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Effects of mechanical vibration on DNA and proteoglycan syntheses in cultured articular chondrocytes

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Abstract The objective of this study was to determine the effects of mechanical vibration loading on DNA and proteoglycan syntheses in cultured rabbit articular chondrocytes. Chondrocyte culture plates were placed in a vibratory apparatus and subjected to a mechanical vibratory load at various frequencies and periods during culture. Mechanical vibration was applied at a sinusoidal waveform of 1.4 *G*-acceleration with frequencies of 200, 300, 400, 800, and 1600 Hz. ^{3}H-thymidine and $H_2{}^{35}SO_4$ incorporation were used to detect radiolabeled DNA and proteoglycan syntheses, respectively. A frequency of 300 Hz showed a time-dependent augmentation of DNA synthesis and gave a maximal increase on day 3 with periodic vibration (8 h per day), and at 72 h or longer with continuous vibration. It also promoted proteoglycan synthesis in long-term culture (from 3 to 15 days) by periodic vibration. However, frequencies above 400 Hz suppressed biosynthesis. These results suggest that mechanical vibration at certain frequencies may modulate the biosynthetic response of articular chondrocytes.

Key words Articular chondrocyte · DNA synthesis · Frequency · Mechanical vibration · Proteoglycan synthesis

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Introduction

Unlike other connective tissues, adult cartilage has no blood vessels and nerves of its own. Accordingly, ordinary wound healing and regeneration in cartilage are notoriously difficult, and involve neovascularization and innervation. It is well documented that constant external mechanical loads are necessary for normal motility and function in joints where cartilage entirely covers the surfaces of bone. When these mechanical conditions cease to exist, the cartilage often degrades. In fact, joints in animal models of osteoarthritis in which surgery is used to change joint motion abruptly initially show adaptation, but subsequently exhibit cartilage deterioration and malfunction.[1] Thus, mechanical loading is an important regulator of chondrocyte metabolism, and appears to be a prerequisite for maintaining normal cartilage matrix properties. Some in vitro studies have shown that a static mechanical load decreased proteoglycan and protein syntheses,[2–9] while a dynamic mechanical load (compression or stretch) stimulated the synthesis of these matrix constituents.[2,8,10–14] The mechanism by which extracellular mechanical signals are transformed into intracellular signals is not fully understood. However, cell surface receptors for matrix molecules are likely to be involved in these response processes.[10]

In vivo and in vitro studies have shown that some dynamic loads, such as mechanical vibration, have a beneficial effect. These studies have shown that loading enhances the tendon fibroblasts of embryonic chicks, promotes the healing of fractures in rabbits and rats, increases the concentration of insulin-like growth factor I (IGF-I) in the tendons and peripheral nerves of rats, and relieves neurogenic or musculoskeletal pain in humans.[1,5–21] While the mechanisms of action for these various effects remain unclear, they likely differ with different modes of vibration.[15,17,20]

In this study, the effects of vibratory load on articular chondrocytes in vitro were assessed by changes in DNA and proteoglycan syntheses as measured by ^{3}H-thymidine and $H_2{}^{35}SO_4$ incorporation, respectively.

Materials and methods

Chemicals

Collagenase (type II), protease (type XIV), chondroitin sulfate, and cetylpyridium chloride were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Rabbit anti-S-100α antibody were obtained from Dakopatts (Glostrup, Denmark). $H_2{}^{35}SO_4$ (1 mCi/ml) and ^{3}H-thymidine (2 mCi/ml) were obtained from NEN Life Science Products (Daiichi Pure Chemical Co., Tokyo, Japan). The culture medium consisted of α-modification of Eagle's minimal essential medium (α-MEM), 10% fetal bovine serum (FBS, Biowhittaker), and 1% antibiotic–antimycotic solution (100 units/ml penicillin G sodium, 100 μg/ml of streptomycin sulfate, and 2 mg/ml neomycin). Culture plates (24-well and 96-well) were purchased from Becton Dickinson Labware (Bedford, MA, USA), 35-mm diameter dishes were from Costor (Cambridge, MA, USA), and 1-well culture chambers were from Nunc Inc. (Roskilde, Denmark). ASC II aqueous counting scintillation was obtained from Amersham (UK). Other reagents used were commercial products of the highest grade available.

Chondrocyte culture

Chondrocytes were isolated from the surface region of the hip and knee joints of 4-week-old male New Zealand rabbits, as described by Shimomura et al.[22] The articular cartilages were dissected out and minced into small pieces of less than 3 mm in diameter. The pieces of cartilage were incubated in 20 ml 0.1% trypsin in EDTA for 15 min at 37°C, washed three times with Ca^{2+}- and Mg^{2+}-free Tyrode's solution [Tyrode (−)], and centrifuged at 800 g for 5 min. Next, the aggregates were digested in 0.2% collagenase in Tyrode (−) and incubated for 3 h at 37°C. The digests were triturated with a 5-ml plastic pipette, and filtered through a nylon sieve (120-μm pore size). The pellet from centrifugation at 800 *g* for 10 min was resuspended in 20 ml α-MEM supplemented with 10% FBS, followed by another centrifugation under the same conditions. This procedure was repeated three times to thoroughly remove collagenase.

For the primary cultures, 8×10^4 cells were seeded in a 25-cm^2 biocoat-collagen type-I flask in the presence of 5 ml α-MEM supplemented with 10% FBS. Cultures were kept in a humidified incubator at 37°C in a 5% CO_2 and 95% air environment. Subconfluent cultures were further subcultured by trypsinization (0.1% trypsin in EDTA).

For assays of DNA synthesis, 3×10^3 chondrocytes from the secondary culture were seeded into the wells of a 96-well plate. For proteoglycan synthesis, 4×10^4 chondrocytes were placed into the wells of a 24-well plate. For morphologic observation, 1×10^5 chondrocytes were plated in a 35-mm-diameter dish. For immunostaining and Alcian blue staining, 8.5×10^4 cells were seeded into a 1-well chamber slide (collagen type-I biocoated). These chondrocytes were grown in the same medium as those for the primary and secondary cultures.

Mechanical vibratory loading

The vibratory stimulation experiments were performed with a specially constructed vibratory system, as shown in Fig. 1. The vibratory unit consisted of an electromechanical vibrator (Diatone ACT-1, Tokyo, Japan) with a platform to hold the culture plate. These culture plates were secured to the platform by double adhesive tape. The vibratory unit was placed in an ordinary CO_2 incubator. Vibrations were delivered by an electromechanical vibrator that was driven by an oscillator (Kenwood AG-203A, Tokyo, Japan). The signal of the oscillator was amplified by a power amplifier (Kenwood VT-171, Tokyo, Japan).

The equipment was set to a sine-wave signal, with a vibration acceleration of 1.4 *G* ($G = 9.81\ m/s^2$). The cultured chondrocytes were subjected to vibratory stimulation at a

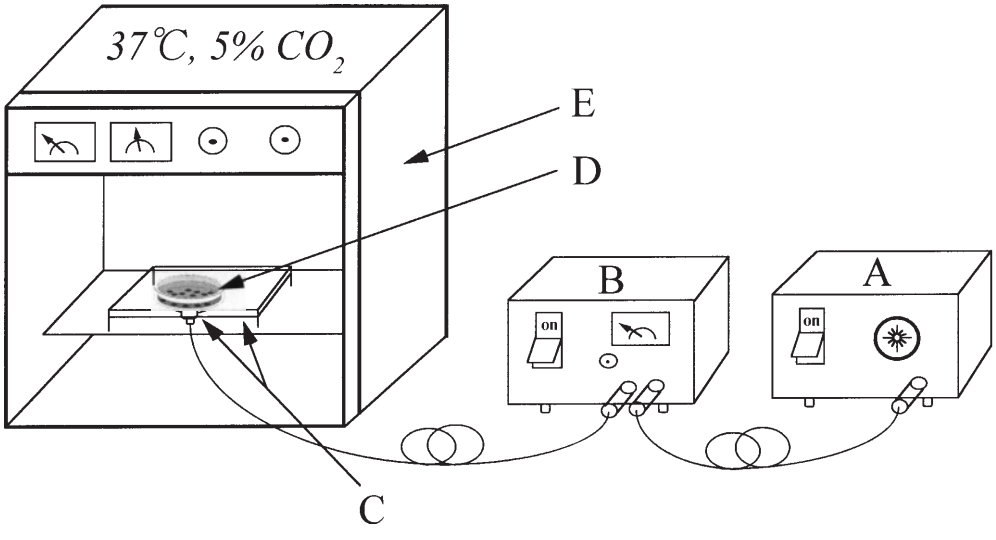

Fig. 1. Schematic illustration of the vibration system. An articular chondrocyte culture plate is fixed onto the platform of the vibrator. The vibrator motion exerts an oscillating force that is transmitted to the articular chondrocytes via the culture plate. *A*, oscillator; *B*, power amplifier; *C*, vibratory unit (vibrator and its platform); *D*, culture plate; *E*, cell incubator. The articular chondrocytes were incubated at 37°C in 5% CO_2 and 95% air

variety of frequencies: 200, 300, 400, 800, and 1600 Hz. Parallel control articular chondrocytes were not vibrated, but were cultured under identical conditions.

Chondrocyte identification

A modified avidin–biotin complex (ABC) technique[23] was used for articular chondrocyte identification. Articular chondrocytes were grown to subconfluency in 1-well chamber slides. After removal of the media and washing with phosphate-buffered saline (PBS), the chondrocytes were fixed in 4% paraformaldehyde (PFA) for 10 min at 4°C. After washing in PBS, they were treated with 0.15% Triton-X100 in PBS for 10 min at room temperature. Nonspecific immunoglobulin binding was blocked with 10% normal rabbit serum. The primary rabbit anticow S-100α antibodies diluted 1:200 in PBS with 2% bovine serum albumin (PBS/BSA) were applied to cultured chondrocyte slides and incubated at room temperature for 1 h in a humidified chamber. After three washes with PBS for 5 min, the slides were incubated with the secondary antibody, a biotinylated antirabbit antibody, at room temperature for 10 min. The slides were washed three times with PBS for 5 min and incubated with streptavidin–peroxidase complex for 5 min. After three washes with PBS for 5 min, immunoreactivity was visualized with chromogen diaminobenzidine (0.025%) in PBS and 0.1% hydrogen peroxide. To show the articular chondrocytes letter, hematoxylin staining was performed.

Alcian blue staining

At each time point, cultured chondrocytes were rinsed with PBS and fixed with 4% paraformaldehyde (PFA) for 20 min. They were then stained with 1% Alcian blue (pH 2.5) for light microscopy.

Measurement of DNA synthesis

DNA synthesis was estimated by measuring the incorporation of ^{3}H-thymidine into cultured articular chondrocytes. Cultures of articular chondrocytes were subjected to vibratory loading at various frequencies and time courses, and then supplemented with 2 μCi/ml ^{3}H-thymidine (10 μl/well) for 16 h of radiolabeling. After removal of the labeling medium and trypsinization for 15 min at 37°C, the detached cells were vacuum-collected on 1.0-μm filters (Toyo Roshi Co., Tokyo, Japan) by a multiple-cell harvester (Labo Mash; Labo Science Co., Tokyo, Japan) and washed with distilled water. The filters were air-dried, and the radioactivity was measured in a scintillation counter (Model LS 6000, Beckman; Palo Alto, CA, USA). Experiments were run in triplicate or quadruplicate, and the mean and SD values were determined.

Measurement of proteoglycan synthesis

The incorporation of $H_2{}^{35}SO_4$ into newly synthesized proteoglycans was measured in both the conditioned medium and the chondrocyte layers using a modified method of Solursh and Meier.[24] When the chondrocytes reached confluence, the cultures were subjected to periodic vibrations (8 h per day) at 300 Hz, 1.4 *G*, for 3–15 days. The vibrated chondrocytes were further exposed to 1 μCi of $H_2{}^{35}SO_4$ in 0.4 ml α-MEM containing 0.5% FBS for 24 h at 37°C. After removal of the ^{35}S-labeling medium, the chondrocytes were washed with 1.0 ml ice-cold PBS. The ^{35}S-labeling medium and the PBS from the washes were combined as the medium fraction. Ice-cold 0.15 N NaOH (1.4 ml) was added to the washed chondrocyte layers, and the cells were immediately scraped off completely with a rubber policeman. The medium and chondrocyte fractions were combined and neutralized with 6 N HCl. To 1.0 ml of the mixture, 2.0 ml 0.2 M Tris-HCl buffer, pH 7.8, containing 5 mM $CaCl_2$ and 4 mg protease XIV were added, and the mixture was further incubated for 15 h at 55°C. Next, 50 μl water containing 5 μg chondroitin sulfate and 1.0 ml 2 mM $MgSO_4$ were added. The polysaccharides were precipitated by the addition of 1.0 ml 1% cethylpyridium chloride, and then the mixture was allowed to stand for 1 h at 37°C. The precipitate was collected on a Millipore filter disc (25-mm diameter, 0.45-μm pore size; Toyo Roshi, Tokyo, Japan) and washed five times with 2.0 ml 1% cetylpyridium chloride–0.02 M NaCl. The filter was air-dried and dissolved in 5 ml ACS II. The radioactivity was measured in a scintillation counter. Experiments were run in quadruplicate.

Statistical analysis

All data are expressed as the mean ± SD. Mann-Whitney's *U* test was used to determine the level of significance. Differences were considered to be significant at $P < 0.05$.

Results

Effects of vibration on the morphogenesis of chondrocytes

As shown in Fig. 2, immunostaining of these cultured cells showed the characteristic morphology of articular chondrocytes. In order to elucidate the influence of vibratory load on the proliferation and differentiation of articular chondrocytes, these cells were subjected to periodic vibratory loading at 300 Hz, 1.4 *G*, for 8 h per day (Fig. 3). In all experimental processes, the vibrated chondrocytes proliferated and differentiated more rapidly as compared with the controls. The chondrocytes exhibited an elongated fibroblastic morphology on day 1 after seeding. On day 3 of culture, the vibrated chondrocytes reached confluency to form a monolayer, whereas the control cells remained sparse. The Alcian blue staining of the cartilage matrix was the same in the two experimental groups (Fig. 4). A piling-up growth of the vibrated chondrocytes appeared on day 6. Their increase in number was accompanied by cell fusion to form a syncytium (Fig. 3). At this time, the Alcian blue staining revealed an accumulation of cartilage matrix (Fig.

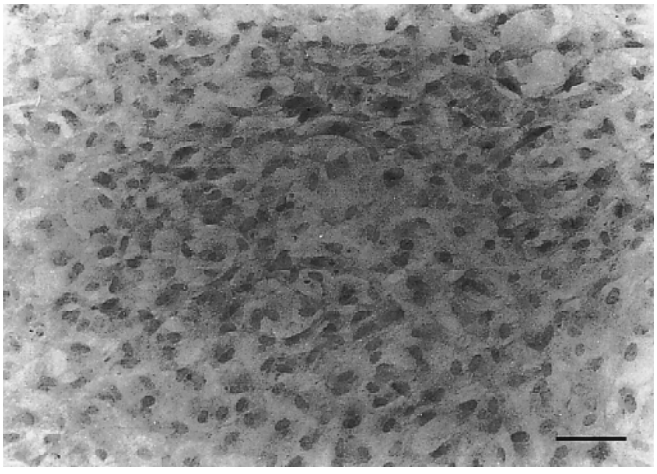

Fig. 2. Cells were cultured in one-well chamber slides, and received a vibration of 300 Hz, 1.4 *G*, with 8 h per day for 3 days. The immunostaining (streptavidin biotin method) with anti-S-100α showed the presence of articular chondrocytes. *Bar* 50 μm

4). On day 12, numerous piles with a cartilage nodule appearance were detected (Fig. 3), and were stained intensely by Alcian blue (Fig. 4) in the vibrated culture.

Effects of vibration on DNA synthesis

Rabbit articular chondrocytes were seeded at a low density (3×10^3 cells/well) into 96-well culture plates, preincubated for 24 h, and then subjected to periodic vibration with 1.4 *G* for 8 h per day. ^{3}H-thymidine incorporation into DNA synthesis was measured in the cells during their logarithmic growth phase within 4 days after the cell inoculation. A mechanical vibratory load at different frequencies led to changes in ^{3}H-thymidine incorporation by articular chondrocytes (Fig. 5). The most ^{3}H-thymidine incorporation was obtained with a frequency of 300 Hz during the growing phase. At a frequency of 300 Hz, a maximal increase was observed on day 3, i.e., from 114% ($P < 0.001$)

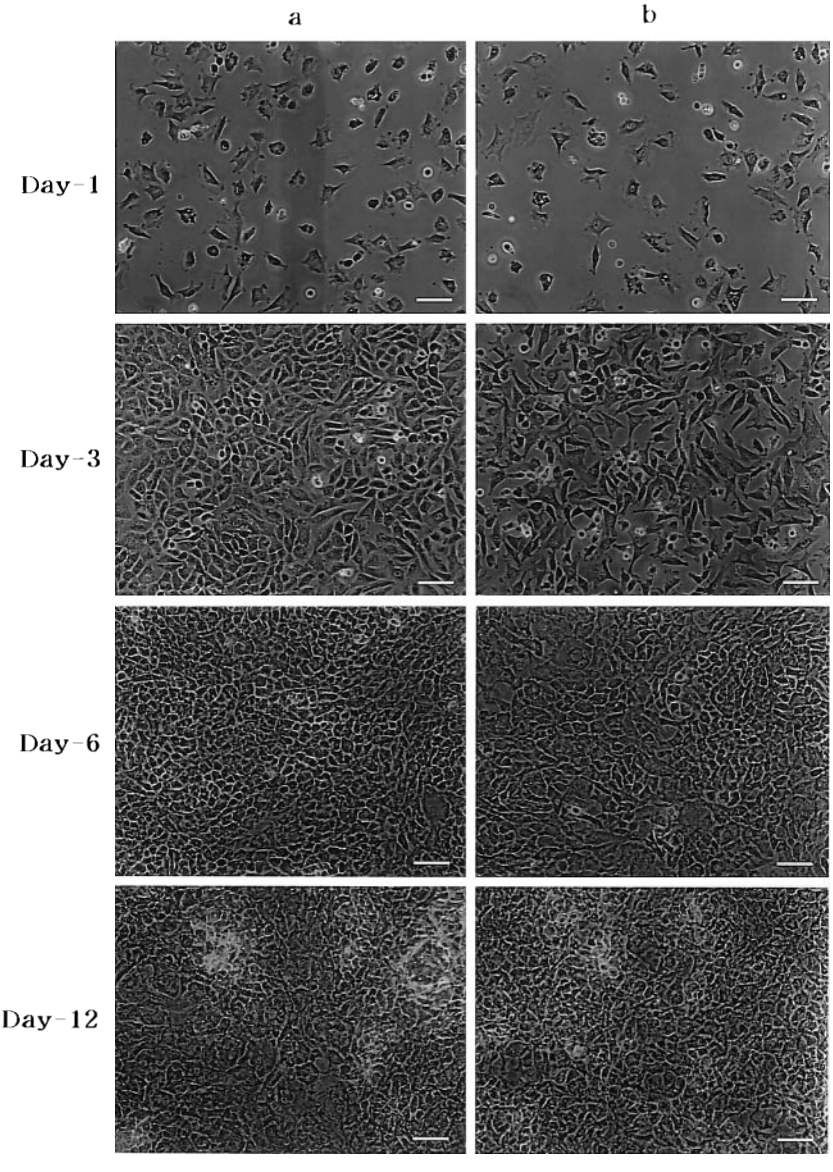

Fig. 3. Influence of mechanical vibration on the morphogenesis of articular chondrocytes. Chondrocytes were cultured in 35-mm-diameter dishes, and subjected to a periodic vibratory load of 300 Hz, 1.4 *G*, for 8 h per day. **a** Cells cultured with vibration; typical cartilage nodules had formed by day 12. **b** Control cells cultured without vibration. *Bar* 100 μm

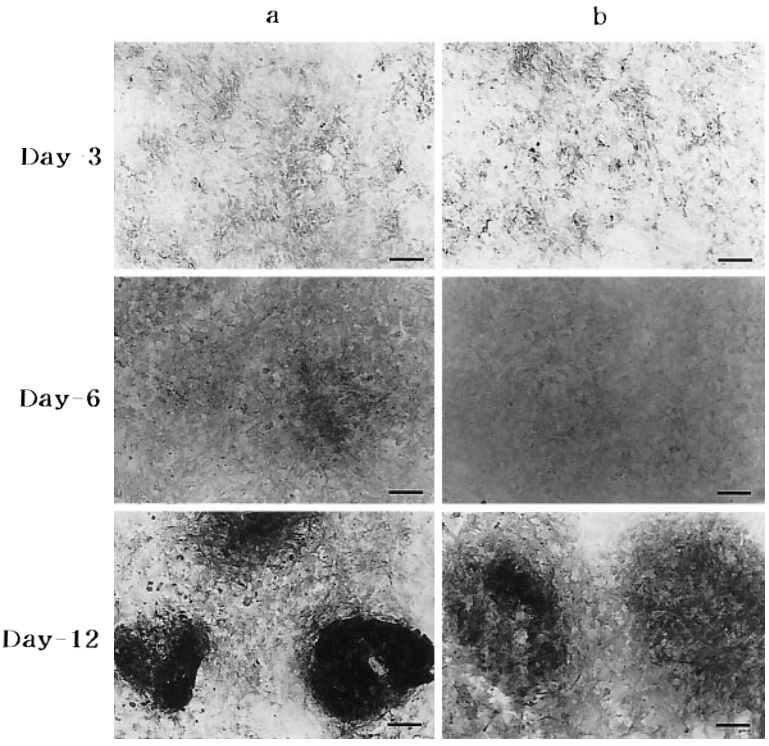

Fig. 4. Influence of mechanical vibration on the cartilage matrix. Articular chondrocytes were cultured in one-well chamber slides, and received a periodic vibratory load of 300 Hz, with 1.4 *G*, for 8 h per day. The cultures were stained with 1% Alcian blue. **a** Cells cultured with vibration. **b** Control cells cultured without vibration. The Alcian blue staining revealed a marked accumulation of cartilage matrix and formation of cartilage nodules in the vibration cultures by day 6 and day 12. *Bar* 100 μm

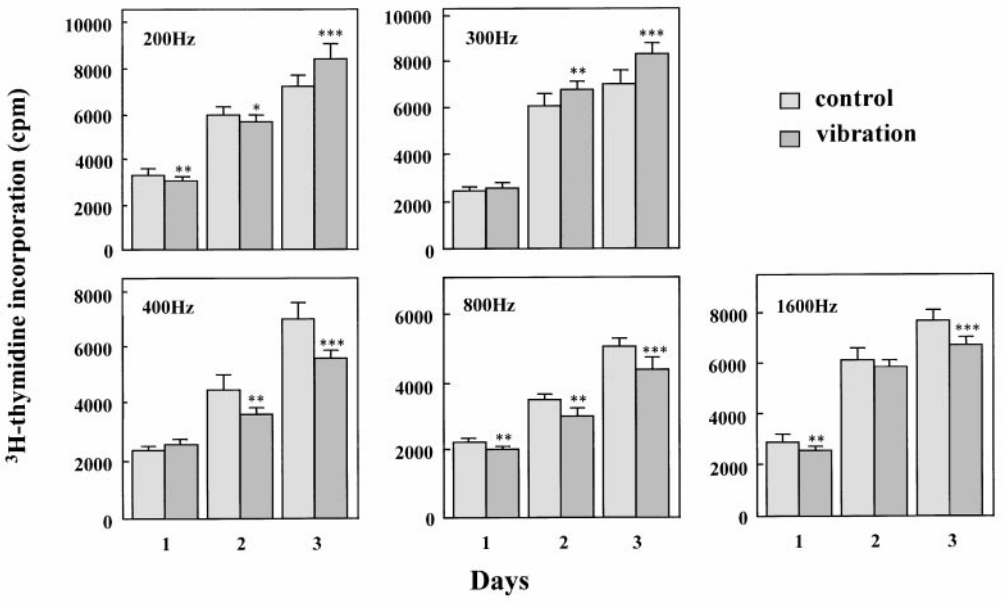

Fig. 5. Effect of periodic vibration load on ^{3}H-thymidine incorporation into articular chondrocytes. Chondrocytes were periodically vibrated at a frequency of 200 Hz, 300 Hz, 400 Hz, 800 Hz, or 1600 Hz, with 1.4 *G*, for 8 h per day. The mean ^{3}H-thymidine uptake is shown. Error bars show the SD (n = 12). Values were considered to be significant at: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

to 125% ($P < 0.001$) of the controls in quadruplicate experiments. At a frequency of 200 Hz, the ^{3}H-thymidine incorporation decreased to 88% ($P < 0.05$) and 94% ($P < 0.05$) of the controls on day 1 and day 2, respectively. Interestingly, however, a significant increase to 119% of the controls ($P < 0.001$ vs. controls) developed on day 3. At a frequency of 400 Hz, a slight increase was obtained on day 1, but with further vibrations the ^{3}H-thymidine incorporation decreased to 82% ($P < 0.01$) and 80% ($P < 0.001$) of the controls. Frequencies above 800 Hz markedly lowered ^{3}H-thymidine incorporation during the 3-day period. These observations indicate that appropriate vibratory loading (300 Hz for 3 days) significantly promoted DNA synthesis.

The relationship between the vibration period and ^{3}H-thymidine incorporation into DNA synthesis is illustrated in Fig. 6. When continuous vibratory loading was applied at 300 Hz, 1.4 *G*, for from 1 to 96 h, the ^{3}H-thymidine incorporation gradually increased and reached a plateau at 72 h.

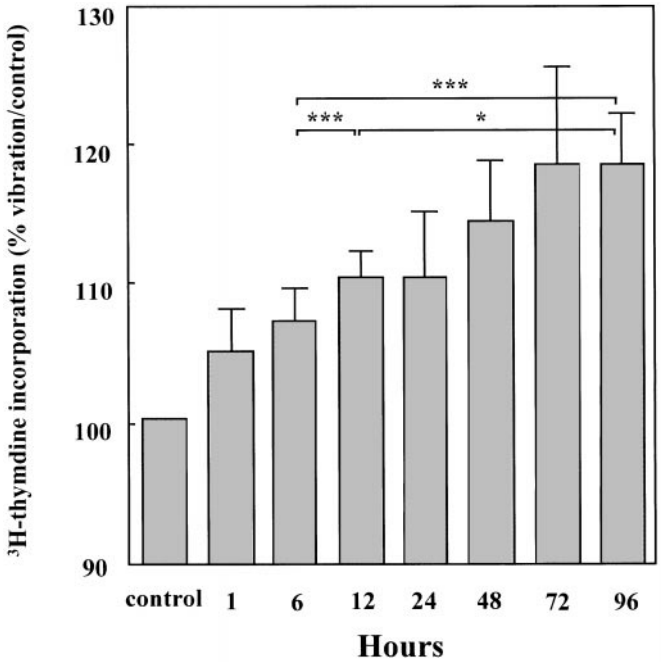

Fig. 6. Effect of continuous vibratory loading on ^{3}H-thymidine incorporation in articular chondrocytes. Chondrocytes were vibrated at 300 Hz and 1.4 *G* for 1, 6, 12, 24, 48, 72, or 96 h. The mean ^{3}H-thymidine uptake as a percentage of the control is shown. Error bars show the SD ($n = 10$). Values were considered to be significant at: $^*P < 0.05$; $^{***}P < 0.001$

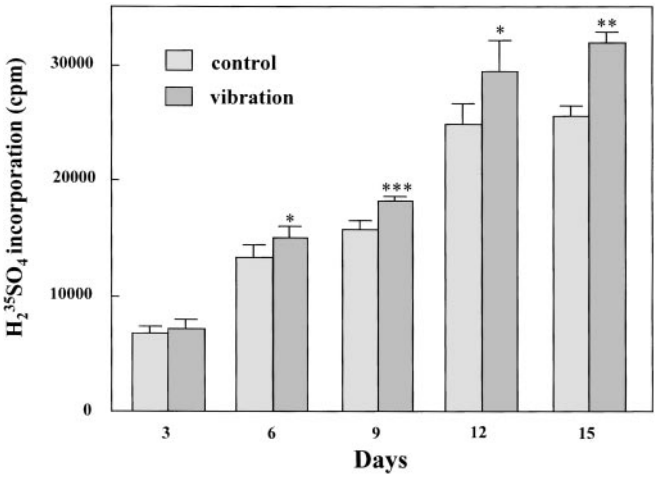

Fig. 7. Effect of periodic vibration loading on $H_2{}^{35}SO_4$ incorporation in articular chondrocytes. Chondrocytes were periodically vibrated at a frequency of 300 Hz with 1.4 *G* for 8 h per day for the time periods indicated. The figure shows the mean $H_2{}^{35}SO_4$ uptake at each time point. Error bars show the SD ($n = 12$). Values were considered to be significant at: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

Effect of vibration on proteoglycan synthesis

Articular chondrocytes were cultured in 24-well culture plates until confluent, and then subjected to periodic vibratory loading at 300 Hz, 1.4 *G*, for 8 h per day. During vibration, the incorporation of $H_2{}^{35}SO_4$ into proteoglycan synthesis gradually increased as compared with the controls (Fig. 7). The rate of $H_2{}^{35}SO_4$ incorporation started to increase slightly on day 3, and reached a maximum on day 15 (126% of control, $P < 0.01$). These findings indicate that a vibratory load of 300 Hz frequency promoted proteoglycan synthesis in a confluent culture of articular chondrocytes.

Discussion

This study demonstrates that vibration promotes the metabolic activity of articular chondrocytes as a function of frequency. A vibration frequency of 300 Hz up-regulates DNA synthesis, while a frequency of 200 Hz or over 400 Hz down-regulates it. At a moderate vibratory loading condition of 300 Hz at 1.4 G for 8 h per day, the incorporation of both ^{3}H-thymidine and $H_2{}^{35}SO_4$ reached a maximal level. On the other hand, a frequency of 200 Hz (except on day 3) or over 400 Hz caused about 10%–20% inhibition. Under the optimal frequency (300 Hz), biosynthetic responses increased with the duration of vibration loading, i.e., 3 days for DNA synthesis, and 15 days for proteoglycan synthesis when periodic vibration was used. We observed that vibrated chondrocytes rapidly entered a confluent stage on day 3 of culture, but the apparent rate of ^{3}H-thymidine uptake only exhibited a modest increase. It is possible that this timing showed that a portion of the chondrocytes had stopped growing. Augmentation of $H_2{}^{35}SO_4$ uptake was enhanced most in the stationary phase at a saturation cell density. In this experiment, used articular chondrocytes exhibited the characteristic features of chondrocytes. Under a vibration of 300 Hz at 1.4 *G* for 8 h per day, cultured chondrocytes reached a confluence and formed cartilage-like nodules faster than the controls, which were found to be S-100α-positive by immunohistochemistry.

It is known that a mechanical vibration load can induce a variety of physical phenomena. Several research reports have shown that a mechanical vibratory load is conducted to fracture or implant sites where micromovement occurs between both fragments, and this may have a beneficial influence on bone regeneration and its healing process.[15,17,25–28] This alternating motion due to mechanical vibration also promotes cartilage formation between bone fragments.[26]

Some investigators have reported that different types of mechanical environments at cartilage explants, such as dynamic mechanical compression and/or stretch loads, can modulate chondrocyte metabolism. These different mechanical models applied frequency ranges below 10 Hz, and resulted in either inhibition or stimulation of chondrocyte biosyntheses according to the dynamic mechanical compression and stretch-loading intensity.[2,8,12,29–31] In our experiment, without compression or stretch loading, a purely mechanical vibration with higher frequency ranges induced similar results, with biosynthetic responses being promoted at moderate vibratory load levels (300 Hz) and inhibited beyond some excessive vibratory load levels (400–1600 Hz).

Sah and co-workers[2,30] have suggested that dynamic compression at 0.1 Hz can cause a stimulation of biosynthesis.

This physical modulation is most likely related to changes in fluid flow, streaming potential, or the cell shape and volume of cartilage explants. De Witt et al.[32] found that cyclic mechanical stretching of 14-day chondrocyte cultures caused a stimulation of proteoglycan and DNA synthesis and cAMP levels; the single test condition of 5.5% stretching at 0.2 Hz was thought to impart compressive forces to the chondrocytes. Lee et al.[33] plated chondrocytes onto elastic membranes and observed a stimulation of glycosaminoglycan (GAG) synthesis when the membranes were stretched at 1 Hz using 1% strain. In our experiment using a sinusoidal acceleratory movement in both the horizontal and vertical planes, a two-dimensional, predominantly vibratory force was conducted to cultures of articular chondrocytes, resulting in the promotion of chondrocyte metabolism. Thus, it is possible that different mechanical loads act to regulate chondrocyte function through a specific signaling pathway, although the physicochemical relevance of the action of mechanical loads for cartilage regeneration remains to be elucidated.

In conclusion, the in vitro mechanical vibration of rabbit articular chondrocytes via sinusoidal waveforms at various frequencies can enhance or depress ^{3}H-thymidine uptake into DNA and $H_2{}^{35}SO_4$ incorporation into proteoglycans. The observed facilitation of the biosynthetic response of chondrocytes by moderate frequency vibration (300 Hz, 1.4 G) is consistent with previous findings in different models of dynamic mechanical load, e.g., cyclic oscillatory compression and oscillatory stretch. These basic findings support a promising simple approach to facilitating the metabolism of articular cartilage, particularly during the regeneration processes.

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References

1. Inerot S, Heinegard D, Olsson SE, Telhag H, Audell L. Proteoglycan alterations during developing experimental osteoarthritis in a novel hip joint model. J Orthop Res 1991;9:658–73.
2. Sah RL, Kim YJ, Doong JY, Grodzinsky AJ, Plaas AH, Sand JD. Biosynthetic response of cartilage explants to dynamic compression. J Orthop Res 1989;7:619–36.
3. Gray ML, Pizzanelli AM, Grodzinsky AJ, Lee RC. Mechanical and physicochemical determinants of the chondrocyte biosynthetic response. J Orthop Res 1988;6:777–92.
4. Schneiderman R, Keret D, Maroudas A. Effects of mechanical and osmotic pressure on the rate of glycosaminoglycan synthesis in the human adult femoral head cartilage: an in vitro study. J Orthop Res 1986;4:393–408.
5. Bayliss MT, Urban JP, Johnstone B, Holm S. In vitro method for measuring synthesis rates in the intervertebral disc. J Orthop Res 1986;4:10–17.
6. Gray ML, Pizzanelli AM, Lee RC, Grodzinsky AJ, Swann DA. Kinetics of the chondrocyte biosynthetic response to compressive load and release. Biochim Biophys Acta 1989;991:415–25.
7. Jones IL, Klamfeldt A, Sandstrom T. The effect of continuous mechanical pressure upon the turnover of articular cartilage proteoglycans in vitro. Clin Orthop Rel Res 1982;165:283–9.
8. Palmoski MJ, Barndt KD. Effects of static and cyclic compressive forces on articular cartilage plugs in vitro. Arthritis Rheum 1984; 27:675–81.
9. Urban JP, Bayliss MT. Regulation of proteoglycan synthesis rate in cartilage in vitro: influence of extracellular ionic composition. Biochem Biophys Acta 1989;992:59–65.
10. Karin H, Lisbet C, Staffan J, James HK, Evy LA. Chondrocyte and chondrosarcoma cell integrins with affinity for collagen type II and their response to mechanical stress. Exp Cell Res 1995;221:496–503.
11. Korver THT, van de Stadt RT, Kiljan E, van Kampen GPJ, van der Korst TK. Effects of loading on the syntheses of proteoglycans in different layers of anatomically intact articular cartilage in vitro. J Rheumatol 1992;19:905–12.
12. Parkkinen TT, Lammi MJ, Helminen HJ, Tammi M. Local stimulation of proteoglycan synthesis in articular cartilage explants by dynamic compression in vitro. J Orthop Res 1992;10:610–20.
13. Banes AJ, Gilbert J, Taylor D, Monbureau O. A new vacuum-operated stress-providing instrument that applies static or variable duration cyclic tension or compression to cells in vitro. J Cell Sci 1985;75:35–42.
14. Banes AJ, Link GW Jr, Gilbert JW, Tran Son Tay R, Monbureau O. Culturing cells in a mechanically active environment. Am Biotechnol Lab 1990;8:12–22.
15. Jancovich JP. The effects of mechanical vibration on bone development in the rat. J Biomech 1972;5:241–50.
16. Sekiya I. Effects of mechanical vibration on tendons in vitro (in Japanese). J Nagoya City Univ Med Assoc 2000;51:87–97.
17. Yasuo U, Joseph E. Different effects of mechanical vibration on bone ingrowth into porous hydroxyapatite and fracture healing in a rabbit model. J Orthop Res 1989;7:559–67.
18. Hansson HA, Dahlin LB. Transient increase in insulin-like growth factor I immunoreactivity in rat peripheral nerves exposed to vibrations. Acta Physiol Scand 1988;132:35–41.
19. Hansson HA, Dahlin LB. Transiently increased insulin-like growth factor I immunoreactivity in tendons after vibration trauma. An immunohistochemical study on rats. Scand J Plast Reconstr Surg 1988;22:1–6.
20. Ryusuke K, Horoshi S. Mechanisms of pain relief by vibration and movement. J Neurol Neurosurg Psychiat 1992;55:282–6.
21. Lurdeberg T. Long-term results of vibratory stimulation as a pain relieving measure for chronic pain. Pain 1984;20:13–23.
22. Shimomura Y, Yoneda T, Suzuki F. Osteogenesis by chondrocytes from growth cartilage of rat rib. Calcif Tiss Res 1975;19:179–87.
23. Hus SM, Raine L, Fanger H. Use of avidin–biotin–peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 1981;29:577–80.
24. Solursh M, Meier S. A conditioned medium (CM) factor produced by chondrocytes that promotes their own differentiation. Dev Biol 1973;30:279–89.
25. Panjabi MM, White AA, Wolf JW Jr. A biomechanical comparison of the effects of constant and cyclic compression on fracture healing in rabbit long bones. Acta Orthop Scand 1979;50:653–61.
26. Rahn BA. Bone healing: histologic and physiologic concepts. In: Sunmer-Smith G, editor. Bone in clinical orthopaedics. Philadephia: WB Saunders; 1982. p. 335–86.
27. Rubin CT, Lanyon LE. Osteoregulatory nature of mechanical stimuli: function as a determinant for adaptive remodeling in bone. J Orthop Res 1987;5:300–10.
28. Carter DR, Wong M. Mechanical stress and endochondral ossification in the chondroepiphysis. J Orthop Res 1988;6:148–54.
29. Larsson T, Aspden RM, Heinegard D. Effects of mechanical load on cartilage matrix biosynthesis in vitro. Matrix 1991;11: 388–94.
30. Kim YJ, Sah RL, Grodzinsky AJ, Plass AH, Sandy JD. Mechanical regulation of cartilage biosynthetic behavior: physical stimuli. Arch Biochem Biophys 1994;331:1–12.
31. Michael DB, Yehezkiel AG, Alan JG, Ernst BH. Mechanical compression modulates matrix biosynthesis in chondrocyte/agarose culture. J Cell Sci 1995;108:1497–508.
32. De Witt MT, Handley CJ, Oakes BW, Lowther DA. In vitro response of chondrocytes to mechanical loading. The effect of short-term mechanical tension. Connect Tiss Res 1984;12:97–109.
33. Lee RC, Rich JB, Kellge KM, Weiman DS, Mathews MB. A comparison of in vitro cellular responses to mechanical and electrical stimulation. Am Surg 1982;48:567–74.