Original Article

Localization of osteopontin and osterix in periodontal tissue during orthodontic tooth movement in rats

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ABSTRACT

Objective: To evaluate the localization of osteopontin (OPN) and osterix in periodontal tissue during experimental tooth movement with heavy force in rats.

Materials and Methods: Nickel-titanium closed-coil springs were used to create a 100 g mesial force to the maxillary first molars. On days 3, 7, 10, and 14 after force application, histological changes in periodontium were examined by immunohistochemistry using proliferating cell nuclear antigen (PCNA), OPN, and osterix.

Results: PCNA-positive cells were found close to the alveolar bone and cementum on both sides. OPN-positive cells were observed along the cementing line of the cementum and bone on both sides and also were visible along with newly formed fibers in the periodontal ligament on the tension side. Osterix-positive cells were strongly detected on the surface of the alveolar bone and cementum on both sides.

Conclusions: During tooth movement, periodontal remodeling occurs on both sides. These results indicate that OPN and osterix may play an important role of differentiation and osteoblasts and cementoblasts matrix formation during periodontal tissue remodeling. (*Angle Orthod.* 2012;82:107–114.)

KEY WORDS: Orthodontic tooth movement; Periodontal tissue; OPN; Osterix

INTRODUCTION

Orthodontic tooth movement is based on force-induced paradental tissue remodeling.[1] Bone resorption by compression-associated osteoclasts and bone deposition by tension-associated osteoblasts are the well-described typical histological characteristics of this process.[2] In the course of tooth movement, root resorption represents a negative side effect. Many factors may be related to root resorption, but the precise etiology remains unknown.[3,4]

Tooth movement within bone in response to orthodontic appliances is an example of mechanical, stress-induced bone remodeling. Appliance-driven tooth movement models have contributed several key findings relative to the function of osteopontin (OPN) during bone remodeling.[5–7] OPN is a major noncollagenous bone matrix glycoprotein that is sialic acid rich and phosphorylated. It was originally isolated from bone and contains an Arg-Gly-Asp (RGD) motif that promotes cell attachment through integrins and CD4.[6] OPN is thought to promote or regulate the adhesion, attachment, and spreading of osteoclasts to the bone surface during bone resorption.[8] During tooth movement, mechanical stress stimulates osteocytes to express OPN to initiate bone remodeling,[6,7,9] resulting in bone resorption and bone apposition.[5] It is also known to be produced by osteoblasts[10–12] and osteoclasts[11,13] as well as osteocytes[6] and is considered to play important roles in bone formation, resorption, and remodeling.[14]

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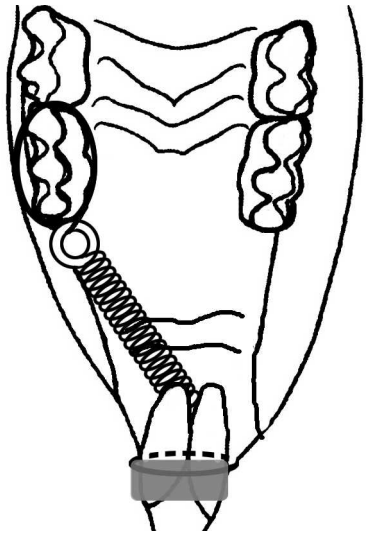

Figure 1. Schematic drawing of an appliance for tooth movement.

Osterix is a zinc-finger–containing transcription factor of the sp family, which is required for bone development and osteoblastogenesis.[15–17] In Osx-null mutant mice, no endochondral or intramembranous bone formation occurs, and arrest in osteoblastic differentiation occurs at a later step than in Runx2/Cbfa1-null mice, indicating that osterix acts as a downstream gene of Runx2 in the osteoblast differentiation signaling pathway.[16] Also, osterix regulates the expression of collagen type I and osteoblastic genes such as OPN, osteocalcin, and bone sialoprotein.[18] However, recent studies have found that osterix is regulated via both Runx2-dependent and -independent mechanisms.[19] Osterix is also expressed in tooth germ mesenchymal cells during tooth development.[16] Osterix triggers the formation and secretion of matrix such as cementum, dentin, and bone, indicating that osterix might be a useful marker of differentiation of cementoblasts, osteoblasts, and odontoblasts.[20]

Previous studies showed that the heavy forces produced less tooth movement and more root resorption compared with light forces. Orthodontic tooth movement with heavy forces led to the increased expression of proteins related to cementum and alveolar bone remodeling.[3,4] In this study, we investigated the immunohistochemical localization pattern of OPN and osterix in periodontal tissue during orthodontic tooth movement with heavy force.

MATERIALS AND METHODS

Experimental Tooth Movement

Twenty Sprague-Dawley male rats (Hanlim Inc, Seoul, Korea; body weight 350 g) were used in this study. Experimental protocols were approved by the Institutional Animal Care and Use Committee of Kyung Hee University.

The rats were acclimatized to the experimental conditions. The application was set under a 50 mg/kg intraperitoneal injection of pentobarbital sodium anesthesia. The posterior end of a nickel-titanium closed-coil spring (Jin Sung, Seoul, Korea) was tied around the maxillary first molar with a 0.09 inch stainless-steel ligature wire (Ortho Classic, McMinnville, OR). The groove was prepared at the cervical line of the upper incisors using a low-speed hand piece. The anterior end of the coil spring was fixed to the groove with a ligature wire, secured by cured composite resin to prevent slippage (Figure 1). The maxillary first molar was moved mesially by applying 100 g of force with the closed-coil spring as described previously.[3,4] The force magnitude was measured with a force gauge (Teclock, Nagano, Japan) in a water bath at 37.5°C. The procedure was performed when the appliance was set and again at the end of the experimental time, producing readings of 99.1 $\pm$ 2.4 g and 102.5 $\pm$ 5.3 g, respectively ($n = 20$).

The amount of tooth movement was determined by the distance between the most posterior point of the posterior border of the maxillary first molar crown and the most anterior point of the anterior border of the maxillary second molar crown during an experimental period. The value of tooth movement for 3, 7, 10, and 14 days was 0.031 $\pm$ 0.004 mm, 0.060 $\pm$ 0.010 mm, 0.104 $\pm$ 0.011 mm, and 0.122 $\pm$ 0.008 mm, respectively.

Tissue Preparation

On days 3, 7, 10, and 14 after orthodontic tooth movement, five rats were sacrificed. The animals were anesthetized and perfused transcardially with 10% formalin, and then the maxilla was immediately dissected and immersed in the same fixative overnight at 4°C. The specimens were decalcified in 10% EDTA (pH 7.4) for 4 weeks and then were dehydrated and embedded in paraffin. Each sample was cut mesiodistally into 7 μm serial sections and prepared for hematoxylin and eosin and immunohistochemistry staining for proliferating cell nuclear antigen (PCNA), OPN, and osterix.

Immunohistochemistry

For immunohistochemistry, a Vectastain ABC kit (Vector Laboratories Inc, Burlingame, Calif) was used, and all procedures were performed according to the manufacturer's instructions. Each section was incubated with anti-PCNA antibody (Serotec, Kidlington, UK), mouse monoclonal to OPN (Santa Cruz Biotechnology Inc, Santa Cruz, Calif), and anti-osterix polyclonal antibody (Abcam, Cambridge, UK) for

Table 1. Immunostaining Intensity of PCNA, OPN, and Osterix at Different Times[a]

	Control			3 Days			7 Days			10 Days			14 Days		
	RT	PDL	AB	RT	PDL	AB	RT	PDL	AB	RT	PDL	AB	RT	PDL	AB
PCNA															
C	†††	††	†††	†	†	†	†††	††	††	††	†††	††††	†††	†††	††††
T	††	††	†††	†	†	††	†	-	†	††	†	††	††	†	††
OPN															
C	††	††	†††	†	†	††	†††	†††	*	†††	†††	††††	††††	†††	††††
T	†††	††	††††	†††	††	†††	††	†	††	††††	†††	††††	††††	†††	††††
Osterix															
C	††	††	†††	††	†	††	††	†	*	†††	†††	†††	††	†	††††
T	†††	††	††††	††	†	†††	†	†	†††	†††	†	††	††††	††	†††

[a] PCNA indicates proliferating cell nuclear antigen; OPN, osteopontin; C, compression side; T, tension side; RT, root; PDL, periodontal ligament; AB, alveolar bone.

- = no staining; † = weak; †† = mild; ††† = moderate; †††† = intense; * = alveolar bone resorption.

30 minutes at room temperature. Negative controls were conducted in the absence of the primary antibody. The sections were incubated in peroxidase substrate solution (SK-4100, Vector Laboratories) until the desired stain intensity had developed. The sections were counterstained with hematoxylin and mounted. The intensity of the staining was scored from 0 to 10 on both sides throughout the entire experimental period and classified into five groups: - = no staining (0), † = weak (1–2), †† = mild (3–5), ††† = moderate (6–8), and †††† = intense (9–10) (Table 1).

Just one examiner performed all procedures to eliminate interexaminer errors. Also, the examiner was blinded to prevent bias, and each staining was repeated three times.

RESULTS

Histological Observation

In this study, the right maxillary first molar served as a control. The periodontal tissues were composed of relatively dense fibers and fibroblasts running from the root surface toward the alveolar bone. Blood capillaries were mostly shown near the alveolar bone in the periodontal ligament (PDL). The alveolar bone and root surfaces with resorption lacunae were relatively smooth (Figure 2A).

PCNA-positive cells were localized in alveolar bone and tooth root surfaces with resorption lacunae (Figure 2B,C). OPN-positive osteocytes and cementocytes were observed along the surface of cementum and alveolar bone (Figure 2D,E). Osterix-positive cells were detected in the PDL of the apical and furcation region, especially near the surface of root and alveolar bone, and also in the odontoblasts (Figure 2F,G).

Histological Observation on the Tension Side During Orthodontic Tooth Movement

The distal side of the distal root of the maxillary first molar served as the tension side. The PDL increased in width, and stretched fibers were observed throughout the entire experimental period. On day 3, osteoclasts lined up in the margin of the alveolar bone adjacent to the PDL, leading to frontal resorption (Figure 3A). On day 7, the crest region of the alveolar bone was absorbed and many blood capillaries were observed in the area (Figure 3B). On day 10, the alveolar bone was partially restored, and the PDL exhibited expanded blood capillaries and coarse fibers (Figure 3C). On day 14, the PDL width and alveolar bone height were almost normal, and many cuboidal osteoblasts were observed on the surface of restored alveolar bone. Root resorption was found on the tooth surface (Figure 3D).

On day 3, PCNA-positive cells were rarely detected (Figure 3E). On day 7, intense reactivity of PCNA was observed within the PDL of the absorbed alveolar bone region (Figure 3F). The PCNA-positive cells were particularly evident in the surface of the forming alveolar bone, although they were well distributed throughout the PDL on days 10 and 14 (Figure 3G,H). On day 3, OPN-positive osteocytes, osteoblasts, and osteoclasts were detected in the alveolar bone (Figure 4A). On days 7 and 10, OPN-positive cells were clearly evident throughout the entire PDL, especially on surfaces of the newly formed alveolar bone and cementum (Figure 4B,C). On day 14, OPN-positive cells were found on the surface of new bony trabeculae extending into the PDL space, cementum surface, and along their cement line (Figure 4D). On days 3 and 7, osterix-positive cells were rarely detected, except in odontoblasts (Figure 4E,F) but on day 10, they were found in the PDL near the absorbed

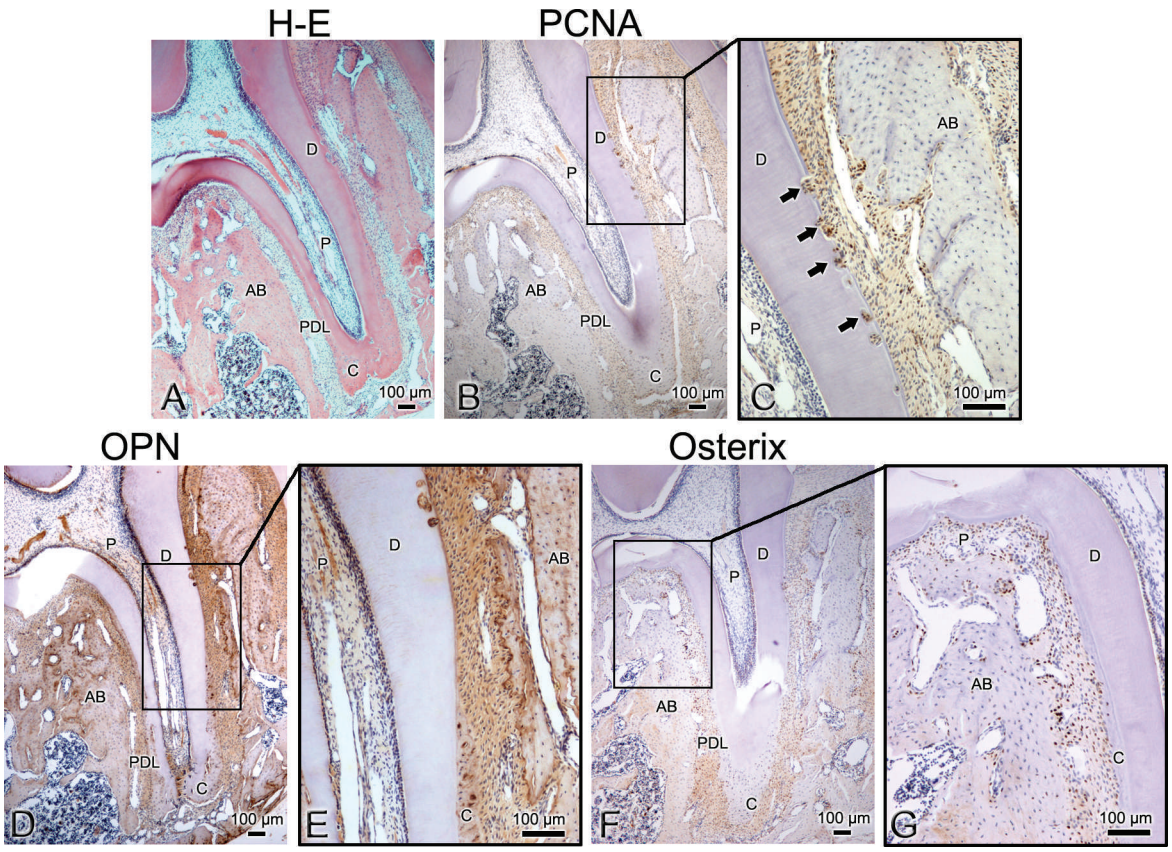

Figure 2. Histology of the right maxillary first molar. (A) Hematoxylin and eosin staining. (B, C) Localization of PCNA. (D, E) Localization of OPN. (F, G) Localization of osterix. (C, E, G) Higher magnification views of the boxes in B, D, and F. Thick arrows indicate the resorption lacuna. AB indicates alveolar bone; C, cementum; D, dentin; P, pulp; PDL, periodontal ligament.

alveolar bone (Figure 4G). On day 14, osterix-positive cells were strongly detected on the surface of the newly formed alveolar bone (Figure 4H).

Histological Observation on the Compression Side During Orthodontic Tooth Movement

The mesial side of the mesial root of the maxillary first molar served as the compression side. The PDL width narrowed, and many condensed cells were found in the compressed PDL. On days 3 and 7, a large number of cellular elements was found in the compressed PDL, and the fiber arrangement became coarse and irregular. Cementoid was observed on the cementum surface, and resorption lacunae with many osteoclasts were found on the surface of the alveolar bone (Figure 5A,B). On days 10 and 14, alveolar bone and cementum were newly formed, and osteoblasts and cementoblasts lined up in the margin of them (Figure 5C,D).

On days 3 and 7, PCNA-positive cells were rarely detected (Figure 5E,F), but on days 10 and 14, they were found on the surfaces of cementum and alveolar bone (Figure 5G,H). OPN-positive osteocytes and cementocytes were present on days 3 and 7 (Figure 6A,B). Immunoreactivity of OPN was also clearly observed in the osteocytes and osteoblasts near the surface of newly formed alveolar bone as well as in cementocytes, cementoblasts, and PDL cells on days 10 and 14 (Figure 6C,D). Osterix-positive cells were mainly observed near the surface of the alveolar bone on days 3 and 7 (Figure 6E,F) and also on the cementum surface on days 10 and 14 (Figure 6G,H).

DISCUSSION

The ultimate aim of orthodontic tooth movement would be to move teeth in the most effective way and at the same time to reduce side effects, such as root resorption. The etiology of root resorption has been studied for several years, but it remains unclear.[4] In this study, we provide the distribution pattern of OPN and osterix during the processes of bone, PDL, and cementum remodeling in response to heavy forces.

Several studies have provided evidence that osteocytes are the most mechanosensitive cells in bone.[21] In the present study, OPN-expressing osteocytes in bone lacunae were localized on both sides in early stages, and then later an extensive localization was observed in osteocytes, osteoblasts, and bone-lining

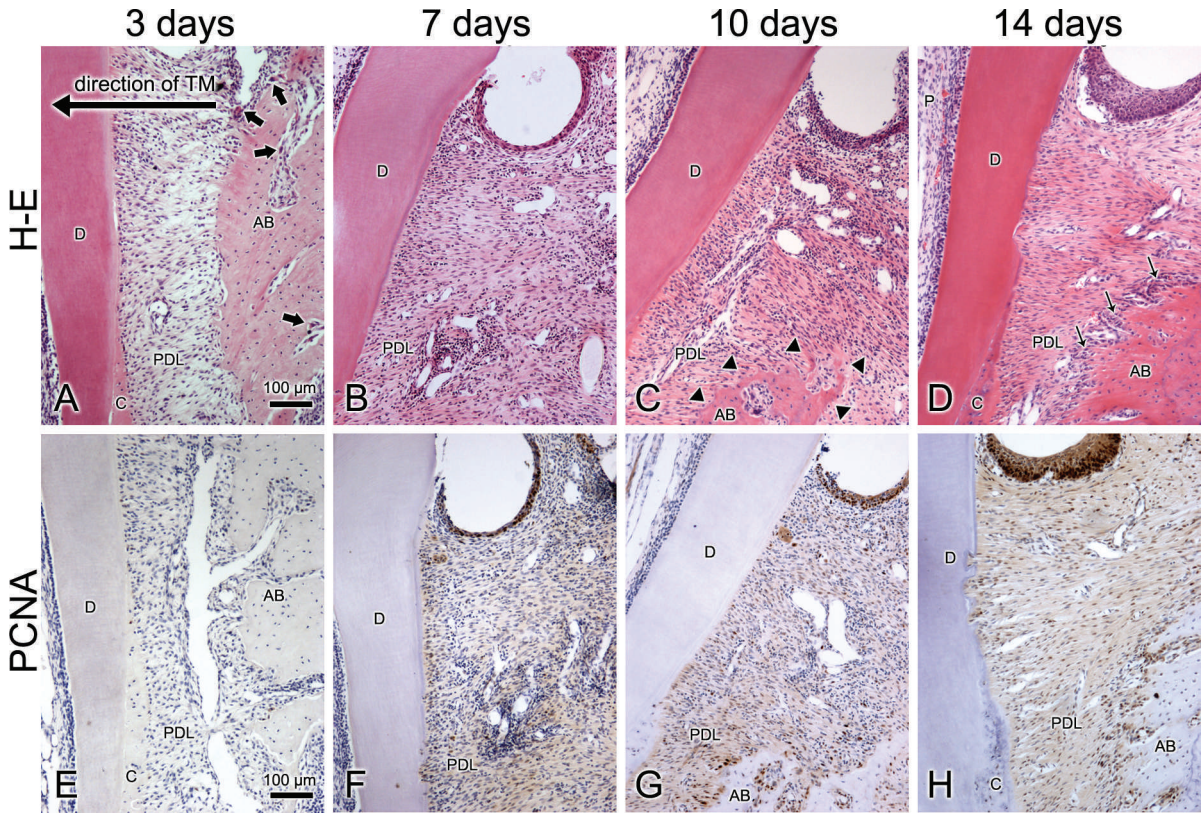

Figure 3. Histological observation on the tension side during orthodontic treatment. (A–D) Hematoxylin and eosin staining. (E–H) Localization of PCNA. Thick arrows indicate the osteoclasts; arrowheads indicate the newly formed alveolar bone; thin arrows indicate the osteoblasts. AB indicates alveolar bone; C, cementum; D, dentin; PDL, periodontal ligament; TM, tooth movement.

cells. These observations are in accord with previous studies that showed that osteocytes are initially responsive to the mechanical stress.[6] We also showed that OPN was detected not only in the cytoplasm of osteocytes and cementocytes but also on the surface of bone and cementum on both sides. OPN-expressing osteocytes and cementocytes resulting from mechanical stress might induce a migration of osteoclastic and odontoclastic precursor cells to the surface of bone and cementum and promote resorption. Therefore, OPN might act as a trigger for bone and cementum remodeling caused by mechanical stress.[6] OPN was also observed in the cement line of bone and cementum on both sides under mechanical stress. It has been proposed that the cement line may contain factors that promote coupling between bone resorption and subsequent bone formation during bone remodeling.[6] Therefore, OPN might possibly be a multifunctional protein that acts as a coupling factor for resorption and formation in the process of bone and cementum remodeling during tooth movement.

Osterix is required for osteoblast differentiation and bone formation and also regulates the expression of osteoblastic genes such as OPN, osteocalcin, and bone sialoprotein.[18,22] In this study, we demonstrated that osterix reactivity was detected in PDL cells of the absorbed alveolar bone region on the tension side and in cuboidal cells of the surface of newly formed alveolar bone and cementum on both sides. OPN reactivity in the cement line and surface of cementum and alveolar bone might be induced by osterix expression. Their strong localization in periodontal tissues when 100 g of heavy force was applied indicates that they might be molecules linking mechanostressing and osteogenic and odontogenic differentiation[20,23,24] and might be involved in bone, PDL, and cementum remodeling during orthodontic tooth movement.

The precise relationship between OPN and osterix during periodontal tissue remodeling in response to heavy force could not be ascertained with the current methodology. Better understating of the OPN and osterix expression might require further research, such as in vitro study or in vivo study using knockout mice.

CONCLUSIONS

- During tooth movement, periodontal tissue remodeling with the resorption and deposition on the surface of alveolar bone and cementum occurs on both sides.

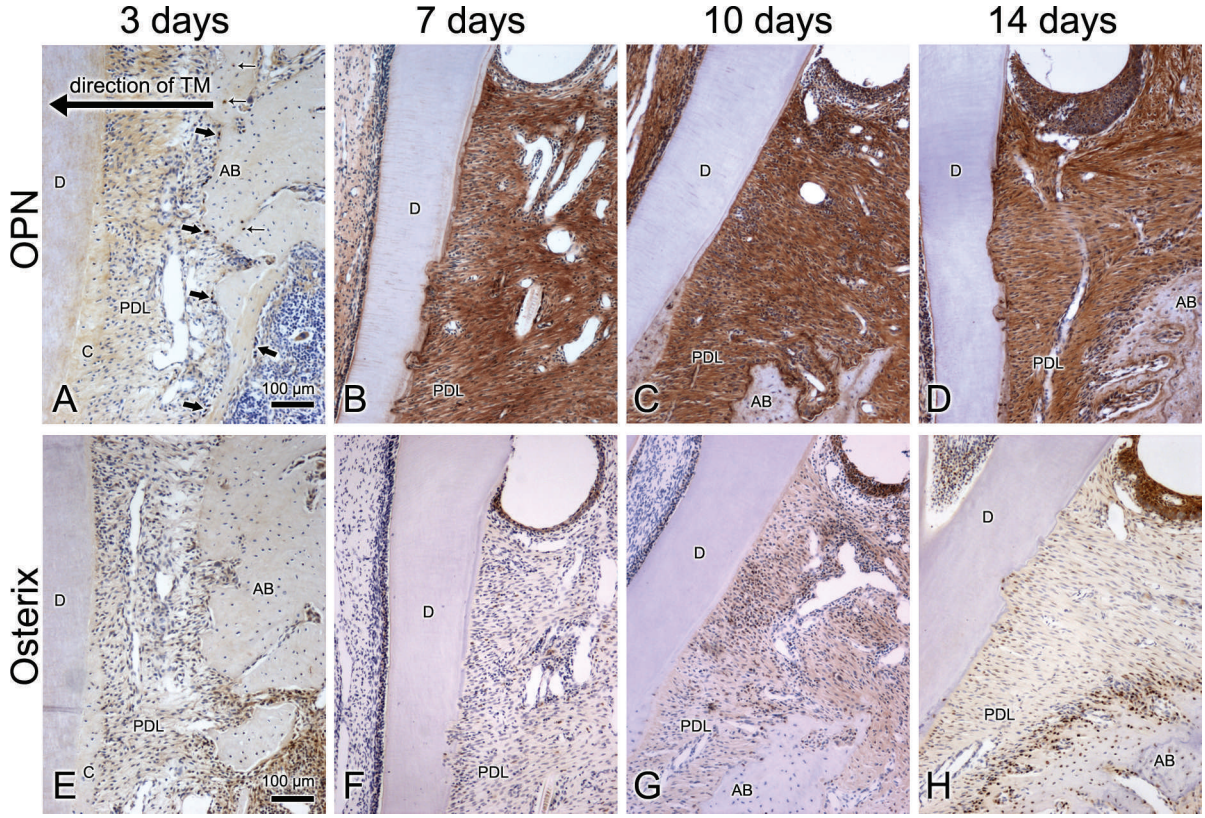

Figure 4. Localization of OPN and osterix on the tension side during orthodontic treatment. Thick arrows indicate the osteoblasts; thin arrows indicate the osteocytes. AB indicates alveolar bone; C, cementum; D, dentin; PDL, periodontal ligament; TM, tooth movement.

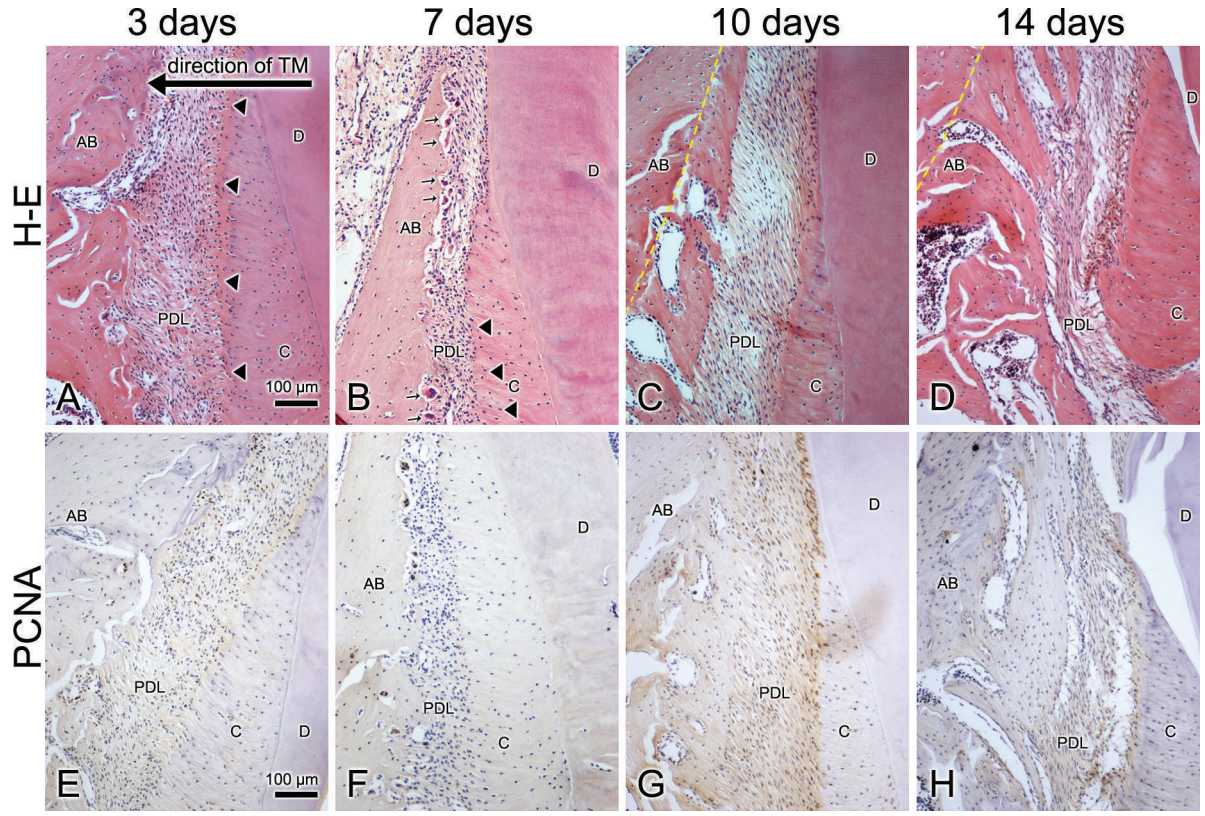

Figure 5. Histological observation on the compression side during orthodontic treatment. (A–D) Hematoxylin and eosin staining. (E–H) Localization of PCNA. Arrowheads indicate the cementoid; thin arrows indicate the resorption lacunae with osteoclasts; yellow dotted lines indicate the boundary of the new alveolar bone. AB indicates alveolar bone; C, cementum; D, dentin; PDL, periodontal ligament; TM, tooth movement.

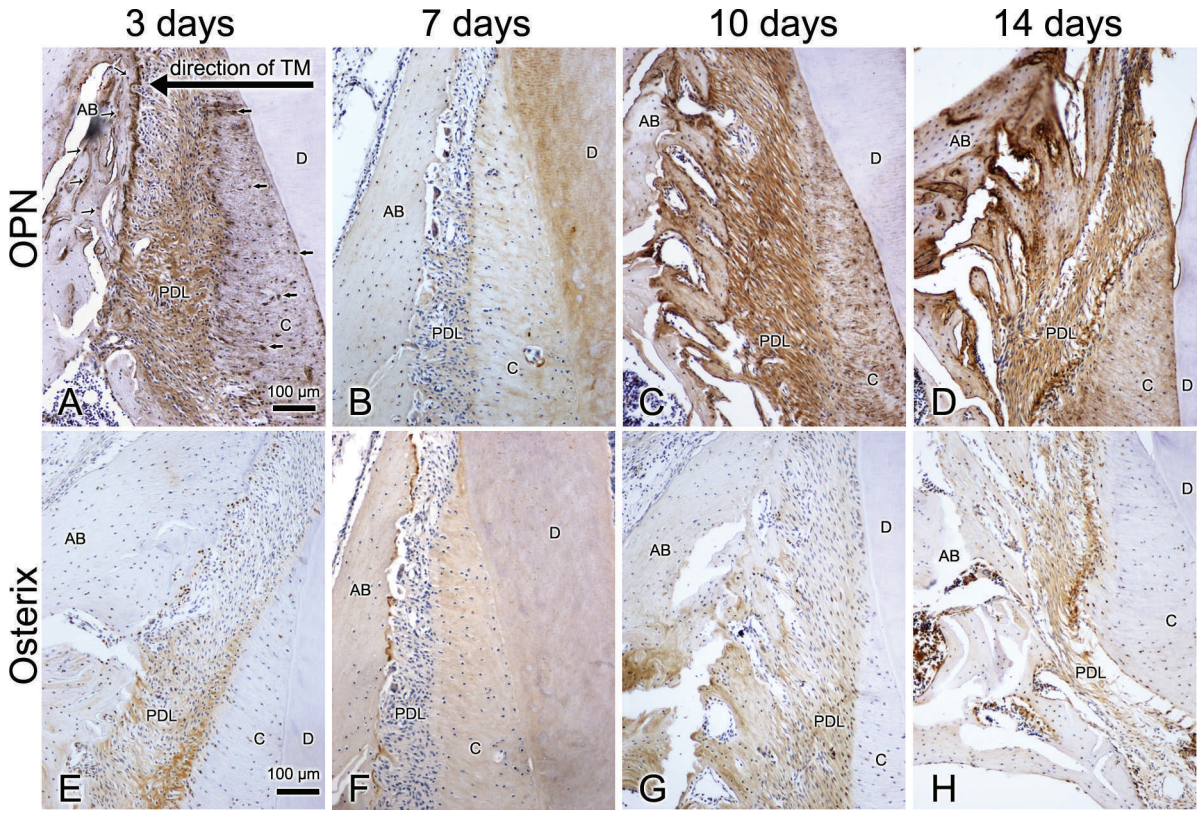

Figure 6. Localization of OPN and osterix on the compression side during orthodontic treatment. Thick arrows indicate the cementocytes; thin arrows indicate the osteocytes. AB indicates alveolar bone; C, cementum; D, dentin; PDL, periodontal ligament; TM, tooth movement.

- OPN was observed in osteocytes at the initial orthodontic stage and in PDL cells, osteoclasts, osteoblasts, cementocytes, cementobalsts, and the cement line of alveolar bone and cementum at the late stage on both sides.
- Osterix was observed especially in cementoblasts and osteoblasts on the surface of cementum and alveolar bone.

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