



# Incisor disocclusion in rats affects mandibular condylar cartilage at the cellular level

G.O. Ramirez-Yañez\*, T.J. Daley, A.L. Symons, W.G. Young

Department of Oral Biology and Pathology, School of Dentistry, The University of Queensland, Qld, Australia

Accepted 18 November 2003

## KEYWORDS

Mandibular condylar cartilage; Incisor disocclusion; Mitotic activity; Chondrocytes maturation; Transforming growth factor-beta1; Alkaline phosphatase; Osteocalcin

**Summary** The effect of altered occlusion on the mandibular condylar cartilage remains unclear. **Objective:** This study investigated the effect of unilateral incisor disocclusion on cartilage thickness, on mitotic activity and on chondrocytes maturation and differentiation in the mandibular condylar cartilage of rats. **Design:** The upper and lower left incisors were trimmed 2 mm every second day in five rats. In other five rats, the incisor occlusion was not altered. Condylar tissues from both sides of each mandible were processed and stained for Herovici's stain and immunohistochemistry for bromodeoxyuridine (BrdU), transforming growth factor-beta1 (TGF- $\beta$ 1), alkaline phosphatase (ALP) and osteocalcin (OCN). Measurements of cartilage thickness and the numbers of immunopositive cells for each antibody were analysed by one-way analysis of variance (ANOVA). **Results:** No significant differences were observed in cartilage thickness after 7 days of unilateral incisor disocclusion. However, the numbers of immunopositive cells for BrdU as a marker of DNA synthesising cells, TGF- $\beta$ 1 as a marker of chondrocytes differentiation, and ALP and OCN as markers of chondrocytes maturation, were significant higher in the cartilage cells on both sides when incisor occlusion was unilaterally altered. Interestingly, alkaline phosphatase was highly expressed on the condylar side of incisor disocclusion, whereas osteocalcin was highly expressed on the side opposite to the incisor disocclusion. **Conclusions:** It is demonstrated that after 7 days, unilateral incisor disocclusion affects the mandibular condylar cartilage at the cellular level by increasing the mitotic activity and by accelerating chondrocytes maturation. Chondrocytes maturation appears more accelerated on the side opposite to incisor disocclusion.

© 2003 Elsevier Ltd. All rights reserved.

## Introduction

Controversy remains as to the effect of an altered occlusion on the mandibular condylar cartilage. Functional changes in some animal models produce an increase in the width of the mandibular cartilage.<sup>1</sup> However, other studies have demonstrated the opposite response.<sup>2</sup> Condylar growth is not

necessarily accompanied by changes in the morphology of the cartilage.<sup>3</sup> This study investigated the effect of altered function on the cells of condylar cartilage.

Endochondral ossification is the process whereby mesenchymal cells differentiate into chondroblasts.<sup>4</sup> These differentiated cartilage cells mature (chondrocytes) and hypertrophy while secreting cartilaginous matrix. The secreted matrix serves as scaffold for mineralisation and for bone remodelling.<sup>5</sup> In cartilage, transforming growth factor-beta1 (TGF- $\beta$ 1) is expressed during proliferation and

\*Corresponding author. Tel.: +61-7-3365-2660;

fax: +61-7-3365-1109.

E-mail address: g.ramirez@uq.edu.au (G.O. Ramirez-Yañez).

differentiation of mesenchymal precursors and stimulates extracellular matrix proteins synthesis.<sup>6,7</sup> Alkaline phosphatase (ALP) and osteocalcin (OCN) are secreted by the chondrocytes and play important roles in the ossification process.<sup>8</sup> Whereas, ALP is associated with the beginning of cartilage matrix mineralisation, OCN associates with chondrocytes hypertrophy.<sup>9–12</sup> OCN presence suggests that hypertrophic chondrocytes developed the phenotypic marker associated with matrix mineralisation similarly to osteoblasts.<sup>9</sup>

The aims of the present study were to determine the effect of unilateral incisor disocclusion on cartilage thickness, on mitotic activity of the cartilage cells and on cartilage cells' differentiation and maturation at the mandibular condylar cartilage.

## Materials and methods

Ten male Lewis rats (7-week-old, weight  $143.6 \pm 2.3$  g) were obtained from the Animal Resources Centre at the University of Western Australia. Ethical approval for the study was obtained from the University of Queensland—Animal Ethics Committee (DENT/361/00). Animals were randomly divided into two groups: experimental and control. In the experimental group, five animals were anaesthetised (Xylazil/Ketamine: 0.1 ml/100 g of body weight), and the incisor occlusion in the rats was altered by trimming with a high speed handpiece both upper and lower left incisors, approximately 2 mm from each incisal edge every second day, to maintain incisor disocclusion. Three trimmings were performed during the experimental time. The right incisors remained untrimmed. In remaining five rats (control group) the occlusion was not altered. The latter group was used as sham-treated controls. They underwent anaesthetising at the same time with the experimental animals, but sham trimming was not performed on the incisors.

On Day 7, rats were injected intraperitoneally with 50 mg/kg of weight of bromodeoxyuridine (BrdU) 2 h prior to sacrifice. Under general anaesthesia (Xylazil/Ketamine), tissues were fixed by perfusion with 2% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffered saline (PBS), and then, animals sacrificed by exsanguination. Both right and left condyles were harvested and postfixed for 4 h in the same fixative. The condyles remained in PBS overnight. On the following day, tissues were placed in EDTA (4.13%; pH 7:47) for approximately 4 weeks until total decalcification was observed by radiography. The condyles were embedded in paraffin, sagittal sectioned (5  $\mu$ m), and mounted for morphological and immunohistochemical studies.

All animals were maintained under identical dietary and lighting regimens. The animals were weighed at the beginning and at the end of the experimental time. They were closely followed up to observe any variation in the dietary habits or general behaviour.

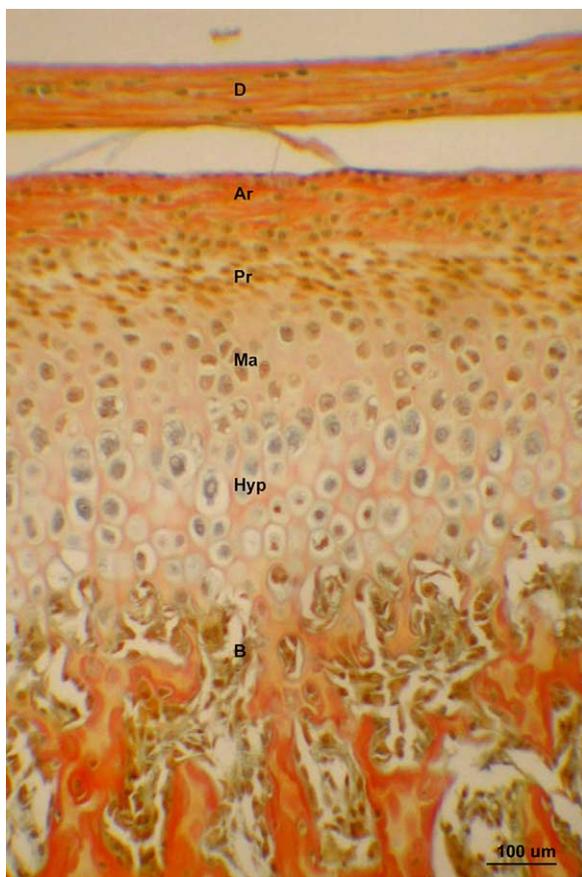
## Histochemistry

Herovici polychrome stain was selected to obtain clear differentiation between mandibular condylar cartilage and the front of endochondral ossification. In brief, after deparaffinisation and hydration, sections were immersed in Celestine Blue dye for 5 min, washed in distilled water and stained with haematoxylin dissolved in 5% aqueous aluminium sulphate solution, with added 4% of aqueous iron chloride and chloride acid for 5 min. Sections were then immersed in Metanil Yellow dye for 2 min and washed in distilled water containing acetic acid, followed by distilled water-containing lithium carbonate. Finally, sections were immersed in Picropolychrome mixture (Methyl blue plus acid fuchsine in glycerol) and washed in 1% acetic acid for 2 min. Afterwards, sections were dehydrated through alcohol, xylened and covered. This staining method produced blue staining of the nuclei in hypertrophied chondrocytes and light pink staining of the extracellular matrix in the hypertrophic layer. The front of endochondral ossification stained as bone matrix red with cell nuclei yellow or brown (Fig. 1).

## Immunohistochemistry

Anti-bromodeoxyuridine antibody (Clone BU-33, B-2531, Sigma) at a dilution of 1:750 was used to determine the cells in the S-phase of the cell mitotic cycle. Anti-TGF-B1 (AF-246-NA, R&D Systems, MN, USA) at a dilution of 1:1600 was used to observe the expression of this growth factor in the cartilage cells of the mandibular condyle. Anti-bone ALP (a gift from Dr. Greg Welowski, Merck Research Laboratories, West Point, PA., USA) at a dilution of 1:200, and anti-OCN (a gift from Dr. Dominique Modrowski, Inserm, Paris, France) at a dilution of 1:300, were used to determine variations in the expression of bone markers by the cartilage cells.

Immunohistochemical staining was performed using the above antibodies as follows: after deparaffinisation, hydration and washing in PBS, the sections were exposed to 3% hydrogen peroxidase for 15 min. Next, the sections were incubated with 1:10 swine serum (code  $\times$  0901, Sigma) for 30 min to block non-specific protein binding. After incubation, excess serum was blotted, and the sections were incubated with the primary antibody for



**Figure 1** Section of the mandibular condylar cartilage stained with Herovici. The different layers of the cartilage are shown: articular (Ar); proliferative (Pr); mature (Ma); and hypertrophic (Hyp). Articular disc (D), zone of endochondral ossification (B). Magnification: 100 $\times$ .

90 min. After twice 3 min washes with PBS containing 0.1% Triton (SIGMA T-9284), the sections were incubated with biotinylated secondary antibody (Biotinylated swine anti-mouse, anti-rabbit, and anti-goat (DAKO LSAB-kit) for 15 min. The sections were rinsed in PBS containing 0.1% Triton and incubated with streptavidin peroxidase complex (DAKO LSAB-kit) for 15 min. The peroxidase activity was visualised with 3,3'-diaminobenzidine (DAB) solution for 3 min and counterstained with haematoxylin for 30 s. Finally, sections were dehydrated through alcohol, cleared in xylene and glass covered. Staining of all sections for each antibody was performed contemporaneously to avoid variations in time, temperature and concentration during the experiment. Positive controls were performed on rat molars sections, where TGF- $\beta$ 1 expression is mainly localised at the apical portion of the root,<sup>13</sup> and ALP and OCN are expressed on the periodontium of rats.<sup>14</sup> For specific negative controls, the primary antibodies were replaced with a non-specific rat IgG. Immunoreactivity was observed for all the

antibodies in the positive controls, whereas the negative controls showed non-immunoreactivity.

### Measurements and cell counts

Prior to decalcification the maximum condylar length (antero-posterior) was determined with a digital calliper ( $2.8 \pm 0.3$  mm). After staining, slides were observed by optical microscopy (Olympus CH2), and the sagittal length of the condyles was measured to ensure that each sagittal section was obtained from the maximum diameter of the condyle in the sagittal plane. Condylar sections measuring between 2.5 and 3 mm in length were used for this study.

Mounted tissue sections were photographed using a digital camera (Nikon Coolpix995, Japan). Digitised pictures of the sections (magnification: 100 $\times$ ) at the central area of the mandibular condylar cartilage were obtained using a light microscope (Olympus CH-2, Japan). Images were transferred to a computer (Pentium III, 1 GHz processor, 256 Mb RAM, 32 Mb video card) and analysed using two different computer softwares. Both computer softwares were calibrated using the image of a 1 mm scale in 10  $\mu$ m units obtained and processed coincidentally with the images of the different stained sections.

### Cartilage thickness

A computerised image analyser (Scion image Beta 4.0.2, Scion Corp, Maryland, USA) was used for this purpose. The total thickness of the cartilage was determined measuring the distance from the superior border of the mandibular cartilage to boundary with the zone of endochondral ossification on the sections stained with Herovici's (Fig. 1). For each condylar section, the average of three measurements was used for statistical analysis.

### Cell counting

For this purpose, an image editor software (Adobe Photoshop 7<sup>TM</sup>) was used on the digitised pictures of sections immunostained for BrdU, TGF- $\beta$ 1, ALP and OCN. An area of 0.122 mm<sup>2</sup> covering the total thickness of the proliferative and the mature layers, as well as part of the articular and the hypertrophic layers constituted each image. The numbers of immunoreactive cells to each antibody present into this area was used for statistical analysis. Both cartilage thickness measurement and cell counting were performed in a blind fashion by two observers. The data from each observer were analysed by Spearman correlation test to determine the level of agreement between the examiners. A high agreement ( $r > 0.95$ ) was observed between the exam-

iners for all the cases. A final average between the results from both examiners was used for statistical analysis.

### Data analysis

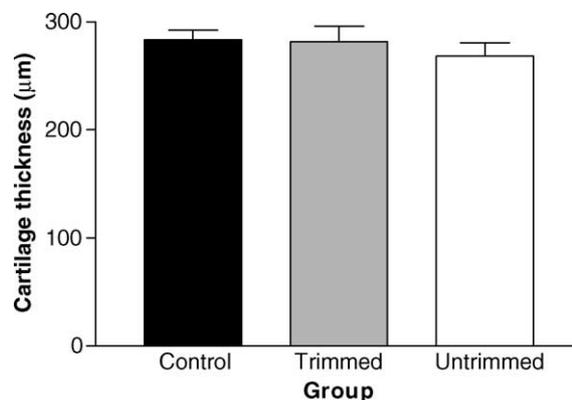
Three groups of condyles were defined for statistical analysis. The first group comprised five condyles from the control group, random chosen regardless of side. The other two groups comprised the condyles from the trimmed (left) and untrimmed (right) sides from the experimental group. The total thickness of the mandibular cartilage, as well as the numbers of immuno-positive cells from the controls, the trimmed side and the untrimmed side for the four different antibodies tested were analysed by one-way analysis of variance (ANOVA). When a significant difference was determined at the 95% level of probability, Newman-Keuls post-test was computed to discriminate the significant difference between the three defined groups. Statistical analysis was performed using Prism 2.1 software (GraphPad Prism Software Inc., San Diego, CA, USA).

### Results

The animals showed no discomfort to incisor disocclusion throughout the experiment. Animals showed some increase in weight over the experimental time ( $1.8 \pm 0.6$  g).

#### Cartilage thickness

No significant differences were observed between the different groups of cartilage sections ( $P > 0.05$ ). However, slightly thinner cartilage was observed in



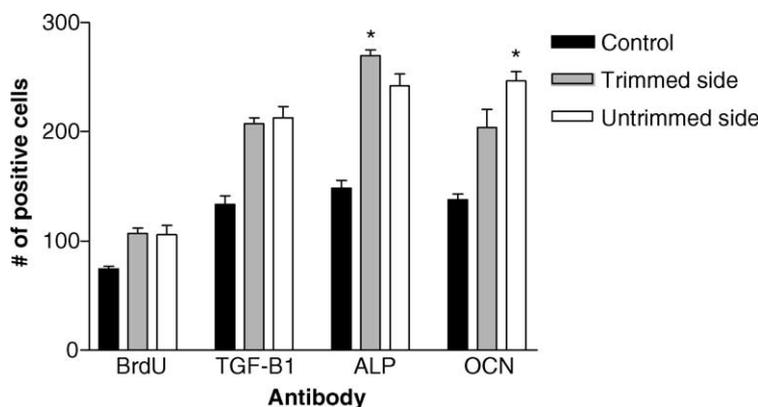
**Figure 2** No significant difference in cartilage thickness is observed in the mandibular condyles between the control group and the trimmed and untrimmed sides in the experimental group. Cartilage thickness is slightly but not significantly reduced in the condyles from the untrimmed side. Mean and SEM are shown.

mandibular condyles of the untrimmed side compared with both control and trimmed side which showed similar cartilage thickness (Fig. 2).

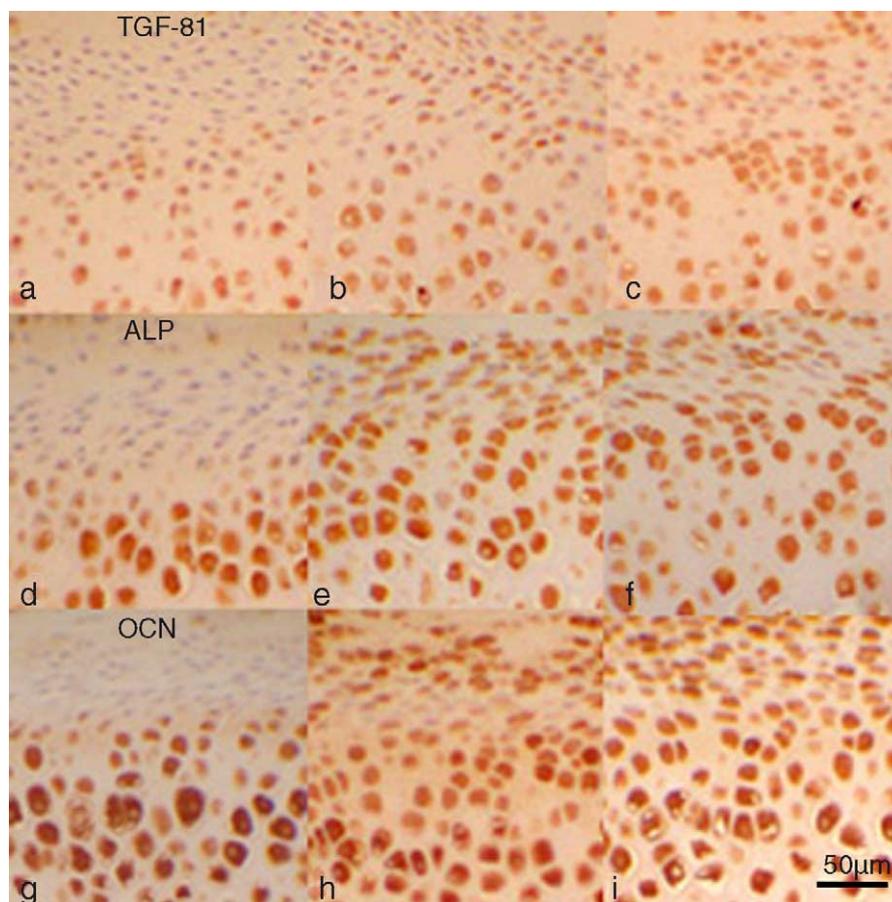
#### Immunohistochemistry

Bromodeoxyuridine as a marker of DNA synthesising cells showed significant differences when the numbers of immunopositive cells on the condylar cartilage from both the trimmed and the untrimmed sides in the experimental group were compared independently with those from the control group ( $P < 0.01$ ). No significant difference was found ( $P > 0.05$ ) when the numbers of BrdU-immunopositive cells from the trimmed and the untrimmed sides were compared within the experimental group (Fig. 3).

Significantly higher numbers of TGF-B1-immunopositive cells were counted in the condyles from



**Figure 3** Significant higher immunoreactivity is observed in the cartilage cells for the different antibodies tested in this study when the incisor occlusion was unilaterally altered in the rat. In the experimental group, ALP expression is significant higher on the trimmed side (\*), whereas OCN is significant higher in the untrimmed side (\*). Mean and SEM are shown.



**Figure 4** Immunohistochemical stain of cartilage sections for TGF- $\beta$ 1, ALP and OCN. Sections from the control group (a, d, and g), from the trimmed (b, e, and h) and the untrimmed (c, f, and i) sides in the experimental group are shown. Magnification: 400 $\times$ .

both trimmed and untrimmed sides in the experimental group compared with the numbers of TGF- $\beta$ 1-immunopositive cells in the cartilage sections from the control group ( $P < 0.001$ ) (Figs. 3 and 4). Again, no significant difference was observed when the trimmed side was compared with the untrimmed side in the experimental group ( $P > 0.05$ ).

Alkaline phosphatase-immunopositive cells were significantly higher in both the trimmed and the untrimmed sides of the experimental group compared with the number of ALP-immunopositive cells in the control group ( $P < 0.001$ ). The trimmed side compared with the untrimmed side within the experimental group showed a significant difference ( $P < 0.05$ ) with significant higher numbers of ALP-immunopositive cells in the trimmed side (Figs. 3 and 4).

Osteocalcin showed significant higher numbers of immunopositive cells in both the trimmed and untrimmed sides of the experimental group than those numbers of OCN-immunopositive cells in the condylar cartilages from the control group ( $P < 0.01$ ). In the experimental group, a significant

difference ( $P < 0.05$ ) was also found when the numbers of OCN-immunopositive cells in the mandibular condylar cartilage from the trimmed and untrimmed sides were analysed. Higher numbers of OCN-immunopositive cells were counted in the condylar cartilage from the untrimmed side (Figs. 3 and 4).

## Discussion

In this study, incisor occlusion was altered in the rats by trimming the incisors on the left side (2 mm each) every second day during 7 days. This modification of the incisor occlusion produced significant modifications at the cellular level. These were significant increases in the mitotic activity as determined by BrdU, alterations in TGF- $\beta$ 1 expression in chondrocytes and in the immunoreactivity to two bone markers (ALP and OCN) as determinants of chondrocytes' maturity. However, after 1 week, no significant changes in the thickness of the mandibular condylar cartilages between groups were observed. A slight reduction in cartilage thickness

on the side opposite to the trimming was not significant but was noted.

Two variables could bias the results, anaesthetising and trimming. Anaesthetising the animals to perform the treatment inactivates the oral function for a period of approximately 4–5 h until animals are totally recovered. Any influence of this variable on the results is avoided by anaesthetising the control animals. Secondly, animals are subjected to a sham treatment, but the grinding procedure is not done. The vibratory action of the bur on the structures of the oral system might influence the cartilage physiology in some way. *In vitro*, mechanical vibration at certain frequencies may modulate biosynthetic response of articular chondrocytes.<sup>15</sup> However, this seems not to be a matter when the effects of vibration on cartilage are evaluated *in vivo*, as high frequencies and previous abnormalities are necessary to produce changes in the cartilage physiology due to transient impulsive forces.<sup>16,17</sup> Therefore, it is considered that the vibratory effect produced by the action of the bur on the oral structures during a short period and performed under high speed three times during the experimental time, does not influence the observed results. If there would be any transient impulsive forces present during the trimming at the temporomandibular joint, they would be of low frequency and are normally absorbed by the temporomandibular joint disc.<sup>18</sup>

What is the meaning of these alterations in the occlusion producing an increase not only in the mitotic activity but also in TGF- $\beta$ 1 expression in the mandibular condylar cartilage? Prior studies have shown that in the early stages of cartilage damage in osteoarthritis, cartilage cell proliferation increases dramatically as a repair response.<sup>19</sup> TGF- $\beta$ 1 stimulates the synthesis of proteoglycans in cartilage<sup>20</sup> and plays an important part in cartilage healing.<sup>21</sup> In control animals, TGF- $\beta$ 1 expression was restricted to the cells of mature and upper hypertrophic layers of the mandibular condylar cartilage. However, when the incisors were disoccluded, cells expressing TGF- $\beta$ 1 were increased with some positive cells being in the proliferative layer. Although the masticatory movements in the rat are bilateral,<sup>22</sup> different responses at the two mandibular condylar cartilages have been observed when the mandible is shifted laterally.<sup>23,24</sup> Regarding BrdU-labelling and TGF- $\beta$ 1 expression, unilateral incisor disocclusion however seems to affect both condyles in a similar way. Therefore, increases in mitotic activity and in TGF- $\beta$ 1 suggest that the mandibular condylar cartilages on both sides are adapting to the new situation at the occlusal level.

Nevertheless, differences were found between the two sides with respect to ALP and OCN in the

experimental group. ALP as a bone marker is an early marker of differentiation of osteogenic lineage cells.<sup>25</sup> Cartilage cells *in vitro* increase ALP activity over the time and this increase correlates with the onset of mineralisation.<sup>10,11</sup> Expression of OCN in chondrocytes correlates with the advanced maturity stage. Its presence suggests that cartilage cells developed phenotypic markers associated with mineralising matrix.<sup>9,12</sup> Interestingly, the numbers of cartilage cells expressing ALP are significant higher on the side of incisor disocclusion than on the opposite side. This may be interpreted either more cells are differentiating from the pool of mesenchymal cells in the proliferative layer. Or, the cells from the mature layer are staying longer in the mature layer in the side of incisor disocclusion. When the expression of ALP is observed in the different cartilage layers it seems that higher numbers of cells are differentiating and maturing from the proliferative layer. However, the numbers of cells positive for OCN are significant higher on the opposite side to incisor disocclusion. As OCN is an indicator of late chondrocytes maturity, it is likely that the cartilage cells are maturing slower on the side of incisor disocclusion and faster on the opposite side. In this context, unilateral incisor disocclusion in the rat affects the mandibular condylar cartilages on both sides by increasing mitotic activity and accelerating chondrocytes maturation. However, chondrocytes maturation appears more accelerated on the side opposite to the incisor disocclusion.

Compressive loading on the mandibular condyle has been shown to reduce the thickness of cartilage *in vitro*<sup>26</sup> and *in vivo*,<sup>27</sup> when loads are present for more than a week. Although, no significant difference in the cartilage thickness was found between groups after 7 days of incisor disocclusion, on the side opposite to the incisor disocclusion cartilage was often thinner (the cartilage thickness at the side of incisor disocclusion was similar to that in the control animals). A reduction in the cartilage thickness may result from a reduction in the number of mesenchymal cells proliferating and differentiating into chondroblasts, or, from a shortened time through the different stages of chondrocytes maturation and hypertrophy. The results from the present study showed that the numbers of BrdU-labelled cells were significantly higher at both sides, which implies a higher mitotic activity. In the same way, TGF- $\beta$ 1 was highly expressed on both sides meaning higher numbers of cells differentiating and maturing. This might imply that cartilage thickness would increase. However, increases in chondrocytes maturation accelerate the endochondral ossification process, which would bring the cartilage thickness to similar values to that in the animals

where incisor occlusion was not altered. Nevertheless, OCN was significantly higher at the side opposite to incisor disocclusion in the experimental animals. This means that chondrocytes maturation is even more accelerated in this side. Therefore, even though incisor disocclusion affects the condylar cartilage at both sides of the mandible at the cellular level, chondrocytes maturation is occurring faster in the cartilage at the side opposite to the incisor disocclusion. Thus, the slight but not significant reduction in cartilage thickness observed on the untrimmed side of the experimental group could be a signal of an initial change in thickness which might show up with a longer time interval, as found by Nakai et al.<sup>26</sup> and Endo et al.<sup>27</sup>

## Conclusions

In this study, the effect of unilateral incisor disocclusion on the mandibular condylar cartilage at both sides was investigated. It is demonstrated that after 7 days, unilateral incisor disocclusion affects the mandibular condylar cartilage, which initially responds at the cellular level by increasing the mitotic activity and accelerating chondrocytes' maturity. Chondrocytes maturation appears more accelerated on the side opposite to the incisor disocclusion.

## Acknowledgements

This study was supported by the Australian Dental Research Foundation (ADRF Grant no. 24/2001). We would like to thank Prof. Mark Bartold and Ms. Huika Li for their support in the immunohistochemistry technique.

## References

- Kantomaa T, Pirttiniemi P. Differences in biologic response of the mandibular condyle to forward traction or opening of the mandible: an experimental study in the rat. *Acta Odontol Scand* 1996;**54**:138–44.
- Yamada K, Kimmel DB. The effect of dietary consistency on bone mass and turnover in the growing rat mandible. *Arch Oral Biol* 1991;**36**:129–38.
- Kiliaridis S, Thilander B, Kjellberg H, Topouzelis N, Zafiriadis A. Effect of low masticatory function on condylar growth: a morphometric study in the rat. *Am J Orthod Dentofacial Orthop* 1999;**116**:121–5.
- Luder HU. Structure and growth activities of the mandibular condyle in monkeys (*Macaca fascicularis*). I. Intracondylar variations. *Am J Anat* 1983;**166**:223–35.
- Shibata S, Suzuki S, Tengan T, Ishii M, Kuroda T. A histological study of the developing condylar cartilage of the fetal mouse mandible using coronal sections. *Archs Oral Biol* 1996;**41**:47–54.
- Cohen MM. Transforming growth factor betas and fibroblast growth factors and their receptors: role in sutural biology and craniosynostosis. *J Bone Miner Res* 1997;**12**:322–31.
- Grimaud E, Heymann D, R dini F. Recent advances in TGF-  effects on chondrocyte metabolism: potential therapeutic roles of TGF-  in cartilage disorders. *Cyt Growth Factor Rev* 2002;**13**:241–57.
- Ben Ami Y, von der Mark K, Franzen A, De Bernard B, Lunazzi GC, Silbermann M. Immunohistochemical studies of the extracellular matrix in the condylar cartilage of the human fetal mandible: collagens and non-collagenous proteins. *Am J Anat* 1991;**190**:157–66.
- Lian JB, McKee MD, Todd AM, Gerstenfeld LC. Induction of bone-related proteins, osteocalcin and osteopontin, and their matrix ultrastructural localisation with development of chondrocyte hypertrophy in vitro. *J Cell Biochem* 1993;**52**:206–19.
- Inoue H, Nebgen D, Veis A. Changes in phenotypic gene expression in rat mandibular condylar cartilage cells during long-term culture. *J Bone Miner Res* 1995;**10**:1691–7.
- Kergosien N, Sautier JM, Forest N. Gene and protein expression during differentiation and matrix mineralisation in a chondrocyte cell culture system. *Calcif Tissue Int* 1998;**62**:114–21.
- Pullig O, Weseloh G, Ronneberger DL, Kakonen SM, Swoboda B. Chondrocyte differentiation in human osteoarthritis: expression of osteocalcin in normal and osteoarthritic cartilage and bone. *Calcif Tissue Int* 2000;**67**:230–40.
- Gao J, Symons AL, Bartold PM. Expression of transforming growth factor-beta1 in the developing periodontium of the rats. *J Dent Res* 1998;**77**:1708–16.
- Li H, Bartold PM, Young WG, Xiao Y, Waters MJ. Growth hormone induces bone morphogenetic proteins and bone-related proteins in the developing rat periodontium. *J Bone Min Res* 2001;**16**:1068–76.
- Liu J, Sekiya I, Asai K, Tada T, Kato T, Matsui N. Biosynthetic response of cultured articular chondrocytes to mechanical vibration. *Res Exp Med* 2001;**200**:183–93.
- Chu ML, Yazdani-Ardakani S, Gradisar IA, Askew MJ. An in vitro simulation study of impulsive force transmission along the lower skeletal extremity. *J Biomech* 1986;**19**:979–87.
- Gemme G, Saraste H. Bone and joint pathology in workers using hand-held vibrating tools: an overview. *Scand J Work Environ Health* 1987;**13**:290–300.
- Beek M, Aarnts MP, Koolstra JH, Feilzer AJ, van Eijden TM. Dynamic properties of the human temporomandibular joint disc. *J Dent Res* 2001;**80**:876–80.
- Dijkgraaf LC, de Bont LG, Boering G, Liem RS. The structure, biochemistry, and metabolism of osteoarthritic cartilage: a review of the literature. *J Oral Maxillofac Surg* 1995;**53**:1182–92.
- Blummenfeld I, Gaspar R, Laufer D, Livne E. Enhancement of toluidine blue staining by transforming growth factor-beta, insulin-like growth factor and growth hormone in the temporomandibular joint of aged mice. *Cells Tissues Organs* 2000;**167**:121–9.
- Wei X, Messner K. Age- and injury-dependent concentrations of transforming growth factor-beta 1 and proteoglycan fragments in rabbit knee joint fluid. *Osteoarthritis Cartilage* 1998;**6**:10–8.
- Weijjs WA. Mandibular movements of the albino rat during feeding. *J Morph* 1975;**145**:107–24.
- Fuentes MA, Opperman LA, Buschang P, Bellinger LL, Carlson DS, Hinton RJ. Lateral functional shift of the mandible. Part I. Effects on condylar cartilage thickness and proliferation. *Am J Orthod Dentofacial Orthop* 2003;**123**:153–9.

24. Fuentes MA, Opperman LA, Buschang P, Bellinger LL, Carlson DS, Hinton RJ. Lateral functional shift of the mandible. Part II. Effects on gene expression in condylar cartilage. *Am J Orthod Dentofacial Orthop* 2003;**123**:160–6.
25. Zernik J, Twarog K, Upholt WB. Regulation of alkaline phosphatase and alpha 2(I) procollagen synthesis during early intramembranous bone formation in the rat mandible. *Differentiation* 1990;**44**:207–15.
26. Nakai H, Niimi A, Ueda M. The influence of compressive loading on growth of cartilage of the mandibular condyle in vitro. *Arch Oral Biol* 1998;**43**:505–15.
27. Endo Y, Mizutani H, Yasue K, Senga K, Ueda M. Influence of food consistency and dental extractions on the rat mandibular condyle: a morphological, histological and immunohistochemical study. *J Craniomaxillofac Surg* 1998;**26**:185–90.