Soft Tissue

1) Effects of low-intensity pulsed ultrasound in repairing injured articular cartilage.

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OBJECTIVE: To investigate the effects of low-intensity pulsed ultrasound in repairing injured articular cartilage. METHODS: Ten adult New Zealand rabbits with bilateral full-thickness osteochondral defects on the cartilage surface of intercondylar fossas were used in this study. The wounds in the left knees were treated with low-intensity pulsed ultrasound as the experimental group. The right knees received no treatment as the control group. All the animals were killed at 8 weeks after injury and the tissues in the wounds were collected for gross appearance grading, histological grading and proteoglycan quantity. RESULTS: The scores of the gross appearance grades, histological grades and the optical density of toluidine blue of the tissues in the experimental group were significantly higher than those of the controls at 8 weeks after injury ($P < 0.05$). CONCLUSIONS: Low-intensity pulsed ultrasound can accelerate the repair of injured articular cartilage.

2) Improved cartilage repair after treatment with low-intensity pulsed ultrasound.

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Low-intensity pulsed ultrasound accelerates bone healing via upregulation of cartilage formation and maturation phases of endchondral bone formation. The current authors evaluated the effect of ultrasound therapy on the repair of full-thickness osteochondral defects. Bilateral, 3.2 mm diameter by 5.0 mm deep osteochondral defects were created in the patellar groove of 106 adult male New Zealand rabbits. The defects were treated with daily low-intensity pulsed ultrasound therapy on the right knee. The left knee was not treated. In Part I, the effect of ultrasound therapy was evaluated at 4, 8, 12, 24, and 52 weeks after surgery. In Part II, the effect of the length of treatment (5, 10, or 40 minutes of

daily ultrasound therapy) compared with standard 20 minute therapy was evaluated. The repair cartilage was evaluated and graded on a standard scale for the gross and histologic appearance. Ultrasound treatment significantly improved the morphologic features and histologic characteristics of the repair cartilage compared with nontreated controls. Earlier, better repair with less degenerative changes at later times was observed in defects treated with ultrasound. Doubling the treatment time to 40 minutes daily significantly increased the histologic quality of the repair cartilage. In the current animal model, daily low-intensity pulsed ultrasound had a significant positive effect on the healing of osteochondral defects.

3) Effects of low-intensity pulsed ultrasound on proliferation and chondroitin sulfate synthesis of cultured chondrocytes embedded in Atelocollagen gel.

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The effects of low-intensity pulsed ultrasound (US) on the proliferation and chondroitin sulfate synthesis of cultured chondrocytes embedded in Atelocollagen gel in vitro were examined. Articular cartilage was harvested from the hip, knee, and shoulder joints of 10-week-old Japanese white rabbits. Chondrocytes isolated by collagenase digestion were embedded in type I collagen gel, Atelocollagen gel, and were cultured in Dulbecco's modified eagle's medium for 3 weeks. The US apparatus, SAFHS, was used to deliver an ultrasound signal with spatial and temporal average intensities of 30 mW/cm(2) (US group). The frequency was 1.5 MHz with a 200-microsecond tone burst repeated at 1.0 kHz. US treatments were administered for 20 min per day under culture dishes, with the medium replaced twice a week. Another group of cells was exposed to sham ultrasound as a control. Cell number, histological findings, synthesis of isomers of chondroitin sulfate, and stiffness of the chondrocyte-collagen gel composites were analyzed. US exposure promoted synthesis of chondroitin sulfate, especially chondroitin 6-sulfate, although it did not significantly enhance cell number and stiffness. In this three-dimensional culture model, these results suggest that US exposure may be clinically useful in improving the quality of chondrocyte-Atelocollagen implants for transplantation into articular cartilage defects. J Biomed Mater Res 59: 201-206, 2002

4) **The influence of pulsed low-intensity ultrasound on matrix production of chondrocytes at different stages of differentiation: an explant study.**

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The proximal and distal parts of sterna of chick embryos represent cartilage undergoing endochondral ossification and hyaline cartilage, respectively. Cartilage explants from both regions were exposed for 20 min to pulsed low-intensity ultrasound (PLIUS) with an intensity of 30 mW. cm(-2) (spatial average-temporal average) at a frequency of 1.5 MHz, with a pulse burst frequency of 1 kHz and burst duration of 200 micros. Histological and immunohistochemical analysis was performed on days 1, 3, 5 and 7 after treatment. An anabolic effect of PLIUS on matrix production was shown by an increase of up to 10% to 20% in quantitative immunohistochemical staining for type II collagen and aggrecan in the two parts of the sternum. PLIUS also increased type X collagen staining by up to 10% in certain regions of the proximal part of the sternum. Staining for type X collagen was negative in the distal part of the sternum in both PLIUS and control groups. These results suggest that PLIUS may stimulate bone formation by increasing hypertrophy of chondrocytes directed to terminal differentiation. However, PLIUS did not induce hypertrophy in hyaline cartilage; moreover, increased matrix synthesis indicates a potential role in cartilage repair.

Low-intensity pulsed ultrasound enhances early healing of medial collateral ligament injuries in rats.

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OBJECTIVE: To investigate the effect of low-intensity pulsed ultrasound exposure on the healing of injured medial collateral ligaments. METHODS: Thirteen male Sprague Dawley rats were used in the study. After surgical transection of the bilateral medial collateral ligaments, the ligament of 1 knee received low-intensity pulsed ultrasound exposure (30 mW/cm2 for 20 minutes daily), whereas no ultrasound was applied to the contralateral knee (control side). Eight rats were killed at 12 days after surgery, and 5 rats were killed at 21 days. The bilateral knees of 5 rats were used for mechanical testing at each of the 2 periods, and 12-day specimens of the remaining 3 rats were prepared for the electron microscopic examination. The knees of 5 additional rats were used to obtain mechanical data of the normal uninjured medial collateral ligament.

RESULTS: On the 12th day, the low-intensity pulsed ultrasound-treated side exhibited significantly superior mechanical properties when compared with the control side in ultimate load, stiffness, and energy absorption ($P < .05$). However, the treatment did not afford any mechanical advantage when tested on the 21st day. The mean diameter of the fibril was significantly larger on the treatment side than on the control side ($P < .05$). CONCLUSIONS: Low-intensity pulsed ultrasound exposure is effective for enhancing the early healing of medial collateral ligament injuries.

5) **Low-intensity pulsed ultrasound accelerates and a nonsteroidal anti-inflammatory drug delays knee ligament healing.**

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BACKGROUND: Low-intensity pulsed ultrasound and nonsteroidal anti-inflammatory drugs are used to treat ligament injuries; however, their individual and combined effects are not established. HYPOTHESES: Low-intensity pulsed ultrasound accelerates ligament healing, a nonsteroidal anti-inflammatory drug delays healing, and the nonsteroidal anti-inflammatory drug inhibits the beneficial effect of low-intensity pulsed ultrasound. STUDY DESIGN: Controlled laboratory study. METHODS: Sixty adult rats underwent bilateral transection of their knee medial collateral ligaments. Animals were divided into 2 drug groups and treated 5 d/wk with celecoxib (5 mg/kg) mixed in a vehicle solution (NSAID group) or vehicle alone (VEH group). One to 3 hours after drug administration, all animals were treated with unilateral active low-intensity pulsed ultrasound and contralateral inactive low-intensity pulsed ultrasound. Equal numbers of animals from each drug group were mechanically tested at 2 weeks ($n = 14$/group), 4 weeks ($n = 8$/group), and 12 weeks ($n = 8$/group) after injury. RESULTS: Ultrasound and drug intervention did not interact to influence ligament mechanical properties at any time point. After 2 weeks of intervention, ligaments treated with active low-intensity pulsed ultrasound were 34.2% stronger, 27.0% stiffer, and could absorb 54.4% more energy before failure than could ligaments treated with inactive low-intensity pulsed ultrasound, whereas ligaments from the NSAID group could absorb 33.3% less energy than could ligaments from the VEH group. There were no ultrasound or drug effects after 4 and 12 weeks of intervention. CONCLUSIONS: Low-intensity pulsed ultrasound accelerated but did not improve ligament healing, whereas the nonsteroidal anti-inflammatory drug delayed but did not impair healing. When used in combination, the beneficial low-intensity pulsed ultrasound effect was cancelled by the detrimental nonsteroidal anti-inflammatory drug effect. CLINICAL RELEVANCE: Low-intensity pulsed ultrasound after ligament injury may facilitate earlier return to

activity, whereas non-steroidal anti-inflammatory drugs may elevate early reinjury risk.

6) **The effects of low-intensity ultrasound on medial collateral ligament healing in the rabbit model.**

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BACKGROUND: Ruptured medial collateral ligaments are capable of healing over time, but biomechanical and biochemical properties remain inferior to normal tissue. Low-intensity ultrasound may improve healing. HYPOTHESIS: Medial collateral ligaments treated with ultrasound will demonstrate superior healing. STUDY DESIGN: Controlled laboratory study. METHODS: Twenty-one late-adolescent male rabbits underwent bilateral ligament transection. One ligament from each rabbit received ultrasound treatment every other day for 6 total treatments. Contralateral ligaments received sham treatments. After 3 or 6 weeks, ligaments were evaluated biomechanically and assayed for collagen concentration and the relative proportions of types I and III collagen. RESULTS: Areas of sonicated specimens were significantly larger (10.6% +/- 4.90%) at 6 weeks. Ultimate load (39.5% +/- 17.0%), ultimate displacement (24.5% +/- 8.0%), and energy absorption (69.1% +/- 22.0%) were significantly higher for sonicated specimens at 6 weeks. No significant biomechanical differences were observed at 3 weeks. The relative proportion of type I collagen was significantly higher in sonicated ligaments at 3 weeks (8.61% +/- 4.0%) and 6 weeks (6.91% +/- 3.0%). No significant differences in collagen concentration were observed at either 3 or 6 weeks. CONCLUSION: Subtle improvement with ultrasound treatment may be apparent by 3 weeks after injury, suggested by increased proportion of type I collagen. Ultrasound appears to improve some structural properties and to modestly increase scar cross-sectional area and type I collagen present at 6 weeks after injury in this model. CLINICAL RELEVANCE: Ultrasound treatments after ligament injury may facilitate earlier return to activities and decrease risk of reinjury.

7) Low-intensity pulsed ultrasound accelerates bone-tendon junction healing: a partial patellectomy model in rabbits.

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BACKGROUND: Low-intensity pulsed ultrasound has been demonstrated to be beneficial for accelerating fracture healing, delayed union, nonunion, and soft tissue repair. HYPOTHESIS: Low-intensity pulsed ultrasound accelerates healing of bone-to-tendon junction repair by promoting osteogenesis and tissue remodeling at the healing junction. STUDY DESIGN: Controlled laboratory study. METHODS: Standard partial patellectomy was conducted in forty-eight 18-week-old rabbits divided into an ultrasound treatment and control group. Daily ultrasound was delivered 3 days after surgery onto the patellar tendon-patella healing junction and continuously up to weeks 2, 4, 8, and 16 postoperatively, when the patella-patellar tendon complexes were harvested for radiographic, histologic, and biomechanical evaluations. RESULTS: Radiographic measurements showed significantly more newly formed bone at the patellar tendon-patella healing junction in the ultrasound group compared with the controls at week 8 (4.91 +/- 2.74 mm(2) vs 2.50 +/- 1.83 mm(2), P < .05) and week 16 (7.22 +/- 2.34 mm(2) vs 4.61 +/- 2.22 mm(2), P < .05) after partial patellectomy. Histologically, the ultrasound group at weeks 8 and 16 showed improved tissue integration, characterized by trabecular bone expansion from the remaining patella and regeneration of fibrocartilage layer at the patellar tendon-patella healing junction. Fluorescence microscopy revealed earlier bone formation in the ultrasound group when compared with the controls at week 8 (1.78 +/- 0.32 vs 1.23 +/- 0.43, P < .01) and week 16 (2.10 +/- 0.67 vs 1.29 +/- 0.35, P < .01). Mechanical testing showed significantly higher failure load and ultimate strength in the ultrasound group (300.2 +/- 61.7 N and 7.10 +/- 1.29 MPa, respectively) as compared with controls (222.3 +/- 65.1 N and 5.26 +/- 1.36 MPa, respectively) at week 16 (P < .05 for both). CONCLUSION: Low-intensity pulsed ultrasound was able to accelerate bone-to-tendon junction repair. CLINICAL RELEVANCE: These results may help establish treatment efficacy for accelerating bone-to-tendon junction repair and facilitating earlier rehabilitation.

8) Low intensity pulsed ultrasound increases the matrix hardness of the healing tissues at bone-tendon insertion-a partial patellectomy model in rabbits.

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BACKGROUND: This study evaluated the low intensity pulsed ultrasound enhancement on matrix hardness of the healing tissues at the bone-tendon junction. METHODS: Sixteen 18 week-old mature female rabbits were used. An established transverse partial patellectomy was performed at the distal one-third of the patella. Animals were then divided according to their body weight into ultrasound group (n = 8) with daily treatment of low intensity pulsed ultrasound and control group (n = 8) without ultrasound treatment. Animals were euthanized at week 8 and 16 postoperatively to evaluate the radiographic new bone formation and the Vickers hardness of the matrix of the healing tissues at the bone-tendon junction. FINDINGS: (1) Comparing with the control group, the anterior-posterior area of the new bone in the ultrasound treated group was found on average to be 3.0 and 3.1 times greater at week 8 and 16, respectively ($P < 0.01$). (2) The Vickers hardness of the new bone in ultrasound group was 11.3% ($P < 0.05$) significantly lower at week 8 but 20.0% ($P < 0.05$) significantly higher at week 16 as compared with that of the control group. (3) The Vickers hardness of the newly regenerated fibrocartilage zone, healing tendon, and cartilaginous metaplasia in ultrasound group was found higher than the control group at both week 8 and 16, but the difference was significant at week 16 only, being 44.1% ($P < 0.05$), 20.1% ($P < 0.01$), and 46.4% ($P < 0.01$) higher, respectively. INTERPRETATION: The preliminary findings suggested for the first time that low intensity pulsed ultrasound treatment resulted in the enhancement of the matrix hardness in new bone, fibrocartilage, cartilaginous metaplasia, and healing tendon at the healing bone-tendon junction. These findings can be extrapolated into clinical practice, i.e. the more rapid healing induced by low intensity pulsed ultrasound, the earlier mobilization of the affected joint. The beneficial effects on prevention of the musculoskeletal deterioration resulting from the prolonged immobilization would be therefore expected.

9) Low-intensity pulsed ultrasound accelerates osteogenesis at bone-tendon healing junction.

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This study was designed to evaluate low-intensity pulsed ultrasound (LIPUS) in acceleration of mineralization and remodeling of the new bone formed at the healing interface of bone-tendon junction. Thirty-two mature New Zealand white rabbits underwent partial patellectomy and direct repair of the patellar tendon and proximal patella. Animals were then divided into LIPUS treatment group (20 min/d, 5 times/wk) and placebo control group and were euthanized at week 8 and 16 postoperatively (n = 8, for each group and time point). The main outcome measures included new bone size and its bone mineral density (BMD). Results showed that the size of new bone was found to be 2.6 and 3.0 times significantly greater in the LIPUS group compared with that of the control group at weeks 8 and 16, respectively. In addition, the LIPUS group showed significantly higher BMD at week 8 than controls, but not at week 16. In conclusion, this was the first experimental study to show that LIPUS was able to enhance osteogenesis at the healing bone-tendon junction, especially before the postoperative week 8. Findings of this study formed a scientific basis for future clinical trials and establishment of indication of LIPUS for enhancing bone-tendon junction repair.

10) Low-intensity pulsed ultrasound accelerates bone-tendon junction healing: a partial patellectomy model in rabbits.

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BACKGROUND: Low-intensity pulsed ultrasound has been demonstrated to be beneficial for accelerating fracture healing, delayed union, nonunion, and soft tissue repair. HYPOTHESIS: Low-intensity pulsed ultrasound accelerates healing of bone-to-tendon junction repair by promoting osteogenesis and tissue remodeling at the healing junction. STUDY DESIGN: Controlled laboratory study. METHODS: Standard partial patellectomy was conducted in forty-eight 18-week-old rabbits divided into an ultrasound treatment and control group. Daily ultrasound was delivered 3 days after surgery onto the patellar tendon-patella healing junction and continuously up to weeks 2, 4, 8, and 16 postoperatively, when the patella-patellar tendon complexes were harvested for radiographic,

histologic, and biomechanical evaluations. RESULTS: Radiographic measurements showed significantly more newly formed bone at the patellar tendon-patella healing junction in the ultrasound group compared with the controls at week 8 (4.91 +/- 2.74 mm(2) vs 2.50 +/- 1.83 mm(2), P < .05) and week 16 (7.22 +/- 2.34 mm(2) vs 4.61 +/- 2.22 mm(2), P < .05) after partial patellectomy. Histologically, the ultrasound group at weeks 8 and 16 showed improved tissue integration, characterized by trabecular bone expansion from the remaining patella and regeneration of fibrocartilage layer at the patellar tendon-patella healing junction. Fluorescence microscopy revealed earlier bone formation in the ultrasound group when compared with the controls at week 8 (1.78 +/- 0.32 vs 1.23 +/- 0.43, P < .01) and week 16 (2.10 +/- 0.67 vs 1.29 +/- 0.35, P < .01). Mechanical testing showed significantly higher failure load and ultimate strength in the ultrasound group (300.2 +/- 61.7 N and 7.10 +/- 1.29 MPa, respectively) as compared with controls (222.3 +/- 65.1 N and 5.26 +/- 1.36 MPa, respectively) at week 16 (P < .05 for both). CONCLUSION: Low-intensity pulsed ultrasound was able to accelerate bone-to-tendon junction repair. CLINICAL RELEVANCE: These results may help establish treatment efficacy for accelerating bone-to-tendon junction repair and facilitating earlier rehabilitation.

11) Effects of low-intensity pulsed ultrasound on tendon-bone healing in an intra-articular sheep knee model.

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PURPOSE: This study reports the mechanical and histologic properties of intra-articular tendon-bone healing with the application of low-intensity pulsed ultrasound (LIPUS) in an ovine knee model. METHODS: A single digital extensor tendon autograft from the right hoof was used as the graft in 89 adult sheep. Femoral fixation was achieved with an EndoButton (Smith & Nephew Endoscopy, Andover, MA) and tibial fixation by tying over a bony post. LIPUS treatment was performed daily for 20 minutes over the femoral and tibial tunnels until sacrifice in all groups, apart from the 26-week group, which was treated only for the first 12 weeks. Histology was performed at 3, 6, 12, and 26 weeks. Mechanical testing was performed at 6, 12, and 26 weeks. RESULTS: The LIPUS-treated group showed increased cellular activity at the tendon-bone interface and general improvement in tendon-bone integration and vascularity. Stiffness and peak load were greater compared with the control group at 26 weeks after surgery (P < .05). CONCLUSIONS: The application of LIPUS appears to improve healing at the tendon-bone interface for soft tissue grafts fixed with a suspensory fixation technique. Histology supports a benefit based on increased integration between tendon and bone and a biologically more active interface,

which would account for the improved mechanical properties. CLINICAL RELEVANCE: The indications of LIPUS may be expanded to include tendon-bone healing, for example, in anterior cruciate ligament reconstruction.

12) Low-intensity pulsed ultrasound accelerates osteogenesis at bone-tendon healing junction.

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This study was designed to evaluate low-intensity pulsed ultrasound (LIPUS) in acceleration of mineralization and remodeling of the new bone formed at the healing interface of bone-tendon junction. Thirty-two mature New Zealand white rabbits underwent partial patellectomy and direct repair of the patellar tendon and proximal patella. Animals were then divided into LIPUS treatment group (20 min/d, 5 times/wk) and placebo control group and were euthanized at week 8 and 16 postoperatively (n = 8, for each group and time point). The main outcome measures included new bone size and its bone mineral density (BMD). Results showed that the size of new bone was found to be 2.6 and 3.0 times significantly greater in the LIPUS group compared with that of the control group at weeks 8 and 16, respectively. In addition, the LIPUS group showed significantly higher BMD at week 8 than controls, but not at week 16. In conclusion, this was the first experimental study to show that LIPUS was able to enhance osteogenesis at the healing bone-tendon junction, especially before the postoperative week 8. Findings of this study formed a scientific basis for future clinical trials and establishment of indication of LIPUS for enhancing bone-tendon junction repair.

13) Pulsed ultrasound treatment accelerates the repair of Achilles tendon rupture in rats.

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A rat tenotomy model was used to investigate the effect of combined conservative management and pulsed ultrasound (PUS) on the repair of tenotomized Achilles tendon. Hemitenotomy of right medial Achilles tendon was performed in 48 rats without suture, and patella tenotomy was performed to mimic immobilization and

limb disuse of an injured limb. PUS and sham PUS were applied to the healing wound for the treatment group and control group for 5 min, 3 times per week for 2 or 4 weeks, respectively. Tensile tests showed that the ultimate tensile strength (UTS) and stiffness of the repaired tendon in the treatment group at 2 weeks reached 48.92+/-8.39% and 62.48+/-32.46% of the contralateral normal tendon strength, which were significantly higher than those of the control group (UTS, 30.36+/-15.46%; stiffness, 33.90+/-17.59; $p<0.05$). At 4 weeks, UTS increased to 77.09+/-15.31% and stiffness to 92.48+/-31.12% in the treatment group, significantly higher than those in the control group (UTS, 54.33+/-18.40%, $p<0.01$; stiffness, 65.02+/-25.48%, $p<0.05$). Light microscopy revealed more regular, denser, and better aligned collagen fibers in the healing scar of the PUS-treated healing tendons. The findings suggested that PUS were able to accelerate the healing of the ruptured tendons. (c) 2005 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res.

14) In vitro effects of therapeutic ultrasound on cell proliferation, protein synthesis, and cytokine production by human fibroblasts, osteoblasts, and monocytes.

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PURPOSE: The aim of this study was to evaluate several in vitro effects of ultrasound that could revert or prevent the hypoxia, hypovascularity, and hypocellularity observed in osteoradionecrosis. MATERIALS AND METHODS: Two different ultrasound machines were evaluated, a "traditional" (1 MHz, pulsed 1:4) and a "long wave" (45 kHz, continuous) machine, tested at various intensities. Ultrasound was applied to human gingival fibroblasts, mandibular osteoblasts, and monocytes. The assays performed were cell proliferation (DNA synthesis), collagen and noncollagenous protein (NCP) synthesis, and cytokine production (ELISA) involving interleukin (IL) 1 beta, IL-6, and IL-8, tumor necrosis factor alpha (TNF alpha), basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF). RESULTS: Both ultrasound machines induced increased cell proliferation in fibroblasts and osteoblasts, between 35% and 52%. The collagen and NCP synthesis were also significantly enhanced to levels up to 112%, the best results being with the 45-kHz machine. The ELISA results showed a slight stimulation of IL-1 beta by all cell types; there was no difference in IL-6 and TNF alpha levels. The angiogenesis-related cytokines evaluated were significantly stimulated: IL-8 and bFGF production was enhanced in osteoblasts, and VEGF production was stimulated in all three cell types. Both ultrasound machines produced the same results, with the recommended intensities being 15 and 30 mW/cm2(SA) for the 45-kHz

ultrasound, and 0.1 and 0.4 W/cm2(SAPA) for the 1 MHz ultrasound. CONCLUSIONS: Therapeutic ultrasound induces in vitro cell proliferation, collagen/NCP production, bone formation, and angiogenesis. These findings support its use in prospective clinical trials for the prevention and treatment of osteoradionecrosis.

15) In vitro evaluation of low-intensity pulsed ultrasound in herniated disc resorption.

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Herniated disc (HD) is often resolved spontaneously without surgical intervention. HD resorption (HDR) is associated with abundant vascularization and infiltration of macrophages (Mphi) into the intervertebral disc (ID), as well as with high levels of matrix metalloproteinases (MMPs). Low-intensity pulsed ultrasound (LIPUS) accelerates bone fracture healing in clinical studies, and angiogenic factors are involved in the mechanism of action. In the present study, we examined the effects of LIPUS on HDR in a rat in vitro HD model. HDR was enhanced by LIPUS as measured by the change in the wet weight of the cultured ID. The secretion of tumor necrosis factor-alpha (TNF-alpha) and macrophage chemoattractant protein-1 (MCP-1) from Mphi into the culture medium was stimulated by LIPUS. LIPUS also enhanced matrix metalloproteinase-3 (MMP-3) maturation. Moreover, many apoptotic cell death were observed in the HDR groups with LIPUS exposure. These results suggest that LIPUS enhanced the HDR via MMP-3 activation through TNF-alpha and MCP-1 pathways. Although animal studies and clinical trial are needed to understand the LIPUS effects on HDR, LIPUS treatment might be an effective treatment for accelerating HDR.

16) Low-intensity ultrasound inhibits apoptosis and enhances viability of human mesenchymal stem cells in three-dimensional alginate culture during chondrogenic differentiation.

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Many studies have investigated optimal chondrogenic conditions, but only a few of them have addressed their effects on cell viability or the methods to enhance it.

This study investigated the effect of low-intensity ultrasound (LIUS), a well-known chondrogenic inducer, on the viability of human mesenchymal stem cells (hMSCs) during chondrogenic differentiation in three-dimensional (3-D) alginate culture. The hMSCs/alginate layer was cultured in a chondrogenic defined medium and treated with transforming growth factor-beta1 (TGF-beta1) and/or LIUS for 2 weeks. Along with chondrogenic differentiation for 2 weeks, the 3-D alginate culture and TGF-beta1 treatment resulted in the decrease of cell viability, which appeared to be mediated by apoptosis. In contrast, co-treatment with LIUS clearly enhanced cell viability and inhibited apoptosis under the same conditions. The effect of LIUS on the apoptotic event was further demonstrated by changes in the expression of apoptosis/viability related genes of p53, bax, bcl-2, and PCNA. These results suggest that the LIUS treatment could be a valuable tool in cartilage tissue engineering using MSCs as it enhances cell viability and directs the chondrogenic differentiation process, its well-known activity.

17) Low-intensity ultrasound stimulation enhances chondrogenic differentiation in alginate culture of mesenchymal stem cells.

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Mesenchymal stem cells (MSCs) are regarded as a potential autologous source for cartilage repair, because they can differentiate into chondrocytes by transforming growth factor-beta (TGF-beta) treatment under the 3-dimensional (3-D) culture condition. However, more efficient and versatile methods for chondrogenic differentiation of MSCs are still in demand for its clinical application. Recently, low-intensity ultrasound (LIUS) was shown to enhance fracture healing in vitro and induce chondrogenesis of MSCs in vitro. In this study, we investigated the effects of LIUS on the chondrogenesis of rabbit MSCs (rMSCs) in a 3-D alginate culture and on the maintenance of chondrogenic phenotypes after replating them on a monolayer culture. The LIUS treatment of rMSCs increased: (i) the matrix formation; (ii) the expression of chondrogenic markers such as collagen type II, aggrecan, and Sox-9; (iii) the expression of tissue inhibitor of metalloprotease-2 implicated in the integrity of cartilage matrix; and (iv) the capacity to maintain the chondrogenic phenotypes in a monolayer culture. Notably, LIUS effects were clearly shown even without TGF-beta treatment. These results suggest that LIUS treatment could be an efficient and cost-effective method to induce chondrogenic differentiation of MSCs in vitro for cartilage tissue engineerin

18) Treatment of human mesenchymal stem cells with pulsed low intensity ultrasound enhances the chondrogenic phenotype in vitro.

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This study examined the effects of low intensity pulsed ultrasound (LIPUS) on human bone marrow-derived mesenchymal stem cells undergoing chondrogenic differentiation. Aggregates of mesenchymal stem cells and mesenchymal stem cells seeded in three dimensional matrices were cultured in a defined chondrogenic medium and subjected to LIPUS for the first 7 days of culture. At 1, 7, 14 and 21 days, samples were harvested for histology, immunohistochemistry, RT-PCR, and quantitative DNA and matrix macromolecule analysis. Cell aggregates with daily treatment for 20 minutes showed no significant differences for proteoglycan and collagen content during chondrogenic differentiation. However ultrasound application for 40 minutes daily resulted in a statistically significant increase of the proteoglycan and collagen content after 21 days in culture. Aggregates treated for 20 minutes daily showed decreased expression of chondrogenic genes at all time points. In contrast, 40 minutes of daily treatment of aggregates resulted in a significant increase of chondrogenic marker genes after an initial decrease at day 7 with time in culture. Ultrasound treated cell-scaffold constructs showed a significant increase of chondrogenic marker gene expression and extracellular matrix deposition. This study indicates that LIPUS can be used to enhance the chondrogenesis of mesenchymal stem cells in cell aggregates and cell-scaffold constructs. We have found a dependency on the specific treatment parameters. We hypothesize that LIPUS can be used for an improved in vitro preparation of optimized tissue engineering implants for cartilage repair. Furthermore this non-invasive method could also be of potential use in vivo for regenerative therapy of cartilage in the future.

19) Low-intensity ultrasound stimulates the viability and matrix gene expression of human articular chondrocytes in alginate bead culture.

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We investigated the effects of low-intensity ultrasound (LIUS) on the activity of human articular chondrocytes isolated from osteoarthritis patients and cultured in the three-dimensional alginate beads. LIUS was treated at 0, 100, 200, and 300 mW/cm(2) for 10 min everyday for 2, 7, or 15 days. LIUS induced the viability of

cells only at day 15 but not until day 7 after treatment, when examined by trypan blue exclusion and LIVE/DEAD(R) assay kit. When examined at day 7, the proliferation of cells was not changed by LIUS in the (3)H-thymine incorporation. The expression of matrix producing proteins (type II collagen and proteoglycan) was clearly induced by 200-300 mW/cm(2) LIUS in the incorporation of radioactivity and Northern blot analysis. Although the expression of MMP-1, a matrix degrading protein, was decreased, that of TIMP-1, an inhibitor of MMPs, was not affected by LIUS. Histological analysis revealed an increase in the number and size of glycosaminoglycan-positive lacunae and cellular organelles, appearing as rough endoplasmic reticulum and mitochondria by LIUS. These results showed that the viability and metabolism of human articular chondrocytes in alginate culture was induced by LIUS treatment, suggesting that they could be a promising autologous source for cartilage tissue engineering.

20) Low-intensity ultrasound stimulation enhances chondrogenic differentiation in alginate culture of mesenchymal stem cells.

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Mesenchymal stem cells (MSCs) are regarded as a potential autologous source for cartilage repair, because they can differentiate into chondrocytes by transforming growth factor-beta (TGF-beta) treatment under the 3-dimensional (3-D) culture condition. However, more efficient and versatile methods for chondrogenic differentiation of MSCs are still in demand for its clinical application. Recently, low-intensity ultrasound (LIUS) was shown to enhance fracture healing in vitro and induce chondrogenesis of MSCs in vitro. In this study, we investigated the effects of LIUS on the chondrogenesis of rabbit MSCs (rMSCs) in a 3-D alginate culture and on the maintenance of chondrogenic phenotypes after replating them on a monolayer culture. The LIUS treatment of rMSCs increased: (i) the matrix formation; (ii) the expression of chondrogenic markers such as collagen type II, aggrecan, and Sox-9; (iii) the expression of tissue inhibitor of metalloprotease-2 implicated in the integrity of cartilage matrix; and (iv) the capacity to maintain the chondrogenic phenotypes in a monolayer culture. Notably, LIUS effects were clearly shown even without TGF-beta treatment. These results suggest that LIUS treatment could be an efficient and cost-effective method to induce chondrogenic differentiation of MSCs in vitro for cartilage tissue engineering.

21) Treatment of human mesenchymal stem cells with pulsed low intensity ultrasound enhances the chondrogenic phenotype in vitro.

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This study examined the effects of low intensity pulsed ultrasound (LIPUS) on human bone marrow-derived mesenchymal stem cells undergoing chondrogenic differentiation. Aggregates of mesenchymal stem cells and mesenchymal stem cells seeded in three dimensional matrices were cultured in a defined chondrogenic medium and subjected to LIPUS for the first 7 days of culture. At 1, 7, 14 and 21 days, samples were harvested for histology, immunohistochemistry, RT-PCR, and quantitative DNA and matrix macromolecule analysis. Cell aggregates with daily treatment for 20 minutes showed no significant differences for proteoglycan and collagen content during chondrogenic differentiation. However ultrasound application for 40 minutes daily resulted in a statistically significant increase of the proteoglycan and collagen content after 21 days in culture. Aggregates treated for 20 minutes daily showed decreased expression of chondrogenic genes at all time points. In contrast, 40 minutes of daily treatment of aggregates resulted in a significant increase of chondrogenic marker genes after an initial decrease at day 7 with time in culture. Ultrasound treated cell-scaffold constructs showed a significant increase of chondrogenic marker gene expression and extracellular matrix deposition. This study indicates that LIPUS can be used to enhance the chondrogenesis of mesenchymal stem cells in cell aggregates and cell-scaffold constructs. We have found a dependency on the specific treatment parameters. We hypothesize that LIPUS can be used for an improved in vitro preparation of optimized tissue engineering implants for cartilage repair. Furthermore this non-invasive method could also be of potential use in vivo for regenerative therapy of cartilage in the future.

22) Molecular mechanisms of low intensity pulsed ultrasound in human skin fibroblasts.

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Soluble factors such as polypeptide growth factors, mitogenic lipids, inflammatory cytokines, and hormones are known regulators of cell proliferation. However, the effect of mechanical stimuli on cell proliferation is less well understood. Here we examined the effect of low intensity pulsed ultrasound (US), which is used to promote wound healing, on the proliferation of primary human

foreskin fibroblasts and the underlying signaling mechanisms. We show that a single 6-11-min US stimulation increases bromodeoxyuridine incorporation. In addition, an increase in the total cell number is observed after sequential US stimulation. US induced stress fiber and focal adhesion formation via activation of Rho. We further observed that US selectively induced activation of extracellular signal-regulated kinase (ERK) 1/2. Inhibition of Rho-associated coiled-coil-containing protein kinase (ROCK) prevented US-induced ERK1/2 activation, demonstrating that the Rho/ROCK pathway is an upstream regulator of ERK activation in response to US. Consequently, activation of ROCK and MEK-1 was required for US-induced DNA synthesis. Finally, an integrin beta(1) blocking antibody as well as a RGD peptide prevented US-induced DNA synthesis. In addition, US slightly increased phosphorylation of Src at Tyr(416), and Src activity was found to be required for ERK1/2 activation in response to US. In conclusion, our data demonstrate for the first time that US promotes cell proliferation via activation of integrin receptors and a Rho/ROCK/Src/ERK signaling pathway.