PERFUSION CO-CULTURE SYSTEM:
FREE PERIOSTEAL GRAFTS MODULATE ARTICULAR CHONDROCYTE MATRIX DEPOSITION
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Introduction  Articular hyaline cartilage has a poor capacity to repair or even regenerate cartilage defects. This has lead to new forms of treatment. One of such new cartilage repair techniques is the autologous chondrocyte transplantation (ACT): Cartilage defects are covered with a periostal membrane and precultured chondrocytes are injected subperiostral. Some authors have postulated that the periostal membrane stimulates transplanted chondrocytes by paracrine effects [1,2]. However, little is known about the influence of periostral grafts on chondrocytes. Thus, this study uses a perfusion co-culture system in order to investigate the modulation of chondrocyte matrix deposition by periostral explants.

Methods  Articular chondrocytes were isolated enzymatically from cartilage of bovine tarso-metatarsal joints and cultured with standard medium in a monolayer on cover glass mounted in special carriers. Periostea from the os metatarsal III of adult cattle was elevated with a punch of 6mm diameter and a periostral elevator. The explants were fixed between two membrane filters and mounted in special ring carriers. Chondrocytes and periostral grafts were transferred into different perfusion chambers which were connected by a silicone tube and a peristaltic pump. Culture medium first entered the chamber with periostral grafts where it remained for 8h and was transferred automatically to the second container containing chondrocytes where it remained for another 8h. The chamber volume of 18ml was changed three times a day. All components of the work line were placed in an incubator (5% CO₂, 37°C). A control setup was performed without periostral explants in the first chamber.

Proliferation  of chondrocytes was estimated by DNA measurement using the intercalation of ethidium bromide (Karsten and Wollenberger) [3]. Proliferation rate was determined by the dimethyl-benzene blue assay. Biosynthetic activity: Chondrocytes were replaced after 10 days of incubation and radio-labeled (10µCi/ml ³H-proline). Collagen type II deposition was measured by ELISA after 10 days of culture and was monitored immunocytochemically (collagen type I and II). Gene expression of collagen type I/II and aggregan mRNA was done by RT-PCR.

Results  Although co-cultured chondrocytes showed a delayed proliferation rate during the first days, the cell numbers were equal in co- and in mono-culture after 10 days. Immunocytochemically collagen type II staining was stronger in mono-culture than in co-culture whereas the staining for collagen type I appeared more intensive in co-cultured chondrocytes and was weak in mono-culture (Fig. 1). Both radio-labeled sulfate incorporation (Fig. 2 left) as well as dimethylene blue assay (inhibition about 34%) showed a decrease of PG content in co-cultures. ³H-proline incorporation decreased significantly by the influence of periostral explants (inhibition about 34%). Quantification of native collagen type II deposition showed less amounts in the extracellular matrix of co-cultured chondrocytes (Fig. 2 right). In contrast preliminary data showed no significant differences in gene expression of collagen type I/II and aggregan in co- and mono-cultured chondrocytes.

Discussion  The present study demonstrates a modulation of chondrocyte matrix deposition by free periostral grafts in vitro. The new established co-culture system allows to optimize culture conditions in consideration of nutritional needs or the continuous elimination of metabolic products [4]. Co-culturing with periostral grafts leads to a delayed proliferation rate of chondrocytes in vitro. The decrease of biosynthetic activity, PG content and extracellular matrix deposition of collagen type II suggests a catabolic effect of periostral explants on articular cartilage. Preliminary RT-PCR analysis showed no significant differences of collagen type I/II and aggregan expression but more experiments have to be done in order to prove whether the modulation of extracellular matrix is regulated posttranscriptionally. Further investigations should clarify the role of periostral in ACT.


Acknowledgments  Thanks to R. Kirsch, C. Kremling and F. Lichte for their excellent technical assistant. This study was supported by the “Verein zur Foerderung der Erforschung und Bekämpfung rheumatischer Erkrankungen Bad Bramstedt e.V.”

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