REGULATION OF AGGRECAN GENE EXPRESSION USING SPECIFIC AND SELECTIVE ELECTRICAL AND ELECTROMAGNETIC SIGNALS

Cross-Reference to Related Applications

The present patent application is a continuation-in-part patent application of U.S. Patent Application Serial No. 10/257,126, filed October 8, 2002, which is the U.S. national phase patent application of PCT/US01/05991, filed February 23, 2001, which, in turn, claims the benefit of the filing date of U.S. Provisional Patent Application Serial No. 60/184,491, filed February 23, 2000.

Field of the Invention

The present invention is directed to a method of regulating aggrecan gene expression in cartilage cells via the application of fields generated by specific and selective electric and electromagnetic signals for the treatment of injured or diseased articular cartilage, as well as a device for generating such signals.

Background of the Invention

The bioelectrical interactions and activity believed to be present in a variety of biological tissues and cells are one of the least understood of the physiological processes. However, there has recently been much research into these interactions and activity regarding the growth and repair of certain tissues and cells. In particular, there has been much research into stimulation by electric and electromagnetic fields and its effect on the growth and repair of bone and cartilage. Researchers believe that such research might be useful in the development of new treatments for a variety of medical problems.

Osteoarthritis, also known as degenerative joint disease, is characterized by degeneration of articular cartilage as well as proliferation and remodeling of subchondral bone. The usual symptoms are stiffness, limitation of motion, and pain. Osteoarthritis is the most common form of arthritis, and prevalence rates increase

markedly with age. It has been shown that elderly patients with self-reported osteoarthritis visit doctors twice as frequently as their unaffected peers. Such patients also experience more days of restricted activity and bed confinement compared to others in their age group. In one study, the majority of symptomatic patients became significantly disabled during an 8-year follow-up period. Massardo et al., Ann Rheum Dis 48: 893-7 (1989).

Nonsteroidal anti-inflammatory drugs (NSAIDs) remain the primary treatment modality for osteoarthritis. It is unknown whether the efficacy of NSAIDs is dependent upon their analgesic or anti-inflammatory properties or the slowing of degenerative processes in the cartilage. There is also a concern that NSAIDs may be deleterious to patients. For example, NSAIDs have well known toxic effects in the stomach, gastrointestinal tract, liver and kidney. However, aspirin inhibits proteoglycan synthesis and normal cartilaginous repair processes in animals. One study in humans suggested that indomethacin might accelerate breakdown of hip cartilage. All adverse effects appear more commonly in the elderly - the very population most susceptible to osteoarthritis.

In the disease commonly known as osteoporosis, bone demineralizes and becomes abnormally rarefied. Bone comprises an organic component of cells and matrix as well as an inorganic or mineral component. The cells and matrix comprise a framework of collagenous fibers that is impregnated with the mineral component of calcium phosphate (85%) and calcium carbonate (10%) that imparts rigidity to the bone. While osteoporosis is generally thought as afflicting the elderly, certain types of osteoporosis may affect persons of all ages whose bones are not subject to functional stress. In such cases, patients may experience a significant loss of cortical and cancellous bone during prolonged periods of immobilization. Elderly patients are known to experience bone loss due to disuse when immobilized after fracture of a bone, which may ultimately lead to a secondary fracture in an already osteoporotic skeleton. Diminished bone density may lead to vertebrae collapse, fractures of hips, lower arms, wrists, ankles as well as incapacitating pains. Alternative nonsurgical therapies for such diseases are needed.

Pulsed electromagnetic fields (PEMF) and capacitive coupling (CC) have been used widely to treat nonhealing fractures and related problems in bone healing since approval by the Food and Drug Administration in 1979. The original basis for the trial of this form of therapy was the observation that physical stress on bone causes the appearance of tiny electric currents that, along with mechanical strain, were thought to be the mechanisms underlying transduction of the physical stresses into a signal that promotes bone formation. Along with direct electric field stimulation that was successful in the treatment of nonunion, noninvasive technologies using PEMF and capacitive coupling (where the electrodes are placed on the skin in the treatment zone) were also found to be effective. Pulsed electromagnetic fields generate small induced currents (Faraday currents) in the highly conductive extracellular fluid, while capacitive coupling directly causes currents in the tissues; both PEMFs and CC thereby mimic endogeneous electrical currents.

The endogeneous electrical currents, originally thought to be due to phenomena occurring at the surface of crystals in the bone, have been shown to be due primarily to movement of fluid containing electrolytes in channels of the bone containing organic constituents with fixed negative charges, generating what are called "streaming potentials." Studies of electrical phenomena in cartilage have demonstrated a mechanical-electrical transduction mechanism that resembles those described in bone, appearing when cartilage is mechanically compressed, causing movement of fluid and electrolytes over the surface of fixed negative charges in the proteoglycans and collagen in the cartilage matrix. These streaming potentials apparently serve a purpose in cartilage similar to that in bone, and, along with mechanical strain, lead to signal transduction that is capable of stimulating chondrocyte synthesis of matrix components.

The main application of direct current, capacitive coupling, and PEMFs has been in orthopedics in healing of nonunion bone fractures (Brighton et al., *J. Bone and Joint Surgery*, 63: 2-13, 1981; Brighton and Pollack, *J. Bone and Joint Surgery*, 67: 577-585, 1985; Bassett et al., *Crit. Rev. Biomed. Eng.*, 17: 451-529 (1989); Bassett et al., *J AMA* 247: 623-8 (1982). Clinical responses have been reported in avascular necrosis of hips in adults and Legg-Perthes's disease in children. Bassett et al., *Clin Orthop* 246: 172-6 (1989); Aaron et al., *Clin Orthop* 249: 209-18 (1989); Harrison et al, *J Pediatr*

Orthop 4: 579-84 (1984). It has also been shown that PEMFs (Mooney, *Spine*, 15: 708-712, 1990) and capacitive coupling (Goodwin, Brighton et al., *Spine*, 24: 1349-1356, 1999) can significantly increase the success rate of lumbar fusions. There are also reports of augmentation of peripheral nerve regeneration and function and promotion of angiogenesis. Bassett, *Bioassays* 6: 36-42 (1987). Patients with persistent rotator cuff tendinitis refractory to steroid injection and other conventional measures, showed significant benefit compared with placebo treated patients. Binder et al., *Lancet* 695-8 (1984). Finally, Brighton et al. have shown in rats the ability of an appropriate capacitive coupling electric field to both prevent and reverse vertebral osteoporosis in the lumbar spine (Brighton et al., *J. Orthop. Res.* 6: 676-684, 1988; Brighton et al., *J. Bone and Joint Surgery*, 71: 228-236, 1989).

More recently, research in this area has focused on the effects stimulation has on tissues and cells. For example, it has been conjectured that direct currents do not penetrate cellular membranes and that control is achieved via extracellular matrix differentiation. Grodzinsky, *Crit. Rev. Biomed. Engng* 9:133 (1983). In contrast to direct currents, it has been reported that PEMFs can penetrate cell membranes and either stimulate them or directly affect intracellular organelles. An examination of the effect of PEMFs on extracellular matrices and *in vivo* endochondral ossification found increased synthesis of cartilage molecules and maturation of bone trabeculae. Aaron et al., *J. Bone Miner. Res.* 4: 227-233 (1989). More recently, Lorich, Brighton et al. reported (*Clin Orthop and Related Research* 350: 246-256, 1998) that signal transduction of a capacitively coupled electric signal is via voltage gated calcium channels, leading to an increase in cytosolic calcium with a subsequent increase in activated (cytoskeletal) calmodulin.

Much research has been directed at studying tissue culture in order to understand the mechanisms of response. In one study, it was found that electric fields increased [^{3}H]-thymidine incorporation into the DNA of chondrocytes, supporting the notion that Na and Ca^{2+} fluxes generated by electrical stimulation trigger DNA synthesis. Rodan et al., *Science* 199: 690-692 (1978). Studies have found changes in the second messenger, cAMP, and cytoskeletal rearrangements due to electrical perturbations. Ryaby et al., *Trans. BRAGS* 6: (1986); Jones et al., *Trans. BRAGS* 6: 51 (1986); Brighton

and Townsend, *J. Orthop. Res.* 6: 552-558, 1988. Other studies have found effects on glycosaminoglycan, sulphation, hyaluronic acid, lysozyme activity and polypeptide sequences. Norton et al., *J. Orthop. Res.* 6: 685-689 (1988); Goodman et al., *Proc. Natn. Acad. Sci.* USA 85: 3928-3932 (1988).

It was reported in 1996 by the present inventors that a cyclic biaxial 0.17% mechanical strain produces a significant increase in TGF-β_1 mRNA in cultured MC3T3-E1 bone cells. Brighton et al., *Biochem. Biophys. Res. Commun.* 229: 449-453 (1996). Several significant studies followed in 1997. In one study it was reported that the same cyclic biaxial 0.17% mechanical strain produced a significant increase in PDGF-A mRNA in similar bone cells. Brighton et al., *Biochem. Biophys. Res. Commun.* 43: 339-346 (1997). It was also reported that a 60 kHz capacitively coupled electric field of 20 mV/cm produced a significant increase in TGF-β_1 in similar bone cells. Brighton et al., *Biochem. Biophys. Res. Commun.* 237: 225-229 (1997). However, the effect such a field would have on other genes has not been reported in the literature.

In the above-referenced parent patent application, entitled "Regulation of Genes Via Application of Specific and Selective Electrical and Electromagnetic Signals," methods were disclosed for determining the specific and selective electrical and electromagnetic signals for use in creating fields for regulating target genes of diseased or injured tissues. The present invention builds upon the technique described therein by describing the method of regulating one targeted gene expression, namely, aggrecan gene expression, through application of a field generated by a specific and selective electrical and electromagnetic signal, for the treatment of cartilage disease (arthritis), cartilage injury, and cartilage defects.

Summary of the Invention

The present invention relates to regulating the aggrecan gene expression in cartilage cells via the application of fields generated by specific and selective electric and/or electromagnetic signals. By performing dose-response curves on the electric field duration, amplitude, frequency, and duty cycle, the optimal signal for up-regulating aggrecan mRNA in articular cartilage chondrocytes was discovered. The optimal signal generated a capacitively coupled electric field with an amplitude of 20 mV/cm, a

duration of 30 minutes, a duty cycle of 50%, a frequency of 60 kHz, and a sine wave configuration. In particular, the present invention relates to up-regulating aggrecan gene expression in cartilage cells via the application of fields generated by such signals.

In a preferred embodiment of the invention, methods are provided to specifically and selectively up-regulate the gene expression of aggrecan mRNA with capacitively coupled electric fields, electromagnetic fields, or combined fields. Osteoarthritis, rheumatoid arthritis, cartilage injury, cartilage defects, and the like are treated with a capacitively coupled electric field of about 20 mV/cm with an electric field duration of about 30 minutes, an electric field amplitude of about 10-20 mV/cm, a frequency of about 60 kHz, a duty cycle of about 50%, and a sine wave configuration that causes the expression of aggrecan mRNA to be up-regulated. In accordance with the method of the invention, a "specific and selective" signal is a signal that has predetermined characteristics of amplitude, duration, duty-cycle, frequency, and waveform that up-regulates the expression of the aggrecan gene (specificity). This allows one to choose different signals to up-regulate aggrecan gene expressions in order to achieve a given biological or therapeutic response (selectivity). The invention further relates to devices employing the methods described herein to generate specific and selective signals that create fields to up-regulate the expression of the aggrecan gene.

In related aspects, the present invention relates to methods and devices for the treatment of osteoarthritis, rheumatoid arthritis, cartilage injury, and cartilage defects. The method of the invention also includes the methodology for determining the "specific and selective" signal for the aggrecan gene by methodically varying the duration of a starting signal known to increase or suspected to increase cellular production of aggrecan. After selecting the optimal duration, the amplitude of the signal is varied for the optimal duration of time as determined by the gene expression of aggrecan. The duty cycle, frequency, and waveform are varied methodically while keeping the other signal characteristics constant. This process is repeated until the optimal signal is determined that produces the greatest increase in the expression of aggrecan .

These and other aspects of the present invention will be elucidated in the following detailed description of the invention.

Brief Description of the Drawings

The present invention will be apparent from the following detailed description of the invention taken in conjunction with the accompanying drawings, of which:

Figure 1 is a graphic representation of aggrecan mRNA expression when articular cartilage chondrocytes are exposed to a 20 mV/cm capacitively coupled electric field for various time durations. As indicated, the optimum aggrecan mRNA production occurred with a signal of 30 minutes duration.

Figure 2 is a graphic representation of aggrecan mRNA expression when articular cartilage chondrocytes are exposed to various amplitudes of a capacitively coupled field of 30 minutes duration. As indicated, the optimum production of aggrecan mRNA occurred with a signal producing an electric field amplitude of 10-20 mV/cm.

Figure 3 is a graphic representation of aggrecan mRNA expression when articular cartilage chondrocytes are exposed to a capacitively coupled electric field of various duty cycles with a duration of 30 minutes (on time) at 20 mV/cm electric field amplitude. As indicated, the optimum duty cycle is 50%.

Figure 4 is a graphic representation of articular cartilage chondrocytes exposed to a capacitively coupled electric field of various frequencies of 30 minutes duration (on time) at 20 mV/cm electric field amplitude with a 50% duty cycle. As indicated, the optimum frequency is 60 kHz.

Figure 5 is a graphic representation of articular cartilage chondrocytes grown for 7 days in culture and then exposed to the optimal capacitively coupled electric field of 20 mV/cm, 50% duty cycle (30 minutes duration on time), at a frequency of 60 kHz, and a sine wave configuration. No interleukin was present in the media of the cultures and, as indicated, hexosamine showed a 3.5 fold increase.

Figure 6 is a graphic representation of articular cartilage chondrocytes grown for 7 days then exposed to the optimal capacitively coupled electric field of 20 mV/cm, 50% duty cycle (30 minutes duration on time), at a frequency of 60 kHz, and a sine wave configuration. Interleukin (10 ug/ml) or IL-β_1 was added to the media at day 7. As indicated, hexosamine showed a 3.5 fold increase despite the presence of interleukin in the media of the cultures.

Figure 7 is a diagram illustrating a device for the treatment of osteoarthritis of the knee, in accordance with a preferred embodiment of the present invention.

Detailed Description of Preferred Embodiments of the Invention

The invention will be described in detail below with reference to **Figures 1-7**. Those skilled in the art will appreciate that the description given herein with respect to those figures is for exemplary purposes only and is not intended in any way to limit the scope of the invention. All questions regarding the scope of the invention may be resolved by referring to the appended claims.

The present invention is based on the discovery that the expression of certain genes can be regulated by the application of fields generated by specific and selective electric and/or electromagnetic signals. In other words, it has been discovered by the present inventors that there is a specific electric and/or electromagnetic signal that generates a field for regulating each gene in bone, cartilage and other tissue cells and that these specific signals are capable of specifically and selectively regulating the genes in such cells. In particular, gene expression governing the growth, maintenance, repair, and degeneration or deterioration of tissues or cells can be regulated in accordance with the invention via the application of fields generated by specific and selective electric and /or electromagnetic signals so as to produce a salutary clinical effect. Such discoveries are useful in the development of treatment methods that target certain medical conditions including bone fractures and defects, osteoarthritis, osteoporosis, cancer and other diseases, as well as for developing devices employing such methods.

As used herein, the phrase "signal" is used to refer to a variety of signals including mechanical signals, ultrasound signals, electromagnetic signals and electric signals output by a device. It is to be understood that the term "field" as used herein refers to an electrical field within targeted tissue, whether it is a combined field or a pulsed electromagnetic field or generated by direct current, capacitive coupling or inductive coupling.

The phrase "remote" is used to mean acting, acted on or controlled from a distance. "Remote" regulation refers to controlling the expression of a gene from a distance. To provide "remotely" refers to providing from a distance. For example, providing a specific and selective signal from a remote source can refer to providing the signal from a source at a distance to tissue or a cell or from a source outside of or external to the body.

The phrase "specific and selective" signal means a signal that produces an electric field that has predetermined characteristics of amplitude, duration, duty-cycle, frequency, and waveform that up-regulate or down-regulate a targeted gene or targeted functionally complementary genes (specificity). This allows one to choose different "specific and selective" signals to up-regulate or down-regulate various gene expressions in order to achieve a given biological or therapeutic response (selectivity).

The term "regulate" means to control gene expression. Regulate is understood to include both up-regulate and down-regulate. Up-regulate means to increase expression of a gene, while down-regulate means to inhibit or prevent expression of a gene.

"Functionally complementary" refers to two or more genes whose expressions are complementary or synergistic in a given cell or tissue.

"Tissue" refers to an aggregate of cells together with their extracellular substances that form one of the structural materials of a patient. As used herein, the term "tissue" is intended to include muscle and organ tissue as well as bone or cartilage tissue. Also, the term "tissue" as used herein may also refer to an individual cell.

"Patient" refers to an animal, preferably a mammal, more preferably a human.

The present invention provides treatment methods and devices that target certain tissues, cells or diseases. In particular, the gene expression associated with the repair process in injured or diseased tissues or cells can be regulated by the application of fields generated by electric signals that are specific and selective for the genes to be regulated in the target tissues or cells. Gene expression can be up-regulated or down-regulated by the application of signals that are specific and selective for each gene or each set of complementary genes so as to produce a beneficial clinical effect. For

example, a particular specific and selective signal may create an electric field that up-regulates a certain desirable gene expression, while the same or another particular specific and selective signal may create an electric field that down-regulates a certain undesirable gene expression. A certain gene may be up-regulated by a field generated by one particular specific and selective signal and down-regulated by a field generated by another specific and selective signal. Those skilled in the art will understand that certain diseased or injured tissues can be targeted for treatment by regulating those genes governing the growth, maintenance, repair, and degeneration or deterioration of the tissues.

The methods and devices of the present invention are based on identifying those signals that generate fields that are specific and selective for the gene expression associated with certain targeted diseased or injured tissue. For example, electricity in its various forms (e.g., capacitive coupling, inductive coupling, combined fields) can specifically and selectively regulate gene expression in targeted tissues or cells in a patient's body by varying the frequency, amplitude, waveform or duty cycle of the applied field for each selected gene. The duration of time exposed to electricity can also influence the capability of electricity to specifically and selectivity regulate gene expression in targeted tissues or cells in a patient's body. Specific and selective signals may generate electric fields for application to each gene systematically until the proper combination of frequency, amplitude, waveform, duty cycle, and duration is found that provides the desired effect on gene expression.

It is to be understood that a variety of diseased or injured tissues or disease states can be targeted for treatment because the specificity and selectivity of an electric field for a certain gene expression can be influenced by several factors. In particular, an electrical field of appropriate frequency, amplitude, waveform and/or duty cycle can be specific and selective for the expression of certain genes and thus provide for targeted treatments. Temporal factors (e.g., duration of time exposed to the electrical field) can also influence the specificity and selectivity of an electric field for a particular gene expression. The regulation of gene expression may be more effective (or made possible) via the application of an electrical field for a particular duration of time. Therefore, those skilled in the art will understand that the present invention provides for

varying the frequency, amplitude, waveform, duty cycle and/or duration of application of an electric field until the electric field is found to be specific and selective for certain gene expressions in order to provide for treatments targeting a variety of diseased or injured tissue or diseases.

Thus, the present invention can provide for targeted treatments because it is possible to regulate expression of certain genes associated with a particular diseased or injured tissue via the application of fields generated by specific and selective signals of appropriate frequency, amplitude, waveform and/or duty cycle for an appropriate duration of time. The specificity and selectivity of a signal generating an electrical field may thus be influenced so as to regulate the expression of certain genes in order to target certain diseased or injured tissue or disease states for treatment. In particular, the present invention provides for the targeted treatment of osteoarthritis, rheumatoid arthritis, cartilage injury, and cartilage defects.

The present invention also provides a device that includes a source of at least one signal specific and selective for aggrecan gene expression. The devices of the present invention can provide for the production of such signals for application to cartilage cells by at least one electrode adapted to apply the field generated by the specific and selective signal.

The device of the present invention is capable of applying a field generated by specific and selective signals directly to diseased or injured tissue and/or to the skin of a patient. The device of the present invention may also provide for the remote application of specific and selective fields (e.g., application of a field at a distance from diseased or injured tissue), although it will be appreciated that capacitively coupled devices must touch the subject's skin. The device of the present invention may include means for attaching the electrodes to the body of a patient in the vicinity of injured or diseased tissue. For example, self-adherent conductive electrodes may be attached to the skin of the patient on both sides of a knee joint afflicted with osteoarthritis as shown in **Figure 7**. As also shown in **Figure 7**, the device 10 of the present invention may include self-adherent electrodes 12 for attaching the device 10 to the body of a patient. For example, the device 10 of the present invention may include electrodes 12 attached to a power unit 14 that has a VELCRO® patch 16 on the reverse

side such that the power unit 14 can be attached to a VELCRO® strap (not shown) fitted around the calf, thigh or waist of the patient.

The device 10 of the present invention can be employed in a variety of ways. The device 10 may be portable or may be temporarily or permanently attached to a patient's body. The device 10 of the present invention is preferably non-invasive. For example, the device 10 of the present invention may be applied to the skin of a patient by application of electrodes adapted for contact with the skin of a patient for the application of fields generated by the predetermined specific and selective signals. Such signals may also be applied via coils in which time varying currents flow, thus producing specific and selective electromagnetic fields that penetrate the tissue. The device 10 of the present invention may also be capable of implantation in a patient, including implantation under the skin of a patient.

The example below will illustrate that the method of the present invention may provide for cartilage growth and repair. Cartilage growth and repair can be stimulated via signals specific and selective for the regulation of expression of aggrecan in cartilage cells so as to stimulate articular cartilage repair in osteoarthritis patients. In particular, the methods of the present invention can provide for the up-regulation of aggrecan genes that repair cartilage. A variety of cartilage cells can be targeted by the methods of the present invention including articular chondrocytes and including articular cartilage, hyaline cartilage, and growth plate cartilage.

The example below further illustrates that the method of the present invention provides for the regulation of gene expression in articular chondrocytes. For example, in the example below, fetal articular chondrocytes have been exposed to a capacitively coupled 60 kHz electrical field of 20 mV/cm for 0.5, 2.0, 6.0 and 24.0 hours. A statistically significant incorporation of $^{35}SO_4$/ug DNA (indicating significant proteoglycan synthesis) was found after only 0.5 hours of stimulation. An identical experiment was repeated and the levels of aggrecan mRNA, the messenger for the major cartilage proteoglycan, monitored. After only 0.5 hours of electrical stimulation there was a significant increase (almost 100%) in aggrecan mRNA. Accordingly, temporal factors may influence the specificity and selectivity of a signal that generates electric fields for regulating gene expression in articular chondrocytes.

Those skilled in the art will understand that a variety of other cartilage diseases and injuries may be targeted for treatment via the method of the present invention.

Those skilled in the art will further understand that the devices of the present invention can be provided in a variety of forms including a capacitively coupled power unit with programmed multiple switchable specific and selective signals for application to one pair or to multiple pairs of electrodes, electromagnetic coils attached to a power unit with switchable multiple specific and selective signals, and an ultrasound stimulator with a power supply for generating specific and selective signals. Generally speaking, device preference is based on patient acceptance and patient compliance. The smallest and most portable unit available in the art at the present time is a capacitive coupling unit; however, patients with extremely sensitive skin may prefer to use inductive coupling units. On the other hand, ultrasound units require the most patient cooperation but may be desirable for use by certain patients.

Example

The invention is demonstrated in the following example, which is for purposes of illustration and is not intended to limit the scope of the present invention.

Materials and Methods

Chondrocyte cultures were prepared from fetal bovine articular cartilage. Chondrocytes (5×10^5 cells/cm^2) were plated onto specially modified Cooper dishes. The cells were grown to seven days with the medium changed just prior to beginning of the experimental condition. The experimental cell cultures throughout these studies were subjected to a capacitively coupled 60 kHz sine wave signal electric field with an output of 44.81 volts peak to peak. This produced a calculated-field strength in the culture medium in the dishes of 20 mV/cm with a current density of 300 μA/cm^2. Control cell culture dishes were identical to that of the stimulated dishes except that the electrodes were not connected to a function generator.

Total RNA was isolated using TRIzol, according to the manufacturer's instructions, and reversed transcription using SuperScript II reverse transcriptase was performed. Oligonucleotide primers to be used in the competitive PCR technique were

selected from published cDNA sequences. Quantitative analysis of PCR products was performed using ScionImage software.

The optimal signal for the desired gene regulation was found systematically as follows. An electrical signal known to increase (or even just suspected to increase) cellular production of a given protein is taken as the starting signal for determining the specific signal for generating the field for the gene expression (mRNA) of that protein. A dose-response curve is first performed by varying the duration of the signal while holding all the other signal characteristics constant (amplitude, duty-cycle, frequency, and waveform) (Figure 1). This determines the optimal duration of the starting signal for the gene expression of that protein. A second dose-response curve is performed by varying the field amplitude for the optimal duration of time (Figure 2). This determines the optimal field amplitude for the optimal duration of time as determined by the gene expression of the protein of interest. A third dose-response curve is then performed, this time varying the duty-cycle from 100% (constant) to 1% or less while holding the optimal amplitude and other signal characteristics constant (Figure 3). A dose-response is repeated a fourth time (varying frequency) (Figure 4) and a fifth time (varying waveform) each time keeping the other signal characteristics constant. By this method an optimal signal is determined for producing the greatest increase in the gene expression of the protein of interest.

Protein expression may be determined by any method known in the art, such as reverse transcriptase PCR, Northern analysis, immunoassays, and the like.

Aggrecan production by articular chondrocytes

Articular chondrocytes were exposed to a capacitively coupled electric field of 20 mV/cm at 60 kHz. The results are illustrated in **Figures 1-4**.

Figure 1 is a graphic representation of aggrecan mRNA expression when articular cartilage chondrocytes (attomole per μl) are exposed to a 20 mV/cm capacitively coupled electric field for various time durations (0.5, 2, 6, and 24 hours). As indicated, the optimum aggrecan mRNA production (almost a two-fold increase) occurred with a signal of 30 minutes duration.

Figure 2 is a graphic representation of aggrecan mRNA expression when articular cartilage chondrocytes (attomole per μl) are exposed to various amplitudes (1, 5,

10, 20, and 40 mV/cm) of a capacitively coupled field of 30 minutes duration. As indicated, the optimum production of aggrecan mRNA occurred with a signal producing an electric field amplitude of 10-20 mV/cm.

Figure 3 is a graphic representation of aggrecan mRNA expression when articular cartilage chondrocytes (attomole per μl) are exposed to a capacitively coupled electric field of various duty cycles (6.7%, 12.5%, 50%, 75%, and 100%) with a duration of 30 minutes (on time) at 20 mV/cm electric field amplitude. As indicated, the optimum duty cycle is 50%.

Figure 4 is a graphic representation of articular cartilage chondrocytes (attomole per μl) exposed to a capacitively coupled electric field of various frequencies (30kHz, 60 kHz, and 120 kHz) of 30 minutes duration (on time) at 20 mV/cm electric field amplitude with a 50% duty cycle. As indicated, the optimum frequency is 60 kHz.

Figure 5 is a graphic representation of articular cartilage chondrocytes grown for 7 days in culture and then exposed to the optimal capacitively coupled electric field of 20 mV/cm, 50% duty cycle (30 minutes duration on time), at a frequency of 60 kHz, and a sine wave configuration. The chondrocytes were exposed to this field for 1 hour per day for 7 days. Control chondrocytes were grown under the same conditions but were not exposed to any electric stimulation. No interleukin, a cytokine that degradates articular cartilage, was present in the media of the cultures. As indicated in **Figure 5**, hexosamine, a protein whose synthesis is stimulated and regulated by aggrecan mRNA and is the main constituent of proteoglycan, showed a 3.5 fold increase when exposed to the applied electric field as compared to the control chondrocytes that were not exposed to the electric field.

Figure 6 is a graphic representation of articular cartilage chondrocytes grown for 7 days then exposed to the optimal capacitively coupled electric field of 20 mV/cm, 50% duty cycle (30 minutes duration on time), at a frequency of 60 kHz, and a sine wave configuration. Interleukin (10 ug/ml) or IL-β_1 was added to the media at day 7. The chondrocytes were exposed to this field for 1 hour per day for 7 days. Control chondrocytes were grown under the same conditions with interleukin in the media but were not exposed to any electric stimulation. As indicated in **Figure 6**, despite the presence of interleukin in the media of the cultures, hexosamine, a protein whose

synthesis is stimulated and regulated by aggrecan mRNA and is the main constituent of proteoglycan, still showed a 3.5 fold increase when exposed to the applied electric field as compared to the control cultures that were not exposed to the electric field.

Figure 7 illustrates a device 10 in accordance with the present invention that is used to treat a patient with osteoarthritis of the knee. As illustrated, two circular, soft conductive, self-adherent electrodes 12 are placed on the skin on either side of the knee at the level of the joint line. The electrodes 12 are attached to a power unit 14 that has a VELCRO® patch 16 on the reverse side such that the power unit 14 can be attached to a VELCRO® strap (not shown) fitted around the calf, thigh or waist. The electrodes 12 may be placed on the skin before the patient goes to bed each evening or any other convenient time. Of course, other suitable types of electrodes 12 may also be used.

The power unit 14 is preferably small (e.g., 6-8 ounces) and powered by a standard 9-volt battery to emit a 5-volt peak-to-peak, 6-10 mAmp, 20 mV/cm, 60 kHz sine wave signal to the electrodes 12 placed on the skin. When this signal is provided approximately 30 minutes per day with the proper duty cycle (50%), it has been shown to significantly up-regulate genes encoding aggrecan. This treatment should prevent or minimize further articular cartilage deterioration as well as to heal articular cartilage that already is damaged or degenerated.

The example described above demonstrates that the expression of the aggrecan gene may be significantly up-regulated to increase the production of proteoglycan in articular cartilage so as to treat arthritis (both osteoarthritis and rheumatoid arthritis), cartilage injury, and cartilage defects. Proteoglycan, along with Type II collagen, is the main constituent of articular cartilage and is degradated and destroyed early in the development of arthritis. The present invention clearly shows that the optimal electric field described in the example can very significantly up-regulate aggrecan mRNA and, hence, increase proteoglycan synthesis, even in the presence of IL-β_1. Those skilled in the art will appreciate that an appropriate electric field, as described herein with capacitive coupling, is also equally effective with any and all electromagnetic systems that produce equivalent, or nearly equivalent, electric field characteristics. Those skilled in the art will also appreciate that more particularized

signal characteristics may be discovered through more experimentation with more data points (e.g., a 50±3% duty cycle for 30±3 minutes), but such relatively minor variations in each of the signal characteristics are believed to be within the level of those skilled in the art given the teachings herein.

Those skilled in the art will also appreciate that numerous other modifications to the invention are possible within the scope of the invention. For example, the optimal field described herein can be applied to any joint via two or more appropriate surface electrodes, in pairs or strips, incorporated in garments, braces, wraps, or casts, and delivered by means of capacitive coupling, inductive coupling (electromagnetic fields), or combined fields. Accordingly, the scope of the invention is not intended to be limited to the preferred embodiment described above, but only by the appended claims.

What is claimed is:

1. A method of specifically and selectively up-regulating the gene expression of aggrecan mRNA in cartilage tissue, comprising the steps of:

generating at least one specific and selective signal that is substantially optimized to up-regulate the gene expression of aggrecan mRNA; and

exposing the cartilage tissue to a field generated by the specific and selective signal for a predetermined duration of time at predetermined intervals so as to up-regulate the gene expression of aggrecan mRNA.

2. The method of claim 1 wherein the generating step comprises the step of selectively varying the amplitude, duration, duty cycle, frequency, and waveform of the specific and selective signal until the gene expression of aggrecan mRNA in the cartilage tissue is substantially optimized.

3. The method of claim 1 wherein the exposing step comprises the step of exposing an articular cartilage chrondrocyte to the field generated by the specific and selective signal for the predetermined duration of time at predetermined intervals of once per day.

4. The method of claim 1 wherein said generating step comprises the step of generating the specific and selective signal at a remote source and said exposing step comprises the step of applying the field generated by the specific and selective signal to the cartilage tissue.

5. The method of claim 4 wherein the exposing step comprises the step of applying the specific and selective signal to electrodes located near the cartilage tissue.

6. The method of claim 5 wherein the exposing step comprises the step of applying the field generated by the specific and selective signal to the cartilage tissue through one of capacitive coupling and inductive coupling.

7. The method of claim 6 wherein the specific and selective signal causes the electrodes to generate one of a capacitive coupling electric field, an electromagnetic field, and a combined field.

8. A method for treating at least one of osteoarthritis, rheumatoid arthritis, cartilage injury, and cartilage defects, comprising the steps of:

generating at least one specific and selective signal that up-regulates the gene expression of aggrecan mRNA; and

exposing cartilage tissue to a field generated by the specific and selective signal for a predetermined duration at predetermined intervals so as to selectively up-regulate gene expression of aggrecan mRNA.

9. The method of claim 8 wherein the exposing step comprises the step of capacitively coupling the field to the cartilage tissue.

10. The method of claim 8 wherein the exposing step comprises the step of applying one of an electromagnetic field and a combined field to the cartilage tissue.

11. The method of claim 8 wherein the generating step comprises the step of generating an electric field having an amplitude of approximately 10-20 mV/cm, a sine wave configuration, a duty cycle of approximately 50%, and a frequency of approximately 60 kHz.

12. The method of claim 11 wherein the exposing step comprises the step of applying the electric field to the cartilage tissue for a duration of approximately 30 minutes every 24 hours.

13. The method of claim 8 wherein the generating step comprises the steps of selectively varying the amplitude, duration, duty cycle, frequency, and waveform of the specific and selective signal until the up-regulation of the gene expression of aggrecan mRNA in the cartilage tissue by the generated field is substantially optimized.

14. The method of claim 13 wherein the exposing step comprises the step of applying the field generated by the specific and selective signal to the cartilage tissue through one of capacitive coupling and inductive coupling.

15. The method of claim 14 wherein the specific and selective signal causes the electrodes to generate one of a capacitive coupling electric field, an electromagnetic field, and a combined field.

16. A device for the treatment of at least one of osteoarthritis, rheumatoid arthritis, cartilage injury and cartilage defects comprising a signal source that provides at least one signal that creates a field specific and selective for up-regulating gene expression of aggrecan mRNA, and electrodes connected to the signal source that receive said at least one specific and selective signal for application of the field to the cartilage tissue for a predetermined duration at predetermined intervals so as to selectively up-regulate gene expression of aggrecan mRNA in said cartilage tissue.

17. The device of claim 16 further comprising a portable power unit that drives said signal source.

18. The device of claim 16 further comprising means for attaching the electrodes to a body of a patient in the vicinity of the cartilage tissue.

19. The device of claim 16 further comprising means for attaching the signal source to a body of a patient.

20. The device of claim 16 wherein the field generated by said at least one specific and selective signal is applied to said cartilage tissue via one of capacitive coupling and inductive coupling.

21. The device of claim 20 wherein the specific and selective signal has a sine wave configuration and generates an electric field having an amplitude of about 10-20 mV/cm at 60 kHz with approximately a 50% duty cycle.

22. A method of treating at least one of osteoarthritis, rheumatoid arthritis, cartilage injury, and a cartilage defect comprising the steps of exposing cartilage tissue to the specific and selective field generated by the device of claim 21 so as to up-regulate expression of aggrecan mRNA in the cartilage tissue.

23. A method of determining a selective signal that generates an electric field that up-regulates aggrecan, comprising the steps of selecting a starting signal known to increase or suspected to affect cellular production of aggrecan, selectively varying a duration of application of said starting signal until an optimal duration that provides an optimal production of aggrecan is found, varying an amplitude of the starting signal for the optimal duration of time until an optimal production of aggrecan is found, and varying a duty cycle of the signal until an optimal production of aggrecan is found.

24. The method of claim 23 comprising the further steps of selectively varying a frequency and waveform of said signal, keeping other signal characteristics constant, until the greatest increase in the gene expression of aggrecan is found.