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Modification of osteoarthritis by pulsed electromagnetic field—a morphological study

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Summary

Objective: Hartley guinea pigs spontaneously develop arthritis that bears morphological, biochemical, and immunohistochemical similarities to human osteoarthritis. It is characterized by the appearance of superficial fibrillation by 12 months of age and severe cartilage lesions and eburnation by 18 months of age. This study examines the effect of treatment with a pulsed electromagnetic field (PEMF) upon the morphological progression of osteoarthritis in this animal model.

Design: Hartley guinea pigs were exposed to a specific PEMF for 1 h/day for 6 months, beginning at 12 months of age. Control animals were treated identically, but without PEMF exposure. Tibial articular cartilage was examined with histological/histochemical grading of the severity of arthritis, by immunohistochemistry for cartilage neoepitopes, 3B3(–) and BC-13, reflecting enzymatic cleavage of aggrecan, and by immunoreactivity to collagenase (MMP-13) and stromelysin (MMP-3). Immunoreactivity to TGFβ, interleukin (IL)-1β, and IL receptor antagonist protein (IRAP) antibodies was examined to suggest possible mechanisms of PEMF activity.

Results: PEMF treatment preserves the morphology of articular cartilage and retards the development of osteoarthritic lesions. This observation is supported by a reduction in the cartilage neoepitopes, 3B3(–) and BC-13, and suppression of the matrix-degrading enzymes, collagenase and stromelysin. Cells immunopositive to IL-1 are decreased in number, while IRAP-positive cells are increased in response to treatment. PEMF treatment markedly increases the number of cells immunopositive to TGFβ.

Conclusions: Treatment with PEMF appears to be disease-modifying in this model of osteoarthritis. Since TGFβ is believed to upregulate gene expression for aggrecan, downregulate matrix metalloprotease and IL-1 activity, and upregulate inhibitors of matrix metalloprotease, the stimulation of TGFβ may be a mechanism through which PEMF favorably affects cartilage homeostasis.
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Key words: Osteoarthritis, Cartilage, Morphology, Pulsed electromagnetic fields.

Introduction

Several new therapeutic strategies have recently been introduced for the treatment of osteoarthritis, including cyclooxygenase-2 inhibitors, tetracycline derivatives, visco-supplementation with various preparations of hyaluronic acid, and oral chondroitin sulfate/glucosamine supplements. While some may provide symptomatic benefit, disease modification has not been established. Two clinical trials have been reported, demonstrating symptomatic benefit with pulsed direct current or low-frequency pulsed electromagnetic field (PEMF) exposure in human osteoarthritis[1,2]. We have demonstrated that exposure to PEMF enhances chondrogenic differentiation and the synthesis of cartilage extracellular matrix proteins[3,4]. This study explores the degree to which PEMF treatment may be disease-modifying in spontaneous osteoarthritis in guinea pigs.

Hartley-strain guinea pigs develop osteoarthritis, beginning at approximately 12 months of age, culminating in eburnation of the medial tibial plateau by 18 months of age[5,6]. The arthritis is characterized by decreases in the contents of aggrecan and collagen, typical of osteoarthritis, increases in collagenases, cartilage fibrillation, and subsequent degeneration. Sclerotic changes in the subchondral bone and osteophytes accompany cartilage loss and eburnation. The proteoglycan neoepitope, 3B3(–), is detected in osteoarthritic cartilage and is a measure of arthritis activity[7]. The development of osteoarthritis in this model has been modified by a number of interventions, including reduction in body weight and tetracycline derivatives[8,9].

PEMF has a number of well-documented physiological effects, including the upregulation of gene expression for, and synthesis of, aggrecan and type II collagen[3,4]. PEMF has been shown to upregulate members of the TGFβ super gene family, and this may be an intermediary mechanism of PEMF activity[10–14]. TGFβ has important regulatory functions in joints, including the stimulation of aggrecan and collagen synthesis, the suppression of the pro-enzyme forms of stromelysin and collagenase, and the suppression of interleukin (IL)-1[15,16]. The upregulation of TGFβ by PEMF may be a mechanism of action of PEMF on the biology of osteoarthritic joints.

Methods

Male 12-month old guinea pigs were allocated randomly into two groups. Ages were known within 1 month. Treated

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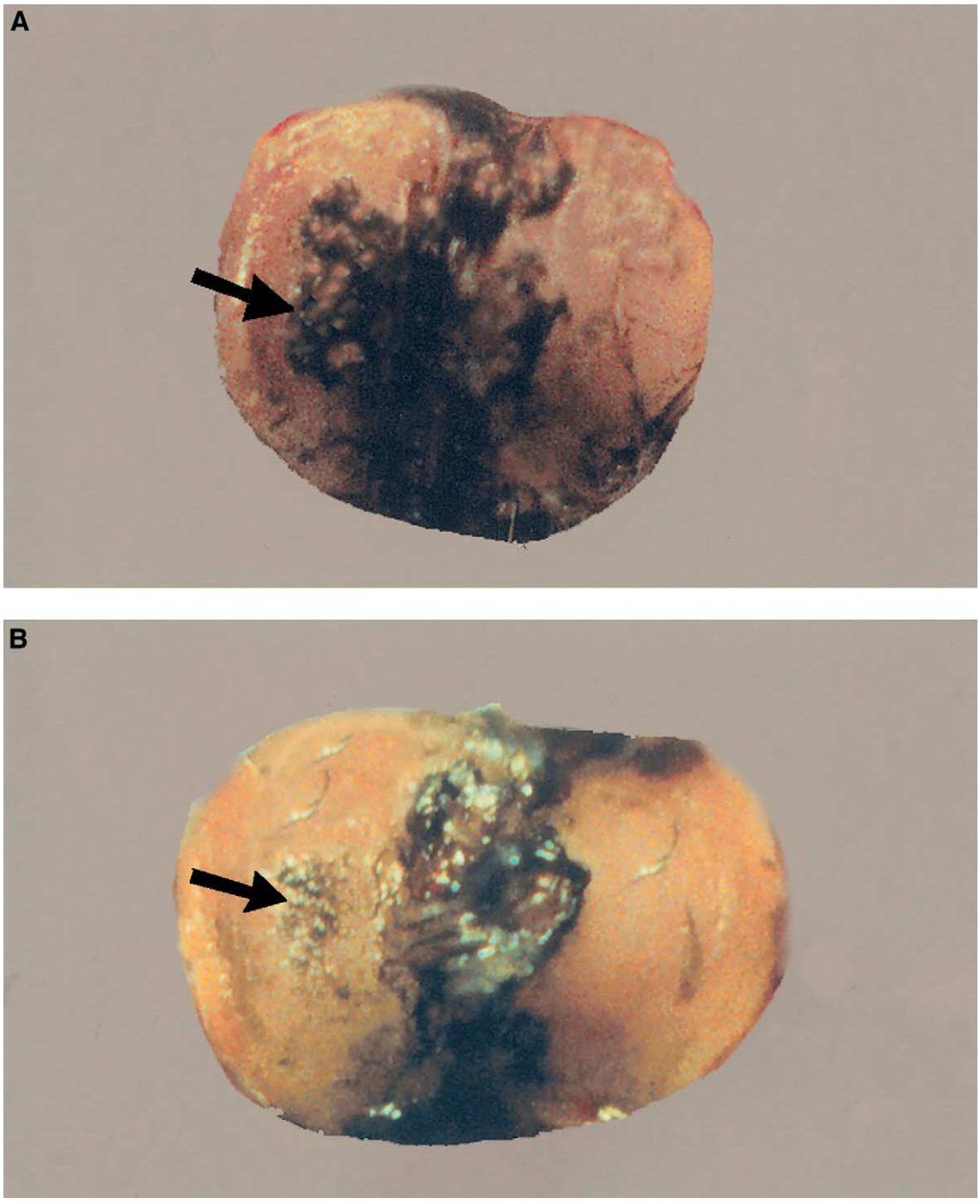


Fig. 1. Gross appearance of cartilage lesions stained with India ink. Arrows indicate cartilage lesions. (A) Control, untreated. (B) PEMF-treated. Lesions from untreated tibial plateaus are approximately 1–5 mm in diameter. When present, lesions in PEMF-treated tibias were smaller and superficial.

animals were exposed to a 1.5 Hz pulse-burst PEMF (EBI, Parsippany, NJ) for 1 h/day for 6 months. Control animals were treated identically, but without exposure to PEMF. All animals remained in standard guinea pig cage environments with food and water, ad lib, and vitamin C supplementation. Animals were sacrificed after 6 months, at 18 months of age. Two separate groups of animals were treated sequentially, representing two replicates of both control and treated animals. One group consisted of 11 animals (six control and five treated). A replicate experiment was carried out with 13 animals (eight control and five treated). One control animal and two experimental animals did not complete the study. Both knees were examined in each animal. A total of 26 control and 16 treated knees were available for study.

The PEMF utilized was similar to the one used clinically for the treatment of fracture repair and is currently being evaluated for the treatment of clinical symptoms of

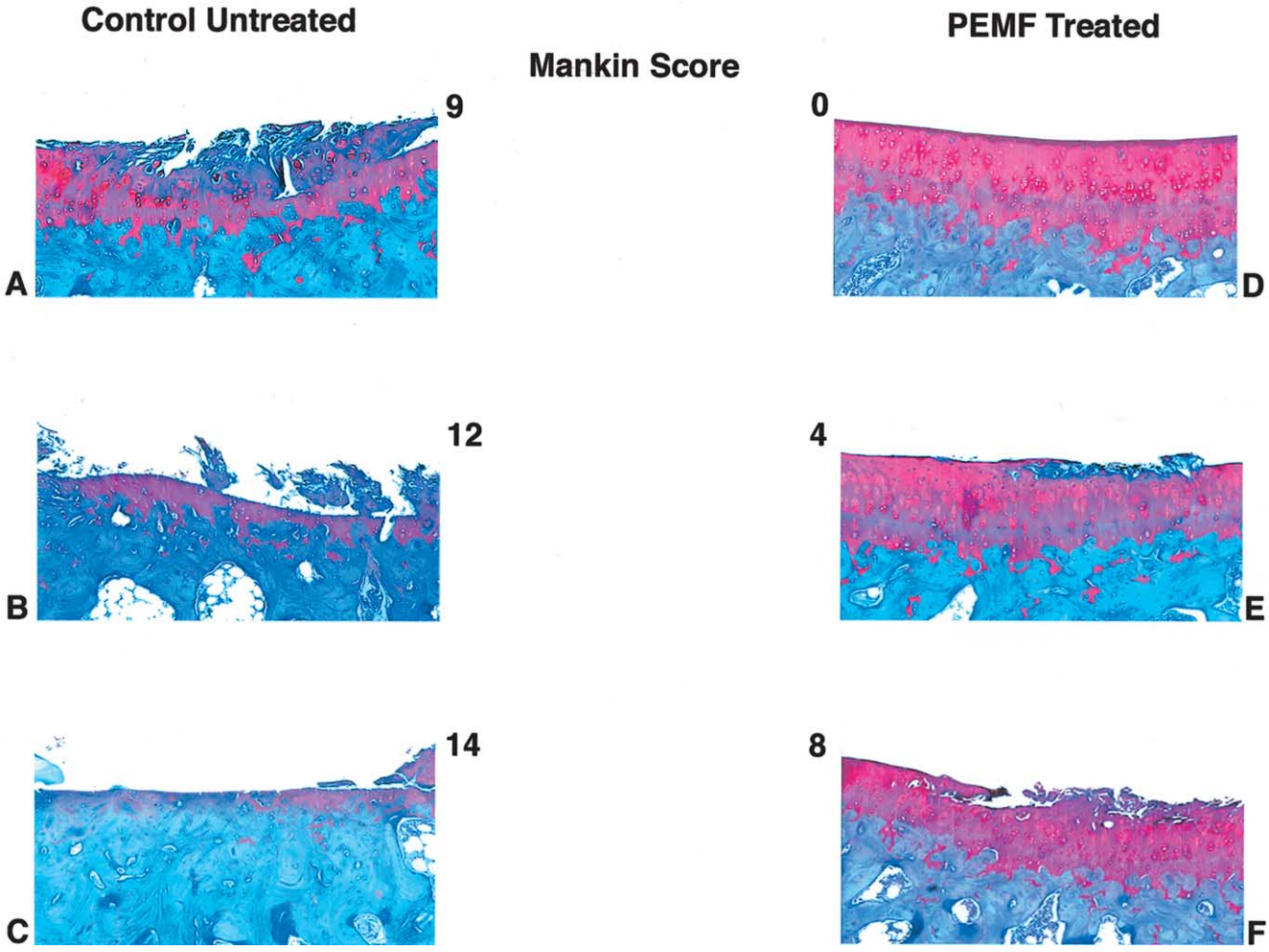


Fig. 2. Histochemical staining of the medial plateau with safranin-O. Examples of control, untreated cartilage (A, mild; B, average; C, severe). Examples of PEMF-treated cartilage (D, mild; E, average; F, severe). Extensive fibrillation and cleft formation to the calcified zone were evident in many control tibias. Complete loss of cartilage and exposure of subchondral bone were commonly observed. No specimens in the PEMF-treated group demonstrated clefts below the transitional zone (4× magnification).

osteoarthritis (EBI). The applied magnetic field consisted of a pulse burst of 30 ms duration, repeated at 1.5 bursts per second with a peak magnetic field of 1.0 G. Fourier analysis of this signal has indicated significant energy content in the low-frequency range, below 75 Hz. Dose response studies in our laboratory with this signal at 1, 4, and 8 h/day of exposure have demonstrated a maximal stimulatory effect on chondrogenesis at 1 h/day (reference 17 and unpublished data). It has been suggested that sham-exposed controls using counterwound coils with the identical electric field be utilized in addition to non-exposed controls in PEMF studies[18]. The magnetic field and, therefore, the induced electric field in the tissue are thus nulled out, while any other possible environmental conditions (e.g., thermal, acoustic, vibrational effects) are not. This is a useful experimental strategy with signals of simple configuration, and one which we have utilized in a study of the effects of continuous sine waves on chondrogenesis[11]. However, with signals of asymmetric shape and rapid repeat rate, such as the one used in this study, sham controls are essentially impossible because of the difficulty in exactly overlaying the signals. If an exact overlay is not achieved, the time-varying magnetic fields are not nulled, other field exposures occur, and unknown induced electric

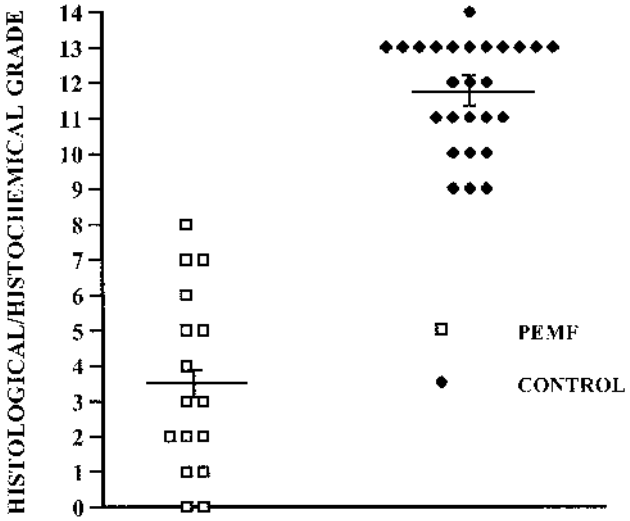


Fig. 3. Histological/histochemical grade. Mean grade of control tibias was 11.7±0.3 compared with 3.5±0.7 of PEMF-treated cartilage (P=0.0001). These grades reflect preservation of cartilage morphology in the PEMF-treated group.

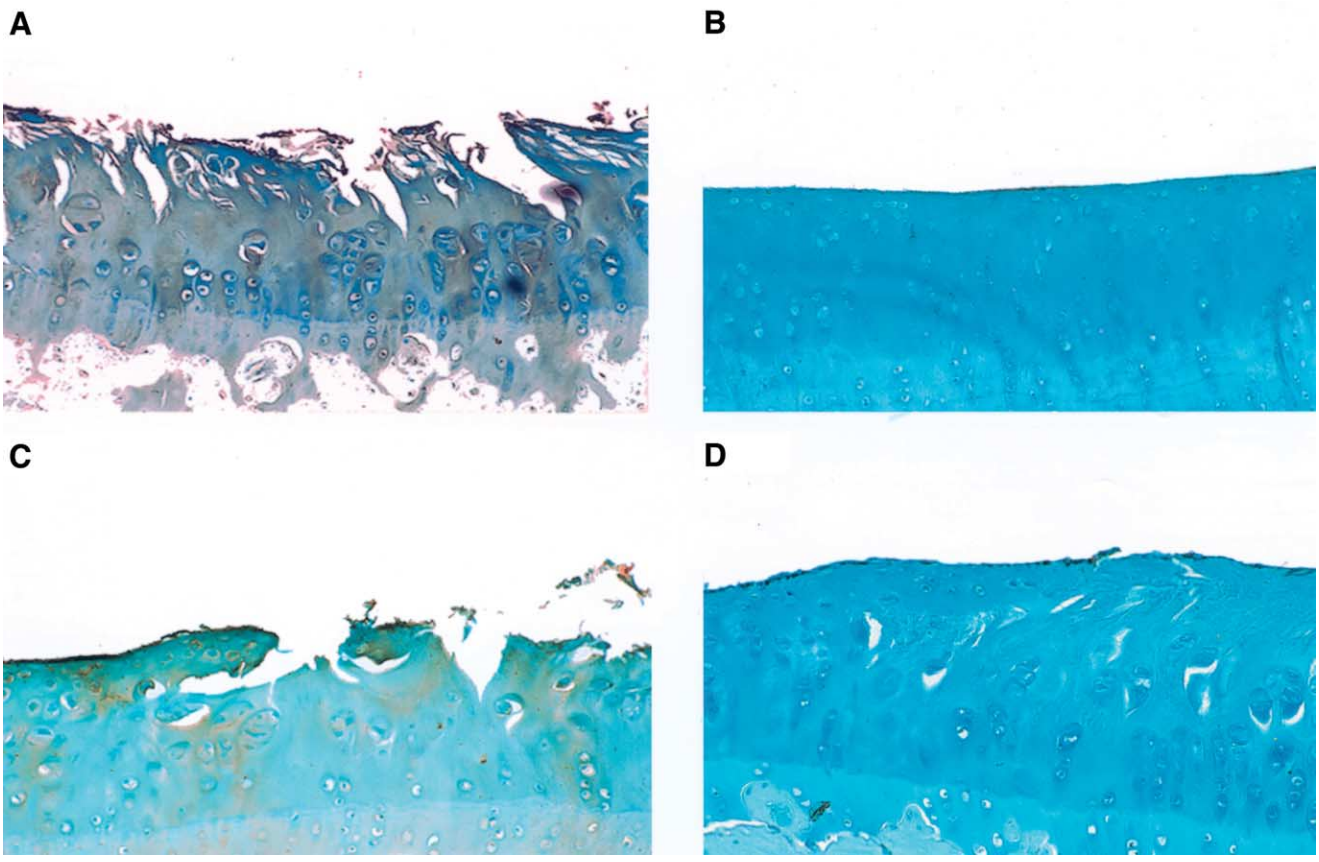


Fig. 4. Immunohistochemistry of the medial plateau for cartilage neoepitopes reflecting aggrecan cleavage. 3B3(–): (A) control, untreated; (B) PEMF-treated. BC-13: (A) control, untreated; (B) PEMF-treated. Immunoreactivity in the extracellular matrix is evident in the untreated controls, but not seen in PEMF-treated cartilage. In addition, there were no immunopositive cells in the PEMF-treated group (10× magnification).

field variables may be introduced. We have, however, studied the major potentially confounding environmental variables on which the suggestion for sham exposure is based, and have demonstrated no difference between treated and control environments. Vibration was measured with an Omega accelerometer at a sensitivity of 0.005 G. Studies were made using a Fourier spectrum analyzer comparing active and sham spectra over a frequency range of 1–5 kHz. No vibration was detected in either active or sham units. Acoustic measurements were made using an active pressure zone microphone with a frequency response from 20 to 18 kHz. No signal was detected in either active or sham units. Temperature was measured with a thermistor probe, and <0.1°C variance was detected between active and sham units.

Both knees from each animal were excised and the tibial plateau cartilage was marked with India ink to identify arthritic lesions. The tibias were fixed in Z-fix (Anatech Ltd) and decalcified with Baxter DeCal solution. The tibias were embedded in paraffin to create a whole mount of the proximal tibia, including both medial and lateral plateaus. Sections (6 μm) were cut in the coronal plane through the mid-portion of the tibial plateau to produce representative serial sections. In the case of tibias with lesions in the medial plateau, the lesions were approximately 1–5 mm in diameter and occurred in the central one-third of the plateau. The serial coronal sections included the lesion. Sections were stained with safranin-O and fast green, and histological/histochemical (Mankin) grades of the medial plateau were determined[19]. In this grading system, 0–6 points are allocated for progressive loss of cartilage structure, 0–3 points for chondrocyte abnormalities, 0–4 points for progressive decrease in safranin-O staining, and 0–1 point for loss of tidemark integrity. Sections were scored without knowledge of their groups, by consensus of two investigators (RKA and DMcKC). On repeated scoring, the intra-observer error was 6.8%; the inter-observer error was 8.2%. Comparison of results from the two experiments demonstrated no significant difference between the replicate groups, so the results were pooled and were expressed as mean±S.E.M. Results were compared for significance by a two-tailed Student's *t*-test.

For immunohistochemistry, 6 μm sections were stained with monoclonal antihuman antibodies conjugated with peroxidase[20,21]. Extracellular matrix was examined with antibodies to the aggrecan neoepitopes, 3B3(–) and BC-13. The antibody to 3B3 was obtained from ICN (69-621-2) and was used with and without chondroitinase digestion. BC-13 was a gift from Dr Bruce Caterson, Cardiff, Wales. These antibodies recognize aggrecan fragments generated by enzymatic cleavage and reflect the severity of the arthritis. Enzyme activity was assessed by immunoreactivity to stromelysin (MMP-3, Calbiochem) and collagenase (MMP-13, Chemicon). Cytokines of interest were examined

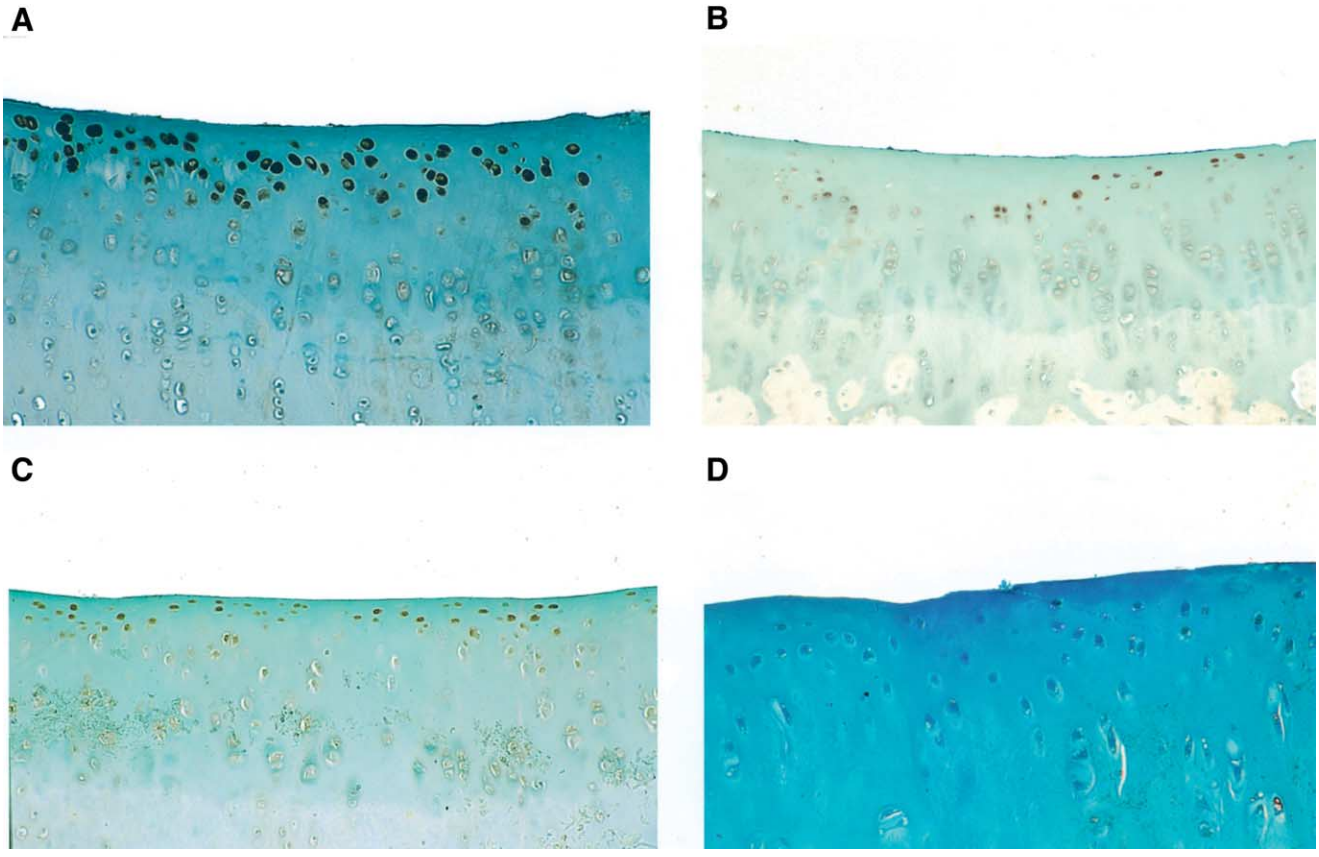


Fig. 5. Immunohistochemistry of the lateral plateau for the matrix-degrading enzymes, stromelysin (MMP-3), and collagenase (MMP-13). MMP-3: (A) control, untreated; (B) PEMF-treated. MMP-13: (C) control, untreated; (D) PEMF-treated. Fewer immunopositive cells were observed in the PEMF-treated specimens (10× magnification). Quantitated data are presented in Table I.

with antibodies to IL-1β (Calbiochem), and TGFβ and IL receptor antagonist protein (IRAP) (both obtained from R&D Systems). The second antibody for MMP-13 was an antirabbit IgG (Vectastain Elite ABC kit), and for TGFβ and IL-1β was antigoat IgG (Biotin, Sigma). The secondary antibody for all other primary antibodies was a mouse polyvalent immunoglobulin (IgG, IgA, IgM, or Biotin). Negative control sections were prepared without primary antibody to check for non-specific binding. Immunopositive cells were counted over a defined area using a microscope grid and are expressed as cells per unit area.

Results

Gross examination of the tibial plateau sections demonstrated cartilage lesions on the medial plateau of all control animals. By contrast, in most specimens, the medial plateaus from the PEMF-treated group exhibited no gross lesions, and those lesions that did occur were smaller than controls (Fig. 1). Histologically, the articular cartilage was thicker in the PEMF-treated tibias (327±13 μm) compared with untreated tibias (108±30 μm; $P=0.0001$). The subchondral bone plate thickness appeared to be greater in the control group, with several control specimens exhibiting large subchondral cysts. Cartilage fibrillation and cleft formation to the calcified zone were evident in many untreated tibias, while no specimens in the treated group showed cleft formation below the transitional zone (Fig. 2). Complete loss of cartilage, in arthritic lesions, was common in untreated, but was not seen in treated, tibias. Histological/histochemical grades of PEMF-treated articular cartilage were significantly lower than controls, reflecting a retardation of the osteoarthritic process (Fig. 3). The mean histological/histochemical grade in the control group was 11.7±0.3 compared with 3.5±0.7 in the PEMF-treated group ($P=0.0001$). Preservation of cartilage matrix by PEMF treatment, observed histochemically, was supported by immunoreactivity to 3B3(–) and BC-13 in the extracellular matrix of the medial plateau cartilage (Fig. 4). Matrix immunoreactivity to these antibodies was decreased by PEMF treatment, suggesting decreased enzymatic cleavage of aggrecan, consistent with matrix preservation.

Immunopositive cells to the various antibodies were observed primarily in the superficial zones of the articular cartilage. Because this tissue was lost in the arthritic lesions of the medial tibial plateau, quantitation of

Table I
Immunopositive cells (per unit area)

	Control	PEMF-treated	% Change	*P*
Collagenase type II	7.2±0.8	0.0±0.0	–	0.01
Stromelysin	13.8±1.9	8.4±1.0	–39	0.02
IL-1β	13.9±2.3	7.2±0.6	–48	0.01
IRAP	10.5±1.0	17.3±1.1	+65	0.003
TGFβ	20.0±3.5	34.4±2.8	+72	0.006

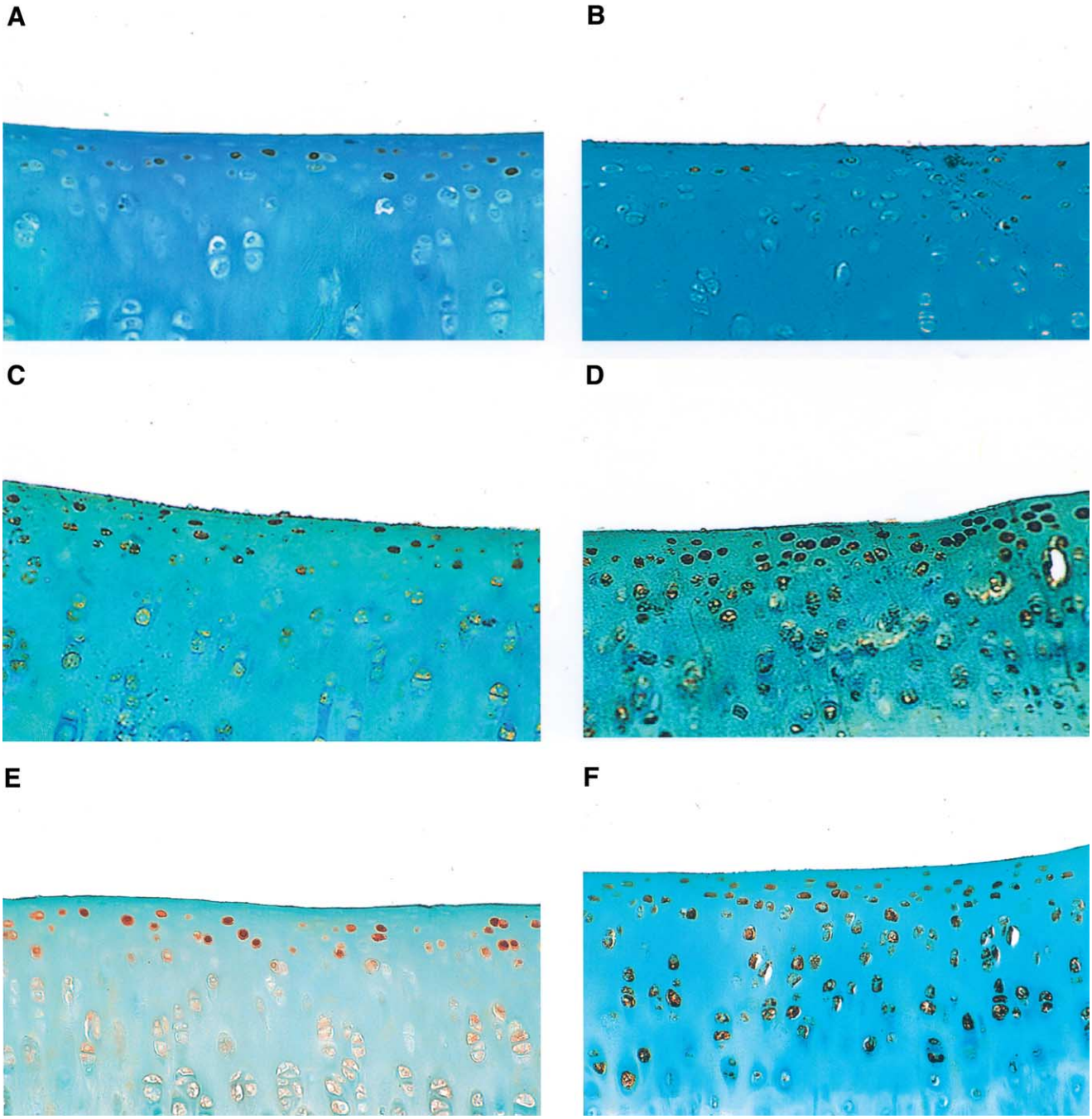


Fig. 6. Immunohistochemistry of the lateral plateau for cytokines. IL-1: (A) control, untreated; (B) PEMF-treated. IRAP: (C) control, untreated; (D) PEMF-treated. TGFβ: (E) control, untreated; (F) PEMF-treated. A reduction in the number of cells immunoreactive to IL-1 and an increase in the number of cells immunoreactive to IRAP and TGFβ were observed in PEMF-treated cartilage (10× magnification). Quantitated data are presented in Table I.

immunopositive cells was performed in the corresponding lateral plateau for each of the treated and untreated tibias. Cartilage from PEMF-treated tibial plateaus demonstrated significant reductions in the number of cells immunoreactive to antibodies for collagenase (MMP-13) and stromelysin (MMP-3) (Fig. 5). The number of cells immunopositive to stromelysin was decreased by 39% by PEMF treatment (Table I). PEMF treatment reduced the number of cells immunopositive for IL-1 and increased the number of cells immunopositive for IRAP and TGFβ (Fig. 6). The number of cells positive to IL-1 was reduced by 48%, while immunoreactivity to IRAP was increased by 65% by PEMF treatment. The number of TGFβ-positive cells was increased by 72% in EMF-treated, compared with control, tibias (Table I).

Discussion

Osteoarthritis in the Hartley-strain guinea pigs resembles the morphologic characteristics seen in human

osteoarthritis. Radiographic features include sclerosis of the subchondral bone plate and osteophytes[22]. Biochemical characteristics include loss of aggrecan and type II collagen and a transient increase in cartilage water content[23]. Collagenases are elevated in the arthritic cartilage[24,25]. Aggrecan neoepitopes appear and reflect the severity of the arthritis[7]. A preliminary examination of animals at 8, 12, and 18 months of age in our laboratory confirmed uniform progression, as reported by other laboratories, culminating in severe osteoarthritic changes, including eburnation, subchondral sclerosis, and occasional osteophytes at 18 months of age.

The data reported in this study demonstrate a reduction in the severity of osteoarthritis and preservation of articular cartilage by exposure to a particular PEMF. This was manifested by significantly lower histological/histochemical grades, reflecting less cartilage destruction in PEMF-treated knees. The preservation of the extracellular matrix was further demonstrated by a reduction in the neoepitopes, 3B3(–) and BC-13. The reduction of immunoreactivity to these antibodies by PEMF treatment indicates less severe enzymatic damage to aggrecan. The number of cells immunopositive to antibodies against collagenase (MMP-13) and stromelysin (MMP-3) was markedly reduced as well, indicating that two of the major cartilage-degrading enzymes were suppressed by PEMF treatment.

The number of cells immunoreactive to TGFβ, IL-β, and IRAP was determined to suggest possible mechanisms of PEMF activity. IL-1 is known to increase the production of MMPs and inhibits the synthesis of aggrecan and type II collagen. IL-1 is present in the synovial fluid and cartilage matrix of osteoarthritic joints. Antagonists of IL-1, including IRAP and TGFβ, are capable of ameliorating some of the IL-1-mediated effects on cartilage matrix degradation[26–28]. IL-1 immunoreactivity was reduced by 48%, while IRAP was increased by 65% by PEMF exposure. TGFβ was increased by 72%. The upregulation of TGFβ has assumed a central position in the hypothesized mechanism of action of PEMF. In other studies in our laboratory, PEMFs have been shown to upregulate TGFβ expression during chondrogenesis[4,11,14]. Depending on the type of PEMF utilized, mRNA for TGFβ was increased by 68–158%; TGFβ protein was increased by 21–25%, coincident with 119–343% increases in the number of cells immunopositive for TGFβ. These observations have been confirmed in MG63 and human fracture non-union cells[12,13]. In these studies, TGFβ has been shown to be elevated, and PGE-2 to be suppressed, by PEMF treatment. Upregulation of TGFβ has also been demonstrated *in vitro* by direct electrical stimulation of MC3T3 cells[29].

TGFβ has been shown to have several important regulatory activities in synovium and articular cartilage. These include: (1) upregulation of gene expression for aggrecan; (2) downregulation of pro-stromelysin and pro-collagenase; (3) upregulation of tissue inhibitors of metalloprotease (TIMP); and (4.) suppression of IL-1 activity. TGFβ has been shown to inhibit IL-1-induced protease activity and subsequent aggrecan degradation[15,27,28]. Treatment with TGFβ blocks IL-1-mediated reduction in aggrecan deposition in the extracellular matrix. These studies have indicated that TGFβ can inhibit the IL-1-mediated catabolic effects on chondrocytes. Together with the upregulation of aggrecan expression, these observations suggest that TGFβ regulates cartilage homeostasis and may result in maintenance of extracellular matrix morphology.

High doses of TGFβ have been shown to have adverse effects in the murine knee[27]. TGFβ injection (100 ng) into joints not only increases proteoglycan synthesis in articular cartilage, but also produces inflammation and synovial hyperplasia. Within 2 weeks after three intra-articular injections, osteophyte formation has been observed. Two months after a series of three injections of TGFβ (200 ng), severe proteoglycan depletion and loss of articular cartilage to the tidemark were observed. We have not observed an increase in osteophyte formation in knees treated with PEMF, even though TGFβ is upregulated. Other studies in our laboratory have demonstrated that sustained increases in TGFβ can be produced above constitutive levels by PEMF of 11 pg/mg tissue, or 0.08 pg/μg DNA[14]. PEMF stimulates sustained moderate increases in TGFβ, sufficient to favorably alter the homeostatic balance of cartilage matrix degradation and synthesis in favor of preservation of cartilage morphology without the induction of local toxicity. The stimulation of TGFβ together with considerations of its regulatory role in joints supports the hypothesis that upregulation of TGFβ expression may be an intermediate mechanism of PEMF modification of osteoarthritis.

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