ORAL DISEASES

Oral Diseases (2009) 15, 573–579. doi:10.1111/j.1601-0825.2009.01597.x

http://www.blackwellmunksgaard.com

ORIGINAL ARTICLE

Comparative study of gene expression during tooth eruption and orthodontic tooth movement in mice

XF Huang[1], YB Zhao[2], FM Zhang[1], PY Han[1]

[1]*Department of Stomatology, Beijing Friendship Hospital, Capital Medical University;* [2]*Department of Periodontology, School of Stomatology, Peking University, Beijing, China*

OBJECTIVE: The aim of this study was to understand tooth eruption by comparing the gene expression during tooth eruption and orthodontic tooth movement (OTM).
MATERIALS AND METHODS: Orthodontic force was applied on maxillary molars for 2, 4, 7 and 14 days to study tooth movement. Mice at PN 0, 7, 10, 15 and 21 were fixed to observe tooth eruption. Comparative study of two procedures was assessed by haematoxylin and eosin, tartrate-resistant acid phosphatase staining and *in situ* hybridization for matrix metalloproteinase (*Mmp*)2, *13*, bone sialoprotein (*Bsp*) and osteocalcin (*Ocn*).
RESULTS: Tartrate-resistant acid phosphatase activity and expression of *Mmp2*, *13* were obviously detectable in the compression region during OTM. They were also identified in the occlusal and apical region of alveolar bone during tooth eruption. Strong expression of *Bsp* and *Ocn* was detectable at the tension side during OTM. These genes were also expressed in the inner lateral region of alveolar bone adjacent to the tooth, but absent in the inner surface of the occlusal and root apical regions during tooth eruption.
CONCLUSION: The process of alveolar bone metabolism during developmental eruption and OTM shares the same mechanism. Internal force, as the orthodontic force for OTM, may be initiating factor for tooth eruption.
Oral Diseases (2009) **15**, 573–579

Keywords: tooth eruption; tooth movement; orthodontic; bone remodelling; periodontal ligament

Introduction

Tooth eruption is a unique developmental and biological process in which the growing tooth migrates vertically from the developing alveolar bone to the functional position in the occlusal plane. Both two opposite aspects of bone metabolism, bone formation (osteogenesis) and bone resorption (osteoclastogenesis) are required for tooth eruption. Without bone resorption, no eruption pathway forms, and the tooth cannot erupt (Wise *et al*, 2002a,b). Without alveolar bone formation, tooth dose not erupt either (Beertsen *et al*, 2002; Bartlett *et al*, 2003). However, the mechanisms that occur during developmental eruption have not been uncovered. Four possible mechanisms for eruption have been mentioned previously as follows: (i) root formation, which induces occlusal movement of the tooth crown; (ii) hydrostatic pressure within the periapical tissues pushing the tooth occlusally; (iii) bony remodelling and (iv) pulling of the tooth in an occlusal direction by the cells and fibres of the periodontal ligament (Ten, 1998; Craddock and Youngson, 2004). Therefore, internal force or motive force mentioned by Wise and King (2008) may be the important factor for tooth eruption.

Orthodontic tooth movement (OTM), a treatment procedure, is an external force-induced tooth movement. Orthodontics takes advantage of the physiological movement of tooth eruption in corrective treatment. On the other hand, numerous theories of tooth eruption may be due to the enormous success of orthodontics in moving teeth with force application. Understanding of the mechanism of OTM will improve the knowledge of tooth eruption. Alveolar bone responds to external mechanic force, and remodels to accommodate tooth movement (Craddock and Youngson, 2004). Bone remodelling is mediated by periodontal tissues during tooth movement, and guides the tooth movement to a new and stable position (Moxham and Berkovitz, 1995; Merzel and Salmon, 2008). Although there is a difference, it seems that the processes of eruption and OTM might be similar. However, the relationship between eruptive tooth movement and OTM has remained unclear yet, and comparative studies of these two kinds of tooth movement have seldom been reported (Wise and King, 2008). In our study, we try to compare the gene expression during two procedures to understand tooth eruption.

Correspondence: Associate Professor Xiaofeng Huang DDS, PhD, Department of Stomatology, Beijing Friendship Hospital, Capital Medical University, No. 95 Yong'an Road, Xuanwu District, 100050 Beijing, China. Tel: 086 010 63138415, Fax: 086 010 63038519, E-mail: hxf1998@hotmail.com
Received 11 March 2009; revised 26 May 2009; accepted 2 June 2009

The direct function of osteoclasts is bone resorption. Acid phosphatase, the marker of osteoclasts can be identified by tartrate-resistant acid phosphatase (TRAP) during tooth eruption and OTM (Beertsen *et al*, 2002; Von *et al*, 2004). Degeneration of extracellular matrix is another character of bone resorption. Matrix metalloproteinases (MMPs) have been identified in periodontium or dental follicle during tooth eruption and OTM (Howard *et al*, 1998; Tervahartiala *et al*, 2000; Tsubota *et al*, 2002; Takahashi *et al*, 2003, 2006; Luan *et al*, 2007). Osteoblasts are related to bone formation. Bone sialoprotein (BSP) and osteocalcin (OCN) have been shown to be specific for mineralization (Chen *et al*, 1992; Domon *et al*, 2001). In our study, we compare the TRAP activity and gene expression of *Mmp2*, *13*, *Bsp* and *Ocn* during tooth eruption and OTM, and find that two processes share the similar osteogenetic and osteoclastogenetic gene expression patterning in the bony microenvironment. As OTM is a force-induced procedure, we suggest that internal force may be one of the initiating factors for tooth eruption during tooth and root development.

Materials and methods

Animals and experimental processing

C57BL6/J mice (the Jackson Laboratory, Bar Harbor, ME, USA) at postnatal day (PN) 0, 7, 10, 15, 21 and 60 were selected as experimental animals. Mice at PN 0, 7, 10, 15 and 21 were used for sagittal section processing directly. Mice at PN 60 were anesthetized with an i.p. injection of pentobarbital sodium (50 mg kg^{-1}) during the setting and adjusting of the orthodontic appliance. According to the method described by Yoshimatsu *et al* (2006), the orthodontic appliance (Autho Instruments Co., Beijing, China) was inserted between the upper incisors and left first molar and fixed with a 0.1 mm stainless wire around both teeth using glass ionomer cement (Shanghai Medical Instruments Co., Ltd, Shanghai, China). The right maxillary first molar was not loaded as the control. After OTM, the maxillars were used for horizontal section process. The protocol for these animal procedures was in accordance with the regulations of Beijing Friendship Hospital and Capital Medical University.

Tissue processing and paraffin section

All mice were killed using CO_2 gas. Tissues from the maxillas at PN 0, 7, 10, 15, 21 and after 2, 4, 7 and 14 days of OTM were fixed with 4% paraformaldehyde for 1 day, decalcified in 5% di-sodium EDTA for 2–4 weeks and dehydrated in a graded series of ethanol. Subsequently, tissues were embedded in paraffin, cut into 6-μm thick sections and prepared for haematoxylin and eosin (H&E) staining, TRAP staining and *in situ* hybridization.

H&E staining and TRAP staining

For general morphology, deparaffinized sections were stained with H&E staining using standard procedures. Detection of TRAP activity in tissue sections was carried out as previously described (Yoshimatsu *et al*, 2006). The sections were incubated in acetate buffer (pH 5.0) containing naphthol AS-MX phosphate (Sigma Chemical, St Louis, MO, USA), Fast Red Violet LB salt (Sigma) and 50 mM sodium tartrate.

In situ hybridization

Samples from mice at PN 0, 7, 10, 15 and samples after 0, 2, 4 and 7 days of tooth movement were paraffin sectioned and mounted following standard procedures. DNA fragments of *Mmp2*, *13*, *Ocn* and *Bsp* (gifts from Dr Yang Chai, USC, USA) were subcloned in vector plasmids. Digoxigenin (DIG)-labelled sense and antisense cRNA riboprobes were synthesized using the DIG RNA Labeling Mix (Roche Molecular Biochemicals, Indianpolis, IL, USA). Paraffin-embedded sections were dewaxed and treated with proteinase K (20 μg ml^{-1}), 0.2 M HCl, acetylated and hybridized overnight at 55°C with DIG labelled probes.

Results

Histological changes in the periodontium after eruptive and OTM

Following root development, the mouse tooth erupts after birth (PN 0) (Figure 1a). Tooth root begins to develop and tooth erupts occlusally at PN 7 (Figure 1b). After PN 7, the tooth continues to erupt and begins to destroy the integrity of alveolar bone at PN 10 (Figure 1c). The tooth erupts into oral cavity at PN 15 (Figure 1d). Root elongation and eruption are almost completed by PN 21 (Figure 1e). During OTM, the periodontal space narrowed on the compression side (mesial side) (Figure 1k–o, u) and became wider on the tension side (distal side) (Figure 1p–u). The periodontal ligament (PDL) on the compression side became an interrupted and acellular structure with hyalinization, but recovered after 14 days of OTM (Figure 1f–u).

Changes of TRAP activity and gene expression during OTM

During the OMT, acid phosphatase, detected by TRAP staining as the chemical marker of osteoclasts, was seldom expressed in periodontium in control (Figure 2a,f). TRAP activity began to be obviously observed on the alveolar bone surface in the compression region on day 4 after OMT (Figure 2k,p, indicated by arrows); however, no activity was observed on the inner bone surface of tension region (Figure 2k). MMP plays an important role in several collagen degradative processes which result in remodelling of hard tissue–soft tissue interfaces (Delaissea *et al*, 1993; Holmbeck *et al*, 1999). *Mmp2* and *13* began to be detectable in the alveolar bone surface of the compression region on day 4 after OTM (Figure 2l,m,q,r, indicated by arrows). However, they were absent in tension side and in the control (Figure 2b,c,g,h,l,m). *Bsp* and *Ocn* are markers of osteoblast. On day 4 after OTM, expression of *Bsp*, which were first identified in the tension side, was stronger than that of the controls (Figure 2d,i,n,s, indicated by arrows). *Ocn* expression was decreased

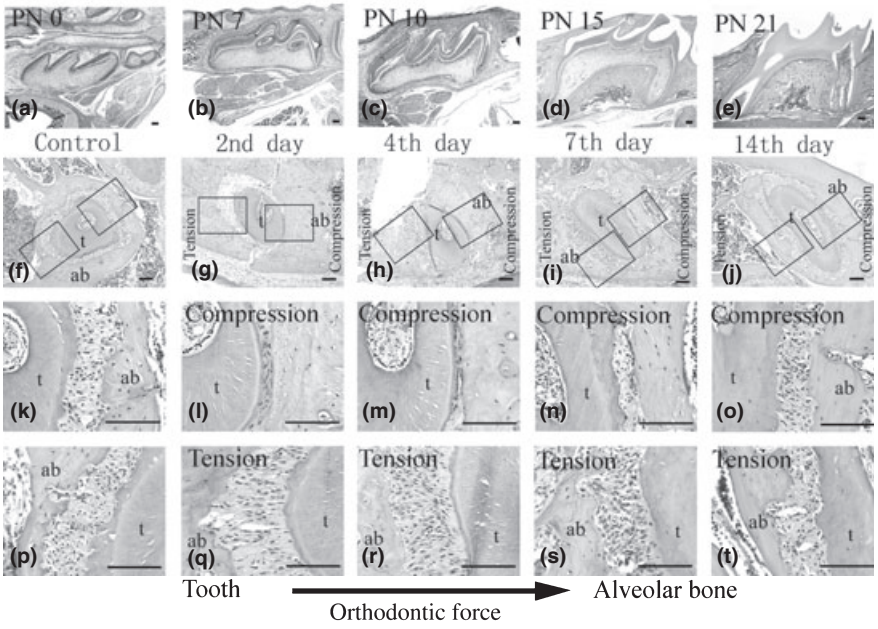

Figure 1 Histological changes in the periodontium during OTM and tooth eruption. The mouse tooth was embedded in alveolar bone after birth (**a**). Tooth developed roots and began to erupt occlusally at PN 7 (**b**). At PN 10, the tooth continued to erupt and began to destroy the integrity of alveolar bone (c). The tooth erupted into oral cavity at PN 15 (**d**). At PN 21, the whole crown of the upper first molar erupted into the oral cavity (**e**). During OTM, the periodontal space narrowed on the compression side (**f–o**) and became wider on the tension side (**p–t**). The PDL on the compression side became an interrupted and acellular structure with hyalinization (**l**, **m**) under the orthodontic force. The periodontal space and histological structure began to recover after 14 days (**j**, **o**, **t**, **u**). The direction of tooth movement caused by orthodontic force was toward the mesial side (indicated by an arrow at the bottom of the figures). ab, alveolar bone; t, tooth. Scale bars = 100 μm

(u)

Perodontal space during orthodontic tooth movement ($\bar{x} \pm s, \mu m$)

	Control	2nd day af OTM	4th day af OTM	7th day af OTM	14th day af OTM
Compression	63.4 ± 3.3	28.4 ± 3.6**	25.1 ± 3.1**	53.3 ± 4.6*	62.1 ± 3.4
Tension	65.1 ± 4.1	121.6 ± 6.1**	102.3 ± 4.9**	86.5 ± 5.2*	67.2 ± 4.0

$n = 5$; *, $P < 0.05$; **, $P < 0.01$

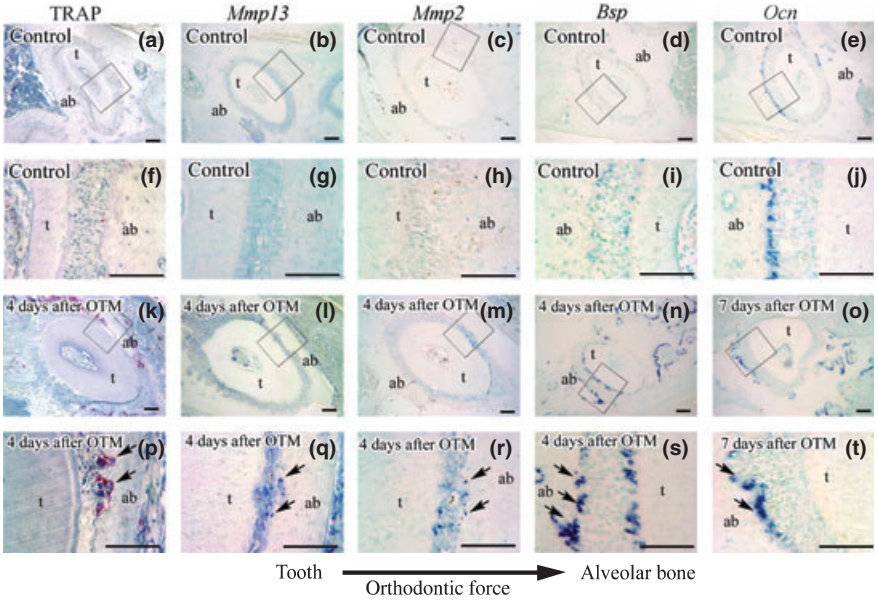

Figure 2 Changes of TRAP activity and gene expression during OTM. TRAP positive cells were seldom expressed in periodontium in control (**a**, **f**). TRAP activity was obviously observed on the alveolar bone surface in the compression region on day 4 after OMT (**k** and **p**, indicated by arrows), but not on the bone surface in the tension region (**k**). *Mmp2* and *13* began to be detected in the alveolar bone surface of the compression region on day 4 after OTM (**l**, **m**, **q**, **r**, indicated by arrows). However they were absent in tension side and in the control (**b**, **c**, **g**, **h**, **l**, **m**). On days 4 and 7, *Bsp* and *Ocn*, first identified after OTM, were strongly expressed in the tension site (**d**, **e**, **i**, **j**, **n**, **o**, **s**, **t**, **r**, indicated by arrows). *Bsp* and *Ocn* expression were not detectable in compression side (**n**, **o**). The direction of tooth movement caused by orthodontic force was toward the mesial side (indicated by an arrow at the bottom). ab, alveolar bone; t, tooth. Scale bars = 100 μm

after OTM (data not shown), however, began to be expressed strongly in tension site on day 7 (Figure 2e,j,o,t, indicated by arrows). *Bsp* and *Ocn* expression was not detectable in compression side (Figure 2n,o).

Changes of TRAP activity and gene expression during tooth eruption

At PN 0, TRAP positive cells can be identified on the surface of the alveolar bone around the tooth (Figure 3a,f,k,p, indicated by arrows). *Mmp2* and *13*, like TRAP activity, were also detected in the alveolar bone around the tooth (Figure 3b,c,g,h,l,m,q,r, indicated by arrows). Expression of *Bsp* and *Ocn* was also detectable, but absent on the inner surface of the alveolar bone (Figure 3d,e,i,j,n,o,s,t, indicated by arrows).

At PN 7 and PN 10, TRAP positive cells could be detectable in the occlusal and apical part of the alveolar bone around the tooth (Figures 4a,f,p and 5a,f,p, indicated by arrows), however, absent on the inner

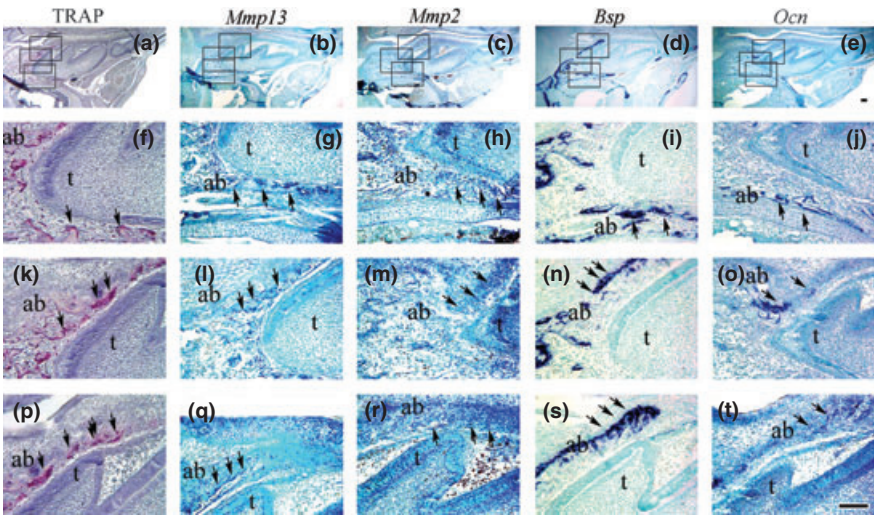

Figure 3 Changes of TRAP activity and gene expression at PN 0. At PN 0, TRAP positive cells could be identified on the surface of the alveolar bone around the tooth (**a**, **f**, **k**, **p**, indicated by arrows). *Mmp2* and *13* were also detected in the alveolar bone around the tooth (**b**, **c**, **g**, **h**, **l**, **m**, **q**, **r**, indicated by arrows). *Bsp* and *Ocn* expression was also detectable, but absent in the inner surface the alveolar bone (**d**, **e**, **i**, **j**, **n**, **o**, **s**, **t**, indicated by arrows). ab, alveolar bone; e, enamel; am, ameloblast. Scale bars = 100 μm

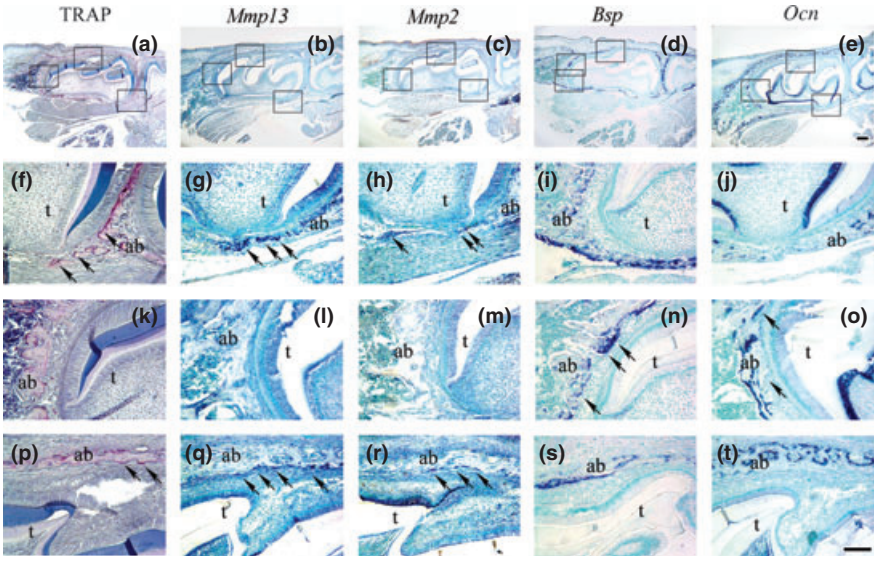

Figure 4 Changes of TRAP activity and gene expression at PN 7. At PN 7, TRAP positive cells were found in the occlusal and apical part of the alveolar bone around the tooth (**a**, **f**, **p**, indicated by arrows), however, absent on the inner lateral surface of alveolar bone (**k**). *Mmp2* and *13* were also detected in the occlusal and apical part of the alveolar bone around the tooth (**g**, **h**, **q**, **r**, indicated by arrows), but not lateral sides (**l**, **m**). The expression of *Bsp* and *Ocn* was absent on the inner surface of occlusal and root apical alveolar bone (**i**, **j**, **s**, **t**), however, they were expressed strongly on the inner surface of lateral sites (**n**, **o**, indicated by arrows). ab, alveolar bone; e, enamel; am, ameloblast. Scale bars = 100 μm

lateral surface of alveolar bone (Figures 4k and 5k). *Mmp2* and *13* were also detected in the occlusal and apical part of the alveolar bone around the tooth (Figures 4g,h,q,r and 5g,h,q,r, indicated by arrows), but not lateral sides (Figures 4l,m and 5l,m). The expression of *Bsp* and *Ocn* was absent on the inner surface of occlusal and root apical alveolar bone (Figures 4i,j,s,t and 5i,j,s,t); however, they were expressed strongly on the surface of lateral alveolar bone (Figures 4n,o and 5n,o, indicated by arrows).

At PN 15, tooth erupts into oral cavity and occlusal alveolar bone is disappeared. TRAP positive cells could be identified in apical two-third of the alveolar bone around the tooth (Figure 6a,f,k,p, indicated by arrows), but absent on the inner surface of occlusal one-third of the bone (Figure 6p). *Mmp2* and *13* were also detected in the apical two-third of the alveolar bone (Figure 6g,h,l,m,q,r, indicated by arrows), but absent on the inner surface of one-third of occlusal bone (Figure 6q,r). On the contrary, the expression of *Bsp* and *Ocn* was detectable on the inner surface of one-third occlusal alveolar bone (Figure 6s,t, indicated by arrows); however, absent on the other surface of alveolar bone around tooth (Figure 6i,j,n,o).

Discussion

The procedure of tooth eruption is accompanied with jaw and root development and becomes complicated. One of the possible factors for tooth eruption is motive force or internal force during root development (Van and McMinn, 1972; Craddock and Youngson, 2004; Wise and King, 2008). However, it has not been known where the initiating force comes from and how the force induces tooth eruption till now. OTM is a treatment procedure, simply induced by mechanical force. It is not difficult to control the orthodontic force and its direction. Comparative study of two processes may help to understand the mechanism of tooth eruption. Modelling and remodelling of the periodontium, especially the

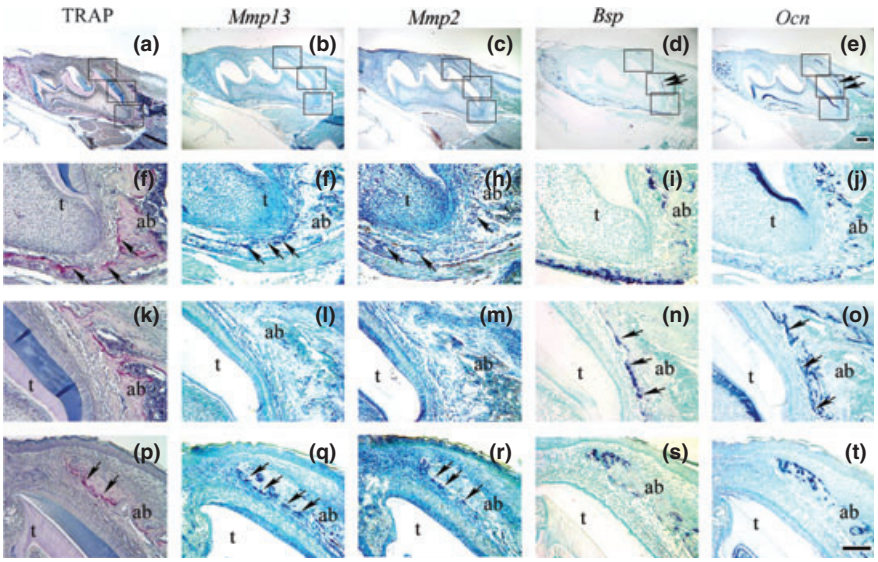

Figure 5 Changes of TRAP activity and gene expression at PN 10. At PN 10, TRAP positive cells were found in the occlusal and apical part of the alveolar bone around the tooth (**a**, **f**, **p**, indicated by arrows), however absent on the inner lateral surface of alveolar bone (**k**). *Mmp2* and *13* were also detected in the occlusal and apical part of the alveolar bone around the tooth (**g**, **h**, **q**, **r**, indicated by arrows), but not lateral sides (**l**, **m**). The expression of *Bsp* and *Ocn* was absent in the inner surface of occlusal and root apical alveolar bone (**i**, **j**, **s**, **t**), however, they were expressed strongly on the surface of lateral sites (**d**, **e**, **n**, **o**, indicated by arrows). ab, alveolar bone; e, enamel; am, ameloblast. Scale bars = 100 μm

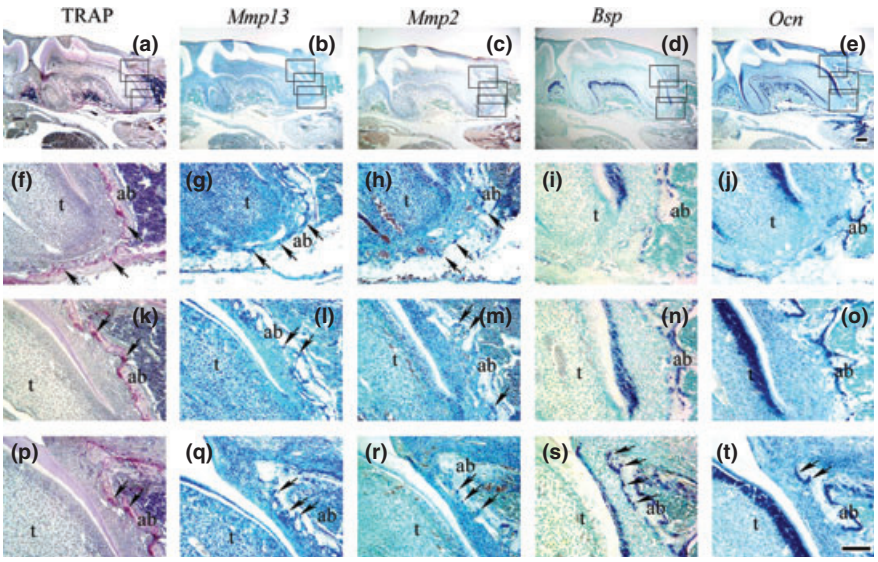

Figure 6 Changes of TRAP activity and gene expression at PN 15. At PN 15, occlusal alveolar bone was disappeared. TRAP positive cells could be identified in apical two-third of the alveolar bone around the tooth (**a**, **f**, **k**, **p**, indicated by arrows), however, absent on the inner surface of occlusal one-third site (**p**). *Mmp2* and *13* were also detected in apical two-third of the alveolar bone (**g**, **h**, **l**, **m**, **q**, **r**, indicated by arrows), but not on the inner surface of occlusal one-third part (**q**, **r**). The expression of *Bsp* and *Ocn* was detectable on the inner surface of one-third occlusal alveolar bone (**s**, **t**), however, absent in the other surface of alveolar bone around tooth (**i**, **j**, **n**, **o**, indicated by arrows). ab, alveolar bone; e, enamel; am, ameloblast. Scale bars = 100μm

PDL and alveolar bone, are essential for tooth movement. Molecules, such as osteoprotegerin, RANKL-RANK system, tumour necrosis factor, interleukin, etc. are related to OTM (Alhashimi *et al*, 2001; Oshiro *et al*, 2002; Tuncer *et al*, 2005; Bletsa *et al*, 2006). After getting the information from OTM, the expression of the same molecules is also detectable during eruptive tooth movement (Wise *et al*, 2000, 2005; Heinrich *et al*, 2005; Liu *et al*, 2005, 2006). The similar fundamental biological processes indicate that they may share the same mechanism for bone metabolism (Wise and King, 2008).

Our study demonstrates that site-specific bone resorption is mediated by ostoeclasts and gene expression of *Mmp2* and *13* during OTM. *In situ* hybridization also showed distinct special patterns of *Bsp* and *Ocn* in the orthodontic tension region of the PDL for bone formation. Then, we observed the *Mmp2*, *13*, *Bsp* and *Ocn* expression during tooth eruption. From PN 0 to 15, distinct special expression patterns of *Mmp2* and *13* were also consistent with the expression of acid phosphatase, the marker of osteoclasts. We also found that *Bsp* and *Ocn* were strongly expressed on the inner surface of the lateral region of alveolar bone without TRAP activities, suggesting that bone formation, as well as bone resorption, is important for tooth eruption (Beertsen *et al*, 2002; Bartlett *et al*, 2003). After tracing osteogenesis and osteoclastogenesis of alveolar bone, we describe bone metabolism during tooth eruption as follows: tooth is embedded in alveolar bone after birth and bone resorption is observed around the tooth; at PN 7, alveolar bone is still intact around the tooth and bone resorption is found on the inner surface of occlusal and apical alveolar bone adjacent to the tooth and bone formation in the lateral sides; at PN 10, parts of occlusal alveolar bone are destroyed and bone resorption and

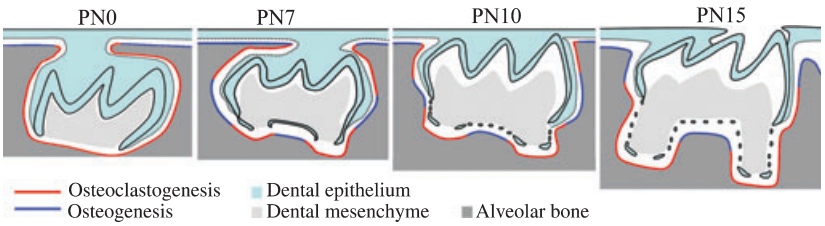

Figure 7 Osteogenesis and osteoclastogenesis during tooth eruption. Tooth is embedded in alveolar bone at PN 0, and bone resorption is observed around the tooth (red line). At PN 7, alveolar bone is still intact around the tooth. There is bone resorption on the inner surface of occlusal and apical alveolar bone adjacent to the tooth (red line) and bone formation in the lateral parts of alveolar bone (blue line). At PN 10, parts of occlusal alveolar bone are destroyed and bone resorption and bone formation is as same as PN7. At PN 15, occlusal alveolar bone are destroyed completely and bone resorption is found in the apical two-third of alveolar bone (red line) and bone formation in the occlusal one-third of alveolar bone and also furcation region around the tooth (blue line)

formation is as same as PN 7; at PN 15, occlusal alveolar bone are destroyed completely and bone resorption is found in the apical two-third of alveolar bone and bone formation in the occlusal one-third of alveolar bone and furcation region around the tooth (Figure 7).

Bones turn over by two distinct and related processes: modelling and remodelling (Frost, 2001; Wise and King, 2008). Bone remodelling is a cyclic process that is a response to the need for continuous repair and renewal of the skeleton throughout life. A remodelling cycle has four phases: activation, resorption, reversal and formation (Frost, 2001). This sequence of events is widely accepted as the way that the skeleton repairs. The histological events in compression sites in OTM are consistent with a remodelling cycle (King *et al*, 1991). Bone modelling is characterized by either bone formation or resorption that is sustained over a specific period of time during skeletal development, where individual bones move in relation to each other and change shape (Wise and King, 2008). The intra-osseous phase at the apical, occlusal and furcation sites of alveolar bone during tooth eruption can be considered to be primarily a process of alveolar bone modelling (Figure 7). However, at the lateral site, bone formation and bone resorption can be found at different stage. Accommodation of the tooth shape during eruption movement might be one of the reasons.

In summary, after comparing the TRAP activity and gene expression during OTM and tooth eruption, we conclude that the process of alveolar bone moulding and remodelling during these two kinds of tooth movement is similar: recruitment of osteoclasts and expression of *Mmp2* and *13* for bone resorption and expression of *Bsp* and *Ocn* for bone formation. Our survey may be useful for a better understanding of bone modelling and remoulding in the process of tooth eruption during tooth and jaw development, and helpful for dentists to provide the most appropriate care in clinic.

Acknowledgements

We thank Dr Yang Chai for the plasmids, Dr Songtao Shi for kind discussion and Yee Hung for critical reading of the manuscript. This work was supported by the Beijing New Star Program, China (2007B54) to Xiaofeng Huang.

Conflicts of interest

There is no conflicts of interest.

Author contributions

Xiaofeng Huang, the corresponding author, designed the research and performed animal experiment. Yibing Zhao prepared the samples and performed the staining. Fangming Zhang and Peiyan Han helped with the experiment.

References

Alhashimi N, Frithiof L, Brudvik P, Bakheit M (2001). Orthodontic movement and de novo synthesis of proinflammatory cytokines. *Am J Orthod Dentofacial Orthop* **119:** 307–312.

Bartlett JD, Zhou Z, Skobe Z, Dobeck JM, Tryggvason K (2003). Delayed tooth eruption in membrane type-1 matrix metalloproteinase deficient mice. *Connect Tissue Res* **44**(Suppl. 1)**:** 300–304.

Beertsen W, Holmbeck K, Niehof A *et al* (2002). On the role of MT1–MMP, a matrix metalloproteinase essential to collagen remodeling, in murine molar eruption and root growth. *Eur J Oral Sci* **110:** 445–451.

Bletsa A, Berggreen E, Brudvik P (2006). Interleukin-1alpha and tumor necrosis factor-alpha expression during the early phases of orthodontic tooth movement in rats. *Eur J Oral Sci* **114:** 423–429.

Chen J, Shapiro HS, Sodek J (1992). Developmental expression of bone sialoprotein mRNA in rat mineralized connective tissue. *J Bone Miner Res* **8:** 987–997.

Craddock HL, Youngson CC (2004). Eruptive tooth movement – the current state of knowledge. *Brit Dent J* **197:** 385–391.

Delaissea JM, Eeckhout Y, Neff L (1993). (Pro)collagenase (matrix metalloproteinase-1) is present in rodent osteoclasts and in the underlying bone-resorbing compartment. *J Cell Sci* **106:** 1071–1082.

Domon S, Shimokawa H, Yamaguchi S (2001). Temporal and spatial mRNA expression of bone sialoprotein and type I collagen during rodent tooth movement. *Eur J Orthod* **23:** 339–348.

Frost HM (2001). Cybernetic aspects of bone modeling and remodeling, with special reference to osteoporosis and whole-bone strength. *Am J Hum Biol* **13:** 235–248.

Heinrich J, Bsoul S, Barnes J, Woodruff K, Abboud S (2005). CSF-1, RANKL and OPG regulate osteoclastogenesis during murine tooth eruption. *Archs Oral Biol* **50:** 897–908.

Holmbeck K, Bianco P, Caterina J *et al* (1999). MT1–MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover. *Cell* **99:** 81–92.

Howard PS, Kucich U, Taliwal R, Korostoff JM (1998). Mechanical forces alter extracellular matrix synthesis by human periodontal ligament fibroblasts. *J Periodont Res* **33:** 500–508.

King GJ, Keeling SD, Wronski TJ (1991). Histomorphometric study of alveolar bone turnover in orthodontic tooth movement. *Bone* **12:** 401–409.

Liu D, Yao S, Pan F, Wise GE (2005). Chronology and regulation of gene expression of RANKL in the rat dental follicle. *Eur J Oral Sci* **113:** 404–409.

Liu D, Yao S, Wise GE (2006). Effect of interleukin-10 on gene expression of osteoclastogenic regulatory molecules in the rat dental follicle. *Eur J Oral Sci* **114:** 42–49.

Luan X, Ito Y, Holliday S *et al* (2007). Extracellular matrix-mediated tissue remodeling following axial movement of teeth. *J Histochem Cytochem* **55:** 127–140.

Merzel J, Salmon C (2008). Growth and the modeling/remodeling of the alveoloar bone of the rat incisor. *Anat Rec* **291:** 827–834.

Moxham BJ, Berkovitz BKB (1995). The periodontal ligament and physiological tooth movements. In: Berkovitz BKB, Moxham BJ, Newman HN, eds. *The periodontal ligament in health and disease*. London: Mosby-Wolfe, pp. 183–210.

Oshiro T, Shiotani A, Shibasaki Y, Sasaki T (2002). Osteoclast induction in periodontal tissue during experimental movement of incisors in osteoprotegerin-deficient mice. *Anat Rec* **266:** 218–225.

Takahashi I, Nishimura M, Onodera K, Bae JW, Mitani H, Okazaki M (2003). Expression of MMP-8 and MMP-13 genes in the periodontal ligament during tooth movement in rats. *J Dent Res* **82:** 646–651.

Takahashi I, Onodera K, Nishimura M, Mitnai H, Sasano Y, Mitani H (2006). Expression of genes for gelatinases and tissue inhibitors of metalloproteinases in periodontal tissues during orthodontic tooth movement. *J Mol Histol* **37:** 333–342.

Ten CAR (1998). *Oral histology: development, structure and function*, 5th edn. Mosby Company: St. Louis, pp. 268–285.

Tervahartiala T, Pirilä E, Ceponis A *et al* (2000). The in vivo expression of the collagenolytic matrix metalloproteinases (MMP-2, -8, -13, and -14) and matrilysin (MMP-7) in adult and localized juvenile periodontitis. *J Dent Res* **79:** 1969–1977.

Tsubota M, Sasano Y, Takahashi I, Kagayama M, Shimauchi H (2002). Expression of MMP-8 and MMP-13 mRNAs in rat periodontium during tooth eruption. *J Dent Res* **81:** 673–678.

Tuncer BB, Ozmeric N, Tuncer C, Teoman I, Cakilci B, Yucel A (2005). Levels of interleukin-8 during tooth movement. *Angle Orthod* **75:** 539–544.

Van HHJ, McMinn RG (1972). Pressure differential favouring tooth eruption in the dog. *Archs Oral Biol* **17:** 183–190.

Von BM, Maltha JC, Von D, Hoff JW, Kuijpers-Jagtman AM (2004). Focal hyalinization during experimental tooth movement in beagle dogs. *Am J Orthod Dentofacial Orthop* **125:** 615–623.

Wise GE, King GJ (2008). Mechanisms of tooth eruption and orthodontic tooth movement. *J Dent Res* **87:** 414–434.

Wise GE, Lumpkin SJ, Huang H, Zhang Q (2000). Osteoprotegerin and osteoclast, differentiation factor in tooth eruption. *J Dent Res* **79:** 1937–1942.

Wise GE, Frazier-Bowers S, D'Souza RN (2002a). Cellular, molecular, and genetic determinants of tooth eruption. *Crit Rev Oral Biol Med* **13:** 323–334.

Wise GE, Yao S, Zhang Q, Ren Y (2002b). Inhibition of osteoclastogenesis by the secretion of osteoprotegerin in vitro by rat dental follicle cells and its implications for tooth eruption. *Archs Oral Biol* **47:** 247–254.

Wise GE, Yao S, Odgren PR, Pan F (2005). CSF-1 regulation of osteoclastogenesis for tooth eruption. *J Dent Res* **84:** 837–841.

Yoshimatsu M, Shibata Y, Kitaura H *et al* (2006). Experimental model of tooth movement by orthodontic force in mice and its application to tumor necrosis factor receptor-deficient mice. *J Bone Miner Metab* **24:** 20–27.