PEMF effects on chondrocyte cellularity and gene expression of the rat distal femoral metaphyseal articular cartilage

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Abstract— **the purpose of the current study was to determine the effect of PEMF on the rat distal femoral joint cartilage, in terms of chondrocytic gene expression level and cellularity. 20 Wistar female rats from two litters were randomly distributed in two groups: 10 rats (Stimulated Group) received PEMF (1 Hz, 30 mT/30 min per day/20 days in a row); the other 10 animals (Control Group) received similar treatment, with the difference that the electromagnetic stimulation was turned off. After the experimental intervention, thin histological sections were analyzed under Clear Field Microscopy in order to quantify chondrocytic cellularity. In addition, specific chondrocyte proteins expression was measure. Statistically significant differences were found in joint cartilage cellularity when MS and Non-MS were compared (101.13 ± 25.61 vs. 69.66 ± 15.55 cells per optical field, respectively; p=0.001). *Collagen XI*, *Sox6* and *Aggrecan* expression levels were also different in magnetically stimulated tissues relative to control. On the other hand RUNX2 and *ALPL* expressions showed no significant differences between groups (*X*-squared test $p < 0.05$). These results are evidence that *in vivo* PEMF stimulation increases the number of well differentiated knee joint cartilage cells in healthy young adult rats. The low gene expression of RUNX2 and *ALPL* supports that the chondrocytic response to PEMF do not correspond to a hypertrophic reaction. These results highlight the possible therapeutic future of PEMF in cartilage injuries, and on its ageing.**

Keywords— **Pulsed electromagnetic fields, joint cartilage cellularity, gene expression.**

I. Introduction

Osteoarthrosis (OA) is the most common musculoskeletal alteration in the adult general population[1, 2]. It is considered a multifactorial disorder having both genetic and environmental components. OA is a non-inflammatory, deforming, degenerative disease where joint cartilage damage is observed. The prevalence of this disease increases with age. Some authors claim that 10% of the population ≥ 60 years of age is affected [3]. The prevalence is greater in women compared to men (34% vs. 31%)[2] .

In recent years, interactions between electromagnetic fields and biological systems have increasingly been studied. The advance in applied electromagnetic knowledge has allowed the study of pulsed electromagnetic fields (PEMF) effects in several musculoskeletal disease conditions. To date, PEMF have a number of well-documented physiological effects on cells and tissues; including the up regulation of gene expression of the transforming growth factor beta super family members, the increase in glycosaminoglycan levels, and an anti-inflammatory action [1, 4, 5, 6, 7]. In previous studies, some authors reported cell proliferation increase of *in vitro* cultured human chondrocytes exposed to different PEMF stimulation paradigms [5, 8]. Therefore, there is a strong rationale supporting the in vivo use of biophysical stimulation with PEMF, better OA treatment.

Objective. The purpose of this study was to determine the effect of pulse electromagnetic fields on joint cartilage in terms of chondrocytic gene expression level and cellularity of the distal femoral metaphysis in the rat.

II. Material and Methods

In a experimental study, twenty female 84 days old Wistar rats from the three litters, average weight of 170.0 ± 24.0 g, were studied. Animals were randomly assigned into two groups: those in the first group received focused pulsed magnetical stimulation on the knee (MS). The second, the control group (Non-MS), was managed similarly, except magnetical stimulation. The study was performed in two phases: in the first PEMF was applied (30 mT/1 Hz/ 30 min/20 days) with a magnetic stimulation system approved for clinical use in depression treatment (Magstim model 220. The Magstim Company Ltd., London, UK). The rats of both groups were soothed by intraperitoneal application of azaperona (Sural, Chinoin S.A. de C.V., Mexico) at 0.5 mg/Kg of weight, then in ventral position and extremities extended, a stimulation coil was placed over the right distal femoral metaphysis, at a separation of 3 cm. PEMF were applied only to MS group animals.

In the second phase, all the animals were euthanized, and knee samples were obtained for histological joint tissue evaluation; 6 µm bone longitudinal sections were obtained

D.A. Jaffray (ed.), *World Congress on Medical Physics and Biomedical Engineering, June 7-12, 2015, Toronto, Canada*,
IFMBE Proceedings 51, DOI: 10.1007/978-3-319-19387-8_253

with a manual microtome (Carl Zeiss Inc., USA), and stained with a hematoxiline-eosine technique [9]. The sections were analyzed in a light clear field microscope (Olympus BX51, Olympus Optical, Co. Japan), with a 40/0.75 objective, in order to quantify the number of chondrocytes per optical field. To determine the expression levels of collagen type XI alpha 2 (Col11a2), (sex determining region Y)-box 6 (Sox6), aggrecan (Acan), runt-related transcription factor 2 (Runx2), and alkaline phosphatase liver/bone/kidney (Alpl) in cartilage samples, specific intron spanning primers were designed. Gapdh was used as constitutive gene.

Table I. Sequence gene primer.

GENE	ACCESSION NUMBER	SEQUENCE	FRAGMENT
Acan	NM_022190	F:CAGAGGAACACACCGAAAGTC R:CGTCCTGAAGTGTCTGTGCTG	190 pb
Alpl	NM_013059.1	F:CATCATCATGTTCCTGGGAG R:GACCTGAGCGTTGGTGTTGT	163 pb
Col11a2	NM_212528	F:CTAAGGGAACATCAGGTGGTG R:ATCCCACTTCTCCTCTCTGG	162 pb
Sox6	NM_001024751	F:CAACAGCAGCAACTTCTACAG R:GGTAGTTATCACCTGGCTTG	196 pb
Runx2	Variant1 NM_001278483 variant2 NM_001278484	F:GATGGTGTTGACGCTGATGG R:CACAACTGGGGAGTGAATGAG	233 pb
Gapdh	AF106860	F:CATCTTCTTGTGCAGTGCCAG R:AACTTGCCGTGGGTAGAGTCA	200 pb

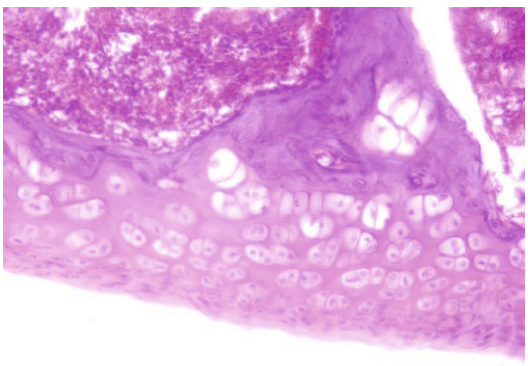
total RNA was isolated by a FFPE RNAeasy Kit. For each sample RNA was reverse transcribed using oligo dT as primer. Real time PCR reactions were performed from 50 ng of DNA. Lab animals were treated according to international ethical regulations.

Descriptive statistical analysis for the data was performed. Paired Student's *t* test was used to determine differences between groups. Significance level was established when $\alpha = 0.05$.

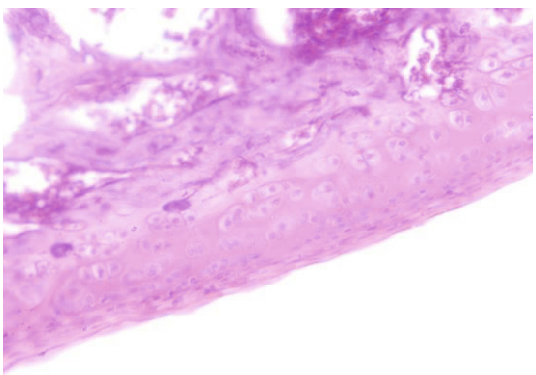
III. Results.

The chondrocytes cellularity of the distal femoral metaphysis joint cartilage histological fields, of both MS and non-MS were 101.13 ± 25.61 vs. 69.66 ± 15.55 cells per optical field, respectively ($p = 0.007$). The figure 1 shows the microscopic differences between stimulated joint cartilage and no-stimulated joint cartilage.

Fig.1. MS and non-MS distal femoral metaphysis chondrocytes. Haematoxylin-eosin stained thin histological longitudinal sections of distal femoral metaphysis joint cartilage of stimulated (a) and no-stimulated (b) rats. Amplification 40X.



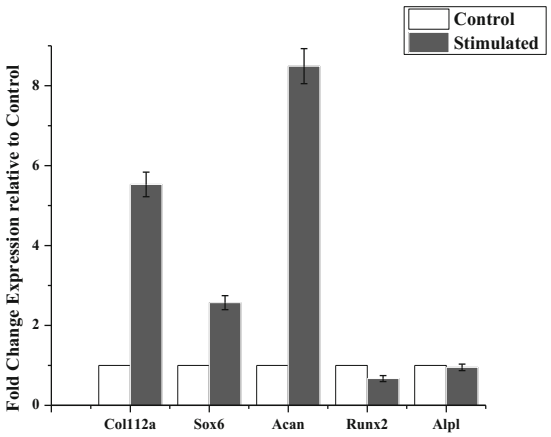
a. 87 chondrocytes per visual field



b. 57 chondrocytes per visual field

The results in graph 1 are the fold difference of proteins expression in MS relative to non-MS, as Mean ± Standard Error (SE) of five independent samples for each group. Differences were evaluated by Paired Student's *t* test and were considered as significant when P< 0.05.

Graph 1. Relative expression of genes in rat bone samples.



IV. Discussion and conclusions

PEMF *in vivo* stimulation of the joint cartilage at the distal femoral metaphysis of healthy rats caused chondrocytes proliferation. This result is similar to Pezzetti et al., [8] and De Mattei et al., [5] *in vitro* observations, both obtained using a different experimental magnetic stimulation paradigm (75 Hz, 2.3 mT), suggesting that several PEMF modalities can induce cartilage remodeling effects

These results are evidence that *in vivo* PEMF stimulation increases the number of well differentiated knee joint cartilage cells in healthy young adult rats. The findings indicate a *trophic* effect upon *in vivo* cartilage joint tissue. The PEMF action mechanisms must be related to the electrical characteristics of the chondrocytes, which start to be investigated and seems related to new matrix production. It is important to characterize different types of cartilage tissue, and diverse stage of development as well as unhealthy tissue responses to PEMF. In any case these observations support a possible application for the development of cartilage tissue in normal and non-traumatic conditions.

The low gene expression of RUNX2 and *ALPL* supports that the chondrocytic response to PEMF do not correspond to hypertrophic reaction. These results highlight the possible therapeutic future of PEMF in cartilage injuries, and on its ageing.

V. References.

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Acknowledgment

This study was support by CONACYT.

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