Original Article

Localization of ODAM, PCNA, and CK14 in regenerating junctional epithelium during orthodontic tooth movement in rats

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ABSTRACT

Objective: To identify the regenerating junctional epithelium (JE) during orthodontic tooth movement in rats.

Materials and Methods: Closed-coil springs were used to create a 20 g mesial force to the maxillary first molars. On days 1, 3, 7, 10, and 14 after force application, histologic changes in JE were examined by immunohistochemistry using proliferating cell nuclear antigen (PCNA), odontogenic ameloblast-associated protein (ODAM), and cytokeratin 14 (CK14).

Results: On day 1, JE was destroyed and lost attachment to the tooth surface. Cell division activity was rarely observed in JE, and ODAM localization was weakly detected in damaged JE. By day 3, regenerating JE had not fully recovered. High cell proliferation activity and CK14 expression started to appear in most basal cells of JE. ODAM expression was reduced and appeared in a small area. By day 7, JE had almost recovered. Cell proliferation activity was still observed in several basal cells of JE, and ODAM expression was detected among JE cells. CK14 was hardly observed in JE except in the basal cells. By days 10 and 14, regenerated JE appeared. ODAM, PCNA, and CK14 expression was similar to that of the control.

Conclusions: Damaged JE might recover rapidly during orthodontic tooth movement because basal cells of the remaining JE, which show higher proliferation activity, are involved in JE regeneration. Reduced ODAM expression during proliferation of JE cells may increase again after JE regeneration is complete. Therefore, ODAM may be associated with the normal function of JE. (*Angle Orthod.* 2014;84:534–540.)

KEY WORDS: Orthodontic tooth movement; Junctional epithelium; ODAM

INTRODUCTION

In orthodontic tooth movement, teeth are repositioned in response to applied mechanical forces that cause remodeling of tooth-supporting tissues, especially of the alveolar bone, periodontal ligament, and gingiva. Stability of the dentogingival junction in gingiva is key to maintaining periodontal health during orthodontic treatment.[1] Although much is known of the reactions of periodontal ligament and alveolar bone to orthodontic force,[2] the corresponding effects of mechanical stimulation on gingiva, especially in the dentoepithelial level, are not so well known.

Gingival epithelium is classified into gingival oral epithelium (OE), sulcular epithelium (SE), and junctional epithelium (JE).[3] JE is a specialized epithelial structure of the dentogingival unit that adheres to an erupted tooth surface and seals off the supporting tissues of the tooth from biohazards and mechanical damage from the oral environment.[4] The gingiva is attached to the tooth surface by an

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epithelial attachment consisting of an internal basal lamina and hemidesmosomes.[5] The components of the epithelial attachment are believed to be produced and renewed by directly attached cells of adjacent JE.[6] However, the exact composition and attachment mechanism to the tooth surface are still not completely understood.

JE is formed as a tooth crown erupts into the oral cavity. It has been proposed that JE, which was originally derived from the reduced enamel epithelium during tooth development, may be replaced in time by JE formed from oral gingival epithelium.[7] Completed JE forms a collar peripheral to the cervical region of the tooth. Injury to the JE can occur through trauma, tooth brushing, flossing, or clinical probing. As JE is located at a strategically important but also delicate site, it may be expected that it should be very well-adapted to mechanical stimulation,[8] but the exact mechanisms that lead to the reformation or regeneration of JE remain unclear.

APIN, which is now called odontogenic ameloblast-associated protein (ODAM),[9,10] was identified in the secretome profile of the rat enamel organ. ODAM is a secretory protein that is synthesized and secreted by secretory ameloblasts.[11] In addition, ODAM is detected when enamel organ cells are reduced and at the interface with a tooth.[10] Among the epithelia of the oral mucosa, only JE expressed ODAM.[9,10] Thus far, the expression pattern during JE regeneration has not been established in detail. In particular, the role of ODAM in regenerating JE is unknown.

Cytokeratins (CKs) are major structural proteins expressed by epithelial cells depending on the stage of differentiation and origin of the epithelium.[12] The CK expression is influenced by interactions with the subepithelial mesenchyme and eventual pathological changes.[13] CK14 is a major keratin expressed by basal cells in a stratifying epithelium. The complex pattern of a CK expression of JE appears to differ from that of any other OE.[14] JE expresses CKs specific for simple epithelia (CK7, 8, 18, and 19), for basal layers (CK5 and 14), and for nonkeratinizing stratified epithelia (CK13 and 16). The gingival epithelium expresses CK5 and CK14 and occasionally CK19 in the basal layer.[12] Proliferating cell nuclear antigen (PCNA) is originally expressed in the nuclei of cells during the DNA synthesis phase of the cell cycle[15] and was used to identify the cell proliferation activity in regenerating JE.

The objective of this study was to investigate the distribution of ODAM compared with that of CK14 used as a differentiation marker for oral epithelial cells and PCNA for cell proliferation in regenerating JE during orthodontic tooth movement.

MATERIALS AND METHODS

Experimental Tooth Movement

Twenty-five 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 environment. The application was set under a 50 mg/kg intraperitoneal injection of pentobarbital sodium anesthesia. According to a review article concerning the optimal force for orthodontic tooth movement, of seven studies on rats, five studied the effect of forces ranging from 20 to 60 cN on the maxillary first molar. Six of the seven studies used tipping forces.[16] Thus, in the present study, the maxillary left first molar was moved mesially by a force of 20 g in the same manner as in our previous study.[17] To limit the influence of interanimal variation in response to metabolic stimuli, a split-mouth design[18] was used, and the untreated contralateral side served as the control.

Tissue Preparation

After orthodontic tooth movement, five rats were killed on days 1, 3, 7, 10, and 14. Fourteen days of a rat's life is equivalent to approximately 1 human year (16.7 rat days = 1 human year).[19] 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% ethylenediaminetetraacetic acid (pH 7.4) for 4 weeks and then dehydrated and embedded in paraffin. Each sample was cut mesiodistally into 7-μm serial sections and prepared for hematoxylin & eosin (H&E) and immunohistochemistry staining.

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 Rabbit anti-rat ODAM antibody (courtesy of Dr J.C. Park), anti-PCNA antibody (Serotec, Kidlington, UK), anti-CK14 antibody (Abcam, Cambridge, UK) for 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, Inc) until the desired stain intensity had developed. The sections were counterstained with hematoxylin and mounted. The same examiner performed all procedures to eliminate interexaminer errors. The examiner

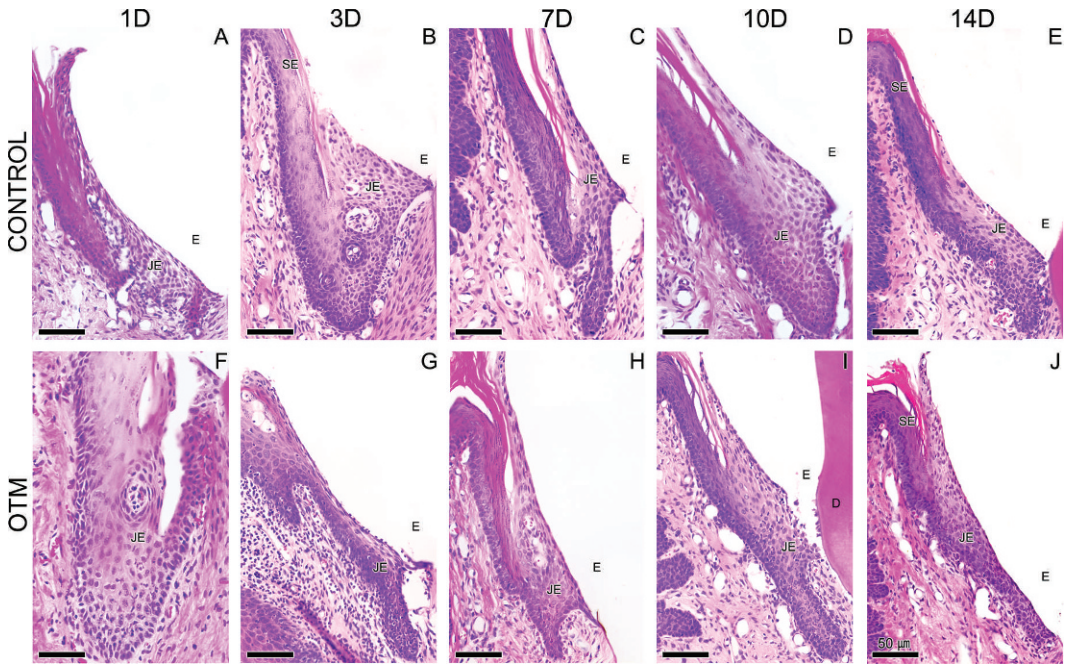

Figure 1. Histology of regenerating JE after orthodontic tooth movement. H&E staining of untreated JE from the mesial side of the right maxillary first molar on (A) day 1, (B) day 3, (C) day 7, (D) day 10, and (E) day 14. H&E staining of JE applied orthodontic force to the mesial side of the left maxillary first molar on (F) day 1, (G) day 3, (H) day 7, (I) day 10, and (J) day 14. JE indicates junctional epithelium; SE, sulcular epithelium; E, enamel space; D, dentin.

was also blinded to prevent bias, and each staining was repeated three times.

RESULTS

Histology of Regenerating JE on the Mesial Side After Orthodontic Tooth Movement

In this study, the mechanically stimulated JE on the mesial side of the left maxillary first molar served as the experimental group, and equivalent sites of the right maxillary first molar served as the control. In the control, the normal dentogingival junction consisted of an SE and JE was observed. JE is a stratified, squamous, nonkeratinizing epithelium. Basal cells rest on an external basal lamina interfacing with the connective tissue. Suprabasal cells that were oriented parallel to the tooth surface showed a flattened shape. The flattened superficial cell layer interfaced with the tooth surface as an actual attachment. The JE showed wide intercellular space and a few neutrophilic granulocytes within the intercellular space (Figure 1A–E).

On day 1 after orthodontic tooth movement, JE destruction by mechanical force was observed in the superficial cells (Figure 1F). On day 3, the intercellular space of the regenerating JE included a number of infiltrating leukocytes (Figure 1G), and JE was still regenerating on day 7 (Figure 1H). On days 10 and 14, the morphology of the reformed JE was similar to that of the control (Figure 1I,J).

Expression of ODAM, PCNA, and CK14 in the Regenerating JE After Orthodontic Tooth Movement

On day 1 after orthodontic tooth movement, the damaged JE were weakly labeled for ODAM in comparison with the control (Figure 2A,D). No immunolabeling of PCNA and CK14 was noted in the JE (Figure 2B,C,E,F). On day 3, ODAM expression was detected in regenerating JE, except for basal cells and suprabasal cells just above them (Figure 3A,D). Intense cell proliferation activity was observed in basal cells of the JE and in suprabasal cells just above them (Figure 3B,E). CK14 expression was observed in OE basal and suprabasal cells as well as in the JE (Figure 3C,F). On day 7, ODAM localization was detected among the regenerating JE cells, except for the basal cells, and in the basal lamina located at the surface of the JE (Figure 4A,D). High cell proliferation activity was still observed in several basal cells of the JE (Figure 4B,E). CK14 localization was observed in the OE and a few basal cells of the JE (Figure 4C,F). On days 10 and 14, ODAM expression was strongly detected in the internal basal lamina and among the regenerated JE cells as in the control. PCNA and

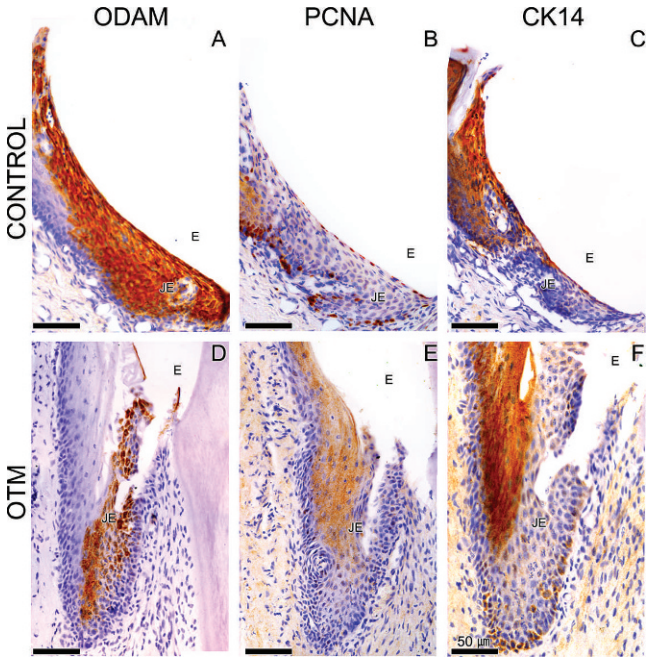

Figure 2. On day 1 after orthodontic tooth movement, expression of ODAM (A and D), PCNA (B and E), and CK14 (C and F) during regeneration of the JE. JE indicates junctional epithelium; E, enamel space.

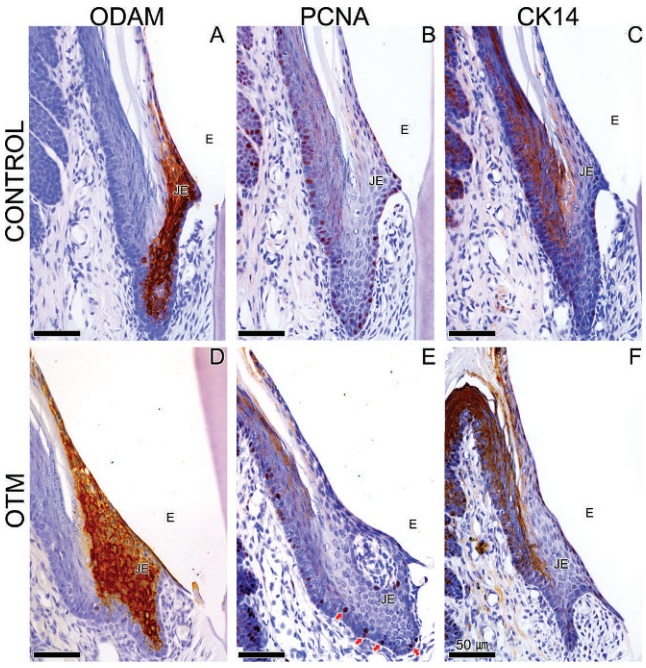

Figure 4. On day 7 after orthodontic tooth movement, expression of ODAM (A and D), PCNA (B and E), and CK14 (C and F) during regeneration of the JE. Arrows indicate the cells showing high proliferation activity in JE. JE indicates junctional epithelium; E, enamel space.

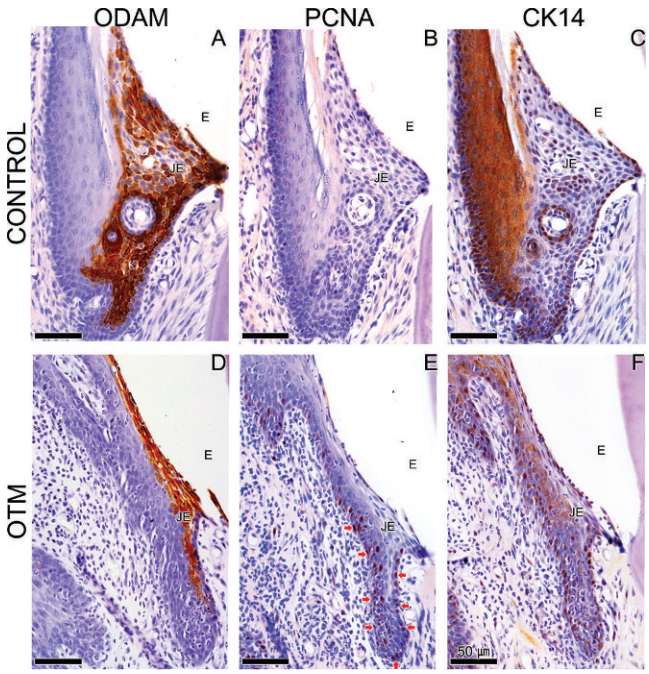

Figure 3. On day 3 after orthodontic tooth movement, expression of ODAM (A and D), PCNA (B and E), and CK14 (C and F) during regeneration of the JE. Arrows indicate the cells showing high proliferation activity in JE. JE indicates junctional epithelium; E, enamel space.

CK14 localization in a few basal cells of the JE was also similar to the control (Figures 5 and 6).

Histology of Regenerating JE on the Distal Side on Day 14 After Orthodontic Tooth Movement

Normal interdental papilla was observed between the right maxillary first and second molars (Figure 7A). On day 14 after orthodontic tooth movement, regenerating JE on the distal side close to the buccal side showed attachment to tooth surface, albeit in a very small area (Figure 7B,C). Regenerating JE at midlevel on the distal side showed a few neutrophilic granulocytes within the intercellular space, but epithelial attachment was not observed (Figure 7D,E). Regenerating JE on the distal side close to lingual JE also showed frail epithelial attachment (Figure 7F,G).

DISCUSSION

In many clinical studies, gingival changes have been reported as unwanted side effects of orthodontic tooth movement. Fixed-appliance orthodontics has been shown to produce deleterious effects on the periodontium, ranging from gingivitis to bone loss.[20] The cementation of orthodontic bands or resin-bonded attachments can evoke local soft tissue response.[21] Orthodontic treatment often leads to changes in oral hygiene habits, which might result in plaque

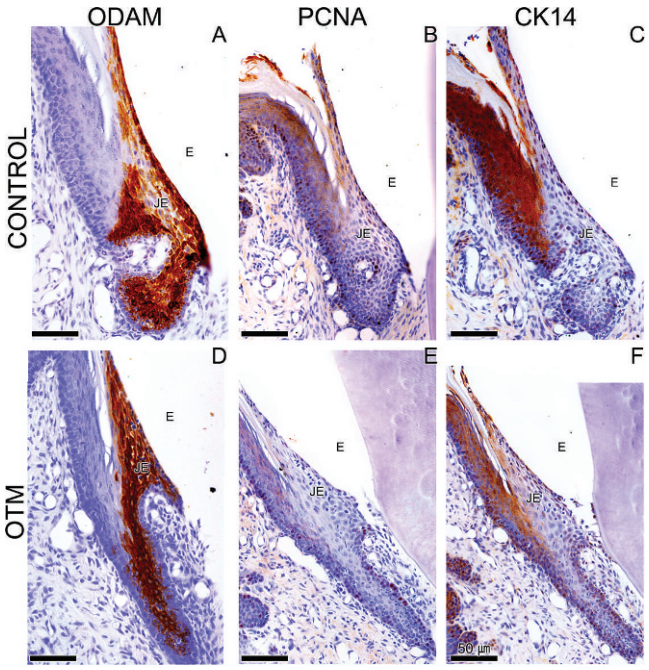

Figure 5. On day 10 after orthodontic tooth movement, expression of ODAM (A and D), PCNA (B and E), and CK14 (C and F) during regeneration of the JE. JE indicates junctional epithelium; E, enamel space.

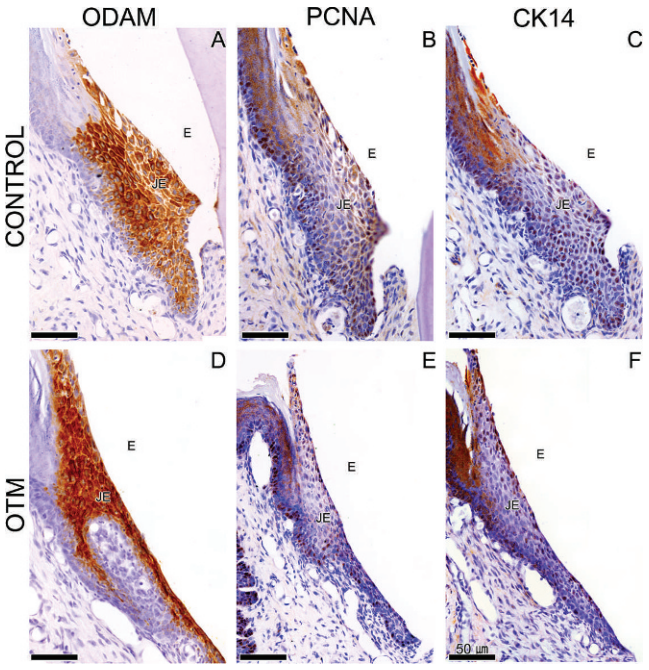

Figure 6. On day 14 after orthodontic tooth movement, expression of ODAM (A and D), PCNA (B and E), and CK14 (C and F) during regeneration of the JE. JE indicates junctional epithelium; E, enamel space.

accumulation and gingival inflammation.[2] Also, in an experimental study, it has been reported that periodontal regenerative surgery before orthodontic treatment is necessary to achieve and maintain periodontal health.[22] Therefore, a study to further understand regeneration of JE is essential for maintaining gingival and periodontal health during orthodontic tooth movement.

It has been reported that JE is readily regenerated from the adjacent SE or OE if it is damaged or surgically excised.[4,8,10] The new JE has all the characteristics of the original tissue, including the same type of CKs and attachment to the tooth that is indistinguishable from the original one.[4] Several studies in rodents have shown that ODAM is one of the early proteins expressed during regeneration.[10] This study describes the expression of ODAM, PCNA, and CK14 in regenerating JE during orthodontic tooth movement in rats. On day 1 after orthodontic tooth movement, JE was degraded, especially the superficial cell layer and so-called internal basal lamina located at the surface. ODAM expression in the JE decreased. On day 3, most basal cells of the regenerating JE showed high proliferation activity. The activity was still observed in several JE basal cells on day 7. Limited ODAM expression showed in several superficial cell layers and then increased again after JE regeneration was almost complete. On days 10 and 14, PCNA expression in JE was hardly detected, as it was in the control. This was consistent with previous studies where mechanical stimulation induced high proliferation in the JE.[1] Also, it can be proposed that repair takes place in the JE itself as the basal cells of the JE are able to undergo cell division and quite rapid turnover when minor gingival injury occurs.[3,4] Although the interdental papilla, including the JE between the first and second molars, was completely destroyed by the ligature wire, not by orthodontic force, regenerating JE was also observed on day 14 on the distal side. It seems that JE is regenerated from the basal cells of the undamaged JE located in the buccal or lingual region. No complete attachment of regenerating JE was evident on the tooth surface at this time. Thus, for successful orthodontic treatment, it may be crucial to have at least a small area of undamaged JE. Recently, it has been reported that OE and epithelial cell rests of Malassez that express ODAM within the periodontal space are associated with JE regeneration after a gingivectomy.[10] Therefore, a better understanding of JE regeneration, especially of the role of ODAM in regenerating JE, might require further research.

Study of orthodontically induced reactions in human JE has been limited. From the present animal study, it was learned that JE is destroyed by the force applied during orthodontic tooth movement in rats, but new JE forms within 10 days. This suggests that JE is a highly

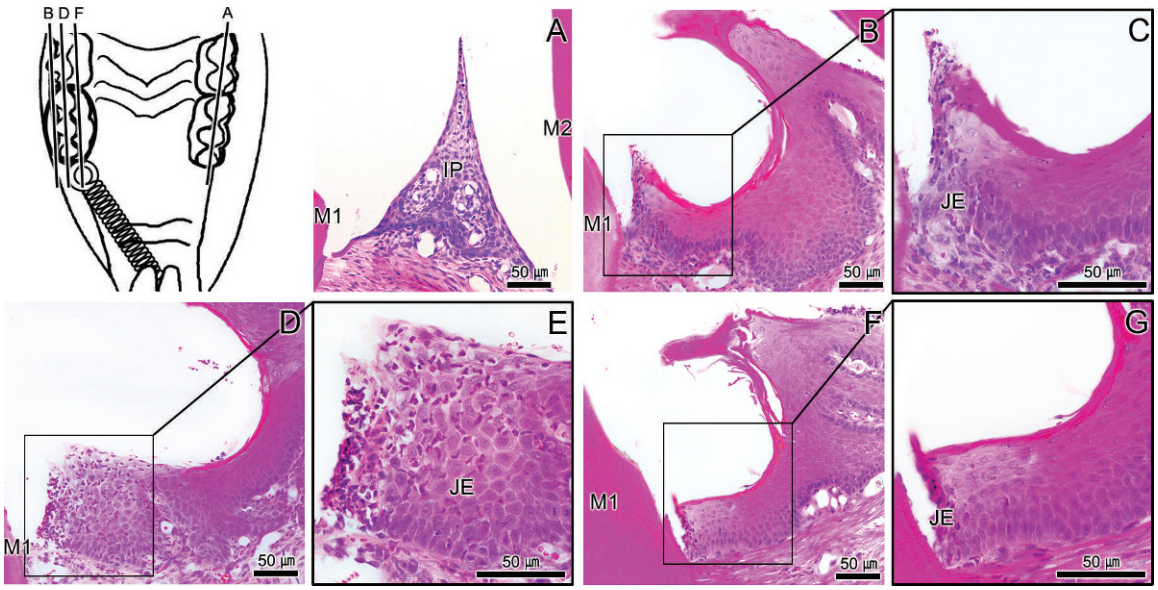

Figure 7. Histology of regenerating JE on the distal side on day 14 after orthodontic tooth movement (H&E staining). Black lines on the schematic diagram show a section plane of (A), (B), (D), and (F). (A) Untreated JE on the distal side of the right maxillary first molar. (B–G) Stimulated JE on the distal side of the left maxillary first molar. (C, E, G) Higher magnification views of the boxes in B, D, and F. M1 indicates first molar; M2, second molar; IP, interdental papilla; JE, junctional epithelium.

dynamic and adaptive tissue that can quickly self-renew from remaining basal cells. The reduced ODAM expression observed during the proliferation of JE cells increased again after JE regeneration was complete (Figure 8). ODAM, protein stemmed from enamel organ, is continuously expressed in JE, although the primary JE derived from the reduced enamel epithelium was replaced by secondary JE formed from OE. Also, when JE was destroyed by orthodontic force, ODAM expression was observed in regenerating and regenerated JE. ODAM appears to be closely related to the normal function of JE, regulating cell status immediately after the proliferation of JE.

Identifying the precise role of ODAM expressed in regenerating JE should help clinicians provide better care for the periodontal health of patients. A better understanding of JE regeneration will require further research to identify the nature of epithelial attachment and cell differentiation of regenerating JE during orthodontic tooth movement with differential forces.

CONCLUSIONS

- JE destroyed on day 1 of the study by forces applied during orthodontic tooth movement was regenerated within 10 days.
- By day 3, cell proliferation activity and CK14 expression had started to appear in most basal cells of the regenerating JE. ODAM expression was reduced and appeared in limited areas of superficial cells but not in the control.
- By day 7, JE had almost completely recovered. Higher cell proliferation activity was still observed in several basal cells of the JE, and ODAM expression had increased in the JE. CK14 was hardly observed in the JE, about the same as in the control.

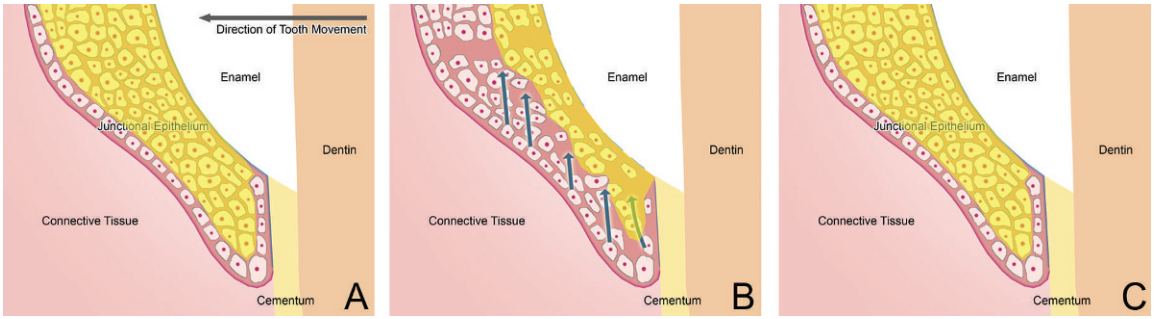

Figure 8. Schematic diagram of JE. (A) JE before orthodontic tooth movement. (B) Regenerating and (C) regenerated JE during orthodontic tooth movement. Arrows indicate the movement of proliferated cells in the basal layer of JE. The light gray area of the JE indicates the ODAM expression (figure in color online only).

- On days 10 and 14, regenerated JE was present. ODAM, PCNA, and CK14 expression was similar to that of the control.

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