

Effects of Pulsed Signal Therapy® on Gene Expression in Three-Dimensional Chondrocyte Cultures

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Introduction:

Pulsed electromagnetic fields (PEMF) have been used widely to treat non-healing fractures and related problems in bone healing since approval by the Food and Drug Administration (FDA) in 1979, with a success rate averaging 70-80% in a wide variety of centers in several countries. A special pulsed magnetic field configuration is used for Pulsed Signal Therapy®. Many patients treated with this Pulsed Signal Therapy® (PST) lost their pains and showed less osteoarthritic symptoms.

To determine the biological effects of PST on cartilage physiology we used a three-dimensional chondrocyte culture as an in vitro model for articular cartilage. Isolated chondrocytes of arthritic cartilage proliferate in monolayer culture. In three-dimensional culture cells redifferentiate again shown by the deposition of cartilage-specific matrix components like collagen type II. Using this cartilage model chondrocytes from different patients were pooled to minimize variability between individual patients.

The aim of our study was to analyze the effects of the Pulsed Signal Therapy (PST)¹ on gene expression in chondrocyte cultures derived from osteoarthritic (OA) articular cartilage.

Material and Methods:

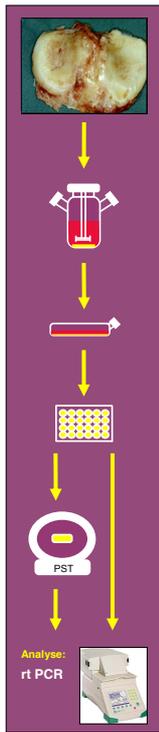
Cartilage specimens were obtained from different patients (mean age 71 years) suffering from coxarthrosis and gonarthrosis, respectively. Chondrocytes also were obtained from healthy cartilage (ND) served as controls.

Chondrocytes were isolated, expanded in monolayer culture and pooled. Cells were cultured three-dimensionally in high-density² for 6 weeks. PST treatment cultures were exposed to one hour of PST daily for 9 consecutive days.

The PST treatment device consisted of a magnetic field generator, an electronic interface, and a system of toroid coils. This produces unidirectional DC elliptical magnetic fields of 10 – 15 Gauss with varying frequencies between 10 and 30 Hz.

Treated as well as untreated OA and ND chondrocytes were cultivated for up to 6 months and were used for subsequent semi-quantitative rtPCR analysis (SYBR Green PCR Core Kit and i-Cycler real-time PCR System).

Expression of the marker genes collagen II and IX, cartilage oligomeric matrix protein (COMP) and aggrecan was measured prior to and directly after PST treatment as well as 3 and 6 weeks, and 6 months after treatment.



gene	Embl database	oligonucleotide (5'→3')	product size (base pairs)
GAPDH	M33197	GGC GAT GCT GGC GCT GAG TAC TGG TTC ACA CCC ATG ACC A	149
type II α1 collagen	X06268	CCG GGC AGA GGC CAA TAG CAG GTT CAA TGA TGG GGA GGC GTG AG	128
type IX α3 collagen	L41162	AAT CAG GCT CTC GAA GCT CAT AAA A CCT GCC ACA CCC CCG CTC CTT CAT	100
COMP	L32137	GGG TGG CCG CCT GGG GGT CTT CTT GCC GCA GCT GAT GGG TCT C	116
aggrecan	X17406	CCA GTG CAC AGA GGG GTT TG TCC GAG GGT GCC GTG AG	146

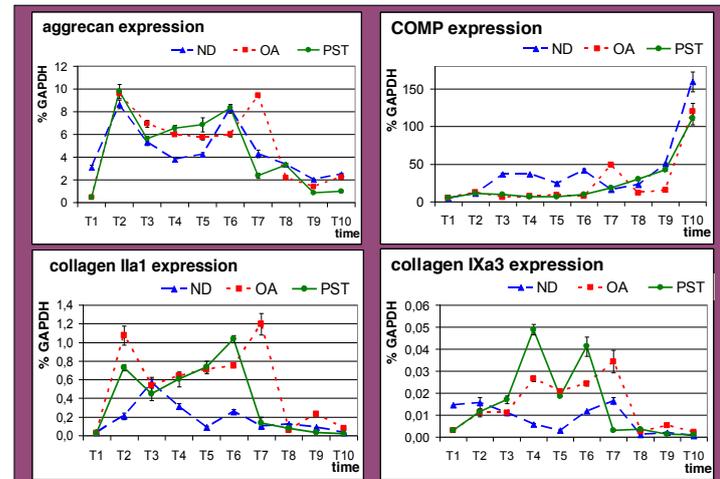
References:

- 1) Trock DH, et al. The effect of Pulsed Electromagnetic Fields in the Treatment of tcoarthritits of the Knee and Cervical Spine. Report of Randomized, Double Blind, Placebo Controlled Trials. *J Rheumatol* 1994 ;21: 1903-1911
- 2) Schulze-Tanzil G, et al. Redifferentiation of dedifferentiated human chondrocytes in high-density cultures. *Cell Tissue Res* 2002;308: 371-397
- 3) Aigner T, et al. Anabolic and Catabolic Gene Expression Pattern Analysis in Normal Versus Osteoarthritic Cartilage Using Complementary DNA-Array Technology. *Arthritis Rheum* 2001;44: 2777-2789

Results:

Compared to the housekeeping gene GAPDH (glyceraldehyde-3-phosphate dehydrogenase), the matrix proteins aggrecan, COMP, type I collagen and type III collagen are very strongly expressed, while the expression profiles of type II and IX collagen are at a very low level (collagen type IX: 0.001 to 0.0001% of GAPDH).

Gene expression of tested matrix proteins is oscillating during the study period. All collagen genes are expressed at a higher level in the osteoarthritic groups than in the normal groups. Concerning articular cartilage chondrocytes, periodic progression of expression in PST-pellets has changed from the progression found in pellets of osteoarthritic chondrocytes to the gene expression profile of normal donor pellets. Type II and IX collagen genes showed a markedly stronger expression in the untreated control pellets than in those treated with PST at the last evaluation dates.



Diagrams: Marker gene expression (expression as percentage of GAPDH expression) in the PST-treated pellets (PST, green) in relation to marker gene expression in the untreated control articular cartilage chondrocyte pellets (OA, red) and in the normal donor chondrocyte pellets (ND, blue). Data values are given as the average ± standard deviation. T1 - before PST treatment (PST), T2 - 5th day of PST, T3 - 9th day of PST, T4 - 2 days after PST, T5 - 4 days after PST, T6 - 6 days after PST, T7 - 9 days after PST, T8 - 3 weeks after PST, T9 - 6 weeks after PST, T10 - 6 month after PST.

Conclusion:

The objective of our study was to investigate the role of PST on chondrocyte matrix formation of adult human articular cartilage. As a hypothesis, PST treatment results in an electromagnetic pulsed field, which may stimulate chondrocytes physiologically to enhance their metabolic activity and the formation of cartilage extracellular matrix. PST apparently had an effect on the expression of chondrocyte marker genes in OA chondrocytes and reverted the expression of selected marker genes towards the expression found in normal chondrocytes.

Strong expression of type II collagen is to be expected in chondrocytes from osteoarthritic cartilage, because in such cartilage, the matrix shows an imbalance between anabolism and catabolism, and the chondrocytes produce increased amounts of matrix proteins.³ However, these are not retained in the extracellular matrix because of enzymatic degradation, and there is a detectable loss of proteoglycans and collagens.

Interestingly, however, in this study the gene expression of matrix proteins in osteoarthritic cartilage seems to be down-regulated by PST. This surprising result may be a first important indication for the possible mode of action of PST on the cytobiological level. If the metabolic balance of the matrix was adequately restored as to bind a sufficient amount of pericellular type II collagen in the cartilage matrix, the gene expression could be reregulated. Since COMP seems to be involved in the cross-linkage of collagens, the slight increase of expression at these later dates may be an indication of improved matrix synthesis. The same applies to type-II-associated type IX collagen. A detailed determination of the expression of molecules linked to the matrix and those involved in its catabolism may provide valuable data to verify this theory.

The surprising results obtained here show that PST has an effect on the level of matrix molecule gene expression. Their interpretation raises new questions that call for more thorough investigations.