Changes of Temporomandibular Joint and Semaphorin 4D/Plexin-B1 Expression in a Mouse Model of Incisor Malocclusion

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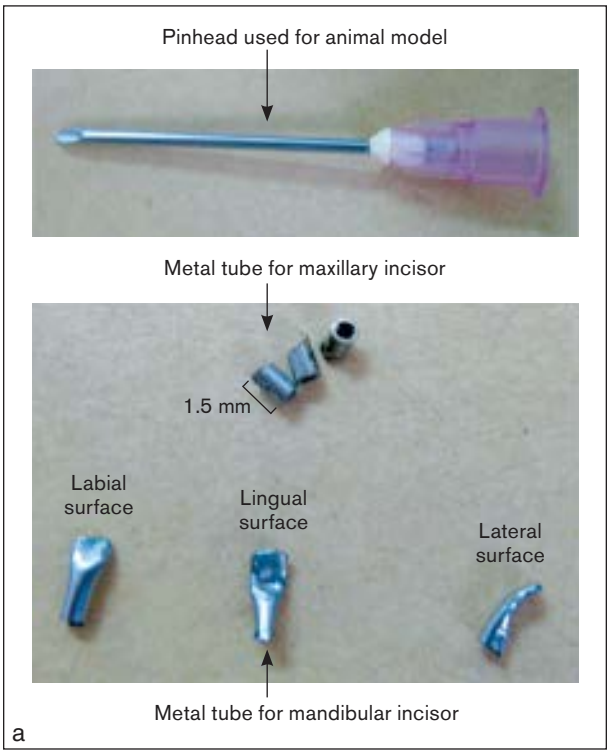
Aims: To investigate the changes in condylar cartilage and subchondral bone of the temporomandibular joint (TMJ) in a mouse model of incisor malocclusion. **Methods:** By bonding a single (single group) or a pair (pair group) of metal tube(s) to the left incisor(s), a crossbite-like relationship was created between left-side incisors in mice. The morphological changes in the TMJ condyles were examined by hematoxylin and eosin and toluidine blue staining. Indices of osteoclastic activity, including tartrate-resistant acid phosphatase (TRAP) staining and macrophage-colony stimulating factor (M-CSF) were investigated by histochemistry or real-time polymerase chain reaction (PCR). The osteoblastic activity was indexed by osteocalcin expression. Expressions of semaphorin 4D and its receptor, Plexin-B1, were detected by real-time PCR. Two-way analysis of variance was used to assess the differences between groups. **Results:** One week and 3 weeks after bonding the metal tube(s), cartilage degradation and subchondral bone loss were evident histologically. Both indices of osteoclastic activity (TRAP and M-CSF) were significantly increased in cartilage and subchondral bone after bonding the metal tube(s). Osteocalcin expression in cartilage was significantly increased at week 3, while its expression in subchondral bone was significantly increased at week 1 but decreased at week 3. The semaphorin 4D expression in cartilage and subchondral bone was significantly decreased at week 1 but significantly increased at week 3. For Plexin-B1 expression, a significant increase was detected in subchondral bone at week 3. **Conclusion:** Bonding a single or a pair of metal tube(s) to left incisor(s) is capable of inducing remodeling in the TMJ, which involved cartilage degradation and alteration of osteoclastic and osteoblastic activity.
J Oral Facial Pain Headache 2014;28:68–79. doi: 10.11607/jop.1082

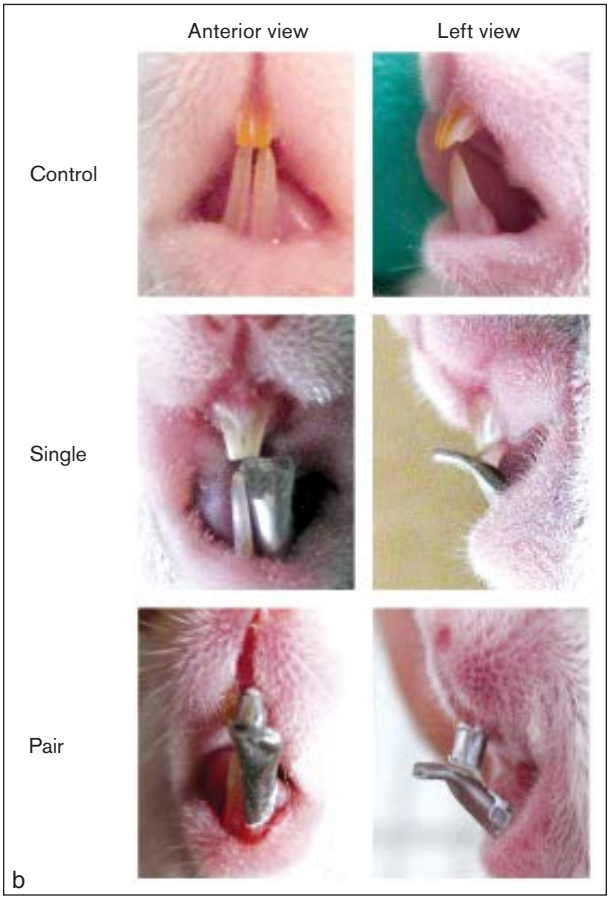
Key words: *cartilage, occlusion, remodeling, semaphorin 4D, temporomandibular joint*

Temporomandibular disorders (TMD) are a widespread orofacial problem, with over 10 million people suffering from TMD in the United States.[1] Generally, TMD have multiple etiologic factors, among which occlusal factors have attracted the most attention. There is still much controversy over the role of an abnormal occlusion in TMD and also its effect on the temporomandibular joint (TMJ).[2] Therefore, the present study first aimed to develop a novel animal model of TMD by changing the occlusal relationship between incisors in mice.

Osteoarthritis (OA) is a severe pathologic change of the TMJ, and is characterized by cartilage degradation and subchondral bone changes.[3,4] Cell death and loss of proteoglycans are two important features, resulting in a decrease in cartilage thickness.[5,6] Subchondral bone changes in OA involve both bone resorption and formation.[7] In human knee and mouse TMJ samples, bone loss has been found in the early phase of OA and attributed mainly to increased osteoclastic activity.[8–11] However, the role of osteoblastic activity in OA, particularly in the TMJ, has seldom been investigated thus far.

Fig 1 *(a)* Pinhead and metal tube used to change the incisal occlusal relationship in the *(b)* single and pair animals; a third group receiving no metal tubes served as the control animals.





Several factors have been shown to be associated with osteoblastic activity. For instance, growth factors such as transforming growth factor-beta (TGF-β), insulin-like growth factor (IGF), and fibroblast growth factor (FGF)-2, -4, and -6, and transcription factors such as runt-related transcription factor 2 (Runx2) and Osterix (Osx) are all inducers of osteoblastic activity and have been extensively investigated previously.[12] However, studies on the inhibitory factors of osteoblastic activity are limited. Recently, a pair of important inhibiting factors of osteoblastic activity, semaphorin 4D (Sema 4D) and Plexin-B, has been shown to inhibit bone formation by suppressing osteoblast differentiation and modulating osteoblast motility.[13,14] However, the expression pattern of Sema 4D/Plexin-B1 during the remodeling of the TMJ has never been investigated before.

Therefore, the present study aimed to investigate the changes in condylar cartilage and subchondral bone of the TMJ in a mouse model of incisor malocclusion. It was hypothesized that occlusal changes involving the incisors could induce TMJ remodeling. A mouse model of anterior dental malocclusion was established by bonding a single or a pair of metal tube(s) on the left incisor(s). Histologic changes in TMJ cartilage and subchondral bone were evaluated. In addition, osteoclastic and osteoblastic activities were investigated by histochemistry and real-time polymerase chain reaction (PCR). Finally, expression changes of Sema 4D and Plexin-B1 in the TMJ were also evaluated.

Materials and Methods

Animals

All operations involving animals were conducted according to the Institutional Animal Care Guidelines and were approved by the Administration Committee of Experimental Animals at the Fourth Military Medical University (FMMU). Sixty BALB/C mice (18 to 20 g), 7 weeks of age, were provided by the Laboratory Animal Center of FMMU. The mice were divided into two experimental groups and one control group (n = 20 in each group), and each group was further subdivided into two groups (n = 10) according to the two evaluation time points (see below).

Experimental Procedures

Metal tubes were made of a pinhead (Shinva Ande, Shangdong, China; inner diameter = 0.61 mm, wall thickness = 0.1 mm) (Fig 1a). The mice were anesthetized by an intraperitoneal injection of 1%

pentobarbital (40 mg/kg). In the first experimental group, a curved metal tube (5 mm long) was bonded to the left mandibular incisor of the mouse with zinc phosphate cement. The occlusal part of the mandibular tube was designed with a 135-degree labial inclination. The angle point of the metal tube was at the same level with the right mandibular incisal edge. The tip of the bonded tube was about 1 mm longer than the right mandibular incisal edge, so that a crossbite-like relationship was created between the left-side incisors (Figs 1a and 1b). This group was termed the "single" group, meaning that a single metal tube was bonded to the mandibular incisor.

The mice in the second experimental group were first treated similarly to those in the single group. In addition, a metal tube with length of 1.5 mm was bonded to the left maxillary incisor. The tip of the bonded tube was at the same level with the right maxillary incisal edge. Thus, not only was a similar crossbite-like relationship to the single group created, but also the compensatory abrasion of the maxillary left incisor was limited (Figs 1a and 1b). This group was termed the "pair" group, meaning that a pair of metal tubes was bonded to the left pair of incisors.

The duration of the operation was within 5 minutes in both the single and pair groups. The mouth opening of the animals was less than 4 mm during the operation. The metal tubes were bonded in the morning, and they were checked in the afternoon to make sure they were still in place. None of the mandibular metal tubes fell off, but the maxillary metal tubes fell off in four mice and were recemented immediately. From the next day on, the metal tube(s) were checked every day, and none was found to have fallen off. The mandibular incisors were forced apart 0.1 mm at the symphysis. The control group was subjected to similar procedures, but no metal tubes were bonded.

The length of the incisors in all three groups was measured after the animals were sacrificed at 0, 1, and 3 weeks after the operation. For incisors without metal tubes, the length from the gingival margin to the incisor edge was measured and recorded as the length of the incisors. For incisors with metal tubes, the length from the maxillary gingival margin to the tip of the metal tube was recorded as the length of the maxillary incisor, and the length from the mandibular gingival margin to the angle point of the metal tube as the length of the mandibular incisor.

After the experimental operation, animals in both experimental groups and the control group were fed a soft diet for 2 days to allow the animals in the experimental group to adapt to the metal tube(s). The soft diet was changed to a standard diet from the third day after the experimental operation. The body weight of the animals was recorded every 3 days after the metal tube bonding.

Tissue Preparation

At 1 and 3 weeks after the operation, the animals were sacrificed under anesthesia. Half of the animals were fixed by transcardiac perfusion of 4.0% paraformaldehyde diluted by phosphate-buffered saline (PBS, pH = 7.4). Tissue blocks containing the TMJs were sampled, further fixed with 4.0% paraformaldehyde at 4˚C for 12 hours, and decalcified with 4% ethylenediaminetetraacetic acid disodium salt (EDTA-2Na) solution for 4 weeks at room temperature. The samples were then dehydrated in ethanol and embedded in paraffin wax. Consecutive slices (5.0 μm thick) were prepared in the sagittal plane for histochemistry.

The other half of the animals were used for real-time polymerase chain reaction (PCR) analysis. After the animals were sacrificed under anesthesia, the cartilage and subchondral bone of the TMJ condyles in each subgroup were carefully dissected and preserved at –80˚C for messenger ribonucleic acid (mRNA) preparation.

Histochemical Staining

To ensure a reliable comparison between the specimens from different subgroups, 15 consecutive tissue sections from the central sagittal plane of each joint were prepared and used for histochemical staining. The sections used for each staining were selected randomly from the central 15 consecutive sections. Hematoxylin and eosin (H-E) and toluidine blue staining (pH 2.5) were adopted for histochemical assessment (three sections for each staining). The integral optical density (IOD) of toluidine blue staining, which could indicate the proteoglycan amount in TMJ cartilage, was measured. Briefly, the surface of the condylar cartilage, defined as the area between the anterior and posterior attachment points of the joints disc to the condyle, was equally divided into the anterior, middle, and posterior thirds, as reported previously.[15] The IOD of toluidine blue staining in the whole cartilage layer, from the superficial layer to the cartilage-subchondral bone interface, at the middle and posterior thirds of each section (Fig 2), was measured using Image-Pro Plus 6.0 software, and the average value of the middle and posterior portions was calculated to represent the IOD of toluidine blue staining for each section. Tartrate-resistant acid phosphatase (TRAP) staining (three sections) was used following the manufacturer's instructions (Sigma 387-A) to identify osteoclasts. Two square regions (0.3 mm × 0.3 mm) under the osteochondral interface were identified and labeled in the middle of the middle and posterior parts of the mandibular condyle (Fig 2). The number of TRAP-positive cells that represent osteoclasts was counted within the two selected square regions on each section. The number of osteoclasts was averaged from the six square regions of three selected tissue sections of each animal.

Sections from all three groups and subgroups were stained simultaneously by histochemistry to ensure a comparable staining background.

Number of Chondrocytes, Cartilage Thickness, and Bone Histomorphometry

The H-E stained sections were observed under a microscope (Leica). The images were analyzed with a computer-assisted image analyzing system (Qwin Plus, Leica Microsystem Imaging Solutions). The number of chondrocytes in the whole cartilage layer at the middle and posterior thirds of each section (Fig 2) was counted with Adobe Photoshop CS3 software, and the average value of the middle and posterior portions was calculated to represent the number of chondrocytes for each section. The middle and posterior portions were further subdivided evenly into four parts. The thickness of the cartilage at the subdividing points of the middle and posterior portions (six values) was measured and averaged. For both the number of chondrocytes and the thickness of cartilage, the averaged value of the data from the three selected sections stained with H-E was calculated to represent the values of that sample (Fig 2).

Bone histomorphometry was performed as reported previously,[8] with a computer-assisted image analysis system from Leica (Qwin Plus) and Adobe Photoshop CS3 software. Two square regions (0.3 × 0.3 mm) under the osteochondral interface were identified and labeled at the middle and posterior portions of the mandibular condyle by using the computer-assisted image analysis system from Leica (Fig 2). Images of the square regions were then analyzed with the Adobe Photoshop CS3 software. Briefly, areas occupied by trabecular bone were painted black, and the areas and perimeters of the black areas were read and recorded as areas and perimeters of the trabecular bone (Tb area and Tb perimeter). All parameters were derived from the areas and perimeters of the trabecular bone by using the formula BV/TV (bone volume per trabecular volume) = Tb area/total area; Tb.Th (trabecular thickness) = Tb area/Tb perimeter; Tb.N (trabecular number) = Tb perimeter/total area; Tb.Sp (trabecular separation) = $\frac{\text{(total area–Tb area)/Tb perimeter}}{1{,}000}$. For calculations of these indices, six square regions from three sections in each animal were used. The values obtained were then averaged for further statistical analysis.

mRNA Preparation, Reverse Transcription, and Real-Time PCR Analysis

The total mRNA in the cartilage and in the subchondral bone was extracted. Reverse transcription of the mRNA to template complementary deoxyribonucleic acid (cDNA) was completed using a TIANScript RT Kit (Tiangen). Primers for targeted genes were designed as follows: osteocalcin (forward, CTGCTCACTCTGCTGACC, reverse, GGACTGAGGCTCCAAGGT); macrophage-colony stimulating factor (M-CSF, forward, CAGCCACTAGCGAGCAAG, reverse, TCGGTGGCGTTAGCATTG); Sema 4D (forward, GTTGATGATCCCGCGAGTTG, reverse, AGATCAGCCTGGCCTTTAGGAA); Plexin-B1 (forward, GGTCCACCTTGATTGCAGGTC, reverse, CACTGCCTGGAATCGCCTTTA); glyceraldehyde-phosphate dehydrogenase (GAPDH, forward, TGTGTCCGTCGTGGATCTGA, reverse, TTGCTGTTGAAGTCGCAGGAG). Genes were analyzed by using the Applied Biosystems 7500 Real-time PCR machine (Applied Biosystems). Each experiment was performed three times and the mean values were derived. The amount of target cDNA, relative to GAPDH, was calculated using the formula $2-\Delta\Delta Ct$ (where Ct refers to cycle threshold). The results were calculated as the relative quantification of the target gene compared to the age-matched control group, which was set at 1.

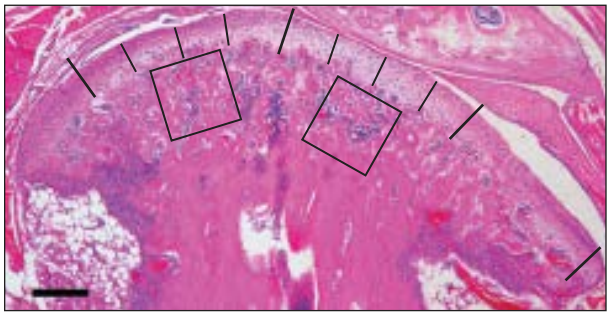


Fig 2 H-E stained TMJ section. The long lines evenly segmented the cartilage into three parts, and the short lines further evenly segmented the middle and posterior parts into four parts. Bar = 200 mm.

Statistical Analysis

All the specimens were measured twice in a blinded fashion by the two observers (X. Zhang and M. Zhang) over an interval of 1 week, and the mean of the two measurements was used for statistical analysis. As there were 10 animals for each group, 20 measurements were obtained by each observer for each group. For each observer, 10 out of these 20 measurements were randomly chosen in order to assess the interrater agreement (Pearson correlation coefficient).

First, descriptive statistics consisting of means ± standard deviation were generated. A two-way analysis of variance (ANOVA) with the factors treatment and time was used to assess group differences. If this analysis revealed a significant overall difference

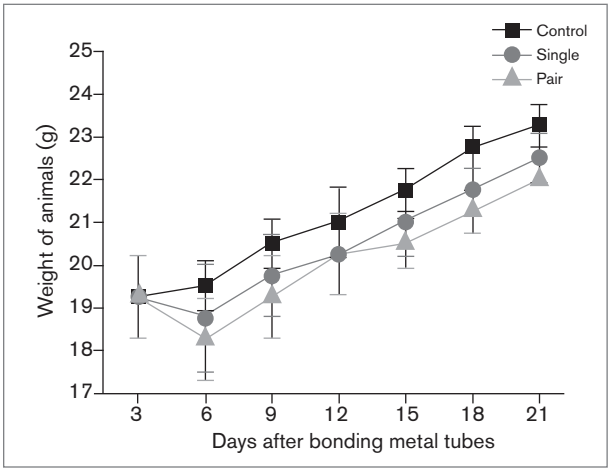


Fig 3 Mean and SD of the animal weights of the three groups.

among the groups, a Tukey post hoc test was applied for pairwise comparisons. A one-way ANOVA was implemented to determine if body weight differed among the groups. All statistical analyses were performed using SPSS 12.0 for Windows. The level of significance was set at $P < .05$ for all statistical tests.

Results

No significant difference in body weight was noticed between the experimental and control groups within the experimental period ($P > .05$) (Fig 3).

Length of the Incisors

After 3 weeks, the maxillary incisors with metal tubes in the pair group were significantly longer than those in the control group ($P < .05$, Fig 4a), but no significant difference was evident in the incisor lengths of the single group both at week 1 and week 3, or in that of the pair group at week 1.

After 3 weeks, the mandibular incisors of the single and pair groups were significantly longer than those of the control group ($P < .05$, Fig 4b).

Histological Observation of Condylar Cartilage

Control group. At both time points, the cells in the condylar cartilage were arranged in a regular pattern (Fig 5) and the proteoglycan stained by toluidine blue was evenly distributed (Fig 6). The number of chondrocytes, the cartilage thickness, the IOD of toluidine blue staining, and the indices of bone histomorphometry did not change between the two time points ($P > .05$) (Figs 7 and 8).

Control vs experimental groups. The number of chondrocytes in both experimental groups (Fig 5) was significantly smaller than that of the control group both at week 1 and week 3, with a larger decrease in the pair group than that in the single group. Moreover, the number of chondrocytes decreased significantly from week 1 to week 3 in both the single and pair groups ($P < .05$, Figs 5 and 7a). No surface changes were noticed in both experimental groups at both time points. The amount of proteoglycan decreased significantly in the cartilage of both the single and pair groups, as indicated by the significant decrease in the IOD of toluidine blue staining at both week 1 and week 3, with less proteoglycan in the joint cartilage of the pair group than in that of the single group at week 1 ($P < .05$, Figs 6 and 8). However, the IOD did not decrease between week 1 and week 3 in the experimental groups ($P > .05$, Figs 6 and 8).

The cartilage of the single and pair groups was significantly thinner than that of the control group ($P < .05$), and the cartilage of the pair group was significantly thinner than that of the single group at both week 1 and week 3 ($P < .05$, Figs 5 and 7b). However, the cartilage in both experimental groups did not become thinner from week 1 to week 3 ($P > .05$, Figs 5 and 7b).

Bone Histomorphometry

Subchondral bone loss was observed in both experimental groups. The bone volume per trabecular volume (BV/TV) was significantly decreased at week 3 in the single group and at both time points in the pair group compared to that in the control group ($P < .05$, Fig 7c). The trabecular separation (Tb.Sp) was significantly increased at week 3 in the single group ($P < .05$, Fig 7c) and at both time points in the pair group compared to that in the control group ($P < .05$, Fig 7c). A significant decrease was found also in the trabecular thickness (Tb.Th) at week 3 in both experimental groups in comparison to that in the control group ($P < .05$, Fig 7c). No significant change in trabecular number (Tb.N) was noticed between the experimental and control groups ($P > .05$, Fig 7c). In both experimental groups, the BV/TV, Tb.Sp, Tb.Th, and Tb.N values did not change significantly between week 1 and week 3 ($P > .05$, Fig 7c).

Osteoclastic Changes

In the subchondral bone, a significant increase in osteoclast number was evident in both experimental groups compared to that in the control group, with a more dramatic change in the pair group than in the single group at both time points ($P < .05$, Figs 9 and 10a). The osteoclast number increased significantly from week 1 to week 3 in the pair group ($P < .05$, Fig 10a), but not in the single group ($P > .05$, Fig 10a).

In cartilage, the mRNA level of M-CSF was significantly higher at week 3 in the single group and at both time points in the pair group than that in the

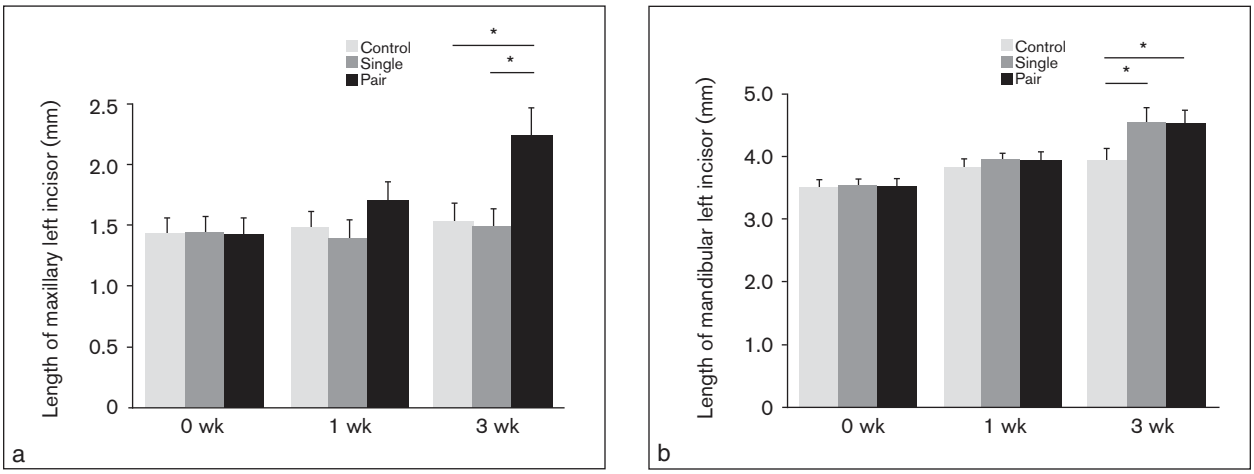


Fig 4 Measurements of the length of the *(a)* maxillary and *(b)* mandibular incisors in each group at each time point. *$P < .05$.

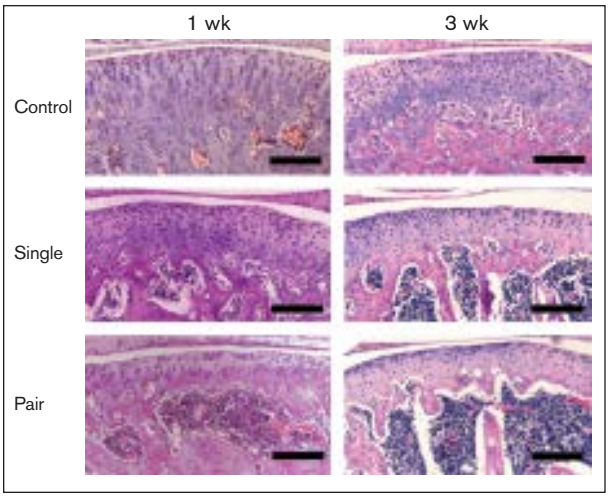


Fig 5 The H-E stained samples show the decrease in the thickness of cartilage in both single and pair groups. Note also the subchondral bone loss in both experimental groups. Bar = 100 mm.

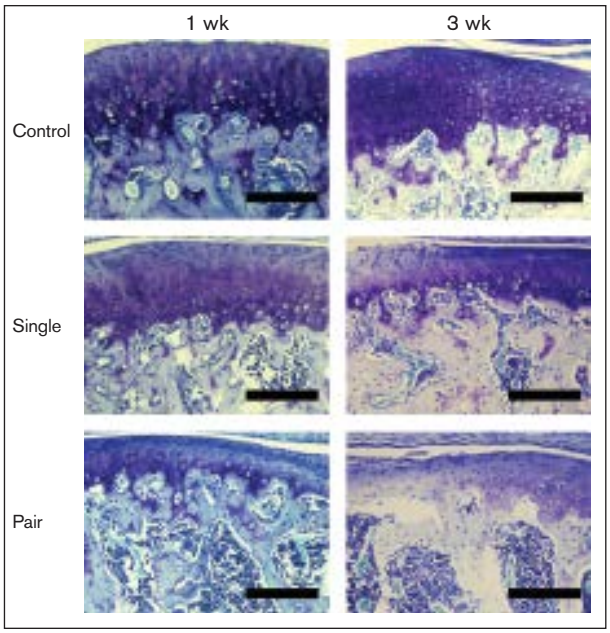


Fig 6 *(right)* Loss of toluidine blue staining in cartilage of both single and pair groups. Bar = 100 mm.

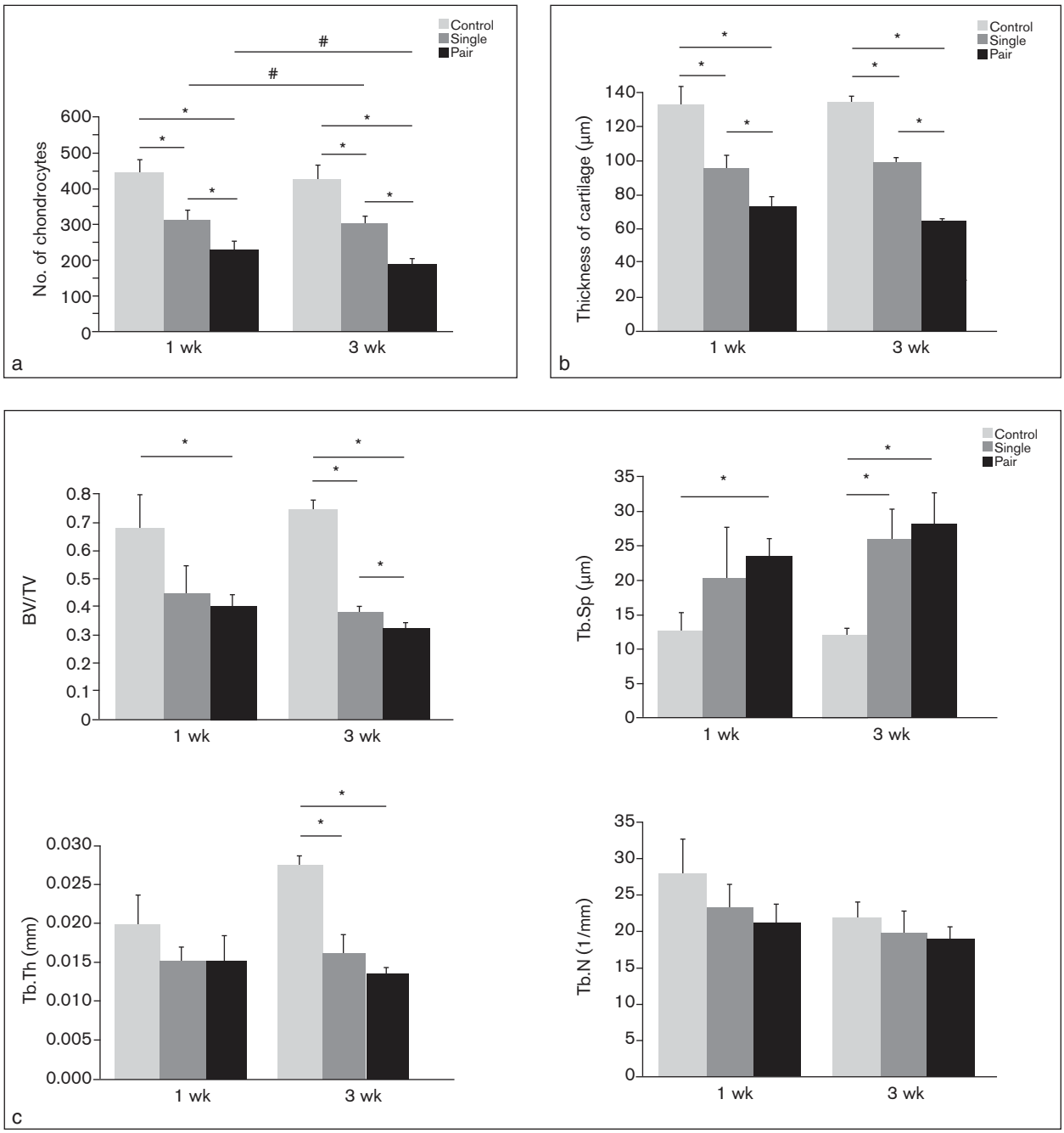
control group ($P < .05$, Fig 10b). The level increased from week 1 to week 3 in the pair group ($P < .05$, Fig 10b), but not in the single group ($P > .05$, Fig 10b), and the level was higher in the pair group than in the single group at week 3 ($P < .05$, Fig 10b).

In the subchondral bone, a significant increase in the mRNA level of M-CSF was observed in both the single and pair groups at both time points ($P < .05$, Fig 10b), with a significantly higher level in the pair group than in the single group at week 3 ($P < .05$, Fig 10b). Moreover, the level increased significantly from week 1 to week 3 in both experimental groups ($P < .05$, Fig 10b).

Osteoblastic Changes

Mature osteoblasts express osteocalcin. In cartilage, a significant increase in mRNA expression of osteocalcin was noticed at week 3 in both experimental groups compared to that in the control group ($P < .05$, Fig 11a), with a significantly higher level in the pair group than in the single group at week 3 ($P < .05$, Fig 11a). The mRNA level of osteocalcin increased significantly from week 1 to week 3 in the cartilage of both experimental groups ($P < .05$, Fig 11a).

In subchondral bone, the mRNA level of osteocalcin was increased at week 1 but decreased at week 3 in both experimental groups ($P < .05$, Fig 11b), with



Figs 7a to 7c Comparison of the histomorphologic findings between the groups and time points. BV/TV, bone volume per trabecular volume; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; Tb.N, trabecular number. *$P < .05$. #$P < .05$ between the two time points.

a significantly lower level in the pair group than in the single group at week 3 ($P < .05$, Fig 11b). The mRNA level of osteocalcin in subchondral bone decreased significantly from week 1 to week 3 in both experimental groups ($P < .05$, Fig 11b).

Sema 4D and Plexin-B1

The mRNA level of Sema 4D in both cartilage and subchondral bone was significantly lower at week 1 ($P < .05$, Fig 12a), but significantly higher at week 3 in both experimental groups than in the control group ($P < .05$, Fig 12a). The changes were more obvious in the pair group than in the single group ($P < .05$, Fig 12a). The mRNA level of Sema 4D increased significantly from week 1 to week 3 in both cartilage and subchondral bone in the single and pair groups ($P < .05$, Fig 12a).

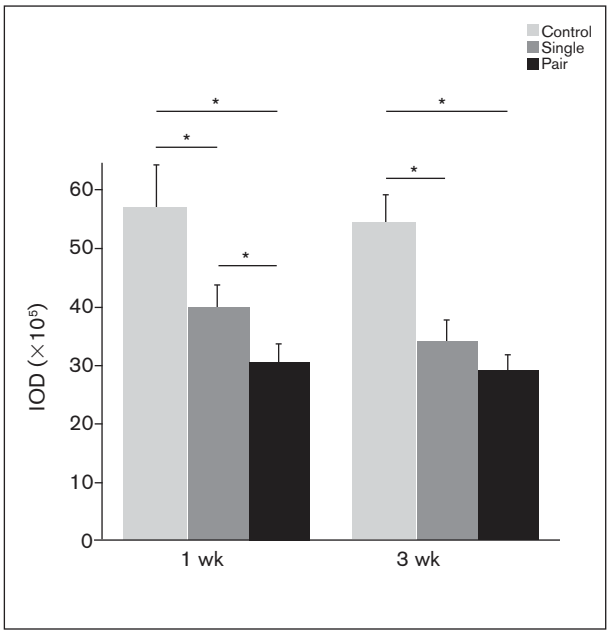


Fig 8 Comparison of the integral optical density (IOD) of toluidine blue staining between the groups and time points. *$P < .05$.

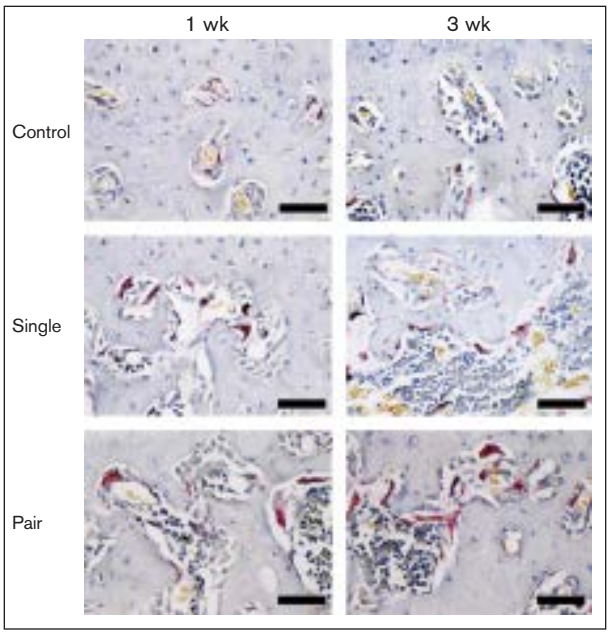


Fig 9 Examples of TRAP staining in the subchondral bone. Arrows indicate the TRAP-positive cells, eg, osteoclasts. Increased TRAP-positive cells are visible in both single and pair groups at both week 1 and week 3. Bar = 25 mm.

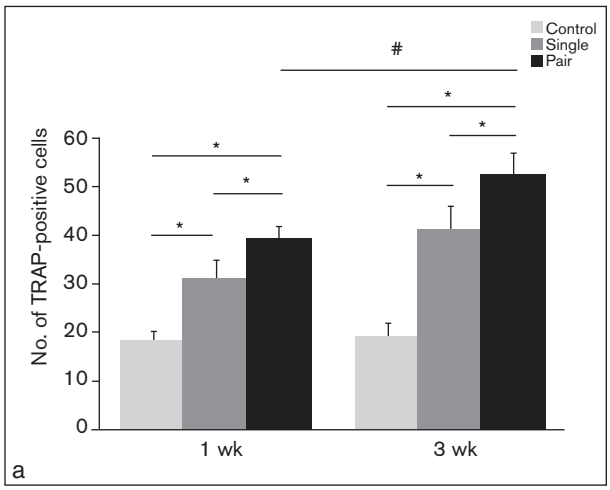
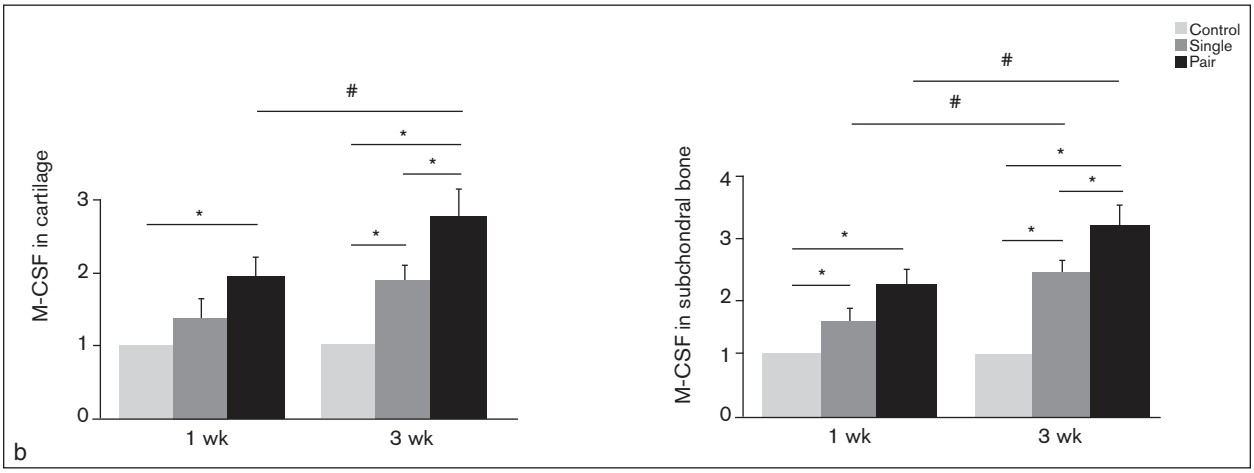


Fig 10 *(a)* Number of TRAP-positive cells and *(b)* mRNA level of M-CSF at week 1 and week 3 for the three groups. *$P < .05$. #$P < .05$ between the two time points.



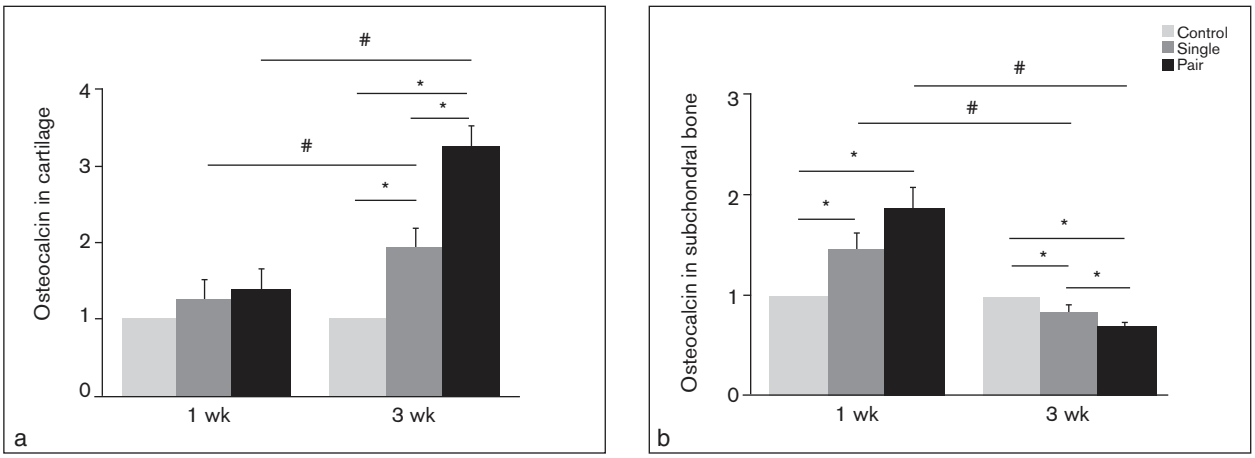


Fig 11 mRNA levels of osteocalcin in the *(a)* cartilage and *(b)* subchondral bone at week 1 and week 3 for the three groups. *$P < .05$. #$P < .05$ between the two time points.

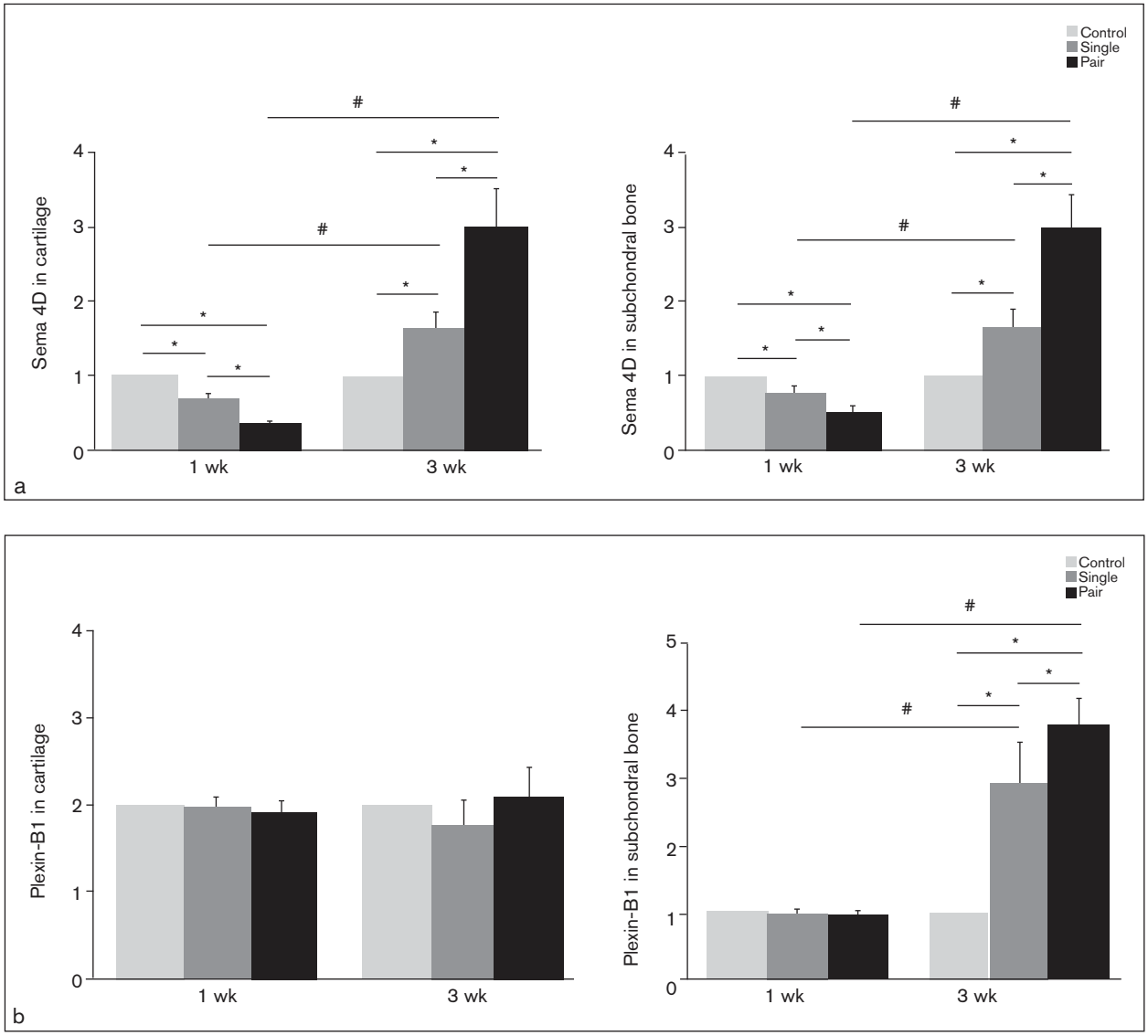


Fig 12 mRNA of of *(a)* Sema 4D and *(b)* Plexin-B1 at week 1 and week 3 for the three groups. *$P < .05$. #$P < .05$ between the two time points.

At week 3, the mRNA level of Plexin-B1 in subchondral bone in the experimental groups was significantly higher than that in the control group, with a higher level in the pair group than in the single group ($P < .05$, Fig 12b). There was a significant increase from week 1 to week 3 in both experimental groups ($P < .05$, Fig 12b). Such differences were not observed in the cartilage ($P > .05$, Fig 12b).

Discussion

The incisor malocclusion induced several cellular and metabolic alterations in the mouse TMJ that for most were more pronounced in the group of animals with a pair of metal tubes than in the group with a single metal tube. Large mouth opening was avoided during the metal tube bonding because of easy exposure of the incisors during the operation. The production of the metal tubes and the bonding operation were standardized, which allowed for reproducible experimental conditions. The results were solid because all samples showed similar changes, including cartilage degradation and subchondral bone loss.

Different animal models could account for differences between studies in histologic changes in the TMJ. For example, a partial discectomy can lead to proteoglycan loss and fibrillation in mouse TMJ condylar cartilage,[5] biglycan/fibromodulin double-knockout to acellular areas, vertical clefts and osteophytes in mouse TMJ condylar cartilage,[16] and mutations in the genes encoding α1 chain of type II collagen or α1 chain of type XI collagen to diminished proteoglycan and fissuring of the TMJ condylar cartilage.[17,18] All these changes were defined by the authors as osteoarthritic changes.[5,16–18] In contrast, histologic changes in the mouse TMJ induced by an experimentally induced abnormal occlusion and altered functional loading were different from the above phenotypes. Cartilage thinning, decreased proteoglycan, and reduced density of subchondral bone have been induced previously by trimming the incisors and soft diet administration, but no splitting or fibrillation had been observed in these studies.[19–21] The alterations induced in these experiments were generally described as alterations or remodeling.[19–21] The present TMJ changes, which were characterized by decreased cartilage thickness and proteoglycan content as well as subchondral bone loss, seem quite similar to the changes caused by trimming of incisors and soft diet administration. Therefore, the histologic and metabolic changes observed in this study were much more similar to remodeling. In addition, the histologic alterations did not proceed from week 1 to week 3. A possible explanation might be that the histologic alterations that occurred during the first week were induced by the initial alteration of the joint biomechanics, to which the TMJ thereafter adapted, so no further changes occurred during the next 2 weeks.

Overloading of the TMJs by an altered occlusion has been reported to be an important etiologic factor for TMJ disorders.[22,23] In previous studies, it has been shown that gradually creating a disordered occlusion in rats could induce mandibular cartilage degradation and subchondral bone resorption.[8,24,25] However, these changes were not constant in all tested animals in these previous studies,[8,24,25] possibly due to the technical difficulties of obtaining a consistent alteration of the posterior occlusion. In the present study, similar changes were found in all mice. This consistency in results may be due either to a more reproducible experiment or to the different animal species. Transgenic techniques in the mouse have been extensively used in studies of molecular mechanisms, making the present mouse model a good candidate in future studies for investigating in-depth mechanisms underlying TMJ remodeling.

Mice are rodents whose incisors are constantly erupting to compensate for occlusal wear. In the present study, the metal tube(s) prevented the left incisor(s) from physiologic wearing, causing an overeruption of the incisor(s) with metal tube(s), with significant longer incisor(s) at week 3 in the animals with metal tube(s). Despite these findings, it is noteworthy that a crossbite-like relationship was successfully established in both the single and pair groups. Therefore, the abnormal crossbite-like relationship and the longer arc of the incisor(s) caused by the bonded metal tube(s) both might have contributed to the TMJ remodeling. In addition, the longer arc of incisors caused by the bonded metal tube might have led to the disruption of jaw growth, and consequently to the TMJ remodeling observed in the present study. Another important feature of mice is that they have three jaw joints, two TMJs and a mobile joint at the symphysis. The metal tubes used in the present study, although only 0.1 mm in wall thickness, forced the mandibular incisors apart 0.1 mm at the symphysis. Although the 0.1-mm separation was considered to be within the physiologic extent of mandibular incisors separation, the possibility exists that this methodological factor may also have influenced jaw mechanics contributing to the TMJ remodeling. Thus, the contribution of these factors, eg, abnormal anterior occlusion, growth of incisors, disruption of jaw growth, and separation of mandibular incisors, to TMJ remodeling needs to be identified in future studies.

Osteocalcin, a major noncollagenous protein in bone, has been found to be mainly expressed by bone osteoblasts.[26] In the present study, the osteocalcin

expression in subchondral bone was increased at week 1 but decreased at week 3 in the experimental groups. The decreased osteocalcin expression at week 3 suggests decreased osteogenesis in subchondral bone, which might be responsible, at least in part, for the subchondral bone loss observed at week 3. Previous studies have shown that osteocalcin expression was not limited to bone but also existed in the condylar cartilage of both normal and osteoarthrotic TMJs, with increased osteocalcin expression in osteoarthritic TMJ cartilage.[27] It has been postulated that the level of osteocalcin expression could be an indicator of OA severity.[26] In addition, the expression of osteocalcin in chondrocytes has been shown to be closely associated with advanced maturity of chondrocytes and endochondral ossification.[28,29] In the present study, osteocalcin expression was increased in the TMJ cartilage in the experimental groups, which might indicate advanced maturity of chondrocytes or endochondral ossification. Studies are currently ongoing to identify the role of increased osteocalcin expression in the TMJ cartilage of animals with an experimentally induced anterior malocclusion.

Sema 4D, a protein expressed by osteoclasts, inhibits the bone-formative function of osteoblasts by binding to its receptor Plexin-B1.[13,14] Thus far, the expression pattern of Sema 4D in the TMJ has never been investigated. In the present study, an initial decrease at week 1 and subsequent increase at week 3 in the expression of Sema 4D, which was paralleled by an increased expression of Plexin-B1 at week 3, was found in subchondral bone in the remodeling TMJ. Therefore, the coincident increase in the expression of Sema 4D and Plexin-B1 at week 3 might explain the subchondral bone loss in the present study. In addition, Sema 4D expression was also increased in the condylar cartilage at week 3 but not that of Plexin-B1. The mechanisms underlying the role of Sema 4D in chondrocyte remain to be identified in future studies.

Conclusion

Bonding metal tube(s) on one or two left incisor(s) induced remodeling in mouse TMJs, which was characterized by cartilage degradation and subchondral bone resorption. The subchondral bone loss involved initially enhanced osteoclastic activity and later depressed osteoblastic activity.

Acknowledgments

The authors thank Ms. Shujing Cai and Zhongying Qiu for technical assistance. This work was supported by the National Natural Science Foundation of China (grants No. 30872870, 30901699, 30801303, and 30973052). The authors reported no conflicts of interest related to this study.

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