Changes in the ratio of type-I and type-II collagen expression during monolayer culture of human chondrocytes

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We compared the changes in the ratio of type-I and type-II collagen in monolayer cultures of human articular chondrocytes (HAC). HAC were isolated from samples of cartilage from normal joints and cultivated in monolayer for up to 46 days. Expression of collagen type-I and type-II was determined by immunocytochemistry, Western blotting, and the nested reverse transcription polymerase chain reaction (RT-PCR), and quantified by real-time PCR.

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The main finding was that expression of the genes encoding collagen type I and II was highly time-dependent and the ratio of collagen type II to I (CII/CI), defined as an index of cell differentiation, was significantly higher (215- to 480-fold) at the beginning of the culture. At the end of the experimental culture time, ratios between 0.1 and 1 were reached.

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Autologous chondrocyte transplantation (ACT) has been introduced as a new option for the biological treatment of patients with focal damage to cartilage. This approach is hampered by the need for the prior cultivation of cells in vitro in order to increase the number of chondrocytes. The underlying mechanisms of proliferation and differentiation of chondrocytes need to be evaluated.

Chondrocytes comprise the single component of adult hyaline cartilage and are to be terminally differentiated cells which maintain the matrix of the extracellular cartilage. The major components of the extracellular matrix synthesised by these specialised cells include highly cross-linked collagen fibrils, primarily consisting of type-II collagen molecules which interact with other cartilage-specific collagens such as type IX and type XI. Large aggregating proteoglycan aggregan, small proteoglycans and other specific and non-specific matrix proteins are expressed at defined stages during development and growth.

Collagen is a multigene family of extracellular structural proteins. Fibrillar collagen is a subfamily of these proteins which contain rigid, rod-like molecules with three α chains folded into a right-handed collagen triple helix. Depending on the type of collagen and on the tissue, triple helices can either be homo- or heterotrimers. Collagen type I is usually an α1(1)2 + α2(1) heterotrimer, while collagen type II is a homotrimer of the form, α1(11)3. Various techniques have been used to describe the synthesis of collagen within chondrocytes including immunocytochemistry, SDS-Page, Western blotting, in situ hybridisation, Northern blotting and the reverse transcription polymerase chain reaction (RT-PCR). These techniques are not sensitive enough to detect low-level gene expression and are not accurate enough to quantify the full range of expression. The non-quantitative technologies are not sensitive enough to detect low-level gene expression. Real-time PCR allows accurate reproducible quantification of mRNA without the need for post-PCR processing. Assessing the metabolic state of chondrocytes is potentially useful for characterising and staging injury to and repair of cartilage.

Our hypothesis was that real-time PCR could identify the quantitative patterns of type-
I and type-II collagen gene transcription of human articular chondrocytes (HAC) grown in monolayer culture.

Materials and Methods

Cartilage specimens. Samples of articular cartilage were collected from the hips of eight patients with no history of joint disease who were scheduled to undergo joint replacement after fracture of the femoral neck. Their mean age was 78.2 years (61 to 87). Non-calciﬁed cartilage from the area superficial to the tidemark was dissected from the bone immediately after surgery. The samples were prepared for cell isolation, ﬁxed in 4% buffered formalin at 4°C for 24 hours for histological analysis or homogenised in Trizol (Life Technologies, Rockville, Maryland) for preparation of mRNA for immunocytochemistry, Western blotting and preparation of RNA. Two sample pools of cells from four patients were used.

Isolation and monolayer culture of human articular chondrocytes. The samples of cartilage, were collected in Dulbecco’s modiﬁed Eagle’s medium (DMEM; Life Technologies) enriched with 30 µg/ml of gentamicin and 5 µg/ml of amphotericin B (Life Technologies). The cartilage was diced into pieces of 1 to 3 mm² and placed in DMEM containing 50 µg/ml of amphotericin B (Life Technologies). The cartilage was diced into pieces of 1 to 3 mm² and placed in DMEM containing 200 U/ml of collagenase (Sigma Chemical, St Louis, Missouri). The tube was capped, covered with Parafilm, placed on an orbital shaker at 25 rpm, and incubated at 37°C for 20 hours. The following day, the digestate was resuspended and centrifuged at 1000 rpm for ten minutes. The supernatant was removed and the pellet gently resuspended with 10 ml of calcium- and magnesium-free phosphate-buffered saline (PBS). The suspension was ﬁltered through a 100 µm mesh into a sterile polypropylene tube. Cells were centrifuged and washed twice more, then counted in a haemocytometer, resuspended in medium and placed into T-75 ﬂasks and chamber slides at a concentration of 1.5 x 10⁴/cm². The growth medium for all cultures was Dulbecco’s modiﬁed Eagle medium with 450 mg/dl of glucose (Life Technologies). The medium was supplemented with 10% fetal bovine serum, 2 g/l of HEPES, 1% L-glutamine, 100 µg/ml of streptomycin and 2.5 µg/ml of fungizone (all from Life Technologies) and 50 mg/l of ascorbic acid (Sigma Chemical). Cells were passaged at 80% conﬂuence by release from the dishes with a solution of 0.1% trypsin and 0.1% EDTA (Life Technologies) in a ratio of 1:3. All the cell cultures were grown in a humidified CO₂ incubator (Cytoperm 8080; Heraeus Instruments GmbH, Hanau, Germany) under 95% air and 5% CO₂, at 37°C. Human ﬁbroblasts isolated from the ﬁbrous hip capsule were cultured as control cells.

Histological analysis. Fixed samples were dehydrated, embedded in parafﬁn and cut into sections 5 µm in size. Slices of cartilage were cut vertically from the surface to the bottom. The sections were stained with haematoxylin and eosin and Safranin O for sulphated glycosaminoglycans (GAG) and were then evaluated based on the score of Mankin et al.¹⁸ by an independent experienced observer.

Immunocytochemistry. The chamber slides of cultivated cells were ﬁxed at intervals of seven days with acetone for ten minutes and stained with antibodies against human collagen type I and type II. The antibody against collagen type I (Southern Biotechnology Associates, Birmingham, Alabama) reacts with conformational determinants of human collagen type I. The MAb anti-collagen type II, clone 2B1.5 (Neomakers, Fremont, California), recognises the α1(11) chain of collagen type II. The epitope is localised to the carboxyl-terminal-one quarter of the macromolecule.¹⁹,²⁰ Briefly, slides were blocked for endogenous peroxidase activity with 2% hydrogen peroxide in PBS for 15 to 20 minutes at room temperature. They were then treated with 1 mg/ml of pepsin (Sigma Chemical) in 0.5 M acetic acid (30 minutes at 37°C). The slides were rinsed with PBS and incubated for 30 minutes at 25°C with normal horse serum diluted 1:10 in PBS. Primary antibodies were diluted 1:100 in PBS/2% bovine albumin (BSA, Sigma Chemical). These were incubated with the sections in a humidified chamber for one hour at room temperature. The slides were washed three times with PBS, after incubation at room temperature in a humidised chamber for one hour with biotin-conjugated horse anti-mouse secondary antibody (Vector Laboratories, Burlingame, California) diluted 1:100 in PBS/2% bovine albumin. They were then treated with avidin-biotin (Vectastain ABC Kit; Vector Laboratories) for 30 minutes, after which they were incubated with alkaline phosphatase and substrate solution (Sigma Chemical) for approximately 10 to 15 minutes until colour developed, rinsed with PBS, counterstained with haematoxylin, rinsed with water, dehydrated, and ﬁnally sealed using Eukitt (Kindler GmBH, Freiburg, Germany). All experiments included one positive and two negative controls. The positive controls were native human articular cartilage and ﬁbroblasts positive for the primary antibody used. The negative controls were PBS only, to test for false binding by the secondary antibody, and normal serum of the sample species from which the primary antibody had been prepared.

Collagen phenotyping and Western blot. For the analysis of pepsin-resistant collagens, the cell layers at days 7, 14, 21, 28, 35, and 42 in monolayer culture were solubilised in an equal volume of serum-free culture medium and 1 M ammonium hydroxide and prepared according to the method of Goldring.²¹ Briefly, solubilised samples were digested with 0.5 mg/ml of pepsin in 0.5 M acetic acid for 16 hours at 4°C and lyophilised. The samples were redissolved in 2 x Laemmli Buffer containing 0.1% β-mercaptoethanol and neutralised with additions of 2 M NaOH of 1 µl to titrate the colour of the bromphenol blue in the sample buffer from yellow-green to blue. The protein content of the samples was determined by the dotMERTIC 1 µl Protein Assay (Chemicon International Inc, Temecula, California) according to the manufacturer’s instructions. Purified human collagen type I (Chemicon International) and type II, prepared from samples of native human articular cartilage were used as negative and positive controls for...
Western blot analysis.²² For SDS Page and Western blot, equal amounts of pepsin-digested protein extracts of cultured chondrocytes were separated on 6% PAA gels. One gel was stained with Coomassie Brilliant Blue R250 for band visualisation. For Western Blot analysis with antibodies against collagen I and collagen II, gels were blotted on Immobilon-P membrane with electrophoretic transfer overnight at 4°C, 56V, and 250 mA in blotting buffer containing 10% methanol. Blots were stained with Ponceau S Red to check the transfer of the proteins. After destaining, the membrane was blocked for 1.5 hours with 3% BSA-0.05% Tween in PBS. It was then incubated for 1.5 hours with an antibody against human collagen II (Oncogene Science, San Diego, California) diluted 1:5000 in PBS with 1% BSA and 0.05% Tween. After washing four times for ten minutes with PBS-0.05% Tween, the membrane was incubated for one hour with the second antibody (goat anti-mouse IgG, horseradish peroxidase conjugated; Pierce, New York), diluted 1:30 000 in the solution mentioned above. The membrane was then washed four times for 15 minutes in the washing solution used above. The blot was developed using a chemiluminescence system (SuperSignal Substrate; Pierce Biotechnology Inc, Rockford, Illinois) according to the manufacturer’s instructions and subsequently exposed to x-ray film (Amersham Biosciences, Freiburg, Germany) to visualise collagen type-II-specific bands.

For detection of collagen type I specific bands, the same blot was stripped under the following conditions. The blot was washed twice for ten minutes in washing buffer. Then, it was incubated for one hour in stripping buffer (100 mM 2-mercaptoethanol, 2% SDS, and 62.5 mM Tris-HCl, pH 6.7) at 50°C. The following steps for preparation of the blot for subsequent blocking were performed according to the SuperSignal Substrate protocol. The blot was blocked under the conditions mentioned above. Subsequently, it was incubated for 1.5 hours with an antibody against human collagen type I (Rockland), diluted 1:10 000 in the dilution buffer. After washing, the membrane was incubated for one hour with the second antibody, diluted 1:35 000 in the dilution buffer. The steps for the development of the blot were the same as mentioned previously.

**RNA preparation.** Total RNA was obtained from pooled cells being cultivated in monolayer culture. Cells were harvested every second day by adding 1 ml of Tri Reagent (Sigma Chemical). Lysis of the cells was performed directly on the culture dish. The cell lysate was transferred into a 2.0 ml microcentrifuge tube and 0.2 ml of chloroform were added. The probes were mixed vigorously and incubated at room temperature for ten minutes. The resulting mixture was centrifuged at 12000 x g for 15 minutes at 4°C. Centrifugation separated the mixture into three phases: a red organic phase (containing protein); an interphase (containing DNA); and a colourless upper phase (containing RNA). The aqueous phase was transferred into a fresh tube and 0.5 ml of isopropanol were added, and the probes were mixed. The sample was incubated at room temperature for five minutes. After incubation, the probes were centrifuged at 12000 x g for ten minutes at 4°C. The RNA precipitate formed a pellet on the side and bottom of the tube. Supernatant was removed and the RNA pellet was washed by adding 1 ml of 75% ethanol per 1 ml of TRI reagent. The RNA was briefly dried and dissolved in 0.5% SDS solution. The purity and amount of RNA were determined by measurement of the OD₂₆₀/₂₈₀ ratio. All samples showed purity indices between 1.5 and 1.8.

**cDNA synthesis.** Total RNA (0.2 to 1 µg) was diluted to a volume of 12.5 µl, and 1 µl of oligo(dt)18 primer (20 µM) was added. The RNA was heated for two minutes at 70°C and quenched on ice. Using an Advantage RT-for-PCR Kit (Clontech; BD Biosciences, Palo Alto, California), a master reagent mix, containing 4 µl of 5 x reaction buffer, 1 µl of dNTP mix (10mM each), 1 µl of MMLV reverse transcriptase, and 0.5 µl of RNase inhibitor, was prepared, added to the RNA/Primer mix, and incubated at 42°C for one hour. The cDNA synthesis was stopped by heating the reaction at 94°C for five minutes. The reaction was diluted by adding 80 µl of DEPC-treated water.

**Nested PCR.** An advantage cDNA PCR Kit (Clontech) was used to perform nested PCR. The cycle parameters proposed by the manufacturer were changed as follows: 94°C 1 minute; 25 x (94°C 30 seconds, 68°C 3 minutes) 68°C 3 minutes. Primer sequences: Col1 forward primer: cgaaggttcccggacacagacg; reverse primer: ggccacagaggtagacacggcttc; nested PCR: forward primer: taggctctgctctggagcttc; reverse primer: gaagccagacagggccacgct; Col2A1: forward primer: gacgcgtgccagccctttgattgc; reverse primer:acttcttcctctcgtctccgatg; nested PCR: forward primer: ctctgccggttgtctc; reverse primer: cacccgtaacacctgctc.

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<th>Gene</th>
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<th>Primers concentration used (nM)</th>
<th>Probe</th>
<th>Probe concentration used (nM)</th>
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<td>AAAGGTGCAACGGTGGAGGCTT</td>
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**Table 1.** Description of the designed primers and probes for quantitative real-time PCR.
Photomicrographs of pooled HAC grown in monolayer culture for 42 days using a monoclonal antibody against collagen type II showing (a to g) staining every seven days during the culture (a, day 0 and g, day 42). The spherical to polygonal cell morphology changed to an elongated fibroblast-like phenotype and collagen type II was detectable until day 21 (d) in monolayer culture. Human fibroblasts were used as controls (h). ABC staining with alkaline phosphatase: x 40.
Fig. 2

Photomicrographs of pooled HAC grown in monolayer culture for 42 days using a monoclonal antibody against collagen type I showing (a to g) staining every seven days during the culture (a, day 0 and g, day 42). Expression of collagen type I was found between day 14 (c) and day 42 (g). Human fibroblasts were used as controls (h). ABC staining with alkaline phosphatase: x 40.
Four Southern blotting DNA probes were separated on a 1.2% agarose gel and transferred to a positively charged nylon membrane (Roche, Basel, Switzerland) using an alkaline transfer procedure and were fixed by baking the membrane at 120°C for 20 minutes. Oligonucleotides were 3' end-labelled using digoxigenin-ddUTP (Roche). Hybridisation was performed overnight at 55°C. Oligonucleotide sequences for hybridisation: CoI1: ccgcatctgcaagtaagtcc; CoI2: gcttgacgctacggtg. Filters were washed 2 x 5 minutes at hybridisation temperature with 50 ml 2xSSC, SDS, 0.1%(w/v) per 100 cm² filter, and 2 x 5 min at hybridisation temperature with 50 ml 0.1xSSC, SDS, 0.1%(w/v) per 100 cm². A DIG Luminescent Detection Kit (Roche) was used to detect DIG-labelled oligonucleotides according to the manufacturer’s instructions.

**Real-time PCR.** Primers and probes of collagen type I and type II were designed using the primer3 program to create oligonucleotides with similar melting temperatures and minimal self-complementarity. To avoid amplification of genomic DNA, the probes were placed at the junction of two exons. Gene specificity of the primers and probes and the absence of DNA polymorphism were confirmed by BLASTN searches. Primers and probes were synthesised from GenXpress (Austria). Primer concentrations were tested for each primer at concentrations of 50 nM, 300 nM, and 900 nM, using the combination which displayed the lowest Ct value. The efficacy of amplification was determined by serial dilutions of cDNA templates, using human fibroblasts as reference tissue for collagen type I and human hyaline cartilage as reference tissue for collagen type II. The description of the designed primers and probes is shown in Table I.

PCR amplification was performed and monitored using an ABI Prism 5700 Sequence Detection System (Perkin-Elmer; Applied Biosystems, Foster City, California). The master mix was based on the Brilliant Quantitative PCR Core Reagent Kit (Stratagene; La Jolla, California). The best results were obtained when using a final concentration of Mg²⁺ of 2.5 mM. Primers and probes were tested and used at concentrations ranging from 50 nM to 900 nM. The thermal cycling conditions comprised the initial steps at 50°C for two minutes and at 95°C for ten minutes. Amplification of the cDNA products was performed with 40 PCR cycles, consisting of a denaturation step at 95°C for 15 seconds and an extension step at 60°C for one minute. All probes were normalised to β-actin, using the pre-developed Taq Man assay (Applied Biosystems). Beta-actin rather than GAPDH was chosen as the reference housekeeping gene, based on a variety of articles which have reported wide variation in the levels of transcription of GAPDH mRNA between individuals and between samples taken from an individual at different time points. All cDNA samples (5 µl in 25 µl) were analysed in triplicate. The final numeric value was calculated as the ratio of the collagens to β-actin and expressed in arbitrary units. Since collagen type II is the typical marker for differentiated chondrocytes in hyaline cartilage, as opposed to collagen type I, which is expressed in dedifferentiated chondrocytes, the ratios of mRNA levels of collagen type II to type I (CII/CI) as a ‘differentiation index’ was defined.

**Statistical analysis.** Descriptive statistical analysis was performed using mean values and the standard error.

**Results**

**Phenotypic characteristics: histology and immunocytochemistry.** The mean Mankin score of the cartilage samples was 1.2 (0.3 to 2.0) and the samples showed few microscopic changes, mostly related to the reduction of Safranin O staining. In monolayer culture, HAC showed extensive morphological changes during the cultivation period. Freshly isolated HAC showed a cell morphology which was either spherical to polygonal and contained a single nucleus which was often eccentric. The cultivated cells had an elongated fibroblast-like phenotype. Immunocytochemical methods showed that the changes in the patterns of expression of cell-associated collagen types occurred around day 21 (14 to 28). Expression of intracellular collagen type II was found in freshly isolated cells and in cells cultivated in monolayer normally until day 21, with a maximum of 28 days (Fig. 1). Synthesis of collagen type I was detectable in cells cultured in monolayer after day 14 and then for the entire observed cultivation period (Fig. 2).
Collagen synthesis: collagen typing with Western blot. The investigation of the synthesised collagen phenotype of HAC grown for different time periods in monolayer culture by Western blot analysis with collagen-specific antibodies showed a synthesis of collagen type II until day 28 (Fig. 3). Detection with collagen type I-specific antibody indicated the beginning of type-I collagen synthesis after day 14 in monolayer culture. At days 7 and 14, only type-II collagen-specific bands appeared whereas at days 35 and 42, only type-I collagen was produced (Fig. 3).

Collagen mRNA expression: nested RT-PCR and real-time PCR. For the analysis of collagen mRNA, HAC in monolayer culture were harvested every second day (0 to 46 days) and nested RT-PCR was performed. A significant expression of collagen type I started at day 12 and continued during the entire cultivation period. A very weak expression was detectable after four days in culture. By contrast, collagen type-II expression was seen during the entire cultivation time in every preparation. As positive and negative controls, cartilage samples and fibroblasts were used. The specificity of the PCR products was confirmed by Southern blotting.

For the quantification of mRNA, real-time PCR was performed using the RNA samples harvested every second day (0 to 46 days) from HAC in monolayer culture. The standard curve for collagen type I was defined with human fibroblasts and the relative value of 1 was assigned to this expression. With regard to the reference gene, β-actin, the relative expression of collagen type I for the entire culture time of 46 days increased 100-fold compared with that at the beginning of the culture (Fig. 6). After the isolation of the HAC (day 0), the relative collagen type-I expression
was $10^4$ lower and increased to levels between $10^{-1}$ and $10^{-2}$ with maximum levels between days 14 and 30. The relative expression of collagen type II was measured over the entire culture period in levels between $1$ and $10^{-3}$ in relation to the reference gene ($\beta$-actin) and was downregulated more than 100-fold. Native cartilage showed a $10^2$ higher expression. During the first days in culture (days 0 to 4), the relative expression was downregulated tenfold and reached the level of 1 after six days. After 16 days, the relative expression of collagen type II decreased continuously to levels between $10^{-2}$ and $10^{-3}$ in relation to the reference gene (Fig. 4).

The collagen II/I ratio as a ‘differentiation index’ showed a sigmoid curve on a logarithmic scale with three phases (Fig. 5). In the first phase, a maximum peak at days 2 to 6 with a 480-fold expression of collagen type II (fourth day) was observed. Between days 8 and 28, the ratio decreased and reached a plateau, with ratios between 0.1 and 1 at the end of the culture time.

**Discussion**

Cultured chondrocytes have served as useful models for studying the differentiation of chondrocytes and the effects of cytokines and growth factors which control the maintenance or suppression of differentiated cartilage phenotypes.\(^8,25\) Freshly isolated human articular chondrocytes express cartilage-specific type-II collagen and continue to do so for several days to weeks in primary monolayer culture.\(^26,27\) During prolonged culture and serial subcultures, these cells begin to express type-I and type-II collagens.\(^11,27\)

A number of tissue-culture studies have shown these cells to be capable of switching collagen synthesis from type II to type I, and *vice versa*, under a variety of experimental conditions.\(^26,33-36\)

In our study, the transition of human articular chondrocytes from the spherical morphology to the flattened morphology of an anchorage-dependent culture was also accompanied by changes in the patterns of collagen expression. Immunocytochemical methods showed that the synthesised cell-associated collagen phenotype changed dramatically between days 21 and 28 in monolayer culture with the replacement of collagen type II. This short interval indicates that most cells had altered their collagen synthesis very quickly.

The collagen phenotype of differentiated chondrocytes is marked by the predominant synthesis of $\alpha_1(11)$ chains, while the de-differentiated phenotype is composed primarily of $\alpha_1(1)$ chains.\(^27\) Switching between the synthesis of these two collagen chains can be properly evaluated by fractionating intact collagen chains with SDS electrophoresis and Western blotting. With the use of specific antibodies, a definitive identification of synthesised collagens is possible in cultures containing a mixture of type-I and type-II collagen.\(^21\) At the beginning of the monolayer culture, the collagen which was present included collagen type II ($\alpha_1(11)$), and was detectable until day 28. The presence of collagen type I was determined by the presence of $\alpha_2(1)$ chains. Collagen type-I synthesis was undetectable in the first two weeks, with very little after 21 days, and predominant after 28 days in culture. With Western blotting, the short interval for the change in collagen type with the replacement of collagen type II between days 21 and 28 in monolayer culture was also observed.

Commonly used methods for the quantification of transcription include Northern blotting and in situ hybridisation,\(^37\) RNAse protection assays,\(^38\) RT-PCR\(^39\) and cDNA arrays.\(^24\) The focus on maximising sensitivity led to the development of ever-more complex procedures. Semi-nested,\(^40\) nested,\(^51\) and even three-step nested\(^42\) RT-PCR techniques increase the sensitivity but compromise the specificity of the reaction.\(^24\)

In our study, we used nested RT-PCR for the qualitative alteration of expression of collagen on the mRNA level. Using this method, collagen type I was detected much earlier in the time course of the cultivation period and started at day 12, compared with protein synthesis. With immunocytochemistry, day 14 was the earliest time-point for the detection of the synthesis of collagen type I. In contrast to the methods for protein typing, the mRNA for collagen type II was found over the entire cultivation period and was not completely replaced by collagen type I. Complete switching and replacement of collagens during the phase of dedifferentiation, as previously described, were not observed.\(^11,27\)

The quantitative changes in the collagen phenotype are currently the best markers for the completion of dedifferentiation and were analysed using real-time PCR.\(^15\) In our study, collagen type I with levels above $10^{-3}$ compared with the reference gene $\beta$-actin was expressed very early starting at day 4 and was the leading collagen type in the second half of the culture period. Compared with the reference gene, the expression increased 100-fold and reached levels between $10^{-1}$ and $10^{-2}$ during the observed culture period. As already observed with nested RT-PCR, collagen type II was measurable during the entire culture time. In the first two weeks, values of 1 were measured which constantly decreased in the following weeks. Overall, expression of collagen type II was downregulated more than 100-fold and reached levels between $10^{-2}$ and $10^{-3}$ in relation to the reference gene. Only in the first days of the monolayer culture was collagen type II reduced. For some specimens of cartilage, in which mRNA was extracted both from the intact tissue and from cells after tissue digestion, PCR analysis showed that the expression of all the genes of interest was markedly and differentially affected by tissue digestion, with differences up to 10 to 15 fold.\(^14\)

The quantitative analysis of the expression of collagen, defined as the CII/C1 ratio, expresses the decrease in collagen type II and the increase in collagen type I. This value was used as a differentiation index to describe the differences between human articular cartilage in normal and osteoarthritic joints.\(^14\) On a logarithmic scale, three periods
of the CII/I ratio of chondrocytes in monolayer culture were observed. The values in the first week formed a plateau with levels between 215-430-fold higher for expression of collagen type II. The maximum peak of 480 was reached at day 4. In this period (days 0 to 8), a differentiated phenotype is present with collagen type II as the leading collagen. The values in this phase are similar to the reported values of normal articular cartilage, with a range of 136.3 to 444.2 and a mean value of 294.6. Between days 10 and 30, the ratio decreased constantly from levels of 28.8 (day 10) to 0.14 (day 30). In this transition phase, collagen type II is nearly completely replaced by collagen type I. The decrease in the CII/I ratio is mainly caused by a change in, and constant downregulation of expression of collagen type II, whereas that of collagen type I changed much less. After day 30, the CII/I ratio reached a steady state with levels around 0.1. The CII/CII ratio can be used to quantify the collagen gene expression in cultured chondrocytes and thus improve the understanding of the process of chondrocyte de- and redifferentiation. Furthermore, this ratio can be used to monitor chondrocytes and their differentiation status in monolayer culture for experimental models or therapeutic use in autologous chondrocyte transplantation.

It should be emphasised that one limitation of our study is that the overall isolation of mRNA was done on pooled cells in culture and therefore the results cannot account for individual differences in the rates of dedifferentiation. The necessity of pooling cells to ensure that sufficient RNA was available limits individual variations in cell growth and differentiation. Because of the heterogeneity of the cell growth as a result of the age of the patient, culture conditions, and individual factors, further studies are needed to evaluate potentially occurring phenotypic changes in certain circumstances. Exploring chondrocyte gene expression in the models of joint injury and repair could help to determine which cellular processes may be suitable targets for therapeutic intervention.

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References


