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ORIGINAL ARTICLE

Mechanical stress induces bone formation in the maxillary sinus in a short-term mouse model

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Abstract
Objectives Clinicians occasionally face the challenge of moving a tooth through the maxillary sinus. The objective of this study was to evaluate tissue remodeling during tooth movement into the maxillary sinus, more specifically as regards to bone formation.
Materials and methods The maxillary first molar of 20 male mice was moved toward the palatal side by a nickel–titanium super elastic wire for 1 to 14 days, and the bone remodeling around the root was evaluated using histomorphometry and immunodetection of bone-restricted Ifitm-like (Bril) protein, a novel marker of active bone formation.
Results When mechanical stress was applied to the tooth, the periodontal ligament on the palatal side was immediately compressed to approximately half of its original width by the tipping movement of the tooth. At the same time, osteoblasts deposited new bone on the wall of the maxillary sinus prior to bone resorption by osteoclasts on the periodontal side, as evidenced by the high level of expression of Bril at this site. As a result of these sequential processes, bone on the sinus side maintained a consistent thickness during the entire observation period. No root resorption was observed.
Conclusions Bone formation on the surface of the maxillary sinus was evoked by mechanotransduction of mechanical stress applied to a tooth over a 2-week period, and was induced ahead of bone resorption on the periodontal ligament side.
Clinical relevance Mechanical stress can be exploited to induce bone formation in the maxillary sinus so that teeth can be moved into the sinus without losing bone or causing root damage.

Keywords Experimental tooth movement · Maxillary sinus · Bone formation · Osteoblast · Bril

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Introduction

When mechanical stress is loaded on a tooth, new bone formation by osteoblasts is activated by the tensile stress on the periodontal ligament (PDL), while there is bone resorption by osteoclasts followed by new bone formation on the compression area [1–3]. This principle is applied during conventional orthodontic treatment. Thus, orthodontic tooth movement can only be achieved when healthy periodontal tissues and sufficient bony support are present in the direction where the teeth will be moved.

Tooth movement through bone-deficient areas (e.g., the maxillary sinus, the atrophic alveolar ridge) is considered a major limitation, and might reduce the alveolar bone height and/or the root length [4–6]. Wehrbein et al. described in their dog experiment [4] and human biopsy study [6] that root resorption and loss of osseous supporting tissue occurred in the basal cortical bone of the nasal sinus after translatory tooth movements. They suggested that the differentiation of osteoblasts required for compensatory subperiosteal bone apposition

may be impaired by the structure and the specific metabolic condition of the mucosa of the maxillary sinus.

In contrast to this common assumption, clinicians occasionally face the formidable challenge of moving a tooth through the maxillary sinus. Previous reports have suggested that a tooth with normal supporting apparatus height can be orthodontically moved through the maxillary sinus while maintaining pulp vitality and bone support and exhibiting normal width of the PDL on both the pressure and tension sides [7, 8]. However, there are few histological reports detailing the periodontal changes and, in particular bone remodeling, during orthodontic tooth movement into the sinus.

In the present study, we moved the maxillary molar palatally and evaluated the remodeling of bone and PDL on the compression area in the maxillary sinus using a mouse experimental tooth movement (ETM) model. To evaluate bone activity, we have immunolocalized bone-restricted Ifitm-like protein (Bril), an osteoblast-specific membrane protein associated with active bone formation [9].

Material and methods

Animal experimental procedures

Twenty male mice (Charles Rivers; St-Constant, QC, Canada) weighing 25±5 g were anesthetized with an intraperitoneal injection of a mixture of 50 mg/kg of ketamine hydrochloride (Ketaset®; Wyeth Canada, St. Laurent, QC, Canada), 5 mg/kg of xylazine (Rompun®; Bayer Inc., Toronto, ON, Canada), and 1 mg/kg of acepromazine maleate (Acevet 10®; Vétoquinol, Lavaltrie, QC, Canada). A nickel–titanium wire, 0.012 in. in diameter, was fixed to the maxillary incisor by means of a composite resin for orthodontic bonding (Transbond®; 3M Unitek, St. Paul, MN), and the left maxillary first molar was moved toward the palatal side with 10-g load as described previously [10], while the untreated contralateral teeth served as control (Fig. 1a). Following experimental manipulations, the animals received an injection of buprenorphine hydrochloride (Temgesic®; Reckitt and Colman, Hull, UK). All animal procedures were approved by the guidelines of the Comité de déontologie de l'expérimentation sur les animaux of Université de Montréal.

Tissue processing

At 1, 3, 5, 7, and 14 days after application of force, animals were anesthetized with 20% chloral hydrate solution (0.4 mg/g body weight; Fisher Scientific, Whitby, ON, Canada) and ketamine hydrochloride (10 mg/kg), and sacrificed by perfusion through the left ventricle with Ringer's lactate (Hospira, Montreal, QC, Canada) for 30 s, followed by a fixative solution consisting of 4% paraformaldehyde (Acros Organics, Morris Plains, NJ) and 0.1% glutaraldehyde (Electron Microscopy Sciences, Washington, PA) in 0.08 M sodium cacodylate (Electron Microscopy Sciences) buffer containing 0.05% calcium chloride (JT Baker, Phillipsburg, NJ), pH 7.2, for 20 min [11]. Maxillae were dissected, and specimens were immersed in the same fixative solution overnight at 4°C and decalcified with Plank–Rychlo's solution [12] consisting of 0.5 M aluminum chloride (Sigma-Aldrich Canada, Oakville, ON, Canada) containing 8.5% hydrochloric acid (Fisher Scientific) and

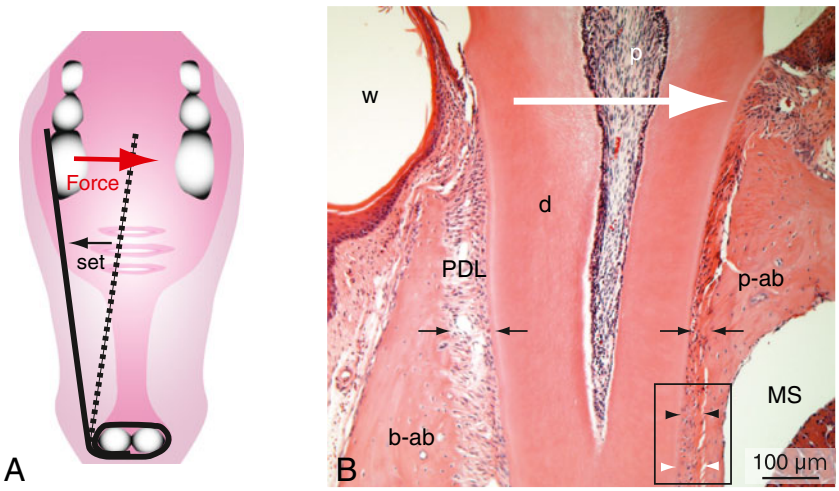

Fig. 1 An experimental tooth movement (ETM) model in the mouse. Schematic illustration of ETM (**a**). The end of a superelastic nickel–titanium wire was initially positioned at the midpalate (*dotted line*) and then activated to the buccal side of the maxillary first molar on the left side (*solid line*). Hematoxylin–eosin staining of the first molar at day 1 after the start of ETM (**b**). The *white arrow* indicates the direction of the tooth movement. The width of the periodontal ligament (*PDL*) was decreased on the compression area and increased on the tension area (*black arrows*). The PDL width about 200 (*black arrowheads*) and 300 (*white arrowheads*) μm from the palate level was used for morphometric analyses. The *boxed area* shows the analyzed area enlarged in Fig. 2. *w* position of wire, *d* dentin, *p* dental pulp, *b-ab* buccal alveolar bone, *p-ab* palatal alveolar bone, *MS* maxillary sinus

5.4% formic acid (JT Baker) for 7 days at 4°C. Decalcified samples were washed for 24 h in 0.1 M sodium cacodylate buffer, pH 7.2, processed for paraffin embedding, and sectioned at 5 μm thickness. For morphological analyses, sections were stained with hematoxylin and eosin.

Immunohistochemistry

Sections were deparaffinized with d-limonene-based solvent (Citrisolv®; Fisher Scientific), rehydrated through descending ethanol series, and washed in distilled water. In order to avoid nonspecific sticking, sections were blocked with 0.01 M phosphate-buffered saline (PBS), pH 7.2, containing 5% skim milk for 30 min at room temperature. After blocking, sections were incubated with an affinity-purified rabbit primary antibody raised against Bril (1:5,000, 3 h, room temperature) [9]. Sections were washed with PBS containing 0.05% (*v*/*v*) Tween 20 (Fisher Scientific), pH 7.4 (0.01 M PBS–Tween 20), followed by treatment with the Dako Envision TM + System, HRP-labeled polymer antirabbit kit (Dako Corporation, Carpinteria, CA) as recommended by the manufacturer. Visualization was performed with 3,3′-diaminobenzidine, and sections were counterstained with 0.5% methyl green (Dako Corporation).

Histomorphometry

Histological examination focused on the bone and PDL surrounding the mesial root of the maxillary first molar at the palatal side. Each group for histomorphometry consisted of four mice, and two areas were evaluated per animal. The first area was about 200 μm from the palate level and the second area about 300 μm away (Fig. 1b). The width of PDL, the total thickness of bone, and the newly formed bone (defined as the distance from the surface of the maxillary sinus to the reversal line) were measured with an image-editing software (Photoshop CS4, Adobe Systems Incorporated, San Jose, CA).

Statistical analysis

The significance of differences in experimental data was analyzed by one-way analysis of variance and the Tukey–Kramer test, and probability levels of $P<0.05$ were considered statistically significant.

Results

Microscopic observations

The nickel–titanium wire provided sufficient orthodontic force to tip the first molar palatally. The width of PDL was significantly decreased on the compression area and increased on the tension area at day 1 after the start of ETM (Fig. 2a). At day 3, bone formation was evident on the maxillary sinus as seen with the increase in bone thickness (Fig. 2b). At days 5 and 7, bone formation on the sinus side and bone resorption by osteoclasts on the PDL side were simultaneously ongoing (Fig. 2c, g). At day 14, there was still evidence of bone formation but the width of PDL was back to the normal range (Fig. 2h) as observed on the contralateral nontreated side (Fig. 2i). No root resorption was observed at any time point (data not shown).

In untreated teeth, expression of Bril was associated with cells on the surface of alveolar bone and cellular cementum at sites where physiological tooth movement occurs (Fig. 2l). At days 3, 5, and 7, Bril was immunodetected in active osteoblasts located on the surface of the maxillary sinus (Fig. 2e, f, j). At day 7, Bril expression was also observed over the surface of bone on the periodontal side, indicating the start of new bone formation (Fig. 2j). Expression was also shown at day 14, which was similar in level to those present in control animals (Fig. 2k, l).

Histomorphometry

The PDL width on the control side was 67.0±7.3 μm. The PDL width significantly decreased on days 1 and 3 after the start of tooth movement, 32.2±11.2 and 30.7±6.2 μm, respectively. After that, the width increased significantly between days 5 and 7, from 47.1±18.5 to 80.7±21.4 μm. The width on day 14 was not significantly different from the control (73.1±10.6 μm, Fig. 3).

The thickness of the new bone increased gradually from days 3 to 14, and the average thickness values (5.2±1.1, 9.3±2.2, 13.3±3.8, and 19.6±2.6 μm) measured at the four time intervals were statistically different. However, the total thickness of the bone showed no statistical difference at any time point (Fig. 3).

Discussion

When mechanical stress was applied to the molar by a nickel–titanium superelastic wire, new bone was first formed on the sinus side of bone which was gradually balanced by resorption on the periodontal side. As a result of these sequential processes, the sinus wall maintained a consistent thickness. Moreover, there was no accentuated root resorption.

The periosteum is the connective tissue membrane surrounding the bone and comprises two layers. The inner layer consists of precursors cells [13] that respond to mechanical stress by differentiating into osteoblasts [14]. The maxillary sinus is pyramidal in shape, and its floor is formed by the alveolar process of the maxilla. The sinus walls are lined by

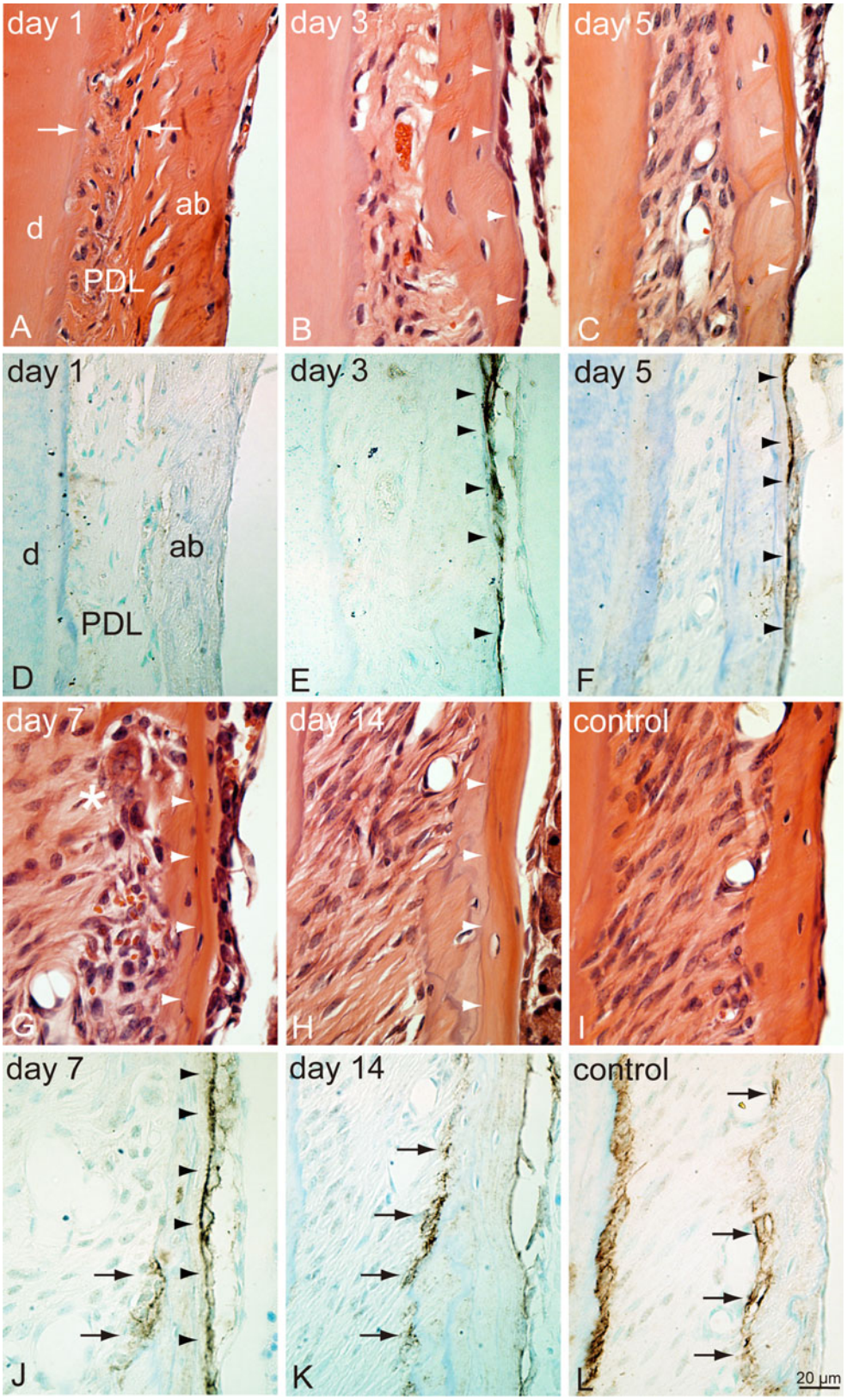

Fig. 2 Bone formation on the maxillary sinus. Hematoxylin–eosin staining (**a**–**c**, **g**–**i**) and immunolabeling for Bril (**d**–**f**, **j**–**l**). At 1 day after the start of experimental tooth movement (ETM), the width of the periodontal ligament (*PDL*) was decreased (**a**, *white arrows*) but no labeling for Bril was detected (**d**). At days 3, 5, and 7, active bone formation was observed on the surface of the sinus (**b**, **c**, **g**, *white arrowheads* indicate the reversal line) and Bril was also expressed in these areas (**e**, **f**, **j**, *blackarrowheads*). At day 7, bone resorption by osteoclasts was shown in the alveolar bone (*ab*) on the PDL side (**g**, *asterisk*). At days 7 and 14 and in control group, Bril expression was observed on the PDL side (**j**–**l**, *black arrows*). In control group, Bril was also labeled on the surface of the acellular cementum (**l**). *d* dentin

a periosteum-like layer referred to as the Schneiderian membrane [15]. In addition, the bone surface is covered, as elsewhere, by some bone lining cells. While a number of molecular pathways are likely implicated in these events, recent studies suggested the presence of mesenchymal stem cells in maxillary sinus membrane which can differentiate

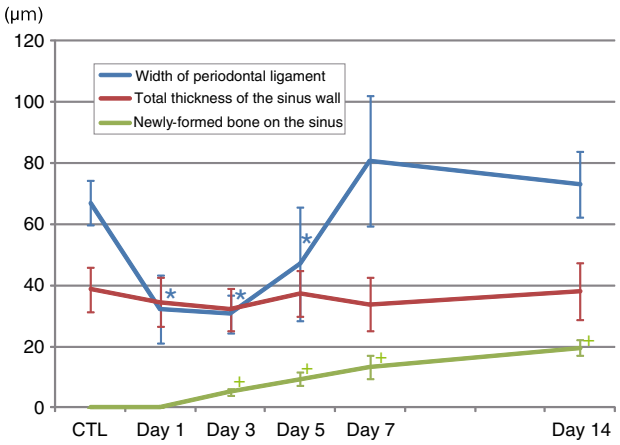

Fig. 3 Linear measurements of the periodontal tissue during the experimental tooth movement (ETM). The mean width of the periodontal ligament was significantly decreased at days 1, 3, and 5 after the ETM (*blue line*, *$P<0.05$). The amount of newly formed bone on the sinus wall was gradually increased from days 1 to 14 (*green line*, +$P<0.05$). However, the total thickness of the sinus wall was consistent during the whole observation period (*red line*)

into osteoblasts under certain osteogenic induction [15, 16]. The surface of the sinus wall is therefore capable of osteogenic activity. Recently, this osteogenic ability has been exploited for sinus floor augmentation [17]. Following elevation of the sinus wall membrane, bone formation around implants inserted into the maxillary sinus is significantly enhanced without any bone grafting [18–21]. In addition, there is bone formation around molar root apices, which have penetrated the bony floor of the maxillary sinus during orthodontic intrusion [22].

Since there was no mechanical stress directly applied to the sinus membrane, we believe that the observed bone formation on the sinus side results from signals originating in the adjacent compressed periodontal tissues (Fig. 4). During compression two mechanical signals are generated. Firstly, the tension normally provided by the PDL is significantly reduced. Secondly, the compression of periodontal soft tissues will presumably exert some force on the alveolar bone surface. Together, these are expected to trigger the osteocyte mechanotransduction system of the alveolar/sinus wall bone [23–25], which ultimately will transmit a stimulatory signal to the bone lining and periosteal cells on the sinus side. The molecular signaling of mechanotransduction is elicited in osteocytes immediately after the application of stimuli [10, 23–25]. Therefore, as our data illustrate, new bone formation is expected to occur soon after ETM. In addition, in order to maintain the overall shape and thickness of the sinus wall, there is a subsequent increase in the frequency of osteoclasts on the periodontal side of the sinus wall from day 3 after ETM and maximal on day 7, an observation consistent with previous quantitative report [10]. As a result of these formation–resorption activities, homeostasis of the sinus wall can be maintained.

While the duration of our study permitted to evaluate just one bone remodeling cycle on the sinus wall and alveolar bone, it is expected that the phenomenon will repeat and will not be exacerbated as long as the same level of mechanical stress is applied. Wehrbein and colleagues have reported cases in which there were root resorption and loss of osseous supporting tissue in the basal bone of the maxillary sinus after orthodontic tooth movements [6]. While the extent of the force may be questioned, other factors such as treatment duration have been associated with root resorption during and after orthodontic tooth movement [26, 27]. Clearly, further studies are warranted to address the long-term consequences of tooth movement into the maxillary sinus.

Recently, temporary anchorage devices have been developed for absolute anchorage in orthodontic treatment [28–30]. They provide evolutionary tooth movement not possible with traditional orthodontic mechanics. Especially, absolute molar intrusion for anterior open bite treatment and group distalization in maxillary and/or mandibular protrusion cases are considered as leading edge innovations as new treatment strategies [29, 31–34]. On the other hand,

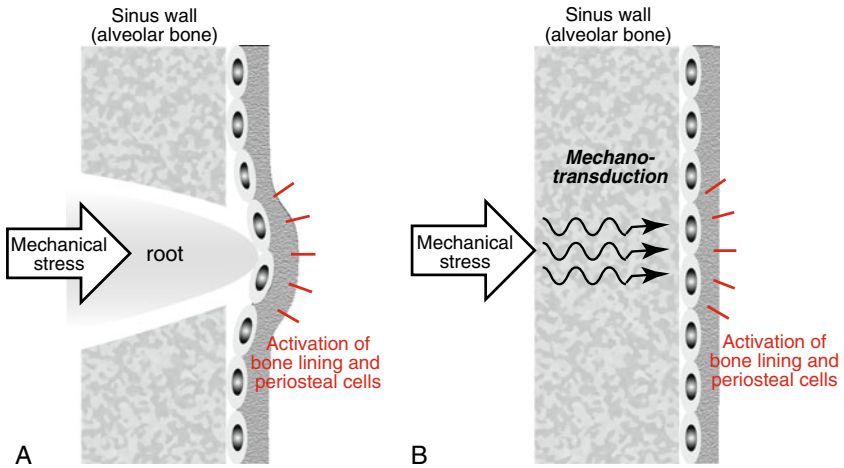

Fig. 4 Schematic illustration of mechanotransduction. **a** Mechanical stress directly applied to bone lining and periosteal cells elicit their osteogenic activity in case of root perforation into the sinus. **b** Mechanotransduction system of the alveolar/sinus wall bone transmits mechanical stress to the bone lining and periosteal cells on the sinus side

with this kind of tooth movement in the maxilla, intrusion of the roots into the maxillary sinus is a major concern. The present study provides histological evidence that the sinus wall is a dynamic structure that responds favorably to mechanical stress, alleviating concerns about tooth movement into the sinus. We believe that such safety should ultimately contribute to expanding the limits of orthodontic treatment.

We have used Bril as a marker of bone formation under mechanical stress. Bril is a novel osteoblast-specific membrane protein which was recently identified from a UMR106 rat osteosarcoma cell line library using a signal–trap screening approach [9]. This protein was formerly classified as interferon inducible transmembrane protein (Ifitm)-5. Screening of cell lines showed high expression of Bril in osteoblasts during onset of matrix mineralization. In situ hybridization and immunohistochemistry revealed that Bril was detected in developing bones and its expression decreased in older bone. In vitro functional analyses revealed that expression of Bril increased with osteoblast differentiation, peaking with matrix production and mineralization [9]. The specific expression of Bril at active bone formation sites demonstrates its usefulness for evaluation of osteogenesis.

In conclusion, bone formation on the surface of the maxillary sinus was evoked by mechanotransduction of mechanical stress applied to a tooth in an ETM model. Osteogenesis was induced ahead of bone resorption on the PDL side, and the bone thickness of the sinus was generally consistent throughout the period of palatal tooth movement. No accentuated root resorption was observed. These results suggest that mechanical stress can be exploited to induce bone formation in the maxillary sinus so that teeth can be moved into the sinus without losing bone support or inducing root resorption.

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Conflict of interest statement The authors declare that they have no conflict of interest.

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