



EFFECTS of PULSED SIGNAL THERAPY on 3-DIMENSIONAL CHONDROCYTE CULTURE

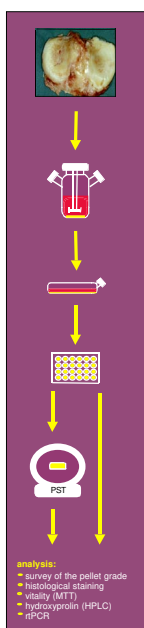
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Introduction:

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.

Material and Methods:



Cartilage and meniscal specimens were obtained from different patients (mean age 71 years) suffering from coxarthrosis and gonarthrosis, respectively.

Chondrocytes were isolated, expanded in monolayer culture and pooled. Long-term cultivation using pellet cultures was performed for subsequent PST treatment and controls. 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.

Deposition of matrix components was verified histological by staining of proteoglycan with Alcian blue and by staining of collagen with Azan. Vitality of chondrocyte cultures was documented by MTT and pellet size was determined by image analysis using Adobe Photoshop®.

The amount of hydroxyproline was measured by HPLC to quantify the content of collagen indirectly.

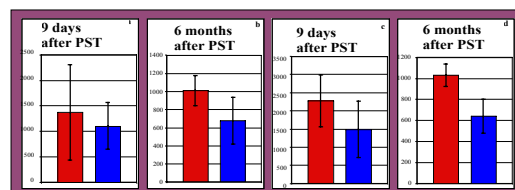
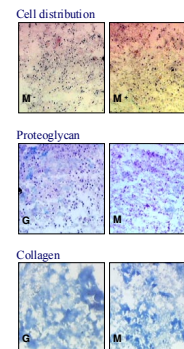
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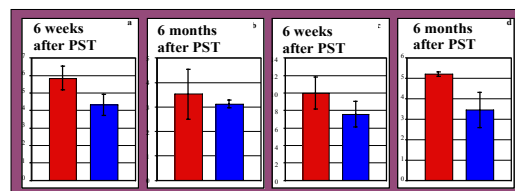
Results:

Histological staining showed production of proteoglycan and collagen. The MTT-test confirmed the cell vitality. Microscopically, at day 9 and 6 months after treatment with PST, chondrocyte pellets appeared to be larger in size compared to chondrocytes not treated with PST. Referring to the amount of hydroxyproline (HPLC), chondrocyte pellets treated with PST showed an increased matrix synthesis of collagen.

Conventional H&E staining of meniscal chondrocytes treated with PST (M') and controls (M). Alcian blue staining of proteoglycan and Azan staining of collagenous matrix components in pellet cultures of human articular (G) and human meniscal chondrocytes (M).



Pellet grade (pixel score and average luminance) of chondrocyte pellet cultures treated with PST (red) and controls (blue). Pellet grade was measured for articular chondrocytes after 9 days (a) and 6 months (b) of PST treatment and for meniscal chondrocytes after 9 days (c) and 6 months (d) after treatment.



Hydroxyproline content (ng/mg wet weight) of chondrocyte pellet cultures treated with PST (red) and controls (blue). Hydroxyproline was measured for articular chondrocytes after 6 weeks (a) and 6 months (b) of PST treatment and for meniscal chondrocytes after 6 weeks (c) and 6 months (d) after treatment.

Discussion:

The objective of our study was to investigate the role of PST on chondrocyte matrix formation of adult human articular and meniscal 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.

In conclusion, regarding the formation of cartilage matrix biochemical and histological analysis revealed a marginal effect of PST on arthritic articular chondrocytes. The present results of this study show a promising approach to evaluate the effects of PST giving rise to mitigation of arthritic diseases. For further analysis of PST effects on chondrocytes, the expression profiles of distinct subsets of extracellular matrix genes (type I, II and type X collagen, aggrecan and link protein) are under investigation to achieve a more profound knowledge of molecular events as a consequence of PST treatment.