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Review

Effects of pulsed electromagnetic fields on articular hyaline cartilage: review of experimental and clinical studies

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Abstract

Osteoarthritis (OA) is the most common disorder of the musculoskeletal system and is a consequence of mechanical and biological events that destabilize tissue homeostasis in articular joints. Controlling chondrocyte death and apoptosis, function, response to anabolic and catabolic stimuli, matrix synthesis or degradation and inflammation is the most important target of potential chondroprotective treatment, aimed to retard or stabilize the progression of OA. Although many drugs or substances have been recently introduced for the treatment of OA, the majority of them relieve pain and increase function, but do not modify the complex pathological processes that occur in these tissues. Pulsed electromagnetic fields (PEMFs) have a number of well-documented physiological effects on cells and tissues including the upregulation of gene expression of members of the transforming growth factor β super family, the increase in glycosaminoglycan levels, and an anti-inflammatory action. Therefore, there is a strong rationale supporting the in vivo use of biophysical stimulation with PEMFs for the treatment of OA. In the present paper some recent experimental in vitro and in vivo data on the effect of PEMFs on articular cartilage were reviewed. These data strongly support the clinical use of PEMFs in OA patients.

Keywords: Articular cartilage; Osteoarthritis; Pulsed electromagnetic fields

1. Introduction

Hyaline articular cartilage is an avascular and specialized functional tissue with low cellularity, high water content, and a dense extracellular matrix (ECM) [26]. The tissue-specific mechanical properties of articular cartilage are dependent on the ECM structure and composition, which accounts for about 90% of cartilage wet weight, and is mainly composed of collagen type II, proteoglycans (PG) (in particular, aggrecan, hyaluronic acid), cations and water [45]. The complex structure of articular cartilage enables this tissue to perform its biomechanical role but appears to hinder repair, as lesions without further surgical treatment often progress into long-term degeneration and osteoarthritis (OA) [17,26]. Even in the absence of injuries, accidents and other joint traumas, articular spontaneous degeneration occurs and prevalence studies indicate that the majority of people over the age of 65 have some form of OA [48].

OA is characterized by a degeneration of hyaline articular cartilage. The breakdown of the cartilage matrix leads to the development of fibrillations, clefts and ulcerations and the disappearance of the full-thickness surface of the joint. This process is accompanied by bone changes with osteophyte formation and thickening of the subchondral bone [43]. Even if changes in the subchondral bone resulting in loss of its stock absorbing capacity could transfer the stress of loading directly to the articular cartilage with secondary changes in the cartilage, OA is usually considered to be a primary disorder of chondrocyte proliferation and function with secondary changes in bone and it is often associated with an inflammatory response [16,30]. Chondrocytes are the single cellular component of hyaline cartilage and are responsible for matrix synthesis and turnover, while the state of the matrix has a

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direct influence on chondrocyte function. The number of chondrocytes, their rate of proliferation, metabolic activity and ability to respond to various stimuli are inversely related to the age of the organism [38,47].

Moreover, cytokines are considered to be an important link in OA since they are produced by cells present in the OA joints and because they are responsible, at least in part, for the changes seen in cartilage damage, synovial membrane, subchondral bone and osteophyte formation [41]. Local release of catabolic cytokines and enzymes such as interleukin 1β (IL-1β), tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6), metalloproteinases (MMP), aggrecanases that is high in OA in response to tissue damage, and inflammation cause a depletion of glycosaminoglycans (GAGs) and suppress type II collagen synthesis [41,45]. Blockade of these cytokines by natural antagonists in OA cartilage, where they are overexpressed, can up-regulate gene expression of the matrix molecules and enhance matrix repair [36]. Furthermore, anabolic cytokines, such as insulin-like growth factor-I (IGF-I), TGFβ-1, inhibit the catabolic effects of pro-inflammatory cytokines [41] and may stimulate useful synthetic processes.

So, controlling chondrocyte death, proliferation, function, response to anabolic and catabolic stimuli, matrix synthesis, matrix degradation and joint inflammation is the most important target of a potential chondroprotective treatment, that is to say a therapy that retards or stabilizes the progression of established OA by altering the underlying pathological processes [1].

Many drugs or substances have been recently introduced for the treatment of OA including cyclooxyganes inhibitors, hyaluronic acid, chondroitin sulfate, and glucosamine sulfate [5,25,53,62]; however the majority of them can relieve pain and increase function, but do not modify the complex pathological processes that occur in these tissues, which are unable to balance undergoing catabolic and anabolic pathways [34]. In addition, such therapy for OA is unlikely to have a direct effect at the subchondral bone level [42,54].

Pulsed electromagnetic fields (PEMFs) have a number of well-documented physiological effects on cells and tissues including the upregulation of gene expression of members of the TGFβ super family, the preservation of ECM integrity of cultured cartilage explants, the increase in GAG levels in embryonic and immature cartilage and in an experimental model of decalcified bone matrix-induced endochondral ossification [2,11,24,32,33,56]. An anti-inflammatory action has also been shown because of a direct effect on adenosine A2a receptors on cell membranes [63]. Several anti-inflammatory drugs are mediated via adenosine receptors and the modulation of the adenosine-receptor-mediated pathway may offer novel methods for treatment of inflammation in the presence of joint diseases [6].

Therefore, there is a strong rationale supporting the in vivo use of biophysical stimulation with PEMFs for the treatment of OA.

In the present paper some experimental (in vitro and in vivo) and clinical studies on the effect of PEMFs on hyaline articular cartilage will be summarized. We searched the English language literature of the MEDLINE database, for the period January 1990 to December 2004 (keywords or title words: PEMFs and cartilage/chondrocytes/osteoarthritis). Articles were included in the review if they were related to the use of PEMFs on hyaline articular cartilage tissue or cells. The search included in vitro, animal and human studies. We excluded reviews and papers with not available full manuscripts. Finally, we excluded papers published before 1990 in an attempt to examine the most up-to-date methodology and outcome measures. We were left with six in vitro, two in vivo and five clinical papers.

2. In vitro studies: PEMF effects on cartilage cell and tissue cultures

The studies summarized in Table 1 on the in vitro effect of PEMFs on hyaline articular chondrocytes and articular cartilagineous tissue have appeared in the literature over the last 10 years [13–15,22,44,52]. They considered different pathogenetic aspects (chondrocyte proliferation, ECM synthesis, secretory activity and inflammation).

More precisely, PEMFs were tested in human and animal monolayer chondrocyte cultures and tissue explants and their effects were investigated by different methodologies. Conflicting observations have been reported and some studies demonstrated a significant effect of PEMFs in increasing articular chondrocyte proliferation [13,44,52], ECM synthesis and PG content in cartilage tissue explants [14] while others demonstrated that PEMFs had no effect on articular chondrocyte GAG synthesis [52].

Some authors also investigated: 1) the role of serum (fetal bovine serum, FBS) in the culture medium, that is to say the availability of growth factors [44]; 2) the contemporaneous administration of anabolic (IGF-1) and pro-inflammatory (IL-1β) cytokines to PEMF stimulation [14,15,22]; 3) the dependence of the PEMF proliferative effects on cell density [13]. Therefore, these studies also permitted testing the PEMF mechanism of action and the therapeutic effect in conditions that simulate an OA inflammatory conditions in vitro. Briefly, it was shown that: 1) the proliferative response of chondrocytes to PEMF exposure is dependent on the amount of serum in the culture medium (0.5 and 10%) [44]; 2) the proliferative response to PEMFs is inversely correlated to the cell density and is strictly growth-stage dependent [13]; 3) PEMFs increased the anabolic effect of IGF-I on cartilaginous bovine explants, and PG synthesis was significantly improved by the PEMF stimulation independently of the absence or presence of serum [44]; 4) when IL-1β was used in vitro to induce cartilage degradation PEMFs stimulated PG synthesis that completely compensated for the IL-1β dependent inhibition of PG synthesis, thereby restoring its basal concentration level [14,22].

Of particular interest, the results of these studies demonstrate that the effect of PEMFs depend on the in vitro model

Table 1
Main in vitro studies on chondrocyte proliferation and ECM synthesis in PEMF stimulated and not stimulated cell and tissue cultures from articular hyaline cartilage (for the period January 1990 to December 2004)

Model	Stimulation	Main results	References
Human articular chondrocytes at low and high density	Frequency 75 Hz Intensity 2.3 mT Pulse duration 1.3 ms Exposure time: 1, 6, 9, 18 h and continuous exposure for 3 and 6 days	PEMFs significantly increased proliferation following 9 and 18 h of exposure in high and low density conditions. PEMFs significantly increased proliferation during the first 3 days of culture in low density conditions. The exposure to PEMFs resulted in a significant increase of proliferation versus control cultures in the first 2 days	[13]
Bovine articular cartilage explants both in basal conditions and in the presence of IL-1β	Frequency: 75 Hz Intensity: 2.3 mT Pulse duration: 1.3 ms Treatment length: 24 h	PG synthesis and the residual PG tissue content were significantly higher in PEMF-exposed explants than in controls. Il-1β induced both a reduction of PG synthesis and an increase in PG release which resulted in a net loss of tissue PG content. In IL-1 treated explants, PEMF increased PG synthesis without modifying PG release.	[14]
Human nasal and articular monolayer chondrocyte cultures in the absence and presence of serum	Frequency: 75 Hz Intensity: 2.3 mT Pulse duration: 1.3 ms Treatment length: 6, 12, 18, 24 and 30 h	Significant increase in proliferation (3H-thymidine incorporation) in cultures exposed to PEMFs in the presence of serum whereas no effects were observed in serum-free conditions.	[15]
Bovine articular chondrocyte monolayers cultures and bovine articular cartilaginous tissue explants in the absence and presence of serum and IGF-I	Frequency: 75 Hz Intensity: 1.5 mT Pulse duration: 1.3 ms Treatment length: 24 h	In cartilage explants, PEMF stimulation significantly increased PG synthesis both in the absence and in the presence of serum. Similarly, IGF-I increased PG synthesis in a dose-dependent manner both in the absence and presence of serum. At all doses of IGF-I, the combined effects of the 2 stimuli resulted additive. No effect was observed on medium PG release. Also in chondrocyte monolayers IGF-I stimulated PG synthesis in a dose-dependent manner, both in the absence and presence of serum, however this was not modified by PEMF exposure.	[44]
Human articular osteoarthritic chondrocytes in alginate and the absence and presence of IL-1 beta	Pulsed signal therapy (PST) Frequency: < 30 Hz Intensity: 10–20 G Pulse duration: 67 ms Treatment length: 3 h/day for 72 h	IL-1 beta induced marked cellular damage observed by TEM analysis and a significant decrease of PG levels. PST stimulation leads to restoration of cell structure and of PG production.	[22]
Human articular cartilage cells	Frequency: 6.4 Hz Intensity: 0.4 mT Pulse width: 230 μs Burst width: 76 ms Treatment length: 5 days	The stimulation promoted cell proliferation but did not enhance GAG synthesis	[52]

adopted (monolayer chondrocyte cultures versus tissue explants), the presence of growth factors in the microenvironment, and environmental constrictors.

3. In vivo studies: PEMF therapeutic efficacy on OA lesions

Because subchondral bone, bone marrow, synovial cells, chondrocytes and synovial fluid all contribute to the development of OA and to the healing of defects of articular cartilage, the use of animal models is essential both to understanding the process of repair and assessing the value of new therapeutic regimens [55]. Many techniques have been employed by researchers to create secondary OA lesions in animals and especially in rabbits, by means of surgical interventions that cause mechanical instability of the knee joint such as meniscectomy, section of anterior or posterior cruciate ligament (ACL, PCL), alone and in combination [29,31,35,45,50,51,64]. However, another animal model which develops spontaneous and asymptomatic age-related OA of the knee is the Dunkin Hartley guinea pigs. The earliest histological signs of OA in Dunkin Hartley guinea pigs appear at 3 months of age in the medial tibial compartment and progress to moderate and severe degenerative changes at 1 year of age [27]. The resulting articular cartilage lesions resemble those found in humans and are also increased by body weight, mechanical load and posture [58].

To date, two in vivo experimental studies into the effect of PEMFs on OA have been performed in the aged Dunkin Hartley guinea pigs [11,21].

Ciombor et al. [11] utilized 12-month-old guinea pigs that were unstimulated for 6 months or stimulated for the same period 1 h/day with PEMFs (pulse-burst of 30 ms duration, repeated at 1.5 bursts per s with a peak magnetic field of 1.0 G, energy below 75 Hz). At the end of the experimental study the Authors excised both knees and studied the mid-portion of the tibial plateau by means of a histological/histochemical score (Mankin score with 0–6 points for cartilage structure; 0–3 points for chondrocyte abnormalities; 0–4 points for pro-

gressive decrease in safranin staining; 0–1 point for loss of tidemark integrity). Collagenase type II, stromelysin, IL-1β, interleukin 1 receptor antagonist protein (IRAP) and TGFβ-1 were also studied by means of immunohistochemistry. Results showed that cartilage was thicker in the medial tibial plateau of guinea pigs stimulated with PEMFs in comparison with not stimulated animals, and the histological/histochemical score was significantly lower in PEMF-treated animal than in Sham-treated animals. Moreover, PEMF treatment significantly reduced immunopositive cells to collagenase type II, stromelysin and IL-1β, while the number of cells immunopositive for IRAP and TGFβ-1 was significantly increased. Ciombor et al. [11] suggested that PEMF stimulation of TGFß-1 might be responsible for the repair mechanism of action. TGFß-1 stimulates PG and collagen II synthesis and also acts as a natural inhibitor capable of directly counteracting pro-inflammatory cytokine production and activity [37,43].

Fini et al. [21] used the same animal model (12-month-old Dunkin Hartley guinea pig), which was stimulated with PEMFs for 3 months 6 h/day (frequency = 75 Hz, intensity of electromagnetic field = 1.6 mT and duty cycle = 1.3 ms). At the end of the study, the entire knee joints were investigated by means of microradiography, a histological/histochemical grading score (Mankin modified by Carlsson with 0–8 points for cartilage structure; 0–3 points for chondrocyte abnormalities; 0–6 points for progressive decrease in toluidine blue staining; 0–1 point for loss of tidemark integrity) and cartilage and bone histomorphometry. Results of the study showed that PEMF-treated animals scored low, indicating a reduction in the progression of chondropathy in comparison with sham-treated animals. The results of medial tibia plateau had the highest histological grading scores in both experimental and control groups, indicating that this site was more susceptible to chondropathy. Regarding cartilage thickness (CT), a significantly higher value was found in the medial tibia plateaus of animals of the PEMF-treated group when compared to other measurement sites of the same group and to the CT value of the medial tibia plateaus of the animals of the Sham-treated Group. As far as bone was concerned, significantly lower values were observed in subchondral bone thickness for the PEMF-treated group in comparison to the sham-treated group when considering each measurement site.

These results have confirmed and extended the observations of Ciombor et al. [11] by showing that PEMFs preserve the morphology of articular cartilage and retard the development of OA lesions in the entire knee of aged osteoarthritic guinea pigs. An anti-inflammatory mechanism of action was also hypothesized on the basis of recent evidence regarding the chondroprotective effect of adenosine agonists selective to the A2a receptor and of the in vitro capability of PEMFs to increase the number of A2a adenosine receptors [63].

4. Clinical studies

Although the different animal models of OA have characteristics similar to the human disease, none of them has proven to be a true model of OA and therefore, any treatment has to be finally tested in clinical trials. PEMF stimulation is still under investigation for use in patients with OA [53]. However, even if different physical parameters and exposure times of stimulation were used, positive results were obtained in clinical studies [28,39,46,60,61].

In 1993, Trock et al. [60] performed a double-blind randomized clinical trial on patients with primary knee OA. Patients were treated with PEMFs (frequency: < 30 Hz; intensity: 10–20 G; 67 ms pulse phase duration) 30 min/day, three to five treatments per week for a total of 18 treatments in 1 month). At different time points, patients were evaluated for pain level, joint motion and tenderness. The actively treated group averaged 34% improvement in the mean value for each variable at each experimental point and 47% improvement by 1 month after treatment. Control patients averaged about 10% and 14% improvement at the same experimental points, respectively.

In 1994, the same authors [61] performed a similar study on the effect of PEMFs in the treatment of patients with knee and cervical spine OA. They observed a strong placebo effect and variability from patient to patient, but they confirmed the statistically significant improvement in pain score, daily activity, knee tenderness and motion for PEMF-treated patients with knee OA. PEMF-treated patients with cervical spine involvement always had a significant improvement by the end of treatment and at follow up.

In 2001, Pipitone and Scott [46] performed a randomized, double-blind, placebo-controlled study on the efficacy of PEMF stimulation in patients with symptomatic knee OA. At 6 weeks, patients were evaluated with different scores (Likert and Euro-Quality of Life). Paired analysis of the follow-up observations on each patient showed a significant improvement in the treated group with regards to pain and disability.

Also Jacobson et al. [28] and Nicolakis et al. [39] observed that PEMF stimulation was safe, reduced impairment in activities of daily life and improved knee function in patients with chronic knee pain due to OA.

5. Discussion and conclusion

In western countries the impact of OA on public health and the significant costs that musculoskeletal conditions generate will be of increasing burden [9]. Our understanding in the treatment of OA evolves as knowledge of the underlying pathophysiology of the condition improves. Previous concepts on OA pathogenesis focused only on the role of chondrocytes in the synthesis and degradation of the ECM. In fact, chondrocytes are the only cell type that constitutes articular cartilage and are responsible for tissue homeostasis, respond to injury and perform the cartilage remodeling process that characterizes OA [30]. More precisely, chondrocyte number, proliferation rate, synthetic and metabolic activity and growth factor responsiveness decrease with the advancing age and in the presence of OA [4]. Cell death and apoptosis during OA lesion progression have also been reported [30].

Newer concepts on OA pathogenesis are related to the role of inflammation that is now well accepted as a feature in OA [9]. Synovitis is common in advanced age involving infiltration of activated B cells and T lymphocytes and the expression of pro-inflammatory cytokines and chemokines is observed in patients with OA in the joints of OA patients and animals [7,23]. With regards to this, IL-1β, TNFα, IL-6, IL-18, IL-17 and leukemia inhibitory factor (LIF) appear to be more relevant to the disease [41,42]. These catabolic cytokines lead to the destruction of joint tissue by stimulating cartilage PG resorption, MMP synthesis and nitric oxide production [43,57]. The purine base adenosine has been shown to limit inflammation through receptor (i.e. A2a)-mediated regulation and suppressing pro-inflammatory cytokines synthesis (TNFα, IL-8, IL-2, IL-6). Adenosine has been reported to reduce inflammation and swelling in several in vivo models of inflammation and also in adjuvant-induced and septic arthritis in animals [8,12,57].

So, a therapy combining an anabolic effect on chondrocytes, a catabolic cytokine blockage, a stimulatory effect on anabolic cytokine production and one that is able to counteract the inflammatory process would be extremely useful for OA treatment.

In vitro studies showed that chondrocyte proliferation and matrix synthesis are significantly enhanced by PEMF stimulation, when investigating also the conditions affecting the PEMF action [13–15,22,44]. A part the importance of physical properties of the fields used (intensity, frequency, impulse amplitude, etc.) and the exposure time, the availability of growth factors, environmental constrictions and the maintenance of the native–cell matrix interactions seem to be fundamental in driving the PEMF-induced stimulation [13,15]. In particular, the interaction between cell membrane receptors and mitogens seems to be one of the molecular events affected by PEMFs [44]. These data are in agreement with results of in vivo studies with a decalcified bone matrix-induced endochondral ossification model and showing that the stimulation of TGFβ-1 may be a mechanism through which PEMFs affect complex tissue behavior and through which the effects of PEMFs may be amplified [13]. In addition, PEMFs are reported to up-regulate mRNA levels for, and protein synthesis of, growth factors resulting in the synthesis of ECM proteins and acceleration of tissue repair [3].

As far as inflammation is concerned, IL-1β is present in high amounts in OA cartilage and is considered to be one of the main catabolic factors involved in the cartilage matrix degradation associated with OA [23]. As previously mentioned, PEMFs in vitro were able to counterbalance efficiently the cartilage degradation induced by the catabolic cytokine [14,22].

Moreover, saturation-binding experiments demonstrated a significant increase in the adenosine A2a receptor density in human neutrophils treated with PEMFs [63]. Activation of adenosine A2a receptors seems to be associated with inhibition of the catabolic cytokines TNFα, IL-6 and IL-8 [6,57]. Although further studies are necessary to determine the direct effect of PEMFs on chondrocyte A2a receptors, a similar mechanism of action could be hypothesized for OA joints on the basis of recent data showing the presence of A2a receptor on chondrocyte membrane, the response of chondrocytes to adenosine and the production of adenosine by chondrocytes [57].

Finally, studies of electrical phenomena in cartilage have suggested also a mechanical–electrical mechanism of action in vivo that resembles the one described in bone, appearing when cartilage is mechanically compressed and causing movement of fluid and electrolytes, leaving unneutralized negative charges in the proteoglycans and collagen in the cartilage matrix. These streaming potentials could work in cartilage and transduce mechanical stress to an electrical (or electromagnetic) phenomena capable of stimulating chondrocyte synthesis of matrix components [60]. The mentioned mechanisms seem to be active also in vivo, so that both in vivo studies performed so far in animals demonstrated important effects of PEMFs in controlling knee OA lesion progression [11,21]. These experimental results were also confirmed by clinical trials [28,39,46,60,61]. In the clinical use the control of the local environment by physical stimuli is achieved by exposing only the specific region/area of interest, and a therapeutic effect by can be activated by delivering locally the optimal effective dose. Thus, the treatment can be performed in the absence of systemic effects and complies with the principle of limiting iatrogenic side effects [20].

In conclusion, experimental and clinical studies suggest that PEMF stimulation would be a promising chondroprotective therapy for OA joints because of an action on chondrocyte metabolism that has been demonstrated in vivo by the enhancing of cartilaginous and subchondral bone tissue properties and in some clinical studies by the amelioration of clinical and radiographic observations. Future experimental studies may be aimed at clarifying the cellular and molecular events which are involved in the tissue response to PEMFs in innovative therapeutic strategies such as tissue engineering. Cell yield, proliferation/expansion and redifferentiation are the main phases of regenerative medicine that are particularly problematic in advanced aged and OA patients [4]. On the basis of the previously mentioned effect of PEMFs on chondrocyte cultures it could be hypothesized that stimulation might accelerate and ameliorate both expansion and redifferentiation. In this modern field of research, it would be important to use cell and tissue cultures from pathological joints. In vitro experimental data have demonstrated the importance of using pathological models to better simulate the clinical conditions [18,19,59], and differences between chondrocytes from healthy and OA joints have been reported [10,49]. Even if it was hypothesized that PEMF effects might be more evident in chondrocytes and cartilage tissue cultures from “old” and OA joints, experimental evidence is required. With regard to in vivo studies, few extensive publications are reported and they are on the same animal model. Therefore, there is the need for other investigations in other animals, such as rabbits and sheep. From a clinical point of view, some

multicentric randomized and double-blind clinical trials are currently being carried out with initial promising results [40].

References

[1] Altman R, Brandt K, Hochberg M, Moskowitz R. Design and conduct of clinical trials in patients with osteoarthritis: recommendations from a task force of the Osteoarthritic Research Society. Osteoarthritis Cartilage 1996;4:217–43.

[2] Aaron RK, Ciombor DMK. Acceleration of experimental endochondral ossification by biophysical stimulation of the progenitor cell pool. J Orthop Res 1996;14:582–9.

[3] Aaron RK, Boyan BD, McK Ciombor D, Schwartz Z, Simon BJ. Stimulation of growth factor synthesis by electromagnetic fields. Clin Orthop 2004;419:30–7.

[4] Barbero A, Grogan S, Schafer D, Heberer M, Mainil Varlet P, Martin I. Age related changes in human articular chondrocyte yield, proliferation and post-expansion chondrogenic capacity. Osteoarthritis Cartilage 2004;12:476–84.

[5] Barbucci R, Lamponi S, Borzacchiello A, Ambrosio L, Fini M, Torricelli P, et al. Hyaluronic acid hydrogel in the treatment of osteoarthritis. Biomaterials 2002;23:4503–13.

[6] Benton HP, MacDonald MH, Tesch AM. Effects of adenosine on bacterial lipopolysaccharide- and interleukin induced nitric oxide release from equine articular chondrocytes. Am J Vet Res 2002;63(2): 204–10.

[7] Borzì MR, Mazzetti I, Marcu KB, Facchini A. Chemokines in cartilage degradation. Clin Orthop Rel Res 2004;427 S:53–61.

[8] Boyle DL, Kowaluk EA, Jarvis MF, Lee CH, Bhagwat SS, Williams M, et al. Anti-inflammatory effects of ABT-702, a novel non-nucleoside adenosine kinase inhibitor, in rat adjuvant arthritis. J Pharmacol Ext Ther 2001;296(2):495–500.

[9] Brooks P. Inflammation as an important feature of osteoarthritis. Bull World Health Organ 2003;81(9):689–90.

[10] Bush PG, Hall AC. The volume and morphology of chondrocytes within non-degenerate and degenerate human articular cartilage. Osteoarthritis Cartilage 2003;11(4):242–51.

[11] Ciombor D, McK Aaron RK, Wang S, Simon B. Modification of osteoarthritis by pulsed electromagnetic field-a morphological study. Osteoarthritis Cartilage 2003;11:455–62.

[12] Cohen SB, Gill SS, Baer GS, Leo BM, Scheld WM, Diduch DR. Reducing joint destruction due to septic arthrosis using an adenosine 2A receptor agonist. J Orthop Res 2004;22:427–35.

[13] De Mattei M, Caruso A, Pezzetti F, Pellati A, Stabellini G, Sollazzo V, et al. Effects of pulsed electromagnetic fields on human articular chondrocyte proliferation. Connect Tissue Res 2001;42(2):1–11.

[14] De Mattei M, Pasello M, Pellati A, Stabellini G, Massari L, Gemmati D, et al. Effects of electromagnetic fields on proteoglycan metabolism of bovine articular cartilage explants. Connect Tissue Res 2003;44(3-4):54–9.

[15] De Mattei M, Pellati A, Pasello M, Ongaro A, Setti S, Massari L, et al. Effects of physical stimulation with electromagnetic field and insulin growth factor-I treatment on proteoglycan sysnthesis of bovine articular cartilage. Osteoarthritis Cartilage 2004;12:793–800.

[16] Dequeker J, Mokassa L, Aerssen J, Boonen S. Bone density and local growth factors in generalized osteoarthritis. Microsc Res Tech 1997; 37:358–71.

[17] Duda GN, Haisch A, Endres M, Gebert C, Schroeder D, Hoffmann JE, et al. Mechanical quality of tissue engineered cartilage: results after 6 and 12 weeks in vivo. J Biomed Mater Res 2000;53:673–7 (Appl. Biomater.).

[18] Fini M, Torricelli P, Giavaresi G, Carpi A, Nicolini A, Giardino R. Effect of L-lysine and L-arginine on primary osteoblast cultures from normal and osteoporotic rats. Biomed Pharmacother 2001;55(4):220–31.

[19] Fini M, Giavaresi G, Borsari V, Giardino R, Nicolini A, Carpi A. Osteoporosis and biomaterial osteointegration. Biomed Pharmacother 2004;58(9):487–93.

[20] Fini M, Giavaresi G, Setti S, Martini L, Torricelli P, Giardino R. Current trends in the enhancement of biomaterial osteointegration: biophysical stimulation. Int J Artif Organs 2004;18(6):291–8.

[21] Fini M, Giavaresi G, Torricelli P, Cavani F, Setti S, Canè V, et al. Pulsed electromagnetic fields reduce knee osteoarthritic lesion progression in the aged Dunkin Hartley guinea pigs. J Orthop Res 2005; (in press).

[22] Fioravanti A, Nerucci F, Collodel G, Markoll R, Marcolongo R. Biochemical and morphological study of human articular chondrocytes cultivated in the presence of pulsed signal therapy. Ann Rheum Dis 2002;61:1032–3.

[23] Goldring SR, Goldring MB. The role of cytokines in cartilage matrix degeneration in osteoarthritis. Clin Orthop Rel Res 2004;427S:S27–36.

[24] Hinsenkamp MG, Rooze MA. Morphological effect of electromagnetic stimulation on the skeleton of fetal and newborne mice. Acta Orthop Scand 1982;196:39–50.

[25] Hinton R, Moody RL, Davis AW, Thomas SF. Osteoarthritis: diagnosis and therapeutic considerations. Am Fam Physician 2002;65:841–8.

[26] Holland TA, Tessmar JKV, Tabata Y, Mikos AG. Transforming growth factor-β1 release from oligo(poly(ethylene glycol) fumarate) hydrogels in conditions that model the cartilage wound healing environment. J Control Release 2004;94:101–14.

[27] Huebner JL, Hanes MA, Beekman B, TeKoppele JM, Kraus VB. A comparative analysis of bone and cartilage metabolism in two strains of guinea-pig with varying degree of naturally occurring osteoarthritis. Osteoarthritis Cartilage 2002;10:758–67.

[28] Jacobson JI, Gorman R, Yamanashi WS, Saxena BB, Clayton L. Low-amplitude, extremely low frequency magnetic fields for the treatment of osteoarthritic knees: a double-blind clinical study. Aletr Ther Health Med 2001;7(5):54–64 (66–9).

[29] Kobayashi K, Amiel M, Harwood FL, Healey RM, Sonoda M, Moriya H, et al. The long-term effects of hyaluronan during development of osteoarthritis following partial meniscectomy in a rabbit model. Osteoarthritis Cartilage 2000;8(5):359–65.

[30] Kuhn K, D'Lima DD, Hashimoto S, Lotz M. Cell death in cartilage. Osteoarthritis Cartilage 2003;12:1–16.

[31] Le Graverand MP, Eggerer J, Mignon E, Otterness IG, Barclay L, Hart DA. Assessment of specific mRNA levels in cartilage regions in a lapine model osteoarthritis. J Orthop Res 2002;20(3):535–44.

[32] Liu H, Lees P, Abbott J, Bee JA. Pulsed electromagnetic fields preserve proteoglycan composition of extracellular matrix in embrionic chick sternal cartilage. Biochim Biophys Acta 1997;1336(2):303–14.

[33] Liu H, Abbott J, Bee JA. Pulsed electromagnetic fields influence hyaline cartilage extracellular matrix composition without affecting molecular structure. Osteoarthritis Cartilage 1996;4(1):63–76.

[34] Mattei JP, Roux H. New potential therapeutic goals: subchondral bone and progression of osteoarthritis. Osteoarthritis Cartilage 1999;7: 329–30.

[35] Messner K, Fahlgren A, Ross I, Andersson B. Simultaneous changes in bone mineral density and articular cartilage in the rabbit meniscectomy model of knee osteoarthrosis. Osteoarthritis Cartilage 2000; 8(3):197–206.

[36] Mix KS, Sporn MB, Brinckerhoff CE. Novel inhibitors of matrix metalloproteinase gene expression as potential therapies for arthritis. Clin Orthop Res 2004;427S:129–37.

[37] Moulharat N, Lesur C, Thomas M, Rolland-Valognes G, Pastoureau P, Anract P, et al. Effects of transforming growth factor-beta on aggrecanase production and proteoglycan degradation by human chondrocytes in vitro. Osteoarthritis Cartilage 2004;12:296–305.

[38] Newman AP. Articular cartilage repair. Am J Sports Med 1998;26(2): 309–24.

[39] Nicolakis P, Kollmitzer J, Crevenna R, Bittner C, Erdogmus CB, Nicolakis J. Pulsed magnetic field therapy for osteoarthritis of the knee-a dopuble-blind sham-controlled trial. Wien Klin Wochenschr 2002;114(15–16):678–84.

[40] Pascarella A, Toro A, Iervolino G, Trinchese GF. Treatment of articular cartilage lesions by PEMF: preliminary report. 3rd European Congress of Sport Traumatology, Madrid, April 1–3. 2004.

[41] Pelletier JP. The influence of tissue cross-talking on OA progression: role of nonsteroidal antiinflammatory drugs. Osteoarthritis Cartilage 1999;7:374–6.

[42] Pelletier JP. The influence of tissue cross-talking on OA progression: role of non-steroidal anti-inflammatory drugs. Osteoarthritis Cartilage 1999;7:374–6.

[43] Pellettier MJ. Pathophisiology of osteoarthritis. Osteoarthritis Cartilage 1999;7:371–3.

[44] Pezzetti F, De Mattei M, Caruso A, Cadossi R, Zucchini C, Carinci F, et al. Effects of pulsed electromagnetic fields on human chondrocytes: an in vitro study. Calcif Tissue Int 1999;65:396–401.

[45] Pfander D, Rahmanzadeh R, Scheller EE. Presence and distribution of collagen II, collagen I, fibronectin, and tenacsin in rabbit normal and osteoarthritic cartilage. J Rheumatol 1999;26(2):386–93.

[46] Pipitone N, Scott DL. Magnetic pulse treatment for knee osteoarthritis: a randomised, double-blind, placebo-controlled study. Curr Med Res Opin 2001;17(3):190–6.

[47] Poole RA. What type of cartilage repair are we attempting to attain? J Bone Jt Surg 2003;85-A(S2):40–4.

[48] Risbud MV, Sittinger M. Tissue engineering: advances in in vitro cartilage generation. Trends Biotechnol 2002;20(8):351–6.

[49] Robertson CM, Harwood FL, Sasho T, Williams SK, Pomerleau AC, Amiel D. Characterization of mature versus aged rabbit articular cartilage: analysis of cell density, apoptosis-related gene expression and mechanisms controlling chondrocyte apoptosis. Osteoarthritis Cartilage 2004;12(11):917–23.

[50] Rogart JN, Barrach HJ, Chichster CO. Articular collagen degradation in the Hulth–Telhag model of osteoarthritis. Osteoarthritis Cartilage 1999;7(6):539–47.

[51] Sah RL, Yang AS, Chen AC, Hant JJ, Halili RB, Yoshioka M, et al. Physical properties of rabbit articular cartilage after transaction of the anterior cruciate ligament. J Orthop Res 1997;15(2):197–203.

[52] Sakai A, Suzuki K, Nakamura T, Norimura T, Tsuchiya T. Effects of pulsing electromagnetic fields on cultured cartilage cells. Int Orthop 1991;15(4):341–6.

[53] Schnitzer TJ. Update of ACR Guidelines for osteoarthritis: role of the Coxibs. J Pain Symptom Manage 2002;23:S24–30.

[54] Schurman DJ, Smith RL, Buckwalter J, Schurman DJ. Osteoarthritis: current treatment and future prospects for surgical, medical, and biological intervention. Clin Orthop 2004;427S:S183–9.

[55] Sellers RS, Peluso D, Morris EA. The effect of recombinant human bone morphogenetic protein-2 (rhBMP-2) on the healing of full-thickness defects of articular cartilage. J Bone Jt Surg 1997;79:1452–63.

[56] Smith RL, Nagel DA. Effects of pulsing electromagnetic fields on bone growth and articular cartilage. Clin Orthop 1983;181:277–82.

[57] Tesch AM, MacDonald MH, Kollias-Baker C, Benton HP. Chondrocytes respond to adenosine via A2 receptors and activity is potentiated by an adenosine deaminase inhibitor and a phosphodiesterase inhibitor. Osteoarthritis Cartilage 2002;10:34–43.

[58] Tessier JJ, Bowyer J, Brownrigg NJ, Peers S, Westwood FR, Waterton JC, et al. Characterisation of the guinea pig model of osteoarthritis by in vivo three-dimensional magnetic resonance imaging. Osteoarthritis Cartilage 2003;11:1–9.

[59] Torricelli P, Fini M, Giavaresi G, Giardino R. In vitro model sto test orthopaedic biomaterials in view of their clinical application in osteoporotic bone. Int J Artif Organs 2004;27(8):658–63.

[60] Trock DH, Bollet AJ, Dyer RH, Fielding LP, Miner K, Markoll R. A double-blind trial of the clinical effects of pulsed electromagnetic fields in osteoarthritis. J Rheumatol 1993;20:456–60.

[61] Trock DH, Bollet AJ, Markoll R. The effect of pulsed electromagnetic fields in the treatment of osteoarthritis of the knee and cervical spine. Report of randomized, double blind, placebo controlled trials. J Rheumatol 1994;21:1903–11.

[62] Uthman I, Raynauld JP, Haraoui B. Intra-articular therapy in osteoarthritis. Postgrad Med J 2003;79(934):449–53.

[63] Varani K, Gessi S, Meriggi S, Iannotta V, Cattabriga E, Spisani S, et al. Effects of low frequency electromagnetic fields on A2A adenosine receptors in human neutrophils. Br J Pharmacol 2002;136:57–66.

[64] Yoshioka M, Coutts RD, Amiel D, Hacker SA. Characterization of a model of osteoarthritis in the rabbit knee. Osteoarthritis Cartilage 1996;4(2):87–98.