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. [i]

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 [ii], 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 at pH 2.5 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®.

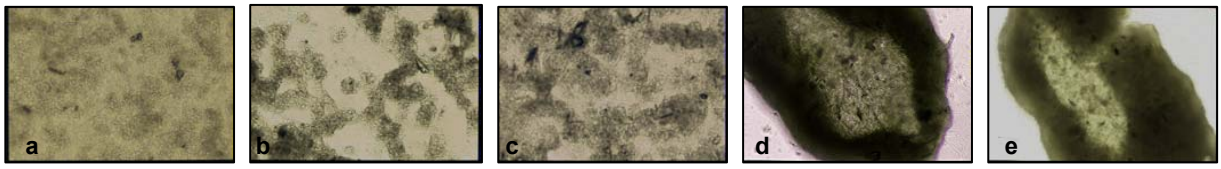
The amount of hydroxyproline was measured by HPLC to quantify the content of collagen indirectly. Samples were incubated for 18h in 0.6 M HCl and resuspended in 100 µl 0.02 M HCl for subsequent HPLC analysis.

Cell growth in monolayers was analyzed by conventional cell counting. The DNA-content was measured photometrically after isolation of genomic DNA with DNAzol according to the manufacturer`s protocol (GibcoBRL).

Results:

Figure 1 exemplarily shows the development of non-treated chondrocyte pellets over the culture period of 8 weeks.

Fig.1:



Development of pellets (articular chondrocytes) without PST over the culture period of 8 weeks (125x magnification) a: after 1 hour; b: after 1 day; c: after 3 days; d: after 3 weeks; e: after 8 weeks

Figure 2 gives an overview of cryo-sections stained by Hämalaun-Eosin. The cytosol of cells is stained blue.

Fig. 2a

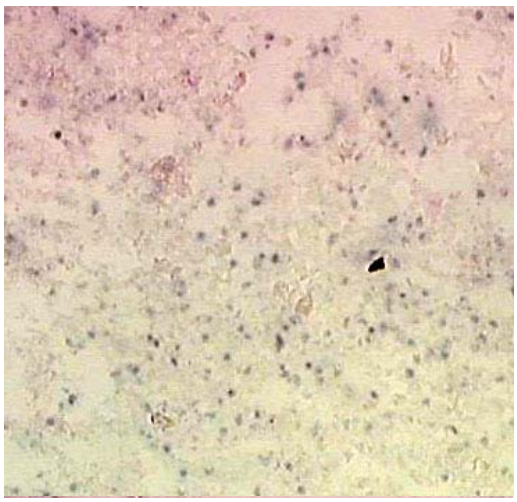
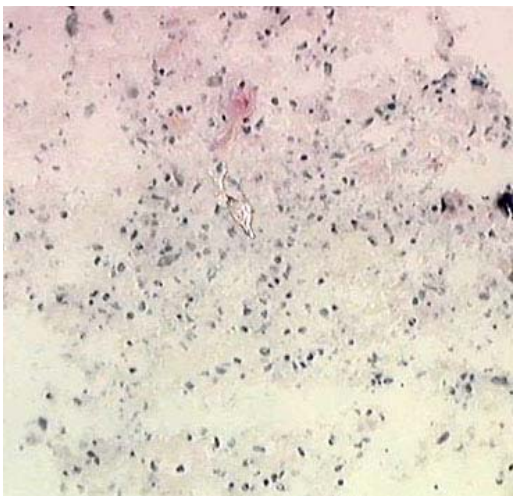


Fig. 2b



Conventional H&E staining of meniscal chondrocytes treated with PST (a) and controls (b)

The MTT-test confirmed the cell vitality (data not shown).

The presence of proteoglycan and collagene within the extracellular matrix of all pellets was demonstrated by histological staining. Figure 3 and 4 show cryo-sections of pellets from articular and meniscal chondrocytes, respectively. Collageneous matrix components are stained blue by Azan (figure 3) and proteoglycan is stained blue by Alcian (Figure 4).

Fig.3: a

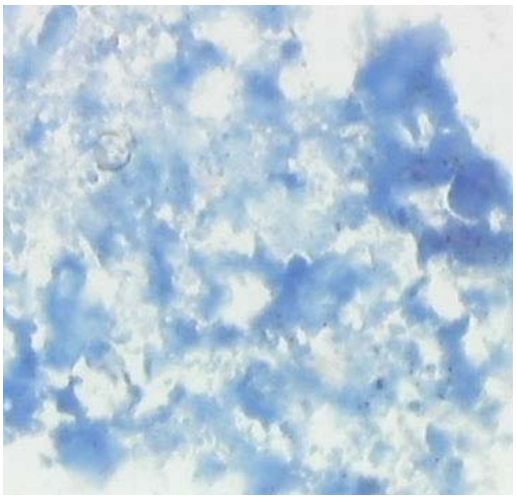
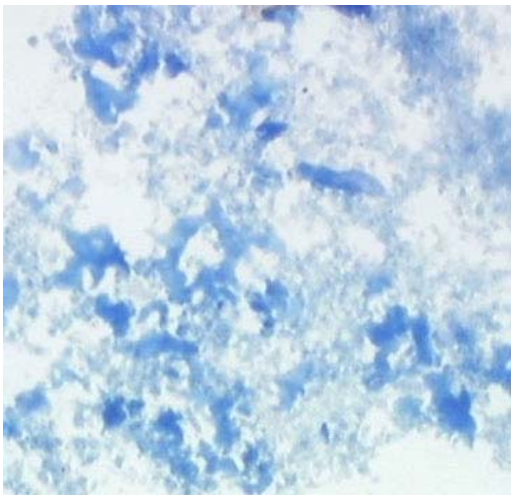


Fig. 3b



Histological staining of collageneous matrix components in pellet cultures of human articular (a) and human meniscal chondrocytes (b).

Fig.4a

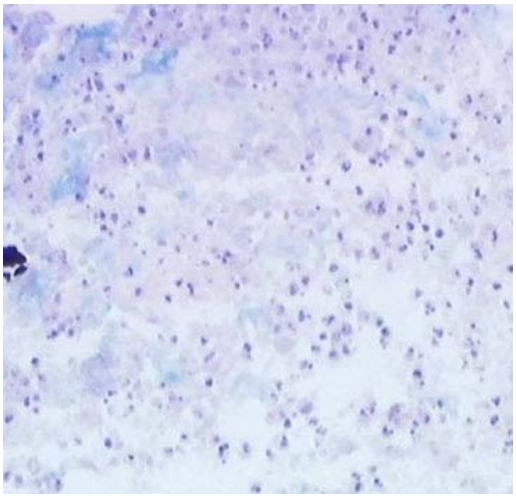
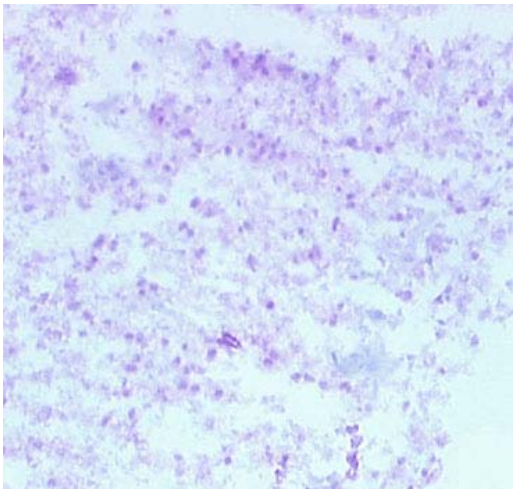


Fig. 4b



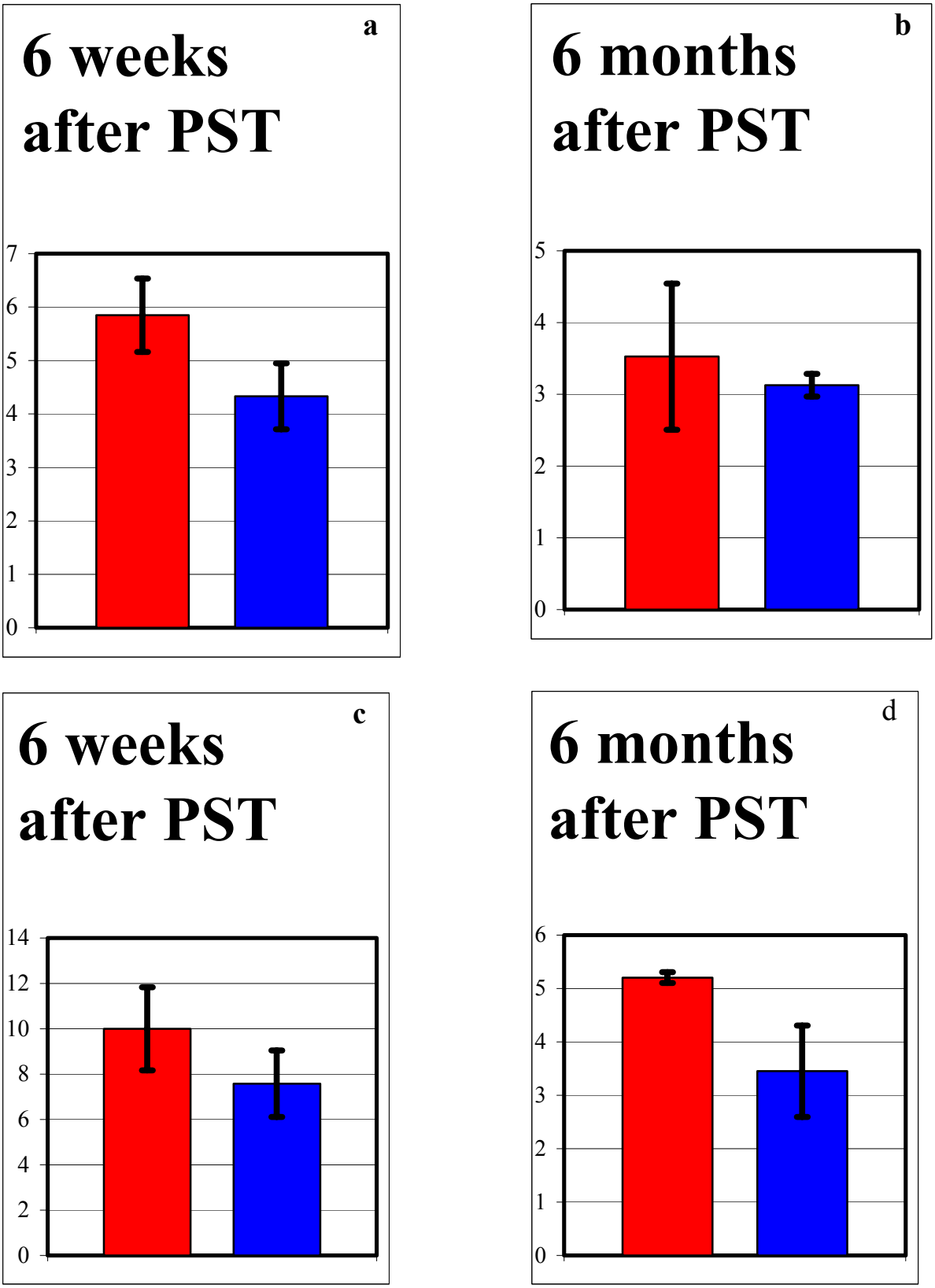
Histological staining of proteoglycan in pellet cultures of human articular (a) and human meniscal chondrocytes (b).

Nine days after PST treatment chondrocyte pellets microscopically appeared to be larger in size compared to chondrocytes not treated with PST. Six month after PST treatment, pellet size of articular and of meniscal chondrocytes were larger compared to controls (data not shown).

Figure 5 demonstrates the comparison of hydroxyproline content (ng/mg wet weight). In all diagrams PST treated pellets are compared with controls. Six weeks after PST, the hydroxyproline content of articular chondrocyte pellets increased. Instead treatment of meniscal chondrocyte pellets resulted in a significantly increase by month six.

Referring to the amount of hydroxyproline (HPLC), chondrocyte pellets treated with PST showed an increased matrix synthesis of collagen.

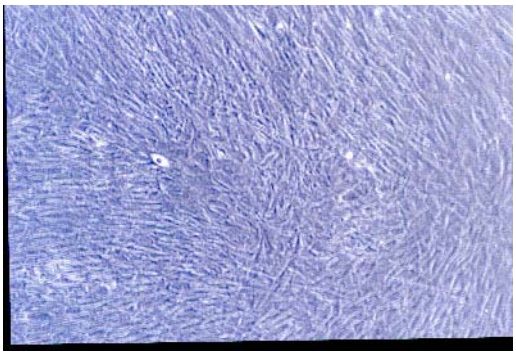
Fig. 5:



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 mensical chondrocytes after 6 weeks (c) and 6 months (d) after treatment.

The PST effect on cell proliferation was tested in monolayer cultures (figure 6) of articular arthritic chondrocytes. Cell number and DNA content remained constant in the proliferation test (data not shown).

Fig.6:



Chondrocytes in monolayer culture

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.

To analyze cartilage matrix formation upon PST treatment, we used a three-dimensional tissue culture system. For engineering of artificial cartilage tissues chondrocytes are isolated due to enzymatic digestion of the extracellular matrix resulting in vital cartilage cells, which are cultured and expanded under conventional cell culture conditions. During these phases of culture and expansion cartilage cells dedifferentiate and proper formation of cartilage matrix is abolished. Chondrocytes are cultured in micromass three-dimensionally, to ensure proper redifferentiation of chondrocytic cells and appropriate cartilage matrix formation in vitro. [iii,iv,v,vi]

Histological analysis revealed the formation of cartilaginous extracellular matrix in PST-treated cultures and controls. On the basis of biochemical analysis, treatment of meniscal and arthritic chondrocyte cultures resulted in an increased deposition of collageneous matrix components. For arthritic articular chondrocytes, only a marginal enhancement of collagen synthesis was documented almost directly after stimulation of artificial cartilage cultures. For meniscal chondrocytes, instead, PST demonstrated a positive effect on matrix formation in the long term up to 6 month after electro-magnetic stimulation.

In conclusion, regarding the formation of cartilage matrix and the growth of chondrocytes, 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.

Keywords:
Pulsed Signal Therapy, cartilage, 3-dimensional chondrocyte culture, condrocytes

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