Perfusion culture system: Synovial fibroblasts modulate articular chondrocyte matrix synthesis in vitro

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ABSTRACT

Objective: To investigate the interactions of chondrocyte metabolism by synovial cells and synovial supernatants in a new perfusion co-culture system.

Methods: Chondrocytes and synovial fibroblasts were obtained from knee joints of slaughtered adult cattle. For experimental studies chondrocytes and synovial fibroblasts were placed together into a perfusion chamber (co-culture) or were placed into two different perfusion culture containers, which were connected by a silicone tube (culturing of chondrocytes with synovial supernatants). A control setup was used without synovial cells. Chondrocyte proliferation was shown by measurement of DNA content. The proteoglycan synthesis was quantified using $^{35}SO_4^{2-}$-labelling and the dimethylmethylene blue assay. ^{3}H-proline incorporation was used to estimate the protein biosynthesis. Type II collagen synthesis was measured by ELISA, furthermore extracellular matrix deposition was monitored immunohistochemically (collagen types I/II). Regarding to the role of reactive oxygen species LDH release before and after stimulation with hydrogen peroxide was measured.

Results: The proliferation of chondrocytes shows an increase in monoculture as well as in co-culture or in culture with synovial supernatants more than fivefold within 12 days. ^{3}H-proline incorporation as a marker for chondrocytes biosynthetic activity decreases in co-culture system and in culture with synovial supernatants. A similar effect is seen measuring total proteoglycan content as well as the $^{35}SO_4^{2-}$ incorporation in chondrocytes. Co-culturing and culturing with synovial supernatants lead to a significant decrease of proteoglycan release and content. Quantification of collagen type II by ELISA shows significant lower amounts of native collagen type II in the extracellular matrix of co-cultured chondrocytes as well as in culture with synovial supernatants. The membrane damage of chondrocytes by hydrogen peroxide is reduced when chondrocytes are co-cultured with synovial fibroblasts.

Conclusion: The co-culture perfusion system is a new tool to investigate interactions of different cell types with less artificial interferences. Our results suggest that synovial supernatants and synovial fibroblasts modulate the biosynthetic activity and the matrix deposition of chondrocytes as well as the susceptibility to radical attack of reactive oxygen species.

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1. Introduction

The physiological function of synovial joints is based on the interactions of different connective tissues. Muscle tissue, adipose tissue, ligaments, blood vessels and nerves are components of synovial joints. However, articular cartilage and synovium are supposed to be the most important tissues both in physiology and pathophysiology. Interactions of chondrocytes and synoviocytes got into the focus of interest, since it has been shown that nutrition of cartilage in particular is dependent on factors produced by synoviocytes (Levick and McDonald, 1995). It is also known that inflammatory processes of the synovium are a part of osteoarthritis and rheumatoid arthritis (Katrib et al., 2002). However, little is currently known about this cellular cross-talk between cartilage and synovium.

In former investigations synovial supernatants were used to study the modulation of chondrocyte metabolism in vitro (Lee et al., 1997), but cellular interactions could not be detected by this experimental setup. Further reports described several other techniques to culture synoviocytes and chondrocytes together. Nevo et al. (1993)

analyzed the adhesion characteristics of chondrocytes cultured separately and co-cultured with synovial fibroblasts. Therefore, chondrocytes were seeded on plates precoated with confluent monolayers of synovial fibroblasts. Methodically, investigations of biochemical changes were impossible since the cell populations could not be separated after co-culture for further investigations. D'Andrea et al. (1998) used the same setup in order to analyze the intercellular calcium signaling between chondrocytes and synovial cells. Another technique of co-culture was reported by Kurz et al. (1999). Chondrocytes were seeded on micro-porous membranes of suitable inserts so interactions between the two different cell types via medium were possible. This setup as well as the setup reported by Huch et al. (2001) allows separation both cell populations for further biochemical investigations. However, both co-culture systems work stationary, i.e. artificial results (because of different pH or different consumptions of nutritives in mono- and in co-culture) are possible.

The aim of this study was to investigate a newly designed perfusion co-culture system that helps to identify mechanisms regarding to the in vivo functions of synovial joints.

The study focuses on the influence of synovial fibroblasts on chondrocyte proliferation, matrix deposition and vulnerability against radical attack by reactive oxygen species. The utilized setup allows to investigate and to compare both the modulation of chondrocyte metabolism by synovial supernatants and by co-culturing with synovial fibroblasts.

2. Material and methods

2.1. Isolation of chondrocytes

Articular cartilage was obtained from tarso-metatarsal joints of slaughtered adult cattle within 10 min of death. Cartilage flakes were washed several times with Hank's balanced salt solution containing antibiotics (penicillin 100 U/ml, streptomycin 100 U/ml) and antimycotics (amphotericin B 2.5 µg/ml) and were cut into small pieces. The incubation with pronase (32 U/ml) and hyaluronidase (2.3 kU/ml) in HBSS for 1 h was followed by a digestion with collagenase (918 U/ml) in Ham's F12 medium containing vitamin C (50 µg/ml), vitamin E (50 µM) and fetal calf serum (10%) for 6 h at 37 °C. The cell suspension was centrifuged at $400 \times g$ for 10 min. The supernatant was discarded and the pellet resuspended in Ham's F12 medium with fetal calf serum (10%), antibiotics, antimycotics and vitamin C/E (see above). Before the cell number was determined using a Thoma chamber the cell suspension was filtered through a gauze filter (pore size 20 µm). Cell vitality was assessed by trypan blue exclusion. Special cell carriers (Minucells®, Germany) were prepared using coverslips (∅ 13 mm) as support material. These carriers were transferred into a 24-well culture plate. Chondrocytes were seeded in Ham's F12 medium containing fetal calf serum, antibiotics, antimycotics and vitamin C/E (see above) in monolayer at a density of 100,000 cells/cm² onto the coverslips. After cell attachment the cell-loaded carriers were transferred into the perfusion chamber system (see below) within 24 h.

2.2. Isolation of synoviocytes

Intimal synovial explants were obtained from the knee of adult cattles within 1 h of death and were rinsed several times with Hank's balanced salt solution containing antibiotics (penicillin 100 U/ml, streptomycin 100 µg/ml) and antimycotics (amphotericin B 2.5 µg/ml). Synovial cells were obtained by digestion with collagenase (918 U/ml) in Ham's F12 medium with fetal calf serum (10%), antibiotics, antimycotics and vitamin C/E (see above) for 8 h
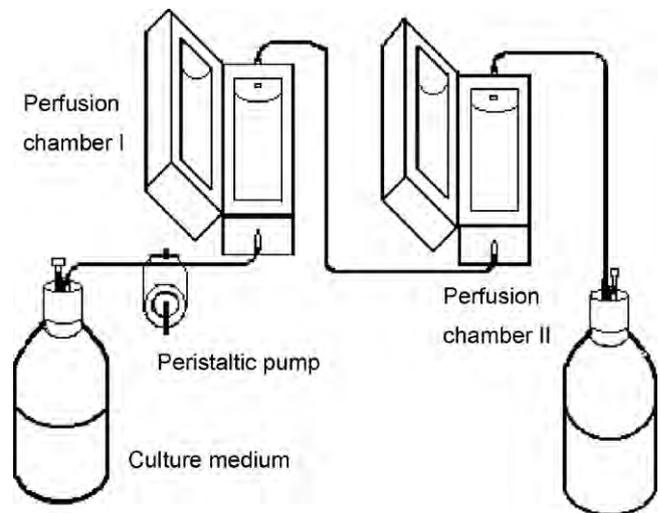
Fig. 1. Common experimental setup. In all groups culture medium entered the first chamber for an incubation of 8 h. Automatically, culture medium was then transferred to the second chamber where it remained for another 8 h. Thereby the whole volume of culture medium of each chamber was changed three times a day.

at 37 °C. Isolated cells were cultured in flasks, passaged several times (5–7 passages) with trypsin (0.25% in PBS) and seeded in Ham's F12 medium containing fetal calf serum, antibiotics, antimycotics and vitamin C/E (see above) onto coverslips (∅ 13 mm) mounted in Minucell carriers (see above for chondrocytes) in a monolayer at a density of 50,000 cells/cm².

2.3. Co-culture of chondrocytes and synoviocytes in a perfusion chamber

For experimental studies three different groups of chondrocyte- and synovial fibroblast-loaded carriers were performed. The common configuration of this setup was composed of two perfusion chambers (Minucells®, Germany) which were connected by a silicone tube (Fig. 1). Culture medium remained in each chamber for 8 h. Then the whole medium of the first chamber was automatically transferred to the second chamber by a peristaltic pump where it remained for another 8 h. The entire volume of each chamber (18 ml) was changed three times a day. All work was carried out in an incubator (37 °C, 5% CO₂).

In protocol A (Fig. 2) the first chamber was left empty whereas the second chamber was fit with chondrocyte loaded carriers. In protocol B synovial fibroblast-loaded carriers were placed in the first chamber and chondrocyte loaded carriers in the second chamber. For protocol C the first chamber was left empty, whereas chondrocyte and synovial fibroblast-loaded carriers were placed together in the second chamber.

2.4. Measurement of DNA-content

DNA content of mono- and co-cultured chondrocytes was measured fluorometrically by intercalation of ethidium bromide. Cells were isolated mechanically and resuspended in 1 ml PBS (test sample). The blank contains 1 ml PBS without cells. 0.5 ml of RNase (5 kU/ml) and 0.5 ml of pronase (0.7 U/ml) were added and incubated at 37 °C for 30 min. Finally, 0.5 ml of ethidiumbromide (25 µg/ml) was added, test samples and blank incubated for another 30 min and measured fluorometrically (Ex 365 nm, Em 590 nm). DNA-content was estimated using a standard curve (using semen of salmon).
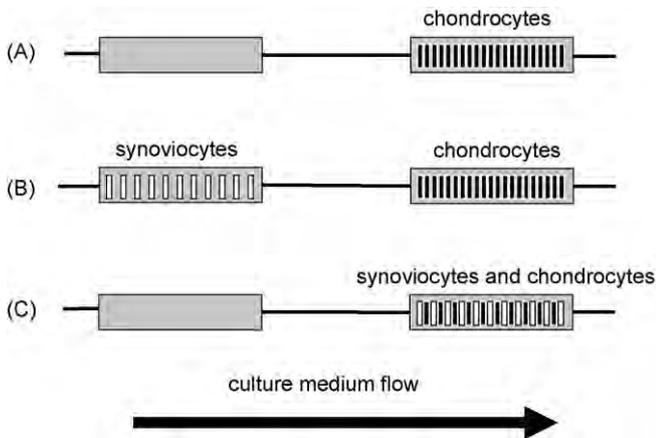
Fig. 2. Three different setups were established. Protocol (A) Monocultured chondrocytes (chamber II), chamber I was left empty; Protocol (B) synovial fibroblasts (Chamber I) and chondrocytes (chamber II) were placed in two different chambers, connected by a silicone tube; Protocol (C) co-culturing of chondrocytes and synovial fibroblasts in a common chamber (chamber II), chamber I was left empty.

2.5. Immunocytochemistry

Coverslips were removed from carrier rings, rinsed three times with Tris-buffered saline (=TBS containing 0.14 M NaCl in 20 mM Tris/HCl buffer; pH 7.4) and fixed with acetone at −20 °C for 10 min. Activity of unspecific peroxidases was blocked by incubation with 0.6% H_2O_2 in methanol for 20 min. Afterwards monolayers were rinsed with TBS again.

Chondrocytes were incubated with the primary antibody (collagen type I: MAB mouse anti-collagen-type I 1:1000 in TBS, Sigma, Germany; collagen type II: MAB mouse anti-collagen-type II 1:1000 in TBS, DSHB Iowa, USA) for 60 min and rinsed three times with TBS. Cells were incubated with the secondary antibody (MAB rabbit anti-mouse IgG HRP-conjugated 1:200 in TBS/1% bovine serum, DAKO, Germany) for 30 min. After rinsing with TBS an incubation with the tertiary antibody (MAB goat antirabbit IgG HRP-conjugated 1:100 in TBS/1% bovine serum) for 30 min followed. Chondrocyte monolayers were rinsed with TBS. Immunoreactivity was demonstrated using diaminobenzidine (DAB-Kit, Vector Laboratories, USA). Cell nuclei were stained with Meyer's hemalum (Merck, Darmstadt, Germany) for 30 s. Controls were done without the primary or secondary antibody.

2.6. Measurement of [³H]-proline incorporation

Chondrocytes of protocols A–C were removed after 10 days of culture. The monolayers mounted by carrier rings were transferred into 24-well plates and rinsed two times with HBSS. The cells were incubated for 8 h in Ham's F12 containing 10 μCi [³H]-proline/ml, fetal calf serum (10%), antibiotics, antimycotics and vitamin C/E (see above). Afterward the supernatant was removed and the monolayers were rinsed three times with PBS including 1 mmol proline for 20 min. The cells were digested at 65 °C in a papain solution (0.1 M Na_2HPO_4, 0.01 M Na_2EDTA, 0.125 mg/ml papain) over night. The next day 200 μl of each sample were mixed with 2 ml of scintillation fluid and measured by a scintillation counter. 500 μl of each sample were used for measurement of DNA-content (see above).

2.7. Measurement of [³⁵S]sulfate incorporation in extracellular macromolecules

Similar to the procedure of [³H]-proline incorporation measurement, chondrocytes of all three setups (A–C) were removed after 10 days of culture. Monolayers were also transferred into 24-well plates and rinsed two times with HBSS. The cells were incubated for 8 h in 500 μl Ham's F12 per well containing 10 μCi [³⁵S]sulfate/ml, fetal calf serum (10%), antibiotics, antimycotics and vitamin C and E (see above). In order to separate the [³⁵S]sulfate incorporated in macromolecules from the free [³⁵S]sulfate the supernatants were centrifuged in centrifugal filter. 200 μl of each macromolecular sample were mixed with 2 ml scintillation fluid and measured using a scintillation counter. Monolayers were rinsed three times with PBS, isolated mechanically and resuspended in 1 ml PBS for the measurement of DNA-content (see above).

2.8. Dimethylene blue assay

After 10 days of culture mono- and co-cultured chondrocytes (setup A/B/C) were transferred into 24-well plates and rinsed two times with HBSS. Cells were incubated for 8 h in 500 μl Ham's F12 per well containing fetal calf serum (10%), antibiotics, antimycotics and vitamin C and E (see above). Supernatants were removed and collected for testing.

Dye solution was prepared mixing 16 μg/ml 1.9 dimethyl-methylene blue, 0.03 M sodium formate and 0.2% formic acid (pH 6.8). For assay standards chondroitin-6-sulfate (10 mg/ml) was solved in PBE (0.1 M Na_2HPO_4, 0.01 M Na_2EDTA in pure water) and different dilutions were used.

900 μl of DMB solution were mixed with 100 μl of test sample, standard or incubation medium respectively. Absorbance was measured at 525 nm in a semi-micro-spectrophotometer. DNA-content of the cell monolayers was determined (see above) and GAG-release calculated as μg/μg DNA.

2.9. ELISA for detection of collagen type II

In order to quantify the amount of collagen type II-protein in cell culture an ELISA kit (Arthrogen-CIA® Capture ELISA, Chondrex, USA) was used. Mono- and co-cultured chondrocytes were removed after 10 days of culture, placed in a 24-well plate and rinsed two times with HBSS. Monolayers were incubated with acetic acid (0.05 M; pH 2.8–3.0). 1/10 volume of pepsin solution (1 mg/ml in 0.05 M acetic acid) was added to the cell layer for digestion at 4 °C over night. Next day 1/10 volume of 1.0 M Tris, 2.0 M NaCl and 50 mM $CaCl_2$ (pH 8.0) were added. The remaining polymeric collagen was monomerized by an incubation started by addition of 1/10 volume of pancreatic elastase (1 mg/ml in TSB; 35 °C; 30 min). After centrifugation the supernatant was used to assay for type II collagen content according to the manufactures manual (Arthrogen-CIA® Capture ELISA system).

2.10. Release of lactate dehydrogenase

Mono- and co-cultured chondrocytes were removed after 10 days of culture and transferred into 24-well plates. The monolayers were washed two times with HBSS and incubated in Ham's F12 for 8 h (without phenol red) in the presence or absence of 1 mM H_2O_2. The release of lactate dehydrogenase was measured in supernatants using a cytotoxicity kit (Boehringer Mannheim, Germany). Maximal release of lactate dehydrogenase was used as a reference value. Therefore, chondrocytes were incubated with Triton X.

2.11. Statistical methods

For statistical analysis Statistica® 6.0 (StatSoft Inc.) was used. All analysis was done by a hospital statistician. For parametric data, Friedmans ANOVA test and Wilcoxon test were used. Significant difference was defined as $p > 0.05$.
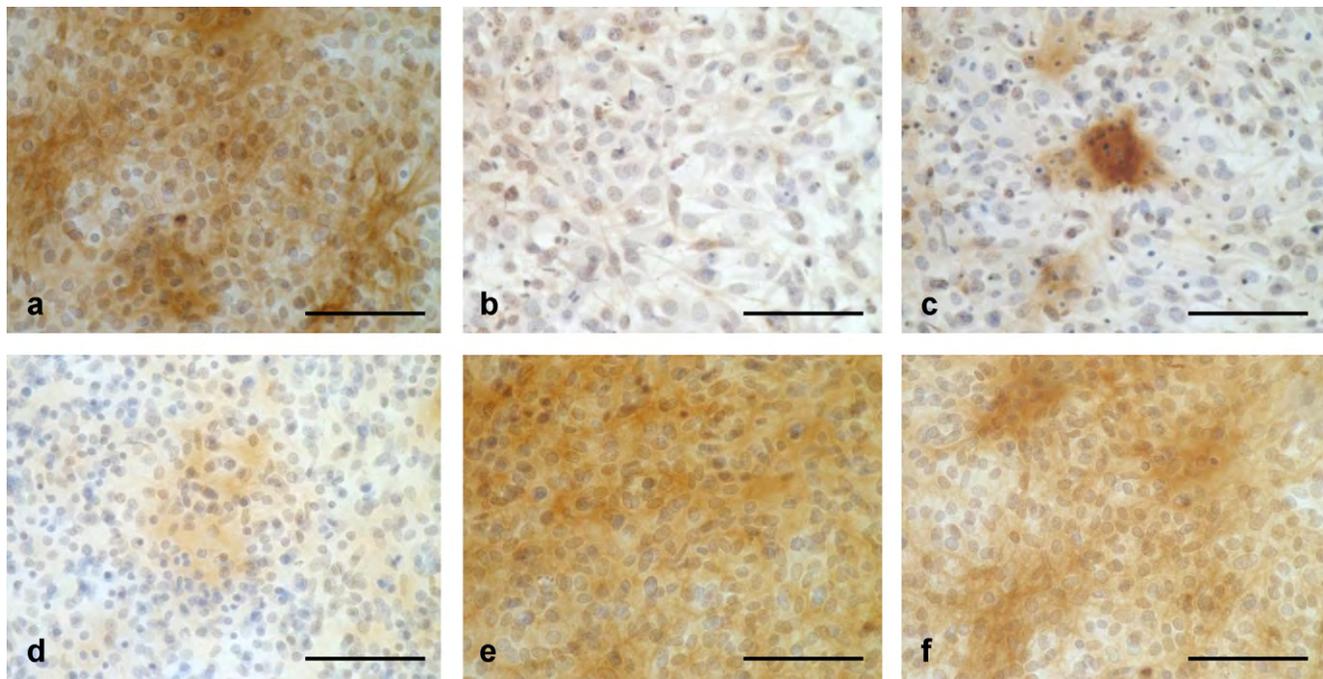
Fig. 3. (a–f) Strong immunostaining of collagen type II in the extracellular matrix of monocultured chondrocytes (a) after 10 days of culture. Chondrocytes cultured with synovial supernatants (b) and chondrocytes co-cultured with synovial fibroblasts showed a much weaker staining. In contrast, only a few cells of monocultured chondrocytes showed a positive immunostaining for collagen type I (d). Chondrocytes cultured with synovial supernatants (e) and co-cultured chondrocytes (f) were strongly immunopositive for collagen type I after 10 days of culture. Scale bars: 100 μm.

3. Results

3.1. Perfusion co-culture system

In order to compare the influence of synovial supernatants and co-culturing with synovial fibroblast on chondrocyte metabolism we established a new perfusion co-culture system. The volume of each perfusion chamber (18 ml) and the period of medium change lead to a 10-fold higher exchange of medium in comparison to experiments in conventional culture dishes with a medium change two times a week. The pH remained stable in mono- as well as in co-cultures (7.39–7.42) during the cultivation period indicating a sufficient supply with oxygen and nutrients. In contrast, cultivation of chondrocytes in a conventional 24-well plate showed significant changes of the pH during a culture period of 3 days (7.21–7.07).

3.2. Morphology and Immunocytochemistry

The morphology of chondrocytes was not influenced by co-culture either with synovial fibroblasts or synovial supernatants. The cells grew in a confluent monolayer and showed the typical polygonal shape. Within the tested time period (12 days) no significant change in morphology was observed. Synoviocytes showed a spindle-shaped fibroblast-like appearance. There were no significant morphological differences between mono- and co-cultured synovial fibroblasts.

Characterization of chondrocyte monolayer was done immunocytochemically after 10 days of culture (Fig. 3a–f). Co-cultured cells (Fig. 3c) showed less collagen type II staining than those grown in monoculture (Fig. 3a). Cells cultured with synovial supernatants (Fig. 3b) only showed sporadic collagen type II staining. In contrast, immunoreactive staining for collagen type I was stronger, when chondrocytes were co-cultured with synovial fibroblasts (Fig. 3f) or with synovial supernatants (Fig. 3e). Monocultured chondrocytes (Fig. 3d) showed less collagen type I staining.

3.3. Proliferation

The DNA content of mono- (A) and co-cultured chondrocytes (C) and cells cultured with synovial supernatants (B) was measured after 1, 2, 5, 8 and 12 days of culture (Fig. 4). Proliferation increased between culture days 5 and 8 in all three setups. After 10 days of culture a confluent cell layer was reached. DNA content showed no significant differences (A → B, $p = 0.32$; A → C, $p = 0.28$).

3.4. Protein biosynthetic activity and extracellular collagen type II deposition

Biosynthetic activity of monocultured chondrocytes or chondrocytes co-cultured with synovial fibroblasts or synovial super-
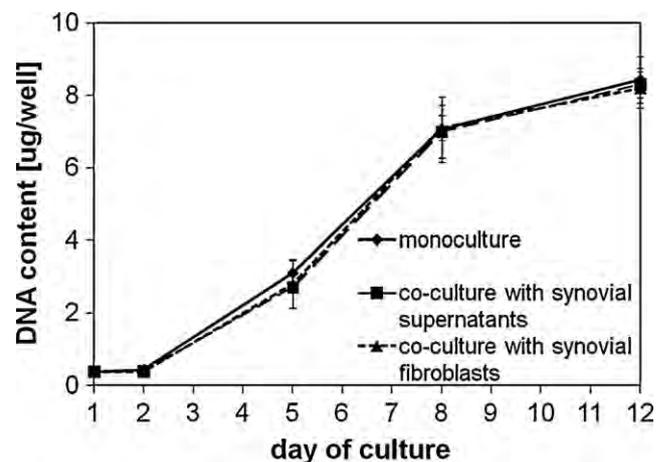
Fig. 4. Proliferation of monocultured (rhombus), co-cultured (triangle) chondrocytes and proliferation of chondrocytes cultured with synovial supernatants (square) was estimated by measuring DNA content after culture days 1, 2, 5, 8 and 12. After 12 days of culture the total DNA content per well in all three groups was equal (A → B, $p = 0.19$; A → C, $p = 0.22$). Shown are mean values (±SD); $n = 5$.
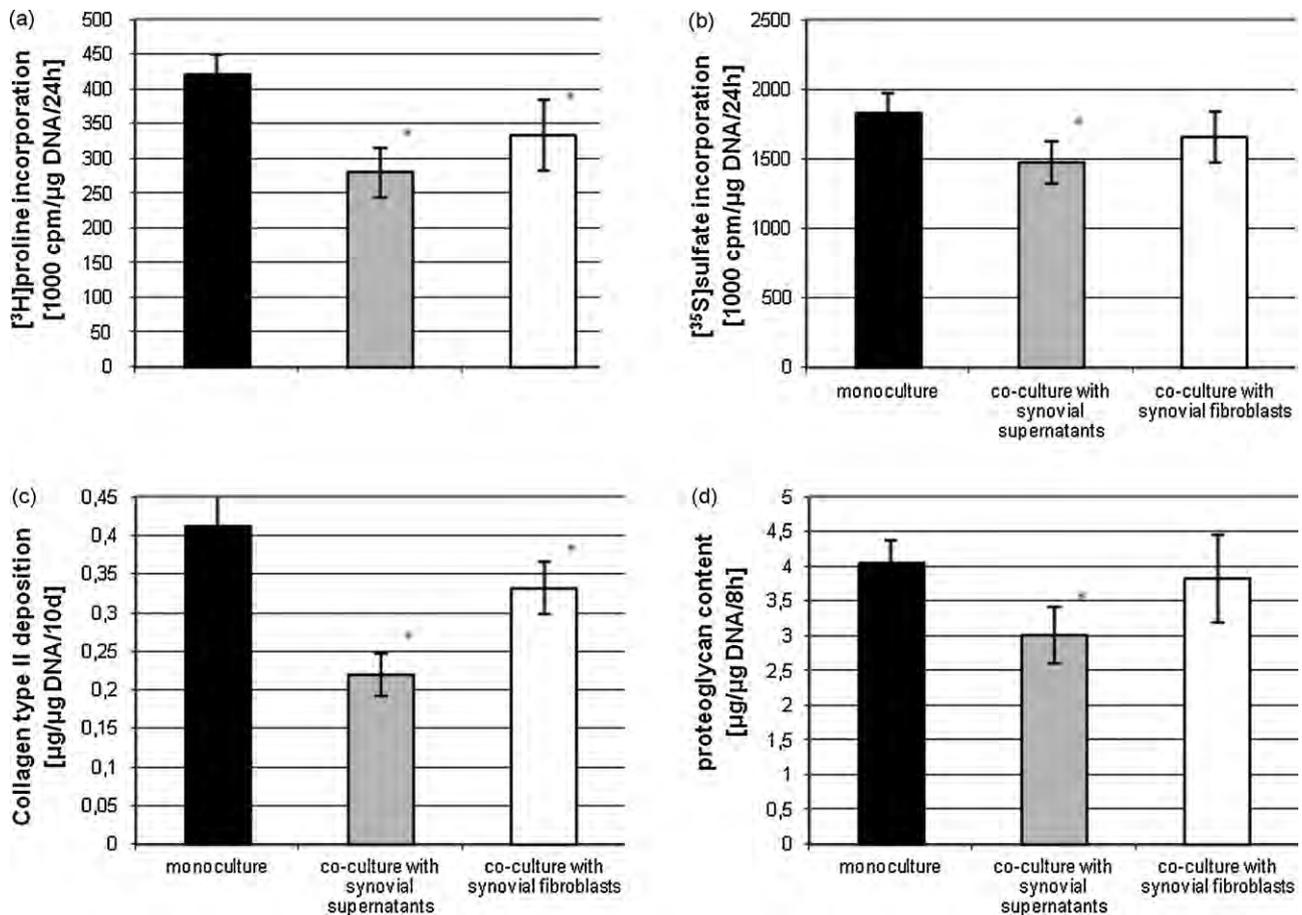
Fig. 5. (a) Biosynthetic activity of chondrocytes measured by incorporation of [³H]proline. After 10 days of culture biosynthetic activity was reduced by synovial cells and even more by synovial supernatants. (b) Extracellular deposition of collagen type II was measured by ELISA. In accordance to the immunocytochemical findings there are lower amounts of collagen type II when chondrocytes are co-cultured with synovial fibroblasts or synovial supernatants. (c and d) Radiolabelled sulfate incorporation and measurement of dimethylene blue assay (DMB) were used to determine proteoglycan content and release. Chondrocytes cultured with synovial supernatants showed a significant decrease in [³⁵S] labelled proteoglycan release (c) and proteoglycan content (d).

natants was measured by incorporation of [³H]proline. After 10 days of culture [³H]proline incorporation in chondrocytes cultured with synovial supernatant was significantly lower (about 33%, $p < 0.05$) than in monocultured cells (Fig. 5a). A significant decrease of proline uptake could also be seen in chondrocytes co-cultured with synovial fibroblasts (about 20%, $p < 0.05$). In concordance with the results of proline incorporation quantification of collagen type II by ELISA after 10 days of culture showed significant lower amounts of collagen type II deposition when chondrocytes were cultured with synovial supernatants (decrease about 56%, $p < 0.002$) or co-cultured with synovial fibroblasts (19%, $p < 0.05$) (Fig. 5b).

3.5. Proteoglycan release and content

Radiolabelled sulfate incorporation and measurement of dimethylene blue assay (DMB) were used to determine proteoglycan content and release. Monocultured chondrocytes showed higher values of [³⁵S]labelled proteoglycan release into the medium and higher levels of proteoglycan content measured by DMB than chondrocytes cultured with synovial supernatants or co-cultured with synovial fibroblasts (Fig. 5c and d). Synovial supernatants caused a decrease in [³⁵S]labelled proteoglycan release about 19% ($p < 0.05$) and proteoglycan content about 24% ($p < 0.05$). Chondrocytes co-cultured with synovial fibroblasts showed a slight decrease without statistical significance.
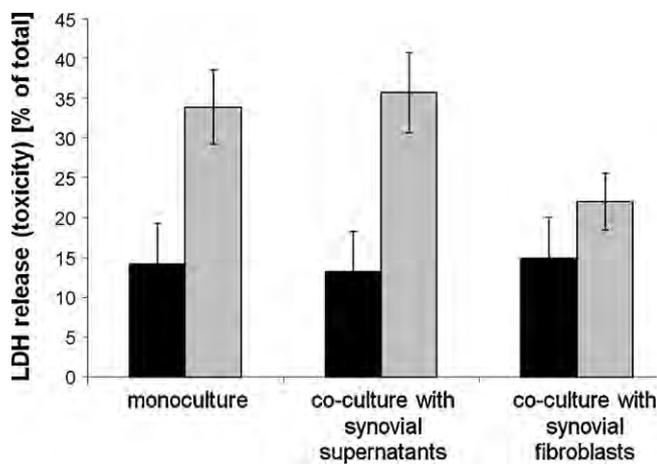
3.6. Release of lactate dehydrogenase

To analyze the toxicity of reactive oxygen species on monocultured and co-cultured chondrocytes the release of lactate dehydrogenase (LDH) was measured. Monocultured chondrocytes showed an increased LDH release after incubation with 1 mM H_2O_2 from 14% (base rate) to 33% of total. Co-cultured chondrocytes showed a similar base rate (15%) but a significant lower LDH release after incubation with H_2O_2 (22%, $p < 0.05$). This effect could not be found in chondrocytes cultured with synovial supernatants (13.5–35%) (Fig. 6).

4. Discussion

Since cartilage is an avascular tissue and the subchondral bone is thought to be an impermeable barrier, chondrocyte nutrition in synovial joints is dependent on diffusion of molecules via the synovial fluid. Generally, to imitate the interactions between cells or different tissues co-culturing might be a more realistic simulation of the in vivo situation (Nevo et al., 1993; D'Andrea et al., 1998; Kurz et al., 1999).

Apart from cultivation of chondrocytes with synovial supernatants only a few reports described co-culturing of chondrocyte and synovial cells. In principle these reports are embarked on two different strategies. One of them is based on culturing chondrocytes and synoviocytes together in a single well in monolayer culture

Fig. 6. Release of lactate dehydrogenase (LDH). Monocultured chondrocytes showed an increased LDH release after incubation with 1 mM H_2O_2 from 14% (base rate) to 33% of total. Co-cultured chondrocytes showed a similar base rate (15%) but a significant lower LDH release after incubation with H_2O_2 (22%, $p < 0.05$). This effect could not be found in chondrocytes cultured with synovial supernatants (13.5–35%).

(Nevo et al., 1993; D'Andrea et al., 1998). This technique did not allow separation of chondrocytes and synoviocytes for further biochemical investigations. Furthermore, these co-culturing models do not represent the in vivo situation, since chondrocytes and synoviocytes are not directly contact with each other in vivo, except for a small zone at the chondral–synovial junction.

Another strategy of co-culturing chondrocytes and synoviocytes was published by Kurz et al. (1999). Both cell populations were separated by a micro-porous membrane that allowed communication via the medium without direct contact of the cells. Cells could be easily separated for further studies. A similar setup was used by Huch et al. (2001) who cultured human osteoarthritic chondrocytes in alginate beads together with monolayers of human synovial fibroblasts. Although these co-culture systems represented a more sophisticated tool to investigate the interaction of chondrocytes and synoviocytes some disadvantages still remained. Specific effects of synoviocytes on chondrocyte metabolism could not be distinguished from non-specific effects as a result of different pH or shortage of nutrients in mono- and co-culture.

Our newly established co-culture system allows investigating the influence of synovial supernatants compared to the influence of synovial cells on chondrocytes. Using a perfusion chamber system (Minuth et al., 2000), a constant pH was provided and the problem of unstable nutrient conditions was minimized by a 10-fold higher exchange-rate of medium compared to conventional culture conditions.

We investigated the proliferation of monocultured chondrocytes compared to cells co-cultured with synovial fibroblasts or cultured with synovial supernatants by measuring the total DNA content. Initially, monocultured chondrocytes proliferate faster than chondrocytes which were influenced by synovial cells or synovial supernatants. However, after a culture period of 10 days chondrocytes in all three experimental setups reached confluence with similar DNA content. In accordance with our results, bovine synovial fluid used as culture medium decreased the thymidine uptake of chondrocytes in vitro (Lee et al., 1997). Components of synovial fluid, such as interleukin-1 and interleukin-6, have been found to be inhibitors of chondrocyte proliferation (Blanco and Lotz, 1995; Iwamoto et al., 1989; Jikko et al., 1998) and even these cytokines were synthesized by unstimulated synovial fibroblasts (Engle et al., 1997; Chen et al., 1998). In contrast to our results, Kurz et al. (1999) found an increase of DNA content and thymidine uptake in chondrocytes co-cultured with synovial fibroblasts. How-

ever, they used a different setup with a synovial cell line consisting predominantly of type B cells (HIG-82, American Type Culture Collection) and their proliferation assay already stopped after 6 days of culture.

Matrix deposition was shown immunocytochemically by detecting of collagen type I and II in the pericellular matrix. In our setup monocultured chondrocytes showed a positive staining for collagen type II even after 10 days of culture. The staining for collagen type I was immunonegative. However, several reports demonstrated a positive staining for collagen type I already after 4–6 days of culture (Norby et al., 1977; von der Mark et al., 1977). For interpretation of these results one should consider the differences between a conventional stationary culture model and a perfusion system. In addition to a stable pH and a constant renewing of nutrient factors perfusion could induce a shear stress that leads to an increase of TGF-beta 1 (Malaviya and Nerem, 2002). TGF-beta 1 again is able to increase collagen type II expression in chondrocytes (Livne, 1994).

Immunostaining of collagen type I and II in co-cultured chondrocytes or chondrocytes cultured with synovial supernatants seemed to be different, since they showed a positive staining for collagen type I even after 5 days of culture and a weaker staining for collagen type II. We reasoned, that synovial fibroblasts as wells as synovial supernatants lead to an earlier dedifferentiation of isolated chondrocytes in vitro.

Additionally, we studied the effect of co-culturing regarding synthesis, release and deposition of extracellular matrix. Collagen type II deposition was significantly reduced by co-cultured chondrocytes and cells cultured with synovial supernatants. Theoretically, two different mechanisms could be responsible for the decrease of collagen type II deposition: a reduction of synthesis as well as a degradation of extracellular matrix. Incorporation of [³H]proline has been described as a tool to estimate biosynthetic activity of chondrocytes and was interpreted as a marker for collagen synthesis in previous reports (Israel et al., 1968). We found a decrease of [³H]proline incorporation in co-cultures as well as in chondrocytes cultured with synovial supernatants. Consistently, Evans et al. (1987) reported an inhibition of [³H]proline incorporation into collagenous proteins by supernatants of an established line of synovial fibroblasts (HIG-82). We conclude that the decrease of collagen type II at least depends on the suppression of protein synthesis partially. On the other hand it is well-known that non-activated as well as activated synovial fibroblasts release cytokines like interleukin-1 and TNF-α and that they are able to synthesize matrix degrading enzymes (like MMPs) (Engle et al., 1997; Legendre et al., 1993; Lin et al., 1996; Ritchlin, 2000). These molecules released by synovial fibroblast are able to degrade the collagen network of chondrocytes directly (MMPs) or indirectly by activating chondrocytes to release neutral proteinases or PGE₂ (Evans et al., 1987). We suggest that the decrease of collagen type II deposition in co-culture or under the influence of synovial supernatants is not merely caused by the suppression of biosynthetic activity but is also due to an enhanced proteolytic activity.

According to the role of synovial fibroblasts regarding collagen type II matrix metabolism we found a decreased release into the medium and a lower content of proteoglycans in the matrix around co-cultured chondrocytes. [³⁵S] sulfate incorporation and total proteoglycan content were significantly reduced by co-culturing and culturing with synovial supernatants. Huch et al. (2001) reported a decrease of proteoglycan content and release of [³⁵S]labelled proteoglycans in human osteoarthritic chondrocytes co-cultured with synovial fibroblast. These results were supported by Evans et al. (1987) showing a decrease of [³⁵S]incorporation of chondrocytes after incubation with synovial supernatants. Obviously, factors which are released by synovial fibroblasts modulate the proteoglycan metabolism as well. Interleukin-1 activates chondrocytes

to synthesize aggrecanase (Sandy et al., 1998; Pratta et al., 2003) and might be involved in proteoglycan degradation.

It is conspicuous, that the decrease of biosynthetic activity, collagen type II matrix deposition, proteoglycan release and GAG content is higher in chondrocytes cultured with synovial supernatants compared to chondrocytes co-cultured with synovial fibroblasts. Particularly with regard to the fact, that the same number of synovial fibroblasts was used in both setups. We suppose that synovial catabolic effects are reduced by co-cultured chondrocytes. However, the methods of protection are still unknown.

The physiological environment of cartilage is characterized by a low oxygen partial pressure (Stockwell, 1983). Thus, we were interested in the interactions between chondrocytes and synoviocytes regarding to radical attack. We have demonstrated that membrane damage by hydrogen peroxide is significantly reduced when chondrocytes are co-cultured with synovial fibroblasts. According to the results of Kurz et al. (1999) this effect is strictly dependent on the presence of synovial cells since the release of lactate dehydrogenase increased in chondrocyte monolayers cultivated with synovial supernatants. Former reports have shown, that interleukin-6 related suppression of matrix degradation is among other metabolic pathways dependent on the inhibition of superoxide production of chondrocytes (Shingu et al., 1994). Synovial fibroblasts as well as TNF-α stimulated chondrocytes are known to be a source of IL-6 (Shinmei et al., 1989). But it remains unknown why this protective effect is dependent on the presence of synovial cells and could not be found in chondrocytes cultured with synovial supernatants.

In conclusion, we established a new co-culture system of synovial fibroblasts and chondrocytes in order to simulate in vivo interactions of both cell types. The setup minimizes artificial interferences of conventional stationary co-culture systems and allows comparing the influence of synovial supernatants and cell-dependent effects of co-cultured synovial fibroblasts on chondrocytes. Our results suggest an overbalance of catabolic effects of synoviocytes on the metabolism of chondrocytes.

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