Immunohistochemical expression of Fibrillin-1 and Fibrillin-2 during tooth development

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ABSTRACT

Background and Objective: Oxytalan fibers are categorized as a microfibril assembly without elastin deposition, and exist as very unique components in the periodontal ligament (PDL). However, little is known about their formation during PDL development. To clarify the mechanisms of oxytalan fiber formation in developing PDL, we performed immunohistochemical analysis to detect the direct expression of fibrillin-1 and fibrillin-2, which are major components of microfibrils.

Methods: Frozen sections of lower molars from mice at several stages of growth were prepared without chemical fixation and decalcification using the film transfer method. Immunostaining was performed with anti-fibrillin-1 and -2, and anti-cytokeratin antibodies.

Results: Fibrillin-1 was not expressed in the dental follicle during the crown forming stage. At post-neonatal day 9, fibrillin-1 expression started with meshwork appearance between the epithelial cells from Hertwig's epithelial root sheath (HERS) at the root dentin surface. Fibirillin-2 was detected much earlier than fibrillin-1 expression. Fibrillin-2 was expressed with a liner appearance, running parallel to the root axis in PDL, and was partially co-expressed with cytokeratin-14 (CK14) expression in HERS. Furthermore, we detected both fibrillin-1 and fibrillin-2 expression in human PDL. Fibrillin-1 was detected in fibers with a vertically oriented root axis in PDL. Fibrillin-2 was widely expressed in PDL, including around the epithelial cells rest of Malassez. Fibrillin-1 and fibrillin-2 were clearly co-expressed in thick fiber structures in human PDL.

Conclusion: Our results suggest that both of fibrillin-1 and fibrillin-2 expression is required to form thick oxytalan fibers in PDL. Based on the expression patterns for

fibrillin-1 and fibrillin-2, they have different functions during tooth root and PDL development. Early expression of fibrillin-2 may regulate dental epithelial cell behavior during root and PDL development.

INTRODUCTION

Oxytalan fibers are composed of pure microfibrils without elastin deposition, and were first described in PDL (1-5). Oxytalan fibers show unique features, such as vertically oriented enclosure of the root axis, perpendicular orientation of collagen fibers, closer proximity to the cementum than to the alveolar bone, and frequent insertion into the root cementum (4,6). These unique features of oxytalan fibers suggest that they play a role in maintaining PDL, similarly to collagen and Sharpey's fibers, between the tooth root and alveolar bone. However, the mechanism of their development remains unclear.

Visualization of oxytalan fibers during PDL development has classically depended on stains such as resorcin-fuchsin (7-10), but this has been insufficient for investigations into the mechanisms of oxytalan fiber formation during PDL development.

Fibrillins are extracellular matrix proteins and are major components of microfibrils. Fibrillin-1 and fibrillin-2, have high homology with regard to overall amino acid components, and have instructive roles of microfibrils in several stages and tissues (11-13). The intrinsic properties of fibrilli-1 and fibrillin-2 have been discussed in the development and maturation stages of microfibril formation. The different spatiotemporal expression of fibrillin-1 and fibrillin-2 during embryogenesis has been examined in several organs (14). For example, fibrillin-2 is expressed earlier than fibrillin-1 in eye, lung and liver development in mice (15-17). In developing ciliary zonule, which is an oxytalan fiber, fibrillin-2 mRNA is expressed first, while fibrillin-1 mRNA expression occurs later. However, fibrillin-1-deficient mice have an intact zonule with fibrillin-2 expression. These observations suggest that the role of fibrillin-1 and fibrillin-2 partially overlap, but differ in temporal and tissue specificity during microfibril development.

With regard to the biological function of fibrillins, numerous molecules, including LTBPs, members of the ADAMTS-like family and fibronectin, have been identified as microfibril-associated proteins (13). Furthermore, fibrillin-rich microfibrils contribute to the extracellular regulation of endogenous TGF-β or BMP activities; thus, microfibrils also act as signal regulators.

According to recent progress in studies of elastic system fibers, interest in the biological function of oxytalan fibers in PDL has also increased. With regard to the biological function of oxytalan fibers in PDL, the molecular interactions between fibrillin-1 and periostin or ADAMTSLβ have been examined using *Fbn1*$^{MgR/MgR}$; fibrillin-1 deficient mice with defects in the periodontal ligament (18,19). Their findings using a model of PDL disease with defects in oxytalan fiber formation demonstrated a therapeutic strategy for systematic connective tissue problems.

We have investigated whether formation of thick oxytalan fibers is required for secretion of fibrillin-1 and fibrillin-2 with MAGP or emilin deposition using periodontal fibroblasts (20-23). We demonstrated that fibrillin-1 suppression did not lead to the formation of fibrillin-2-positive thick fibers, whereas fibrillin-2 suppression led to the formation of fibrillin-1-positive thin fibers, but not thick fibers using nonpigmented ciliary epithelial cells, which also compose oxytalan fibers (24,25).

However, there is little information on how fibrillin-1 and fibrillin-2 form microfibrils during periodontal ligament development in vivo. Studies into the spatial and temporal expression of fibrillin-1 and fibrillin-2 are thus necessary.

In this present study, we investigated the biological mechanisms of oxytalan fiber formation by tracing fibrillin-1 and fibrillin-2 expression during tooth development using immunohistochemical analyses.

MATERIALS AND METHODS

The protocol for these experiments was reviewed and approved by the Animal Committee and Research Ethics Committee of the Fukuoka Dental College, and informed consent was obtained from all tissue donors (No. 239).

Histological analysis preparation

Fiber staining

In order to visualize elastic system fibers, mandibular bone was dissected from mice at P10. After fixation and decalcification, paraffin sections were prepared and stained with resorcin-fuchsin solution (Muto Pure Chemicals, Tokyo, Japan) after 10% oxone (Wako, Osaka, Japan) treatment, followed by counterstaining with 1% Orange G (Wako, Osaka, Japan).

Immunohistochemistry

ICR mice were prepared at embryonic stage 14.5 (E14.5), newborn (NB), and at postnatal days 5 (P5), 9 (P9) and 12 (P12). Mouse head or mandibular bones were dissected without any chemical fixation or decalcification, and were embedded in super cryo-embedding medium, and rapid-frozen using the hexane-dry ice method. Samples were cut with a cryostat into 6-μm sections for immunohistochemistry. Mouse root-forming stage samples were cut using the film transfer method (Leica Microsystems, Wetzlar, Germany) (26).

Human erupted lower second premolars extracted for orthodontic reasons were also prepared and cut without chemical fixation and decalcification using the film transfer method for immunohistochemistry.

Primary antibodies used were rabbit anti-fibrillin-1 polyclonal (Abcam Japan, Tokyo

Japan), rabbit anti-fibrillin-2 polyclonal (Elastin Products Co., Owensville, MO, USA), mouse anti-cytokeratin 14 monoclonal antibodies (Abcam Japan, Tokyo Japan). Immunoreactions were visualized on sections with anti-IgG antibody conjugated with Alexa Fluor™ 488 and 594 (Molecular probes, Eugene, OR, USA), and were then counterstained with DAPI (Vector Laboratory, Burlingame, CA, USA). As negative controls, sections were incubated with secondary antibody only. For double staining of fibrillin-1 and fibrillin2, mouse anti-fibrillin-1 (Thermo Fisher Scientific, Fremont, CA, USA) was used in human samples.

RESULTS

Fibrillin-1 is not expressed in tooth germ, but fibrillin-2 is expressed in dental papilla and follicle during crown-forming stage

The appearance of elastic fibers at P10 was confirmed using Resorcin-fuchsin staining (Fig. 1A). Thick, vertically oriented fibers were detected in PDL, and the tip of these fibers was observed attaching to the surface of root dentin (Fig. 1B). In addition, numerous thin fibers were located at the apical end of the developing root (Fig. 1C). Next, in order to examine the expression of fibrillin-1 and fibrillin-2 during tooth development, we performed immunohistochemical analysis in mouse tooth germ at E14.5 and NB. Fibrillin-1 was not expressed in tooth germ (Fig. 1E), but fibrillin-2 expression was observed in the dental papilla and follicle beginning at E14.5 (Fig. 1F). In later stages, fibrillin-1 was detected in alveolar bone and the tendon of masseter muscle, but not in the dental follicle at new bone (NB) (Fig. 1H and 1K). In contrast, fibrillin-2 expression was strongly observed in the dental papilla and dental follicle (Fig. 1I and L).

Fibrillin1 expression starts in periodontal ligament at root surface during root-forming stage

Next, we examined fibrillin-1 expression during the root-forming stage. Fibrillin-1 expression was not detected in PDL at P5 (Fig. 2B and D). At P9, finally, the meshwork expression of fibrillin-1 was visualized in PDL (Fig. 2F and H, arrows). This expression was detected initially at the labial side of the root dentin surface between fragmented epithelial cells from HERS. Double-staining of fibrillin-1 and cytokeratin 14 (CK14) was performed in order to clearly detect staining in epithelial cells (Fig. 2I). Fibirillin-1

expression was seen in the extra-cellular region between CK14-positive epithelial cells on the surface of the root dentin (Fig. 2J, K). Fibrillin-1 expression was not detected in the HERS region (Fig. 2L). At P12, a meshwork appearance of fibrillin-1 expression was observed on both the lingual and labial sides of PDL (Fig. 2M). Some epithelial cells with CK14 staining located on the root dentin surface were close to fibrillin-1 fibers (Fig. 2N, P). According to these results, fibrillin-1 fibers start to form among the interrupted epithelial cells on the root surface at P9. Expression becomes more widespread during development of PDL.

Fibrillin-2 is expressed in developing PDL, including Hertwig's epithelial root sheath

At P5, fiber-like expression of fibrillin-2 was seen in the dental papilla and dental follicle (Fig. 3A). Fibrillin-2-positive thin fibers were short, but were parallel to the root axis (Fig. 3B). At this stage, these fibrillin-2 fibers were localized near epithelial cells, such as HERS. Partial double staining of fibrillin-2 and CK14 was observed in the HERS region at P5 (Fig. 3D). At P9 and P12, fibrillin-2 fibers were elongated with parallel orientation along the root axis (Fig. 3E, I). The shape of fibrillin-2-positive fibers was different from that of fibrillin-1-positive fibers, but both were located on the surface of root dentin (Fig. 3F, G, J, K). During root development, the area of fibrillin-2 expression in dental papilla became limited at the apical end of the developing root region. Interestingly, HERS cells were observed to be wrapped with fibrillin-2 fibers (Fig. 3H and L).

Both fibrillin-1 and fibrillin-2 are present in thick oxytalan fibers in PDL of human premolars

Next, we examined the expression of fibrillins in mature human PDL. Erupted second premolars were extracted for orthodontic reasons. Tooth root with PDL tissue was sectioned for immunohistochemical analysis (Fig. 4A-C). Fibrillin-1-expressing fibers were observed in PDL with parallel orientation next to the root (Fig. 4D). Fibrillin-2 was expressed widely in PDL. On the alveolar bone side, fibrillin-2 expression was seen in non-fiber structures (Fig. 4E), while fibrillin-1 and fibrillin-2 were clearly co-expressed in thick fibers on the root side of PDL (Fig. 4F). Epithelial cell rests of Malassez were detected by cytokeratin 14 staining (Fig. 4G), and fibrillin-2 expression was clearly co-expressed with cytokeratin 14 expression (Fig. 4I). These results indicate that fibrillin-2 is expressed in epithelial cells in PDL.

DISCUSSION

Mutation in the fibrillin-1 gene causes Marfan syndrome (MFS:OMIM 154700), which is associated with deformities in the skeletal, pulmonary and cardiovascular systems, and orofacial manifestations, such as ectopia lens and high palatal arch (27-30). On the other hand, mutation in the fibrillin-2 gene is related to congenital contractual arachnodactyly (CCA:OMIM 121050), which has similar phenotypes as MFS (31). These two syndromes show partially overlapping phenotypes. For example, the severe periodontitis phenotype has been reported in MFS, but not in CCA (32, 33). To maintain PDL, fibrillin-1 is a critical factor in oxytalan fiber formation and it is necessary to compare the spatiotemporal expression of fibrillin-1 and fibrillin-2 during PDL development.

The localization of oxytalan fibers in PDL has been described in several articles. In most of these cases, fiber staining methods such as aldehyde-fuchsin or resorcin-fuchsin were used to detect oxytalan fibers (7,9,34). Discussion of the biological functions of these fibers to date has been limited to morphological observations. Therefore, clarifying the direct expression of fibrillin-1 and fibrillin-2, which are major components of microfibrils, is necessary. However, because of chemical fixation, demineralization or paraffin wax often masks the sensitive of epitopes of antigens, and it has been difficult to clearly show fibrillin expression. In present study, we successfully detected fibirlllin-1 and fibrillin-2 expression using the film transfer method, which does not require fixation or demineralization.

During tooth crown and root development, fibrillin-2 expression in the dental papilla and follicle was detected at E14.5, which is earlier than fibrillin-1 detection. This indicates that only fibrillin-2 homotypic fibers exist in the early crown and root

developing stage. Therefore, the previous observation of short and thin fibers with aldehyde or resolcin-fuchsin staining in the early stages of tooth development apparently consists only of fibrillin-2 (7,9). This is also observed in ciliary zonule development in mice (35). Fibrillin-2 is predominantly expressed during the embryonic period, whereas fibrillin-1 expression is initiated during late embryogenesis and dominates the juvenile and adult periods. The ciliary zonule is also categorized as oxytalan fibers. Fibrillin-2 may thus have an important role in the initiation of oxytalan fiber development.

Furthermore, we focused on where fibrillin expression started. To investigate the fibrillin expression related to dental epithelial cells for the PDL development, we performed double staining for both fibrillins and CK14. The biological significance of HERS in tooth root formation is known, but its role in fiber formation during PDL development has not been thoroughly investigated. According to our observations, fibrillin-1 positive fibers were present on the surface of root dentin where continuous HERS elongation was interrupted. Fibrillin-2-positive fibers also were seen around the elongated and entwined HERS. We believe HERS and cementoblasts participate in the initiation of oxytalan fibers during root development, and this may be one why the tip of oxytalan fibers is present in cementum.

Because immunohistochemical analysis is insufficient for assessing the production of fibrillins in epithelial cells, further investigation by in situ hybridization is needed to clarify whether dental epithelial cells or cementoblasts are able to directly produce fibrillins.

In contrast with the developmental stage, elongated thick oxytalan fibers with both of fibrillin-1 and fibrillin-2 expression were observed in human PDL. This observation

supports our previous conclusions regarding the necessity of both fibrillin-1 and firillin-2 expression to form thick oxytalan fibers in vitro (23,25). Single expression of fibrillin-2 with a non-fiber structure was widely seen in human PDL. Fibrillin-2 may therefore have an original function unrelated to oxytalan fiber formation. Interestingly, fibrllin-2 was observed in Malassez epithelial cells in human PDL. There have been several reports showing that epithelial cell rests of Malassez are not silent, and that they have the potential to proliferate and differentiate in response to environmental factors. Together with fibrillin-2 expression in the developing stage, Malassez epithelial cells have the potential to regenerate oxytalan fibers when PDL is damaged.

In this study, the localization and distribution of fibrillin-1 and fibrillin-2 in PDL were clarified during the developmental and maturing periods. The present finding will be helpful for understanding the mechanisms of degradation and regeneration of oxytalan fibers.

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References

1. Fullmer HM, Lillie RD. The oxytalan fiber: a previously undescribed connective tissue fiber. *J Histochem Cytochem* 1958;**6**:425-430.
2. Fullmer HM. Observations on the development of oxytalan fibers in the periodontium of man. *J Dent Res* 1959;**38**:510-518.
3. Bock P, Stockinger L. Light and electron microscopic identification of elastic, elaunin and oxytalan fibers in human tracheal and bronchial mucosa. *Anat Embryol (Berl)* 1984;**170**:145-153.
4. Sims MR. Oxytalan fiber system of molars in the mouse mandible. *J Dent Res* 1973;**52**:797-802.
5. Tsuruga E, Nakashima K, Ishikawa H, Yajima T, Sawa Y. Stretching modulates oxytalan fibers in human periodontal ligament cells. *J Periodontal Res* 2009;**44**:170-174.
6. Sculean A, Karring T, Theilade J, Lioubavina N. The regenerative potential of oxytalan fibers. An experimental study in the monkey. *J Clin Periodontol* 1997;**24**:932-936.
7. Inoue K, Hara Y, Kuroda N, Sato T. Development of the oxytalan fiber system in the periodontal space of rat incisors. *Ann Anat* 2013;**195**:475-483.
8. Ganburged G, Suda N, Saito M, Yamazaki Y, Isokawa K, Moriyama K. Dilated capillaries, disorganized collagen fibers and differential gene expression in periodontal ligaments of hypomorphic fibrillin-1 mice. *Cell Tissue Res* 2010;**341**:381-395.
9. Inoue K, Hara Y, Sato T. Development of the oxytalan fiber system in the rat molar periodontal ligament evaluated by light- and electron-microscopic analyses. *Ann*

Anat 2012;**194**:482-488.

10. Strydom H, Maltha JC, Kuijpers-Jagtman AM, Von den Hoff JW. The oxytalan fibre network in the periodontium and its possible mechanical function. *Arch Oral Biol* 2012;**57**:1003-1011.
11. Olivieri J, Smaldone S, Ramirez F. Fibrillin assemblies: extracellular determinants of tissue formation and fibrosis. *Fibrogenesis Tissue Repair* 2010;**3**:24.
12. Lin G, Tiedemann K, Vollbrandt T, Peters H, Batge B, Brinckmann J, Reinhardt DP. Homo- and heterotypic fibrillin-1 and -2 interactions constitute the basis for the assembly of microfibrils. *J Biol Chem* 2002;**277**:50795-50804.
13. Ramirez F, Sakai LY. Biogenesis and function of fibrillin assemblies. *Cell Tissue Res* 2010;**339**:71-82.
14. Davis MR, Summers KM. Structure and function of the mammalian fibrillin gene family: Implications for human connective tissue diseases. *Mol Genet Metab* 2012;**107**:635-647.
15. Beene LC, Wang LW, Hubmacher D, Keene DR, Reinhardt DP, Annis DS, Mosher DF, Mecham RP, Traboulsi EI, Apte SS. Nonselective assembly of fibrillin 1 and fibrillin 2 in the rodent ocular zonule and in cultured cells: implications for Marfan syndrome. *Invest Ophthalmol Vis Sci* 2013;**54**:8337-8344.
16. Zhang H, Hu W, Ramirez F. Developmental expression of fibrillin genes suggests heterogeneity of extracellular microfibrils. *J Cell Biol* 1995;**129**:1165-1176.
17. Quondamatteo F, Reinhardt DP, Charbonneau NL, Pophal G, Sakai LY, Herken R. Fibrillin-1 and fibrillin-2 in human embryonic and early fetal development. *Matrix Biol* 2002;21:637-646.
18. Saito M, Kurokawa M, Oda M, Oshima M, Tsutsui K, Kosaka K, Nakao K, Ogawa M,

Manabe R, Suda N, Ganjargal G, Hada Y, Noguchi T, Teranaka T, Sekiguchi K, Yoneda T, Tsuji T. ADAMTSL6 β Protein Rescues Fibrillin-1 Microfibril Disorder in a Marfan Syndrome Mouse Model through the Promotion of Fibrillin-1 Assembly. *J Bio Chem* 2011;**286**:38602-38613.

19. Ganburged G, Suda N, Saito M, Yamazaki Y, Isokawa K, Moriyama K.Dilated capillaries, disorganized collagen fibers and differential gene expression in periodontal ligaments of hypomorphic fibrillin-1 mice. *Cell Tissue Res* 2010;**341**:381-395.
20. Tsuruga E, Irie K, Sakakura Y, Yajima T. Expression of fibrillins and tropoelastin by human gingival and periodontal ligament fibroblasts in vitro. *J Periodontal Res* 2002;**37**:23-28.
21. Tsuruga E, Irie K, Yajima T. Gene expression and accumulation of fibrillin-1, fibrillin-2, and tropoelastin in cultured periodontal fibroblasts. *J Dent Res* 2002;**81**:771-775.
22. Fujita T, Tsuruga E, Yamanouchi K, Sawa Y, Ishikawa H. Microfibril-Associated Glycoprotein-1 Controls Human Ciliary Zonule Development *InVitro*. *Acta Histochem. Cytochem* 2014;**47**:11-17.
23. Nakatomi Y, Tsuruga E, Nakashima K, Sawa Y, Ishikawa H. EMILIN-1 regulates the amount of oxytalan fiber formation in periodontal ligaments in vitro. *Connent Tissue Res* 2011;52:30-35.
24. Kawagoe M, Tsuruga E, Oka K, Sawa Y, Ishikawa H. Matrix metalloproteinase-2 degrades fibrillin-1 and fibrillin-2 of oxytalan fibers in the human eye and periodontal ligaments in vitro. *Acta Histochem Cytochem* 2013;**46**:153-159.
25. Yamanouchi K, Tsuruga E, Oka K, Sawa Y, Ishikawa H. Fibrillin-1 and fibrillin-2

are essential for formation of thick oxytalan fibers in human nonpigmented ciliary epithelial cells in vitro. *Connect Tissue Res* 2012;**53**:14-20.

26. Kawamoto T. Use of a new adhesive film for the preparation of multi-purpose fresh-frozen sections from hard tissues, whole-animals, insects and plants. *Arch Histol Cytol* 2003;**66**:123-143.
27. Ramirez F, Dietz HC. Fibrillin-rich microfibrils: Structural determinants of morphogenetic and homeostatic events. *J Cell Physiol* 2007;**213**:326-330.
28. Judge DP, Dietz HC. Marfan's syndrome. *Lancet* 2005;**366**:1965-1976.
29. Suda N, Shiga M, Ganburged G, Moriyama K. Marfan syndrome and its disorder in periodontal tissues. *J Exp Zool B Mol Dev Evol* 2009;**312B**:503-509.
30. Pyeritz RE. The Marfan syndrome. *Annu Rev Med* 2000;**51**:481-510.
31. Putnam EA, Zhang H, Ramirez F, Milewicz DM. Fibrillin-2 (FBN2) mutations result in the Marfan-like disorder, congenital contractural arachnodactyly. *Nat Genet* 1995;**11**:456-458.
32. De Coster PJ, Martens LC, De Paepe A. Oral manifestations of patients with Marfan syndrome: a case-control study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002;**93**:564-572.
33. Shiga M, Saito M, Hattori M, Torii C, Kosaki K, Kiyono T, Suda N. Characteristic phenotype of immortalized periodontal cells isolated from a Marfan syndrome type I patient. *Cell Tissue Res* 2008;**331**:461-472.
34. Kondo Y, Sawada T, Shibayama K, Inoue S. Elastic system fibers in rat incisor periodontal ligament--immunohistochemical study using sections of fresh-frozen un-demineralized tissues. *Acta Histochem* 2011;**113**:125-130.
35. Shi Y, Tu Y, De Maria AD, Mecham RP, Bassnett S. Development, Composition , and

Structural Arrangements of the Ciliary Zonule of the Mouse. *Invest Ophthalmol Vis Sci* 2013;54:2504-2515.

Figure legends

Figure 1.

Fibrillin-1 and fibrillin2 expression in crown development stage. (A-C) Lower first molars at PN10 were stained with Resorcin-fuchsin. (B, C) Higher magnification appearance of PDL and apical end of developing root of boxes in A. (D-L) H&E staining, Fibrillin-1 and Fibrillin-2 in first lower tooth germ at E14.5 (D-F) and NB (G-I). (J-L) Higher magnification of buccal cervical loop regions showed in (G-I). Arrows indicate thick fibers in PDL. Arrowheads indicate fiber in cementum. Bar=200 μm (A, D-I), 50 μm (B, C, J-L). D; Dentin E; Enamel PDL; Periodontal ligament DE; Dental epithelium DM; Dental mesenchyme DP; Dental Pulp AB; Alveolar Bone CL; Cervical Loop,

Figure 2.

Fibrillin-1 expression in root development stage. (A-D) H&E and Fibrillin-1 expression at P5. (C, D) Higher magnification of boxes in A and B, respectively. (E-H) H&E and Fibrillin-1 expression at P9. (G, H) Higher magnification of boxes in E and F, respectively. (I-P) Expression of Fibrillin-1 (green), Cytokeratin 14 (red) and H&E staining at P9 (I-L) and P12 (M-P). (J, K) Higher magnification of boxes in I and J, respectively. (N, P) Higher magnification of box in M. Bar=200 μm (A, B, E, F, I), 100 μm (M), 50 μm (C, D, G, H, J, L, O, P), 25 μm (K, N) DP; Dental Pulp AB; Alveolar Bone D; Dentin PDL; Periodontal ligament

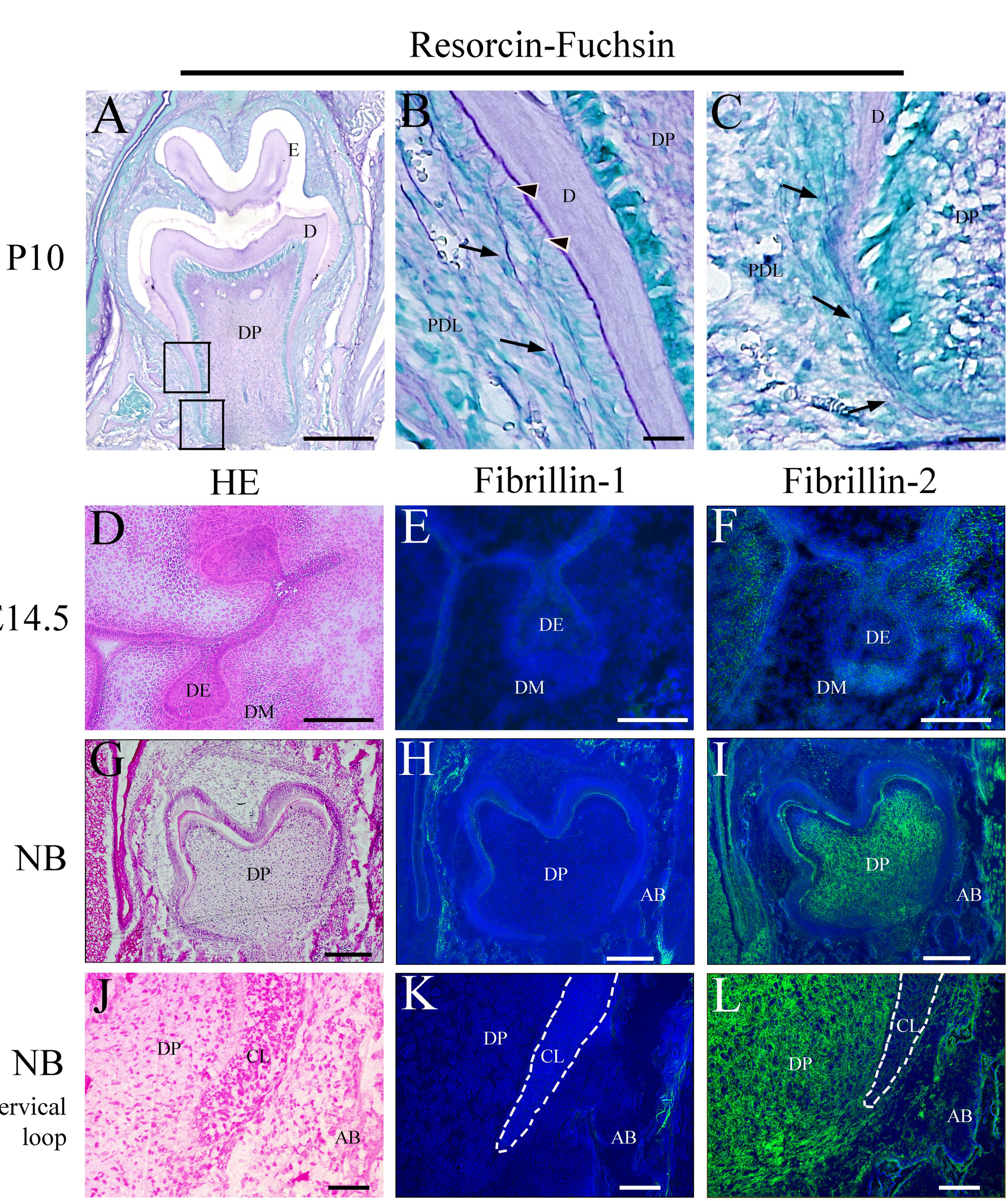
Figure 3.

Fibrillin-2 expression in root development stage. (A-D) Fibrillin-2 and Cytokeratin

expression at P5. (B-C) Higher magnification of box in A. (E-L) Double-staining of Fibrillin-2 (green) and Cytokeratin 14 (red) at P9 (E-H) and P12 (I-L). (F-H) Higher magnification of box in E. (L) Higher magnification of box in I. Arrow indicates Fibrillin-2-positive fibers. Bar=200 μm (A, E, I), 50 μm (B-D, F-H, J-L) DP; Dental Pulp AB; Alveolar Bone D; Dentin HERS; Hertwig's epithelial root sheath

Figure 4.

Fibrillin-1 and fibrillin-2 expression in human PDL. (A) Appearance of extracted second premolar. (B,C) H&E staining of root and PDL. (D-F) Fibrillin-1 (red) and fibrillin-2 (green) expression in human PDL. (G-I) Cytokeratin (red) and fibrillin-2 (green) expression in human PDL. Bar=500 μm (B), (C) 50 μm (D-F), 20 μm (G-I)



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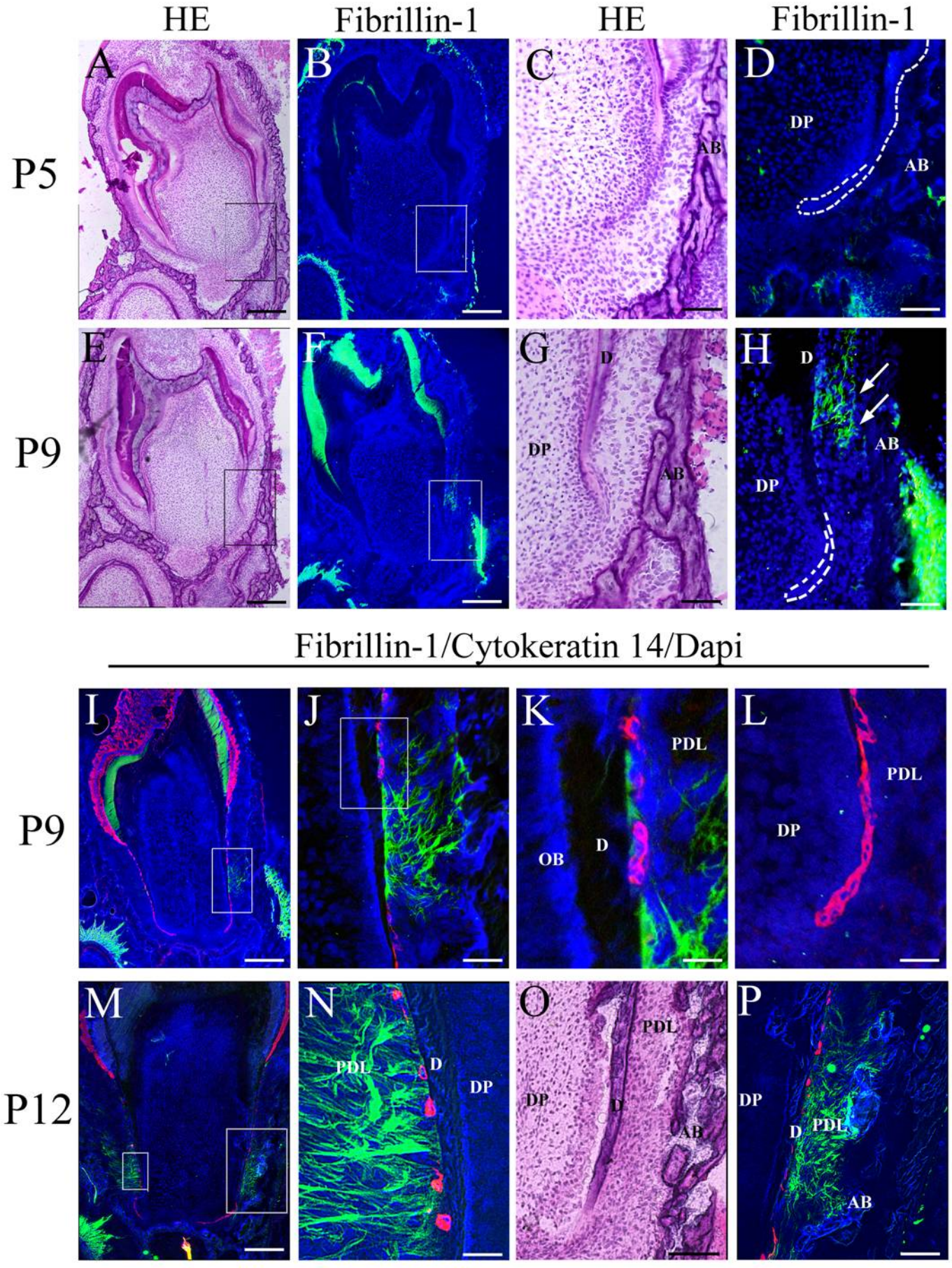

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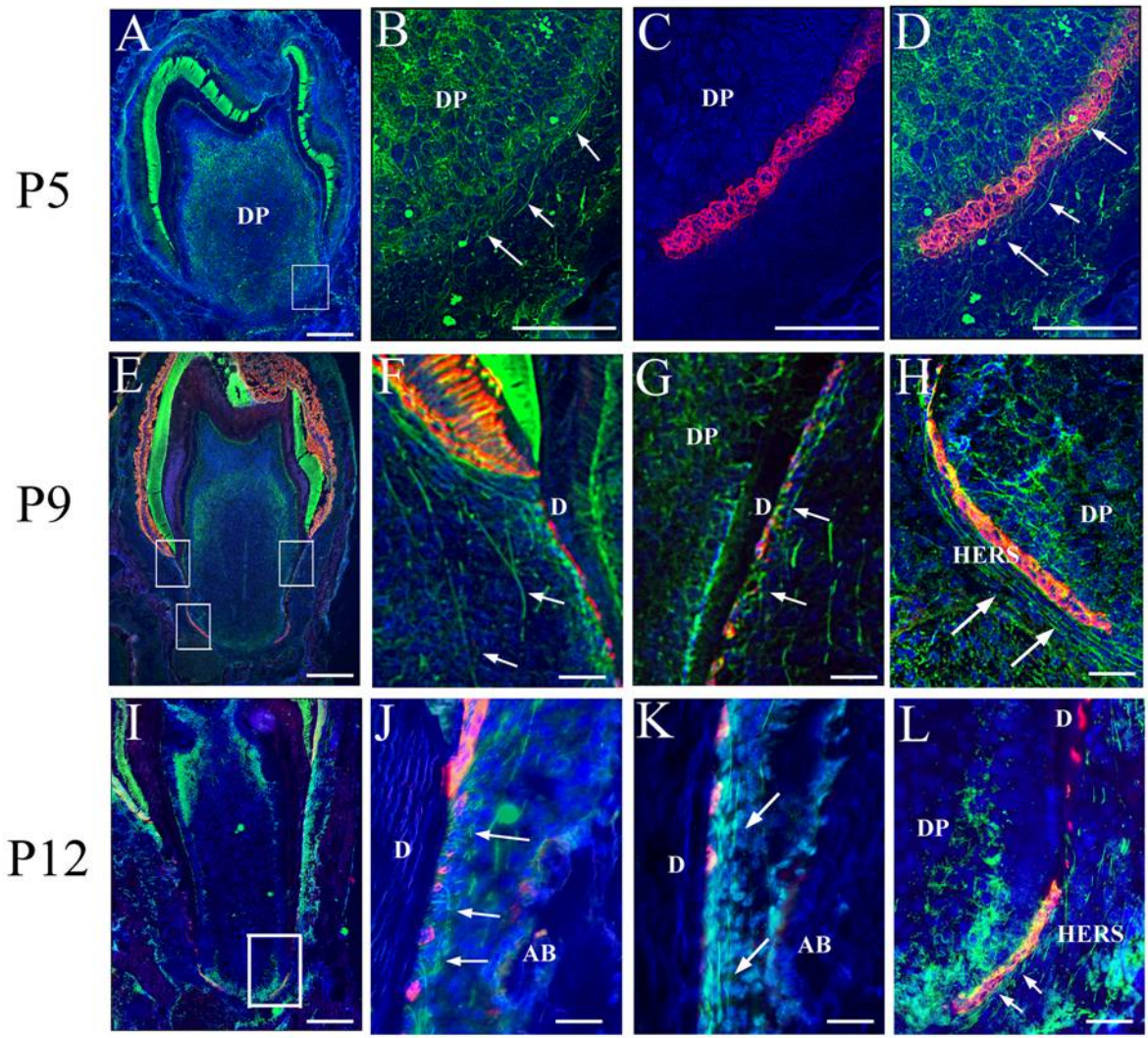

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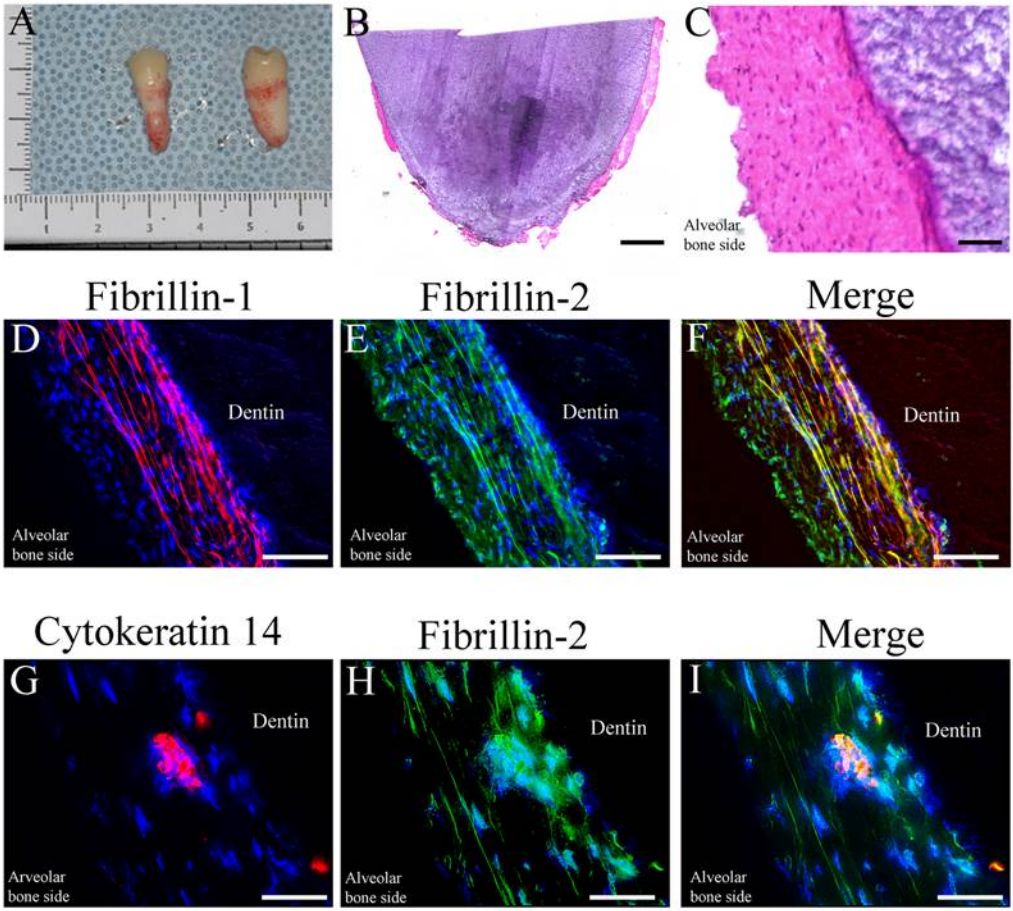

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