 MCB, vol.8, no.1, pp.43-60, 2011

Effect of Mechanical Pressure on the Thickness and Collagen Synthesis of Mandibular Cartilage and the Contributions of G Proteins

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Abstract: To investigate the role of mechanical pressure on cartilage thickness and type II collagen synthesis, and the role of G protein in that process, in vitro organ culture of mandibular cartilage was adopted in this study. A hydraulic pressure-controlled cellular strain unit was used to apply hydrostatic pressurization to explant cultures. The explants were compressed by different pressure values (0 kPa, 100 kPa, and 300 kPa) after pretreatment with or without a selective and direct antagonist (NF023) for the G proteins. After 4, 8 and 12 h of cell culture under each pressure condition, histological sections of the explants were stained with hematoxylin-eosin to investigate the thickness of the cartilage. Immunohistochemical staining was used to observe type II collagen expressions. The results showed that a hydrostatic pressure of 100 kPa significantly reduced the thickness of the proliferative layer in condylar cartilage without affecting the thickness of the transitional layer. Hydrostatic pressures of 100 kPa and 300 kPa significantly enhanced the synthesis of type II collagen. G proteins are involved not only in the proliferation and differentiation of condylar cartilage regulated by prolonged pressure, but also in the process of collagen production in condylar cartilage stimulated by pressure.

Keywords: Mechanotransduction; Mandibular condylar cartilages; Thickness; Collagen; G protein

The temporomandibular joint (TMJ) is alone among all human joints in maintaining the ability of lifelong reconstruction (1, 2). Biomechanical factors resulting from the functional activity of the mandibular joint are thought to influence the

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growth of the condyle, especially of pressure-sensitive articular cartilage (3). The chondrocytes can receive mechanical, ionic and osmotic signals and integrate those with the effects of other stimulation factors such as hormones, genetic modulators and environmental signals to regulate its metabolism, which is indispensable for the TMJ to maintain its normal structural relationship and regular function (4,5). For many years, biomechanical studies of TMJ stress and biochemical studies of TMJ reconstruction have been the focus of attention in the TMJ field. More and more studies have shown that it is the load on the TMJ that results in remodeling (6-15). The condyle, and especially the covering made of pressure-sensitive articular cartilage, undertakes the most important remodeling work. However, the method by which it translates mechanical signals into essential biological signals has not been fully elucidated. G proteins present a diverse and versatile group of signaling molecules that sense and initiate the intracellular signal cascade for mechanotransduction and play a primary role in the mechanotransduction of strain in many cells (16). Activation of G proteins has been shown to activate the inositol phosphate pathway as well as store-operated calcium influx (17).We have confirmed the participation of G proteins in mechanoreception by mandibular condylar chondrocytes (MCCs) under pressure (18), but the precise role of G protein in the proliferation and secretion ability of the mandibular cartilage under pressure remains unknown.

By employing cell culture, we have found that applying mechanical pressure causes ultrastructural changes, upregulation of aggrecan, increased nitric oxide secretion and decreased PGF1a composition in MCCs (11). However, we do not think that the results of monolayer cell culture can completely represent the biomechanical reactions seen in vivo. As we know, mandibular cartilage belongs to the category of secondary cartilage, which incorporates several kinds of chondrocytes such as prechondrocytes, functional chondrocytes, hypertrophic chondrocytes and further calcified chondrocytes. These are neatly arranged from the surface to the center of the condyle in accordance with their maturity (19, 20). Therefore, it is easy to see that although we speak of using a single cell type for in vitro cultured chondrocytes, the sample will inevitably contain a mixture of several different subpopulations, which have different proliferation and secretion abilities and may also have different sensitivities to mechanical stimulation. Additionally, a monolayer culture of chondrocytes excludes the crosstalk between chondrocytes at different maturation stages and the interactions between the chondrocytes and the extracellular matrix, which could be very important for mechanotransduction (21). Therefore, it is necessary to verify our results with cellular investigations at the organ level.

Thus, the present study was performed to investigate the influence of mechanical pressure on different layers of cartilage, which reflect the dynamic changes of chondrocytes due to differentiation and maturation, and on type II collagen synthesis.

Furthermore, we explore the role of G protein in the biomechanical regulation of cartilage growth and function.

1 Materials and methods

1.1 Organ culture of mandibular condylar cartilage

One-week-old Sprague-Dawley (SD) rats were anesthetized with ether, and bilateral mandibular condylar cartilage explants were obtained and standardized to 2.0 mm in length using a stereomicroscope. Cartilage explants were cultured according to the method of Copray et al (22,23). Immediately after explantation, the cartilage samples were collected in a Petri dish containing culture medium at 37˚C. Once four condyles had been collected, each was transferred to a Trowell-type organ culture dish (Falcon 3037, B-D Cockeysville, MD, U.S.A.) and placed on a stainless-steel grid at the gas-medium interface. Each culture dish contained 1.0 ml of culture medium that consisted of serum-free Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY) supplemented with penicillin/streptomycin (100 μg) and ascorbic acid (150 μg). These explant blocks were then used for subsequent histological and biochemical experiments.

1.2 Mechanical pressurization of the organ culture

To apply pressures to cultured MCCs, a hydraulic pressure-controlled cellular strain unit similar to the model developed by Yousefian et al. (24) was used. Hydrostatic pressurization is a frequently used modality to compress cells, tissues and explanted cultures. The technique includes both negative (vacuum) and positive pressurization. Hydrostatic compression is attractive for several substantial reasons: the simplicity of the equipment, the spatial homogeneity of the stimulus, the ease of using a multiple loading configuration (via manifolding) and the ease of delivering and transducing either static or transient loading inputs. In addition, there is no physical impediment to metabolite transport processes between the culture layer and the nutrient medium. During the experiment, the culture dishes were placed into the sample compartment of a closed culture chamber placed in a 37˚C incubator, and pressure was generated by continuously compressing the gas (2% CO_2in air) in the chamber (at 98% humidity). We could exert accurate, adjustable and consistent hydraulic pressure on in vitro cultured organs, which has proven effective in modulating the ultrastructure and secretion capability of in vitro cultured mandibular condylar chondrocytes (MCCs) (9, 11 and 18).

1.3 Experimental groups

A total of 72 cartilage explants from 36 1-week-old SD rats (Animal Centre of the Fourth Military Medical University , Xi'an, China) were divided into two groups: one group was cultured with serum-free DMEM, whereas the other was treated for 4 h with 10 μM 8,8′-(Carbonylbis(imino-3,1- phenylene)) bis-(1,3,5- naphthalenetrisulfonic acid), abbreviated NF023, which is known to be a selective and direct antagonist for the alpha subunits of the inhibitory G proteins Gi and Go (Calbiochem, San Diego, CA). The explants were further grouped by exposure to three different pressure conditions: 0 kPa, 100 kPa and 300 kPa. After 4, 8 and 12 h of culture under each of the pressure conditions, 4 explants from each pressure and culture condition were fixed and sectioned for further examination.

1.4 Histology and analytical procedures for cartilage depth and thickness

At the end of the culture period, the cartilage explants were fixed in a solution consisting of 0.4% aminoacridin in 4% formaldehyde/70% ethanol. The explants were embedded in paraffin wax and serially sectioned into 5-μm slices in the sagittal plane. The sections were stained with hematoxylin-eosin to investigate the depth and thickness of different layers of the cartilage. Histologically stained sections were examined under a light microscope (Leica DM 2500, Wetzlar, Germany). Image acquisition was performed with a Leica DFC490 system (Leica, Wetzlar, Germany). Morphometric measurements on histological sections were made with electronic measuring scale software developed by Dr. Jun Cao of the School of Stomatology of the Fourth Military Medical University; the measurements were performed along three standard axes in the mid-sagittal portion of the condylar cartilage. The mandibular condylar cartilage was typically organized into fibrous, proliferative, transitional and hypertrophic layers. The thicknesses of the proliferative layer, which is mainly made of prechondroblasts (the only cellular component with potential proliferation ability), and the transitional layer, which mainly consists of differentiating chondroblasts, were measured along each of three axes (Figure 1), and the average of those values was recorded as the final depth of each layer on one section. When a boundary between the adjacent layers was unclear, the middle of the transition region was taken as the boundary.

1.5 Immunohistochemical staining of type II collagen and image analysis

To stain for collagen II, sections were incubated in 3% H_2O_2 for 15 min at room temperature followed by antigen retrieval with 1 mg/ml protease E (Sigma-Aldrich) for 10 min. Sections were then blocked with 5% goat serum. Sections were incubated with anti-collagen II primary antibody (1:100 dilution, Sigma Chemical

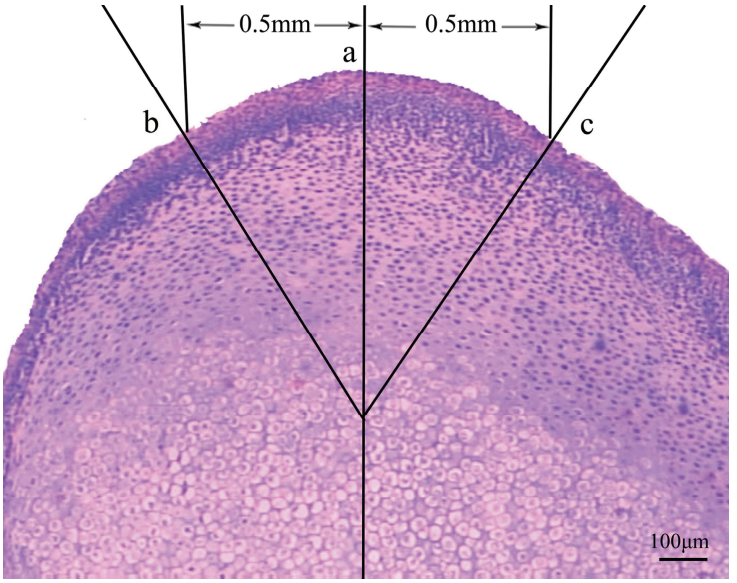

Figure 1: On the sagittal central section of the explanted mandibular cartilage, a central line (a) was drawn through the most protruding point of the condyle surface. Bilaterally, 0.5 mm apart from the midline, lines (b) and (c) were determined normal to the cartilage surface. The mean depths of the proliferative layer and transitional layer of the mandibular cartilage were calculated by averaging the three values from the three axes of (a), (b) and (c).

Co., St Louis, MO) overnight at 4˚C followed by a biotinylated secondary antibody (1:200 dilution, Sigma) for one hour at room temperature. The antibodies were stained for visualization using 3,3-diaminobenzidine tetrahydrochloride (DAB) (Sigma). Negative controls were processed using PBS in place of the primary antibody. Sections were incubated in normal goat serum for 4 h prior to staining to minimize non-specific antibody interactions. To quantify the reaction product as effectively as possible, the section was not counterstained. Five ISH slides were chosen randomly from each group for examination under the light microscope (Leica DM 2500, Wetzlar, Germany). Images were captured using a Pixera PVC100C digitalcamera (Pixera Corp., Los Gatos, CA) under 10x (low power) magnification. The mean gray value of each image was computed and analyzed by Image J v1.31 analysis software (National Institutes of Health, USA). The same image field was measured ten times at 60-min intervals.

1.6 Ultrastructure observation

The condyles of 100 kPa/1 h pressure group, 300 kPa/1 h pressure group and control were fixed with 4% glutaraldehyde and 1% osmic acid. The samples were embedded in Epon812, sectioned by LKB-V ultramicrotome (LKB, Bromma, Sweden), and stained with uranyl acetate and lead citrate. The ultrastructures of the muscles were then observed with transmission electron microscopy (JEM-100SX, JEOL Company, Japan).

1.7 Statistical analysis

Statistical analysis was performed using SPSS software version 11.0 (SPSS, Chicago, IL, USA). All data acquisition and analysis procedures were performed blind. Results are expressed as the mean $\pm$ standard deviation (SD). Statistical comparisons of the means were performed by one-way analysis of variance. When significant main effects were found, specific comparisons between subgroups were made with Bonferroni's modification of Student's t-test as a post hoc test. Resulting p-values of less than 0.05 were considered significant.

2 Results

2.1 The morphology of condylar cartilage layers in the control group

As shown in Figure 2, the condylar cartilages from one-week-old newborn rats had a smooth surface with distinct layers, which are the fibrous layer, proliferative layer, transitional layer, hypertrophic layer and calcified layer in order from the top to the bottom of the cartilage. The fibrous layer consisted of parallel arrays of collagen and 4-5 rows of cells. The proliferative layer had 5-8 rows of small round cells in a dense arrangement and had a greater thickness in the center than at the two ends of the sagittal section. The transitional layer was significantly thicker than the proliferative layer and had a nearly crescent shape. Its cellular density was lower than that of the proliferative layer. The cells in the transitional layer were round or oval in shape and their longitudinal axes were aligned parallel to the surface of the cartilage. Abundant extracellular matrix was observed surrounding these cells. The hypertrophic layer consisted of large round cells with deeply stained nuclei and clear cytoplasm. The number of cell rows was not uniform across the hypertrophic layer. The bottom of the hypertrophic layer was adjacent to the calcified cartilage layer, which is connected to the subchondral bone. No significant changes were observed in the thickness of the proliferative layer or the transitional layer during the 12 hours of culture ($p > 0.05$) for the control group.

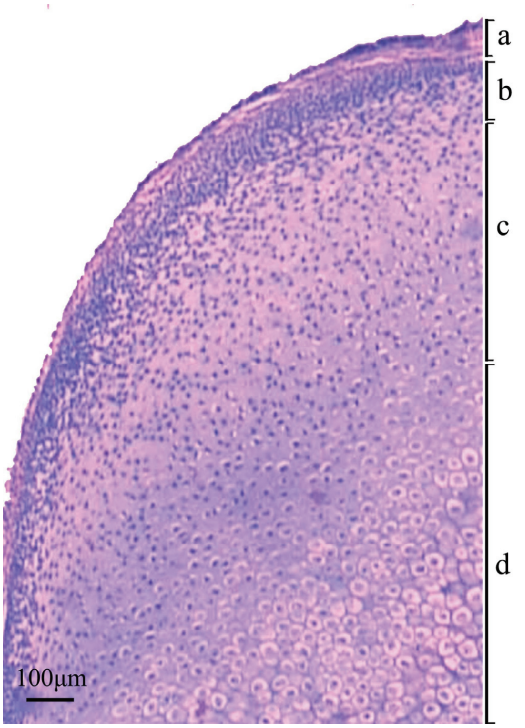

Figure 2: Sagittal central section of the in vitro cultured mandibular cartilage stained with hematoxylin and eosin. Four layers are indicated: a: fibrous layer, b: proliferative layer, c: transitional layer and d: hypertrophic layer.

2.2 The effect of mechanical stimulus on the thickness of condylar cartilage

No significant changes were detected in the thickness of the proliferative layer at different time points ($p > 0.05$) (Figure 3). After 8 and 12 hours of culture under a pressure of 100 kPa, the thickness of the proliferative layer of condylar cartilage was significantly reduced compared to the control group ($p < 0.05$ and $p < 0.01$, respectively). No significant changes in the thickness of the proliferative layer of condylar cartilage were observed at different time points between the control group and the group treated with a pressure of 300 kPa ($p > 0.05$). Eight hours after the addition of G protein antagonist, the thickness of the proliferative layer under a pressure of 100 kPa remained at a similar level to that of the groups without antagonist treatment ($p > 0.05$), but it was significantly lower than that of the control group ($p < 0.01$). However, after 12 h, the thickness of the proliferative layer was significantly higher than that of the group without antagonist treatment ($p < 0.01$)

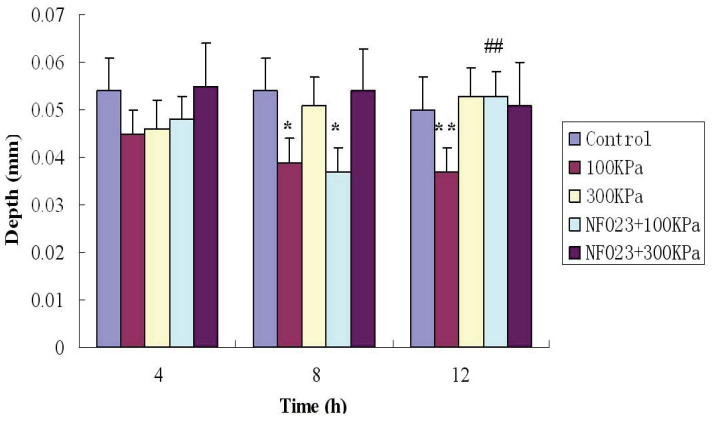

Figure 3: Effects of mechanical pressure on the thickness of the proliferative layer. Under a compression of 100 kPa for 8 or 12 hours, the thickness of the proliferative layer in the condylar cartilage decreased significantly when compared with the control group. After the addition of G protein antagonist, the thickness of the proliferative layer was still remarkably lower than the control group, but returned to the level of the control group at 12 h. Condylar cartilage treated with a compression of 300 kPa did not display significant changes in the thickness of the proliferative layer at different time points. Asterisks indicate a difference between the experimental groups and the control (*$p < 0.05$, **$p < 0.01$). "#" indicates a difference between the mechanical pressure groups pre-treated with or without G protein antagonist at the same pressure value (# $p < 0.05$, ## $p < 0.01$).

and returned to the level of the control group ($p > 0.05$).

The thickness of the transitional layer did not exhibit significant changes after 4, 8, or 12 h ($p > 0.05$, Figure 4), even in the presence of pressures of 100 kPa and 300 kPa ($p > 0.05$). The addition of G protein antagonists did not create significant differences between the treated groups and the control group at different time points ($p > 0.05$).

2.3 Type II collagen expression in the condylar cartilage of the control group

As shown in Figure 5 from a sagittal view, type II collagen was mainly expressed at the junction between the transitional layer and the hypertrophic layer. The expression in the proliferative layer and in most of the transitional layer was relatively weak. In the control group, the positive staining, which indicates the presence of type II collagen, was primarily located in the cytoplasm with less staining in the extracellular matrix.

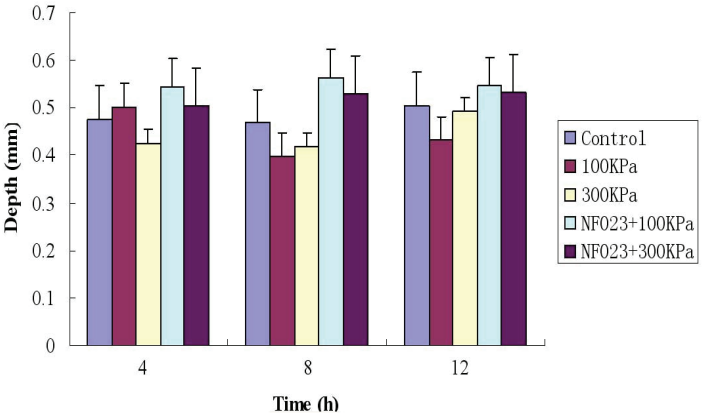

Figure 4: Effects of mechanical pressure on the thickness of the transitional layer. Compressions of 100 kPa and 300 kPa did not cause significant changes in the thickness of the transitional layer. Following the addition of G protein antagonist, the treatment group exhibited no significant differences at different time points when compared with the control group.

2.4 The effects of mechanical stimulus on the synthesis of type II collagen in condylar cartilage

As shown in Figure 6, type II collagen expression was significantly higher at 4, 8, and 12 h after compression at 100 kPa and 300 kPa, compared to the control group ($p < 0.05$). No significant difference was observed at different time points between the two groups treated with compression ($p > 0.05$). At 4 and 8 h after the addition of G protein antagonists, the level of type II collagen expression was significantly lower than in the group without G protein antagonists, but it was still higher than that of the control group at different time points ($p < 0.05$). When the duration of action of the G protein antagonists was extended to 12 hours, the expression level of type II collagen reduced significantly when compared to the groups without antagonist treatment ($p < 0.01$) and recovered to a level similar to the control group ($p > 0.05$).

2.5 The effects of mechanical pressure on the ultrastructure of condylar cartilage

As shown in Figure 7, when cartilages were pressed at 100 kPa for 12 h, the chondrocytes showed better proliferative and metabolic condition compared to control group. Euchromatin level increased, the nucleolus located in periphery of karyon,

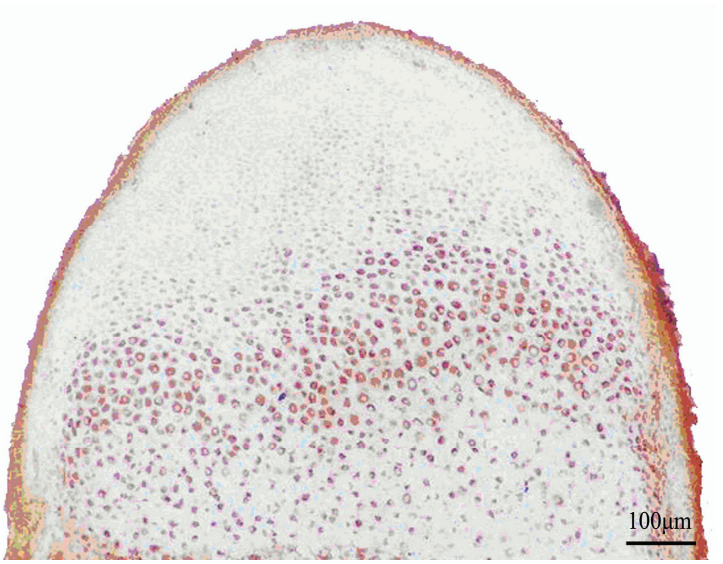

Figure 5: Expression of collagen II in the mandibular cartilage explant. Type II collagen expression was mainly detected at the junction between the transitional layer and the hypertrophic layer. The expression in the proliferative layer and most of the transitional layer was relatively weak. The positive staining was primarily located in the cytoplasm with less staining in the extracellular matrix.

rough endoplasmic reticulum dilated as marked by black arrow in Panel b. The homologous cell group can be seen in certain area. When pressed under 300 kPa for 12 h, the chondrocytes showed similar condition as the 100 kPa group. The most obvious change was about pericellular matrix circle (as marked by black arrow in Panel 6), which was composed of abundant amount of proteoglycan and mesh arrangement of collagen fibers.

3 Discussion

3.1 Normal structures of condylar cartilage

Mandibular condylar cartilage is a secondary cartilage that grows strictly in an appositional mode: mesenchymal progenitor cells differentiate into functional chondroblasts, which subsequently mature to hypertrophic chondroblasts. The layered distribution of a variety of cells in condylar cartilage reflects the maturation process of chondrocytes (19,20). The cells in the proliferative layer are mainly undifferentiated cells with a similar morphology to mesenchymal cells. They are small round

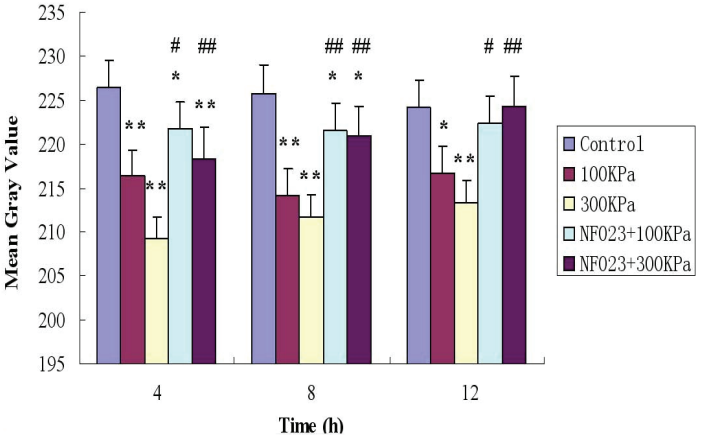

Figure 6: Effects of mechanical pressure on the collagen II expression of *in vitro* cultured mandibular cartilage. Hydrostatic pressures of 100 kPa and 300 kPa markedly stimulated the expression of type II collagen in condylar cartilage. At 4 h and 8 h after the addition of G protein antagonists, the level of type II collagen expression was significantly lower than that of the group without G protein antagonists, but it was still higher than that of the control group at different time points. When the duration of action of the G protein antagonists was extended to 12 hours, the expression level of type II collagen recovered to a level comparable to that in the control group. Asterisks indicate a difference between the experimental groups and the control (*$p < 0.05$, **$p < 0.01$). "#" indicates a difference between the mechanical pressure groups pre-treated with or without G protein antagonist at the same pressure value (# $p < 0.05$, ## $p < 0.01$).

cells in a compact and dense arrangement. Previous studies have shown that this layer is the only region in cartilage that possesses the capabilities of division, proliferation and differentiation (22, 23). The cells in this layer cannot only produce additional undifferentiated cells through proliferative division, but are also able to differentiate into chondroblasts and chondrocytes in the deep layers of cartilage. The number of cells in this layer can change with the location of the cartilage, the age, and other factors. The transitional layer, also known as the chondrogenic layer, is beneath the proliferative layer and is mainly composed of chondroblasts. Chondroblasts are larger than the cells in the proliferative layer and have an oval shape. Their longitudinal axes are parallel to the surface of the cartilage, and the cellular density is relatively lower than other layers. Chondroblasts can further differentiate into chondrocytes in deep cartilage layers but have no proliferative capability. The hypertrophic layer and the calcified cartilage layer are under the

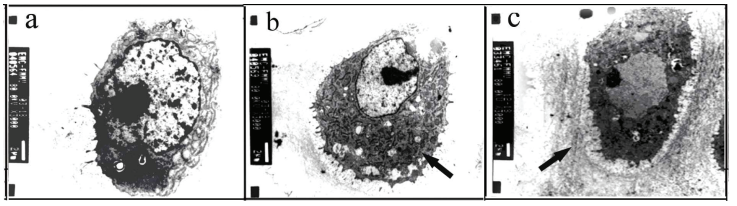

Figure 7: Ultrastructure of mandibular cartilage under TEM (N = 6). (a) Cells of the control developed well with abundant viscid cytoplasm and big nucleus, without any sign of apoptosis. (b) After been pressed under 100 kPa for 12 h, the cells showed increased euchromatin, clearer nucleolus, increased microvilli and distinctive dilated rough endoplasmic reticulum (black arrow indicated). (c) Under the pressure of 300 kPa for 12 h, the cells showed not only the same changes as 100 kPa group, but also more distinct pericellular zone around the cells (black arrow indicated) (magnification, 3000×; bar = 2 μm).

transitional layer and are mainly composed of mature and calcified chondrocytes. Neither of these types is capable of proliferation or differentiation.

3.2 Simulation of compressive Stimulation

The pressurization device used in this study exerted relatively accurate and controllable static pressure on the cartilage by changing the air pressure. During cartilage compression, the pressure applied to the cartilage was similar to the hydrostatic pressure on deep tissue. Cartilage tissue did not deform under these conditions, which avoids the potential interference caused by cartilage deformation and focuses only on the observation of the effect of pure pressure on cartilage. As to the pressure value, it is not possible for us to know the real pressure value that the condylar cartilage endured in vivo, so the values in the in vitro simulation experiment varied by a great deal to account for the measurements of different mechanical instruments and different researchers. However, the intra-articular pressure is always used as the practical standard. In the TMJ, the range of intra-articular pressure with normal occlusion in the intercuspal position (ICP) ranges from 0.5 to 1.5 kPa. Because the intra-articular cavity of the TMJ is very small and the relative functional parts (the fossa, disc and condyle) are in close contact with each other, the real pressure that the cartilage endures should be much higher than the intra-articular pressure. The results of our finite element analysis showed that when a normal occlusion was in the ICP, the compressive stress in the condylar cartilage was about 0.3 MPa (25). From our previously reported cell experiments, the form and function of the cells could be greatly affected when placed under too great of a pressure (11). Therefore,

we set the pressure values at 300 kPa and a relatively lower level of 100 kPa.

Like many other synovial joints, the TMJ enables large relative movements. These motions, sometimes combined together, result in static and dynamic loading in the TMJ. Static loading occurs, for example, during clenching, grinding, and bruxism; dynamic loading occurs during, for example, talking and chewing. Basically, three types of loading can be distinguished: compression, tension, and shear. During natural loading of the joint, combinations of these basic types occur on the articulating surfaces. In articular cartilage, collagen forms a three-dimensional network and thus impacts its form, stability and tensile strength and resistance to outside stress and strain (26). When cartilage is loaded by compression, the low permeability of the collagen network impedes the interstitial fluid to flow through the collagen network (27). Besides, during joint loading the cartilage layers are also sheared to adapt their shape to the incongruent articular surfaces. Excessive shear can cause a fatigue, which irreversibly may lead to damage of cartilage. Previous study demonstrated that the shear behavior of the mandibular condylar artilage was dependent on the frequency and amplitude of the applied shear strain (28). Taking into account of the complexity of the shear force and possible cross-effect with the pressure, in the present, we just applied pure pressure onto the in-vitro cultured cartilage. Further, the exact effects of shear force or even a reasonable form of composite force with both pressure and shear force still need further studies.

3.3 Compressive stimulation and cartilage remodeling

Compressive signals resulting from articular functioning regulate proliferation, differentiation and synthesis of extracellular matrix components during development of the condyle (19, 20 and 29). The results of the present study showed that the thickness of the proliferative layer did not change significantly during 12 hours under a pressure of 300 kPa. However, a static pressure of 100 kPa for 8 hours or more significantly decreased the thickness of the proliferative layer in condylar cartilage to a value lower than that of the control group or the group treated with a pressure of 300 kPa. It suggested that although the pressure has the proliferation promoting potential just as the TEM observation supported, the relatively lower pressure may provide the cells with much stronger capability of differentiation into a more mature stage, which was supported by the TEM observation of increased microvilli and distinctive dilated rough endoplasmic reticulum compared to that of the control. Further, ultrastructure of the cartilage under a relatively higher pressure showed a distinct sign of clear pericellular zone, that is, chondron formed, which was proved stiffer and having higher deformation at rupture and higher rupture forces (30). It indicated the cells being in a much more stable niche, which could be one of the reasons for the little change in the thickness of the proliferative

layer under the higher pressure. The formation of obvious chondron was also consistent with the immunohistological results that expression of collagen II under the relatively higher pressure showed the highest. The results also showed that static pressures of 100 kPa and 300 kPa did not affect the thickness of the transitional layer significantly. This may be due to the different mechanical environment experienced by the chondrocytes in different cartilage layers under the same pressure conditions, which can cause a variety of physical and chemical changes in cartilage, including morphological changes of chondrocytes and matrix (31) and changes in the static pressure gradient inside cartilage tissue can affect fluid flow, streaming potential, current (32), the water capacity of the matrix, fixed charged density, mobile ion concentration and osmotic pressure (33,34). However, these changes due to the pressure are not uniform across the different parts of different cartilage layers at different time points; it is a dynamic process. In addition, different chondrocytes exhibit different reactivity to compressive stimulation. Undifferentiated cells are more sensitive to compressive stimulation because they are actively proliferating and differentiating, whereas chondroblasts exhibit lower mechanical sensitivity than undifferentiated cells because of their inability to proliferate. This result is consistent with the results from the study conducted by Wang et al. on stem cells (35).

3.4 Compressive stimulation and collagen synthesis

Cartilage can maintain its intact structure within the complex mechanical environment of the body. Achieving this depends on the perception of external mechanical stimulation by the chondrocytes, which can cause cartilage to adjust its anabolic metabolism and change the composition of the extracellular matrix. Type II collagen synthesis is regulated by a variety of compressive stimulations as well. Previous studies demonstrated that the synthesis of type II collagen is closely related to the type, intensity, frequency, duration and other parameters of the pressure (36-38). Excessive static pressure can promote collagen expression over a short time but eventually suppress collagen expression after a longer duration (39). In the present study, the effect of sustained hydrostatic pressure on the collagen expression in different cartilage layers was observed at different time points. The results showed that static pressures of 100 kPa and 300 kPa significantly enhanced the synthesis of type II collagen after 4 hours. In addition, it was revealed that the ability of chondrocytes to synthesize collagen depends on the pressure intensity. Under higher static pressure, chondrocytes displayed a stronger collagen synthesis capability, which is consistent with previously published reports. These changes may be caused by the effects of pressure on the activity of chondrocytes. Previous studies have shown that compressive stimulation of certain intensity can promote

the transition of chondrocytes to the active phase (40). Cells in the active phase have strong synthesis activity, which enhances the synthesis of collagen. However, collagen synthesis is a complex process. The specific mechanisms underlying its regulation by pressure are still unclear.

3.5 Effects of G proteins on the regulation of condylar cartilage proliferation and collagen synthesis by mechanical stimulation

G proteins present a diverse and versatile group of signaling molecules that sense and initiate the intracellular signal cascade for mechanotransduction and play a primary role in the mechanotransduction of strain in many cells (41). Our results indicate that G protein is involved in the regulation of chondrocyte proliferation and collagen synthesis by hydrostatic pressure. A protein antagonist for Gi/Go completely reversed the thickness reduction of the proliferative layer caused by a 12 hours at a pressure of 100 kPa and returned it to the control level. Gi/Go protein antagonists can also partially block the stimulative effects of 100 kPa and 300 kPa static pressure on type II collagen expression at 4 h and 8 h. The antagonist also completely reversed the effects of 12 hours of 100 kPa and 300 kPa static pressure on type II collagen expression and returned the collagen expression level to the control level. These results indicate that G proteins are involved in regulating the proliferation and differentiation of condylar cartilage under pressure. Furthermore, the stimulatory effect of pressure on cartilage collagen synthesis after 8 hours is partially G protein-dependent (other signaling molecules in addition to G protein are involved in the regulatory process). However, with the duration of the pressure extended to 12 hours, the regulatory effect of pressure on collagen synthesis in condylar cartilage exhibits a complete G protein-dependency. We note that the antagonist used in this experiment is not specific for a single G protein; therefore, conclusions cannot be drawn on which G protein contributed to this effect. In addition, Ozaki and others show that G protein activation is related to the threshold of pure pressure (42). For the two static pressure values used this experiment, it has yet to be studied whether Gi/Go protein exists in two different activation states.

4 Conclusions

A hydrostatic pressure of 100 kPa significantly reduced the thickness of the proliferative layer in condylar cartilage without affecting the thickness of its transitional layer. Hydrostatic pressures of 100 kPa and 300 kPa significantly enhanced the synthesis of type II collagen. G proteins are involved not only in the proliferation and differentiation of condylar cartilage regulated by long pressure duration but also in the process of collagen production in condylar cartilage stimulated by pressure.

Acknowledgement: This work was sponsored by the Nature Science Foundation of China (Nos. 30000035 and 30670518). We thank the Department of Oral Anatomy and Physiology, School of Stomatology, Fourth Military Medical University, for technical support.

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