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The effects of static pressure on chondrogenic and osteogenic differentiation in condylar chondrocytes from temporomandibular joint

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ABSTRACT

Objective: The goal of the study was to investigate the production of collagen, type II, alpha 1 (COL2A1), SOX9, alkaline phosphatase (ALP), runt-related transcription factor 2 (Runx2), Indian hedgehog (Ihh), and periarticular cell-derived parathyroid hormone-related protein (PTHrP) in mandibular condylar chondrocytes under static pressure stimuli.

Methods: Mandibular condylar chondrocytes separated from rabbit temporomandibular joint (TMJ) were treated with a static pressure of 100 kPa for 0, 1, 2, 3, and 4 h by an in-house-designed pressure chamber. A CCK-8 kit was used to analyze the cell viability. The production of COL2A1, SOX9, ALP, Runx2, Ihh, and PTHrP was detected by Western blot or real-time polymerase chain reaction (PCR). Changes in cell morphology were observed by scanning electron microscopy.

Results: Compared with the control group (0 h), the cytoplasmic processes of treated chondrocytes obviously increased and elongated, and the cell viability of pressurized chondrocytes were 91.13% (1 h), 103.41% (2 h), 103.47% (3 h), and 104.94% (4 h), respectively. The exposure of condylar chondrocytes to a static pressure of 100 kPa for 3–4 h resulted in a significant increase in COL2A1, SOX9, ALP, and Runx2. After a static pressure loading of 100 kPa, the activation of Ihh and PTHrP was also observed.

Conclusions: Mandibular condylar chondrocytes adapt to alterations of the microenvironment. Ihh and PTHrP are sensitive to static pressure. Our findings suggest that static pressure accelerated the chondrogenic and osteogenic differentiation of condylar chondrocytes, which may influence the pathological progress of temporomandibular diseases.

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1. Introduction

Temporomandibular disorder (TMD) is a common disease in the oral and maxillofacial region. Orofacial pain, joint sounds, and abnormal mandibular movement are common clinical manifestations of TMD.[1,2] The aetiology and pathological mechanisms of TMD are still unclear. Usually, the condyle bears the pressure from jaw movements, and the disc plays an important role in buffering and dispersing pressure. When the disc loses its normal function, the physical microenvironment of the temporomandibular joint (TMJ) will be destroyed and the intra-articular pressure will abnormally elevate.[3,4] Condylar degenerative changes resulting from articular disc displacement (ADD) is possibly associated with the change in pressure on the mandibular condyle. Pressure is an essential physiological factor in maintaining the stability of the joint.[5] It has been found that hydrostatic pressure induced the alteration of the cytoskeleton and production of bone morphogenetic-related proteins of synovial fibroblasts, which may influence the pathological condition of TMD.[6] Appropriate intra-articular pressure contributes to maintaining the health of the articular cartilage, whereas abnormal pressure may cause irreparable damage of the articular cartilage.[7,8] Previous studies have mainly focused on changes in the productions of synovial fibroblasts or bone marrow mesenchymal stem cells (BMSCs) under static pressure.[9,10] Zhang et al.[11] discovered that mechanical pressure could affect intracellular calcium release and cytoskeletal structure in rabbit condylar chondrocytes. However, how the mandibular condylar cartilage responds to pressure loading is still poorly understood.

SOX9 is a primary and essential transcription factor of chondrocyte differentiation from the early stages onward, which plays an important role in the process of chondrogenesis.[12,13] As a downstream target protein of SOX9, collagen, type II, alpha 1 (COL2A1) is an important component of the cartilage matrix. The main function of COL2A1 is to resist stress.[14] Therefore, the expression of COL2A1 and SOX9 are chosen as chondrogenic differentiation indexes in this study. Runt-related transcription factor 2 (Runx2) is an important transcription factor necessary for osteoblast differentiation and bone formation.[15,16] Alkaline phosphatase (ALP) is mainly expressed in osteoblasts and hypertrophic chondrocytes.[17] They are commonly used as markers for osteogenic differentiation. Previous studies have demonstrated that Indian hedgehog (Ihh) is the most active reactive factor to mechanical loading. It regulates chondrocyte proliferation and rates of chondrocyte maturation in cooperation with periarticular cell-derived parathyroid hormone-related protein (PTHrP). The Ihh–PTHrP feedback pathway plays an important role in regulating the process of endochondral ossification in the mandibular condylar cartilage.[18–21]

In the present study, the expression of COL2A1, SOX9, Runx2, ALP, Ihh, and PTHrP production under the static pressure of 100 kPa was detected, so as to explore the effects of static pressure on chondrogenic and osteogenic differentiation and the production of Ihh and PTHrP in mandibular condylar chondrocytes.

2. Materials and methods

2.1. Cell isolation and culture

First, cartilage tissues from the condyles were harvested aseptically from five 4-week-old New Zealand white rabbits. The tissues were washed thrice with phosphate-buffered saline (PBS), minced meticulously, and digested with 0.25% trypsin (Gibco, New York, USA) for 30 min, and subsequently digested with 0.1% collagenase (Serva, Heidelberg, Germany) in growth medium (Hyclone, Logan, UT, USA) supplemented with 10% foetal bovine serum (FBS) (Sijiqing, Hangzhou, China), 100 μg/mL penicillin, and 100 μg/mL streptomycin (Sigma, St.Louis, USA). Following incubation at 37 °C in a humidified atmosphere of 5% CO_2, the chondrocytes were collected at intervals of 4 and 12 h by centrifugation, respectively. They were washed once with sterilized PBS. Finally, the condylar chondrocytes were resuspended and replated, respectively, in culture six-well plate with a suitable concentration of 2×10^4 cells/mL. For the duration of the chondrocyte culture, the medium was changed every 3 days. In this study, P2 of condylar chondrocytes was used in the following experiments.

2.2. Immunocytochemical staining of COL2A1 and SOX9

Slides of P2 of condylar chondrocytes were washed three times with PBS and subsequently fixed with 4% paraformaldehyde for 15 min at room temperature. The primary antibody for immunodetection was polyclonal rabbit anti-COL2A1 (1:100) (BioWorld, USA) and anti-SOX9 (1:150) (Abcam, Cambridge, UK). Bound primary antibodies were detected with goat anti-rabbit immunoglobulin conjugated with horseradish peroxidase (ICH, USA) and visualized by diaminobenzidine (DAB) (Dako, Glostrup, Denmark). The nucleus was stained with haematoxylin for 3 min.

2.3. Application of static pressure

Static pressure was applied to the monolayer of P2 condylar chondrocytes by an in-house-designed computer-controlled pressure chamber (Fig. 1). This system was based on Smith's design description[22] and manufactured by The Fourth Military Medical University. It can apply from −50 to 250 kPa of static pressure or from 0.1 to 5 Hz of dynamic pressure to treated cells at a constant temperature. The control centre automatically regulated the infusing and outgoing premixed gas through normal pressure amortization and negative pressure amortization to maintain an accurate pressure in the heat-sealed chamber.

P2 of condylar chondrocytes was seeded in the culture six-well plate at a concentration of 2×10^4 cells/mL, 2 mL per well. After 3 days of culture, the chondrocytes reached 70–80% confluence, and the chondrocytes were transferred to the pressurizing vessel. In addition, the growth medium was changed 12 h before the treatment. Static pressure was applied at 100 kPa for 0, 1, 2, 3, and 4 h at 37 °C. Chondrocytes treated for 0 h were chosen as non-pressurized controls. All specimens were harvested after pressure loading immediately.