

Effects of Selective Alveolar Decortication on the Periodontal Complex

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Research Article

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Abstract

Background

Modification of bone turnover has been reported following selective alveolar decortication but the molecular signals in the periodontal ligament space (PDL) remain unanswered. The objective of this study was to understand how selective alveolar decortication affects the biological reactions in the periodontal ligament.

Methods

Selective alveolar decortication in wild-type mice (n=25) was performed on mandibular right buccal cortical plate adjacent to the mandibular right third molar and euthanized at 3, 7, 14 and 28 days. We also performed selective alveolar decortication in Lrp5^{ACT} (n=5) mice and Ad-Dkk1 treated mice (n=5), and euthanized at 7 days. The periodontium around the mandibular third molars were examined using histology, immunohistochemical analyses for osteogenic markers, TGF- β , RANKL, TRAP and alkaline phosphatase activity.

Results

The expression of osteogenic markers in the wild-type PDL was maintained during healing time period after selective alveolar decortication. Increased osteoclast activity in the wild-type mice was observed at 3 and 7 days after selective alveolar decortication. The PDL in Lrp5G171V (Lrp5^{ACT}) mice and adenovirus Dkk1 (Ad-Dkk1) treated mice also showed insignificant changes in the expression of osteogenic markers following selective alveolar decortication. In Lrp5^{ACT} mice where there was a reduction of bone resorption, selective alveolar decortication caused a dramatic increase in osteoclast activity.

Conclusions

Selective alveolar decortication affects only bone turnover, but not the expression of osteogenic markers in the PDL.

Background

Selective alveolar bone decortication has been performed as a means to accelerate tooth movement.¹ The theory behind this procedure is that decortication can induce demineralization of the alveolar bone that surrounds the tooth root, leading to a softening of the mineralized matrix that offers less resistance to tooth movement. This demineralized condition accelerates tooth movement until the alveolar bone remineralizes, and thus reduces treatment time by 4-6 months.² During this demineralization and

reminerlization, it is postulated that osteoblast and osteoclast populations alter in numbers and cause an osteopenic effect, which leads to a regional acceleratory phenomenon (RAP).^{3,4} RAP consists of four phases: activation, resorption, reversal, and formation.⁵ In the activation phase, receptor activator of nuclear factor kappa-B ligand (RANKL) cooperates with receptor activator of nuclear factor kappa-B (RANK), leading to the differentiation of preosteoclasts into mature osteoclasts.⁶ Bone resorption continues for about 2 weeks. In the reversal phase, secretion of osteoid by osteoblasts predominates.

Performing a localized alveolar decortication without tooth movement can provide insights into the biologic mechanisms taking place in the periodontal ligament space (PDL). Decortication of both buccal and lingual bone plates results in increased bone resorption and bone formation in rats with peaks at 3 weeks⁷. While alteration in bone turnover has been reported, the underlying response in the PDL following the localized alveolar decortication remains unclear. The aim of this study was to investigate the biologic reactions in the PDL space and the alveolar bone after selective alveolar decortication as the function of time. We combined this procedure with a genetic approach to modulate Wnt signaling: we used Lrp5G171V (Lrp5^{ACT}) mice, where bone mass is markedly increased due to reduced bone resorption⁸ and adenovirus Dkk1 (Ad-Dkk1) treated mice that have reduced bone mass due to increased resorption.⁹

Materials And Methods

Generation of Selective Decortication

Twenty-five 10 weeks-old male mice (C57BL/6) were used for this study. A regular hard diet and water *ad libitum* were supplied. Following anesthesia with ketamine/xylazine (87.5/12.5 mg/kg, respectively), the right cheek was shaved and an incision was made along the mandibular ramus, and the buccinators muscle was elevated until the molar alveolus was found. About 10.0 mm up from the mandibular base (the deepest point of the mandibular base concavity), a hole with a diameter of 1.0 mm was created through the surrounding tissues on the buccal surface of mandibular alveolar ridge on the distal side of the mandibular third molar. A handpiece with #331 bur (diameter 1.0mm, head length 1.6mm) was used to form the hole that does not penetrate the underlying trabecular bone (Fig. 1). The region was irrigated, and the skin was closed with use of a suture. Mice were sacrificed at multiple time points including 0, 3, 7, 14, 28 days after decortication (PD0, PD3, PD7, PD14, PD28: 5 mice for each group).

Generation of Mouse Strains

The generation of Lrp5G171V mice (here referred to as Lrp5^{ACT} mice) were described previously;¹⁰ five 2 month-old mice were analyzed 7 days after decortication. The generation of Ad-Dkk1 treated mice were described previously;¹¹ five 2 month-old mice were analyzed 7 days after decortication. Selective decortication was performed and sacrificed at PD7.

Sample Preparation and Processing

Right mandibular molar areas in mice were harvested and placed in 4% paraformaldehyde for one night at 4°C. Demineralization of samples were processed in a microwave using 19% EDTA for fourteen days. After process of decalcification, specimens were dehydrated using an ascending ethanol series and paraffin embedding was followed. Longitudinal sections were cut in an 18-micron thick and collected on Superfrost-plus slides for analysis. Equivalent levels and planes of section in the distal root of the mandibular third molar were selected from each of the mouse for analysis.

Histology

Movat's pentachrome staining¹² was performed. Alkaline phosphatase (ALP) staining¹³ and tartrate-resistant acid phosphatase (TRAP) activity was performed using standard procedures.

Immunohistochemistry

After deparaffinization of tissue sections, endogenous peroxidase activity was dampened using 3% hydrogen peroxide and then washed using phosphate buffered saline (PBS). With 5% goat serum (S-1000, Vector, Burlingame, CA), slides were blocked for 60 minutes at room temperature. The assigned primary antibody was added and incubated for one night at 4°C, then washed using PBS. Incubated with biotinylated secondary antibodies (BA-x, Vector) for 30 minutes, samples were washed using PBS. An avidin/biotinylated enzyme complex (PK-4000, Vector) was combined and incubated for 30 minutes. A 3,3' diaminobenzidine substrate kit (SK-4100, Vector) was used to detect color. Antibodies included *Osterix* (OriGene, Rockville, MA; 1:2000 dilution), *Runx2* (OriGene, Rockville, MA; 1:2000 dilution), Osteopontin (LF 175, National Institutes of Health, Bethesda, MD; 1:4000 dilution), RANKL (LabVision AB, Värmdö, Sweden; 1:100 dilution) and TGF-beta (TGF-β) (Abcam plc. Waltham, MA; 1:200 dilution).

Ethics

All procedures and the housing and care of mice were performed in accordance with animal welfare based on an approval of Stanford University Institutional Animal Care and Use Committee (protocol #13146). The study was reported in accordance with ARRIVE guidelines.

Results

Influence of Decoration on the Tissue Architecture of PDL

Histologic examination of the mandibular periodontal complex revealed that compared to PD0 (Fig. 2A,2A'), decortication did not noticeably alter the fibrillar structure of the PDL at PD3 (Fig. 2B,2B'), PD7 (Fig. 2C,2C'), PD14 (Fig. 2D,2D') and PD28 (Fig. 2E,2E') in the wild-type mice. Higher magnification images of the periodontal complex showed that the periodontal ligament consists of numerous cells, probably fibroblasts within and between the fiber bundles. We turned to analyses of the alveolar bone osteogenesis and homeostasis in the PDL for a deeper understanding of how decortication affected the alveolar bone and the PDL with increasing healing time.

Influence of Decoration on the Osteogenic factors in the PDL

Cells of the periodontal ligament are osteogenic in nature.¹⁴ In PD0, *Osterix* expression was observed close to cementum in the PDL space. Compared to its expression in PD0 (Fig. 3A), *Osterix* expression on the decortication side was not altered in the PDL space of PD3 (Fig. 3B). With increasing healing time, *Osterix* expression on the decortication side maintained throughout the PDL space (Fig. 3C-E). Runx2 expression followed the same pattern: on the intact side, Runx2 expression was observed throughout the PDL space (Fig. 3F); on the decortication side, Runx2 expression was maintained with increasing healing time (Fig. 3G-J). Osteopontin was expressed in both cementoblasts and osteoblasts of PD0 (Fig. 3K). Similarly, Osteopontin expression was maintained at decortication sides with increasing healing time (Fig. 3L-O). Following decortication of the alveolar bone, osteogenic factors in the PDL space maintained the expression on the decortication side until PD28.

A previous report suggests that expression of osteogenic factors in the PDL space could be related with TGF- β .¹⁵ We investigated the possible relationship between the osteogenic factors and TGF- β in the PDL space after selective decortication in the surrounding alveolar bone. As reported, TGF- β was observed in the PDL space at PD0 (Fig. 3P),¹⁶ and its expression was maintained during the healing period (Fig. 3Q-T).

Influence of Decoration on the Bone Turnover

ALP activity in PD0 was nearly undetectable in the PDL (Fig. 4A), while ALP activity on the decortication side was gradually increased in the PDL space with increasing healing time (Fig. 4B-E). Using TRAP staining we visualized a level of osteoclast activity on PD0 (Fig. 4F). TRAP activity at both PD3 and PD7 was noticeably increased (Fig. 4G,4H) compared to that in the intact side (Fig. 4F). TRAP activity in the decortication side at PD28 returned to the level seen on PD0 (Fig. 4I,4J).

Osteoclast activity is regulated by the combined actions of the osteoprotegerin (OPG) receptor and its ligand, RANKL. Expression of RANKL on the decortication side of periodontal complex (Fig. 4K-O) followed the same trend observed with TRAP activity. Compared with PD0, particularly high levels of RANKL were observed in the PDL at PD3 (Fig. 4L). We turned to analyses animal models with gain- and loss-of-function of Wnt signaling to better understand how high bone mass and loss of bone mass phenotypes affected the periodontium at PD7.

Influence of Decoration on the PDL Homeostasis and Bone Turnover in animal models with gain- and loss-of-function of Wnt signaling

We observed differences in the expression of osteogenic transcription factors in the PDL of Lrp5^{ACT} and Ad-Dkk1 treated mice after creation of decortication. For Lrp5^{ACT} mice, expression of *Osterix* (Fig. 5A) on the decortication side of the PDL space was slightly increased throughout the PDL space compared to its expression on the intact side (Fig. 5A). For Lrp5^{ACT} mice, expression of Runx2 (Fig. 5C) on the

decortication side of the PDL space was slightly elevated, close to cementum, compared to its expression on the intact side as well (Fig. 5C). For Lrp5^{ACT} mice, expression of OPN (Fig. 5E') on the decortication side of the PDL space was also elevated, close to alveolar bone, compared to its expression on the intact side (Fig. 5E). ALP activity in the PDL of Lrp5^{ACT} was slightly increased on the decortication side, close to alveolar bone (Fig. 5G, G'). For Ad-Dkk1 treated mice, expression of *Osterix* was not changed on the decortication side of the PDL space compared to the intact side (Fig. 5B, B'). Expression of Runx2 was equivalent between the intact and decortication sides of Ad-Dkk1 treated mice (Fig. 5D, D'). Expression of Osteopontin in the decortication side was partly observed in the cementum and alveolar bone of Ad-Dkk1 treated mice (Fig. 5F, F'). ALP level was not altered on the decortication side of Ad-Dkk1 treated mice compared to the intact side (Fig. 5H, H').

Compared to the intact side of Lrp5^{ACT} mice, osteoclast activity was significantly increased on the decortication side of Lrp5^{ACT} mice (Fig. 5I, I'). Even in Lrp5^{ACT} mice where higher Wnt signal is associated with decreased osteoclast activity, creation of decortication in the surrounding bone caused dramatic increase of osteoclast activity. In Ad-Dkk1 treated mice, strong expression of osteoclast activity in the intact side was also observed in the decortication side (Fig. 5J, J').

Discussion

Little is known how decortication in alveolar bone causes any alteration of homeostasis in the PDL space. Decortication of alveolar bone did not alter the tissue architecture in the PDL space (Fig. 2). The expression of osteogenic factors was also maintained on the decortication side until PD 28 (Fig. 2). Stimulation by decortication in the alveolar bone may influence cell signaling in the PDL space. One of the possible candidates for cell signaling is TGF- β , which involves in growth, proliferation and differentiation of osteoblasts.^{17,18} In the same context, PDL cells were more differentiated upon elevation of TGF- β -receptor III,¹⁵ and TGF- β -receptor II knock-out mouse exhibited defects in proliferation and differentiation of osteogenic cells.¹⁷ TGF- β expression in the PDL cells was demonstrated,¹⁶ and it was also expressed in the PDL space before creation of decortication (Fig. 3P). Here, we found expression of TGF- β in the PDL space was continuously maintained until PD 28 (Fig. 3Q-T). Along with the expression of osteogenic factors, the expression of TGF- β was maintained on the decortication side until PD 28.

Following decortication, ALP activity was gradually increased with increasing healing time (Fig. 4A-E). Osteoclast activity was highest at PD3 (Fig. 4G) and returned to baseline at PD 28 (Fig. 4J). Thus, we observed different phase of bone turnover: a resorption phase at PD 3 and a formation phase at PD 28. At the early time period, bone resorption mostly occurred and then bone formation exceeds bone resorption. Later, a phase of mineralization followed, which is consistent with a previous study where the widespread formation of alveolar bone occurred at PD 21 after decortication.^{5,19} An increase of bone formation in response to decortication has also been observed in the rabbit tibia at 4 weeks.^{3,20}

We sought clues into alteration of molecular reaction in the PDL space of high bone mass and loss of bone mass phenotypes following creation of decortication. Previously reported, the expression of osteogenic markers is high in an environment where Wnt signaling is up-regulated.²¹ Expression of osteogenic factors in the PDL space of Lrp5^{ACT} mice was slightly elevated at PD 7 (Fig. 5). Upon decortication, amplified Wnt signal was positively associated with increase of osteogenic factors expression in the PDL space of Lrp5^{ACT} mice. One question that remains to be answered is whether up-regulated Wnt signaling can significantly enhance the expression of osteogenic factors, especially ones related to mineralization, in the Lrp5^{ACT} mice later time period. Expressions of osteogenic factors in Ad-Dkk1 treated mice, however, were not altered following decortication in the surrounding alveolar bone at PD 7.¹¹

The Lrp5^{ACT} showed alveolar bone accumulation with either unaltered bone resorption²² or decreased bone resorption.²³ Although the physiologic mechanism is not fully understood, decreased bone resorption in the Lrp5^{ACT} is due to reduced number and/or function of osteoclasts by altering the OPG to RANKL ratio.²⁴ Here, selective decortication caused a dramatic increase of osteoclast activity in Lrp5^{ACT} mice, which was also observed in Lrp5^{ACT} mice after creation of injury.¹¹ Upon speculation of the reason for increased osteoclast activity, inflammatory reaction in the Lrp5^{ACT} mice after selective decortication could exceed a threshold tolerance for expression of Wnt signaling during osteoclastogenesis.²⁵

Abbreviations

PDL

periodontal ligament space

Lrp5^{ACT}

Lrp5G171V

Ad-Dkk1

adenovirus Dkk1

RANKL

receptor activator of nuclear factor kappa-B ligand

RANK

receptor activator of nuclear factor kappa-B

ALP

Alkaline phosphatase

TRAP

tartrate-resistant acid phosphatase

PBS

phosphate buffered saline

D

dentin

AB

alveolar bone.

Declarations

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Authors' contributions

Defining the study aims: WHL, JL; performing the experiments: WHL; analyzing and discussing the data: JL, JA; creating the figures: JA; writing the manuscript: JL,JA; revised the manuscript: WHL.

All authors contributed to the writing of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

All procedures and the housing and care of mice were performed in accordance with animal welfare based on an approval of Stanford University Institutional Animal Care and Use Committee (protocol #13146).

Consent for publication

Not applicable.

Conflict of interest

The authors declare no competing interests.

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Figures

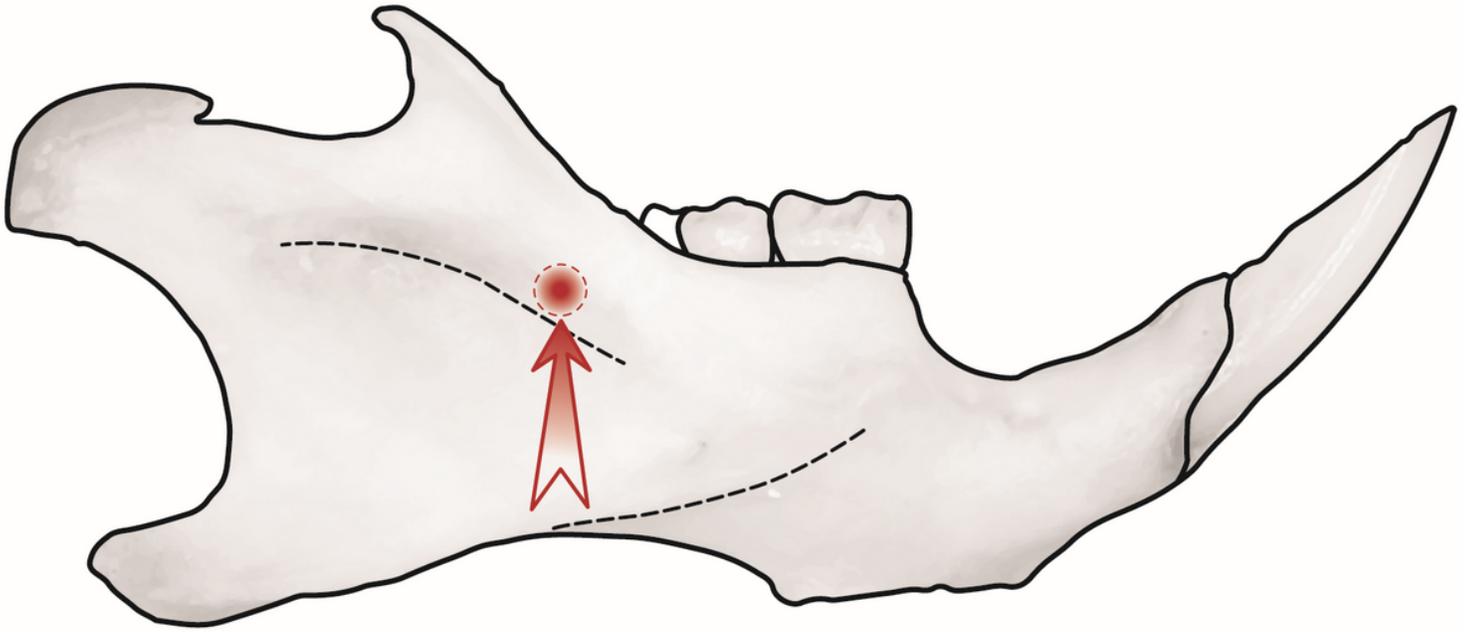


Figure 1

Figure 1

Decortication was created on the right side of mandible. About 10 mm from the mandibular base, a hole with a diameter of 1.0 mm (red point) was created on the buccal surface of mandibular alveolar process near the mandibular third molar.

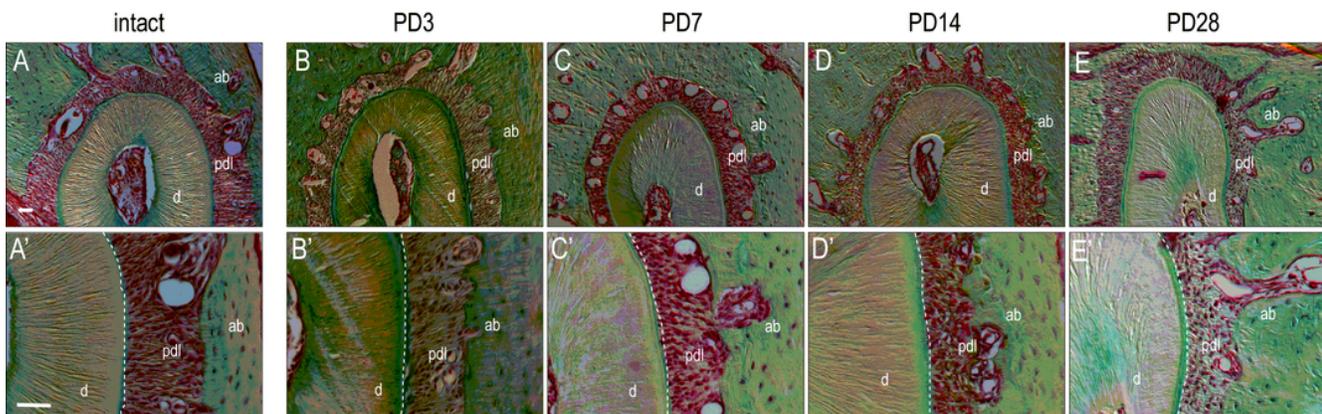


Figure 2

Figure 2

Tissue architecture of the PDL was not affected by decortication. Pentachrome staining of tissue sections through the right mandibular third molar distal root of adult mice from (A, A') PD 0, (B, B') PD 3, and (C, C') PD 7 (D, D') PD 14 (E, E') PD 28 of defect sides. Abbreviations: d, dentin; ab, alveolar bone; pdl, periodontal ligament. All scale bars are 500 μ m.

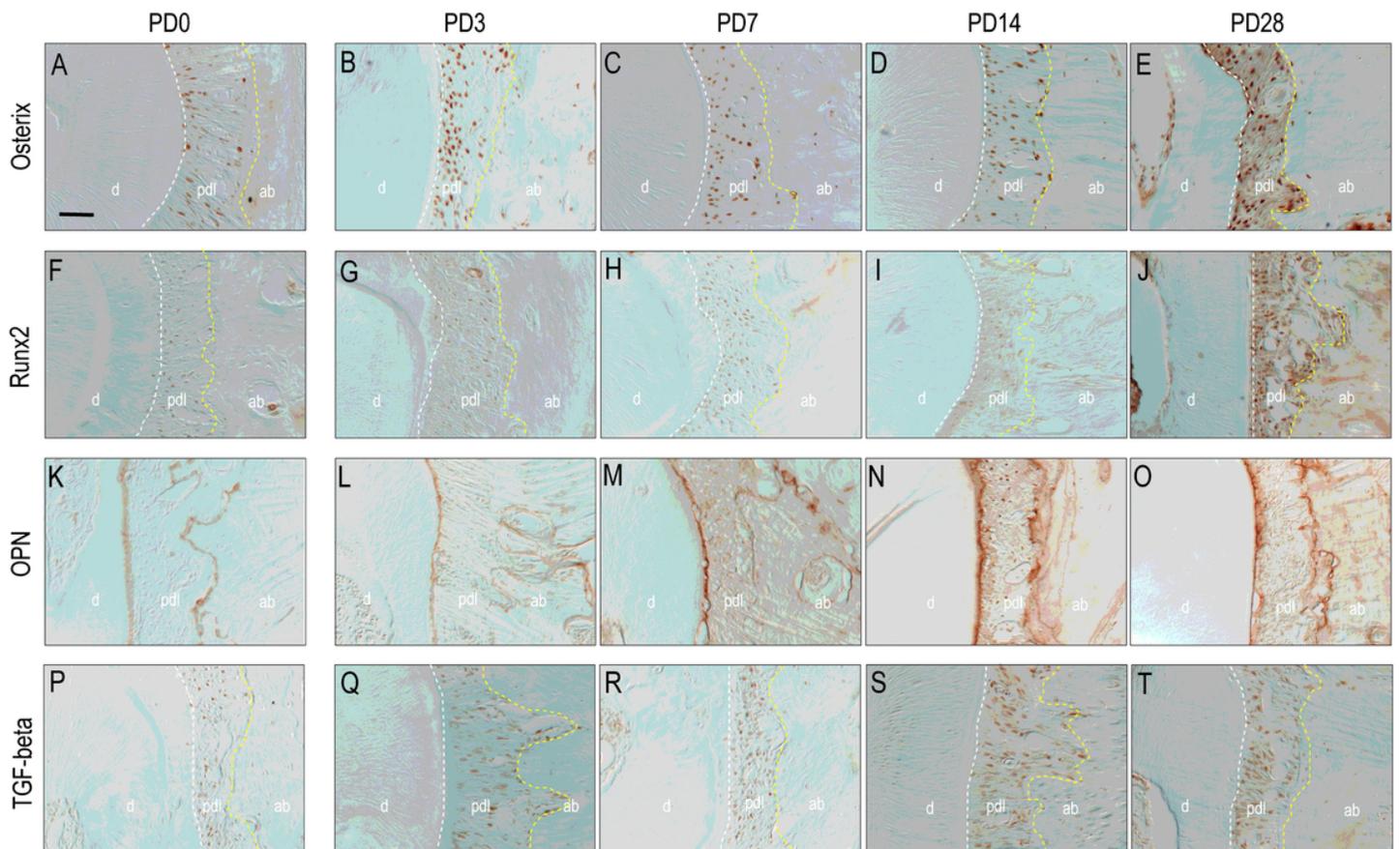


Figure 3

Figure 3

Expression of Osteogenic factors in the PDL space was not influenced by decortication. *Osterix* immunostaining shown on tissue sections through the mandibular third molar roots from (A) PD 0, (B) PD 3, and (C) PD 7 (D) PD 14 (E) PD 28 of defect sides. (F-J) Runx2 immunostaining shown on tissue sections from the same categories. (K-O) Osteopontin immunostaining shown on tissue sections from the same categories. (P-T) TGF- β immunostaining shown on tissue sections from the same categories. Abbreviations: d, dentin; ab, alveolar bone; pdl, periodontal ligament. All scale bars are 500 μ m.

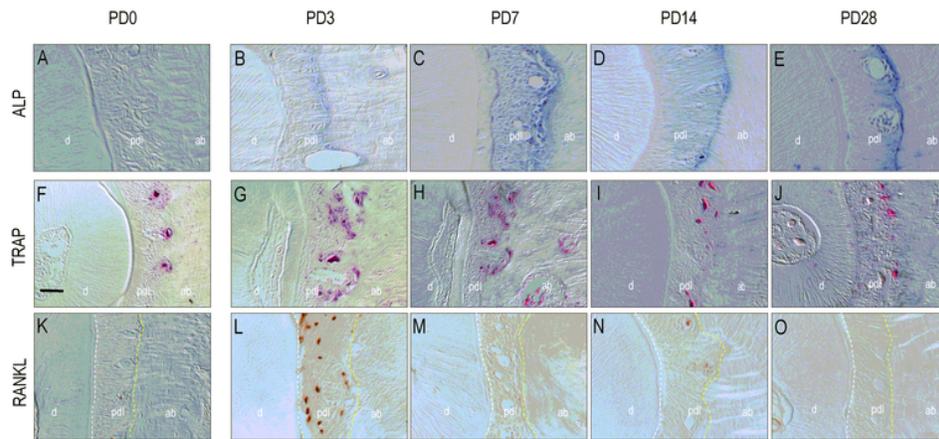


Figure 4

Figure 4

Alveolar bone osteogenesis and osteoclast activity was impacted by decortication. (A-E) ALP staining shown on tissue sections from PD 0, PD3, PD7, PD14, PD28 of defect sides, respectively. (F-J) TRAP activity shown on tissue sections through the mandibular third molar roots from the same categories. (K-O) RANKL Ligand immunostaining on tissue sections through the mandibular third molar roots from mice in the same categories. Abbreviations: d, dentin; ab, alveolar bone; pdl, periodontal ligament. All scale bars are 500 μ m.

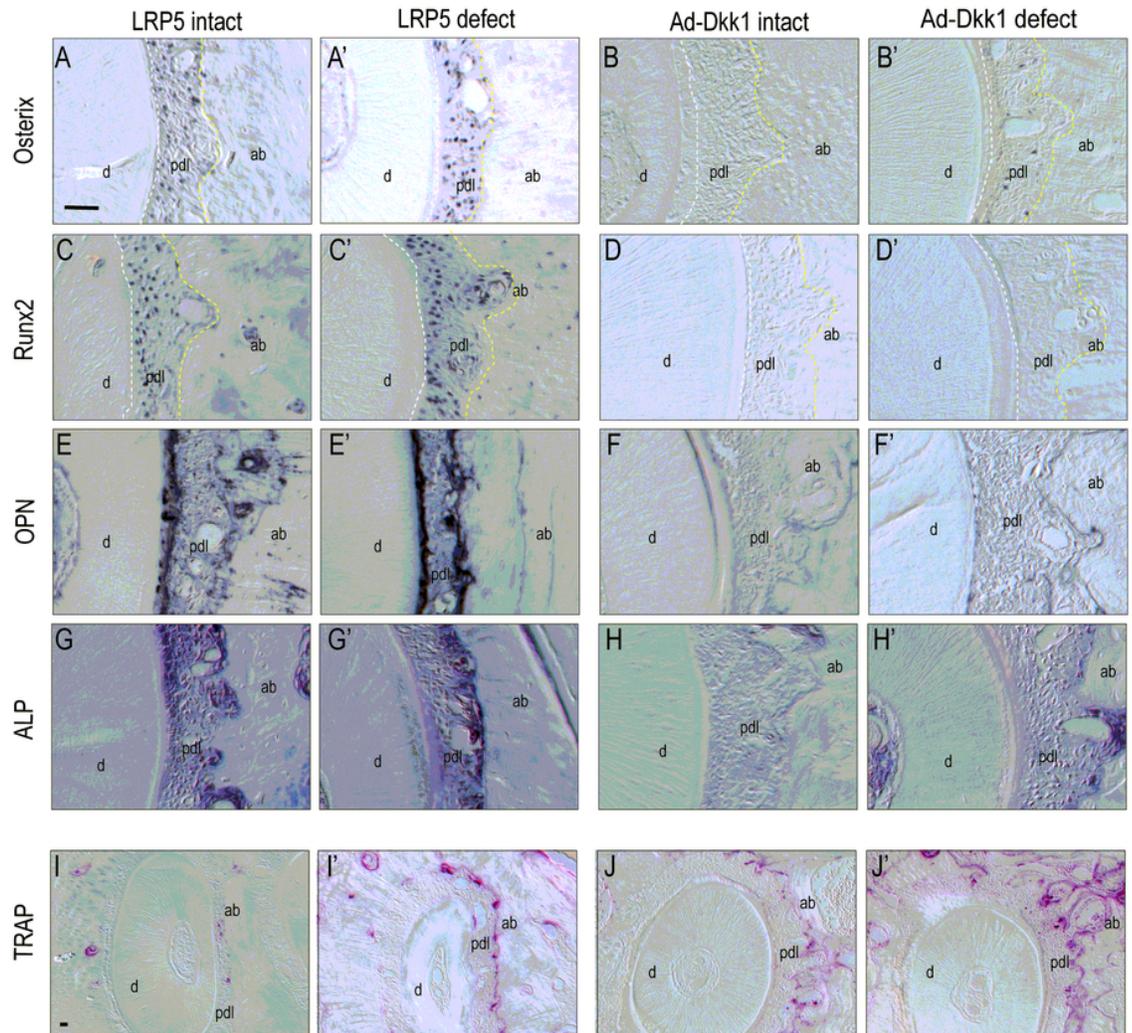


Figure 5

Figure 5

PDL homeostasis and osteoclast activity were influenced by decortication in animal models with gain- and loss-of-function of Wnt signaling. *Osterix* immunostaining shown on tissue sections through the mandibular third molar roots from of (A) intact side of *Lrp5*^{ACT} mice, (A') defect side of *Lrp5*^{ACT} mice at PD 7, (B) intact side of Ad-Dkk1 treated adult mice (B') defect side of Ad-Dkk1 treated adult mice at PD 7. (C, C', D, D') Runx2 immunostaining shown on tissue sections from the same categories. (E, E', F, F') Osteopontin immunostaining shown on tissue sections from the same categories. (G, G', H, H') ALP activity shown on tissue sections from the same categories. (I, I', J, J') TRAP activity shown on tissue sections from the same categories. Abbreviations: d, dentin; ab, alveolar bone; pdl, periodontal ligament. All scale bars are 500 μ m.

