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Mechanism in favorable prognosis of pediatric condylar fractures managed by closed procedures: an experimental study in growing rats

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Abstract – The purpose of this study was to investigate the underlying mechanism in favorable prognosis following pediatric condylar fractures managed by closed procedures. Seventy-five 1-month-old male Wistar rats were used in this experiment. Unilateral medially rotated condyle fracture in growing rats was adopted as the condyle fracture model to investigate the mechanism in favorable healing of pediatric condylar fractures. The entire fracture healing process was investigated. The rotated subcondylar fractures in young rats healed by means of callus formation, with simultaneous and prompt repositioning of the condyle. The positive outcome in these condyle fractures was also associated with active cell proliferation potential in the condyle, as well as the condyle's remodeling capability. The growth potential and remodeling capability of the condyle during the growing period might be the intrinsic factor for favorable healing following pediatric condylar fractures managed by closed procedures.

The proportion of condylar fractures is higher in children than adults who accounts for the majority of mandibular fractures among children (1). There is a great consensus that the closed management is advocated for such fractures (2–5). In general, they are unilateral and subcondylar with medial deviation (6–9), and managed by closed procedures with favorable prognosis (3, 6, 10), an observation that is supported by some experimental studies (11–13).

It is noted that a unilateral medially rotated condyle fracture can heal with favorable outcome by means of callus formation, with simultaneous and prompt repositioning of the condyle. The condyle forms the very cornerstone of mandibular morphology and function, and injuries to the mandibular condyle in children may adversely affect both growth and development of the jaws and the occlusion (14, 15).

The usually favorable prognosis of a pediatric condylar fracture not only means the normal union of fractured fragments but also morphologic structure recovery of the condyle without growth disturbances. This might be ascribed to compensation by continuous condyle growth (3, 16, 17) and remodeling (10, 18) in the growing period. However, there is a lack of experimental studies regarding this opinion. The purpose of this study was to study growth potential and remodeling capability of condylar fractures in growing rats.

Material and methods

Experimental animals

Seventy-five 1-month-old male Wistar rats with an average body weight of 70.02 g were used in the experiment. They were separated at random into three groups: experimental group (suffering from condylar fracture, $n = 55$); sham-operated group (suffering from condylar periosteum exposure without condylar fracture, $n = 10$); blank group (without any operation intervention, $n = 10$).

Operation procedure and sacrifice

A unilateral medially rotated condyle fracture in growing rats was adopted as the condyle fracture model. Anesthesia was achieved by means of an intramuscular

ketamine hydrochloride injection. Each right preauricular area was shaved and prepared with antiseptic solution. An approximate 10 mm preauricular skin incision was made directly below the zygomatic arch over the right side. Blunt dissection was performed through the masseter muscle, and then the condylar process was exposed. In the experimental group, the condylar fracture was made with mosquito forceps. Fracture completeness was confirmed by condyle fragment mobility. Next, the condyle fragment was displaced in the medial direction before the wound was closed. The sham-operated animals underwent the same procedure, but were limited to condylar periosteum exposure. Rats in the blank group were received no operation intervention.

The 10 rats from the experimental group were sacrificed 24 hours, 1 week, 2, and 4 weeks after the operation. The other 15 rats in the experimental group were sacrificed 12 weeks after the operation, in which five rats chosen by random underwent bone tissue fluorescence labeling. The 10 rats in the sham-operated group were sacrificed 12 weeks after the operation. Two rats from the blank group were sacrificed 24 h, 1 week, 2, 4, and 12 weeks after the operation.

Evaluation of the body weight

The body weights were monitored and recorded while the rats were sacrificed. The body weights in the sham-operated group were monitored and recorded 24 h, 1 week, 2, 4, and 12 weeks after the operation. Statistical evaluation using Student's t test was performed to evaluate the significant difference between the experimental group's mean body weight values and the sham-operated group 24 h, 1 week, 2, 4, and 12 weeks after the operation. A P value of <0.05 was considered statistically significant.

Observation of X-ray photograph

After the rats from both the sham-operated group and experimental group were sacrificed, the whole skulls were obtained and fixed in 4% paraformaldehyde for 48 h. They were then radiographed to evaluate the mandible's deviation degree, referring to the methods described by Luz & de Araujo (12). Radiograms were taken from directly above. Angular measurements of mandible deviation were performed on the radiograms, with reference to the angle (angleα) formed by the intersection of the mandibular midline and the line between both tympanic bullae. Student's t test was used to evaluate the significant difference between the mean values of experimental and sham-operated groups at the 12th week after the operation, with the level of significance set at $P < 0.05$.

Histological observation by Mallory's trichrome staining

The right TMJ from all the specimens were obtained, decalcified, dehydrated, and embedded in paraffin for histological observation by Mallory's trichrome staining. Histological observation focused on fracture healing and TMJ changes. All specimens were cut continuously in the coronal plane, and the midcondylar sections were prepared for histological staining.

Polychrome sequential labeling and fluorescence microscopy observation

The polychrome sequential labeling was used to establish chronologically oriented condyle growth and fracture healing characteristics in growing rats. To perform polychrome sequential labeling, five rats were randomly chosen from the experiment group. The polychrome sequential labeling was performed by intraperitoneal injection alternated every week between calcein (1% in 2% $NaHCO_3$ solution, 5 ml kg^{-1} body weight) and alizarin (1% in 2% $NaHCO_3$ solution, 20 ml kg^{-1} body weight) until 12 weeks after the operation. All specimens undergoing polychrome sequential labeling were fixed, dehydrated, and embedded in acrylic resin. The resin blocks were cut along the coronal plane of the condyle. The sections were then fixed on an acrylic carrier and polished to a thickness of approximately 40 μm for fluorescence microscopy observation.

Histological observation by Safranin O-fast green staining

To investigate condylar cartilage histological changes, safranin O-fast green staining (19) was performed on the midcondylar sections of both the experimental and blank group. The histological sections of the condyle from the blank group rats served as controls. The thickness of the chondrocyte layer, identified by Safranin O staining in red color, was measured according to the method described by Rabie et al. (20), and the measurements were carried out in the condyle's center region.

Immunohistochemical observation of proliferating cell nuclear antigen

Immunohistochemical observation of proliferating cell nuclear antigen (PCNA) (21) was performed on the midcondylar sections from the experiment group to investigate the expression status of special antigens in the proliferation of condyle chondrocyte layer. The histological sections of the condyle from the blank group were served as the controls. When the cell nucleus was clearly stained brown it was treated as a positive cell. The results were represented by a PCNA labeling index (PI). Three highly magnified (×400) fields including a center region, along with regions in both the lateral and medial poles of the condyle were observed. One hundred cells were counted in each field. Next, the positive cell numbers obtained from each region were added. PI = (PCNA positive cell number/300) × 100%.

Tartrate-resistant acid phosphatase staining

To investigate bone remodeling during condyle fracture healing, osteoclasts with the characteristic of TRAP activity were identified with a staining kit (Sigma, St. Louis, MO, USA) following the manufacturer's instructions (22). The TRAP staining was performed on the midcondylar sections of the experimental group. The

histological sections of the condyle from the blank group were served as control.

Results

General condition and body weight change

No animal died accidentally in the postoperative period. Healing after the operation progressed uneventfully. The means and SD for body weights of the rats from both the sham-operated group and experimental group at each time point are shown in Fig. 1. The animals in the sham-operated group continued to grow with weight increase. The experimental group's weight decreased after the first week, and then recovered and increased from the second week on.

Statistical evaluation between mean body weight values of the experimental group and sham-operated group at each time point revealed significant difference at the first, second, and fourth week after the operation; however, there was no significant difference at the 12th week.

Observation of X-ray photograph

The mandible deviation to the right side was observed in the experimental group. Mean angleα values (Fig. 2) indicated mandible deviation degree severity. Twenty-four hours after the operation, the mandibles showed obvious deviation. In addition, the mandible deviation degree increased at the first week after the operation. The deviation degree reached its maximum at the second week, and then reduced at the fourth week. At the 12th week, the deviation degree became indistinct. Overall, statistical evaluation between the mean values of the two groups at the 12th week revealed no significant difference.

Histological observation by Mallory's trichrome staining

At the 24th hour after the operation, condylar process fractures were confirmed in the experimental animals, and the medial displacement of the fractured fragment took place under traction by the lateral pterygoid muscle (Fig. 3a). The articular disc remained attached to the condylar head, but there was acute inflammation along

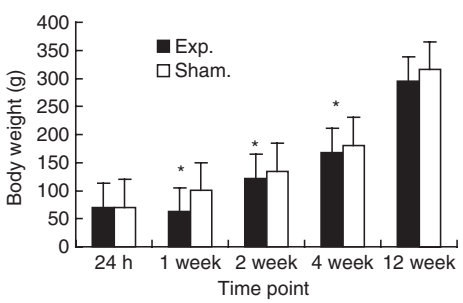

Fig. 1. The body weights of the rats from the sham-operated and experimental groups at each time point. Values are mean ± SD. Significant difference between the two groups is marked with asterisks (*$P < 0.05$).

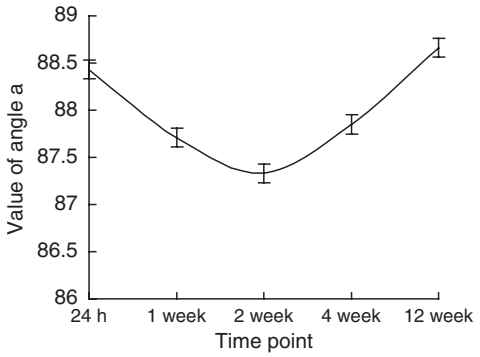

Fig. 2. The mean values of angleα for experimental group rats. The mean values of angleα reached the minimum at the second week, and the mean value of angleα became normal at the 12th week.

the articular capsule and adjacent muscle fibres. At the first week, the fractured fragment was less rotated than before, and cartilaginous tissue formation occurred between the fractured condyle and the ramus (Fig. 3b). At the second week, there was abundant callus presented in the fracture site, which composed of both cartilaginous and osseous tissue (Fig. 3c). Simultaneously, the rotated condyle had been promptly repositioned. At the fourth week, the condyle recovered to the normal position in the temporal fossa, with articular disc interposition, and callus remodeling was also completed (Fig. 3d). However, the condyle was not in line with the mandible ramus. At the 12th week, the fractured condyle was in line with the mandible ramus, and the condyle and TMJ presented a normal structure (Fig. 3e), although the condyle neck seemed to be 'thicker' than the normal condyle in the blank group (Fig. 3f).

Polychrome sequential bone labeling and fluorescence microscopy observation

During the fracture healing process, the new bone formation occurred mainly around the fracture site, whereas the growth line of the new bone formation was regular and well ordered. Before the new bone formation, the fractured condyle had almost reduced to its normal site. The new bone then formed around the fracture site layer upon layer (Fig. 4).

Histological observation by Safranin O-fast green staining

At the 24th hour after the operation, the thickness of the condyle chondrocyte layer was unchanged, with clear tissue arrangement. In addition, there was no cartilaginous tissue formation in the fracture site. At the first week, the chondrocyte layer's thickness enlarged, and there was cartilaginous tissue formation in the fracture site. The tissue arrangement in the chondrocyte layer was clear, but the proliferative zone became thick. At the second week, the cartilage cells actively proliferated, and the chondrocyte layer became thicker, which was visible in both the proliferative and hypertrophic zones (Fig. 5). Tissue arrangement in the chondrocyte layer was irregular. Further, the boundary between the hypertro-

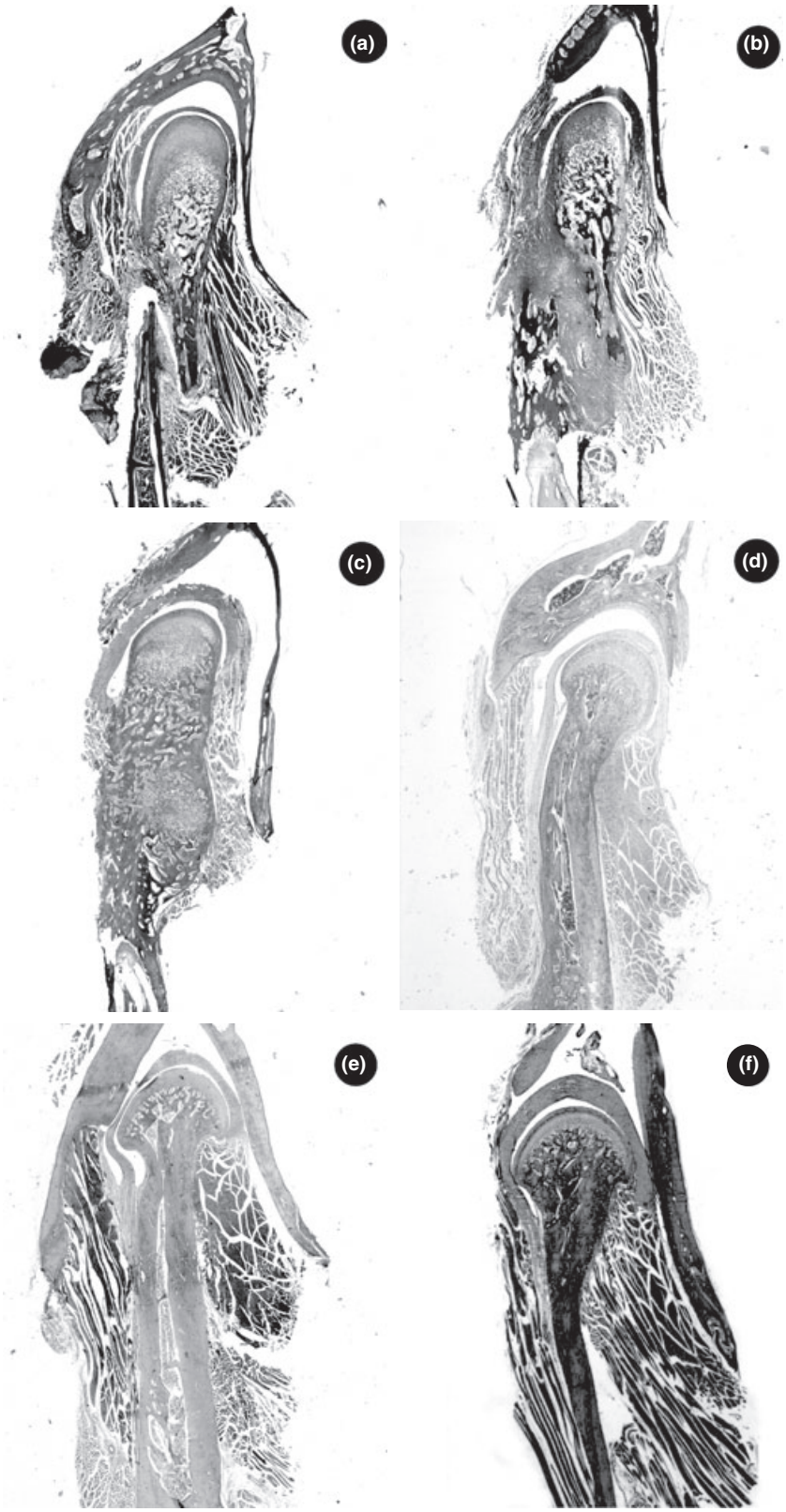

Fig. 3. The healing process of unilateral medially rotated condyle fractures in growing rats. At the 24th hour, a unilateral medially rotated condyle fracture was verified (a). At the first week, the fractured fragment was less rotated, and cartilaginous tissue formation occurred on fracture site (b). At the second week, the rotated condyle had promptly repositioned, with abundant callus presented in the fracture site (c). At the fourth week, the condyle fracture was indistinct with the completion of callus in the fracture site, but the condyle was not in line with the mandible ramus (d). At the 12th week, the condyle fracture had healed completely, and the condyle presented a normal structure (e). The normal condyle of the blank group at the 12th week (f). Original magnification ×2.0.

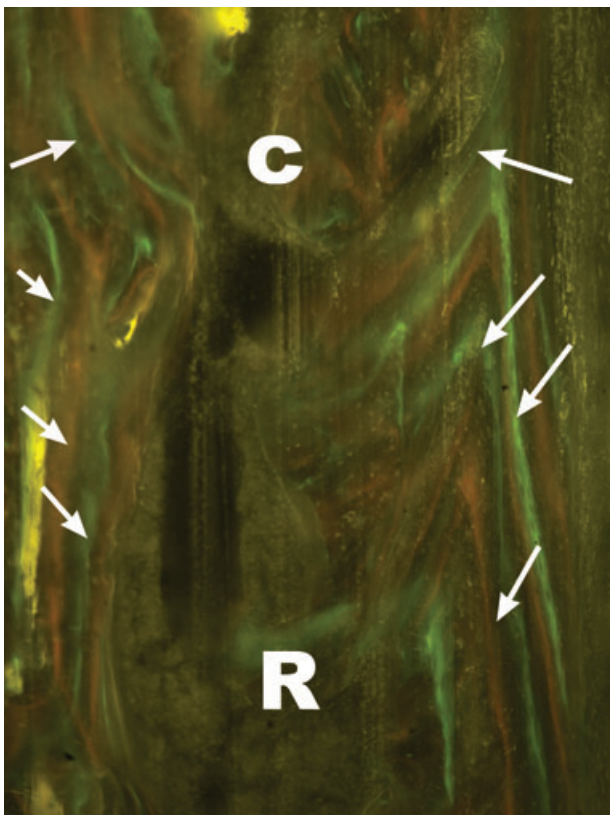

Fig. 4. Before the new bone formation, the fractured condyle had almost reduced to the normal site, and then the newly formed bone was regular and well ordered. C, condyle; R, ramus; arrow, growth line. Original magnification ×10.

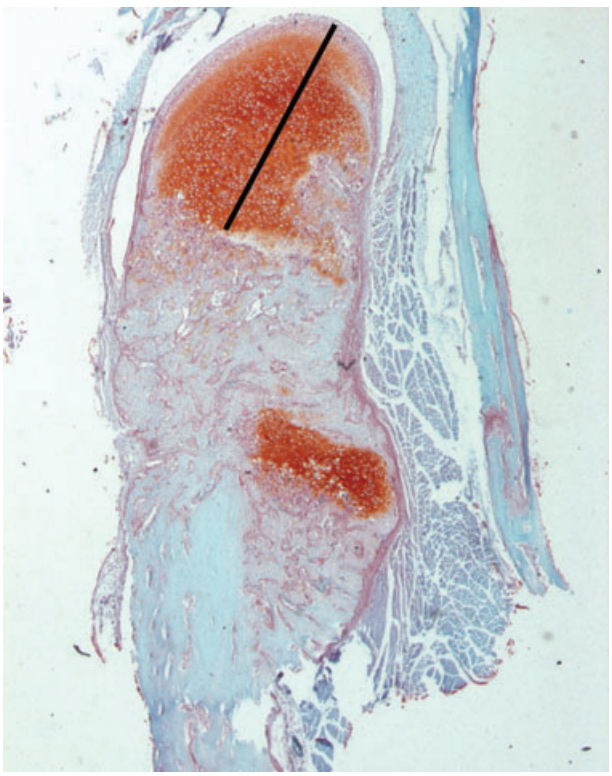

Fig. 5. Histological observation on condylar cartilage change by Safranin O-fast green staining. The thickest condyle chondrocyte layer was presented at the second week after the operation. The straight line refers to the thickness of the chondrocyte layer. Original magnification ×3.2.

phic zone and erosive zone was unclear, and some proliferated cells in the hypertrophic zone even broke through the boundary and entered into the erosive zone. In addition, there was active cartilaginous tissue inside the fracture site callus. At the fourth week, tissue arrangement in the condyle chondrocyte layer was almost normal, but the chondrocyte layer was thicker than normal. The fracture site was united and lacked cartilaginous tissue formation. At the 12th week, the tissue arrangement in the chondrocyte layer was regular with normal cartilage thickness, which possessed no difference when compared with the normal chondrocyte layer thickness, which is presented in Fig. 6.

Immunohistochemical observation of proliferating cell nuclear antigen

The PCNA-labeled cells were identified in the condylar cartilage layers by the overlying brown deposits of DAB in the cell nucleus. At all time points after the operation, PCNA-labeled cells were visible in cartilage layer cells, yet the area and degree of expression varied with time points.

At the 24th hour after the operation, the PCNA expression of cartilage layer in the experimental rats was similar to the blank group. PCNA-labeled cells were mainly visible in the proliferative zone. At the first week, active PCNA-labeled cells appeared in the hypertrophic

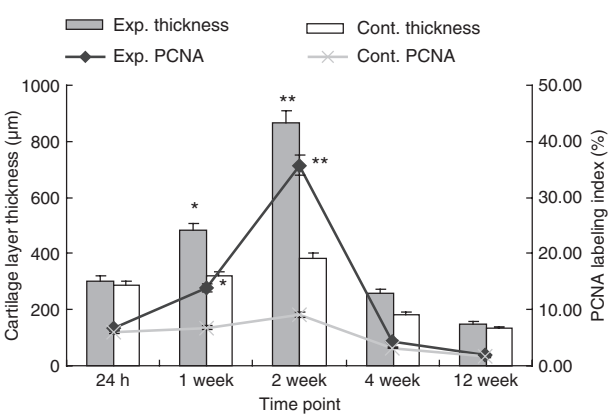

Fig. 6. The thickness of the chondrocyte layer and PCNA labeling index of the condyles from control and experimental groups. Values are mean ± SD. Significant difference between two groups is marked with asterisks (*$P < 0.05$; **$P < 0.01$).

zone, whereas the number of labeled cells also increased in the proliferative zone. At the second week, labeled cells with heavy PCNA expression were visible in both zones. At the fourth week, the PCNA expression degree resumed to normal levels. Labeled cells were located in both zones, but mainly in the proliferative zone. At the 12th week, PCNA expression in the experimental rats was comparable with the blank group, whereas there

were less PCNA-labeled cells in the hypertrophic zone. The values of PI (mean ± SD) are presented in Fig. 6.

Tartrate-resistant acid phosphatase staining

At the 24th hour after the operation, the condyle was under normal bone remodeling conditions. Osteoclasts were located in the trabecular bone. There was no TRAP reaction in the fracture site. At the first week, active bone remodeling occurred in both the trabecular and cortical bone of the condyle, including the fracture site. However, there was no TRAP reaction seen in the fracture site's callus. At the second week, the fracture site callus displayed a strong TRAP reaction, whereas there were plenty of osteoclasts with strong TRAP reaction observed in the trabecular bone (Fig. 7). At the fourth week, TRAP reaction was still seen in the trabecular and cortical bone of the condyle. At the 12th week, the condyle resumed normal bone remodeling conditions, presenting weak TRAP reaction in the trabecular bone and no TRAP reaction in the cortical bone.

Discussion

In the present experiment, from the change of trends in body weight and the mandible's deviation degree severity in the experimental animals, we can presume a favorable prognosis in these condylar fractures. In addition, the histological observations, including Mallory's trichrome staining and polychrome sequential bone labeling, did reveal the favorable condyle fracture prognosis, which also demonstrated that rotated subcondylar fractures in young rats could heal by callus formation, with simultaneous and prompt condyle repositioning.

In the study of 15 years follow up on condylar fractures conservatively treated, in children no major growth disturbances were observed and in most cases, there were no signs of the earlier fracture and the function of the masticatory system was good (23). As for condylar fractures, most authors believe the closed treatment is sufficient in pediatric patients, but remains controversial for adult patients. The therapeutic goal in adult patients differs from that in growing patients, as in children the condyle is a major growth center for the mandible (24). Chatzistavrou & Basdra (3) presented five cases of isolated condylar fractures in children who were treated solely by a closed orthopedic approach and obtained a favorable prognosis. Therefore they deemed that the successful healing in these patients is because the condyle possesses growth potential during childhood. During the healing process of rotated subcondylar fractures in the present experiment, the condyle growth potential in young rats was remarkable. By the specific Safranin O-fast green staining, during the initial stages of condylar fracture healing, the thickness of the condyle chondrocyte layer increased, especially in the proliferative zone. As far as the results in our experiment were concerned, the PCNA expression revealed the active proliferation status in the cells of the condyle chondrocyte layer. The expression of PCNA is associated with the proliferation status in cells (25). All these results confirm the key role of condyle cartilage's growth potential in the healing process of rotated subcondylar fractures during the growing period. We suppose that the proliferation in the chondrocyte layer supports new bone formation in the fractured condyle, which contributes to the continuous and simultaneous condyle growth when a condyle fracture heals.

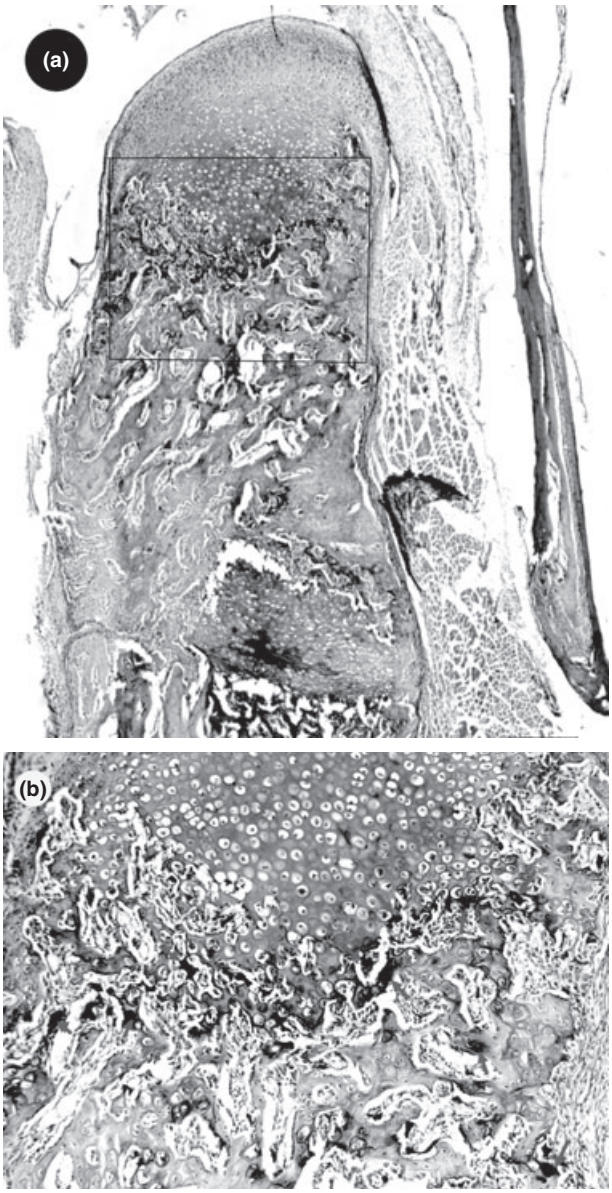

Fig. 7. At the second week after the operation, the condyle presented strong TRAP reaction in the callus of the fracture site and trabecular bone (a and b). Arrow: osteoclasts. Original magnification: a (×4.0); b (×25).

Remodeling processes in the TMJs of children (3- to 11-years old) sustaining condylar fractures were observed that there was a completely return to normal skeletal relations in most joints (26). In the present experiment, we also observed the important role of remodeling in the condyle (including trabecular and cortical bone of the condyle) for gradual recovery of the condyle morphologic structure while the fracture

healed. During the fracture, healing the appearance of osteoclast indicates the remodeling in the bone (27). The specific TRAP staining is the common index reflects the cell function of osteoclast (28). During the growing and adult period, the mandibular condyle undergoes adaptive remodeling. Therefore, when the condyle fracture occurred (the 24th hour after the operation) and the condyle fracture healed (the 12th week after the operation), there was remodeling activity in the trabecular bone, which was consistent with phenomenon of the normal condyle. At the second week, there was a strong TRAP reaction seen in the trabecular bone of the condyle, as well as the callus of the fracture site. This suggests that the major changes in the condyle had transformed from bone formation to bone remodeling. At the fourth week, bone formation had almost ceased, but the remodeling of the condyle's cortical bone was obvious. All these results indicate that bone remodeling contributes to the morphologic recovery of the traumatized condyle during the fracture healing process.

In clinic, some patients' sequelae subsequent to condylar fractures can also occur in mandibular asymmetric deformities, functional disturbances, and even TMJ ankylosis (4, 14, 29, 30). From clinical experience (31–34), it is believed that the damage to the disc and its attachments to the condyle head should be considered an important factor in condyle fracture healing. Unilateral medially rotated condyle fractures in growing rats were adopted as the condyle fracture model in the present experiment, and this type of condyle fracture model retained the fibrous attachments of capsule and disc to the lateral and medial poles of the condyle. The fibrous attachments of capsule and disc aided by muscular movement, might serve to gradually reposition of the fractured fragment in the early condyle fracture-healing stages. Therefore, one possible theory relating to the favorable healing of condylar fractures during the growing period can be supposed. The undamaged TMJ capsule and disc aid the reduction and realignment of a displaced condylar fracture. On the contrary, the unilateral medially rotated condyle fracture in the present experiment did not produce traumas targeting cartilage of the condylar head. There is evidence that suggests trauma targeting condylar head may cause ankylosis, growth retardation, and resultant facial malformations (35). Taken together, we deduce that undamaged fibrous attachments of the capsule and disc, and unbroken cartilage of the condylar head, are macroscopical qualification for the favorable prognosis of condyle fractures during the growing period.

Based on the results from the present experiment, we can conclude that the growth potential and remodeling capability of a condyle during its growing period might be the intrinsic factor for the favorable prognosis of condyle fracture managed by closed procedures. Extrapolating this mechanism to clinical practice, it appears that pediatric condylar fractures could be managed by closed procedures and obtain an encouraging prognosis, as long as there was no damage to the fibrous attachments of the capsule, disc, and condyle's cartilage. We should believe that these condyle fractures during the growing period will be compensated by continuous condylar growth and remodeling.

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