactions for Vimentin (1-4b), and negative for Actin (1-4c). Positive reactions for CD34 are observed in spindle cells, as well as in undifferentiated mesenchymal cells around the myogenic blood vessels, which intersperse into connective tissues (1-4d) (×200).

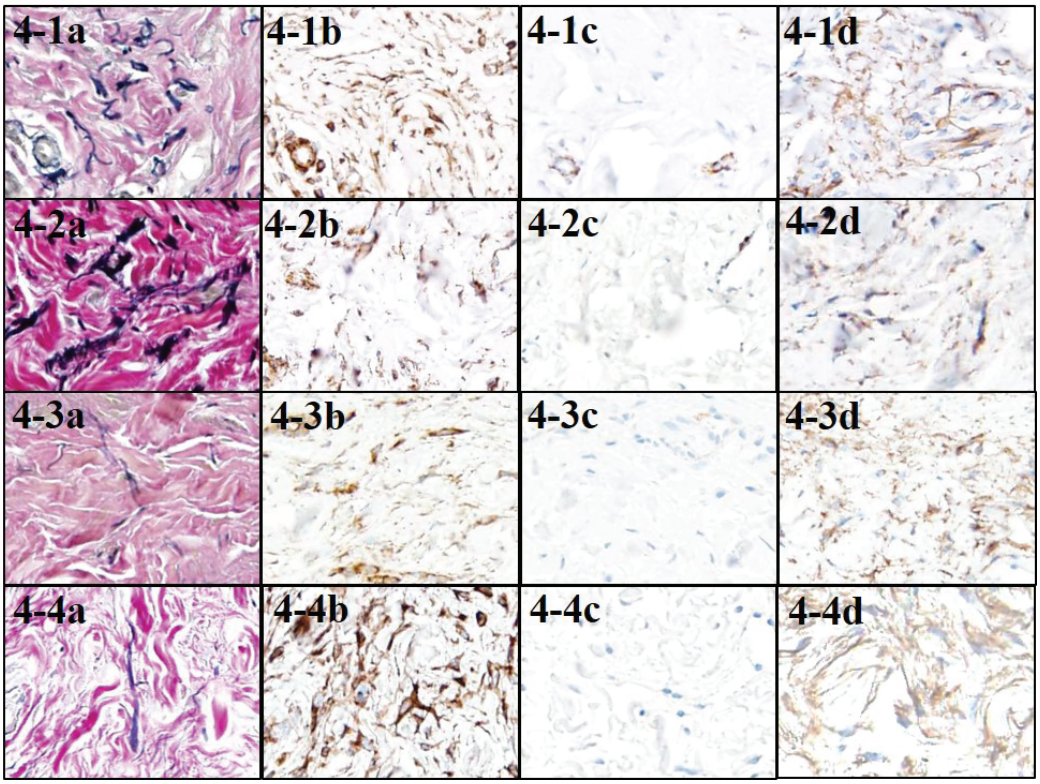

Fig. 5 EvG and immunohistochemical staining of cases in EF- group

Cases without elastic fibers (EF− group) include fibroma of the dorsal surface of tongue (1a-d), fibroma of the gingiva (2a-d), and fibrous and fibromatous epulis (3a-d). Cytoplasm of spindle cells in all of cases show positive reactions for Vimentin (1-3b), and negative for Actin (1-3c) and CD34 (1-3d) (×200).

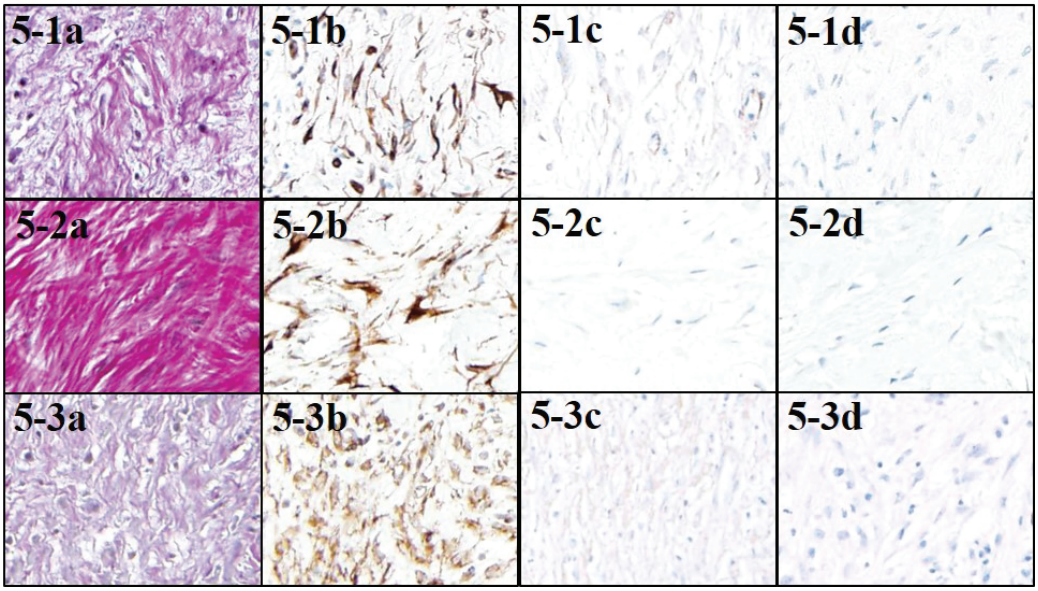

Fig. 6 Comparison of quantity of elastic fibers between control and OFH

There are significantly greater quantities of elastic fibers in the central area of the dorsal surface of tongue in control than in fibroma ($^{*}p < 0.05$) (Mann-Whitney U test).

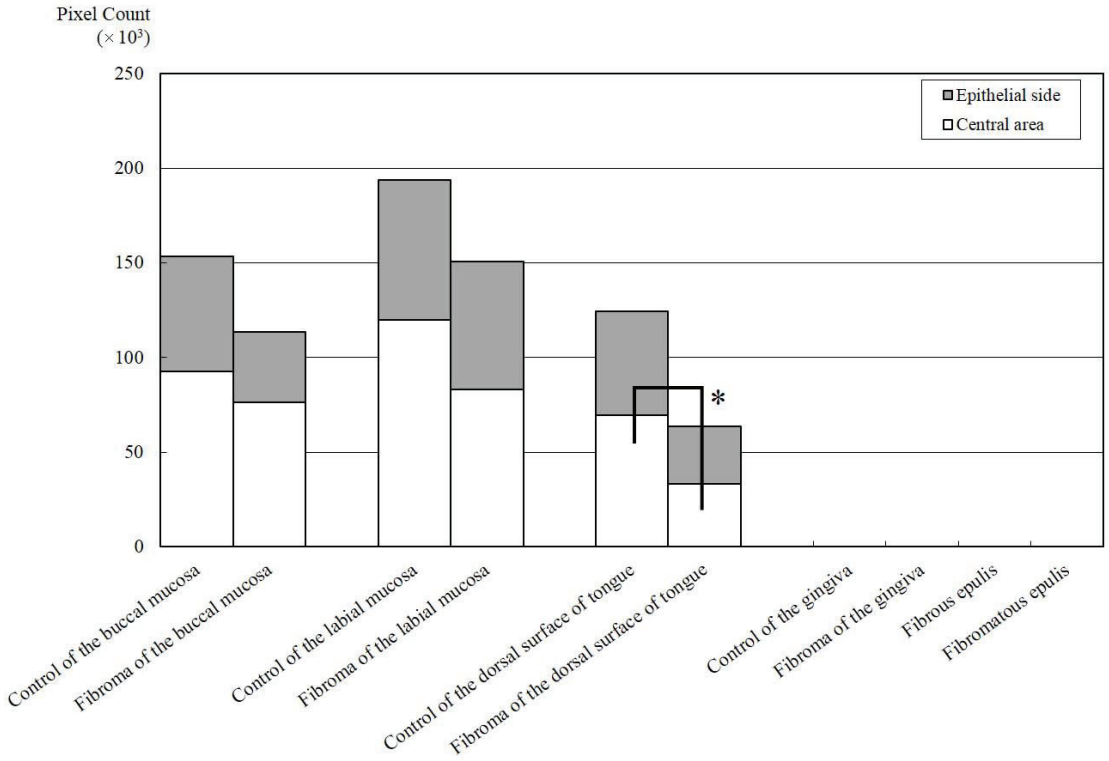

Fig. 7 Comparison of quantity of elastic fibers among OFH in the same area

Significant differences are observed in each area, and the quantity of elastic fibers is particularly marked in fibroma of the labial mucosa. The lesion with the second greatest quantity of elastic fibers is fibroma of the buccal mucosa, while fewer elastic fibers are seen in fibrous epulis and fibromatous epulis (Kruskal-Wallis H test).

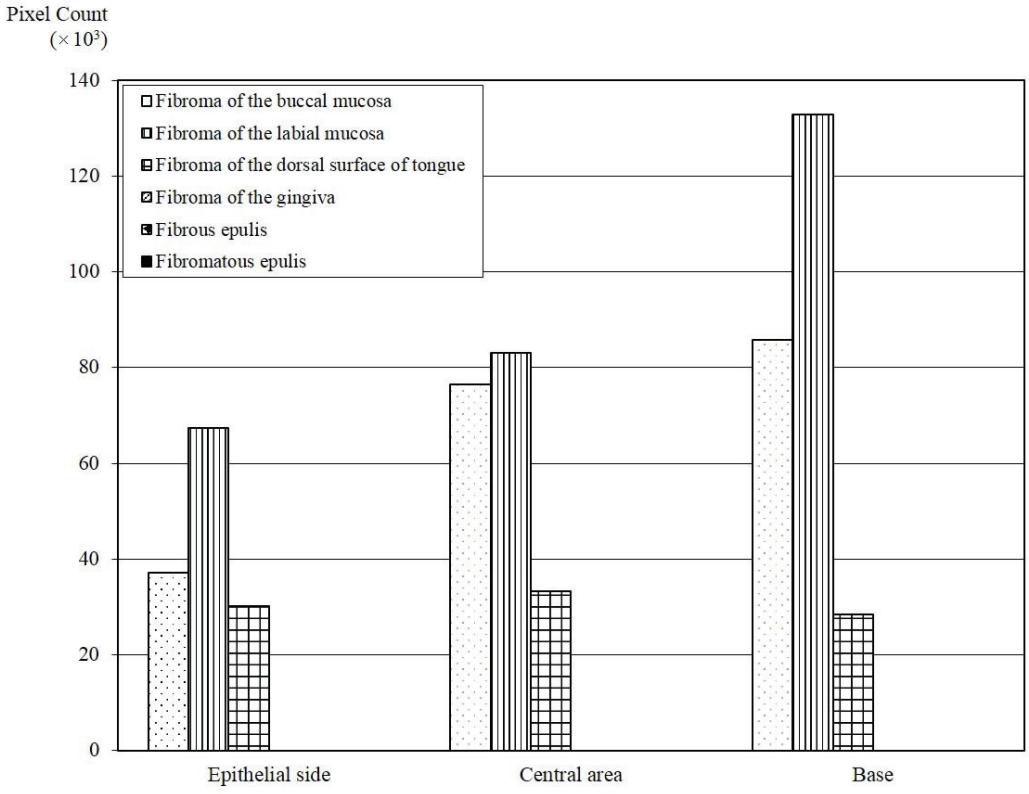

Fig. 8 Comparison of quantity of elastic fibers among 3 areas in the same case

Significant differences are observed in fibroma of the buccal mucosa ($^{***}p < 0.001$) and fibroma of the labial mucosa ($^{**}p < 0.01$). Elastic fibers are mainly distributed in the base ($^{**}p < 0.01$) (Friedman test).